

**Pharmacognostic Evaluation and Green Synthesis of
Silver/Gold Nanoparticles of *Sageretia thea* (Osbeck) Johnst,
for Biological Screening**



Ph. D Thesis

By

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**A THESIS SUBMITTED TO THE DEPARTMENT OF BOTANY,
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Chapter # 01

INTRODUCTION

1.1 HISTORY OF MEDICINAL PLANTS

Allah says in the Holy Quran “I have created all the things in the universe for the welfare of mankind and mankind is created for my worship (Al-Quran Surah Nahl, Ayat #12). From the above saying it is clear that all the materials on earth are created for benefit of man and man has to explore these materials through knowledge. From the day first the main struggle of humans was to discover things (plant and animals) for food and treatment of disease. The use of different plant parts and drugs derived from plants used for the treatment of diseases goes back to pre historic times. The Greeks, the Romans, the Egyptians and the people of subcontinent (Pakistan and India) developed their material medica respectively. The Greek medicine was further developed by Muslim Arab and Romans (Ackerknecht, 1973). Later on the plants were classified into two categories, medicinal plants and food plants. The use of plant parts and different extracts derived from plants for the curement of different disease is called herbalism. WHO defined medicinal plants as “A whole plant or its organs contains such type of substances which have therapeutic value and is also good for synthetic drugs production. Many plants naturally synthesize useful substances as primary & secondary metabolites, in which secondary metabolites are more useful for human and animal health maintenance. These substances also play role in health maintenance as well as a defense mechanism for plants itself against herbivores, microbes and insects (Lai and Roy 2004). From the history of medicines it is clear that from the very beginning human has been dependent on plant for health maintenance (Bensky *et al.*, 1993). Plants use as a medicine in both eastern and western cultures dates back to 6,000 year ago (Solecki and Shanidar, 1975). The founder of Greek’s pharmacy and medicine Hippocrates has mentioned in his writing about 400 items as a medicinal substances.

1.2 Importance of Medicinal Plants

In ancient civilization the plant extracts were considered as a cure for different disease (Grabely and Thiericke, 1999). Today 50% of all the medicines are obtained from natural products in which 25% are from higher plants (Cragg and Newman, 2005).

1.3 Pharmacognosy

The term Pharmacognosy was first used about 200 years ago by C. A. Sydlar (Kinghorn, 2001). "A molecular science that explores naturally occurring structure activity relationship with a drug potential" Bruhn and Bohlin (1997). According to "American Society of Pharmacognosy,, Pharmacognosy is the study of chemical, physical and biochemical properties of natural drugs, their potential, drug substances, origin and the discovery of new drugs from natural sources. The study of multicellular and unicellular organism including fungi, bacteria, aquatic organisms, aquatic plants, terrestrial plants and animals, all come in pharmacognostic circle. In broad sense, different parameters like herbal remedies, botanical dietary supplements and searching for new chemical constituents from biological sources used as drugs is "Pharmacognosy" (Cardellina, 2002; Tyler, 1999).

1.4. PHARMACOGNOSTIC STUDY

Pharmacognosy helps us to know the past history, collection, cultivation, drying, preservation, storage, commerce and uses of drugs obtained from biological source (Evans, 2002; Wallis, 2005). Morph-histological study is the preliminary step towards identification and standardization of plants and plants derived drugs (Youngken, 1950).

1.4.1. Macro and microscopic evaluation

Pharmacognostic study helps in identification, authentication and quality assurance of the plant material which contribute to its safe use as a medicine. Different pharmacognostic parameters are used for the identification and standardization of plant material like size, shape, surface and some organoleptic feature like taste, odor, colour etc (Patel *et al.*, 2006). Some microscopic parameters are also used for identification and standardization of crude drugs e. g, leaf constants like vein islet, vein termination number, stomatal

index, palisade cell ratio, trichomes types etc (Anonymous, 1998: Trease and Evens, 2002). Various workers carried out macro-morphological study like Gersbach, (2002), *Prostanthera ovalifolia* leaves ; Taur *et al.*, (2010), *Clitoria ternatea* leaves; Saraswathy *et al.*, (2009), *Cassia auriculata* Linn leaves; Li *et al.*, (2009), *Bupleurum scorzonerifolium* Willd root; Essiett *et al.*, (2010), *Diodia scandens* Sw stem. In the present study, various pharmacognostic parameters of *S. thea* will be worked out.

1.4.2. Microchemical tests

The great importance of medicinal plants is to the health of individual and communities. The importance of medicinal plants is due to the presence of some chemical substances called as active constituents which produce a definite physiological action on the human body. These chemical constituents of medicinal plants are mostly secondary metabolites in the form of alkaloids, saponins, glycosides, volatile oils, tannins, phenolics compound and flavonoids (Hill, 1952; Tyler *et al.*, 1999). These bioactive phytochemicals are investigated in plants through preliminary phytochemical screening tests and are used for the treatment and prevention of various ailments (Tanaka *et al.*, 2002). Various workers like (Musa *et al.*, (2006), *Gisekia pharnacioides*; James and Emmanuel, (2010), *Euphorbia hetrophylla*; Kamboj and Saluja (2010), *Bryophyllum pinnatum*; Kumar and Hemalatha (2010), *Alangium lamarckii*) have carried out microchemical tests on various plants. As no such work is reported on *S. thea*, so in the present work various microchemical tests will be performed on *S. thea*.

1.4.3. Fluorescence test

Different crude when viewed under UV light drugs show different fluorescence at different wavelengths. This is due to the presence of different chemical constituents in the drug. If the drugs do not show fluoresce it is first treated with different reagents which convert them into fluorescence derivatives. The fluorescence analysis of drugs is helpful for identification of crude drugs as well as for the detection of adulterations in whole or powder drugs (Reddy and Chaturvedi, 2010; Ansari *et al.*, 2006; Wallis, 1985). Many researchers worked out fluorescence analysis on different medicinal plants like (Jarald

and Jarald, 2007; Tian *et al.*, 2006) *Polygonum cuspidatum*; Hussain *et al.*, (2011) *Hygrophila auriculata*). As no such fluorescence analysis of *S. thea* has been carried out, so in the present work the leaf, stem and root powder material will be evaluated for fluorescence analysis in order to establish pharmacognostic parameters for this plant.

1.4.4. Elemental analysis

Deficiency of vital and trace elements in human can occur even under the most practical dietary conditions and in many diseased statuses. About 40 different types of elements have been detected in plants and animals. All of these elements play a combating role against various diseases. In human being the deficiency of Ca cause tooth decay, hypertension, paralysis and increase cholesterol level (Soetan *et al.*, 2010). The deficiency of Mg causes semi coma, diabetes mellitus and neurological disturbances (Ahmed and Chaudhary, 2009). For protein and carbohydrate metabolism K is an important element. Plants not only provide energy, but at the same time also supply nutrients to the consumers including man. Nutrients are essential for normal body functioning (Clemens *et al.*, 2004). Medicinal plants is a rich source of organic and inorganic compounds, and most of medicines contain one or more individual elements, therefore provide therapeutic action of the medicine (Singh *et al.*, 1996). Each medicinal plant has its own elemental composition and having pharmacologically important phytoconstituents (Hoffman *et al.*, 1998; Dingman, 2002). The screening of the actual bioactive elements of plants origin and assessment of elemental composition of the widely used medicinal plants is highly essentials (Saiki *et al.*, 1990). In human metabolic reaction macro, micro and trace elements play important role. Trace elements play a combating role in curing of various diseases. In medicinal plants these elements form active compound which is responsible for both their medicinal and toxic properties (Rajurkar and Damame. 1998). Some medicinal plant has toxic elements such as Pb, Cd, which are toxic for health (Garcia *et al.*, 2000; Lekouch *et al.*, 2001; Lo'pez *et al.*, 2000).

1.4.5. Proximate analysis

Proximate and nutritional analysis of medicinal plants plays important role in assessing their nutritional significance. Evaluating the nutritional significant of medicinal plants help to understand the medicinal use of that plants (Pandey *et al.*, 2006). All of these

nutrients like carbohydrate, fats, protein, lipids etc are important for physiological function of human body. The quality and quantity of protein in the seed are important factors in the selection of plants for nutritive value and plant improvement programs (Siddique, 1998). The studies related with therapeutic plants aim to characterize the active components of plants for its therapeutic properties (Naidu *et al.*, 1999; Ferreira *et al.*, 2003).

1.5. NANOTECHNOLOGY

The word “nano” is used to indicate one billionth of meter. Nanoparticles are clusters of atoms in the size range of 1–100 nm. Nano” is a Greek word synonymous to dwarf meaning extremely small. Nanotechnology is a field that has an impact in all spheres of human life. The term Nanotechnology was coined by Professor Norio Taniguchi of Tokyo Science University in the year 1974. Nanotechnology is grouped in to organic nanoparticles and inorganic nanoparticles. The organic nanoparticles include carbon while inorganic include metals like silver and gold. Metallic nanoparticles are a remarkable biomedical agent so there is a growing interest in metallic nanoparticles because they provide superior material properties with functional versatility. Nanotechnology is defined as the field that applies the nanoscale principles and techniques to understand and transform biosystems and which uses biological principles and materials to create new devices and systems integrated from the nanoscale (Ali *et al.*, 2011). It has emerged up as integration between biotechnology and nanotechnology for developing biosynthetic and environmental friendly synthesis of nanomaterials. The goal of improving nanomedicine is for human health (Soloviev, 2007; Chatterjee *et al.*, 2011). Nanomaterials exhibit unique physical, chemical, optical, electrical, magnetic, mechanical, thermal, dielectric, and biological properties, different from those of bulk materials due to their distinct size and shape-dependent characteristics (Schmid, 1992). Due to these reasons nanotechnology is one of the upcoming areas of research in the modern field of material science. The use of nanoparticles also has some general advantages. They have been reported to reduce drug-associated adverse effects and ensure biocompatibility (De-Jong and Borm 2008), to increase the intracellular penetration (Couvreur *et al.*, 1992) and to enhance the pharmacological activity (Fonseca

et al., 2002). The small size, customized surface, improved solubility, and multifunctionality of nanoparticles are opening many doors and creating new medical applications. Indeed, their novel properties offer the ability to interact with complex cellular functions (Singh and Lillard, 2009). Nanoparticulate systems have the potential to improve topical drug release (Zhao *et al.*, 2009), to increase the efficacy of drug delivery in the eye (Wadhwa *et al.*, 2009), to reduce the phagocytic uptake in general (Avgoustakis, 2004) and particularly in the respiratory tract (Buxton 2009), or even to improve brain drug delivery (Khalil and Mainardes 2009; Mistry *et al.*, 2009). Future developments depend on identification of clinically relevant targets and on raising targeting efficiency of the multifunctional nanocarriers (Debbage, 2009). Applications of nanoparticles and nanomaterials are emerging rapidly on various fields because nanoparticles show completely new properties such as size, distribution and morphology of the particles (Kaviya *et al.*, 2011). Chemical methods used for the synthesis of nanoparticles are too expensive and also involve the use of toxic, hazardous chemicals that are responsible for various biological risks. So there is a need to develop environmental friendly processes through green synthesis and other biological approaches. Synthesis of nanoparticles using plant extracts is the most adopted method of green, eco-friendly production of nanoparticles and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites (Ankamwar *et al.*, 2005). In the present research work the silver and gold nanoparticles of *Sageretia thea* will be synthesized to evaluate their biological efficacy.

1.5. 1. Green Synthesis of Nanoparticles

Gardea-Torresdey and co-workers for the first time reported the formation of gold and silver nanoparticles by living plants (Gardea-Torresdey *et al.*, 2002; Gardea-Torresdey *et al.*, 2003). Their protocol for the synthesis of nanoparticles by plant extracts exemplifies the promising application of the green synthesis of metal nanoparticles. Currently different types of plants are investigated for the synthesis of nanoparticles. Recently it has been reported that phytochemicals play major role in reduction of metallic nanoparticles. The responsible phytochemicals have been reported as flavones, terpenoids, aldehydes,

ketones, amides and carboxylic acids in the light of IR spectroscopic studies. Xerophyte, hydrophytes and mesophytes also play a role in NPs synthesis. Xerophytes contain emodin, anthraquinone which undergo redial tautomerization leading to the formation of NPs. Mesophytes contain cyperoquinone, dietchequinone, and remirin and it was suggested that increasing temperature result activation of quinine which leading to particle size reduction. Similarly hydrophytes contain Catechol and protocatechaldehyde due to which took hold a promis for NPs synthesis. Song *et al.*, (2009) studied the effect of temperature on NPs synthesis and concluded that lower temperature polydispersed particles were obtained while a higher temperature supported the formation of smaller and spherical particles.

1.5.1.i. Silver Nanoparticles (AgNPs)

Silver is one of the basic elements that make up our planet. Silver is naturally occurring but rare element, slightly harder than gold but highly ductile and malleable. Silver posses highly antimicrobial property due to its large surface area which have better contact to microbes. Silver is the metal of choice as they have the potential to kill microbes affectively (Vadlapudi and Kaladhar 2014). Recently it has been known that AgNPs have promising microbial efficacy as they hold the promise of killing target microbes both extra-cellularly and intra-cellularly. The application of silver nanoparticles is increasing in number. According to Bourne Research silver nanoparticles is one of the fastest growing products in nanotechnology. It has been known for long time that silver based compound is highly toxic to a wide range of bacteria and improve the function of refrigerators, washing machines and food container. As compared to Ag metal silver nanoparticles are more effective because of the high surface fraction of Ag nanoparticles which lead to high antimicrobial activity. In the recent research it has been demonstrated that silver nanoparticles have the ability to attack viruses like HIV-1 to kill infected cells and prevent transmission (Elechiguerra *et al.*, 2005).

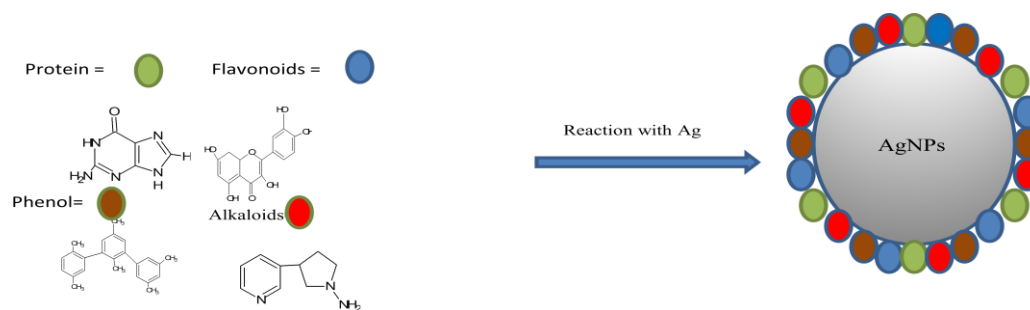


Figure 1. Different functional groups present in plant extract capped over the surface of Ag and reduce it by stabilizing the reaction medium.

1.5.1.ii. Gold Nanoparticles (AuNPs)

The fascinating story with this metal is it play important role in every area of human existence, because it's a way of expressing wealth, love, emotion, religious devotion and also causes of battles and wars. Gold is a biocompatible metal and in ancient time colloidal gold was used as a drinkable solution because it has curative properties for various diseases. In recent years a new type of fascination with gold has been emerge in modern science. Scientist have found a new interest in gold and divided it into minuscule grains such as gold nanoparticles. In 1987 it was discovered that gold nanoparticles with size smaller than 5 nm is excellent catalyst. Spherical nanoparticles smaller than the wavelength of light at certain frequency induces a resonant coherent oscillation of free electrons of the gold nanoparticles result scattering of electromagnetic radiation in visible range (about 520 nm) in resonance with the Plasmon frequency give intense colour and optical properties (Mie., 1908). Gold is a good antibacterial but the effect depends on the size of particle (Dror-Ehre *et al.*, 2009). AuNPs play a vital role in nanotechnology as biomedicines because of surface conjugation with microbes. The important function of gold is the delivery of gene therapy, protein and nucleic acid etc (Vadlapudi and Kaladhar 2014).

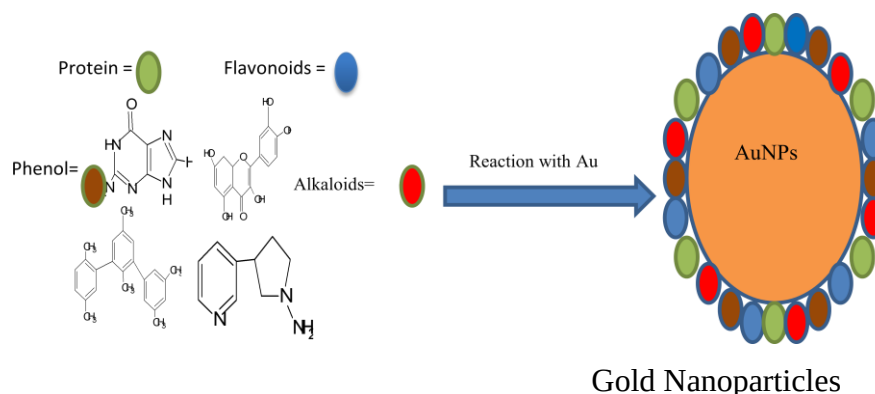


Figure 2. Different functional groups present in plant extract chapped over the surface of Au and reduce it by stabilizing the reaction medium.

1.5.2. Characterizations of Nanoparticles

1.5.2.i. UV-Visible Spectroscopy (UV)

UV-Vis spectroscopy can be comprehended as absorption spectroscopy in the spectral region of ultra-violet and visible spectra. It uses light in visible and near-UV range. Ultraviolet and visible light are strongly energetic and promote outer electron to higher energy level, and UV-Vis spectroscopy is mostly applied to molecules in solution form. The UV-Vis spectra have broad applications that are of limited use for sample identification but are very useful for quantitative measurements. The UV-Vis range spans the range of human visual acuity of approximately 400-850 nm, UV-Vis spectroscopy is useful to characterize the absorption, reflectivity and transmission of a variety of technologically important materials, such as pigments, coatings etc. Deuterium discharge lamp for UV measurements and a tungsten-halogen lamp for visible and near infra-red measurements is used as a light source in UV-Vis spectra. The commercial UV-Vis absorption spectrometers use one of three overall optical designs a fixed or scanning spectrometer with a single light beam and sample holder, a scanning spectrometer with dual light beams and dual sample holders for simultaneous measurement of baseline and sample of interest, or a non-scanning spectrometer with an array detector for simultaneous measurement of multiple wavelengths. In single and dual beam spectrometers the light from a lamp is dispersed before reaching the sample cell, while in array detector all wavelengths pass through the sample and the dispersing elements in

between the sample and array detector. To obtain the absorbance vs wavelength curve of silver and gold nanoparticles dual beam spectrophotometer was used in this research.

1.5.2.ii. X-Rays Diffraction Spectroscopy (XRD)

X-ray diffraction is generally used for the identification of crystalline material and provides information on unit cell dimensions also. X rays diffraction is an analytical technique used for studying crystalline structure and atomic spacing as well. XRD also used for the identification of unknown crystalline like inorganic compound and minerals etc. The important application of XRD is crystalline material characterization, purity test of samples and dimension of unit cell determination. For this experiment XRD helped the presence of silver/gold and its dominant planes verification.

1.5.2.iii. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was first introduced by Baron Manfred von Ardenne in 1938 (Tholen and Yao, 2003). But in Berlin air raid in 1944 von Ardenne microscope was destroyed unfortunately and he did not return to the field after the Second World War (Tholen, 1990). Scanning electron microscopy (SEM) is a sensitive technique in which electrons are uses instead of light to form an image. So simply SEM is a type of electron microscope that produce images of a sample by scanning it with a focused beam of electrons. To produce an image the electron beam is generally scan in a raster scan and then the position of beam is combined with the detected signals. Electron gun at the top of the microscope produce a beam of electrons, through the microscope the electron beam follows a vertical route. The beam then travel through electromagnetic fields and lenses, these lenses focus the beam down towards the sample. When a beam of electrons hit the sample electron and X-Rays emitted from the sample. Backscattered electrons, X-Rays and secondary electrons are collected by detectors and then convert them into a signal that is sent to the screen that produced final image of the surface. SEM play important role in research area of material science and nanotechnology. The main advantage of SEM over traditional microscope is SEM allows more of a specimen to be in focus at one time. The surface morphology of silver and gold nanoparticles was studied by means of this.

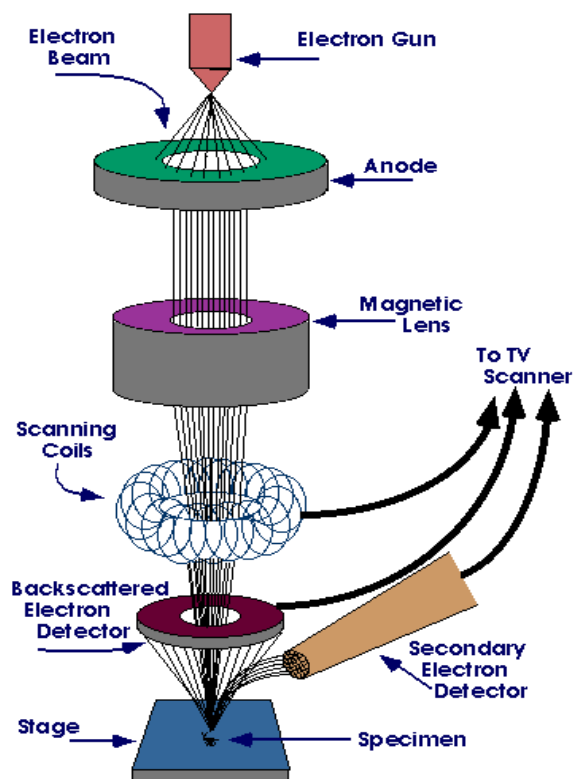


Figure 3. TYPICAL SEM FUNCTIONING

1.5.2.iv. Energy Dispersive Spectroscopy (EDX)

Energy dispersive X-Rays spectroscopy (EDX) is a sensitive technique that provides the elemental curve as output. Generally the EDX technique is used in conjugation with the scanning electron microscopy (SEM). The EDX technique is specially used for the detection of X-Rays emitted from the sample by bombardment through electron beam for characterizing the elemental composition of the sample. To determine the presence of an element in the sample the X-Rays energy values of sample from the EDX spectrum are compared with known characteristic X-Rays energy values. Elements ranging from Beryllium to Uranium can be detected through EDX spectroscopy. EDX spectroscopy helped to verify the presence of silver and gold in the sample and its percentage as well.

1.5.2.v. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infra red spectroscopy is a useful technique used for the identification and characterization of organic chemicals as well as some inorganic include adhesive, polymers, resins, paints, coatings and drugs etc in a whole range of applications. For

isolating and characterizing organic contamination FT-IR is a powerful tool. FT-IR prove that most molecule absorb light in the infra-red region of electromagnetic spectrum. This absorption is the same the bond present in the molecules. The frequency range is usually from 4000-50 cm^{-1} and measured as wave numbers. First the background emission spectra of the IR source are recorded which is then followed by emission spectrum of the IR source with the sample. The ratios of both these spectra the sample spectra and background spectra are directly proportional to the sample absorption spectra. The resultant absorption spectrum from the bond shows the presence of different functional groups and chemical bonds present in the sample. FT-IR is particularly important for the identification of organic molecular groups and compounds due to range of functional groups, all of these have characteristic frequency in the range of infra red. FTIR has emerged as a valuable tool for understanding the involvement of surface functional groups in metal interactions. This technique was applied to determine the groups that were present in various parts of *Sageretia thea* extract and to predict their role in the synthesis of silver and gold nanoparticles (Philip, 2009 and Tripathy *et al.*, 2010).

1.6 Pharmacology

1.6.1. Antimicrobial Activity

Bacteria cause many diseases in humans including bacterial pneumonia, cholera, Leprosy, whooping cough, tetanus and lyme disease. Tuberculosis (TB) is also Bactria causing disease leading death of humans. Some bacterial disease dispersed in water and food is paratyphoid fever, typhoid fever and bacillary dysentery while Typhus spreads through insect's vector in rodents and humans. It has been reported that *Strep pneumoniae* infection is very common among patients with Aids (Simberkoff *et al.*, 1984). Bacteria not only causing infection in humans, animals and plants also, for example *Pseudomonas cichorii* cause dark brown spots on inner leaves of *Lettuce* and also causing diseases on cabbage, tomato, celery and Chrysanthemum. To control bacterial growth and infection various nanoparticles have been synthesized biologically, in all of these nanoparticles AgNPs have been proved the most effective as they have efficacy against bacteria, virus and microorganism (Jaidev and Narasimha, 2010; Li *et al.*, 2010; Saravanan *et al.*, 2011). The effect of AgNPs different on gram positive

bacteria and gram negative bacteria, because gram positive bacteria have thick wall due to peptidoglycan whereas gram negative have a layer of polysaccharides. Banker *et al.*, (2010) reported that the Banana peel extract mediated AuNPs have shown efficient effect towards most of the tested bacterial and fungal culture. Ramamurthy *et al.*, (2013) also describe the effect of Au and Ag nanoparticles against various bacteria strains using *Solanum torvum*. Ali *et al.*, (2011) also reported that AuNPs of *Mintha piperita* leaf was also effective against human pathogens like *Staphylococcus aureus* and *Escherichia coli*.

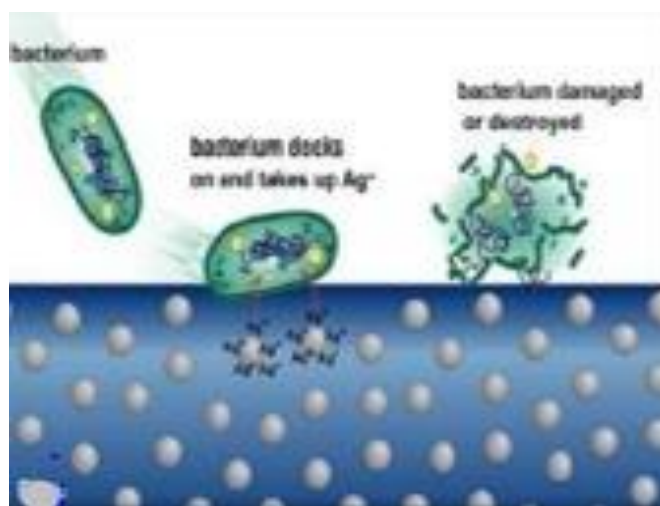


Figure 4. Effect of Silver Nanoparticles on Bacteria growth

1.6.2. Antileishmanial Activity

Leishmaniasis is a group of tropical disease and one of the serious health problems worldwide. A disease caused by a number of species of protozoan parasites belonging to genus *Leishmania*. *Leishmania* follows a digenic life cycle, completed in two hosts. The flagellated extracellular promastigote form occurs in sand fly. The non flagellated and intramacrophagic form, amastigote occur in vertebrate host. Sand fly when bite a vertebrate host, injects promastigotes to its body. The promastigotes are engulfed by host macrophages, where they are transformed into amastigote form. Sand flies of genera *Phlebotomus* and *Lutzomyia* act as invertebrate vectors in Old world and New world

respectively. These sand flies mostly inhabit tree holes and human habitations during wet season. While in dry season they are likely to be found in animal burrows, which is their main breeding place (Sereno *et al.*, 1998; Basimike and Mutinga 1997; Dostalova and Volf, 2012). Currently the only confirmed reservoir host is dog. However crab eating fox, opossum, domestic cat and black rat are suspected reservoir hosts of human leishmaniasis. About 12 million people in 80 countries and two to three million cases of *Leishmania* every year were recorded (WHO 1996). Various drugs have been used for the treatment of *Leishmania* such as pentavalent, antimonial etc but they are very toxic to Humans as well as expensive. Some time drug use for the treatment of *Leishmania* is ineffective against several species of *Leishmania* which is another disadvantage of that drug. So the Green synthesis of Ag and Au nanoparticles is an important step for the treatment of *Leishmaniasis* (Angeli *et al.*, 2008; Swai *et al.*, 2009 and Debbage, 2009). Antilarval and antibacterial effect of Au and Ag ion has been reported (Tokumaru *et al.*, 1974; Oka *et al.*, 1994; Oloffs *et al.*, 1994). On the other hand nanoparticles has great chemical reactivity and capable of producing Reactive Oxygen Species (ROS). The host cell for *Leishmania* parasite can produced large amount of ROS which is then prevented by *Leishmania* by inhibition of the enzymatic mechanism of host cell responsible for ROS production. Therefore in order to inhibit *Leishmania* parasites ROS must be produce through Au and AgNPs instead of enzymatic way, because nanoparticles have the potential to produce ROS independently of the host cell and use as an effective agent in the treatment of *Leishmaniasis*.

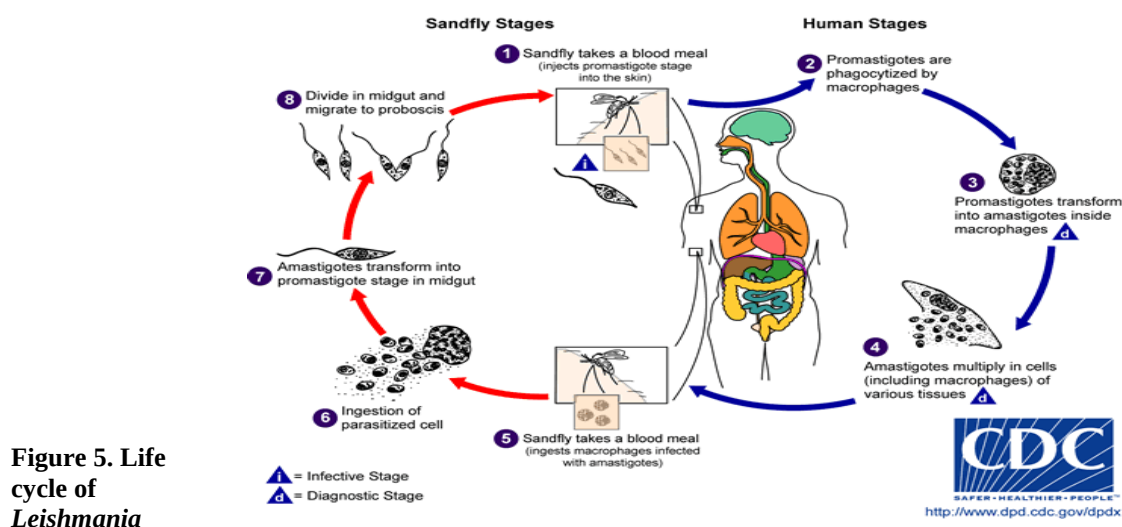


Figure 5. Life cycle of *Leishmania*

species

1.6.3. Analgesic Activity

Pain control is one of the most important therapeutic priorities (Rang *et al.*, 2003). The unpleasant sensory and emotional experience associated with actual or potential tissue damage is called pain. It is a warning signal in nature and causes a lot of discomfort and lead to many adverse effects (Raquibul *et al.*, 2010). Search for the analgesic compound is the priority of pharmaceutical industries (Mattison *et al.*, 1988). The analgesic compound available in the market is having undesirable effects such as gastrointestinal bleeding, ulceration, respiratory distress, nausea etc (Laurence *et al.*, 1997; Mate *et al.*, 2008). So to control these affects there is a search for such bioactive compounds derived from natural products used as an alternative of analgesics and which have little or no side effects. Natural products especially medicinal plants are believed to be an important and traditionally used as analgesics (Eisner, 1990). Medicinal plants contain cocaine and morphine and traditionally used as a pain killer but the search for new pain killer compound is still an important strategy. Nanotechnology can also be used to optimize drug formulations, increasing drug solubility and altering the pharmacokinetics to sustain the release of the drug, thereby, prolonging its bioavailability (Ghrab *et al.*, 2009; Kaya *et al.*, 2009). In the modern material science the synthesis of silver and gold nanoparticles and its application as analgesics is seen as fruitful research. Since the effect of Ag and AuNPs did not evaluate for analgesic effect. In the present study the analgesic potential of silver and gold nanoparticles of *S. thea* will be evaluated to detect its analgesic efficacy.

1.6.4. Cytotoxic Activity

The discovery of new and efficient drugs against various cell diseases like cancer and skin etc are made by screening of active compounds from various parts of plants (Amara *et al.*, 2008). But now a day's cancer is the leading cause of death worldwide (Garrett and Workman, 1999). Cancer is a major health problem and arises from a one single cell. A significant part of population especially children is affecting by a major type of cancer called Leukemia (Canadian cancer society 2005). The transformation of normal cell into tumor cell occurs in multistage process. Diagnosis of tumors in the human body was very difficult. New agents and treatment has been studied; however all the treatments have

many limitations due to their side effects (Robert and Jarry, 2003). So a new therapeutic agent is required which is more active and produce less side effect as compared to cytotoxic agent already used. Nanotechnology is interdisciplinary research field involving biology, chemistry, medicines and has potential for detection and treatment of cancer (Cai and Chen 2007). Recently, nanoparticles are also used to overcome this problem. The nanoscale devices can easily enter the cells and they made an interaction with DNA, proteins, enzymes and cell receptors. The nanoparticles can detect the cancer disease in a very small volume of cells or tissue. Nanoparticles are smaller in size as compared to biological molecule like enzyme, antibodies and receptor. These nanoparticles can interact with these biomolecules both on the surface and also inside the cell. Geetha *et al.*, (2013) and Cai and Chen, (2008) also carried out Green synthesis of AuNPs for their anticancer potential. According to the literature no such activity is recorded for *S. thea*. So in the current study various parts of *S. thea* will be evaluated for their anticancer/cytotoxic efficacy.

1.6.5. Larvicidal Activity

Mosquitoes are important vector of human disease. Every year millions of people kill by spreading serious diseases such as malaria, dengue fever, yellow fever, Chikungunya fever, and so forth (Morens and Fauci, 2013). To control mosquito causing disease it is essential to control mosquito populations. Most of the insecticides are chemically synthesized causing environmental pollution as well as kill non target organisms. Due to these limitations a new method is necessary to replace these chemically synthetic insecticides. Currently biological control is an eco-friendly approach for mosquito control. In modern science nanotechnology is a renewable source for the production of nanoparticles. Green synthesis of nanoparticles is a clean, non toxic and environmentally acceptable method for preparation of metal nanoparticles. Hence the need to use green synthesized silver and gold nanoparticles for the control of disease vectors. Targeting the larval stage control is a critical point in eradicating mosquitoes. Soni and Prakash, (2012) synthesized gold nanoparticles (AuNPs) from a fungus *Aspergillus niger* and used it

against three mosquito species *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*. Similarly Rajakumar and Rahuman, (2011) synthesized nanoparticles from aqueous extract of *Eclipta prostrata* against fourth instar larvae of filariasis vector while Velayutham *et al.*, (2013) investigate green synthesis of silver nanoparticles using bark aqueous extract of *Ficus racemosa* and studied its larvicidal activity. No such record was found of *S. thea* for mosquito control so in the present investigation it will be evaluated against mosquito's larvae.

1.6.6. Antioxidant Activity

The production of energy for many biological processes oxidation is an essential in many living organisms, but the uncontrolled production of oxygen with free radical damage cell and their functions. As a result of oxidation new free radical formation which causes damage of cell and DNA, tissue loosening, cancer cell formation and genetic disorder etc. Many synthetic antioxidants is used such as propyl gallate, butylated hydroxyanisole, butylated and hydroxytoluene for the controlled of cell damages in human body however recent research investigate that all of these synthetic antioxidant is health hazard and cause damage to liver cells (Grice, 1988). Thus the utilization of natural antioxidant is essential to protect human body from free radical and control heart diseases, cancer, and arthritis process (Nandita and Rajini, 2004). In recent years potential compounds of antioxidant have been searched in various parts of medicinal plants (Ramarathnam *et al.*, 1995). Different secondary metabolites like flavonoids, phenol, tannins and lignin play important role in normal growth of plant as well as defensive against various infection and injuries (Heldt, 1997). In the present research work various parts of *S. thea* have been investigated for their antioxidant potential, because past history demonstrates that *S. thea* is a rich source of flavonoids and phenolic contents (Chung *et al.*, 2009) and posses strong antioxidant potential (Chung *et al.*, 2004).

1.7. Rhamnaceae (Family)

1.7.1. Description

The family *Rhamnaceae* consists of 58 genera and about 900 species (Qaiser and Nazimuddin, 1979). In Pakistan this family is represented by 6 genera and 21 species.

The plants are trees or shrubs, leaves simple, inflorescence axillary cymes, racemes and spikes. Flowers are green, yellow or blue, hermaphrodite rarely unisexual, actinomorphic. Calyx tubular, 5-rarely 4- or 6- lobed, Petals 5 rarely 4 or 6, Stamens opposite to the petals, ovule 1 in each locus, seed with a large erect embryo (Yilin and Carsten, 1979).

1.7.2. Distribution

In world this family is most commonly distributed in tropical and sub tropical regions (Kaiser and Nazimuddin, 1979).

1.7.3. Importance

Several species of *Rhamnaceae* have the antihyperlipidemia, hypnotic-sedative, hypothermic and laxatives effects (Yilin and Carsten, 1979). For the relief of constipation the dried bark of *Rhamnus fragnula* and dried ripe berries of *Rhamnus catharticus* has been used in traditional medicines (Khare, 2007).

1.8. GENUS (*Sageretia*)

1.8.1. Description

Sageretia genus consists of about 35 species. ***S. horrida*** shrubs erect about 3 meter tall. Branchlets short, with red-brown spines, sparsely pubescent when young, glabrous when old. ***S. pycnophylla*** shrubs evergreen, erect, about 2 m tall, spinescent. Branchlets opposite or subopposite, red-brown or black-brown, puberulent. ***S. paucicostata*** shrubs or rarely small trees, erect, to 6 m tall. Young branches yellow tomentose, glabrescent; branchlets opposite or subopposite, spinescent. ***S. lucida*** shrubs scandent, unarmed or with spines. Leaves alternate or subopposite; petiole 8–12 mm, glabrous; leaf blade adaxially shiny. ***S. gracilis*** Shrubs, erect or scandent, spinescent. Leaves alternate or subopposite; stipules subulate, 1–2 mm; petiole 5–14 mm, glabrous or sparsely puberulent. ***S. camelliifolia*** shrubs erect, ca. 4 m tall, unarmed. Branchlets gray-brown, glabrous, longitudinally striate. Stipules subulate, caducous; petiole 5–7 mm, glabrous. ***S. brandrethiana*** Shrubs, spinescent. Branchlets opposite or subopposite, gray-brown, glabrous or sparsely pubescent, often terminating in a spine. Leaves alternate; petiole 1.5–2.5 mm, densely white tomentose. ***S. lijiangensis*** Shrubs spinescent. Branchlets opposite or subopposite, brownish, densely tomentose, apex spinescent. Leaves alternate or subopposite, dimorphic. ***S. yilini*** Shrubs unarmed. Branchlets subopposite or

alternate, densely whitish tomentose. Petiole 2–3 mm, whitish tomentose, leaf blade elliptic, 1–1.5 × 0.7–1 cm, thinly leathery, abaxially densely whitish tomentose, glabrescent when old. ***S. rugosa*** Shrubs unarmed, scandent or erect, ca. 4 m tall. Juvenile branches and branchlets ferruginous tomentose or densely puberulent. ***S. omeiensis*** shrubs scandent, unarmed. Branchlets gray-brown, canescent or ferruginous tomentose. Leaves subopposite or alternate; petiole 7–11 mm, canescent or ferruginous tomentose. ***S. laxiflora*** shrubs scandent or erect, to 10 m tall, armed. Branchlets white or yellow tomentose, or puberulent; old branches glabrescent, conspicuously longitudinally striate, with stout spines. ***S. hamosa*** shrubs evergreen, scandent, armed. Branchlets grayish brown or dark brown, with incurved, hook like stout spines, glabrous or puberulent at base only, sometimes yellow-brown pubescent. ***S. randaiensis*** shrubs small. Branchlets dark brown, slender, sparsely yellow brown hairy or subglabrous; old branches often opposite to branchlets, with hooklike, deflexed spines. ***S. melliana*** shrubs evergreen, scandent, spinescent. Branchlets terete, brown-yellow puberulent. Leaves generally opposite; petiole 4– 8 mm, strongly canaliculate, puberulent or glabrous. ***S. subcaudata*** shrubs scandent or erect, ca. 1.5 m tall. Branchlets dark brown, glabrous or sparsely puberulent. Leaves subopposite or alternate. ***S. henryi*** shrubs scandent, rarely small trees, ca. 2.5 m tall, unarmed or spinose; branchlets red-brown, glabrous; old branches grayblack. ***S. pedicellata*** shrubs scandent, spinose. Branchlets gray, longitudinally striate; branches subopposite, densely brownish puberulent (Flora of China).

1.8.2. Distribution

About 35 species: mainly in South East Asia, a few species in Africa and North America; 19 species (15 endemic) in China.

1.8.3. Importance

The fruit of some species is edible. The leaves are used as a substitute for tea. Strongly antioxidant, several species are very popular in Bonsai gardening.

1.9. *Sageretia thea* (Osbeck) Johnst

1.9.1. Description

Sageretia thea (Osbeck) Johnst (*Rhamnaceae*) commonly called Chinese Sweet Plum and Poor Man's Tea is a spinescent, evergreen tender shrub or semi-evergreen climber

like shrubs. Shrubs scandent or erect, to 3 m tall, armed. Branchlets slender, alternate or subopposite, brownish, terminating in a spine, finely tomentose when young. Leaves opposite at basal nodes to alternate; petiole 2–7 mm, puberulent; leaf blade abaxially pale green, adaxially green, usually elliptic, oblong, or ovate-elliptic, rarely ovate or nearly orbicular, 2–4.5 × 0.7–2.5 cm, papery, abaxially glabrous or pubescent on veins, sometimes tomentose and glabrescent, adaxially glabrous, lateral veins 3–5(–7) pairs, conspicuously prominent abaxially, base rounded or subcordate, margin serrulate, apex acute, obtuse, or rounded. Flowers yellow, sessile, fragrant, usually 2- to few fascicled in terminal or axillary lax spikes or paniculate spikes; rachis 2–5 cm, sparsely puberulent. Calyx tube sparsely pubescent, shallowly cup-shaped, sepals triangular, ca. 1 mm. Petals spatulate, shorter than sepals, apex 2-fid, often reflexed. Disk fleshy, glabrous, distinctly thickened around ovary. Ovary 3-loculed, with 1 ovule per locule; style very short; stigma 3-fid. Drupe black or purple-black at maturity, subglobose to obovoid, ca. 5 mm in diam., with 1–3 pyrenes; mesocarp fleshy, sour-tasting. Seeds flat, emarginate at both ends. Fl. Jul–Sep, fr. Mar–May of following year.

1.9.2. Distribution

Sageretia thea is a member of family *Rhamnaceae* native to Asia and warmer areas of North America. Recently it has been extensively grown in China and Japan for use as a bonsai. In Korea, it grows along the southern seashore areas.

1.9.3. Importance

The plant has been used as folk medicine for the treatment of hepatitis (roots and stems) in China (Wu *et al.*, 1987; Xu *et al.*, 1994). Traditionally, its leaf has been used as tea materials in Korea. Past studies have demonstrated that the water extract of the leaves possessed strong antioxidative (Chung *et al.*, 2004) and moderate HIV-1 protease inhibitor activities. *S. thea* contain flavonoids and phenol contents and responsible for antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects. Leaves contain rich source of flavonoids and may be used as functional foods and dietary supplements (Chung *et al.*, 2009). Flavonoids-O-glycosides are also abundant in *S. thea* leaves and having antioxidant potential (Shen *et al.*, 2004). Edible pigments derived

from the fruit of *S. thea* can be used as colouring agent in food materials (Hong *et al.*, 2009).

AIMS AND OBJECTIVES OF THE STUDY

Following objectives were set and achieved through the Pharmacognostic Evaluation and Green Synthesis of Silver/Gold Nanoparticles of *Sageretia thea* (Osbeck) Johnst, for Biological Screening.

The purpose of my work is to study morpho-histological and nanoscale activities of various parts of *S. thea*. The specific objects of my research work is given below

1. For correct identification of selected plant parts histological study was carried out that will be helpful towards taxonomic classification and differentiation of this plant from their closed allied species.
2. Anatomical study of leaf with special reference to vein termination numbers, vein islet number, stomatal type, stomatal number, palisade ratio and stomatal index was undertaken which helped in correct identification of drugs derived from this plant.
3. Phytochemical analysis of leaf, stem and root were carried out to assess the chemical composition of this plant and to explore their pharmacological potentials
4. Elemental analysis was carried out to explore Pharmacognostically active secondary metabolites present in different parts of the plant.
5. Various characterizations like (XRD, SEM, EDX, FTIR and UV-Vis spectroscopy) were completed for synthesized Ag/AuNPs which confirmed its synthesis, absorbance, size, crystalline nature and presence of different functional groups.

6. The synthesized Ag/AuNPs were assessed for different biological activities like Antifungal, Antibacterial, Antioxidant, Larvicidal, Cytotoxic, Antileishmanial and Analgesic activity. The response of the tested sample showed the efficacy of synthesized Ag/AuNPs.

Chapter # 02

REVIEW OF LITERATURE

2.1 PHARMACOGNOSTIC STUDY

Saraswathy *et al.*, (2009) carried out pharmacognostic and microscopic evaluation of *Cassia angustifolia* Vahl powder the microscopic study of the leaves revealed presence of paracytic stomata and hair on lower epidermis with long trichomes and prism shaped crystal of calcium oxalate. The present investigation suggests that it is useful for identification and standardization of drug. Li *et al.*, (2009) studied anatomical features of *Bupleurum scorzonerifolium* Willd. *B. scorzonerifolium* roots and reported four developmental stages in the formation and growth of roots of these plants. Idu *et al.*, (2009) carried out comparative morphological and anatomical comparison of the two species *Jatropha curcas* and *jatropha tanjorensis*. Transverse section of leaf and stem of these plants revealed that trichomes were present in *J. tanjorensis* but absent in *J. curcas*, there was few but thick palisade parenchyma of *J. tanjorensis* while more but thinner in *J. curcas*. Gautam *et al.*, (2010) worked on powdered and anatomical sections of the *Toona ciliata* bark. Externally bark are Grey to reddish-brown in colour, strong odour, bitter taste, rough and hard, double quill and curved curvature. The transverse section revealed the presences of periderm, cortex, Sclerides, medullary rays and phloem fiber. These findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant. Taur & Patil (2010) studied anatomical features of *Clitoria ternatea* L. (Family: *Fabaceae*) leaf and reported the presence of paracytic stomata on upper and lower epidermis and thick layer of cuticle covered by trichomes. Essiett *et al.*, (2010) studied anatomical features of *Diodia scandens* Sw (*Rubiaceae*) and reported four types of stomata. The morphological study revealed that the leaf was compound and has glabrous surface. Such studies serve as an important parameter in leaf materiel standardization which confirms its quality

formulation and distinguish it from other closely related species. Yadav *et al.*, (2011) carried out macroscopic and microscopic study of *Madhuca longifolia* (Family *Sapotaceae*) and *Madhuca longifolia*. The leaf transverse section showed the presence of single layered epidermis with thin cuticle, vascular bundles, uniseriate trichomes, cortex and pith. The transverse section of leaf showed the presence of *paracytic* stomata on both surface while the stem transverse section showed the presence of xylem, cortex, cork, phloem and pith. Ahirrao *et al.*, (2011) studied microscopic and physical constants of *Vitex negundo* Linn. Leaves including palisade number, Stomatal Index, Vein-Termination, Vein-Termination and Palisade cells Ratio. Zunjar *et al.*, (2011) demonstrated that leaves of *Carica papaya* Lin have no trichomes but abundant calcium oxalate crystals. The leaf study had 31.56 ± 3.41 stomatal index, 3-4 vein termination number and 12.65 ± 1.57 palisade ratio. The powder drug study revealed the presence of calcium oxalate crystal and starch grains, ash value and moisture contents were also studied as these parameters are useful for identification and standardization of drugs. All of these characters created a set of diagnostic parameters for the study of different species. Gandagule *et al.*, (2013) demonstrated the pharmacognostic and phytochemical investigation of *Ziziphus xylopyrus* leaves. The leaf study revealed the presence of stomata, covering trichomes, sclerenchyma, collenchyma, epidermal cells and vascular strands. Shraddha *et al.*, (2014) studied the pharmacognostic evaluation of *Vitex purpurascens* leaves. Various trichomes unicellular covering trichomes, multicellular, sessile glandular trichomes, collapsed covering trichomes, knee-shaped trichomes and other characters like pericyclic fibers, annular xylem vessels and anisocytic stomata were recorded.

2.2 PHYTOCHEMICAL SCREENING

Mojab *et al.*, (2003) carried out the phytochemical screening of fifty five plants belonging to different families for the presence of tannins, alkaloids flavonoids and saponins they also reported medicinal uses of these plants. Musa *et al.*, (2006) screened leaf powder of *Gisekia pharnacioides* for phytochemical analysis which indicated the presence of alkaloids, resins, tannins, cardiac glycosides and flavonoids. Onocha *et al.*, (2008) screened hexane extract of *Acalypha hispida* for phytochemical investigation. The results revealed the presence of carbohydrates, flavonoids, phenols and alkaloids.

Shivalingam *et al.*, (2009) reported that *Nothosaerva brachiata* Wight and *Aerva lanata* are used for the treatment of urinary calculi. Phytochemical screening test of *N. brachiata* Wight showed the presence of Tannins, Carbohydrates, Sterols and Glycosides which were similar to *A. lanata*, so it is concluded that *N. brachiata* Wight is used as a substituent for *A. lanata*. James & Emmanuel, (2010) screened fresh and dry leaf powder of *Euphorbia hetrophylla* for phytochemical analysis and detected the presence of fats, protein, carbohydrates, alkaloids, tannins, flavonoids and saponins. Protein, fats and carbohydrates were also present in fresh leaf but in lower quantity as compared to dry powder. Kamboj & Saluja (2010) demonstrated that *Bryophyllum pinnatum* Kurz (Crassulaceae) has different pharmacological potential and also possesses medicinal values. The phytochemical analysis of the extract confirmed the presence of gum, mucilage, phenolic, tannins, alkaloids, carbohydrates, glycoside and lignin's also suggested that this type of study is useful for monograph of the plant and standardization. Kumar & Hemalatha (2010) found that *Alangium lamarckii* Thwaites is very useful for many ailments. Preliminary phytochemical studies showed the presence of phenols, alkaloids, tannins, phytosterols, triterpenoids, carbohydrates and amino acids. Kumar *et al.*, (2010) carried the phytochemical screening of *Erythrina indica*. Lam. root and reported that it contained protein, carbohydrates, amino acid, alkaloids, flavonoids, tannins and phenolics. Rao *et al.*, (2011) reported the phytochemical screening of chloroform, Petroleum ether, and methanolic extracts of the leaves of *Tephrosia purpurea*, *Alternanthera sessilis*, and *Clitoria ternate*. The phytochemical investigation showed that total content of flavonoid and phenols were high in *C. ternata* than *T. purpurea* and minimum in *A. sessilis*. Total tannins were high in *T. purpurea* while low in *A. sessilis*, alkaloids were high in *C. ternata* is followed by *T. purpurea* and *C. ternata*. All of these secondary metabolites had strong antioxidant effect. Menon & Latha (2011) studied *Coleus forskohlii* extracts by using different organic solvents for secondary metabolites and confirmed the presence of saponins, protein, tannins, alkaloids, glycosides, phenols, carbohydrates and cardiac glycosides. Savithramma *et al.*, (2011) reported that the phytochemical screening of medicinal plants which are used in traditional medicines. The qualitative phytochemical screening of these plants confirmed the presence of secondary metabolites like steroids, steroids, triterpenoids, anthraquinons, leucoanthocyanins,

tannins, flavonoids, lignin's and phenols which showed that these plants have the potential of curing different disease and can be used for various medicinal purposes. Bulbul *et al.*, (2013) investigated the preliminary phytochemical screening test of methanolic extract of *Polygonum lapathifolium* stems. The phytochemical screening showed the presence of amino acid & protein, alkaloid, phytosterols, diterpens and flavonoids. Similarly Gandagule *et al.*, (2013) demonstrated the pharmacognostic and phytochemical investigation of *Ziziphus xylopyrus* leaves. Phytochemical evaluation showed presence of alkaloids, carbohydrates, steroids and sterol, glycosides, saponins, flavonoids, phenolic compounds, triterpenoids. Ogbonna, *et al.*, (2013) carried out phytochemical and proximate analysis of *Tetracarpidium conophorum* root and leaf. The phytochemical analysis revealed that both leaf and root contained that *tannins*, *alkaloids* and *saponins* while *anthraquinones*, *Cardiac glycosides*, *ellagic acid* and *caffeinic acid* were absent from both parts.

2.3. ELEMENTAL ANALYSIS

Sodipo *et al.*, (2008) carried out the elemental analysis of *Solanum macrocarpum* Linn and reported the presence of S, Na, K, Fe, Mg, Zn, Ca, Cu, Mn, P, As and Cr in various part of the plant. Adnan *et al.*, 2010 assess the elemental property of humid and sub-humid regions of North-west Pakistan and reported some macro and micro elements in these regions. Ata *et al.*, (2011) worked on elemental composition of different medicinal plants of Pakistan. It was investigated that the level of essential elements was higher than trace elements. Zhang *et al.*, (2011) conduct the elemental investigation of *Paris polyphylla*. In this investigation it was estimated that the elements found in the order of Ca > K > Mg > Fe > Na > Cu > Mn > Zn > Cr while Cr, Cu, Ca and Mn level was affected by some environmental condition. It was concluded that the elemental composition of *P. polyphylla* was different from other species of the same genera. Fatima *et al.*, (2013) performed the elemental analysis of *Anethum graveolens*, *Sisymbrium irio* and *Vernonia anthelmintica*. The analysis showed that *A. graveolens* contained high level of K while *S. irio* contained high level of Fe, Na and Mn. In *V. anthelmintica* Cl, Co, Zn and Cr concentration was very high as compared to Fe.

2.4. PROXIMATE ANALYSIS

Read (1946) reported fat, tannin, sugar, pectin and cellulose in *Persicaria maculosa*, Irvine (1952) reported carbohydrate, ash and fat in *Polygonum hydropiper*, Duke & Ayensu (1985) reported water, protein, fat, carbohydrate, ash and fiber in *Polygonum bistorta*. Gupta (1962) reported protein, fat, carbohydrate, ash and water in *Chenopodium album*. Khodzhaeva *et al.*, (2002) reported the concentration of lipids, proteins, flavonoids and carbohydrates in the aerial part of the *Rumex confertus*. Shah *et al.*, (2014) investigate that root and stem of *S. thea* contained high amount of carbohydrate concentration while leaf contain high amount of protein, fat and moisture. Stem contained high concentration of crude fibers and ash.

2.5. FLUORESCENCE STUDY

Different powder drugs of different parts of plant give different fluorescence in visible light and under ultraviolet radiation; therefore fluorescence evaluation is used for identification of plant and powder drugs (Jarald & Jarald, 2007). Many researchers have worked out fluorescence studies of different medicinal plants for identification like, Tian *et al.*, (2006) reported the florescence analysis of *Rheum* species and *Polygonum cuspidatum* and Hussain *et al.*, (2011) florescence study of *Hygrophila auriculata* K. Schum.

2.6. SILVER NANOPARTICLES

2.6.1. Characterization of Silver Nanoparticles

Mallikarjuna *et al.*, (2011) carried out the synthesis of silver nanoparticles by reducing the silver ions by leaf extract of *Ocimum sanctum*. Various characterizations like XRD, SEM, TEM, EDX, FTIR and UV-Vis spectrophotometer revealed the synthesis of SNPs while the FTIR results identify the possible biomolecules which are responsible for the stabilization of Silver nanoparticles. Solgi & Taghizadeh (2012) synthesized the silver nanoparticles using the peel extract of *Punica granatum* and petal extract of *Rosa damascene*. UV-visible spectrum showed absorption peak at 380-500 nm while other characterizations like XRD, FTIR and SEM confirmed the synthesis of AgNPs with an average particle size estimated 21 nm. Pavani *et al.*, (2013) demonstrated that Ipomea species is traditionally used for nutritional, medicinal, ritual and agriculture. In the present investigation synthesis and characterization of silver nanoparticles using

methanolic extract of *Ipomoea indica* flowers. The access synthesis of NPs UV-Vis spectroscopy was carried out. To confirm synthesis of NPs various characterizations like Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD) studied with average particle size range from 10 to 50 nm. Similarly Awwad *et al.*, (2013) also synthesized silver nanoparticles from aqueous solution of silver nitrate using carob leaf extract (*Ceratonia siliqua*) in a single pot process. Iravani & Zolfaghari (2013) carried out the green synthesis of silver nanoparticles of *Pinus eldarica* bark extract. Various parameters like pH, temperature and substrate concentration was also studied. The size of the particles was measured through TEM analysis in the range of 10-40 nm. Kudle *et al.*, (2013) carried out synthesis of nanoparticles by biological way. The synthesis was conducted by mixing 1 Mm solution of silver nitrate with leaf and root extract of *Phyllanthus reticulatus*. After incubation the change in colour from pale yellow to reddish brown indicated the formation of NPs. The synthesized NPs were characterized by UV-Visible Spectroscopy, FTIR and their morphology was determined by Transmission Electron Microscope (TEM). To find out the biological effect of NPs antibacterial activity were also performed.

2.6.2. Microbial activity of Silver Nanoparticles

Kim *et al.*, (2007) demonstrated that the antimicrobial effect of Ag salt is well known but the effect of AgNPs on microorganisms has not been clear. Therefore the synthesized AgNPs were tested against yeast, *Escherichia coli*, and *Staphylococcus aureus*. The result suggests that the silver NPs can be used as effective growth inhibitor for various microorganisms. Pal *et al.*, (2007) investigated the antibacterial properties of differently shaped silver nanoparticles against the gram-negative bacterium *Escherichia coli*. Energy-filtering transmission electron microscopy images revealed that silver nanoparticles undergoes a shape dependent interaction with gram-negative bacteria *E. coli*, it is proposed that triangular AgNPs showed strongest biocidal action against *E. coli* as compared to spherical and rod-shaped nanoparticles. Jaina *et al.*, (2009) worked on the synthesis of silver nanoparticles through papaya fruit extract. Various characterizations using UV-Vis absorption spectroscopy, FTIR, XRD and SEM. X-ray diffraction and SEM analysis showed the average particle size of 15 nm. The antifungal potential of SNPs were also evaluated which showed highly toxicity against human pathogens.

Krishnaraj *et al.*, (2010) conducted the synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its activity against water born bacterial pathogen. The results recorded from various characterizations like UV–Vis spectrum, scanning electron microscopy (SEM), X-ray diffraction (XRD) and energy dispersive spectroscopy (EDS) strongly support the synthesis and characterization of silver nanoparticles. The size of the silver nanoparticles was measured 20–30 nm from resolution transmission electron microscopy (HRTEM) analysis. Further the Ag NPs showed good inhibitory property against *Escherichia coli* and *Vibrio cholerae*. Elumalai *et al.*, (2010) lay out biologically inspired synthesis of silver nanoparticles of leaf extract of *Euphorbia hirta*. He demonstrated that the biological way for the synthesis of NPs is cost effective and eco friendly as compared to chemical synthesis. The synthesized nanoparticles were characterized through UV-Vis spectroscopy and the antifungal potential was also conducted. Elumalai *et al.*, (2010) synthesized silver nanoparticles using aqueous extract of *Euphorbia hirta* (L) leaves. To access the formation of UV-visible spectroscopy was carried out. Various characterizations like SEM analysis revealed that nanoparticles are polydispersed with a size ranging from 40 nm to 50 nm. The microbial efficacy showed that NPs were effective against *B. cereus* and *S. aureus*. Nabikhan *et al.*, (2010) carried out the synthesis of silver nanoparticles of *Sesuvium portulacastrum* L. and its antimicrobial activity. The synthesis of Ag NPs were confirmed through different characterizations which revealed that callus extract could be able to produce silver nanoparticles, better than leaf extract. The size of particles varied from 5-20 nm within spherical in shaped as evident by Transmission Electron Microscopy (TEM). Fourier transforms infrared (FTIR) spectroscopy measurement confirmed the presence of aromatic rings, germinal methyls and ether linkages, flavones and terpenoids which was responsible for the stabilization of the silver nanoparticles. So the present work highlighted the efficacy of tissue culture-derived callus species for the synthesis of antimicrobial silver nanoparticles. Savithramma *et al.*, (2011) conducted the synthesis of silver nanoparticles of stem bark extracts of *Boswellia ovalifoliolata* and *Shorea tumbuggaia* and leaf extract of *Svensonia hyderabadensi*. The change of colour and UV-spectroscopy confirmed the synthesis of Ag NPs. The antifungal and antibacterial activity of SNPs revealed that the stem and bark SNPs were toxic towards *Klebsiella*, *Aspergillus*

and *Pseudomonas*, *Fusarium* respectively, while the leaf extract of *S. hyderabadensis* showed toxic effect towards *Pseudomonas* and *Rhizopus* species. So the results indicate that the silver nanoparticles may have an important advantage over conventional antibiotics. Savithramma *et al.*, (2012) evaluated the stem, roots, stem, bark and leaves of *Shorea tumbuggaia* for the synthesis of silver nanoparticles (SNPs) for antimicrobial activity. The formation of Ag NPs was confirmed by AFM and UV-Vis spectroscopy. Further the anti-microbial efficacy has also been evaluated. The results showed that the SNPs were more toxic toward bacterial species than that of fungal species. So it is concluded that the SNPs may have important advantage over conventional antibiotics to which the bacteria got resistance. Behera *et al.*, (2013) demonstrated that green synthesis of silver nanoparticles using microorganisms, plants and plant extracts is cost effective, nontoxic and ecofriendly method. In the present investigation green synthesis of silver nanoparticles using microbial source is carried out. Various characterizations like UV-visible spectroscopy for surface Plasmon resonance, Scanning electron microscopy (SEM) for morphology, Energy Dispersive X-ray Spectroscopy for crystalline size (EDS) and Dynamic light scattering (DLS) analysis for elemental detection were carried out. The antibacterial potential of synthesized NPs was also evaluated. Geoprincy *et al.*, (2013) used the *Nelumbo lucifera* leaves for the synthesis of silver nanoparticles. For various parameters like absorbance, stabilization of bonds, particle sizes in terms of nanometer and the particle shapes were checked by various characterizations like UV spectroscopy; Fourier Transform Infra-Red spectroscopy analysis, X-Ray Diffraction analysis; Scanning Electron Microscopy and High Resolution Transmission Electron Microscopy were assessed. The significance of SNPs was confirmed by antimicrobial activity against gram positive and gram negative bacteria and some pathogenic fungus. Caroling *et al.*, (2013) synthesized the silver nanoparticles by green chemistry approach. The synthesis of SNPs was carried out by using the aqueous extract of Broccoli florets (*Brassica Oleracea* L. var. Italica. Nanoparticles were characterized by UV-VIS, FT-IR, XRD, SEM, TEM and EDAX analysis. The UV-Vis analysis showed surface plasmon resonance at 425nm while the presence of phenolic groups and active protein were identified by FT-IR analysis. Average particle size in the range of 40-50nm was confirmed by SEM, TEM and XRD. The presence of Silver was tested by EDAX

analysis. The antifungal activity of SNPs found effective against human pathogens like, *Klebsiella Pneumonia*, *Staphylococcus Saprophyticus* and *Escheria Coli*. Gnanajobitha *et al.*, (2013) synthesized the silver nanoparticles from the flower extract of *Hortensis*. The changing of colour and surface plasmon absorption band obtained at 460nm indicated the synthesis of AgNPs. The nanoparticles were characterized by Scanning electron microscope (SEM), energy-dispersive microanalysis (EDX), X-ray diffraction spectroscopy (XRD), fourier transform infra-red (FTIR). Further antimicrobial activity of gram positive (*B. subtilis*) and gram negative (*K. planticola*) bacteria were also evaluated.

2.6.3. Cytotoxic Activity of Silver Nanoparticles.

Devi *et al.*, (2012) synthesized silver nanoparticles from aqueous extract of *Gelidiella* sp. The NPs showed absorbance band at 435 nm through UV-Vis Spectroscopy. Various characterizations like FTIR, EDX and SEM analysis were carried out for synthesized NPs. the silver NPs was assessed for anticancer activity against Hep-2 (Human laryngeal) cell lines. Devi & Bhimba, (2012) synthesized AgNPs by Seaweed *Ulva lactuca* *In vitro* extract. The UV-Vis spectroscopy showed absorbance peak at 434nm. SEM, TEM and XRD analysis confirmed that the nanoparticles were spherical in shape, with average size 20-56nm. The synthesized NPs were then applied for anticancer activity. Sulaiman *et al.*, (2013) carried out green synthesis of silver nanoparticles *Eucalyptus chapmaniana* leaves extract. UV-Vis analysis showed SPR band at 413nm XRD analysis revealed that the AgNPs were crystalline in nature with face centered cubic structure. These NPs were then utilized against the viability of HL-60 cells in a dose dependent manner. Ranjitham *et al.*, (2013) synthesized silver nanoparticles by using Cauliflower floret extract as a reducing agent. The surface Plasmon resonance supports the formation of NPs synthesis which showed UV-Vis peak at 425 nm. Various characterizations like FTIR, EDX, TEM, XRD and SEM analysis were carried out to evaluate the NPs formation. Further these nanoparticles efficiently showed reduced viability and increased cytotoxicity on MCF-7 breast cancer cell line in a dose dependent manner.

2.6.4. Antileishmanial Activity of Silver Nanoparticles

Allahverdiyev *et al.*, (2011) chemically synthesized two types nanoparticles, UV-exposed AgNPs and non UV-exposed AgNPs then carried out the Antileishmanial activity against amastigotes. The results concluded that both UV-exposed and non-UV-exposed Ag-NPs demonstrated antileishmanial effects by inhibiting growth, metabolic activity, and infectivity of promastigotes by preventing survival of amastigotes inside host cells. Shah *et al.*, (2014) carried out Antileishmanial activity by extract of *J. dolomiaea*. The ethyl acetate fraction” was more active against Leishmania. Similarly Tahir *et al.*, (1998) carried out antileishmanial activity of Sudanese plants. Literature review showed that no such nanoparticles potential have been studied for antileishmanial activity, so in the present research work the potent of silver nanoparticles will be evaluated against leishmanial.

2.6.5. Larvicidal Activity of Silver Nanoparticles

Rajakumar and Rahuman, (2011) demonstrated that mosquitoes transmit serious diseases in humans. The larval control was carried out by applying natural products through synthesized nanoparticles from aqueous extract of *Eclipta prostrate* against fourth instar larvae of filariasis vector, *Culex quinquefasciatus* say and malaria vector, *Anopheles subpictus* Grassi (Diptera: Culicidae). The maximum efficacy of synthesized AgNPs was recorded against *C. quinquefasciatus* and against *A. subpictus*. This is a new approach to control mosquito larvae. Velayutham *et al.*, (2013) investigated green synthesis of silver nanoparticles using bark aqueous extract of *Ficus racemosa* and its larvicidal activity against fourth instar larvae *Culex quinquefasciatus* and *Culex gelidus*. Soni and Prakash, (2012) conducted the synthesis of silver nanoparticles and characterized it through UV-Vis spectroscopy and transmission electron microscopy (TEM). The synthesized nanoparticles were tested against larvae and pupae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. The investigation showed that 100% lethality showed by AgNPs against *Cx. Quinquefasciatus* larvae while *An. stephensi* and *A. Aegypti* were found less susceptible to the synthesized AgNPs. Rawania *et al.*, (2013) carried out an eco-friendly green synthesis of AgNPs using green berry extract of *Solanum nigrum* L. The synthesized NPs were used against of *Culex quinquefasciatus* and *Anopheles stephensi*. Various characterizations such as UV, Vis spectroscopy, XRD, EDX, TEM, FTIR were carried out for AgNPs which determined that the NPs was spherical to

polyhedral in shape with size of 50–100 nm. These results suggest that the synthesized AgNPs of *S. nigrum* have the potential to be used as an ideal eco-friendly compound for the control of the mosquito larvae. Velayutham *et al.*, (2013) investigated larvicidal bioassay of synthesized silver nanoparticles utilizing aqueous bark extract of *Ficus racemosa* and tested against fourth instar larvae of filariasis vector. Various characterizations were carried out for synthesized nanoparticles which revealed that NPs are uniform and rod shaped with 250.60 nm in size. The LD₅₀ value showed that AgNPs were more active against larvae. Suresh *et al.*, (2014) conducted an experiment on the green synthesis of silver nanoparticles using aqueous extract of *Delphinium denudatum*. Various characterizations carried out for the size and shape detection of NPs which determined that the NPs were spherical in shape and size of the particle was 85 nm. The silver nanoparticles of *Delphinium denudatum* showed significant larvicidal efficacy against second instar larvae of dengue vector *Aedes aegypti*. Suganya *et al.*, (2014) investigates the effect of synthesized silver nanoparticles of leaf extracts of *Leucasaspera*. The formation of NPs was confirmed by various characterizations. The results showed that the maximum efficacy was observed in synthesized AgNP from leaf extracts against the fourth instar larvae of *A. aegypti* as compared to crude solvent extracts thus making them an effective combination for controlling *A. aegypti*.

2.6.6. Analgesic Activity of Silver Nanoparticles.

Afroz *et al.*, (2013) evaluated the ethanolic extract of *Caesalpinia pulcherrima* for analgesic activity. The crude extract showed significant writhening inhibition in mice induced by acetic acid as compared to standard Diclofenac sodium. Singh *et al.*, (2013) studied the analgesic potential of various medicinal plants like *Ricinus communis* Linn. *Salix alba* L., *Sida cordifolia* L., *Sylibium marianum* L., *Spilanthes acmella* Murr and *Tripterygium wilfordii* Hook f. All the plants showed varying degree of analgesic property. The present literature studied showed that no such analgesic activity have been reported on nanoparticles, so in the present study the analgesic potential of various parts of *S. thea* will be evaluated.

2.6.7. Antioxidant Activity of Silver Nanoparticles.

Bunghez *et al.*, (2012) reported that the silver nanoparticles of *Hyacinthus orientalis* L. and *Dianthus caryophyllus* L. act as a promising tool for antioxidant activity. The bioreduction of Ag^+ to Ag^0 was investigated by UV-Vis spectra, FTIR and DLS analysis. The results indicated that AgNPs have good antioxidant potential. Mohamed *et al.*, (2013) prepared the silver nanoparticles using *Chenopodium murale* leaf extract. For the confirmation of silver nanoparticles various analysis were carried out such as X-Rays diffraction analysis (XRD) and Transmission Electron Microscopy (TEM). The synthesized NPs were in the range of 30-50 nm. The AgNPs showed significant antioxidant activity as compared to *C. muralei* leaf extract or silver alone. Ranjitham *et al.*, (2013) synthesized silver nanoparticles by using Cauliflower floret extract as a reducing agent. The surface Plasmon resonance supports the formation of NPs synthesis which showed UV-Vis peak at 425 nm. Various characterizations like FTIR, EDX, TEM, XRD and SEM analysis were carried out to evaluate the NPs formation. To check the antioxidant potent of AgNPs DPPH assay were carried out which revealed that AgNPs showed significant result as compared to standard Vitamin C. Selvi and Sivakumar, (2014) carried out the biological synthesis of silver nanoparticles of *F. oxysporum* for antioxidant potential. The free radical scavenging activity of AgNPs was found to be higher than that standard confirmed in the present investigation. Giri *et al.*, (2014) screened *J. adhatoda*, *A. vulgaris* and *P. guajava* for synthesis of AgNPs. The antioxidant activity of both crude extract and AgNPs were evaluated. The antioxidant activity showed that *J. adhatoda* was found low antioxidant which is then followed by *A. vulgaris* and *P. guajava* was found to be highly antioxidant.

2.7. GOLD NANOPARTICLES

2.7.1. Characterization of Gold Nanoparticles (AuNPs)

Devi & Bhimba, (2012) synthesized AgNPs by Seaweed *Ulva lactuca* invitro extract. The UV-Vis spectroscopy showed absorbance peak at 434nm. SEM, TEM and XRD analysis confirmed that the nanoparticles were spherical in shape, with average size 20-56nm. Pandey *et al.*, (2012) synthesized highly stable gold nanoparticles using fruit peel of

Momordica charantia extract. UV-Vis spectroscopy verified the formation of Au NPs. XRD and TEM analysis revealed that NPs are 10-100 nm in size. Gopinath *et al.*, (2013) worked on the gold nanoparticles of *Terminalia arjuna*. Different characterization such as UV-Vis spectroscopy showed absorbance peak at 530nm while SEM and XRD analysis showed spherical NPs with average size ranges from 20-60nm. EDX and FTIR spectroscopy showed the presence of metallic Au and reducing agents amines and amides. Suman *et al.*, (2014) worked on the synthesis of gold nanoparticles of *Morinda citrifolia* extract. UV-vis spectroscopy, XRD, FTIR, FE-SEM, EDX and TEM were performed to confirm the formation of Au NPs. UV-vis spectrum showed peak at 540 nm, XRD revealed that particles are cubic structure. The FTIR results explain that protein is responsible for NPs formation while SEM and TEM analysis revealed that NPs are spherical in shape with ranges from 12.17-38.26 nm. Yasmin *et al.*, (2014) synthesized gold nanoparticles using *Hibiscus rosa-sinensis*. The synthesis of AuNPs was confirmed from SPR value at 520 nm. The average particle size about 16-30 nm were confirmed by Transmission Electron Microscopy (TEM). Fourier Transform Infrared Spectroscopy (FTIR) showed the presence of alkaloids and flavonoids which play a vital role in nanoparticles synthesis. Gopalakrishnan & Raghu, (2014) synthesized gold nanoparticles of Milk Thistle (*Silybum marianum*) seed extract. The formation of gold nanoparticles and stabilization of medium were confirmed by UV-Vis spectroscopy. The size of NPs was calculated by XRD and HTEM analysis. The confirmation of biomolecules responsible for the reduction and stabilization of nanoparticles were conducted by FTIR analysis.

2.7.2. Microbial Activity of Gold Nanoparticles.

Ali *et al.*, (2011) conducted an experiment for the biosynthesis of gold nanoparticles and its antibacterial activity. The synthesis of gold nanoparticles by *Mentha piperita* extract was confirmed by UV-Vis spectroscopy. Various characterizations like FTIR, XRD, SEM and EDX analysis were carried out which revealed that the NPs are spherical in shape with 150nm in size. The microbial activity of AuNPs was evaluated against clinically isolated human pathogens, *Staphylococcus aureus* and *Escherichia coli*. Nagaraj *et al.*, (2012) carried out green synthesis of gold nanoparticles of *Caesalpinia*

pulcherrima. UV-Vis spectroscopy indicates the formation of AuNPs. TEM analysis revealed particle size 10-50nm. As compared to standard antibiotic the gold nanoparticles of *Caesalpinia pulcherrima* showed good efficacy. Geethalakshmi and Sarada, (2012) prepared gold nanoparticles with root extract of *Trianthema decandra*. Various shapes of NPs were confirmed by Field emission-scanning electron microscopy. XRD analysis showed average 33-65 nm particle size. EDX analysis showed the presence of metallic gold. The synthesized nanoparticles showed significant microbial activity. Jayaseelan *et al.*, (2013) worked on seed extracts of *Abelmoschus esculentus* for the synthesis of gold nanoparticles (AuNPs). Different characterizations and antifungal activity were performed which confirmed the synthesis of AuNPs. The characterized peak for UV-visible spectrum at 536 nm whiles the crystalline nature and 62 nm in size was confirmed from XRD. Similarly FTIR clearly showed that the extracts containing OH as a functional group act in capping the nanoparticles synthesis. FESEM images revealed that all particles were spherical with a narrow size range of 45–75 nm. The antifungal activity against various fungi like *Puccinia graminis tritici*, *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* revealed that the AuNPs were highly effective against *P. graminis* and *C. albicans*. So it is confirmed that the AuNPs of *Abelmoschus esculentus* have high antifungal efficacy and it can be used in antifungal drugs for various fungal diseases. Vijayakumara *et al.*, (2013) carried out the biosynthesis, characterization and antibacterial activity of silver nanoparticles using *Artemisia nilagirica* extract. Different characterizations like scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDX) were carried out which determined the size of the particles as 70–90 nm and (EDX) analysis confirmed the presence of silver. The results of microbial activity revealed that the susceptibility to AgNPs was different for each microorganism. Ahmed *et al.*, (2014) used *Salicornia brachiata* for the synthesis of gold nanoparticles. Various characterizations were carried out to assess the formation of AuNPs. The NPs produced peak at 532 nm due SPR. SEM and XRD analysis revealed that AuNPs are polydispersed and 22 to 25 nm in size. To check antibacterial efficacy of the synthesized AuNPs were applied for antibacterial activity against various bacterial strains.

2.7.3. Cytotoxic Activity of Gold Nanoparticles

Cai *et al.*, (2008) summarized the application of nanospheres, nanocages, nanoparticles and nanoshells against cancer cells. The summarized application revealed that gold nanoparticles hold the promise for a “magic gold bullet” against cancer. Raghunandan *et al.*, (2011) explored the efficacy of silver nanoparticles of guava and clove plant extract against four different cancer cell lines for human colorectal adenocarcinoma, human kidney, human chronic myelogenous, leukemia, bone marrow, and human cervix. It is suggested that flavonoids were responsible for synthesis of gold NPs and as compared to guava NPs clove buds NPs were more efficient towards anti-cancer activities. Joshi *et al.*, (2012) explore the anticancer potential of chloroquine gold nanoparticles against MCF-7 breast cancer cells. Different concentrations of gold nanoparticles were treated with MCF-7 cells. The viability was assessed by trypan blue. Flow cytometry analysis revealed that at the major pathway of cell death was necrosis, which was mediated by autophagy. Geetha *et al.*, (2013) disclosed the biosynthesizing capacity of *Couroupita guianensis* extract gold nanoparticles. In the this investigation gold NPs were synthesized and various characterizations such as UV-Vis spectroscopy, FTIR, XRD, SEM and TEM analysis were conducted to evaluate the morphological feature as well as the particle size of the newly synthesized NPs. The NPs were further investigated by disclosing the applications like MTT assay, DNA fragmentation, apoptosis by DAPI staining, and comet assay for DNA damage. Lokina & Narayanan, (2013) carried out bio-inspired synthesis of gold nanoparticles of grapes fruit extract. The surface Plasmon resonance showed the formation of AuNPs at 5nm. The FTIR analysis showed the presence of Polyphenols in grapes extract which is responsible for NPs formation. The synthesized NPs showed significant anticancer activity against HeLa cell lines.

2.7.4. Antileishmanial Activity of Gold Nanoparticles

Kayser & Kiderlen, (2001) performed *In vitro* leishmanicidal activity by naturally occurring chalcones and concluded that natural compounds are considered good and affluent source of bioactive compounds having antileishmanial ability. Similarly Chan-Bacab and Rodriguez, (2001) evaluate the potent of natural products for leishmanicidal activity. Grecco *et al.*, (2010) isolated compound from the leaves of *Baccharis retusa* and applied it against leishmania. The flavonoids constituents present in *Baccharis retusa* leaves have strong anti leishmanial efficacy. Literature survey revealed that no such

nanoparticles potential have been studied for antileishmanial activity, so in the present investigation the efficacy of gold nanoparticles will be evaluated against leishmania.

2.7.5. Larvicidal Activity of Gold Nanoparticles.

Soni and Prakash, (2012) synthesized gold nanoparticles (AuNPs) from a fungus *Aspergillus niger*. The larvicidal potential of these NPs was evaluated against three mosquito species *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* and reported that the fungal NPs have larvicidal potential. Gopinath *et al.*, (2013) worked on the gold nanoparticles of *Terminalia arjuna*. Different characterization such as UV-Vis spectroscopy showed absorbance peak at 530nm while SEM and XRD analysis showed spherical NPs with average size ranges from 20-60nm. EDX and FTIR spectroscopy showed the presence of metallic Au and reducing agents amines and amides. The synthesized NPs were then evaluated for control of malarial vector against *Anopheles stephensi*.

2.7.6. Analgesic Activity of Gold Nanoparticles.

Afroz *et al.*, (2013) evaluated the ethnanolic extract of *Caesalpinia pulcherrima* for analgesic activity. The crude extract showed significant writhing inhibition in mice induced by acetic acid as compared to standard Diclofenac sodium. Singh *et al.*, (2013) studied the analgesic potential of various medicinal plants like *Ricinus communis* Linn., *Salix alba* L., *Sida cordifolia* L., *Sylbium marianum* L., *Spilanthes acmella* Murr., *Tripterygium wilfordii* Hook f. All the plants showed varying degree of analgesic property. The present literature studied showed that no such analgesic activity have been reported on nanoparticles, so in the present study the analgesic potential of various parts of *Sageretia thea* will be evaluated.

2.7.7. Antioxidant activity of Gold Nanoparticles

Song *et al.*, (2009) used leaf extracts of two plants *Magnolia kobus* and *Diopyros kaki* for the synthesis of gold nanoparticles. The synthesis of gold nanoparticles were characterized by different characterization like UV-visible spectroscopy, energy-dispersive X-ray spectroscopy (EDS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), Fourier-transform infrared spectroscopy (FTIR). SEM and TEM images showed pentagons, and hexagons structures of NPs (size, 5–300 nm) were formed

at lower temperatures while smaller spherical shapes were obtained at higher temperatures. Sane *et al.*, (2013) used *Betel* leaf, *Hibiscus* leaf, *Lantana camara* and nuts of bitter guard for synthesis of gold nanoparticles (GNPs) by green biogenic approach. Gold nanoparticles were synthesized by thermal reduction (microwave irradiation) of chlorauric acid (HAuCl₄) using aqueous extracts of plant materials mentioned above. The formation of gold nanoparticles was confirmed by using UV-Visible spectra with absorbance peaks obtained between 500 nm to 600 nm. Bio-reduction of 0.5 mM and 1mM HAuCl₄ by varying gold salt and extract ratio were investigated. About 26 to 63nm AuNPs have been recorded. Muthuvel *et al.*, (2014) carried out environmentally benign biological process for the synthesis of gold nanoparticles using *Solanum nigrum* leaf extract as reducing agent. Biosynthesized AgNPs showed strong DPPH radical scavenging activity as compared to the aqueous leaf extract of *S. nigrum*. So the present report suggests that AuNPs of *Solanum nigrum* leaf have strong potential as antioxidant and act as a promising candidate against cancer cells.

Chapter # 03

MATERIALS AND METHODS

The pharmacognostic and pharmacological study of *Sageretia thea* was carried out with following the below procedure

3.1. COLLECTION AND PRESERVATION

3.2. LEAF SURFACE MECROSCOPY

3.2.1. Vein termination number

3.2.2. Vein islet number

3.2.3. Palisade ratio

3.2.4. Stomatal frequency and stomatal index

3.3 PHYSICOCHEMICAL STUDY

3.3.1. Powder drug florescence study

3.3.2. Proximate and nutritional analysis of various parts

3.4 PHYTOCHEMICAL SCREENING OF LEAF, STEM, ROOT

3.4.1 Detection of carbohydrates

3.4.2 Detection of Protein

3.4.3 Detection of alkaloid

3.4.4 Detection of Phytosterols and triterpenoids

3.4.5 Detection of phenol

3.4.6 Detection of flavonoid

3.4.7 Tannins

3.7.8 Detection of saponins

3.4.9 Detection of fats and oil

3.5. GREEN SYNTHESIS OF SILVER/GOLD NANOPARTICLES OF *S. THEA*

3.5.1. Characterization of Silver/Gold Nanoparticles

3.5.1. i. UV-Visible Spectroscopy (UV)

3.5.1. ii. Scanning Electron Microscopy (SEM)

3.5.1. iii. X-Rays Diffraction Analysis (XRD)

3.5.1. iv. Energy Dispersive Spectroscopy (EDX)

3.5.1. v. Fourier Transform Infra Red Spectroscopy (FTIR)

3.5.1.vi. Transmission Electron Microscopy (TEM)

3.5.1.vii. Dynamic Scattering spectroscopy (DLS)

3.6. PHARMACOLOGY

3.6.1. Cytotoxic activity

3.6.2. Antifungal activity

3.6.3. Antioxidant activity

3.6.4. Larvicidal activity

3.6.5. Antimicrobial activity

3.6.6. Analgesic activity

3.6.7. Antileishmanial activity

3.1. COLLECTION AND PRESERVATION

Fresh specimens of *S. thea* at their leafy stages were collected from District Dir, Khyber pakhtunkhwa, Pakistan. The collected parts of the plant were cleaned, washed with tap water, separated and dried in shade. The dried specimens were next powdered by electric grinder and used for different tests i.e. powder drug study, microchemical tests and for synthesis of nanoparticles. Morphological and anatomical characters of the fresh parts of the plant were studied and rest of the plant parts were cut into small pieces and dried in shade at about 25 ± 3 °C and powdered with the help of electric grinder and meshed. Powders were preserved in airtight bottles to protect from the effect of humidity, light and attack of insects/pathogens (Kala *et al.*, 2011).

3.2. LEAF SURFACE MICROSCOPY

The following epidermal microscopic parameters of the leaf of *S. thea* worked out using light microscope.

3.2.1. Vein Islet Number

The ultimate division of vein in small area of photosynthetic tissue of leaf is known as **vein islet** and the number of that vein in per square mm of the leaf is called **vein-islet number** (Trease and Evans, 2002).

Material Required

Leaves, slides, cover slip, sharp razor, microscope

Chemicals Required

Sodium hypochlorite, Hydrochloric acid,

Procedure

Fresh leaves of *S. thea* were boiled with 100 % concentrated chloral hydrate solution to decolorize the leaves. Those leaves which were still unclear after this treatment were next treated successively with sodium hypochlorite and 10 %

hydrochloric acid to remove coloring material and calcium oxalate by following the standard procedure (Trease & Evans, 2002). The decolorized leaves were cut in to 1 sq mm piece and mounted on microscopic slides. For determination of vein islet number, the slides were observed under Labomed microscope provided with overhead digital IVU 3100 camera. Those areas which were completely enclosed by veins were counted while excluding those areas from counting which were incompletely enclosed or cut by top or left hand sides or cut by other two sides.

3.2.2. Vein Termination Number

The free termination of vein-let and their number per square mm of photosynthetic tissue of leaf is called **vein termination number**.

Procedure

For determination of vein termination number the same procedure was carried out as used for vein islet number but in this method the number of blindly ended veins were counted under Labomed microscope by following the procedure of Trease & Evans, (2002), Wallis (2005).

3.2.3. Palisade Ratio

The total number of palisade cells present beneath a single upper epidermal cell of leaf is known as **palisade cell ratio**. Palisade cell ratio is a constant value for each species and not affected by geographical factors, therefore it is helpful for identification of species (Trease & Evans, 2002).

Procedure

For determination of palisade ratio 2 mm square piece of leaf were decolorized by boiling with 100% concentrated solution of chloral hydrate. The cleared pieces of leaf were observed under the Labomed microscope provided with overhead digital IVU 3100 camera. First a number of group of four epidermal cells were selected and then the palisade cells lying beneath each group was focused by slight rotation of fine adjustment of microscope and the palisade cells were counted. The total number of palisade cells beneath each group was dividing by 4 which give the palisade ratio (Trease & Evans, 2002).

3.2.4. Stomatal number and stomatal index

The total number of stomata per square mm of epidermis on each surface of a leaf is known as **stomatal number** while the percentage of stomata to the total number of epidermis is called **stomatal index** (Trease & Evans, 2002).

Procedure

Fresh leaves were immersed in water to prevent these from desiccation. Epidermis was peeled off from both surface of fresh leaf with the help of sharp raizer blade. The peeled surface was placed on a microscopic slide and mounted with glycerin and the following parameters were studied under microscope with 100x.

Presence and absence of stomata on each epidermis, number of epidermal cells,

Type of stomata,

Size of stomatal pore,

Size of guard cells and number of stomata per square mm magnification

By using the above data the stomatal index of the leaves of *S. thea* was calculated with the help of following formula (Chaudhary and Imran, 1997; Trease and Evans., 2002).

$$I = \frac{S}{S+E} \times 100$$

Where as

I= Stomatal index

S= Number of stomata per unit area

E= No. of epidermal cells per unit area.

Statistical evaluation

Statistical evaluation of the epidermis and stomata including mean, standard error, variance and coefficient of variance were also calculated from the above data (Chaudhary & kamal, 2004).

3.3. PHYSICOCHEMICAL STUDY

3.3.1. Powder Drug Flourescence study

Powder drug of different parts of plant give different fluorecence under ultraviolet radiation; therefore florescence evaluation is used for identification of plant and powder drugs (Jarald & Jarald, 2007).

Apparatus required

UV tube (254 nm, 366 nm), glass slide, dropper

Chemicals required

50% HCl	50% H ₂ SO ₄
50% HNO ₃	Picric acid
10% FeCl ₃ solution	NaOH in water
NH ₃ solution	NaOH in ethanol
Iodine solution	

Procedure

The fluorescence analysis of dried powder of leaf, stem and root of *S. thea* was carried out by treating 1 gm dried powder of each part with different chemicals (50% HCl, 50% H₂SO₄, 50% HNO₃, picric acid, 10% FeCl₃ solution, NaOH in water, NH₃ solution, NaOH in ethanol and Iodine solution) and each treated sample was observed under ordinary light and then under UV light of both long and short wave lengths (Brain & Turner, 1975; Evans, 2002).

3.3.2. PROXIMATE AND NUTRITIONAL ANALYSIS

Sampling

Each sample in powdered form were weighed and digested in a 5: 1:0.5 mixtures of nitric acid for 24 hours and then add perchloric acids for three hours. The solution was heated till the appearance of white fumes and then filtered. The entire filtrate was diluted suitably with distilled water by following (Khan *et al.*, 2011). The dilute filtrate solution was used for elemental analysis.

Atomic absorption spectroscopy measurements (AAS)

Macro and micronutrients were determined using Perkin Elmer; Analyst 700, single beam atomic absorption spectrometer and the data was obtained in parts per million (ppm), (1ppm=1mg/kg). Calibration curve was established using working standards of 1000 ppm for each element. Laboratory procedures for the determination of macro and micronutrients were used as outlined by Shah *et al.*, (2009) for plant samples.

Proximate analysis

Proximate analysis of the each samples for moisture, total ash, fiber, fats, proteins and carbohydrates were determined by following AOAC methods (Anonymous, 1990). The moisture and ash were determined using weight difference method (Haro *et al.*, 1968; Boussama *et al.*, 1999 and Das *et al.*, 1997). The determination of proteins in terms of nitrogen was done by micro Kjeldahl method involving digestions, distillation and

finally titration of the sample (Pearson, 1976). The nitrogen value was converted to protein by multiplying to a factor of 6.25. The lipid content of the samples was done using Soxhlet type of the direct solvent extraction method. The solvent used was petroleum ether (Folch *et al.*, 1957). The fiber was also determined by the method described by (Haro, 1968, Boussama *et al.*, 1999). The total carbohydrates were determined by difference method [100 - (proteins + fats + moisture + ash in percentage)] (Muller and Tobin 1980), while the energy values were determined by using the following formula,

$$\text{K calories/100 g} = 9 (\text{crude fats (\%)}) + 4 (\text{carbohydrate (\%)}) + \text{protein (\%)})$$

All the proximate values were calculated in percentage (AOCS, 2000: Okwu and Morah, 2004).

Statistical analysis:

Data obtained was statistically analyzed using statistical package i.e., Cohort V-6.1 (Co-stat-2003). Each experiment was repeated three times and values expressed are means $\pm$ standard deviation.

3.4. PHYTOCHEMICAL SCREENING TEST

Preparation of extract by using organic solvent

To derive extract from leaf, stem and root of *S. thea* 500 g of each sample was soaked separately in 3 liters of ethanol for 15 days and kept these on electric magnetic stirrer model no Corning PC-420D. The extract were then filtered and evaporated through rotary evaporator under reduced pressure. Each extract of the three samples were collected and used for qualitative analysis, as given below.

Qualitative analysis

For the detection of different secondary metabolites (carbohydrates, proteins, alkaloids, phytosterols, triterpenoids, phenols, tannins, saponins, flavonoids, fixed oil and volatile oil) various qualitative screening test were conducted for each part of *S. thea*.

3.4.1 Detection of carbohydrates

- **Molisch's test:** The extract solution was taken in test tube and few drops of alcoholic α -naphthol were added. Then through the side wall of the test tube 0.2 ml of concentrated H_2SO_4 was added. The appearance of purple to violet colour ring at the junction confirms the presence or absence of carbohydrates (Trease & Evans, 2002).
- **Benedict's test:** Few drops of Benedict's reagent were added to extract of each sample and then boiled it on water bath. The presence of reducing sugar was detected by the appearance of reddish brown precipitate (Trease & Evans, 2002).
- **Fehling's test:** Same volume of Fehling's A (Copper Sulphate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) were added with few drops of sample solution of each extract and then boiled. The appearance of brick red precipitate of cuprous oxide confirms the presence of reducing sugar (Trease & Evans, 2002).

3.4.2 Detection of Protein

- **Millon's test:** Each sample of respective extract was treated with 2 ml of Millon's reagent. The appearance of white precipitate in solution which turned red by heating confirmed the presence of protein (Trease & Evans, 2002).
- **Ninhydrin test:** Extract solution of each sample was taken in test tube and added 0.2% of Ninhydrin solution in it and then boiled. The appearances of violet colour indicate the presence of protein otherwise not (Kumar & kiladi, 2009).

3.4.3 Detection of alkaloids

- **Mayer's test:** Few drops of Mayer's reagent were added to each sample extract. The appearance of reddish brown precipitate confirmed the presence of alkaloids (Khandelwal, 2004).
- **Wagner's test:** Few drops of Wagner's reagent were added to each extract solution. Presences of reddish brown precipitate detect the presence of alkaloids (Khandelwal, 2004).

- **Hager's test:** For the detection of alkaloids, few drops of Hager's reagent were added in sample of each extract. The formation of yellow precipitate confirms the presence of alkaloids (Khandelwal, 2004).

3.4.4 Detection of Phytosterols and triterpenoids

- **Libermann-Burchard test:** Few drops of acetic anhydride were added to each sample extract then boiled and cooled. From the side of test tube wall concentrated sulphuric acid was added. If a brown ring is formed at the junction of two layers, then above the ring green color indicated the presence of sterols while lower deep layer showed the presence of triterpenoids (Harborne and Baxter, 1999).

3.4.5 Detection of phenol

- **Ferric chloride test:** 2 ml of ferric chloride solution was taken in a test tube to which 2 ml of extract solution added. The bluish green colour of the solution indicates the presence of phenol otherwise not (Dahiru *et al*, 2006).

3.4.6. Detection of flavonoids

- **Alkali test:** Few drops of sodium hydroxide were added with extract solution of each sample. The appearance of yellow to red precipitate confirms the presence of flavonoids (Kokate, 1994).

3.4.7. Tannins

- **Ferric chloride test:** To an extract, FeCl_3 was added. The formations of yellow to red precipitate detect the presence of tannins (Kokate, 1994).
- **Alkali test:** Sodium hydroxide was added to an extract solution. Tannins were detected by the appearance of yellow to red precipitate (Kokate, 1994).

3.4.8. Detection of saponin

- **Frothing test:** In a test tube 5 ml of an extract solution was taken and then vigorously shake it. On surface of solution the froth formation indicate the presence of saponins (Chaouche *et al*, 2011).

3.4.9. Detection of fats and oil

- **Spot test:** Powder drug of each sample was individually pressed between two filter papers. Existence of oily spots on filter paper shows the presence of fixed oil (Gomathi et al., 2010).

3.5. SYNTHESIS OF SILVER AND GOLD NANOPARTICLES

Chemicals

The silver nitrate (AgNO_3) and Gold chloride (HAuCl_4) were purchased from (E. Merck. D-6100 Darmstedt, F.R. Germany.

Preparation of Extracts

For preparation of extract different parts of *S. thea* ground by using electric grinder to 60 mesh diameter powders. Five hundred grams (500 g) powder of each sample was soaked in 1500ml of 70% ethanol for 15 days. The extract of each part was filtered through Whatman filter paper No. 1823 and then evaporated through rotatory evaporator at 40°C and preserved in refrigerator at 4°C for nanoparticles synthesis.

Preparation of Silver Nitrate and Gold Chloride solutions

Analytical grade silver nitrate (AgNO_3) and Gold chloride (HAuCl_4) were purchased from (E. Merck. D-6100 Darmstedt, F.R. Germany. Stock solution 1 mM of AgNO_3 and HAuCl_4 in distilled water was prepared according to the following calculations

For AgNO_3 :

The molecular weight of AgNO_3 :

$$[\text{Ag}-107.87, \text{N}-14, \text{O}-16] = 107.87 + 14 + (16 \times 3) = 169.87$$

Therefore, Molar mass of $\text{AgNO}_3 = 169.87 \text{ g}$

Calculations:

Density	Volume	Required amount of Silver nitrate (AgNO ₃)
1Mm	1000	(169.87/1000) g

For HAuCl₄ :

The molecular weight of HAuCl₄:

[H- 1.007+Au- 196.96+ Cl-35.5×4=142= 339.78 g

Therefore, Molar mass of HAuCl₄= 339.78 g

Calculations:

Density	Volume	Required amount of AgNO ₃
1Mm	1000	(339.78 /1000) g

Synthesis of Nanoparticles

700 ppm extract solution of each sample with 1mM aqueous solution of silver nitrate and gold chloride in different ratios by volume for synthesis of Ag/AuNPs were mixed and stirrer for 4 hours till the colour of solution completely change into yellow brown.

3.5.1. Characterizations

3.5.1.i. UV-vis spectrophotometer

The reduction of silver/gold ions were confirmed by measuring the UV–vis spectrum of the reaction mixture against distilled water ethanol mixture as a blank. The Spectral analysis was done using Uv-1602 Double Beam UV/Vis spectrophotometer BMS Biotechnology Medical Services at a resolution of 1 nm from 300 to 800 nm. The selected ratio was freeze dry for further characterizations.

3.5.1.ii. FT-IR Spectroscopy

PerkinElmer spectrometer FT-IR SPECTRUM ONE in the range of 4000–0 cm⁻¹ at a resolution of 4 cm⁻¹ was used. The sample was mixed with KBr procured from Merck chemicals. Thin sample pellet was prepared by pressing with the Hydraulic Pellet Press and subjected to FT-IR analysis. FTIR spectroscopy was carried out in Pakistan institute of engineering and applied sciences (PIEASE) Islamabad, Pakistan.

3.5.1.iii. X-RAY DIFFRACTION (XRD)

Resulting solutions of the synthesized SNPs dried at 80 °C for X-ray powder diffraction measurements. XRD measurements were carried out on a JEOL JDX 3532 X-ray diffractometer. The pattern was recorded by Cu-K α 1 radiation with λ of 1.5406 Å and nickel monochromator filtering the wave at tube voltage of 40 kV and tube current of 30mA. The mean particle diameter of Ag/AuNPs was calculated from the XRD pattern according to the line width of the maximum intensity reflection peak. The size of the nanoparticles was calculated through the Scherrer's equation (Patterson, 1939).

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where D is the average crystal size, K is the Scherrer coefficient (0.89), λ is the X-ray wavelength ($\lambda=1.5406$ Å),

2θ is Bragg's angle, β cor the corrected of the full width at half maximum (FWHM) in radians, and β sample and β ref are the FWHM of the reference and sample peaks, respectively.

3.5.1.iv. SCANNING ELECTRON MICROSCOPY (SEM)

Morphology of the sample was found out using FE-SEM (JSM-5910-JEOL-JAPAN). A pinch of dried sample was coated on a carbon tape. It was again coated with gold with auto fine coater (Spi-module sputter coater) and then the material was subjected to analysis.

3.5.1.v. ENERGY DISPERSIVE X-RAY SPECTROSCOPY (EDX)

The particles were isolated by centrifugation of 20 ml of suspension in deionised water containing Ag nanoparticles for 20 min at 10,000 rpm. The pellets were collected and were dried in the oven at 50 °C to remove any excess water. The sample was collected in the powder form and was used for EDX analysis. Ag/AuNPs were dried and drop coated on to carbon film. EDX analysis was then performed using the Oxford inca 200 SEM instrument equipped with a Thermo EDX attachment.

3.6. PHARMACOLOGY

3.6.1. LARVICIDAL ACTIVITY

Material Required

Plant extracts, AgNO₃, HAuCl₄, Mosquitoes larvae, yeast and dog biscuits, double-distilled water and multivial tray.

Mosquito culture

A. aegypti larvae were collected from University of Peshawar, Pakistan. The larvae were kept in plastic trays containing tap water and were maintained, in the laboratory. All the experiment was carried out at 32°C. Larvae were fed with yeast and dog biscuits in the 4:1.

Larvicidal Activity of Ag/Au Nanoparticles

Larvicidal bioassay was carried out according to WHO (1996). Thirty field-collected late fourth instar larvae were taken for each replicates in 249 ml of water and 1.0 ml of aqueous plant extract with different concentration (5 to 30 mg/mL). The control was set up with dechlorinated tap water. After 24 h of exposure, the number of dead larvae were counted and the percentage mortality was calculated. Synthesized Ag/AuNP of leaf, stem and root extracts toxicity tests were conducted using a multi concentrations test, consisting of a control and different concentrations of Ag/AuNP of leaf, stem and root extracts. Each test was performed by placing 30 mosquito larvae into 200 ml of sterilized double-distilled water with nanoparticles into a multivial tray. Nanoparticles solutions were diluted using double-distilled water as a solvent according to the desired concentrations (2 to 12 mg/mL). Each test included a set control group (Silver nitrate/Gold chloride and distilled water) with three replicates for each individual concentration. Mortality was assessed after 24 h to determine the acute toxicities on fourth instars larvae of *A. aegypti* mosquitoes. Number of survivors was counted and % mortality rate was determined using formula:

$$\% \text{ Mortality} = 100 - \frac{\text{Number of larvae alive in test sample}}{\text{Number of larvae alive in control}} \times 100$$

3.6.2. Antioxidant Activity of Ag/Au Nanoparticles

Material Required

Plant extracts, HAuCl₄, AgNO₃, UV-Spectroscopy, DPPH (1,1-diphenyl-2-picrylhydrazyl), Test tubes, stand, Ascorbic acid, and pipette.

Procedure

The DPPH free radical scavenging assay was conducted based on the method of Chang *et al.*, (2002). One milliliter of 0.1 mM DPPH (in ethanol) was added to different concentrations (10, 20, 40, 60, 80, 100 µg/ml) of ethanolic extract of each sample and its Ag/AuNPs. The reaction mixture was shaken and incubated in the dark for 30 min. The absorbance at 517 nm was measured against a blank (ethanol). Ascorbic acid (Vitamin C) was used as the standard. The lower absorbance of the reaction mixture indicated a higher percentage of scavenging activity. The percentage of scavenging of free radicals was determined by the following formula:

$$\% \text{Antioxidant potential} = \frac{\text{Absorbance of control (nm)} - \text{Absorbance of test (nm)}}{\text{Absorbance of control (nm)}}$$

3.6.3. Antibacterial Activity of Ag/Au Nanoparticles

Material Required

All analytical reagents used in the study were of analytical grade. Nutrient agar for bacterial culture and Mueller–Hinton broth and agar for antimicrobial activity were purchased from and were purchased from Merck, Pakistan.

Antibacterial Activity

The antibacterial activity was carried out against *E. coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseud. aeruginosa* and *B. pumilus*.

Preparation of inoculums

Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to Mueller–Hinton broth (MHB) tubes that were then incubated without agitation for 24 h at 37 °C. The cultures were diluted with fresh Mueller–Hinton broth to achieve optical densities corresponding to 0.5 (i.e., 10⁵–10⁶ CFU/ml using MacFarland's standard).

Agar well-diffusion method

Mueller–Hinton agar plates were swabbed on three axes with a sterile cotton-tipped swab that was first dipped in the freshly prepared diluted culture. A 6 mm hole was bored aseptically with a sterile cork borer. The agar plugs were taken out carefully, without disturbing the surrounding medium. The holes were filled with ethanolic extract and Ag/AuNPs and allowed to stand for 1 h for the perfusion of the nanoparticles. The plates were kept for further incubation at 30 °C for 24 h. After incubation, the Petri dishes were evaluated for antibacterial activity, which was measured in terms of the diameter (in millimeters) of the inhibition zone. The standard antibacterial agent used was streptomycin (2 mg mL⁻¹).

3.6.4. Antifungal Activity of Ag/Au Nanoparticles

Material Required

Plant extracts, HAuCl₄, AgNO₃, Fungal strains, Petri plates, Agar Agar medium

Procedure

Dose dependent antifungal activity of the synthesized Ag/AuNPs was carried out by well diffusion method. The fungal strains were grown for 72 hours in potato dextrose broth (PDB). Microorganism was prepared by spreading 100 μ L of revived culture (containing 10⁴ cells ml⁻¹) on potato dextrose agar (PDA) (Hi Media) media with the help of spreader. Various concentrations of Au NPs (10, 20, 30, 40 and 50 μ L) were added to the centre of the well with 7 mm of dia. The amphotericin B and distilled water were used as a positive and negative control for the antifungal assay. The petriplates were incubated at 25 °C for 72 h in incubator during which activity was evidenced by the presence of a zone of inhibition (mm) surrounding the well (Jayaseelan *et al.*, 2012).

3.6.5. Cytotoxic Activity of Ag/Au Nanoparticles

The cytotoxic activity of Ag/AuNPs of leaf, stem and root extract of *S. thea* were carried out as follows by McLaughlin *et al.*, (1993).

Activity Requirement

Synthesized Ag/AuNPs of leaf, stem and root extract, Brine shrimp eggs, Sea Salt (38 g/L of D/W, pH 7.4), Hatching tray with perforated partition, electric lamp to attract brine-shrimp larvae, Micro pipette (5, 50, 500 μ l), Vials tray, vials, ethanol.

Preparation of Brine solution

Brine solution was prepared by dissolving thirty eight grams (38 g) of sea salt in one liter of distilled water and then filtered.

Hatching Techniques

The hatching tray was divided into unequal two parts with a center perforated partition. The tray was half filled with filtered brine solution. In smaller part of the tray 25 mg of brine shrimps eggs were sprinkled and it was covered with black paper while the other larger half was left uncovered. For hatching of shrimp's eggs the tray was kept at 25°C. To illuminate the open portion of the tray a lamp was suspended above the tray. When the eggs hatched it will be noted that the larvae will be moving from the covered dark portion toward the enlightened portion through the perforation of the partition. Brine shrimp *Artemia salina* cysts were used for cytotoxicity assay (McLaughlin *et al*, 1993). Briefly, *Artemia salina* cysts of 1 gm were aerated in 1 L capacity of glass jar containing 3.2 % of saline water (3.2 gm NaCl in 100 ml of distilled water). The jar was aerated constantly for 48 h at room temperature (25-29 °C). After hatching, active free-floating nauplii was collected from bright illumination and were used for the bioassay. Experiment was performed in 2 ml sterile eppendorf with 3.2 % of saline water. A negative control (without Ag/AuNPs) was also included for the experimental setup. Each nauplii were transferred with the addition of desired concentration of Ag/AuNPs. The experimental setup was allowed to remain 24 h in darkness and nauplii were counted after incubation time. Percentages of mortality, LD₅₀ for tested concentration of Ag/AuNPs were determined using Finny computer program for LD₅₀ values determination with 95% confidence intervals (D.J. Finney).

3.6.6. Antileishmanial Activity of Ag/Au Nanoparticles

***Leishmania tropica* KWH₂₃ culture**

Leishmania tropica clinical field isolate KWH₂₃ (originally isolated, studied, preserved and cultured in a culture flask containing M199 growth medium supplemented 10% heat inactivated Fetal Calf Serum (HI-FSC), 100ug/ml Penicillin, 100 ug/ml Streptomycin, 50 ug/ml Kenamycin and 5 ug/ml of Hemin. Culturing is done under required aseptic conditions in bio level two culture lab. The culture was placed inside incubator under anaerobic conditions at 24-26 °C and regularly observed and counted

daily upto 10 days with help of Neubauer Haemocytometer. The promastigotes showed log phase increase in growth and the culture bulked up in medium. Subpassage was done by spinning the culture in Sigma Centrifuge at 2000 rpm for 12 minutes. The pellet obtained was resuspended into 8-10 ml fresh growth medium to obtain 1×10^5 cells/ml dilutions for experiment. The unused promastigotes were cryopreserved (using standard protocol) in liquid nitrogen for further studies.

Preparation of Different Concentrations of Silver and Gold Nanoparticles

Six different concentrations of AuNPs and AgNPs were taken by serial dilutions from stock solution in 0.5 % DMSO before experiment. The required concentrations were 150 μ M, 100 μ M, 50 μ M, 20 μ M, 10 μ M, and 5 μ M. The serial dilution of AuNPs and AgNPs were performed by using the formula;

$$V_1 = M_2 V_2 / M_1$$

Anti-Promastigote Activity of Au/AgNPs of leaf, stem and root of *S. thea*

In vitro assay was used for anti-leishmanial activity of Au/Ag nanoparticles. The experiment was performed in 96 wells flat bottom plates. The promastigotes were counted in culture suspension by using Improved Neubauer Haemocytometer. From viable promastigotes bulk culture (4×10^6 parasites/mL), 1×10^5 promastigotes/well in 200 μ L fresh M-199 medium were seeded in 96-well plates. Each 96 wells plate needed approximately 20mL promastigotes culture. The above mentioned six different concentrations of Au/AgNPs were applied in first six rows while the last row was kept as negative control treated with 0.5% DMSO only. Each concentration of a given sample was applied in triplicate. The 96 well plate was then incubated at 26°C for 48 hours. After 48 hours, the number of promastigotes in each well (treated and control) was counted microscopically using Improved Neubauer Haemocytometer.

Statistical Analysis

After counting percent inhibition for each concentration of a given compound in triplicate was calculated by using formula:

$$\text{Percent inhibition} = \left(\frac{\text{No. of control promastigotes} - \text{No. of treated promastigotes}}{\text{No. of control promastigotes}} \right) \times 100$$

The 50% inhibitory concentration (IC₅₀) was determined by linear regression analysis using Biostatistics software.

3.6.7. Analgesic Activity of Ag/Au Nanoparticles

Material Required

Eddy's hot plate, 5 cc syringe, charcoal, silver nitrate (AgNO₃) and gold chloride (HAuCl₄), plant extract.

Animals

Swiss albino mice were obtained from National Institute for Health (NIH) Islamabad, Pakistan. The animals were maintained in colony cages at 25±2 °C, relative humidity 50–55% maintained fewer than 12 h light and 12 h dark cycles. The animals were fed with Standard animal feed. All the animals were acclimatized to the laboratory conditions prior to experimentation.

Hot plate method

The hot plate method for analgesic activity was first time developed by Woolfe and MacDonald (1944). The paws of mice are very sensitive to the temperature which is not damaging the skin. The response of mice to temperature is in the form of withdrawal of the paws and jumping (Eddy and Leimback, 1953; Toma et al., 2003). The mice were kept on Eddy's hot plate at a temperature of 55±2 °C. To avoid damage to the paw skin a time period of 15 second was observed. Reaction time and response of the mice will be noted by using stopwatch. Dichlofenac sodium was used as a positive control. The Silver and gold nanoparticles of ethanolic extract of various parts of *S. thea* were injected intraperitoneally at a concentration of 100, 300 and 500 mg/kg.

Chapter # 04

RESULTS AND DISCUSSION

4.1. Systematic position

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Rhamnales
Family: *Rhamnaceae*
Genus: *Sageretia*
Species: *thea*
Botanical name: *Sageretia thea*
Local Name: Munmanra (Pashto)

4.2. LEAF SURFACE MICROSCOPY

Microscopical studies help in identification, authentication and quality assurance of plants material which contribute to its safe use as a medicine. Similarly anatomical parameters are used for the identification and standardization of plant material (Patel *et al.*, 2006). The leaf surface studies are also used for identification and standardization of crude drugs like vein islet, vein termination number, stomatal index, palisade cell ratio, trichomes types etc (Anonymous, 1998: Trease & Evans, 2002). In the current research work the anatomical study of *S. thea* leaf has been carried out (Fig. 06).

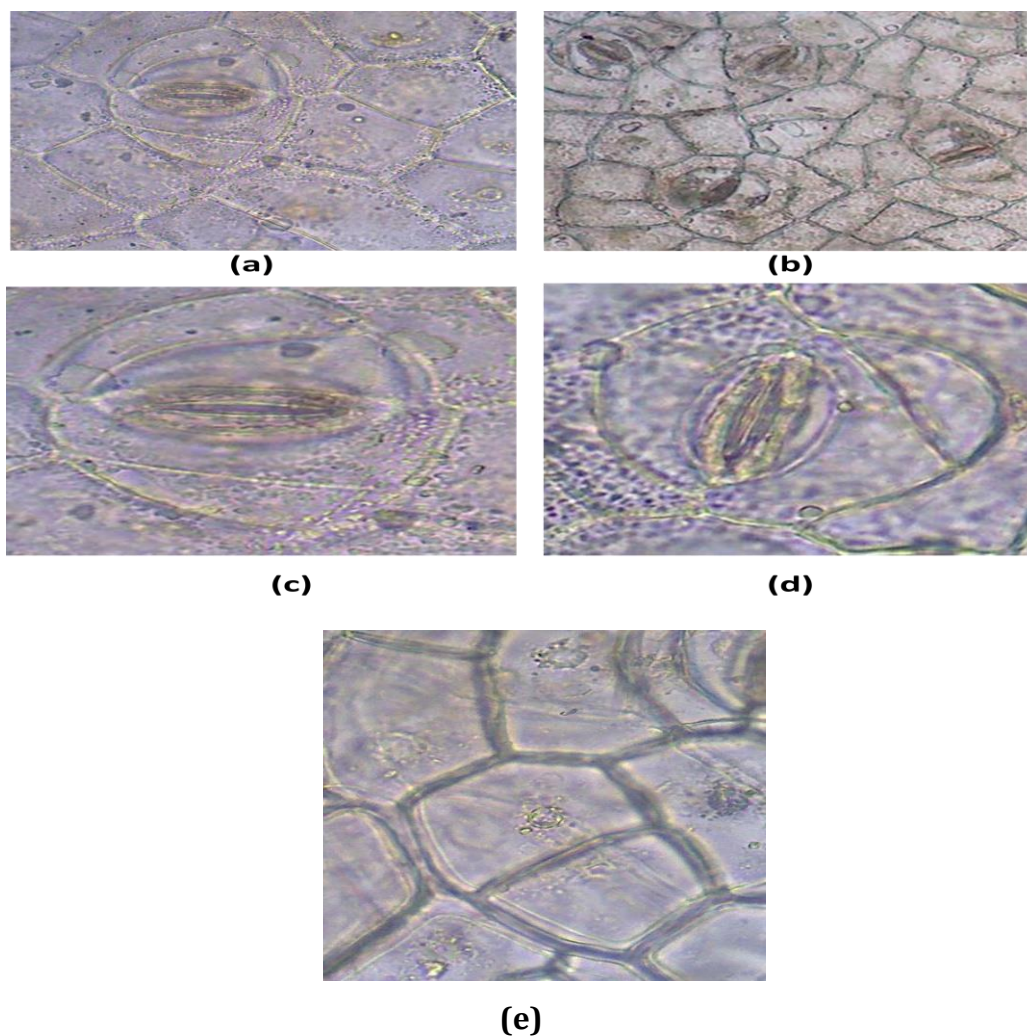


Figure 6. Key: a. Stomata on adaxial surface, b. Stomata on abaxial surface, c. Stomata closed on adaxial surface, d. Stomata open on abaxial surface. e. Vein islet and vein termination.

Table 1. Leaf Epidermal Study

Parameters	<i>S. thea</i> leaf	
	Range	Mean
Stomatal frequency (upper Epidermis)	6-11	8
Stomatal index (upper Epidermis)	2-3	2
Stomatal frequency (Lower Epidermis)	19-25	21
Stomatal index (Lower Epidermis)	7-11	12
Vein Islet Numbers	14-17	15
Vein Termination Numbers	21-30	27
Palisade Ratio (adaxial surface)	4-8	6
Palisade Ratio (abaxial surface)	4-9	5

4.3. Fluorescence Analysis

Different crude drugs when viewed under UV light show different fluorescence at different wavelengths. This is due to the presence of different chemical constituents in the drug. If the drugs do not show fluorescence it is first treated with different reagents which convert them into fluorescence derivatives. The fluorescence analysis of drugs is helpful for identification of crude drugs as well as for the detection of adulterations in whole or powder drugs (Reddy and Chaturvedi, 2010; Ansari *et al.*, 2006; Wallis, 1985). The fluorescence study of the powder of leaf, stem and root of *S. thea* was carried out with different reagents under visible and ultra violet lights. The UV light (UV 366) was used for the fluorescence character of powder drugs. The observations showed marked variation in coloration based on the part used, nature of reagents and wavelength light. The fluorescence study results are given in table (Table. 2). Many researchers have worked out fluorescence analysis of different medicinal plants like *Kirganelia reticulata* (Shruti *et al.*, 2013); *Polygonum cuspidatum* (Tian *et al.*, 2006) and *Hygrophila auriculata* (Hussain *et al.*, 2011). The results of present study have a great similarity with the findings of these workers.

Table 2. UV & Visible Fluorescence Study of Leaf, Stem and Root Powder of *S. thea*

Powder Treatment	Leaf		Stem		Root	
	Visible light	UV 366	Visible light	UV 366	Visible light	UV 366
Untreated	Dull green	Dark green	Green	Light green	Yellow	Whitish
NH ₃	Green	Black	Green	Dark green	Orange	Brown
Tape water	Dark green	Light green	Spring green	Grass green	Dry grass yellow	Pale yellow
Picric acid	Yellowish	Brown	Yellow	Brownish	Yellow	Yellow
50% HNO ₃	Yellow red	Brick red	Brick red	Dark brown	Brown	Reddish brown
Ethanol	Light Green	green	Yellowish	Spring green	Orange yellow	Yellow
50% HCL	Gray	Gray	Light red	Dark brown	Brick red	Light red
n-hexane	Green	Dark green	Light green	Grass green	Orange	Whitish
50% H ₂ SO ₄	Black	Black	Black	Brown black	Black	Dark black
Methanol	Light greenish	Light red	Yellowish	Dark Red	Light brown	Dark brown

4.4. Phytochemical screening

Each part of plant contains pharmacologically active compounds which are isolated from plant sources through a useful preliminary phytochemical screening method. Fruits and vegetables are also used for the treatment of different diseases like cancer, cardiovascular disease and immune dysfunction, the positive influence which attributed it, is the presence of secondary metabolites (Gupta *et al.*, 2013). These active constituents are used for the treatment of various diseases. Flavonoids have been used from ancient times for the treatment of various human diseases (Havsteen, 1983). Phenolics, flavonoids and flavonol content have antioxidant potential (Gupta *et al.*, 2013). Saponins, tannins and triterpenoids are used for treatment of various human ailments like mouth disease, headache and chest pain (Ghazanfar, 1994). In the present study, qualitative phytochemical screening was carried out using ethanolic extracts of leaf, stem and root of *S. thea* (Table 3). These results indicate that *S. thea* is a rich source of both primary (carbohydrate) and secondary metabolites. Saponins were present in leaves and stem but not detected in root. Alkaloids, Phytosterols and Triterpenes, Tannins, Flavonoids and Glycosides were detected in all the three parts while fixed oil were absent in all of these parts. Some phytochemist carried out phytochemical analysis of various parts of plants like Rao *et al.*, (2011) carried out phytochemical screening of ethanolic extracts of *Tephrosia purpurea*, *Alternanthera sessilis*, and *Clitoria ternata* and detected flavonoids, phenols, alkaloids and tannins in the leaves of these plants. Menon and Latha, (2011) worked out on different organic solvent extracts of *Coleus forskohlii* and reported a wide range of active compounds like saponins, protein, tannins, alkaloids, glycosides, carbohydrates, Phenols and cardiac glycosides. The preliminary phytochemical screening test revealed that a wide range of bioactive secondary metabolites are present in leaf, stem and root of *S. thea* which make it a multi- action drug and can be used for various medicinal purposes.

Table 3. Phytochemical Study of Leaf, Stem and Root of *S. thea*

S. No	Biomolecules	Leaves	Stem	Root
1	Carbohydrates	+	+	+
2	Phenols	+	+	+

3	Alkaloids	+	+	+
4	Saponins	+	+	-
5	Phytosterols	+	+	+
6	Tannins	+	+	+
7	Flavonoids	+	+	+
8	Glycosides	+	+	+
9	Fixed oil	+	-	-
10	Triterpenes	+	+	+

Note: Present =+ and Absent=-

4.5. Proximate Analysis of Leaf, Stem and Root of *S. thea*

In current results the moisture content was 11.9% in leaf, 8.3% in stem and 9.7% in root. Ash was 4.07% in leaf 6.3% in stem and 5.8% in root. Crude fibers were 16.4 % in leaf, 19.8% in stem and 17.1% in root. Fats in leaf were 8.4%, in stem was 3.8% and in root was 7.0%. The protein was 20.0% in leaf, 16.2% in stem and 10.6% in root. Carbohydrate was 55.63% in leaf while 65.35% in stem and 66.87% in root. The highest energy values were recorded in leaf (378.12 Kcal/100 g) which was followed by stem (360.6 Kcal/100 g) and root (373 Kcal/100 g) showed in (Table 4). The results revealed that the plant was good source of carbohydrate and to some extent protein and fibers. The bi-variable correlation co-efficient simply calculated by using the average for a replication of three observations and created a relationship between significance and non significance values. The correlation exhibited that there is a strong correlation between fats and energy level (0.999 Kcal/100 g) and it can observe that a species having high fats contents will also have high energy value, but a negative correlation also exist between fibers and energy level (-0.995 Kcal/100 g) (Table 5). Some other workers also detected proximate analysis of various medicinal plants like Read (1946) reported fat, tannin, sugar, pectin and cellulose in *Persicaria maculosa*, Duke & Ayensu, (1985) reported water, protein, fat, carbohydrate, ash and fiber in *Polygonum bistorta*, Irvine (1952) reported carbohydrate, ash and fat in *Polygonum hydropiper*, Khodzhaeva *et al.*, (2002) reported the concentration of lipids, proteins, flavonoids and carbohydrates in the aerial part of the *Rumex confertus*, Gupta, (1962) reported protein, fat, carbohydrate, ash and water in *Chenopodium album*. The author stated that root and stem of *S. thea* contained

high amount of carbohydrate concentration while leaf contain high amount of protein, fat and moisture. Stem contained high concentration of crude fibers and ash.

Table 4. Proximate analysis of Leaf, Stem and Root of *S. thea*

Parts	Carbohydrate %	Protein %	Fats %	Fibers %	Ash %	Moisture %	Energy value (Kcal/100g)
Leaf	55.63	20.00	8.4	16.4	4.07	11.9	378.12
Stem	65.35	16.25	3.8	19.8	6.3	8.3	360.6
Root	66.87	10.63	7.0	17.1	5.8	9.7	373

Table 5. Correlation coefficients among various proximate parameters

	Carbohydrate	Protein	Fats	Fibers	Ash	Moisture	Energy value
Carbohydrate	1						
Protein	-0.871	1					
Fats	-0.644	0.185	1				
Fibers	0.560	-0.081	-0.994	1			
Ash	0.942	-0.657	-0.862	0.804	1		
Moisture	-0.867	0.511	0.939	-0.898	-0.983	1	
Energy value	-0.634	0.172	0.999*	-0.995	-0.855	0.934	1

4.6. ELEMENTAL ANALYSIS

Deficiency of vital and trace elements in human can occur even under the most practical dietary conditions and in many diseased statuses. About 40 different types of elements have been detected in plants and animals. All of these elements play a combating role against various diseases. In human being the deficiency of Ca cause tooth decay, hypertension, paralysis and increase cholesterol level (Soetan *et al.*, 2010). The deficiency of Mg causes semi coma, diabetes mellitus and neurological disturbances (Ahmed and Chaudhary, 2009). For protein and carbohydrate metabolism K is an

important element. In current results elemental analysis showed that Na was present in all of the three parts, the highest concentration was found in stem followed by root and leaf (Table 6). Mg was observed in a range of 16.41mg/L to 11.59mg/L. Leaf and root have the same concentration of K while stem have low concentration. The highest concentration of Ca was recorded in root which is then followed by stem and leaf. Similarly like macro elements the microelements were also found in low and high concentration in all of these three parts. Zn, Pb and Cu were present in high concentration in leaf which is then followed by stem and root. Cd was higher in stem low in root and moderate in stem while Co and Ni both were higher in leaf while low concentration was found in root and stem respectively as shown in (Table 6). Many other researchers also screened out some medicinal plants for proximate analysis like (Sodipo *et al.*, 2008) carried out the elemental analysis of *Solanum macrocarpum* Linn. and reported the presence of S, Na, K, Fe, Mg, Zn, Ca, Cu, Mn, P, As and Cr in various part of the plant. Adnan *et al.*, (2010) assesses the elemental property of humid and sub-humid regions of North-west Pakistan and reported some macro and micro elements in these regions.

Table 6. Elemental Analysis of Leaf, Stem and Root of *S. thea*

Part	Macro-elements								Micro-elements											
	Na		Mg		K		Ca		Zn		Pb		Cu		Cd		Co		Ni	
	Mean (mg/L)	SD	Mean (mg/L)	SD	Mean (mg/L)	SD	Mean (mg/L)	SD	Mean (mg/L)	SD	Mean (mg/L)	SD	Mean (mg/L)	SD	Mean (mg/L)	SD	Mean (mg/L)	SD	Mean (mg/L)	SD
Root	27.19	4.30	16.41	0.73	27.29	1.04	121.41	5.28	0.097	0.0038	0.064	0.004	0.012	0.0014	0.005	0.0035	0.015	0.0187	1.023	0.0487
Stem	34.71	4.19	11.59	0.57	26.51	1.21	109.96	3.26	0.159	0.0022	0.088	0.0103	0.025	0.0011	0.088	0.0103	0.016	0.0027	1.015	0.0204
Leaves	23.44	3.10	12.81	0.83	27.07	1.53	96.57	2.83	0.190	0.0030	0.102	0.0372	0.042	0.0025	0.028	0.0079	0.078	0.0073	1.052	0.0130

4.7. GREEN SYNTHESIS OF SILVER AND GOLD NANOPARTICLES

4.7.1. Synthesis of Silver Nanoparticles

When aqueous solution of each extract of *S. thea* was added to 1 mM solution of AgNO₃, change in colour from colorless to yellowish red was observed in about 10 min. The final color deepened with constant stirring for about 4 h. The reduction of silver ions and the formation of stable nanoparticles occurred rapidly within four hours of reaction (Fig. 8). In our current study a brown red colour was produced when extract was added to silver nitrate solution indicating the formation of silver nanoparticles. At various concentrations the intensity of colour change depends upon size of AgNPs synthesized. The gradual formations of AgNPs due to bioreduction of Ag⁺ ion by *S. thea* extract. The colour changes during the synthesis of silver nanoparticles is due to the collective oscillation of free conduction of electrons induced by interacting electromagnetic field which is called surface Plasmon peak (Smitha *et al.*, 2009). Shankar *et al.*, (2003) reported that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. Mulvaney, (1996) also demonstrated that change in colour is due the excitation of the Surface Plasmon Resonance (SPR) effect and the reduction of AgNO₃. The results of our present study are in consistent with their report.

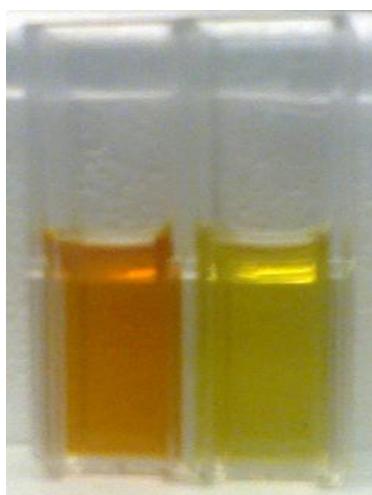


Figure 7. Synthesis of Silver Nanoparticles from *S. thea*. The change in colour indicates the reduction of silver.

4.7.2. CHARACTERIZATION OF SILVER NANOPARTICLES

4.7.2.i. UV-Visible Spectroscopy of silver Nanoparticles of Leaf Extract

The exposure of plant extract to AgNO_3 result the reduction of AgNO_3 into AgNPs indicates the formation of silver nanoparticles is due to Surface Plasmon Resonance. The change in colour (Brownish-yellow) is the first indication of nanoparticles formation (Fig. 09). In current research work the reduction of silver nitrate into silver ions is further confirmed by UV-Visible spectroscopy. UV-Visible spectra of reaction media taken with different concentrations which explicit that surface Plasmon Resonance (SPR) are found between 400-415nm. Each concentration give different absorbance peak but 1:30 give maximum absorbance with λ_{max} at 410 nm which is a green shifted. Many researchers carried out UV-visible spectroscopy of AgNPs and reported the absorption peak in the range of 400-500nm. Similarly Krishnaraj *et al.*, (2010) analyzed AgNPs using *Acalypha indica* leaf extracts through UV-Visible spectroscopy and recorded intense peak at 420 nm. Solgi & Taghizadeh (2012) synthesized the silver nanoparticles using the peel extract of *Punica granatum* and petal extract of *Rosa damascena*. UV-visible spectrum showed absorption peak at 400-450 nm. Similarly Goodarzi *et al.*, (2014) conducted UV-Vis spectroscopy of AgNPs of plant extract and reported absorption peak at 410nm. The investigations of all these researchers are in consistency to our findings. From our research work it is concluded that biomolecules present in leaf of *S. thea* responsible for the reduction of silver nitrate in to silver ions and formation of AgNPs.

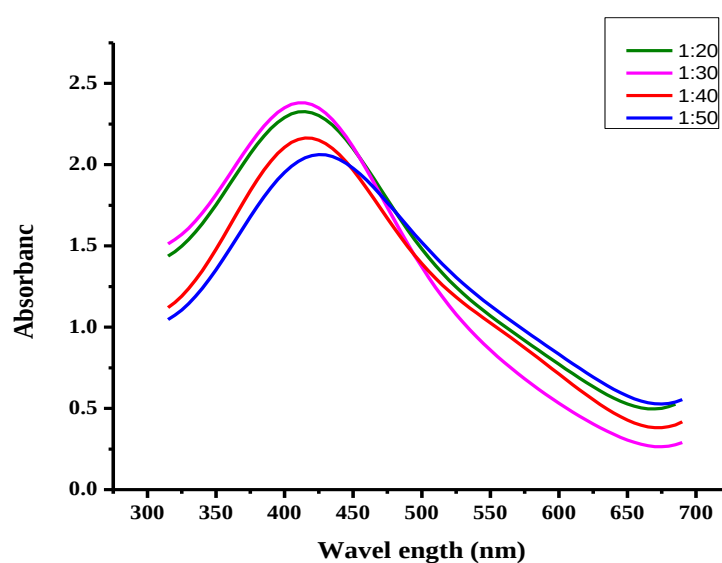


Figure 8. Optimization through UV-Visible Spectroscopy of different concentrations of leaf extract mediated silver nanoparticles showed as the concentration of AgNO_3 solution increased the absorbance

of peak also increased. But after specific range the absorbance decreased due to unavailability of functional groups.

4.7.2.ii. UV-Visible Spectroscopy of Silver Nanoparticles of Stem Extract

The change of colour from colorless to yellowish brown is the initial indication of reduction of Ag^+ . After constant stirring for about 4 h the intensity of colour increased from yellow to red brown this is directly proportion to stirring. This may be due to Surface Plasmon Resonance (SPR) effect and the reduction of Ag^+ (Mulvaney, 1996). Metal nanoparticles have free electrons, which gives Surface Plasmon Resonance (SPR) absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. Different ratios of synthesized nanoparticles were analyzed by UV-visible spectroscopy to optimize a specific ratio with maximum absorbance. All the synthesized ratios give intense peak in the range of 400 to 450 nm. Ratio 1:2 gave an intense peak at 425 nm with maximum absorbance (Fig. 10). One of the possible ways of Ag reduction is the secondary metabolite present in plant which may be responsible for the reduction of Ag^+ ions and synthesis of AgNPs. Secondary metabolites like flavonoids, alkaloids and phenols are present in *S. thea* stem (Shah *et al.*, 2013). All these secondary metabolite are responsible for reduction of silver. Caroling *et al.*, (2013) reported that UV-Vis analysis of AgNPs showed surface plasmon resonance at 425nm. Gnanajobitha *et al.*, (2013) also obtained the surface plasmon absorption band at 460nm indicated the synthesis of AgNPs of flower extract of *Hortensis*. Similarly Kannan *et al.*, (2013) also obtained a characteristic absorption peaks at 422 and 425 nm for fresh and dried *C. capitatum* in the spectrum confirmed the formation of silver nanoparticles. So it is confirm that our results are entirely in consistency with early research.

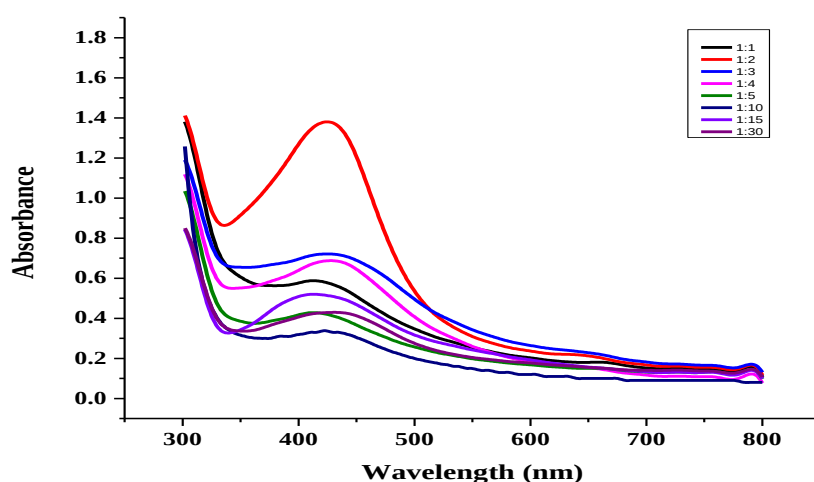


Figure 9. Optimization through UV-Visible Spectroscopy of different concentrations of stem extract mediated silver nanoparticles showed as the concentration of AgNO₃ solution increased the absorbance of peak also increased. But after specific range the absorbance decreased due to unavailability of functional groups.

4.7.2.iii. UV-Visible Spectroscopy of Silver Nanoparticles of Root Extract

In present study silver nanoparticles were synthesized using root extract of *S. thea*. After constant stirring for about 4h the colour of solution change to brown-yellow indicate the formation of silver nanoparticles. The intensity of brown colour increased in direct proportional to stirring. After addition of root extract a rapid change in colour start and after 4h stirring a brownish colour indicate the initial formation of AgNPs. The change in colour is due to excitation of Surface Plasmon Resonance (SPR) effect and reduction of silver nitrate into silver ions. The formation of AgNPs was confirmed by UV-Visible Spectroscopy. Different concentrations showed different absorptions but a characteristic peak which showed maximum absorbance at 435nm is 1:5 as shown in (Fig. 11). Biomolecules present in plant extract is responsible for the reduction of silver nitrate and formation of AgNPs. Ahmad *et al.*, (2011) concluded three possible ways for the formation of AgNPs. The first is the secondary metabolites present in plant extract is responsible for NPs formation, second is biogenic route is the energy or electron released during Glycolysis (photosynthesis) for conversion of NAD to NADH led to transformation of AgNO₃ to form AgNPs and third is release of an electron during formation of ascorbate radicals from ascorbate reduces the silver nitrate and convert it into silver ions. Many other researchers also carried out the UV-Visible spectrum of AgNPs like Behera *et al.* (2013) carried out green synthesis of AgNPs and reported absorbance peak at 440nm. Similarly another worker like Kaur *et al.* (2013) synthesized AgNPs using *S. aromaticum* extract and reported the formation of AgNPs at λ_{max} of 440 nm. So it is concluded that the spectra obtained from the AgNPs of *S. thea* root extract are in agreement with other researchers.

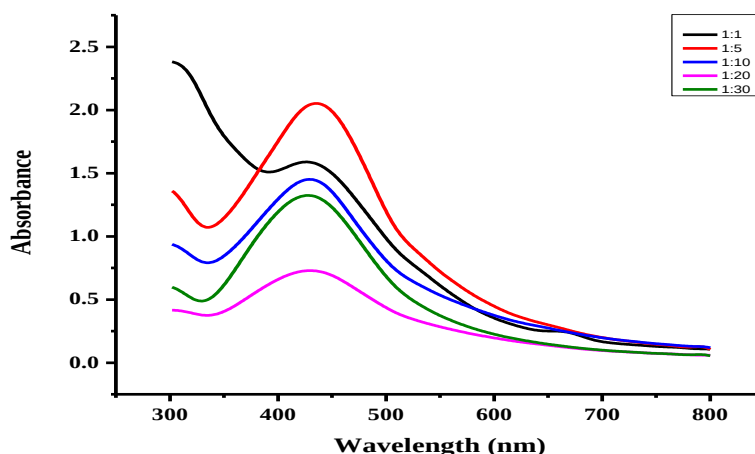


Figure 10. Optimization through UV-Visible Spectroscopy of different concentrations of root extract mediated silver nanoparticles showed as the concentration of AgNO₃ solution increased the absorbance of peak also increased. But after specific range the absorbance decreased due to unavailability of functional groups.

4.7.2.iv. X-Ray Diffraction Analysis of Silver Nanoparticles of leaf, Stem and Root Extract

The size and nature of synthesized silver nanoparticles were studied through XRD analysis. The XRD pattern showed numbers of Bragg reflections that may be indexed on the basis of the face-centered cubic structure of silver. The nanocrystal showed distinct peak at 2θ -38.1°, 44.2° and 64.4° which were indexed to (1 1 1), (2 0 0) and (2 2 2) respectively with matching card number JCPDS no 1- 1170 (Fig. 12). The XRD analysis showed that the synthesized NPs were face cubic structure. The average particle size of the silver nanoparticles formed in this process was estimated from the Debye-Scherrer equation by determining the width of the (1 1 1) Bragg's reflection (Borchert *et al.*, 2005). The estimated mean size of the particle was 13 nm. The XRD patterns describe here are similar with earlier reports (Das *et al.*, 1997). Without this some other undifferentiated peaks 2θ -14.4° and 16.9° also arise due to organic impurities in sample.

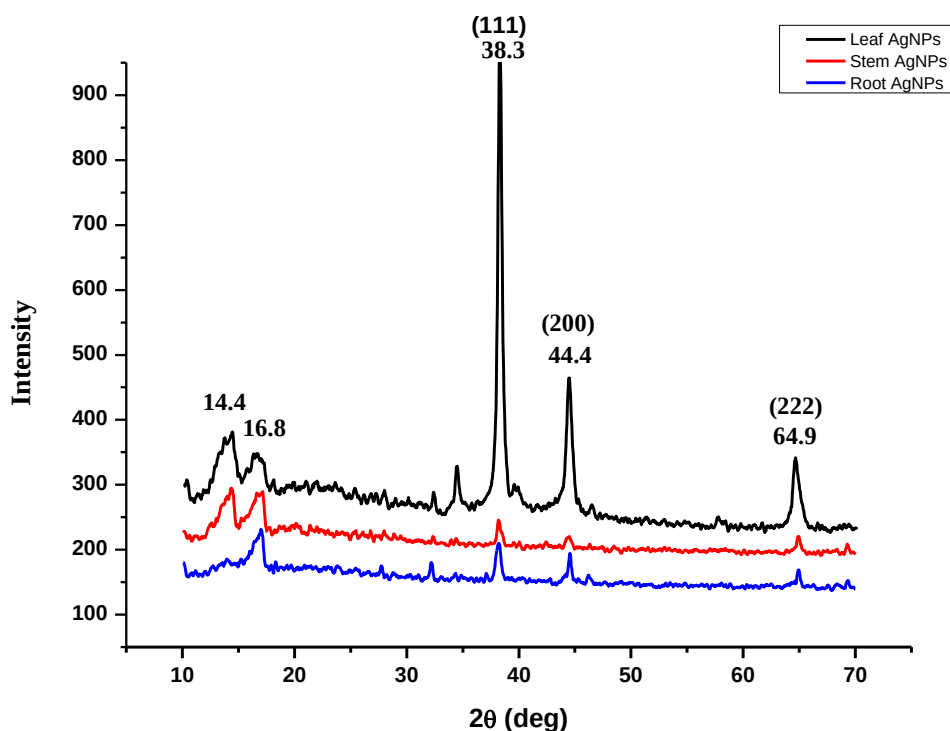


Figure 11. X-Ray Diffraction Analysis of Silver Nanoparticles of Leaf, Stem and Root Extract

4.7.2.v.Scanning Electron Microscopy of Silver Nanoparticles of Leaf Extract of *S. thea*

The formation of silver nanoparticles (AgNPs) their distribution, shape and size measurement carried out by Scanning Electron Microscopy (SEM). The synthesized AgNPs of leaf extract of *S. thea* were spherical in shape, equally distributed in medium and approximately uniform in size with diameter in the range of 10-15nm (Fig. 13). The aggregation of the nanoparticles indicates that they were in direct contact, but were stabilized by a capping agent (Panneerselvam *et al.*, 2011). SEM morphology revealed that AgNPs are Spherical in shape and dispersed in solution and may also bind onto the surface of the cells. Jaina *et al.*, (2009) also carried out SEM analysis of silver nanoparticles of papaya fruit extract and calculated average particle size about 15 nm. Similarly Elumalai *et al.*, (2010) synthesized silver nanoparticles using aqueous extract of *Euphorbia hirta* (L.) leaves and reported that SEM analysis revealed that nanoparticles are polydispersed with a size ranging from 40 to 50 nm. Caroling *et al.*, (2013) analyzed AgNPs of *Brassica oleracea* extract through SEM and measured average particle size in the range of 40-50nm. So all of these researchers not only provide information but also their research work supports our present investigation.

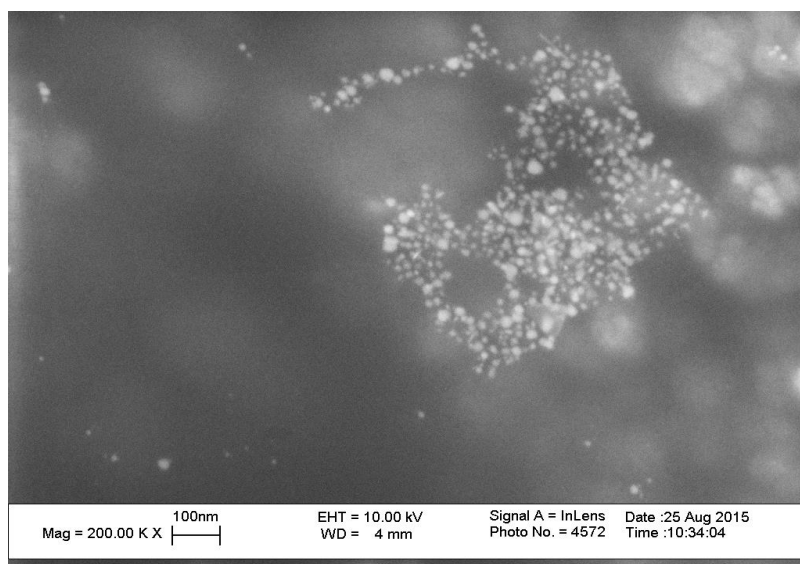


Figure 12. Scanning Electron Microscopy of AgNPs of Leaf Extract

4.7.2.vi. Scanning Electron Microscopy of Silver Nanoparticles of Stem Extract

SEM analysis shows that the nanoparticles are uniform in size about 33nm (Fig. 14). All the particles are equally distributed and spherical shaped. The shape of nanoparticles is due to capping of silver. The uniform spherical size shows that there is very small aggregation in particles. Some others workers also reported spherical shape of AgNPs such as Awwad *et al.*, (2013) synthesized AgNPs using *Ceratonia siliqua* leaf extract. Solgi & Taghizadeh (2012) also analyzed AgNPs through SEM analysis and reported spherical shape NPs about 21nm in size. Elumalai *et al.*, (2010) analyzed AgNPs of aqueous extract of *Euphorbia hirta* (L.) leaves through and revealed that nanoparticles are polydispersed with a size ranging from 40 nm to 50 nm. Similarly Jaina *et al.*, (2009) carried out SEM analysis of silver nanoparticles through papaya fruit extract and reported 15nm average particle size.

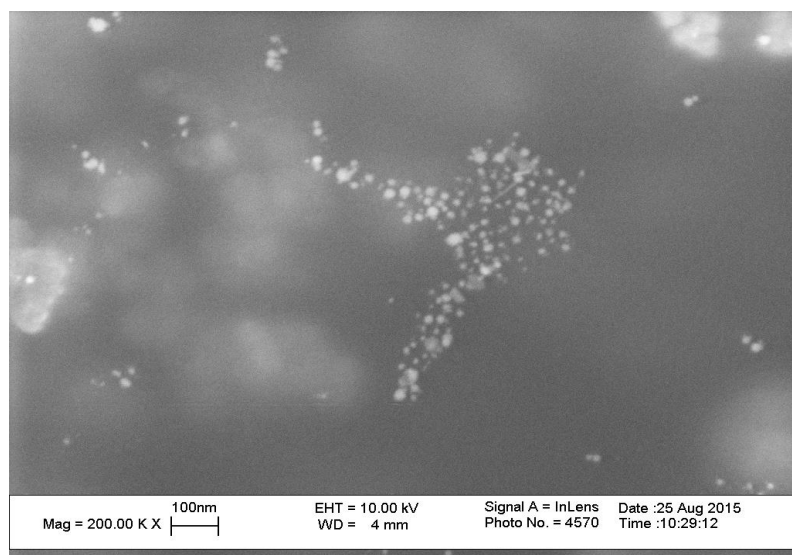


Figure 13. Scanning Electron Microscopy of AgNPs of Stem Extract

4.7.2.vii. Scanning Electron Microscopy of Silver Nanoparticles of Root Extract

To study the morphology, size and shape of synthesized AgNPs of root extract of *S. thea* SEM analysis were carried out. SEM at different magnification shows that nanoparticles are spherical or near to spherical in shape and dispersed in medium with a little aggregation with 15nm average particle sizes (Fig. 15). SEM analysis revealed that all the particles are equally distributed and uniform in size. Researchers related to nanoparticles synthesis always interested in size of NPs therefore some researchers like Solgi & Taghizadeh (2012) analyzed AgNPs of *Punica granatum* for SEM and find out average particle size 21nm, similarly Pavani *et al.*, (2013) synthesized silver nanoparticles using methanolic extract of *Ipomoea indica* and calculated average particle size range from 10 to 50 nm through SEM analysis. Our research work is in consistency to the previous work and their work supports our SEM analysis.

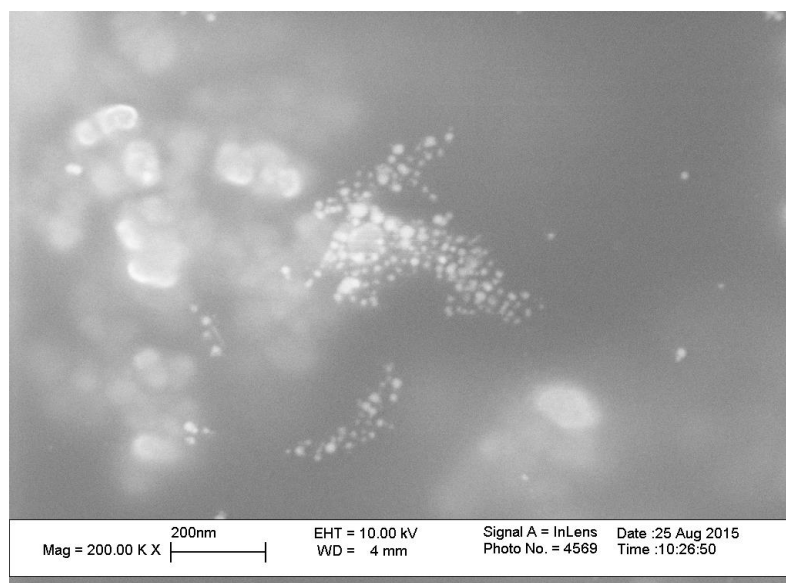


Figure 14. Scanning Electron Microscopy of AgNPs of Root Extract

4.7.2.viii. Energy Dispersive Analysis of Silver Nanoparticles of Leaf Extract

A strong silver peak in EDX spectrum confirms the presence of elemental silver in synthesized nanoparticles of leaf extract of *S. thea*. The metallic peak of silver in EDX analysis is at 3KeV (Fig. 16). According to Magudapathi *et al.*, (2001) the silver nanoparticles shows a typical optical peak at 3KeV due to Surface Plasmon Resonance. In EDX analysis Ag along with O, Na, Ca and Cl as the mixed components present in the reaction medium. A strong silver signal along with oxygen that may be originated from the biomolecules bound to the surface of the nanoparticles (Song *et al.*, 2009). These biomolecules were chapping over the silver nanoparticles. The other elements like carbon, oxygen indicate the presence of plant extract in AgNPs. Similarly Krishnaraj *et al.*, (2010) also showed the presence of intense peak of Ag in EDX analysis at 3KeV confirmed the presence of elemental silver in silver nanoparticles using *Acalypha indica* leaf extracts. So the present investigation is also in favour of all these findings and it is confirmed that due surface Plasmon resonance AgNPs showed a characteristic peal at 3KeV.

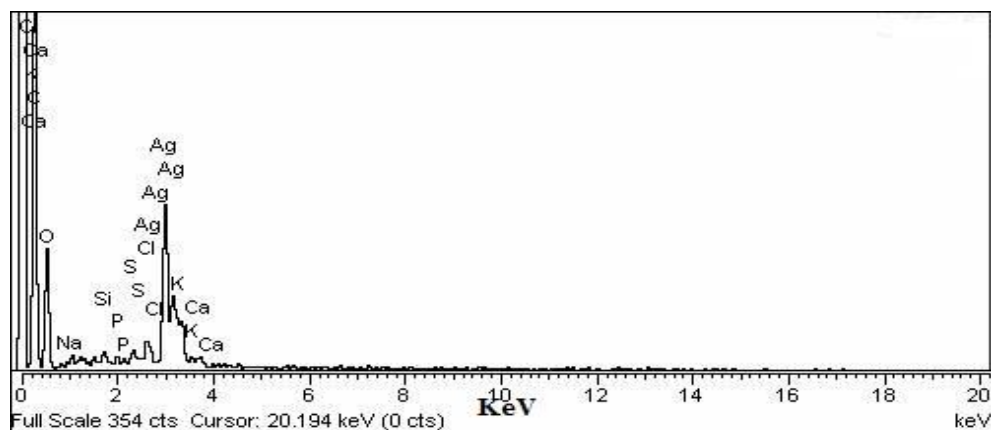


Figure 15. Energy Dispersive Analysis of Silver Nanoparticles of Leaf Extract

4.7.2.ix. Energy Dispersive Analysis of Silver Nanoparticles of Stem Extract

From the Energy Dispersive Absorption Spectroscopy (EDX spectrum) confirmed the presence of signal characteristic of silver it is clear that stem of *S. thea* have weight 0.50% due to Surface Plasmon Resonance (Magudapathy *et al.* 2001). The AgNPs show absorption band peak approximately at 3KeV along with Ca and O (Fig. 17). The spectral signals for Ca, K and S were also observed that some other organic molecules were also absorbed on the surface of silver nanoparticles. Some researchers also find out EDX analysis of AgNP and confirmed a distinct peak of elemental silver in silver nanoparticles at 3 KeV. According to Prasad *et al.*, (2012) AgNPs shows an absorption peak at 3KeV due to Surface Plasmon Resonance (SPR). Kannan *et al.*, (2013) also reported EDX of AgNPs of *Codium capitatum* and showed a characteristic peak at 3Kev. The findings of all these researchers confirmed that AgNPs shows a strong peak at 3KeV, so the entire EDX analysis supports our current findings.

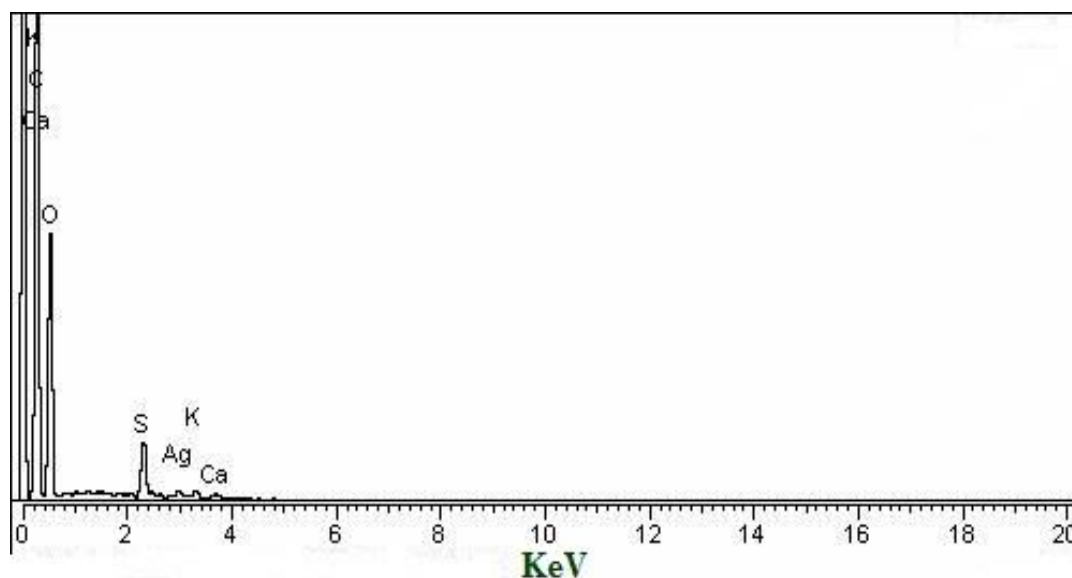


Figure 16. Energy Dispersive Analysis of Silver Nanoparticles of Stem Extract

4.7.2.x. Energy Dispersive Analysis of Silver Nanoparticles of Root Extract

Elemental mapping of silver nanoparticles carried out to confirm the presence of silver in synthesized nanoparticles. Identification peak of Ag at 3keV appeared along with O, Cl, Mg, Ca and Na peaks with low density (Fig. 18). These biomolecules present in root extract and bound to the surface of AgNPs result in the formation of NPs. The C and O signals were likely due to X-ray emission from carbohydrates/proteins present in plant extract (Nagajothi and Lee, 2011). The elemental mapping also shows the Quantitative analysis of AgNPs. Ag, O, Mg, Na and C elements exhibited weight percentages of 3.45%, 38.23%, 0.25%, 0.19% and 55.69% respectively. Ahluwalia *et al.*, (2014) analyzed EDX analysis of AgNPs of *Trichoderma harzianum* and reported 3KeV of silver region due to surface plasma resonance. Some other researchers like Krishnaraj *et al.*, (2010) and Kannan *et al.*, (2013) also carried out AgNPs analysis and concluded that 3KeV is the silver peak region. In the light of the previous research work our present analysis is also in agreement to them.

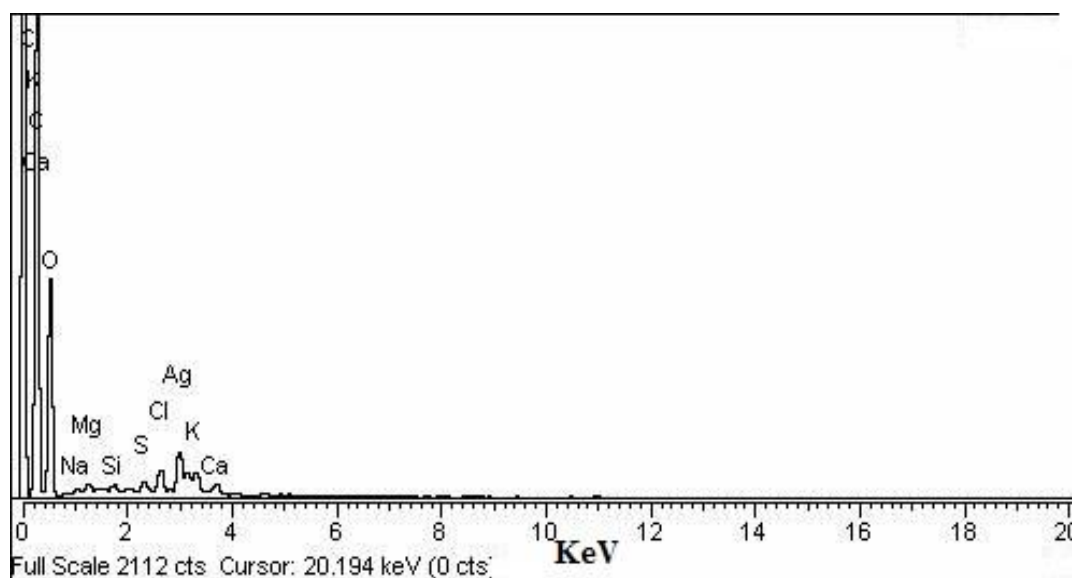


Figure 17. Energy Dispersive Analysis of Silver Nanoparticles of Root Extract

4.7.2.xi. Fourier Transform Infrared Spectroscopy of Leaf Extract and AgNPs of *S. thea*

To identify the potential biomolecules in leaf extract of *S. thea*, FTIR analysis was carried out. These biomolecules are responsible for the reduction of silver nitrate and the formation of silver ions as well as the formation of AgNPs of *S. thea* leaf. The FTIR spectra of untreated leaf extract and AgNPs is depicted in (Fig. 19). The untreated leaf extract shows absorption bands at 3315, 2917, 1639, 1313 and 1043 cm^{-1} . The strongest absorption band was observed at 1639 cm^{-1} indicates the presence of flavonoid compounds in leaf extract. This may be responsible for the reduction of metal ion to metal nanoparticles (Heneczowski *et al.*, 2001). Band originated at 1539 cm^{-1} in AgNPs showed a slight change from 1639 to 1539 cm^{-1} represent the presence of amide II group, as would be expected due to origin of these samples from plant extract. Amide II group is due to carboxyl stretch and N-H deformation vibrations in the amide linkages of the proteins in it (Caruso *et al.*, 1998). Comparison between spectra of untreated sample to the treated sample AgNPs revealed only minor changes in the positions as well as on the magnitude of the absorption bands. The absorption band at 1539 cm^{-1} was absent as compared to untreated one with stronger bands at 1639 cm^{-1} and 1043 cm^{-1} . The prominent appearance of amino II group in AgNPs indicated that silver nanoparticles are bound to proteins through free amine groups. The FTIR spectroscopy of AgNPs was carried out by different scientist to investigate the correct mechanism of NPs formation. Nabikhan *et al.*, (2010) carried out (FTIR) spectroscopy measurement of silver nanoparticles of *Sesuvium portulacastrum* L, it was concluded that the

presence of aromatic rings, germinal methyl and ether linkages, flavones and terpenoids are responsible for the stabilization of the silver nanoparticles. Similarly Caroling *et al.*, (2013) synthesized the silver nanoparticles of *Brassica oleracea* by green chemistry approach. The FTIR analysis showed the presence of phenolic groups and active protein in silver nanoparticles of *Brassica oleracea* which play a role in stabilizing medium and nanoparticles synthesis. Literature survey suggests that our research findings are absolutely right and secondary metabolites present in *S. thea* leaf stabilize the medium and synthesized AgNPs.

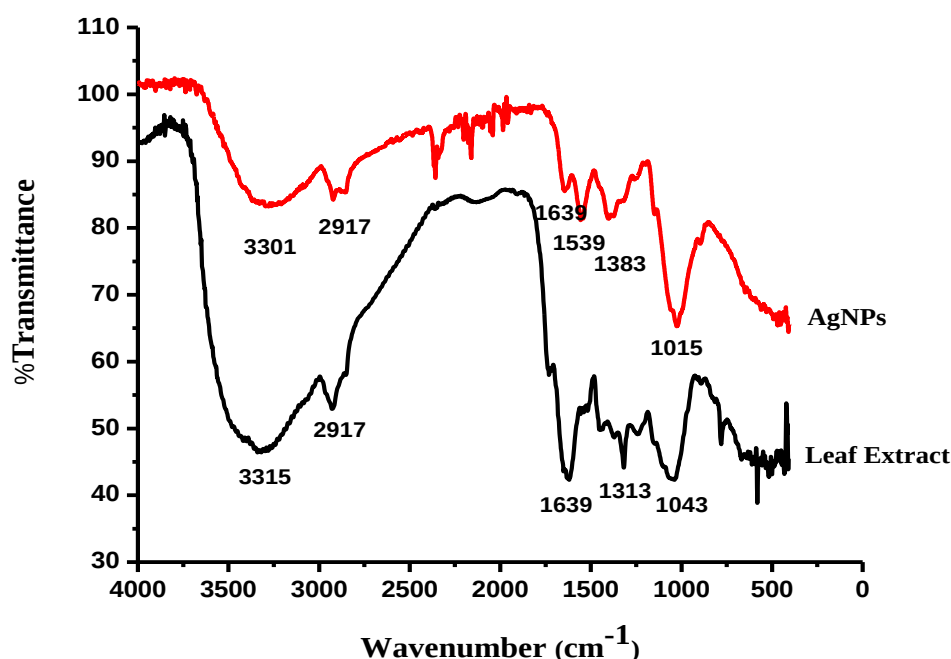


Figure 18. FTIR Spectroscopy of Leaf Extract and AgNPs of *S. thea*

4.7.2.xii. Fourier Transform Infrared Spectroscopy of Stem Extract and AgNPs of *S. thea*

For the identification of biomolecules in the stem of *S. thea* which were responsible for reducing of Ag⁺ FTIR spectroscopy was carried out. Absorption peaks observed in stem extract were at 3329, 2917, 1736, 1624, 1328 and 1029cm⁻¹ (Fig. 20). The strongest absorption bands at 1624cm⁻¹ can be assigned carbonyl peak (C=O stretching) indicating carboxylate content in plant based samples. The closer examination of AgNPs of stem extract shows the disappearance of absorption band at 1736 and 1328 cm⁻¹ as compared to stem

extract. The absence of this band reveals that fatty acids and lipids are present in stem extract and responsible for the synthesis of nanoparticles by capping over silver. While a slightly change at 1440cm^{-1} represent the presence of Amide III protein group which also stabilize the medium and formation of metallic silver take place. The synthesized nanoparticles were surrounded by amide bond of proteins arising from carbonyl group of stretching in proteins and secondary metabolites like phospholipids and cholesterol having functional groups. The FT-IR analysis revealed that protein has the strongest ability of chapping silver nanoparticles to prevent agglomeration and stabilized medium. The reduction range of silver nitrate is $1540\text{-}1242\text{cm}^{-1}$. The carbonyl group present at peak 1624 cm^{-1} proved that flavonoids and terpenoids could be absorb on the surface of Ag nanoparticles by interaction through carbonyl groups in the absence of legating agent in sufficient concentration. The investigation of such functional groups was also carried out by many research workers like Daniel *et al.*, (2011). They analyzed the FTIR spectroscopy of AgNPs of *Ocimum tenuiflorum* and identified various functional groups like carbonyl and amide II group responsible for AgNPs synthesis. Similarly Saranyaadevi *et al.*, (2014) synthesized AgNPs of leaf extract of *Capparis zeylanica* and carried out FTIR spectroscopy. The results indicates that phenolic compounds, Alkaloids, flavonoids and fatty acids are present in leaf extract and reduced silver by stabilizing the medium.

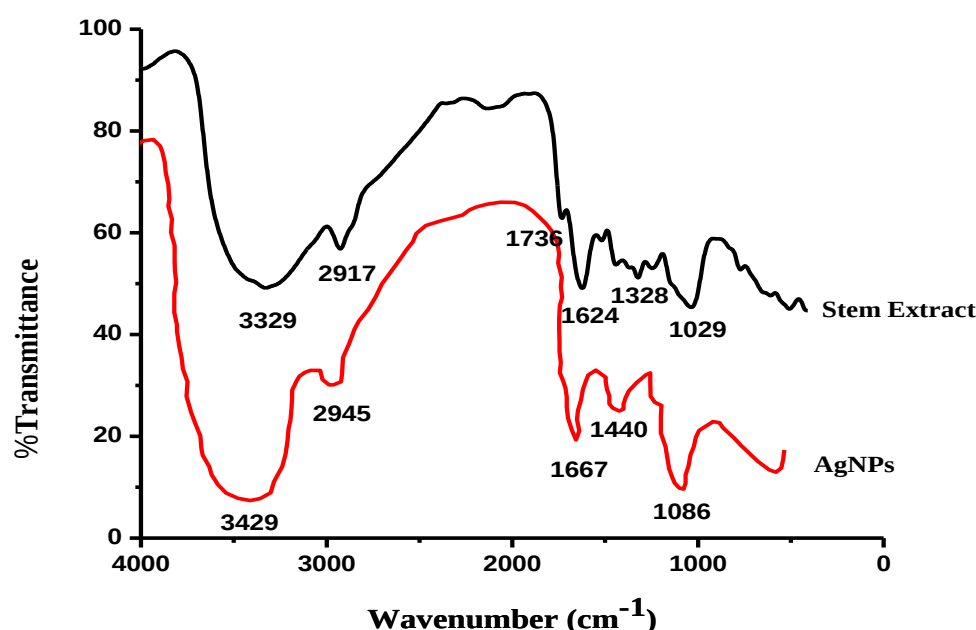


Figure 19. FTIR Spectroscopy of Stem Extract and AgNPs of *S. thea*

4.7.2.xiii. Fourier Transform Infrared Spectroscopy of Root Extract

Fourier Transform Infrared Spectroscopy (FTIR) is a simple reproducible technique and also nondestructive to the tissue, and only small amounts of material (micrograms to nanograms) with a minimum sample preparation are required. In present research work FTIR spectroscopy of Root extract (untreated) and AgNPs of *S. thea* root were carried out. The results obtained after analysis showed that the absorption bands of untreated sample are at 3329, 2917, 1696, 1610, 1496, 1228 and 1000 cm^{-1} . A broad band in both samples was detected at 3329 and 3358 cm^{-1} represent the presence of Amide A group while a sharp band was recorded at 1000 cm^{-1} reveal the presence of protein amide I absorption. Comparative study of both sample showed that a slight change was noted at 1494 cm^{-1} bent to 1310 cm^{-1} due to Amide III component with protein. An absorption peak at 1696 cm^{-1} showed Amide I group due to the stretching of C=O band and weakly coupled stretching of C-N and bending of N-H bond (Fig. 21). The above discussion conclude that Amide I, II, III functional groups are present in root extract of *S. thea* and these protein components capping and agglomerate over silver and convert it into metallic silver and formation of silver nanoparticles takes place by stabilizing the reaction medium. Some other researchers also worked on FTIR spectroscopy of AgNPs and reported various functional groups present in plant extract which bind on the surface of silver and reduce it metallic silver. Donda *et al.*, (2013) analyzed silver nanoparticles using extracts of *Securinega leucopyrus* by FTIR and reported the presence of Carboxyl (-C=O), hydroxyl (-OH) and Amine (-NH) groups. Similarly Geoprincy *et al.*, (2013) recorded a literature survey of *S. torvum* leaf extract and its AgNPs and demonstrate the presence of Amide I and II bands respectively and concluded that amide band I is due to the stretch mode of the (-CO) carbonyl group coupled to the (-NH) amide linkage while the amide II band is due to the N-H stretching modes of vibration in the amide linkage. Some other functional groups like alcohols and phenols (O-H), carboxylic acids and its derivatives (C=O) and Chloroalkanes (CX) were also reported in AgNPs of *Dioscorea bulbifera* tuber extract. The literature study revealed that the natural metabolites present in plant extract are responsible for AgNPs synthesis and support our current findings of FTIR spectroscopy of AgNPs of *S. thea* root extract.

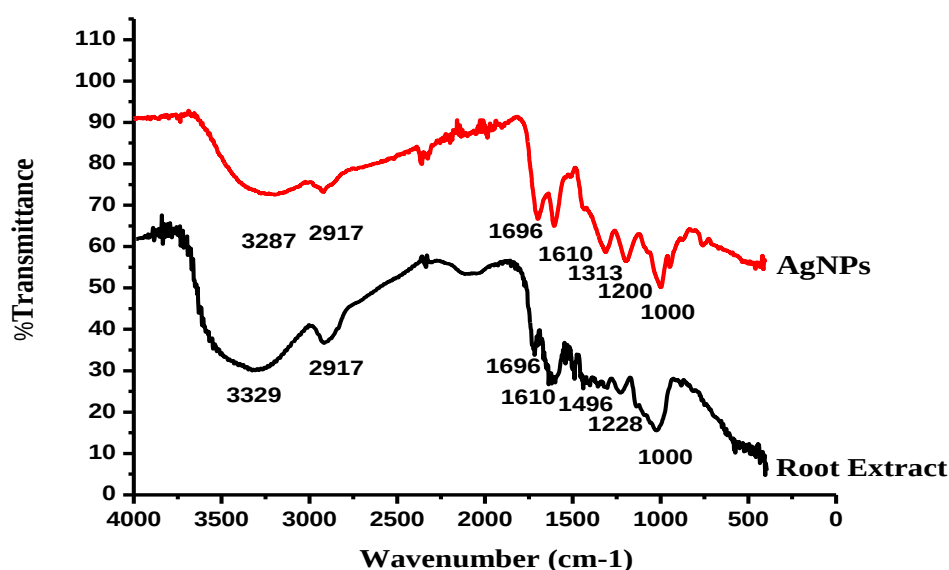


Figure 20. FTIR Spectroscopy of Root Extract and AgNPs of *S. thea*

4.8. BIOLOGICAL SCREENING OF SILVER NANOPARTICLES

4.8.1. Antibacterial Activity of Silver Nanoparticles

Bacteria cause many diseases in humans including bacterial pneumonia, cholera, Leprosy, whooping cough, tetanus and lyme disease. Tuberculosis (TB) is also Bactria causing disease leading to death of humans. Bacteria not only causing infection in humans and animals but in plants also, for example *Pseudomonas cichorii* cause dark brown spots on inner leaves of *Lettuce* and also causing diseases on cabbage, tomato, celery and chrysanthemum. In current work we investigate and compared the antibacterial effect of crude extract of leaf, stem and root of *S. thea* and its mediated silver nanoparticles against both gram positive and negative bacterial strain (*Escherichia coli*, *Staphylococcus aureus*, *B. pumilus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*). The bactericide potential of leaf, stem and root extracts and its AgNPs was evaluated in comparison to negative control as DMSO, silver nitrates and positive control as streptomycin (marketed antibacterial drug). The zone of inhibitions in millimeter measured for various bacterial strains are summarized in (Table 07). The zone of inhibition measured for AgNO₃ was 9.4±0.6 mm, 11.7±0.7 mm, 8.2±0.5 mm, 12.1±0.4 mm and 10.3±0.6 mm, exhibited 32.75, 48.20, 30.04, 32.88 and 39.02 % antibacterial potential comparison to streptomycin against *Escherichia coli*, *Staphylococcus aureus*, *B. pumilus*, *Pseudomonas aeruginosa* and *Klebsella pneumoniae* respectively. DMSO remained effective against all bacterial strains. Leaf extract show low antibacterial potential with 16.7±0.4mm, 7.1±0.3 mm, 15.6±0.3 mm, 12.8±0.2mm and 13.3±0.3mm zone

of inhibition against *Escherichia coli*, *Staphylococcus aureus*, *B. pumilus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* respectively as compared to Streptomycin. While leaf extract mediated AgNPs showed strong antibacterial efficacy with 30.7 ± 2 mm, 14 ± 0.2 mm, 21.7 ± 0.2 mm, 23.5 ± 0.8 mm and 24.7 ± 0.2 mm. The same experiment is carried out for stem crude extract and AgNPs. As compared to standard drug Streptomycin crude stem extract show low antibacterial activity with percent inhibition 53.38%, 49.59%, 63.92%, 35.02% and 60.08% against *Escherichia coli*, *Staphylococcus aureus*, *B. pumilus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* respectively. The same bacterial strains are tested by stem extract mediated AgNPs. The AgNPs showed good antibacterial activity against all of these strains with 29 ± 0.4 mm, 24 ± 0.3 mm, 23.0 ± 0.5 mm, 22.4 ± 0.7 mm and 21.9 ± 0.7 mm zone of inhibition. The low zone of inhibition showed the resistance of bacterial strains against crude stem extracts as well as the low passage of macromolecules of crude drugs through bacterial membrane. The root extract mediated AgNPs is also evaluated for its antibacterial potential. The results indicated that as compared to stem crude extract root crude extract showed good antibacterial activity while low antibacterial activity as compared to AgNPs. The percent inhibition of crude extract with zone of inhibition is 82.09%, 49.59%, 63.92%, 35.02% and 60.08% with 21 ± 0.9 mm, 19.1 ± 0.2 mm, 17.3 ± 0.3 mm, 20.4 ± 0.3 mm and 18.9 ± 0.3 mm zone of inhibition. The same root crude drug mediated AgNPs showed good results against these strains as compared to crude root extract while low antibacterial potential in comparison to standard streptomycin. The zone of inhibition of AgNPs confirmed that AgNPs is more effective against bacterial strains with 26.03 ± 0.3 mm, 28.4 ± 0.7 mm, 18.02 ± 0.6 mm, 16.03 ± 0.3 mm and 21.07 ± 0.8 mm against *Escherichia coli*, *Staphylococcus aureus*, *B. pumilus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* respectively. In light of above results we can conclude that plant extract mediated AgNPs showed additive antimicrobial property than extract alone. The chemical antibacterial drugs caused some serious problems both in animals and humans. These kinds of limitation can be overcome by using natural products mediated AgNPs. The effect of AgNPs different on gram positive bacteria and gram negative bacteria, because gram positive bacteria have thick wall due to peptidoglycan whereas gram negative have a layer of polysaccharides. Pal *et al.*, (2007) investigated the antibacterial properties of differently shaped silver nanoparticles against the gram-negative bacterium *Escherichia coli*. Kim *et al.*, (2007) demonstrated that the antimicrobial effect of Ag salt is well known but the effect of AgNPs on microorganisms has not been clear. Therefore the synthesized AgNPs were tested against yeast, *Escherichia coli*, and *Staphylococcus aureus*. Banker *et al.*, (2010) reported that the Banana peel extract

mediated AgNPs have shown efficient effect towards most of the tested bacterial and fungal culture. Ramamurthy *et al.*, (2013) also described the effect of Ag nanoparticles against various bacteria strains using *Solanum torvum*. The antibacterial mechanism of AgNPs is due to binding of these nanoparticles with the wall as of bacteria as well interacting with cytoplasmic and nuclear components and hindering its growth (Morones *et al.*, 2005).

Table 7. Antibacterial Activity of Leaf, Stem and Root Extract of *S. thea* and AgNPs

Treatments		Zone of Inhibition in millimeter (% of the standard)				
		<i>E. coli</i>	<i>Staph. aureus</i>	<i>B. pumilus</i>	<i>Psed. aeruginosa</i>	<i>Kleb. pneumoniae</i>
DMSO		-	-	-	-	-
AgNO ₃		9.4±0.6 (32.75)	10.7±0.7 (48.20)	7.6±0.5 (30.04)	12.1±0.4 (32.88)	10.3±0.6 (39.02)
Streptomycin		28.1±1.2 (100)	20.4±0.7 (100)	25.5±1.5 (100)	35.4±0.4 (100)	24.8±0.9 (100)
Leaf	Extract	16.7±0.4 (59.43)	7.1±0.3 (34.80)	15.6±0.3 (61.17)	12.8±0.2 (36.15)	13.3±0.3 (53.62)
	AgNPs	30.7±2 (109.25)	14±0.2 (68.62)	21.7±0.2 (85.09)	23.5±0.8 (66.38)	24.7±0.2 (99.59)
Stem	Extract	21±0.9 (53.38)	19.1±0.2 (49.59)	17.3±0.3 (63.92)	20.4±0.3 (35.02)	18.9±0.3 (60.08)
	AgNPs	29±0.4 (103.2)	24±0.3 (117.64)	23.0±0.5 (90.19)	22.4±0.7 (63.27)	21.9±0.7 (88.30)
Root	Extract	23.07±0.1(82.09)	25.8±0.3(126.47)	17.02±0.2(66.74)	13.9±0.7(39.26)	19.1±0.3(77.01)
	AgNPs	26.03±0.3(92.63)	28.4±0.7(139.21)	18.02±0.6(70.66)	16.03±0.3(45.28)	21.07±0.8(84.95)

4.8.2. Anti fungal Activity of Silver Nanoparticles

Nanotechnology is responsible for the production of plant extract mediated metallic nanoparticles. The synthesized nanopartciles have various applications but the most highlight application is antimicrobial activity against fungus, bacteria and virus. In curret investigation antifungal effects of AgNPs was assessed on the bases of the zone inhibitions. The synthesized AgNPs showed antifungal activity using well diffused method. The testing samples *Candida albicans*, *Aspergillus flavus*, *Aspergillus paraciticus*, *Fusarium solani* and *Aspergillus niger* displayed different zone inhibition. The maximum zone of inhibition was whown by leaf extract mediated AgNPs against *Aspergillus flavus* with (84.29±0.83) mm zone of inhibition Which was then followed by root extract mediated AgNPs against *Aspergillus niger* with zone inhibition (87.51±1.17 mm). Stem extract mediated AgNPs showed highest activity against *Aspergillus niger* with (58.13±0.46 mm) zone of inhibition. Leaf extract AgNPs showed maximum inhibition against *Aspergillus flavus* while lowest inhibition against *Fusarium solani*, similarly stem extract AgNPs and root extract AgNPs displayed maximum inhibition against *Aspergillus niger* while lowest inhibition against *Fusarium solani* by stem AgNPs and against *Aspergillus paraciticus* by root AgNPs. After these results we can conclude that as compared to crude leaf extracts of leaf, stem and root of *S. thea* synthesized AgNPs of the same extract showed significant results (Table 08). DMSO is used as a standard in present research work. Different plant extracts and its synthesized

nanoparticles have been used against various fungal strains through different researchers like Mallmann *et al.*, (2015) carried out antifungal activity of greenly synthesized silver nanoparticles. The antifungal investigation of ribose mediated silver nanoparticles revealed strong antifungal potential and represents an alternative for fungal infections, The synthesized nanoparticles showed significant activity against fungi. From this discussion we conclude that synthesized AgNPs of *S. thea* are capable of rendering high antifungal efficacy and can be used in preparation of drugs used in fungal diseases.

Table 8. Antifungal Activity of AgNPs using Leaf, Stem and Root Extracts of Sageretia thea

Treatments		Percent inhibition of mycelia growth				
		<i>Candida albicans</i>	<i>Aspergillus flavus</i>	<i>Aspergillus paraciticus</i>	<i>Fusarium solani</i>	<i>Aspergillus niger</i>
DMSO		-	-	-	-	-
Leaf	Extract	57.44±0.45	68.72±1.19	29.54±0.39	12.39±0.19	66.67±0.71
	AgNPs	68.71±0.37	84.29±0.83	50.00±0.63	30.58±0.37	60.27±1.39
Stem	Extract	40.09±0.65	32.67±0.52	9.22±0.22	-	39.44±0.37
	AgNPs	54.66±0.49	51.11±0.40	45.18±0.61	37.56±0.22	58.13±0.46
Root	Extract	43.68±0.52	59.38±0.62	68.90±1.57	47.10±0.31	52.36±1.22
	AgNPs	73.90±0.48	62.52±1.39	49.53±0.71	63.78±0.52	87.51±1.17

4.8.3. Anti-Promastigote Activity of Silver Nanoparticles of Leaf, Stem and Root of *S. thea*

Leishmaniasis is a group of tropical disease and one of the serious health problems worldwide. A disease caused by a number of species of protozoan parasites belonging to genus *Leishmania*. *Leishmania* follows a digenic life cycle, completed in two hosts. The flagellated extracellular promastigote form occurs in sand fly. The non flagellated and intramacrophagic form, amastigote occur in vertebrate host. Though both the amastigote and promastigote forms show differences, they do have similarities in their metabolic machinery and pathways. So the target against the first form could be relevant against the second one. In the current study the effect of various concentrations of AgNPs of leaf, stem and root extract of *S. thea* were evaluated against promastigote. The result shows different lethality rate at different concentration. The lethality rate is dose dependent as well as concentration of AgNPs increase the cell viability value decrease as shown in (Fig. 22, Table 09). The highest effect on metabolic activity of promastigote was seen at 150µg/ml for all three parts. There is variation in these parts their effect is also different against promastigotes. The leaf extract AgNPs shows more lethality at 150µg/ml followed by root extract AgNPs and stem extract

AgNPs. Leaf extracts mediated AgNPs showed 21.402, 55.49 and 220.06 LD₅₀, LD₇₀ and LD₉₀ respectively. Moderate lethality were shown by root mediated AgNPs which are 26.49, 74.70 and 334.44 respectively. Lowest mortality rate was shown by stem extract mediated AgNPs with LD₅₀, LD₇₀ and LD₉₀ values are 32.02, 91.92 and 422.41 respectively. The difference in effect is due to accumulation of various secondary metabolites in different parts of the plant. A number of researchers investigated the effect of AgNPs against bacterial and leishmanial cells. They investigated that AgNPs binds to sulfur and phosphate containing compounds and because of this property they can damage the bacterial cell wall by impairing sulfur containing protein. AgNPs damage sulfur containing enzymes and phosphorus containing DNA which leads to the inhibition of ATP synthesis. Another antileishmanial mechanism is the release of silver ions (Song *et al.*, 2006). These ions have the capacity of producing ROS to which the leishmania parasites are known to be very susceptible. These ions form complexes with cysteine group of enzymes and prevent the enzymatic functions of protein. Similarly Butkus *et al.*, (2004) explored for the first time the synergistic effects of silver ions and UV light together on a RNA virus, and demonstrated that RNA viruses were not affected by UV light only but by silver ions and UV light giving rise to inactivation of viruses together. So in the light of the previous literature it is concluded that for the first time plant extract AgNPs were determined to possess antileishmanial effects against leishmanial parasites through examining their effects on various cellular parameters of promastigote. The results demonstrate that AgNPs synthesized by plant extract is the future alternative to the current antileishmanial drugs. Further due to lack of enough resources and budget we were pushed to skip the *in vitro* assay against amastigotes.

Table 9. Anti-Promastigote Activity of Silver Nanoparticles of Leaf, Stem and Root of *S. thea*

Concentrations (µg/ml)		% inhibition (mean)	IC ₅₀	IC ₇₀	IC ₉₀	Chi-square value
Leaf AgNPs	5	30.32	21.402	55.491	220.063	13.521
	10	36.34				
	20	40.33				
	50	47.18				
	100	85.33				
	150	97.22				

Stem AgNPs	5	25.98	32.021	91.9282	422.411	11.750
	10	30.17				
	20	34.16				
	50	40.28				
	100	76.39				
	150	88.27				
Root AgNPs	5	28.21	26.495	74.705	334.448	11.893
	10	32.33				
	20	38.33				
	50	43.14				
	100	80.05				
	150	91.55				
DMSO (0.5%)		-	-	-	-	-

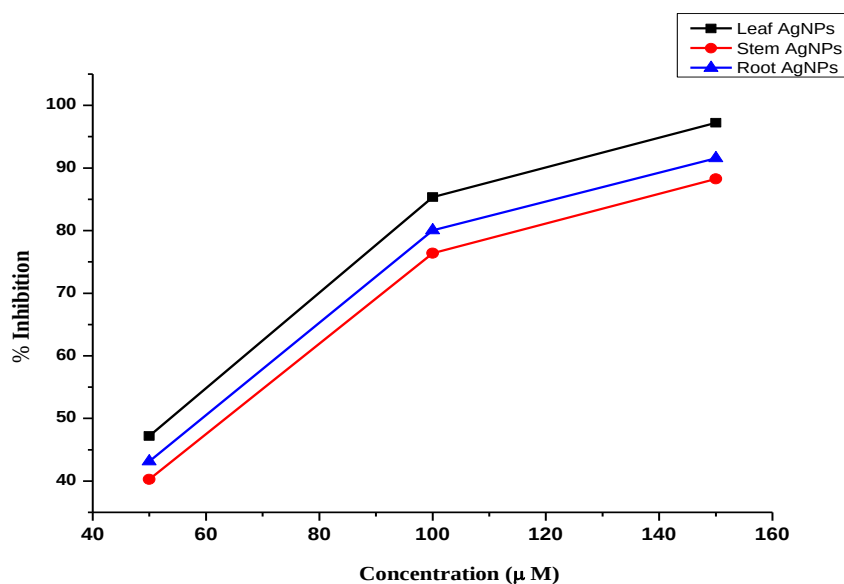


Figure 21. Anti-Promastigote Activity of AgNPs of Leaf, Stem and Root of *S. thea*

4.8.4. Cytotoxic Activity of Leaf Crude Extracts and Silver Nanoparticles of *S. thea*

Nanoparticles are smaller in size as compared to biomolecules and can interact with these molecules both on the surface and also inside the cell. In current cytotoxic activity greenly synthesized nanoparticles and crude extract of *S. thea* leaf were evaluated. Different concentrations showed different effect highest lethality rate of crude extract were 40% at

30µg/ml while lowest mortality rate were recorded at 5µg/ml about 6.7%. Silver nanoparticles of leaf extract show significant mortality rate at 30µg/ml about 83% while lowest mortality rate was 20% at 5µg/ml (Fig. 23). The LD₅₀, LD₇₀, LD₉₀ values for crude extract was 48.058, 93.12 and 242.35 respectively. The comparative study of both crude extract and AgNPs of leaf extract revealed that AgNPs have highest cytotoxic potential with LD₅₀, LD₇₀, LD₉₀ values 14.05, 24.96 and 57.29 respectively (Table 10). From the above discussion it is concluded that silver nanoparticles have great efficacy against brain shrimps larva and can be used to control cell growth and reduce tumor formation. The size of silver nanoparticles is smaller as compared to bulk counter parts and can easily pass through the biological membrane. The response of brain shrimps larva is similar to that of common body cells and can be used against cancer cells also. Different scientist performed cytotoxic activity using AgNPs of different medicinal plants, like Devi *et al.*, (2012) synthesized silver nanoparticles from aqueous extract of *Gelidiella* sp. The synthesized AgNPs were assessed for cytotoxic activity against Hep-2 (Human laryngeal) cell lines, similarly Ranjitham *et al.*, (2013) synthesized silver nanoparticles by using Cauliflower floret extract as a reducing agent. Various characterizations were carried out to confirm the formation of AgNPs and then applied against cancer cells which showed reduced viability and increased cytotoxicity on MCF-7 breast cancer cell line in a dose dependent manner. The literature studied support our current research work and concluded that AgNPs of leaf extract were more effective against cells that reduce its viability by killing cells can be used as anticancer.

Table 10. Cytotoxic Activity of Leaf Crude Extract and Silver Nanoparticles of *S. thea*

Conc. (µg/ml)	T. No. of Larvae	No. of survivors	% mortality	LD ₅₀	LD ₇₀	LD ₉₀	Regression Line	χ ² (p)
	Leaf ethanolic extract							
0	30	30	0.00	48.058	93.12	242.35	Y=1.93 +1.82 X	4.47 (0.34)
5	30	28	6.7					
10	30	27	10.0					
15	30	26	13.4					
20	30	24	20.0					
25	30	20	33.4					
30	30	18	40.0					
	Silver Nanoparticles of Leaf							
0	30	30	0.00	14.05	24.96	57.29	Y=1.59 + 2	1.69

5	30	24	20.0					(0.79)
10	30	19	36.7					
15	30	15	50.0					
20	30	12	60.0					
25	30	10	66.7					
30	30	05	83.3					

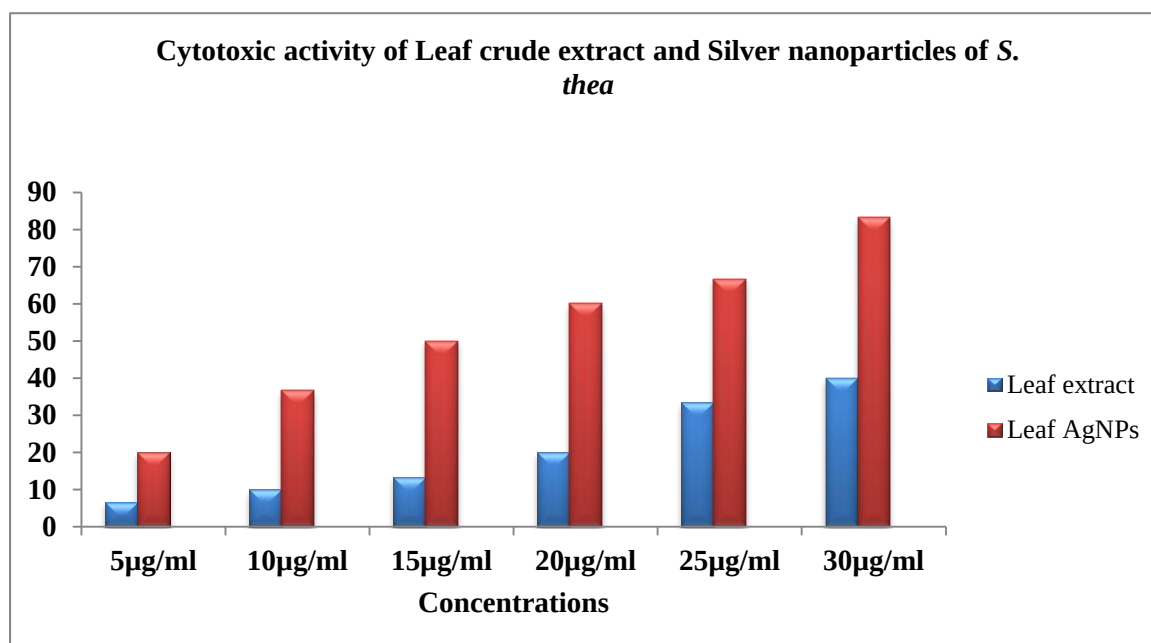


Figure 22. Cytotoxic Activity of Leaf Crude Extract and Silver Nanoparticles of *S. thea*

4.8.5. Cytotoxic Activity of Stem Crude Extract and Silver Nanoparticles of *S. thea*

The discovery of new and efficient drugs against various cell disease like cancer and skin disease etc are made by screening of active compounds from various parts of plants (Amara *et al.*, 2008). For detection of general toxicity of active compound derived from various parts of the plant, brine shrimps (*Artimia salina*) bioassay has been used for the last 30 years (Persoone and Wallis, 1987). In the present study the cytotoxic activity of crude ethanolic extract of stem and AgNPs of stem extract of *S. thea* were evaluated. The percent mortality of crude extract was significant at 30µg/ml while the lowest mortality rate were recorded at 5µg/ml with LD₅₀, LD₇₀ and LD₉₀ values is 59.56, 116.47 and 307.20 respectively while regression line and *chi*-square value was $Y = 1.81 + 1.80X$ and 2.17 (0.70) as shown in (Table 11). The effect of AgNPs were also evaluated and concluded that the percent mortality increased as the concentration of dose increased. Significant mortality about 70% was recorded at 30µg/ml while lowest mortality rate was recorded at 5µg/ml with LD₅₀, LD₇₀ and

LD₉₀ values are 15.61, 29.06 and 71.40 respectively (Fig. 24). The results revealed that the effect of AgNPs is more toxic as compared to crude drug. Different scientists studied the effect of AgNPs against brain shrimps. Kumar *et al.*, (2012) evaluated the effect of AgNPs of *Sargassum ilicifolium* against brain shrimps larva and reported that AgNPs have significant lethality against brain shrimps (*Artemia salina*). Similarly Devi & Bhimba, (2012) synthesized AgNPs by Seaweed *Ulva lactuca* *In vitro* extract. The synthesized AgNPs were assessed for cytotoxic potential against cancer cells. From the above discussion it is concluded that AgNPs of *S. thea* have great effect against brain shrimps larva, as the response of shrimps larva is similar to that of common body cell so we can use it as anti cancer and also for cell growth control to prevent cancer and tumor formation.

Table 11. Cytotoxic Activity of Stem Extract and Silver Nanoparticles of *S. thea*

Extract Conc. (µg/ml)	T. No. of Larvae	No. of survivors	% mortality	LD ₅₀	LD ₇₀	LD ₉₀	Regression Line	χ ² (p)
	Stem ethanolic extract							
0	30	30	0.00	59.56	116.47	307.20	Y= 1.81+1.80X	2.17 (0.70)
5	30	29	3.33					
10	30	27	10.00					
15	30	27	10.00					
20	30	24	20.00					
25	30	23	23.33					
30	30	20	33.33					
	Silver nanoparticles of stem							
0	30	30	0.00	15.61	29.06	71.40	Y= 2.68+1.94X	1.91 (0.75)
5	30	24	20.00					
10	30	20	33.33					
15	30	17	43.33					
20	30	12	60.00					
25	30	11	63.33					
30	30	07	76.67					

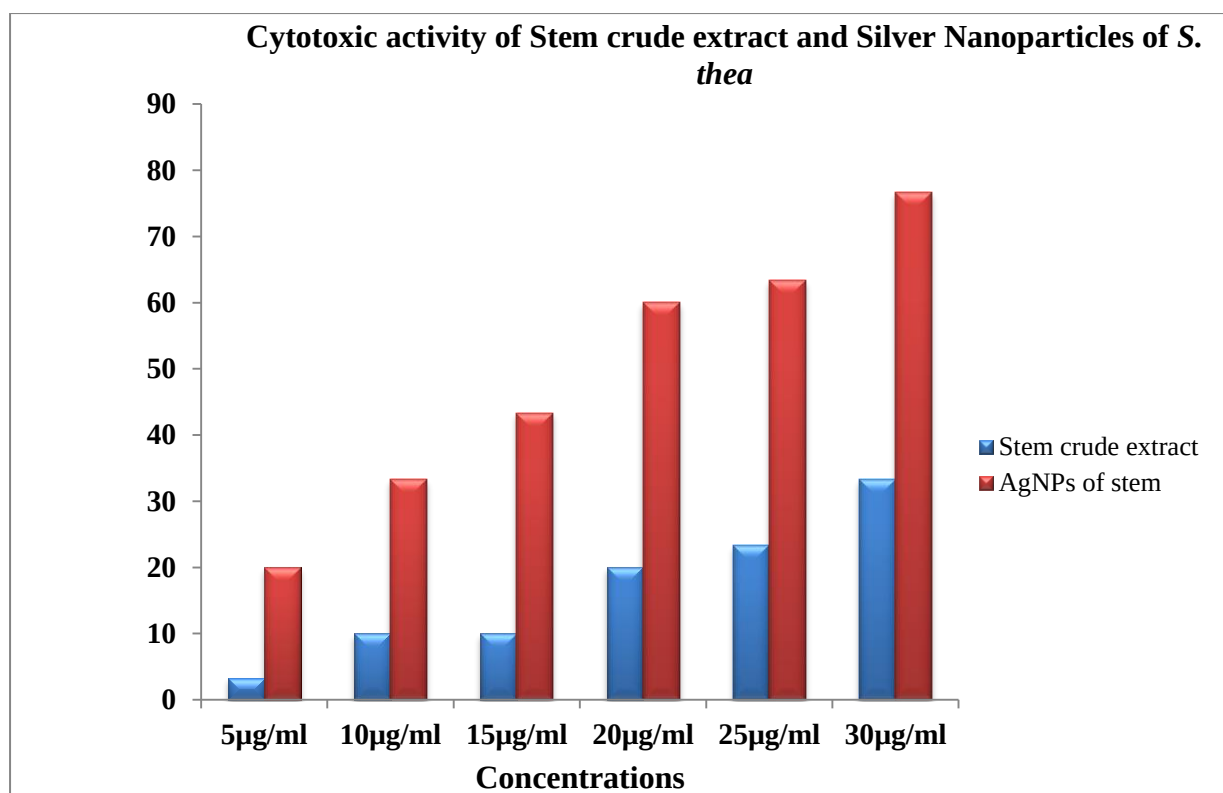


Figure 23. Cytotoxic Activity of Stem Extract and Silver Nanoparticles of *S. thea*

4.8.6. Cytotoxic Activity of Root crude Extract and Silver Nanoparticles of *S. thea*

The transformation of normal cell into tumor cell occurs in multistage process. Diagnosis of tumors in the human body was very difficult. The nanoparticles can detect the cancer disease in a very small volume of cells or tissue. Nanoparticles are smaller in size as compared to biological molecule like enzyme, antibodies and receptor. These nanoparticles can interact with these biomolecules both on the surface and also inside the cell. The present research work also explained the comparative effect of root crude extract and silver nanoparticles (AgNPs) of root extract of *S. thea* on brain shrimps larva. The root extract showed highest mortality rate about 36% at 30µg/ml with LD₅₀, LD₇₀ and LD₉₀ values are 69.75, 182.56 and 733.86. AgNPs showed significant percent mortality at 30µg/ml about 70% (Fig. 25) with LD₅₀, LD₇₀ and LD₉₀ values 17.47, 32.47 and 79.55 respectively (Table 12). The comparative study of both samples showed that the effect of AgNPs of root extract is more significant as compared to crude extract. Different research workers used AgNPs for cytotoxic activity like Sulaiman *et al.*, (2013) carried out green synthesis of silver nanoparticles *Eucalyptus chapmaniana* leaves extract and utilized against the viability of HL-60 cells in a dose dependent manner.

Table 12. Cytotoxic Activity of Root crude Extract and Silver Nanoparticles of *S. thea*

Extract Conc. (µg/ml)	T. No. of Larvae	No. of survivors	% mortality	LD ₅₀	LD ₇₀	LD ₉₀	Regression Line	χ^2 (p)
Root ethanolic extract								
0	30	30	0.00	69.75	182.56	733.86	$Y=2.69 + 1.25 X$	2.39 (0.66)
5	30	27	10					
10	30	26	13.3					
15	30	25	16.6					
20	30	23	23.3					
25	30	21	30.0					
30	30	19	36.6					
Silver Nanoparticles of Root								
0	30	30	0.00	17.47	32.47	79.55	$Y=2.58 + 1.24 X$	0.52 (0.97)
5	30	25	16.6					
10	30	21	30.0					
15	30	17	43.3					
20	30	13	56.6					
25	30	12	60.0					
30	30	09	70.0					

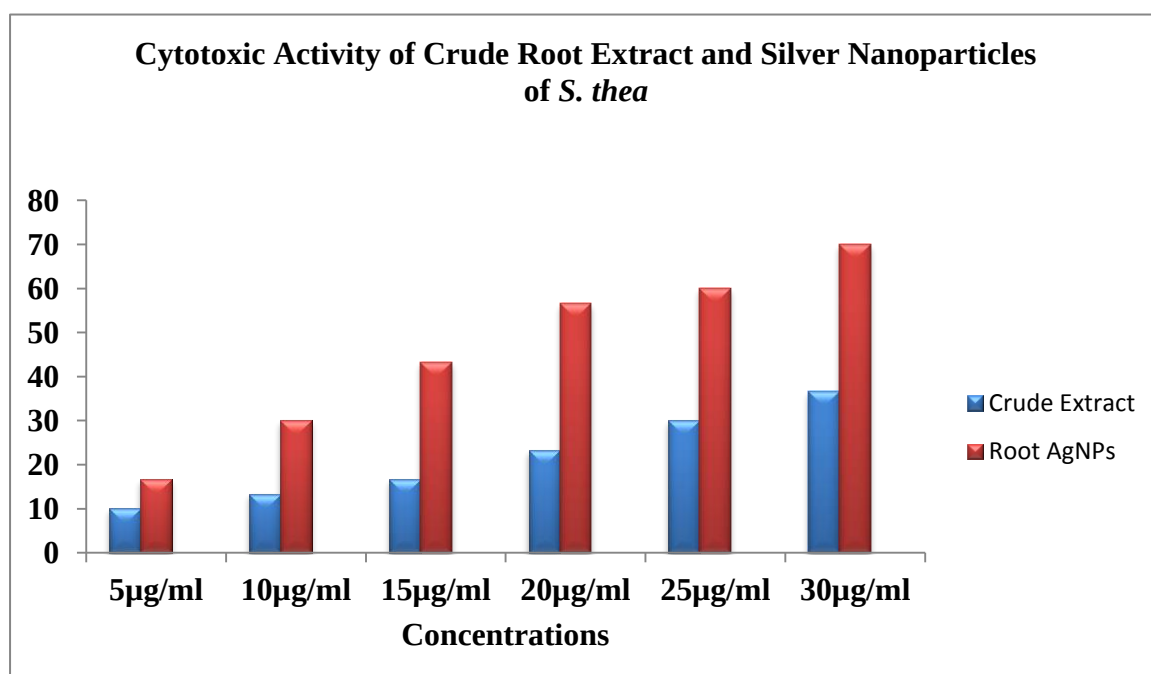


Figure 24. Cytotoxic Activity of Root crude Extract and Silver Nanoparticles of *S. thea*

4.8.7. Antioxidant Assay of Silver Nanoparticles of Leaf Extract of *S. thea*

4.8.7.i. DPPH Scavenging Assay

Free radicals are molecules which contain one or more unpaired electron but there is no specific location for these unpaired electrons. DPPH is a stable compound and it will be reduced by accepting hydrogen or electron. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron stealing reaction. The antioxidant nutrients themselves don't become free radicals by donating an electron because they are stable in either form. The reducing activity of AgNPs of leaf extract of *S. thea* spectroscopically quantified by changing its colour from purple to yellow. Percent of inhibition of AgNPs and leaf extract are 86% and 75% at concentration of 250 μ l respectively, while A. acid showed 94% inhibition at the same concentration as shown in (Fig. 26). The high antioxidant activity of AgNPs is due to the large surface area of silver nanoparticles. The phenol contents present in plant extract absorb onto the active surface of leaf mediated silver nanoparticles. Due to high surface area to volume ratio of AgNPs generate a tendency to interact and scavenge these free radicals. The comparative study of test sample and standard ascorbic acid showed that A. acid possess more reducing power which is then followed by AgNPs and leaf extract (Fig. 26). Our results showed that DPPH scavenging assay is dose dependent. In plants the phenolic compound are one of the major group of compounds which have antioxidant property. They have the ability to donate electron and scavenge reactive oxygen species (ROS) (Cook and Samman, 1996). These compounds are hydrogen donor of the form AH that act through: $AH + R^{\bullet} \rightarrow RH + A$ where the free radical $A^{\bullet}$ is stable and not to propagate the free radical chain reaction and stabilize the structures by resonance. According to recent research on the basis of kinetic analysis of reaction between phenol and DPPH is in fact behaves like electron transport (ET) reaction (Foti *et al.*, 2004). These antioxidant compounds might absorb onto the active large surface of silver and reduce it. The present investigation revealed that leaf extract mediated AgNPs act as strong reducing agent by fast electron transfer step from phenoxide anion to DPPH. The oxidation of low density lipoprotein in blood contributes heart disease. Plants, fruits and vegetables are a good source of phytochemicals which reduce the oxidation of compound and lowered risk of various diseases like cancer, heart disease and some neurological disease. Some synthetic antioxidants like butylated hydroxytoluene, butylated hydroxyanisole have also been use to control lipid oxidation, but in food industry scientist are interested to uncover natural antioxidants. So the overall results concluded that medicinal plants have antioxidant efficacy that make it therapeutically important.

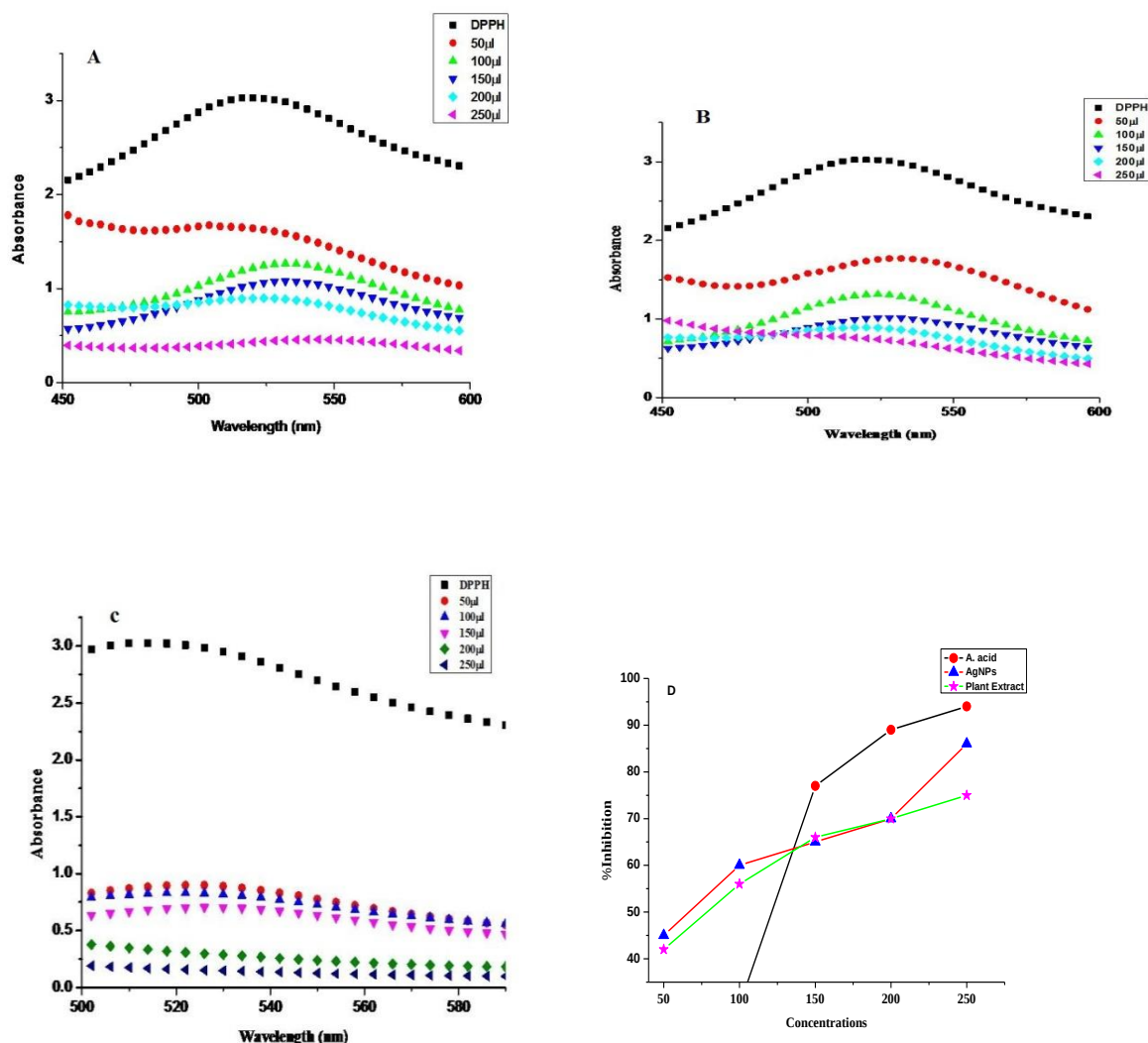


Figure 25. DPPH Scavenging Activity of leaf crude extract, AgNPs and Ascorbic acid. (A) Interaction of various concentrations of AgNPs with DPPH at 518 showing good antioxidant activity (nm). (B) Interaction of various concentration of leaf crude extract with stable free radical DPPH showing modest antioxidant activity at 518 (nm). (C) Interaction of various concentration of Ascorbic acid with DPPH showing highest antioxidant potential at 518(nm). (D) Percent inhibition of A. acid, AgNPs and leaf extract.

4.8.7.ii. Dye Protection Assay

Secondary metabolites derive from plants such as phenol, flavonoids and terphenoids play an important role as natural antioxidant. In biological system the origin of reactive oxygen species (ROS) is often consider as the reaction of hydrogen per oxide or superoxide generate enzymatically or nonenzymatically with iron. In present Dye Protection Assay the antioxidant potential of AgNPs were evaluated. We explored the antioxidant efficacy of AgNPs of leaf extract as well as the absorbance of untreated dye and absorbance of treated with AgNPs were analyzed. The percent inhibition of treated dye with AgNPs was calculated by using (eq. 1). The treated dye with AgNPs has more antioxidant potential as compared to

untreated dye. Here the hydroxyl radical was produced by Fenton reaction. The AgNPs of leaf extract showed low absorbance as compared to untreated dye. In this reaction the low absorbance showed that Fenton reaction is inhibited by iron chelation with secondary metabolites of leaf extract. Biologically the hydroxyl radical ($\cdot\text{OH}$) are widely generated when Fe (II) react with H_2O_2 (Fenton reaction). But the mixture of these two compounds has some disadvantages in an antioxidant assay. Some antioxidants have chelating property and when mixed with Fe (II) they form chelators by altering the Fe (II) activity. Thus it becomes difficult to distinguish that the antioxidant is good OH scavenger or metal chelators. Antioxidants derived from natural products act as pro-oxidants by reducing Fe (III) to Fe (II) and produce $\cdot\text{OH}$ generation catalytic. So it is possible to deactivate free metal ions Fe (II) formation by chelation to prevent hydroxyl radical formation or by converting H_2O_2 to harmless H_2O and O_2 by catalase. When metal chelators bind Fe (II) then they remain inert towards H_2O_2 . Secondary metabolites derived from natural products containing metal chelators and act as a defensive antioxidant. The leaf extract of *S. thea* consists of phenolic compounds which are a class of antioxidant compounds and terminate the free radicals as shown in (Fig. 27). The FTIR spectroscopy of leaf extract showed (Fig.19) that the strongest absorption band was observed at 1639 cm^{-1} indicates the presence of flavonoid compounds in leaf extract which showed best chelating property. Flavonoids are a class of compounds which have hydroxyl groups and are responsible for antioxidant effect. The reduction of reactive oxygen species can be achieved by chelating metal ions with these natural phytochemicals.

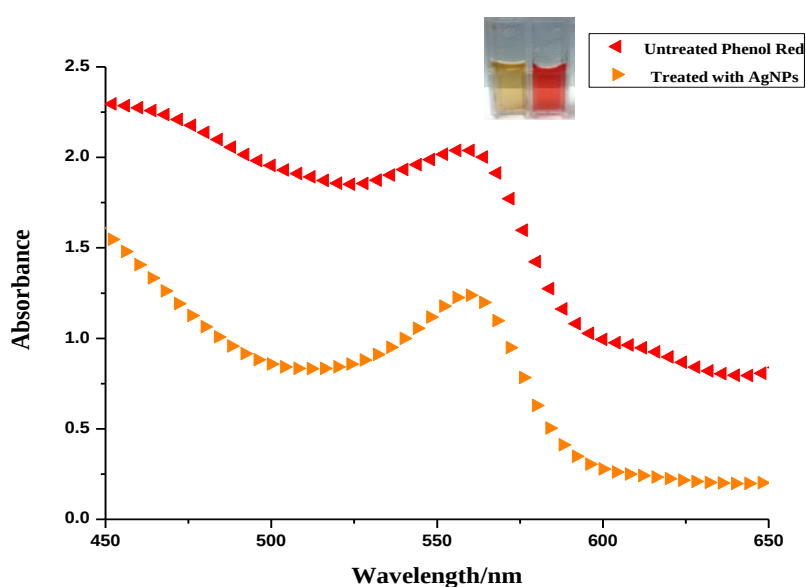
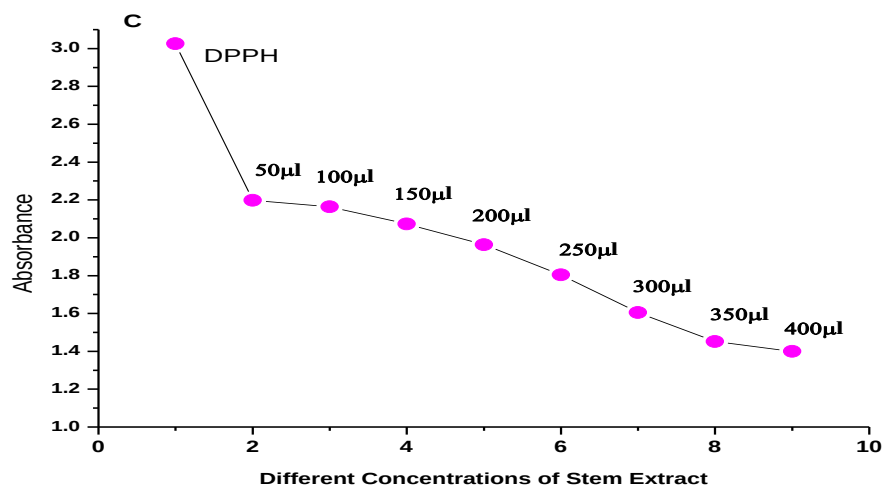
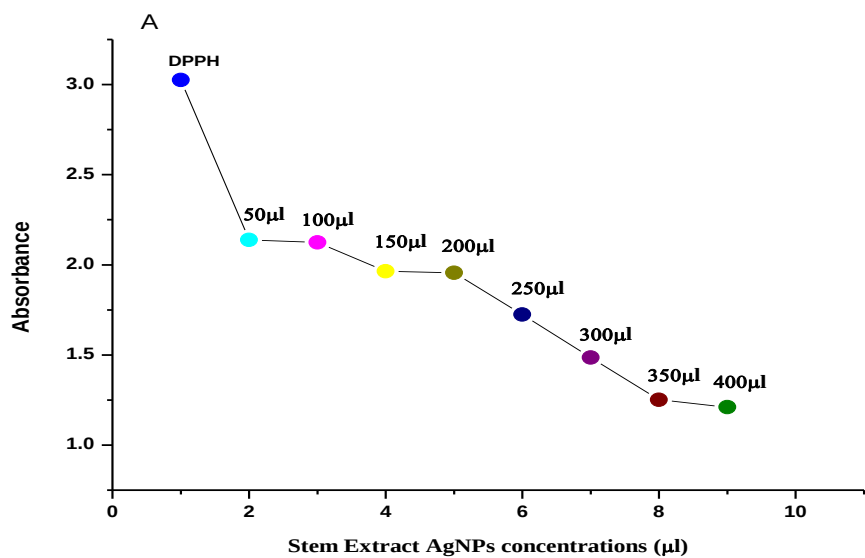


Figure 26. Dye protection Assay of AgNPs by $\cdot\text{OH}$ generation by hydrogen peroxide

4.8.8. DPPH Scavenging Activity of AgNPs of Stem Extract of *S. thea*

Free radicals are molecules which containing one or more unpaired electron but there is no specific location for these unpaired electrons. DPPH is a stable compound and it will be reduced by accepting hydrogen or electron. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron stealing reaction. The antioxidant nutrients themselves don't become free radicals by donating an electron because they are stable in either form. The reducing property of AgNPs of stem extract of *S. thea* spectroscopically quantified by changing its colour from purple to yellow. Percent of inhibition of AgNPs and stem extract are 59.6% and 56.6% at concentration of 400 μ l respectively, while A. acid showed 97.3% inhibition at the same concentration as shown in (Fig. 28C). The high antioxidant activity of AgNPs is due to the large surface area of silver nanoparticles. The phenol contents present in plant extract absorb onto the active surface of stem mediated silver nanoparticles. Due to high surface area to volume ratio of AgNPs generate a tendency to interact and scavenge these free radicals. Dipankar and Murugan, (2012) reported similar results for AgNPs of *Iresine herbstii* leaf aqueous extracts. The comparative study of test sample and standard ascorbic acid showed that A. acid possess more reducing power which is then followed by AgNPs and crude stem extract (Fig.28D). Our results showed that DPPH scavenging assay is dose dependent. In plants the phenolic compound are one of the major group of compounds which have antioxidant property. They have the ability to donate electron and scavenge reactive oxygen species (ROS) (Cook and Samman, 1996). These compounds are hydrogen donor of the form AH that act through: $AH + R^{\bullet} \rightarrow RH + A$ where the free radical $A^{\bullet}$ is stable and not to propagate the free radical chain reaction and stabilize the structures by resonance. According to recent research on the basis of kinetic analysis of reaction between phenol and DPPH is in fact behaves like electron transport (ET) reaction (Foti *et al.*, 2004). These antioxidant compounds might absorb onto the active large surface of silver and reduce it. The present investigation revealed that leaf extract mediated AgNPs act as strong reducing agent by fast electron transfer step from phenoxide anion to DPPH. The oxidation of low density lipoprotein in blood contributes heart disease. Plants, fruits and vegetables are a good source of phytochemicals which reduce the oxidation of compound and lowered risk of various diseases like cancer, heart disease and some neurological disease. Some synthetic antioxidants like butylated hydroxytoluene, butylated hydroxyanisole have also been use to control lipid oxidation, but in food industry scientist are interested to

uncover natural antioxidants. So the overall results concluded that medicinal plants have antioxidant efficacy that make it therapeutically important.



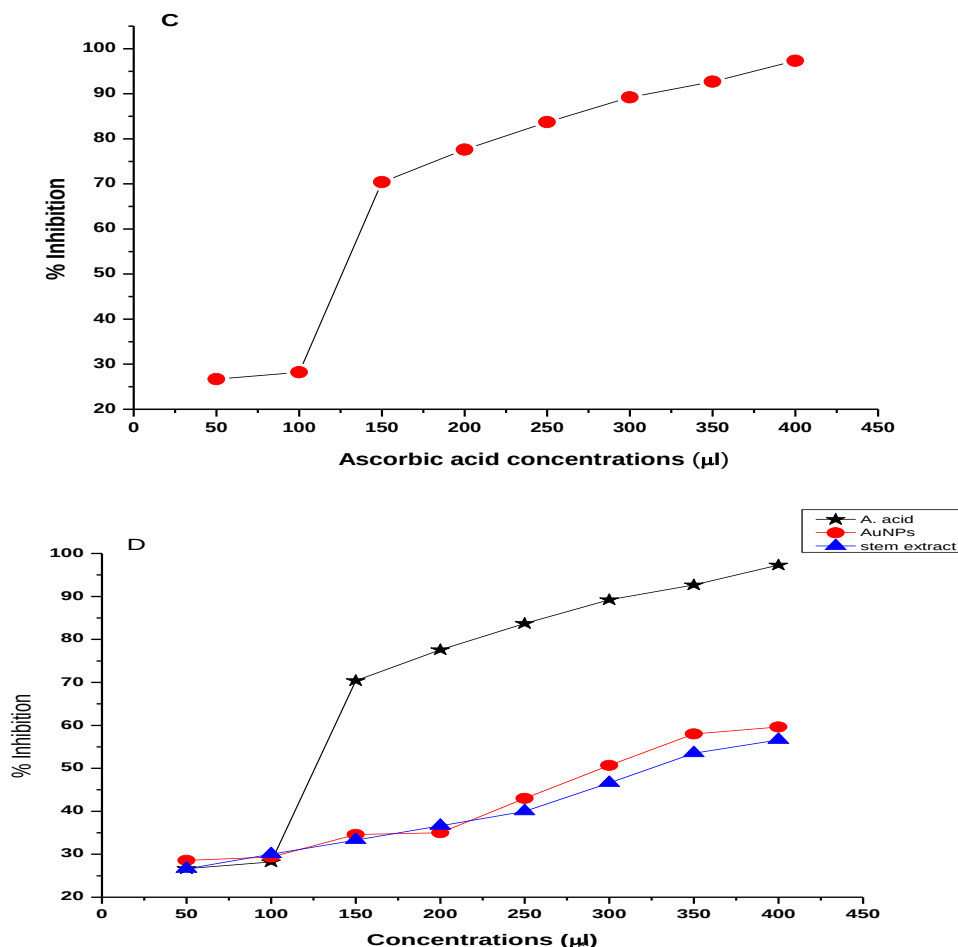


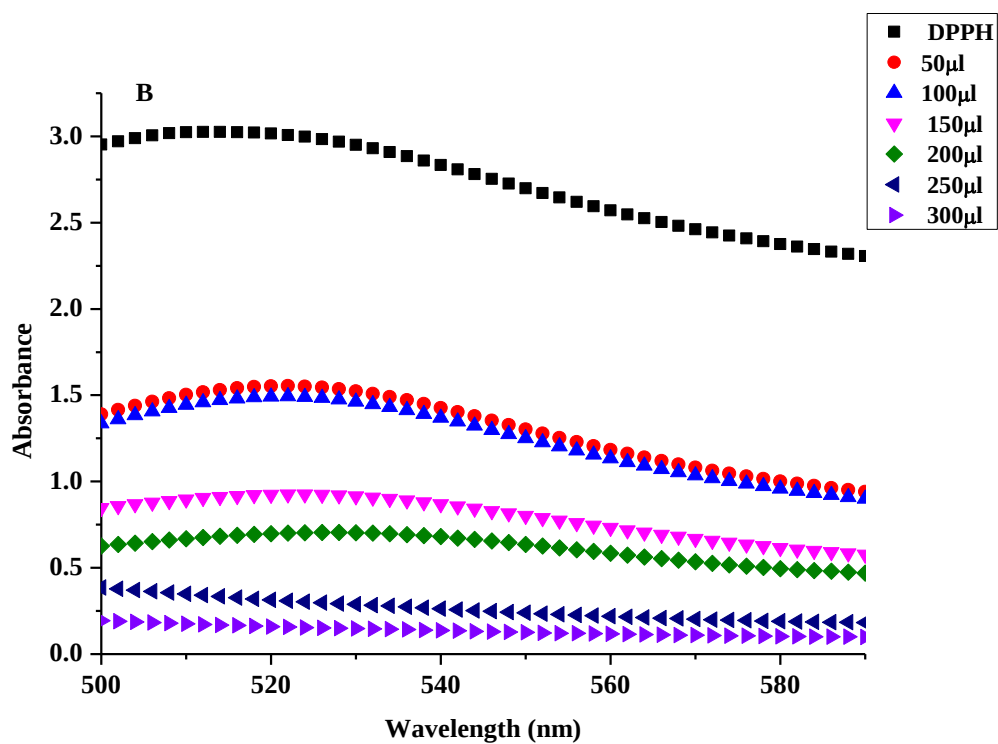
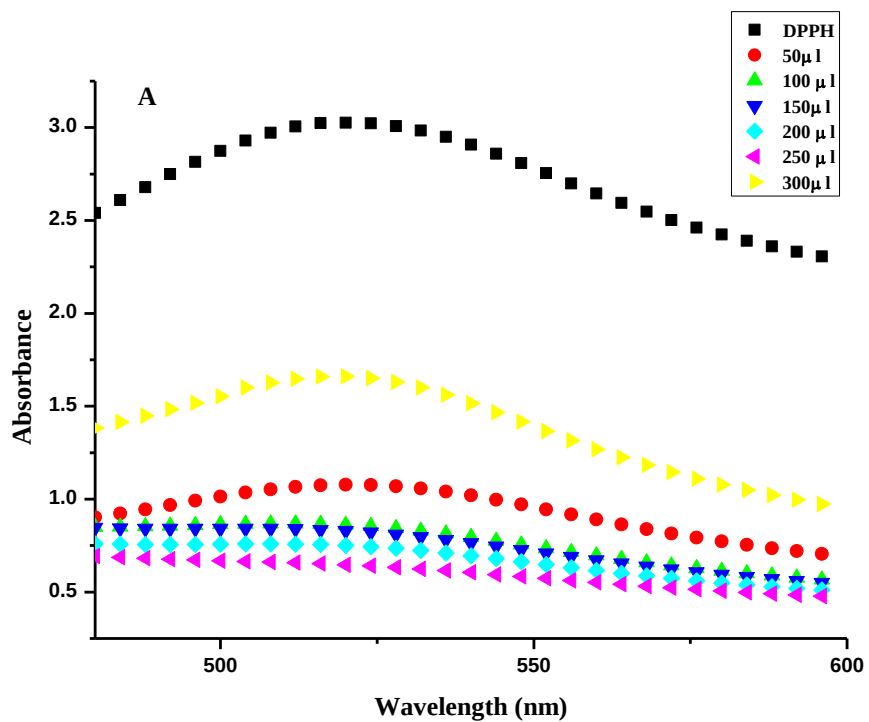
Figure 27. DPPH scavenging activity of stem mediated AgNPs, A. acid and crude stem extract of *S. thea*. (A) Stem extract mediated AgNPs showed increase in concentration is inversely proportional to absorbance. (B) Stem crude extract showed different absorbance at different concentrations. (C) Different concentrations of Ascorbic acid showed different absorbance at 518 (nm). (D) Percent antioxidant potential of stem mediated AgNPs, A. acid and crude stem extract of *S. thea*.

4.8.9. Antioxidant Activity of AgNPs of Root Extract of *S. thea*

4.8.9.i. DPPH Scavenging Activity of AgNPs of Root Extract of *S. thea*

DPPH is a stable compound and it will be reduced by accepting hydrogen or electron. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron stealing reaction. The antioxidant nutrients themselves don't become free radicals by donating an electron because they are stable in either form. The reducing potential of AgNPs of root extract of *S. thea* was spectroscopically quantified by changing its colour from purple to yellow. In present research work the percent inhibition of AgNPs as shown in (Fig. 29) revealed that inhibition is dose dependent. The highest inhibition is 89% recorded at 300μl. Ascorbic acid is run as a standard which showed 89.2% antioxidant potential at the same

concentration. The UV-spectroscopic analysis showed that AgNPs have more reducing power as compared to crude root extract. The high antioxidant capacity is due to the presence of phenolic compound in plant extract. The FTIR spectroscopic analysis revealed absorption peak at 3358 cm^{-1} and 1696 cm^{-1} showed Amide A and Amide I group due to the stretching of C=O band and weakly coupled stretching of C-N and bending of N-H bond. Another strong absorption bands at 1000 and 1610 cm^{-1} were also recorded which showed the presence of protein amide I and phenolic compounds. The reaction mechanism revealed that these metabolites optimized the reactive oxygen molecules level. Different oxygen reactive species produced in cell like hydrogen peroxide, hypochlorous acid and free hydroxyl radicals. All of these oxygen species are highly reactive and damage cell by oxidizing DNA and cause mutation while protein destruction lead to enzymatic inhibition. These natural metabolites have the capacity to donate electron and scavenge reactive oxygen species. There is no free radical formation by donating electrons because they are naturally donor. These compounds are hydrogen donor of the form AH that act through: $\text{AH} + \text{R}^{\bullet} \rightarrow \text{RH} + \text{A}^{\bullet}$ where the free radical $\text{A}^{\bullet}$ is stable and not to propagate the free radical chain reaction and stabilize the structures by resonance. According to recent research on the basis of kinetic analysis of reaction between phenol and DPPH is in fact behaves like electron transfer (ET) reaction (Foti *et al.*, 2004). Many synthetic antioxidants are also use as an antioxidant but in food industries scientist are interested to uncover the antioxidant potential of natural products. The worthy point of this technique is that plants are easily available, free from side effects and safe to handle. The results conclude that the AgNPs of root extract of *S. thea* have strong antioxidant efficacy which make it therapeutically important.



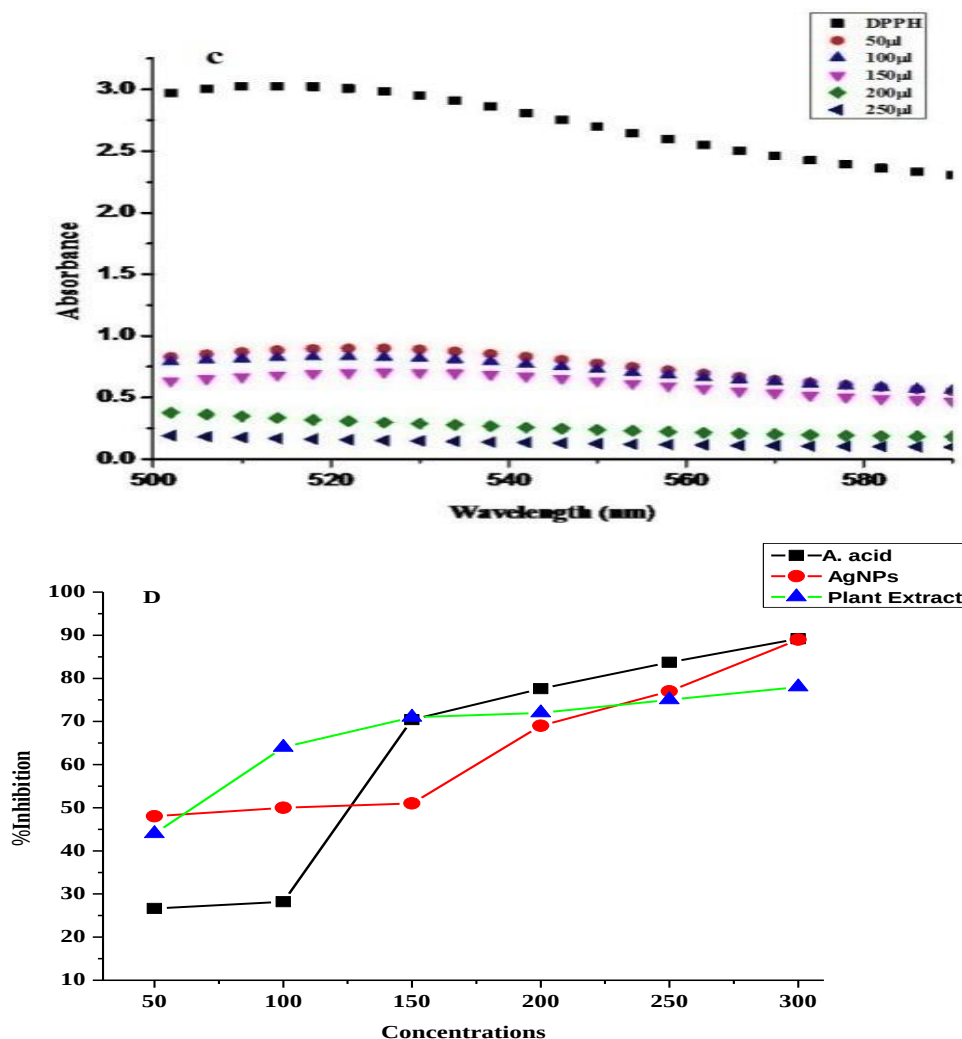


Figure 28. DPPH Scavenging Activity of AgNPs of Root Extract of *S. thea*

4.8.9.ii. Dye Protection Assay of Root Extract mediated AgNPs

Oxidation is the fundamental essence of many living organisms. The energy produced during oxidation is used as a fuel for biological processes. The production of reactive oxygen species and its accumulation leads to oxidative stress and building several diseases in body. Once the oxidation chain begins, it finally results the disruption of living cell which lead to cancer. The oxidation of low density lipoprotein in blood contributes heart disease (Barnham, 2004). In the current research work H_2O_2 and $FeCl_2$ are used for hydroxyl radical production. Here the $\cdot OH$ is generated by Fenton chemistry, $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\cdot + OH^-$. The H_2O_2 oxidized Iron Fe^{+2} to Fe^{3+} , but some time it become difficult to distinguish that the antioxidant is good OH scavenger or metal chelator. Because some antioxidants have chelating property and when mixed with Fe (II) they form chelators by altering the Fe (II) activity. To prevent hydroxyl radical formation it is possible to deactivate free metal ions

formation by chelating or by converting H_2O_2 into H_2O and O_2 by catalase. The metal chelators bind Fe (II) then it remains inert towards H_2O_2 . The antioxidant activity of AgNPs revealed that the plant extract mediated AgNPs work as a good $\cdot\text{OH}$ scavenger. The dye treated with root extract mediated AgNPs showed low absorbance and more $\cdot\text{OH}$ scavenging potential. Percent inhibition of AgNPs treated dye is 70% calculated by (Eq.1). Inhibition in both cases was dose dependent. FTIR spectroscopy showed (Fig. 30) the absorption band at 1000 and 1610 cm^{-1} represents the presence of protein amid I and phenolic compound. Natural products consist of these compounds and have strong antioxidant efficacy. Secondary metabolites derived from natural products containing metal chelators and act as a defensive antioxidant. The current investigation is in consistency with previous finding that like phenolic compound proteins compounds also have reducing and antioxidant efficacy. We concluded that root extract mediated silver nanoparticles have potential to use as an antioxidant.

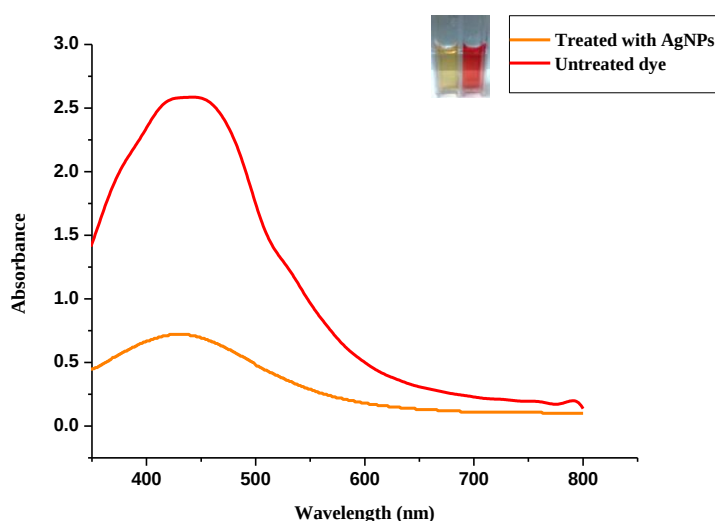


Figure 29. Dye Protection Assay of AgNPs

4.8.9.iii. Hydrogen peroxide Radical scavenging Assay of Root extract mediated AgNPs

In chemical terms H_2O_2 is poorly reactive and does not oxidize most biological molecules readily. The problems comes when H_2O_2 convert reactive hydroxyl radical ($\text{OH}\cdot$) by interaction with transition metal or UV light indiscriminately (Ueda *et al.*, 1996). Human body intake hydrogen peroxide directly from environment through inhalation or skin contact with crops leaves. Hydrogen peroxide can directly produced by a range of oxidase enzymes or by peroxisomal pathway for β -oxidation of fatty acids (Halliwell *et al*, 1999, Chance *et al*, 1979 and Reddy and Rao, 1989). Different beverages like green, black tea and coffee consist

of H_2O_2 (Long et al, 1999 and Fujita *et al*, 1985). Addition of hydrogen peroxide to cell leads to transition metal ion-dependent $\text{OH}^\bullet$ mediated oxidative DNA damage as well as liberating iron from heme proteins (Halliwell and Gutteridge, 1990). To control oxidative stress different compound are isolated from plants. The plant extract consist of phenolic compound are responsible for antioxidant activity. In present investigation we evaluate plant mediated AgNPs and root crude extract hydroxyl radical scavenging efficacy. In our work we compared the hydrogen peroxide scavenging assay of root extract mediated AgNPs and root crude extract. Extract mediated AgNPs showed 80% inhibition while in same concentration root crude extract showed 75% inhibition rate shown in (Fig. 31). The comparative study revealed that AgNPs have more $\text{OH}^\bullet$ radical scavenging power. The antioxidant compound absorbs onto the surface of particles and high surface area to volume ratio of nanoparticles generates the tendency to interact and scavenged these free radicals. Root extract consist more phytochemicals but due to unavailability of large surface area it remain inert towards $\text{OH}^\bullet$ scavenging.

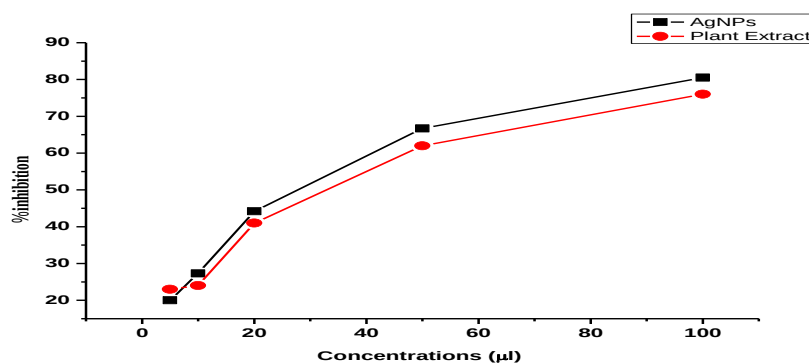


Figure 30. Hydrogen peroxide radical scavenging assay of AgNPs

4.8.10. Analgesic potential of leaf extract of *S. thea* and its AgNPs

Pain is officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain is a disabling accompaniment of many medicinal conditions and pain control is one of the most important therapeutic priorities (Rang *et al.*, 2003). Analgesic is drug used to treat or reduce pain but some synthetic compounds have been developed and are associated with serious adverse effects such as gastrointestinal bleeding, ulceration, additive potential, drowsiness, respiratory distress, nausea etc (Mate *et al.*, 2008). Therefore there is a need to search a bioactive compound from natural products especially from medicinal plants used for alternative analgesics with little or no side effects. In current ethanolic extract of leaf of *S. thea* and its derived AgNPs were screened out for pain relieving potential in swiss albino mice using hot plate method. After administration of leaf extract and leaf extract mediated AgNPs the mean latency time (minutes) at different

time intervals (0, 30, 60 and 90 Minutes) were recorded. After 30 min of saline administration, the Control group (Group I) showed mean latency time of 2.37 ± 0.11 seconds, whereas the Group II received Diclofenac sodium (standard) showed a significant average latency time of $7.11^{**} \pm 0.22$ seconds compared to the control condition. Both leaf extract and its derived AgNPs showed significant and dose dependent analgesic property as compared to control condition. After 30 min of drug administration maximum mean latency time 2.37 ± 0.19 Sec was observed for leaf derived AgNPs at a dose of 300mg/kg of body weight followed by 2.37 ± 0.17 Sec at 200mg/kg and 2.28 ± 0.21 Sec at 100 mg/kg of body weight. Leaf extract mediated AgNPs showed 70.04%, 87.48% and 95.08% analgesia at 100, 200 and 300mg/kg respectively as compared to standard Diclofenac sodium. Significant results were observed after 60 min drugs administration for all doses of ethanolic leaf extract and its derived AgNPs in comparison to normal saline doses. Maximum latency time $8.91^{**} \pm 0.18$ was observed for diclophenac sodium followed by $7.88^{**} \pm 0.21$ sec. at 300mg/kg, $7.39^{**} \pm 0.30$ representing 82.85% analgesia and $5.67^{**} \pm 0.22$ sec. at 200 and 100 mg/kg at body weight representing 88.34% and 63.57% analgesia to the absolute Diclofenac sodium as shown in (Table 13). Similar response of mice in term of paw flick latency time was observed after 90 minutes of drugs administration. Generally synthesized silver nanoparticles using leaf extract showed more efficacy of analgesic effect as compared to ethanolic crude leaf extract. Many plants containing flavonoids have been shown to have analgesic, diuretic and anti-inflammatory actions (Okuda, 1962). Leaf extract of *S. thea* also contain flavonoids and phenols and have strong analgesic potential (Shah *et al.*, 2013).

Table 13. Analgesic Potential of Leaf Extract of *S. thea* and its AgNPs

Dose (mg/kg,b.w)	Mean Latency time (seconds)±SEM						
	IFT (0 min)	30 min		60 min		90 min	
	Normal saline						
10 ml/kg	2.46±0.17	2.37±0.11		2.44±0.20		2.39±0.14	
	Diclofenac sodium						
10	2.43±0.13	7.11**±0.22	100.00	8.91**±0.32	100.00	12.09**±0.57	100.00
	Leaf extract						
100	2.34±0.18	4.21*±0.27	59.21	4.98**±0.17	55.83	5.87**±0.30	48.55
200	2.27±0.09	5.12**±0.27	72.01	6.16**±0.25	69.06	6.91**±0.29	57.15

300	2.22±0.14	5.75**±0.22	80.87	7.15 **±0.39	80.16	7.81**±0.31	64.60
AgNPs from leaf extract							
100	2.28±0.21	4.98*±0.13	70.04	5.67**±0.22	63.57	7.93**±0.33	65.59
200	2.37±0.17	6.22**±0.19	87.48	7.39**±0.30	82.85	8.89**±0.31	73.53
300	2.37±0.19	6.76**±0.32	95.08	7.88 **±0.21	88.34	9.73**±0.42	80.48

4.8.11. Analgesic Potential of Stem Extract of *S. thea* and its AgNPs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain relieving. However long term administration may induce gastro-intestinal ulcers, bleeding and renal disorders. Therefore researchers searched all over the world new analgesic drugs which lacking those effects. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects such as pain relieving (Gupta *et al.*, 2006). In present investigation analgesic property of crude stem extract and its mediated AgNPs were evaluated. After administration of stem extract and stem extract mediated AgNPs the mean latency time (seconds) at different interval (0. 30, 60 and 90 Minutes) were recorded. After 30 min of saline administration, the Control group (Group I) showed mean latency time of 2.37±0.11seconds, whereas the Group II received diclophenac sodium (standard) showed a significant average latency time of 7.11**±0.22 seconds compared to the control condition. Stem extract mediated AgNPs showed significant effect 6.67**±0.39 at a dose of 300 mg/kg with 93.81% analgesia as compared to Diclofenac sodium. As compared to crude stem extract AgNPs showed significant effects at 200 and 100mg/kg with 78.90 and 62.45% analgesia respectively. After 60 minutes oral administration of stem extract mediated AgNPs and crude stem extract showed significant results. AgNPs at 300mg/kg showed 8.45**±0.43 second latency rate for 60 minutes with 94.73% analgesia as compared to standard Diclofenac sodium, but as the concentration of doses reduced latency rate also reduced. Mean latency of AgNPs after 60 minutes at 200 mg/kg and 100 mg/kg showed 78.90 second and 62.45 second producing 72.76% and 66.03% respectively as compared to standard Diclofenac sodium. After 90 minutes the mice analgesic responses were observed as 12.09**±0.57 second for declophenac sodium which is then followed by AgNPs of stem extract with 9.36**±0.31 second at 300mg/kg body weight. As compared to crude root extracts at 100 mg/kg and 200mg/kg AgNPs showed more analgesia with 60.63% and 65.76% respectively. The current investigations showed that as compared to crude root extract AgNPs showed

more analgesia (Table 14). The significant pain reduction of both plant extract and AgNPs might be due to the presence of analgesic principles acting with prostaglandin pathways. It was found that the analgesic property of AgNPs is due to the presence of analgesic constituents present in stem extract. Stem extract of *S. thea* consist of alkaloids and flavonoids and these secondary metabolites have strong analgesic potent. Flavonoids are also known for triggering prostaglandins responsible for peripheral nociceptive perception (Fernanda *et al.*, 2002). So our present findings are in consistency with the previous findings.

Table 14. Analgesic Potential of Stem Extract of *S. thea* and its AgNPs

Dose (mg/kg,b.w)	Mean Latency time (seconds)±SEM						
	IFT (0 min)	30 min	60 min	90 min			
	Normal saline						
10 ml/kg	2.46±0.17	2.37±0.11	2.44±0.20	2.39±0.14			
	Diclophenac sodium						
10	2.43±0.13	7.11**±0.22	100.00	8.92**±0.32	100.00	12.09**±0.57	100.00
	Stem extract						
100	2.44±0.21	3.89*±0.10	54.71	4.14*±0.18	46.41	5.31**±0.20	43.92
200	2.31±0.16	4.50**±0.28	63.29	5.17**±0.22	57.96	6.73**±0.30	55.67
300	2.29±0.10	5.18**±0.25	72.86	6.22 **±0.27	69.73	7.32**±0.31	60.55
	AgNPs from Stem extract						
100	2.38±0.19	4.44*±0.22	62.45	5.89**±0.19	66.03	7.33**±0.33	60.63
200	2.27±0.21	5.61**±0.30	78.90	6.49**±0.26	72.76	7.95**±0.31	65.76
300	2.33±0.13	6.67**±0.39	93.81	8.45**±0.43	94.73	9.36**±0.31	77.42

4.8.12. Analgesic potential of Root extract of *S. thea* and its AgNPs

Analgesics are defined as the substances which decreases pain sensation by increasing pain threshold to external stimuli. Steroidal and non steroidal chemicals are used as a analgesic. The use of these steroidal drugs as analgesic becoming highly dangrous due to their multiple side effects. Therefore a need arises for the development of new analgesic drugs from natural sources with more powerful effects and less side effects as a substitute for chemical agents. Therefore natural plants are used as analgesic agents because plants are easily available and safe to handle. In our present investigation ethanolic root extract and its AgNPs is used for

analgesic activity. After 30 minutes of administration of root extract mediated AgNPs showed latency rate $6.55^{**}\pm 0.32$ and $5.32^{**}\pm 0.2$ seconds at a dose of 300 mg/kg and 200 mg/kg followed by ethanolic root extract at a dose of 300mg/kg with $5.13^{**}\pm 0.22$ seconds as compared to standard Diclofenac sodium. Significant result was recorded after 90 minutes of oral administration of crude root extract and its AgNPs. At 300mg/kg AgNPs showed 80.81% while crude root extract showed 70.06% analgesia which is then followed by AgNPs with 61.95% analgesia at a dose of 200 mg/kg as compared to diclophenac sodium with $12.09^{**}\pm 0.57$ second latency rate (Table 15). The analgesic nature of plant extract is due to the presence of alkaloids and flavonoids because flavonoids are responsible for peripheral nociceptive perception. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Ferdous *et al.*, 2008). The significant pain reduction of both the root extract and AgNPs might be due to the presence of analgesic principles acting with the prostaglandin pathways.

Table 15. Analgesic Potential of Root Extract of *S. thea* and its AgNPs

Dose (mg/kg,b.w)	Mean Latency time (seconds)±SEM						
	IFT (0 min)	30 min		60 min		90 min	
	Normal saline						
10 ml/kg	2.46±0.17	2.37±0.11		2.44±0.20		2.39±0.14	
	Diclofenac sodium						
10	2.43±0.13	7.11**±0.22	100.00	8.92**±0.32	100.00	12.09**±0.57	100.00
	Root extract						
100	2.39±0.09	4.11*±0.14	57.81	4.67**±0.21	52.35	6.79**±0.32	56.16
200	2.46±0.18	4.84**±0.21	68.07	5.62**±0.19	63.00	7.13**±0.20	58.97
300	2.28±0.12	5.13**±0.22	72.15	5.98**±0.32	67.04	8.47**±0.38	70.06
	AgNPs from Root extract						
100	2.44±0.16	4.62**±0.13	64.98	5.11**±0.31	57.29	6.88**±0.21	56.91
200	2.31±0.09	5.32**±0.29	74.82	6.13**±0.37	68.72	7.49**±0.23	61.95
300	2.37±0.26	6.55**±0.32	92.12	7.34**±0.27	82.29	9.77**±0.36	80.81

4.8.13. Larvicidal Activity of Leaf, Stem and Root crude Extract of *S. thea* and its AgNPs

Mosquitoes are important vectors of human diseases. It kills million of people every year especially in tropic areas. Control of mosquito's population is the only option to reduce mosquito causing diseases like malaria, filariasis, dengue and chikungunya. Different chemicals are used for mosquitoes control but it cause environmental pollution. So in the

present research leaf crude extract and AgNPs of *S. thea* leaf were evaluated for larvicidal activity. The results indicate that synthesized AgNPs of leaf extract showed highest mortality at 10µg/ml which is 73.3% as compared to crude leaf extract which is 63.3%. The lowest mortality rate at 5µg/ml for AgNPs is 26.6% and 16.6% for crude leaf extract. The results indicate that the activity is dose dependent for AgNPs with LD₅₀, LD₇₀ and LD₉₀ values are 15.76, 33.97 and 103.08 respectively. While for crude extract LD₅₀, LD₇₀ and LD₉₀ values are 24.3, 50.7 and 136.9 respectively (Table 16). The larvicidal activity of stem and root extracts of *S. thea* and its AgNPs were also carried out using fourth instars larva of *Aedes aegypti*. The comparative study revealed that the lethal effect of AgNPs is more as compared to crude extract. The crude ethanolic stem extract showed 40.0 percent mortality while AgNPs showed 70.0% at 30 µg/ml (Table. 16) with 1.39+2.65X of regression line and 3.58 (0.04) of *chi*-square value. The percent inhibition rate of AgNPs of root extract also showed significant inhibition rate as compared to crude root extract. The LD₅₀, LD₇₀ and LD₉₀ values for root ethanolic extract are 66.6, 176.03 and 716.86 while for AgNPs of root extract it is 24.17, 61.20 and 234.47 respectively. The previous knowledge about the effect of silver nanoparticles and their larvicidal efficacy was studied by different researchers, like Ramos *et al.*, (2012) showed that the effects of latex from *C. procera* is highly toxic to the egg hatching and larvae of *A. aegypti*, similarly Salunkhe *et al.*, (2011) evaluated the effect of AgNPs of filamentous fungus *Cochliobolus lunatus* against *A. aegypti* and reported the LD₅₀ and LD₉₀ values. From the above discussion it is concluded that AgNPs is more toxic than crude extract and can be use for the lethality of mosquitos' to prevent chronic diseases like dengue and malaria.

Table 16.A. Larvicidal Activity of Leaf Crude Extracts of *S. thea* and its AgNPs

Extract Conc. (µg/ml)	T. No. of Larvae	No. of survivors	% mortality	LD ₅₀	LD ₇₀	LD ₉₀	Regression Line	χ ² (p)
	Leaf Ethanolic Extract							
0	30	30	0	24.3	50.7	136.9	Y=1.64+2.72	3.76 (0.05)
5	30	25	16.6					
10	30	23	23.3					
15	30	20	33.3					
20	30	18	40.0					
25	30	15	50.0					
30	30	11	63.3					
	Silver Nanoparticles of Leaf							
0	30	30	0	15.76	33.97	103.08	Y=1.57+3.11	0.05 (4.09)
5	30	22	26.6					
10	30	20	33.3					

15	30	17	43.3					
20	30	14	53.3					
25	30	11	63.3					
30	30	08	73.3					

Table 17.B. Larvicidal Activity of Stem Crude Extracts of *S. thea* and its AgNPs

Extract Conc. (µg/ml)	T. No. of Larvae	No. of survivors	% mortality	LD ₅₀	LD ₇₀	LD ₉₀	Regression Line	$\chi^2(p)$
Stem Ethanolic Extract								
0	30	30	0	47.6	112.9	393.5	Y=1.39+2.65X	3.58 (0.04)
5	30	27	10.0					
10	30	25	16.6					
15	30	24	20.0					
20	30	21	30.0					
25	30	19	36.6					
30	30	18	40.0					
Silver Nanoparticles of Stem								
0	30	30	0.0	20.16	46.12	152.58	Y=3.10+1.46X	4.03 (0.40)
5	30	23	23.4					
10	30	21	30.0					
15	30	18	40.0					
20	30	17	43.4					
25	30	14	53.4					
30	30	09	70.0					

Table 18.C. Larvicidal Activity Root Crude Extracts of *S. thea* and its AgNPs

Extract Conc. (µg/ml)	T. No. of Larvae	No. of survivors	% mortality	LD ₅₀	LD ₇₀	LD ₉₀	Regression Line	$\chi^2(p)$
Root Ethanolic Extract								
0	30	30	0	66.6	176.03	716.86	Y=1.24+2.73X	3.54 (0.03)
5	30	27	10					
10	30	26	13.3					
15	30	24	20.0					
20	30	23	23.3					
25	30	21	30.0					
30	30	19	36.6					
Silver Nanoparticles of Root								
0	30	30	0	24.17	61.20	234.47	Y=1.29+3.20X	3.99 (0.04)
5	30	23	23.3					
10	30	22	26.6					
15	30	19	36.6					
20	30	18	40.0					
25	30	15	50.0					

30	30	11	63.3					
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4.9. GREEN SYNTHESIS OF GOLD NANOPARTICLES

4.9.1. Synthesis of AuNPs of Leaf Extract of *S. thea*

The reduction of metal ions during exposure to leaves, stem and root extract of *S. thea* and the appearance of ruby red colour is clearly indicates the formation of gold nanoparticles. Change in colour is due to surface Plasmon resonance of metal nanoparticles (Rajeshkumar *et al.*, 2013). Reduction of gold ions to gold nanoparticles is a time taking process. A rapid colour change after the addition of plant extract but the concentration of colour is directly proportional to stirring and uniform heat. After 4h of stirring the colour deepen to brown red indicate the AuNPs formation and uniform heat prevent the particles from aggregation.

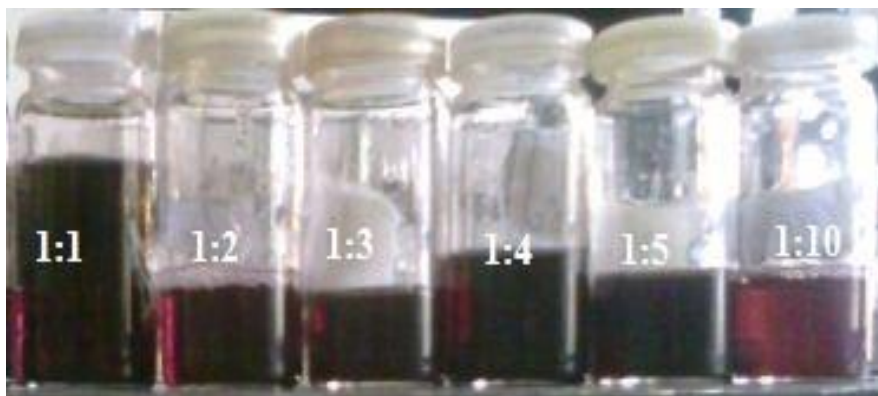


Figure 31. Optimization of AuNPs of leaf extract of *S. thea*

4.9.2. Optimization of Gold Nanoparticles

The process of optimization of gold nanoparticles was carried out by keeping constant plant extract solution while changing volume of gold chloride solution (1mM). Various absorption spectra were taken for various concentrations (Gold solution from lower to higher concentration). At specific ratio maximum absorbance recorded which showed that plant extract plays an important role in controlling the size and shape of nanoparticles. As the concentration of gold solution increase the absorbance of AuNPs also increase but after specific volume of gold solution the absorbance decreased due to the unavailability of functional groups in leaf, stem and root extracts for gold ions leads to the competition between extracts and metal ions for reduction process (Fig. 32).

4.9.3. CHARACTERIZATION OF GOLD NANOPARTICLES

4.9.3.i. UV-Visible Spectroscopy of Gold Nanoparticles of Leaf Extract (AuNPs)

Formation of AuNPs was confirmed by UV-Visible spectral analysis. An electromagnetic wave imposing on Au surface has a certain penetration depth more than <50 nm. The electron on the surface of metal is the most significant and their collective oscillations are termed surface plasmons (SPs). AuNPs having free electrons give rise to a surface Plasmon resonance (SPR) absorption band, due to the combine vibration of electron of metal nanoparticles in resonance with the light wave. Various concentrations of AuNPs of ethanolic leaf extract were used to optimize a specific ratio which gives maximum absorbance. Each concentration of AuNPs gives different absorbance peaks but 1:3 gives maximum absorbance at 535nm (Fig. 33). Narrow sharp peak revealed that the nanoparticles are spherical in shape, small in size and poly dispersed with no aggregation. As well as particle size increase Plasmon band frequency decrease and band shift to longer wavelength. Plant extract play a major role as it is responsible for the synthesis of nanoparticles. It is believed that natural products extract acts a reducing agent for the generation of metal nanoparticles. Some other researchers also conduct UV-Vis analysis of AuNPs of different plant extracts. Research findings of these investigator suggest that the absorbance range for AuNPs is in the range of 500-600nm. Devi & Bhimba, (2012) synthesized AuNPs by Seaweed *Ulva lactuca* Invitro extract and showed absorbance peak at 534nm, similarly Gopinath *et al.*, (2013) worked on the gold nanoparticles of *Terminalia arjuna* and reported UV-Vis spectroscopy showed absorbance peak at 530nm. Suman *et al.*, (2014) worked on the synthesis of gold nanoparticles of *Morinda citrifolia* extract and found absorbance band at 540nm through UV-Visible spectral analysis. The literature review confirms that our spectral analysis is in consistency with previous findings.

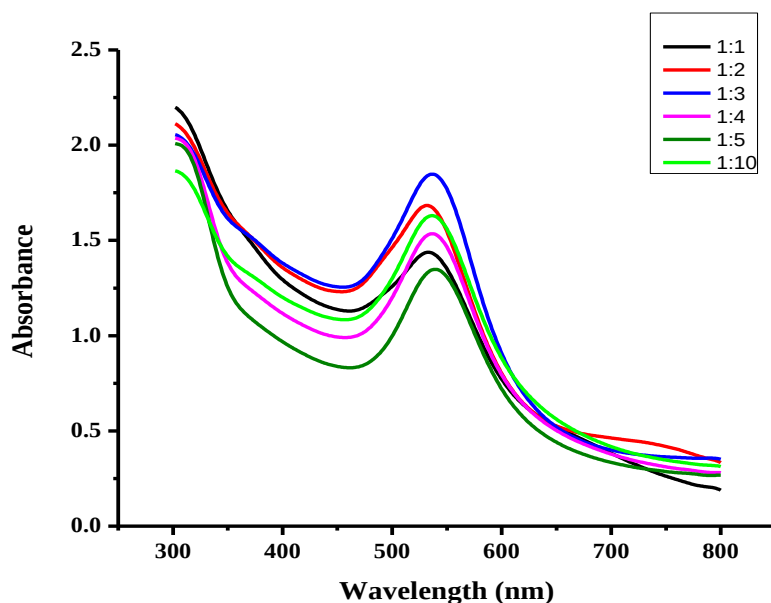


Figure 32. UV-Visible Spectroscopy of Gold Nanoparticles of Leaf Extract

4.9.3.ii. UV-Visible Spectroscopy of Gold Nanoparticles of Stem Extract

The nanoparticles synthesis reaction started after the exposure of stem extract. Initial indication of AuNPs confirmed from ruby red colour of AuNPs solution. After constant heat and stirring the colour deepens indicate complete formation of AuNPs. The synthesized AuNPs of various concentrations of stem extract of *S. thea* were further analyzed through UV-Vis spectroscopy. UV-vis spectroscopy is usually the first technique which is used in characterization of metallic nanoparticles because of surface Plasmon resonance (SPR) phenomenon (Smitha *et al.*, 2009). Different concentrations give different peaks in 500 to 580nm range but maximum absorbance was recorded for 1:2 at absorbance peak at 546nm which is a typical absorbance range for AuNPs due surface Plasmon vibration (Fig. 34). Surface plasmon resonance is a collective excitation of the electrons in the conduction band around the nanoparticles surface. Electrons conform to a specific vibration mode by particle size and shape. Therefore, metallic nanoparticles display characteristic optical absorption spectra in the UV-vis region (Fayaz *et al.*, 2009). Some other workers also conclude that formation of nanoparticles is due to SPR, like Suman *et al.*, (2014) worked on the synthesis of gold nanoparticles of *Morinda citrifolia* extract and obtained absorbance peak at 540nm. Yasmin *et al.*, (2014) synthesized gold nanoparticles using *Hibiscus rosa-sinensis* and reported absorbance band at 520nm. The research work of these researchers proved that the

absorbance range for AuNPs is from 500 to 600nm, so their findings support our current investigation.

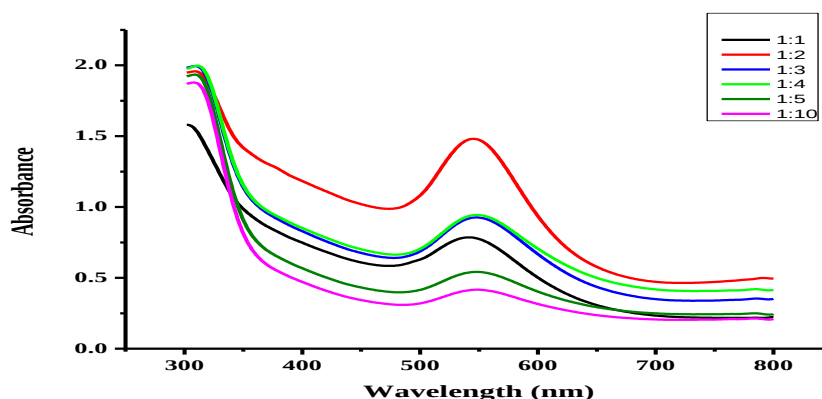


Figure 33. UV-Visible Spectroscopy of Gold Nanoparticles of Stem Extract

4.9.3.iii. UV-Visible Spectroscopy of Gold Nanoparticles of Root Extract

When the root extract was subjected to HAuCl_4 the biosynthesis of AuNPs started after few minutes. The initial indication of AuNPs formation is the colour change reaction. It was observed that pale yellowish colour solution of HAuCl_4 turned ruby red colored solution indicated the formation of AuNPs (Fig.32). Optimization of synthesized AuNPs of root extract of *S. thea* showed that different concentration gives different absorbance spectra with different frequency and different particles size. After optimization of different concentration it is concluded that 1:1 and 1:3 gives maximum absorbance at 546nm but smooth and sharp peak were observed at 1:3 due to surface Plasmon resonance (Fig. 35). The current UV-Vis analysis revealed that as the concentration of gold solution increase particle size also increase which lead to increase the intensity of peak but after specific volume the intensity of peak decrease and become more broaden. The low intensity and shifting of band towards high wavelength indicate low frequency of particles. To check the particle shape, size and position various researchers carried out UV-Vis spectral analysis of AuNPs of plant extracts. The research work of different scientists showed that the absorbance range for AuNPs is from 500-600nm visible range due to surface Plasmon resonance. Sane *et al.*, (2013) used *Betel* leaf, *Hibiscus* leaf, *Lantana camara* and nuts of bitter guard for the synthesis of gold nanoparticles (GNPs) by green biogenic approach. The formation of gold nanoparticles was confirmed by using UV-Visible spectra with absorbance peaks were obtained between 500 nm to 600 nm. Gopinath *et al.*, (2013) worked on the gold nanoparticles of *Terminalia arjuna*. The UV-Vis analysis showed absorbance peak at 530nm, similarly Ahmed *et al.*,

(2014) used *Salicornia brachiata* for the synthesis of gold nanoparticles. The NPs produced peak at 532 nm due to SPR with particles size about 25nm. So all researchers showed that the peak range for gold nanoparticles is from 500-600nm is due to surface Plasmon resonance. In our current spectral analysis of AuNPs of root extract also showed absorbance peak in this range which indicate that this is a correct and convenient method for the synthesis of AuNPs of plant extract.

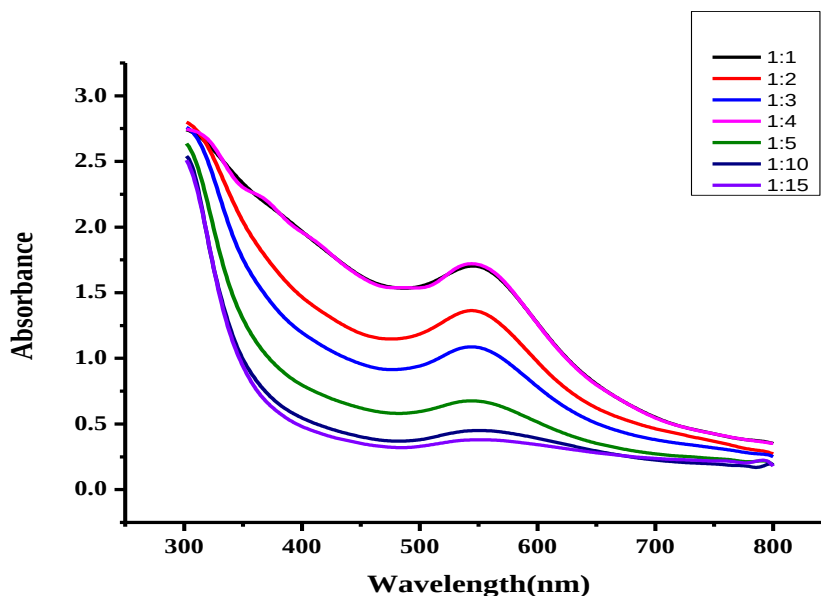


Figure 34. UV-Visible Spectroscopy of Gold Nanoparticles of Root Extract

4.9.3.iv. X-Ray Diffraction Analysis Gold Nanoparticles of Leaf Extract

X-ray diffraction is generally used for the identification of crystalline material and provides information on unit cell dimensions also. In our research work XRD analysis of AuNPs were carried out to find the crystalline nature of AuNPs of leaf extract of *S. thea*. The powder X-Ray diffraction analysis revealed that all the AuNPs were crystalline in nature. The X-ray diffraction spectra (Fig. 36) showed intense peaks at 2θ position, corresponding to (111), (2 0 0), (2 2 0) and (3 1 1) Bragg's planes and denoted the cubic structure of AuNPs (Shankar *et al.*, 2003). The XRD patterns which match with the database of JCPDS file no. 04-0784, indicate that all types of synthesized AuNPs were of pure crystalline nature. The Debye–Scherrer's equation was used to calculate the size of the AuNPs on the basis of the FWHM of the (111) Bragg's reflection arising from the diffractograms (Borchert *et al.*, 2005). The average crystallite size of AuNPs was calculated about 18nm. To find out crystalline nature of AuNPs of different plants researchers carried out XRD analysis. The XRD pattern of their

work showed that the AuNPs are crystalline in nature. Anuradha *et al.*, (2014) carried out XRD analysis of AuNPs of *Pistia stratiotes* and reported crystalline size in the range of 19.8 to 22.1nm. Suman *et al.*, (2014) worked on the synthesis of gold nanoparticles of *Morinda citrifolia*. The XRD analysis showed that all the AuNPs synthesized from *Morinda citrifolia* were face centered cubic and crystalline in nature with a size about 15nm. The literature study reflect that our XRD analysis are also in line with similar results like crystalline nature, intense peak at 38.2° and reflection of cubic structure and supports our present XRD analysis of AuNPs.

4.9.3.v.X-Ray Diffraction Analysis Gold Nanoparticles of Stem Extract

A structural analysis of gold nanoparticles prepared from stem extract of *S. thea* by X-Rays Diffraction Analysis (XRD). XRD pattern of derived AuNPs shows three intense peaks at 2θ value 38.3° , 44.5° and 64.9° for stem extract of *S. thea*. On the bases of angular position of Bragg peaks at (1 1 1), (2 2 2), (2 2 0) and (3 1 1) indicates cubic structure of gold nanoparticles (Fig. 36). The XRD pattern which match with the database of JCPDS files no. 04-0784 clearly indicates that gold nanoparticles synthesized by stem extract of *S. thea* are crystalline in nature. Debye–Scherrer's equation was used to calculate the size of the AuNPs on the basis of the FWHM of the (111) Bragg's reflection. The average grain size of gold nanoparticles is 18 nm. So it is confirmed that stem extract of *S. thea* gold ions into crystalline gold nanoparticles. Different researchers carried out XRD pattern for different plant extract synthesized nanoparticles. The results of their XRD analysis shows crystal structure gold nanoparticles. Parida *et al.*, (2011) biosynthesized gold nanoparticles of *Allium cepa* and calculated average particle size about 22 nm. Pavani *et al.*, (2013) analyzed gold nanoparticles of *Allium cepa* by XRD analysis. The XRD pattern shows that all the nanoparticles were cubic in shape and pure crystalline in nature with grain size ranging from 13 to 16 nm. Our results also show cubic structure and crystalline nature of synthesized gold nanoparticles. So XRD analysis of gold nanoparticles of stem extract of *S. thea* is in consistency with previous XRD characterization of gold nanoparticles.

4.9.3.vi. X-Ray Diffraction Analysis Gold Nanoparticles of Root Extract

X-Ray Diffraction Analysis (XRD) is crystalline material characterization, purity test of samples and dimension of unit cell determination. In present investigation XRD technique is

applied to find out crystalline nature as well particle size of synthesized gold nanoparticles of root extract of *S. thea*. Three intense peaks at 2θ values 38.2° , 44.5° , 64.7° and 77.8° with Bragg's planes values (1 1 1), (2 2 2), (2 2 0) and (3 1 1) were recorded (Fig. 36). The XRD patterns which match with the database of JCPDS file no. 04-0784, indicate that synthesized AuNPs were cubic shape and pure crystalline nature. The Debye–Scherrer's equation was used to calculate the size of the AuNPs on the basis of the FWHM of the (111) Bragg's reflection arising from the diffractograms. The average crystallite size of AuNPs was calculated about 19nm. Pandey *et al.*, (2012) synthesized highly stable gold nanoparticles using fruit peel of *Momordica charantia* extract. Through XRD analysis reported 10-100nm gold nanoparticles size of *Momordica charantia* fruit peel extract. Nagaraj *et al.*, (2012) reported 10-50nm size of gold nanoparticles of *Caesalpinia pulcherrima*. Vijayakumara *et al.*, (2013) determined the size as 70–90 nm of gold nanoparticles of *Artemisia nilagirica* extract. The literature survey indicates that all the synthesized gold nanoparticles size is in the range of 1-100nm. So in the light of previous XRD characterization it is concluded that our results are accurate and have strong consistency with the findings of these researchers.

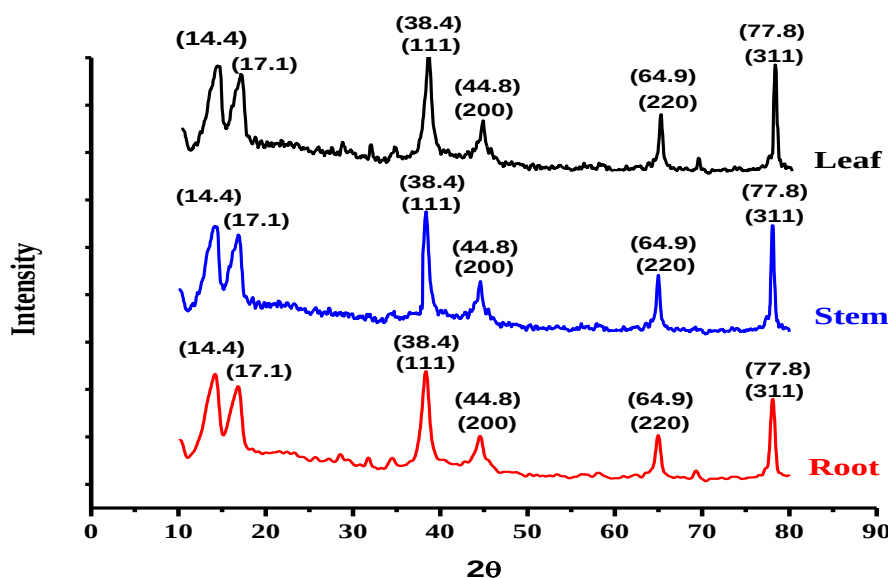


Figure 35. X-Ray Diffraction Analysis (XRD)

4.9.3.vii. Scanning Electron Microscopy Gold Nanoparticles of Leaf Extract

Scanning electron microscopy (SEM) is a sensitive technique in which electrons are used instead of light to form an image. So simply SEM is a type of electron microscope that produce images of a sample by scanning it with a focused beam of electrons. In the present research work scanning electron microscopy of AuNPs of leaf extract of *S. thea* was carried

to find out the particles shape and morphology. The SEM results revealed that most of the nanoparticles are spherical in shape while some changes from spherical to rod shaped with an average particle size about 20-22nm (Fig. 37). Gold nanoparticles having large surface energy coalesce to give thermodynamically favored bulk particle. In the absence of any repulsive forces the van der Waals forces between two metal nanoparticles lead toward coagulation of nanoparticles. So for spatial confinement of nanoparticles it is essential to stabilize the particles. This can be achieved by using a capping agent or suitable functional group. Leaf extract of *S. thea* contained various secondary metabolites which act as capping agent and bind on the surface of particle and steric stabilization is achieved. These organic molecules reduce the chances of particle aggregation and prevent the particles from coming close to each other. Different researchers performed SEM analysis of AuNPs of plant extracts. Ahmed *et al.*, (2014) synthesized AuNPs of *Salicornia brachiata* and calculated that average particle size was about 22 to 25 nm. Gopinath *et al.*, (2013) also prepared AuNPs of *Terminalia arjuna* and reported spherical NPs with average size ranges from 20-60nm. Our current analysis revealed that SEM analysis of gold nanoparticles of leaf extract showed similar results to the previous investigations and our results are in consistency to their findings.

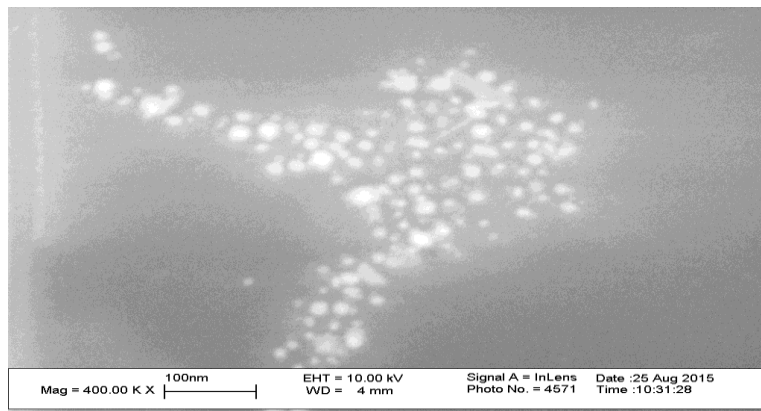


Figure 36. Scanning Electron Microscopy AuNPs of Leaf Extract

4.9.3.viii. Scanning Electron Microscopy Gold Nanoparticles of Stem Extract

Scanning Electron Microscopy (SEM) is a type of electron microscope that produced images of a sample by scanning it with a focused beam of electrons. The scanning electron microscopy of gold nanoparticles of stem extract of *S. thea* was carried to find out the morphology of synthesized AuNPs. The main aim of this investigation is the shape and size confirmation of AuNPs. The SEM analysis revealed that the AuNPs are irregular in shape. Some are spherical while some change from spherical to elongate in shape. The shape of

nanoparticles depends on the binding of biomolecules on the surface of Au. The capping of these biomolecules reduces the size of particles and converts it in to spherical shape. The SEM analysis showed the average particle size about 33nm as shown in (Fig. 38). The morphological study indicates that different functional groups bind on the surface of Au and stabilize it which leads to the formation of metallic Au. Some researchers chemically synthesized AuNPs in which different types of chemical agents and legends are used for Au stabilization. But in green synthesis plant extract act as a stabilizing agent because various secondary metabolites present in plant extract which act both as a stabilizing agent as well as reducing agent. Some other researchers like Nagaraj *et al.*, (2012) carried out green synthesis of gold nanoparticles of *Caesalpinia pulcherrima* and calculate particle size about 10 -50 nm. Jayaseelan *et al.*, (2013) worked on seed extracts of *Abelmoschus esculentus* for the synthesis of gold nanoparticles (AuNPs). SEM spectroscopy showed that nanoparticles were spherical in shape and about 47-75 nm in size. Similarly Vijayakumara *et al.*, (2013) carried out the biosynthesis AuNPs of *Artemisia nilagirica* extract and reported 70-90 nm average particle size. The research findings of these researchers showed that our present analysis of AuNPs of stem extract is similar to their investigation.

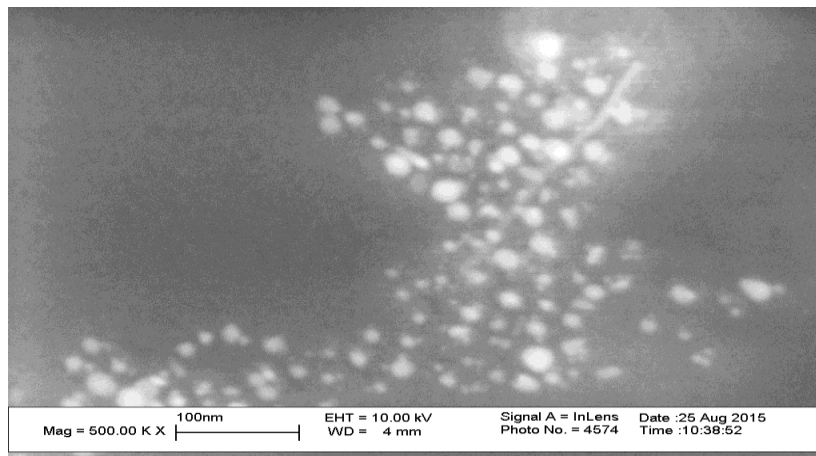


Figure 37. Scanning Electron Microscopy AuNPs of Stem Extract

4.9.3.ix. Scanning Electron Microscopy Gold Nanoparticles of Root Extract

The topographic features of biosynthesized AuNPs were analyzed in order to evaluate the size, shape and morphology using Scanning Electron Microscopy (SEM). The ingredients present in root extract of *S. thea* reduced the size of synthesized gold nanoparticles and spherical shape AuNPs were formed (Fig. 39). The preliminary phytochemical tests suggests that plants contain different secondary metabolites like alkaloids, saponins, glycosides,

volatile oils, tannins, phenolics compound and flavonoids (Hill, 1952; Tyler, 1999). All of these chemical constituents have reducing power. These metabolites chapped over the surface of ionic Au^{+3} and reduce it to Au^0 . During the reduction process the size of gold nanoparticles reduces and spherical shape nanoparticles formed. The shape, size and morphology of gold nanoparticles depend on the presence of these metabolites in plant extract. So the gold nanoparticles of root extract of *S. thea* showed AuNPs with spherical morphology and about 27nm in size as shown in (Fig. 40). The functional groups which reduce ionic gold also stabilize the reaction medium. Arunachalam *et al.*, (2013) carried out synthesis of gold nanoparticles of *Aegle marmaloes* and reported triangle shape gold nanoparticles. Elia *et al.*, (2014) synthesized gold nanoparticles using plant extract. The SEM analysis shows that all the nanoparticles were polydispersed with a size about 100nm, similarly Muniyappan and Nagarajan, (2014) also prepared gold nanoparticles through green approach and reported 20 nm particles size. The previous study showed that gold nanoparticles were mostly spherical in morphology due to capping of functional groups. So our current SEM images strongly support by previous scanning microscopy.

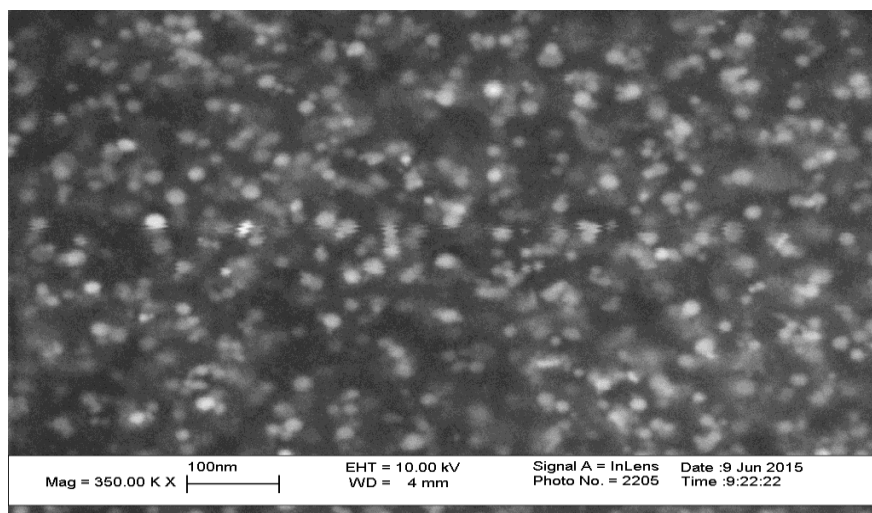


Figure 38. Scanning Electron Microscopy AuNPs of Root Extract

4.9.3.x. Energy Dispersive Analysis Gold Nanoparticles of Leaf Extract

Energy dispersive X-Rays spectroscopy (EDX) is a sensitive technique that provides the elemental curve as output. In the present analysis the presence of gold atom in gold nanoparticles were confirmed by energy dispersive spectroscopy (EDX). The optical absorption peak for gold nanoparticles of leaf extract of *S. thea* was observed at 2.3keV (Fig. 40) which is a typical absorption range for AuNPs absorption region for gold nanoparticles.

The strong signal of gold nanoparticles in this region is due to surface Plasmon resonance. The current energy dispersive spectroscopic profile of gold nanoparticles of leaf extract showed strong gold signals at 2.3 and 9.7 keV. Some other elements like Ca, K, Cl and C are also present the presence of these trace elements are due to leaf extract. Literature survey revealed the location of gold nanoparticles peak in this range also. Geethalakshmi & Sarada, (2012) prepared gold nanoparticles with root extract of *Trianthema decandra*. The EDX analysis showed the presence of metallic gold at 0.30, 2, 2.2, 2.4, and 9.7 keV. Similarly Ali *et al.*, (2011) conducted an experiment for the biosynthesis of gold nanoparticles. The EDX spectroscopic study of AuNPs showed a strong and sharp peak of gold at 2.3keV. The results of these researchers showed that the absorption of metallic Au peak at this range is in consistency to their work.

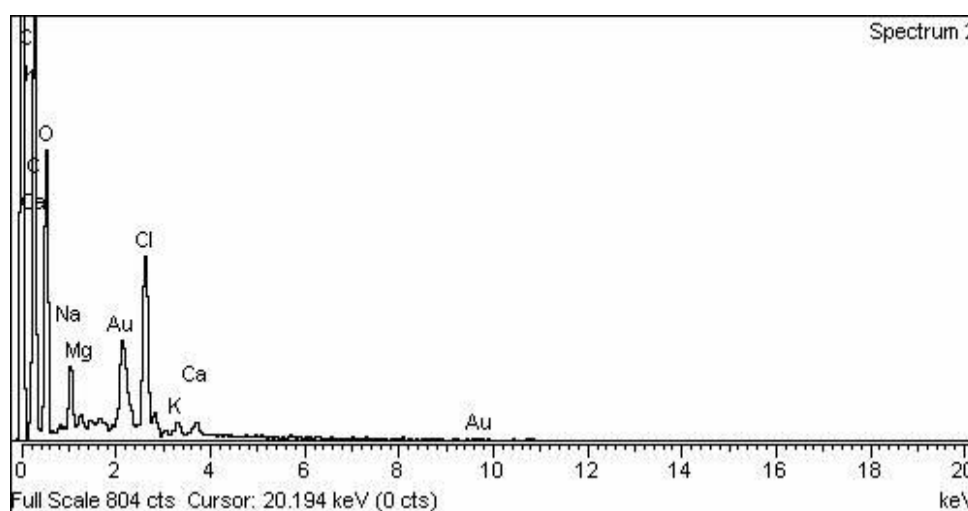


Figure 39. Energy Dispersive Analysis Gold Nanoparticles of Leaf Extract

4.9.3.xi. Energy Dispersive Analysis Gold Nanoparticles of Stem Extract

To confirm the presence of Au in gold nanoparticles of stem extract of *S. thea* Energy Dispersive Spectroscopic (EDS) technique was carried out. Metallic gold showed a sharp peak at 2.2keV (Fig. 41). Some other gold signals were also detected at 8.2, 9.7 and 11. 2keV are because of gold coating over sample. Weak signals of trace elements like Ca, K, Mg, Na and C were also present in synthesized gold nanoparticles. The trace elements are originated from biomolecules bound to the surface of gold nanoparticles. C, Mg, Na are present in plant abundantly. The position of gold peak at keV value is strongly supported by previous research work like Song *et al.*, (2009) used leaf extracts of two plants *Magnolia kobus* and *Diopyros kaki* for the synthesis of gold nanoparticles. In synthesized nanoparticles the

presence of metallic gold was confirmed by energy-dispersive X-ray spectroscopy (EDS). The EDX analysis showed intense peak at 2.3 keV. Jayaseelan *et al.*, (2013) worked on seed extracts of *Abelmoschus esculentus* for the synthesis of gold nanoparticles (AuNPs). The EDX analysis of AuNPs of *Abelmoschus esculentus* revealed the presence of strong signals at 3.0 and 3.8 keV for gold while weak signals for other elements like O, C, K, Mg and Ca. These weak signals could have been arisen from X-ray emission from macromolecules like proteins/enzymes bound to the NPs or in the vicinity of the particles. These biomolecules also present in stem extract of *S. thea* which gives week signals at various energy level. From the above discussion it is concluded that our research findings are similar to the work of previous researchers. So the present analysis proved the synthesis of AuNPs of stem extract of *S. thea*.

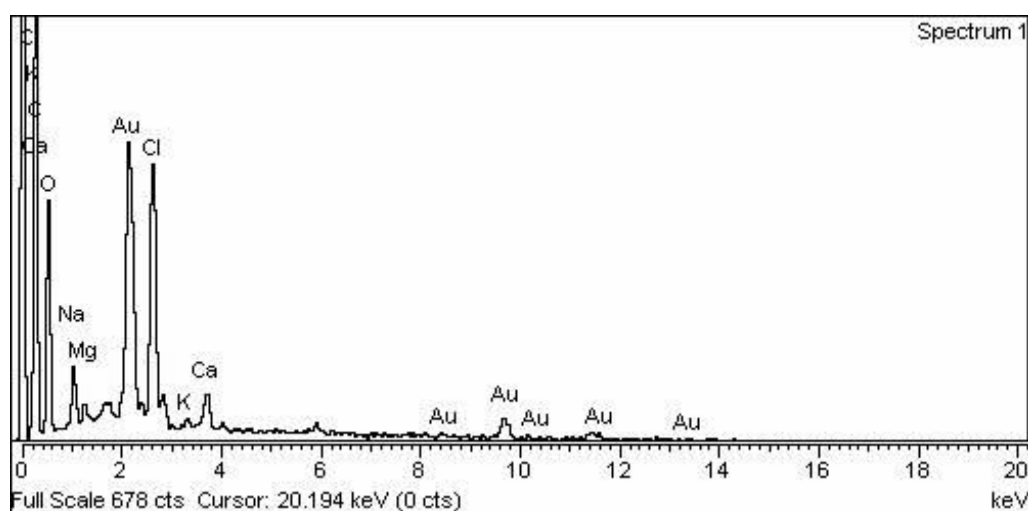


Figure 40. Energy Dispersive Analysis Gold Nanoparticles of Stem Extract

4.9.3.xii. Energy Dispersive Analysis Gold Nanoparticles of Root Extract

The Energy Dispersive Analysis (EDX) technique is specially used for the detection of X-Rays emitted from the sample by bombardment through electron beam for characterizing the elemental composition of the sample. To determine the presence of an element in the sample the X-Rays energy values of sample from the EDX spectrum are compared with known characteristic X-Rays energy values. To confirm the presence of Au in synthesized gold nanoparticles of root extract of *S. thea*, XRD analysis carried out. The optical absorption peak for gold is 2.2keV due to surface Plasmon resonance which is a typical range for gold. Other trace elements like Ca, C, K and Na also give peak at different keV as shown in (Fig. 42). These biomolecules present in plant extract due to the presence of various functional groups like protein, alkaloids and flavonoids etc. These secondary metabolites capped over the

surface of ionic gold (Au^{+3}) and reduce it. XRD then confirmed the presence of bioactive metallic gold (Au^0). Bankar *et al.*, (2010) synthesized Banana peel extract mediated synthesis of gold nanoparticles. The EDX analysis showed that intense peak at 2.3keV which is a typical gold range. Elia *et al.*, (2014) synthesized gold nanoparticles using plant extract. The confirmation of gold was detected by EDX analysis. The EDX analysis showed the presence of gold at 2.2keV position. So the EDX analysis of root mediated gold nanoparticles of *S. thea*, also showed Au peak in this range which is in consistency with previous EDX result of gold nanoparticles.

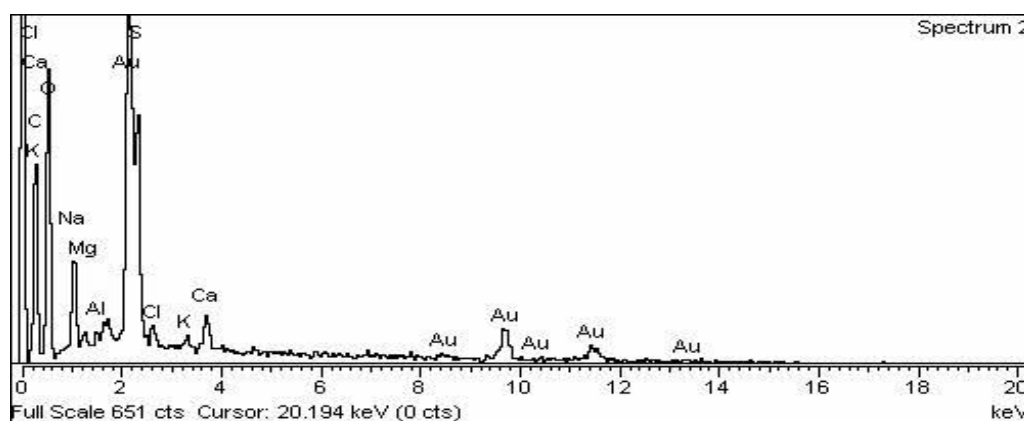


Figure 41. Energy Dispersive Analysis Gold Nanoparticles of Root Extract

4.9.3.xiii. Fourier Transform Infrared Spectroscopy of Leaf Extract and AuNPs

The FTIR analysis was carried out to find the capping material present in the particles which enabled the synthesis. FTIR analysis was used for the characterization of the extract and the resulting nanoparticles. FTIR absorption band of ethanolic extract before and after reduction of Au are shown in (Fig. 43). Absorbance band of leaf extract are observe in the region of 0 to 4500 cm^{-1} are 4053, 3411, 1517, 1259, 999 and 709 cm^{-1} while AuNPs of gold nanoparticles of leaf extract of *S. thea* showed absorbance band at 4053, 3411, 965 and 678 cm^{-1} . The absorbance band at 4053 and 3411 cm^{-1} represent the presence of O-H and N-H stretching vibrations and Stretching O-H asymmetric respectively. While absorbance band at 709 and 678 cm^{-1} also showed OH group. Two absorbance band at 1253 and 1517 cm^{-1} were present in leaf extract while absent in gold nanoparticles of leaf extract. The functional groups present at position 1517 cm^{-1} represent the presence of Amid I group while 1259 cm^{-1} represent phosphate I. The FTIR results indicate that protein is present in plant extract responsible for the formation of AuNPs. Amid I present in plant extract due to presence of protein capping on the surface of gold and reduce it. FTIR analysis revealed that protein present in plant extract has stronger ability to bind metals and form metal nanoparticles. So

this is confirmed that biological molecules have functional groups which facilitate the formation of Au nanoparticles in aqueous medium.

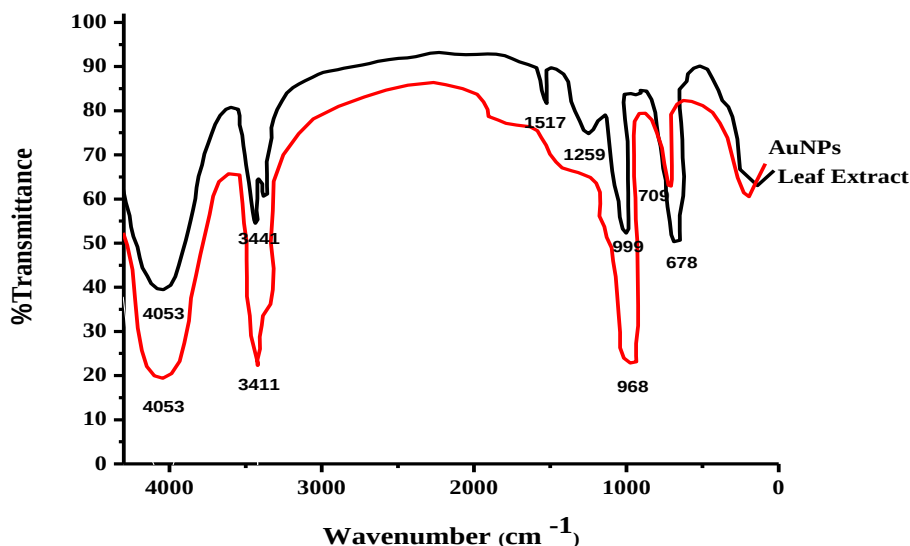


Figure 42. Fourier Transform Infrared Spectroscopy of Leaf Extract and AuNPs

4.9.3.xiv. Fourier Transform Infrared Spectroscopy Gold Nanoparticles of Stem Extract of *S. thea*

Fourier Transform Infrared Spectroscopy (FTIR) was used to characterize the successful functionalization of Gold nanoparticles. The FTIR spectra of both synthesized nanoparticles of stem extract of *S. thea* and ethanolic extract of stem as shown in (Fig. 44). It has found that some peaks obtained by stem extract have been repeated in FTIR spectrum of functionalized gold nanoparticles. The characteristic peaks for O-H & N-H stretching vibrations and for O-H stretching (water) were observed at 3616 and 3003 cm^{-1} respectively. Both of these peaks remain same for both samples. Some characteristics peaks of stem extract observed at 1482, 1260, 1140 and 722 cm^{-1} . The absorbing peak at 1482 cm^{-1} showed the presence of C-H coupled with a ring vibration of Guanine. Another absorbance peak 1140 cm^{-1} was also observed in stem extract spectra which represent the presence of oligosaccharide C-OH stretching band, the oligosaccharides is due to the presence of carbohydrates in plant extract. The comparison observation of both spectra showed the disappearance of band after the bioreduction may be due to the fact that starch and protein are mainly responsible for reduction of gold ions where they themselves get oxidized and leading to a broad band at

1140 and 1482 cm^{-1} for reduction of gold ions. Some other researchers also carried out FTIR analysis of AuNPs and plant extract. Jain *et al.*, (2009) synthesized AuNPs of *papaya* fruit extract and reported absorbance band at 1226 cm^{-1} due to C-O group of polyols and the fact is that the polyole is mainly responsible for the reduction of gold ion. Similarly Parida *et al.*, (2013) analyzed AuNPs of *Elettaria cardamomum* L. extract through FTIR spectroscopy. IR report suggests that typical peaks at 621 cm^{-1} , which are observed in functionalized AuNPs pattern close to 602 cm^{-1} , signify the presence of R-CH group. This result suggested that the biological molecules possibly perform dual function of formation and stabilization of gold nanoparticles.

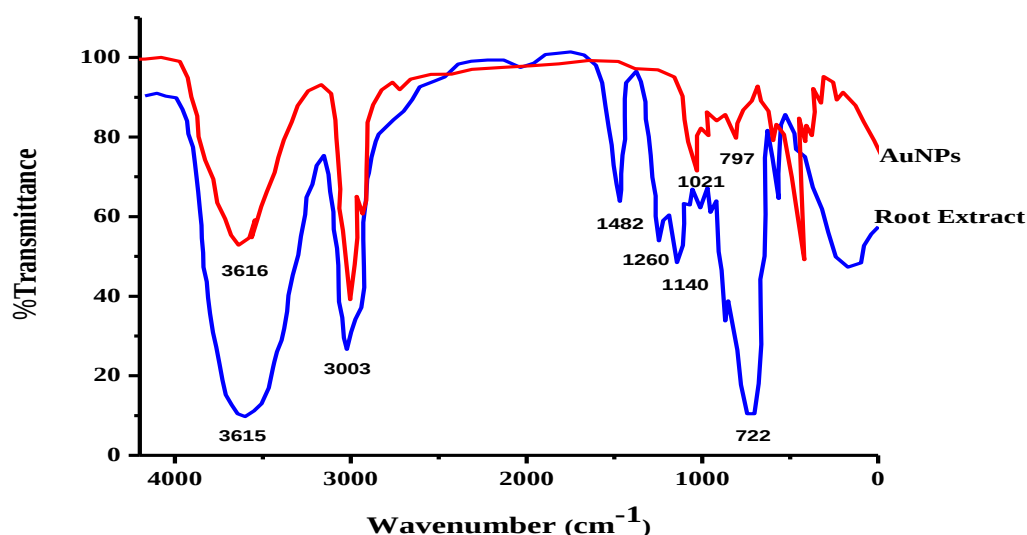


Figure 43. Fourier Transform Infrared Spectroscopy Gold Nanoparticles of Stem Extract

4.9.3.xv. Fourier Transform Infrared Spectroscopy of Root Extract

Fourier transform infrared spectroscopy (FTIR) analysis was performed to identify the biomolecules localized on the surface and responsible for the reduction of gold solution. In the present research work FTIR measurement of both root extract and AuNPs of root extract of *S. thea* were carried out to identify various functional groups responsible for reduction of Au^+ ions and capping of the bio-reduced gold nanoparticles synthesized by the root extract. The major peaks in the FTIR spectrum of AuNPs were observed at 3780, 3180, 1646, 1185 and 961 cm^{-1} the following units shows the presence of various functional groups present in root extract. The presence of O-H & N-H stretching vibrations at 3780 and Amide I at 1646 represent the presence of protein in plant extract. C-O deoxyribose, C-C bond was also

confirmed by FTIR analysis. The same functional groups were also detected in synthesized nanoparticle of root extract of *S. thea* but some of the functional groups like Amide and Deoxyribose were present in crude root extract while absent in synthesized nanoparticles. The similar results were also reported by different researchers using plant extract as a reducing and stabilizing agent like Vigneshwaran *et al.*, (2007) demonstrated that aromatic and aliphatic amines are also used as a reducing agent for the reduction of gold chloride and stabilizing medium. FTIR spectroscopy was also carried out by other research workers like Barman *et al.*, (2013) concluded that the peak ($1,720\text{ cm}^{-1}$) due to the acid groups present in tomato extract is missing in the AuNPs spectrum which confirms that these groups are responsible for reduction. Suman *et al.*, (2014) analyzed AuNPs of root extract of *Morinda citrifolia* and reported that amide I and carbonyl groups are present in reaction mixture and this protein might be responsible for AuNPs synthesis by stabilizing medium. The current findings are also in agreement of the above mentioned reports that the root extract of *S.thea* have the efficacy of reduction and stabilization of AuNPs.

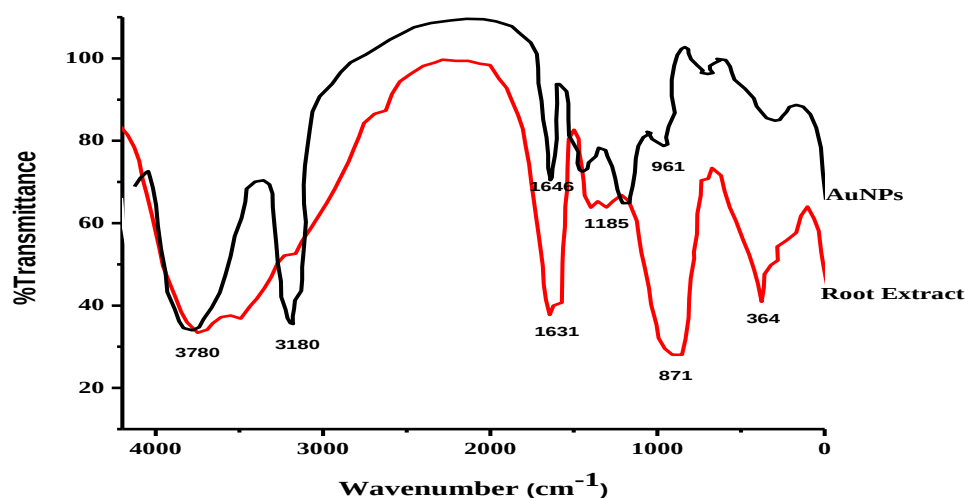


Figure 44. Fourier Transform Infrared Spectroscopy of Root Extract

4.10. BIOLOGICAL ACTIVITIES OF GOLD NANOPARTICLES

4.10.1. Antibacterial Activity of AuNPs of Leaf Extract of *S. thea*

In the current investigation the antibacterial effect of AuNPs was evaluated as shown in (Table 17). According to the Clinical and Laboratory Standard Institutes (2006) guidelines bacterial pathogens is resistant against (Streptomycin) which is used as a positive control in this study. AuNPs in this study showed strong antibacterial activity against all bacterial pathogens. Metallic gold is comparatively more effective as compared to crude extract. In

current antibacterial investigation leaf, stem and root extracts of *S. thea* and its mediated AuNPs are used against bacterial strains. The comparative study of all the crude extract and AuNPs suggested that synthesized AuNPs have more efficacies against bacterial strains. A positive charge on Au⁺ is necessary for electrostatic attraction between positive charges of Au⁺ and a negative charge of plasma membrane of microorganisms (Hamouda *et al.*, 2001). The AuNPs enter into the cell of microorganism by creating a hole in bacterial cell wall caused the leakage of cell contents and coagulation of protein which lead to cell death (Rai *et al.*, 2010). Crude leaf extracts showed low antibacterial potential as compared to streptomycin while leaf extract mediated AuNPs showed strong activity as compared to crude leaf extract. The effectiveness of AuNPs is directly proportional to zone of inhibition. Leaves extract mediated AuNPs showed 23.4±0.1mm for *E. coli*, 11±0.2 mm for *Staph. Aureus*, 19.3±0.6mm for *B. pumilus*, 20.5±0.8mm for *Psed. aeruginosa* and 17.1±0.6mm for *Kleb. pneumonia*. Similar results were also recorded for stem and root extracts and its mediated AuNPs. Stem extract showed more antibacterial behavior against *Staph. aureus* as compared to standard streptomycin and crude stem extract. The percent inhibition of both stem and root extract are 53.38%, 49.59%, 63.92%, 35.02% and 60.08% for stem and 82.09%, 126.47%, 66.74%, 39.26% and 77.01% for root against *E. coli*, *Staph. aureus*, *B. pumilus*, *hsed. aeruginosa* and *Kleb. pneumonia* respectively. While the same extract mediated AuNPs showed 24±0.6mm, 23±0.4mm, 22.0±0.3mm, 21.5±0.3mm and 20.3±0.4mm zone of inhibition for stem extract mediated AuNPs and 22.01±0.2mm, 27.6±0.2mm, 17.06±0.2mm, 15.05±0.4mm and 21.02±0.3mm zone of inhibition for root extract mediated AuNPs. Many of the phytometabolite present in plants have antimicrobial activity. The flavonoids, phenols and tannins present in plant disrupt bacterial cell wall followed by leakage of cell content. *S. thea* consists of all these phytochemicals and have antimicrobial potential (Shah *et al.*, 2013). Many researchers carried out antibacterial activity using different plant extracts like Ali *et al.*, (2011) conducted an experiment for the biosynthesis of gold nanoparticles and its antibacterial activity. Similarly Nagaraj *et al.*, (2012) carried out green synthesis of gold nanoparticles of *Caesalpinia pulcherrima* and evaluated it for antibacterial potential. In light of the above discussion we conclude that leaf, stem and root extract mediated AuNPs have strong efficacy against microbes and can be use to control microbial diseases in humans, animals as well as in plants.

Table 19. Antibacterial Activity of AuNPs of Leaf, Stem and Root Extract of *S. thea* and its AgNPs

Treatment s		Zone of Inhibition in millimeter (% of the standard)				
		<i>E. coli</i>	<i>Staph. aureus</i>	<i>B. pumilus</i>	<i>Psed. aeruginosa</i>	<i>Kleb. Pneumonia</i>
DMSO		-	-	-	-	-
AgNO ₃		9.1±0.7 (31.75)	10.7±0.7 (48.20)	7.6±0.5 (30.04)	12.1±0.4 (32.88)	10.3±0.6 (39.02)
Streptomycin		28.1±1.2 (100)	20.4±0.7 (100)	25.5±1.5 (100)	35.4±0.4 (100)	24.8±0.9 (100)
Leaf	Extract	16.7±0.4 (59.43)	7.1±0.3 (34.80)	15.6±0.3 (61.17)	12.8±0.2 (36.15)	13.3±0.3 (53.62)
	AuNPs	23.4±0.1 (83.27)	11±0.2 (53.92)	19.3±0.6 (75.68)	20.5±0.8 (59.59)	17.1±0.6 (68.95)
Stem	Extract	21±0.9 (53.38)	19.1±0.2 (49.59)	17.3±0.3 (63.92)	20.4±0.3 (35.02)	18.9±0.3 (60.08)
	AuNPs	24±0.6 (85.40)	23±0.4 (112.74)	22.0±0.3 (86.27)	21.5±0.3 (60.73)	20.3±0.4 (81.85)
Root	Extract	23.07±0.1 (82.09)	25.8±0.3 (126.47)	17.02±0.2 (66.74)	13.9±0.7(39.26)	19.1±0.3 (77.01)
	AuNPs	22.01±0.2 (78.32)	27.6±0.2 (135.29)	17.06±0.2 (66.90)	15.05±0.4(42.51)	21.02±0.3 (84.75)

4.10.2. Anti fungal Activity of Gold Nanoparticles

Nanotechnology is responsible for the production of plant extract mediated metallic nanoparticles. The synthesized nanopartciles have various applications but the most prominent application is antimicrobial activity against fungus, bacteria and virus. In current investigation antifungal effects of AuNPs was assessed on the basis of the zone inhibitions. The synthesized AuNPs showed antifungal activity using well well diffused method. The testing samples *Candida albicans*, *Aspergillus flavus*, *Aspergillus paraciticus*, *Fusarium solani* and *Aspergillus niger* displayed different zone inhibition. The maximum zone of inhibition was shown by leaf extract mediated AuNPs against *Aspergillus flavus* with (83.39±0.73) mm zone of inhibition followed by root extract mediated AgNPs against *Aspergillus niger* with (81.51±1.25 mm) zone inhibition. Stem extract mediated AuNPs showed highest activity against *Candida albicans* with (53.54±0.46 mm) zone of inhibition. Leaf extract AuNPs showed maximum inhibition against *Aspergillus flavus* while lowest inhibition against *Fusarium solani*, similarly stem extract AuNPs and root extract AuNPs displayed maximum inhibition against *Aspergillus niger* while lowest inhibition against *Fusarium solani* by stem AuNPs and against *Aspergillus paraciticus* by root AuNPs. We can

concluded that as compared to crude leaf extracts of leaf, stem and root of *S. thea* synthesized AuNPs of the same extract showed significant results as given in (Table. 18). DMSO is used as a standard in present reaserch work. Different plant extracts and its synthesized nanoparticles have been used against various fungal strains through different researchers like Jayaseelan *et al.*, (2013) worked on seed extracts of *Abelmoschus esculentus* for the synthesis of gold nanoparticles (AuNPs). The synthesized nanoparticles showed significant antifungal activity and concluded that the seed extract mediated gold nanoparticles of *Abelmoschus esculentus* can be used as an alternative of fungal drugs. We conclude from the above discussion that synthesized AuNPs of *S. thea* are capable of rendering high antifungal efficacy and can be used in preparation of drugs for in fungal diseases.

Table 20. Antifungal Activity of AuNPs using Leaf, Stem and Root Extract of *S. thea*

Treatments		Percent inhibition of mycelia growth				
		<i>Candida albicans</i>	<i>Aspergillus flavus</i>	<i>Aspergillus paraciticus</i>	<i>Fusarium solani</i>	<i>Aspergillus niger</i>
DMSO		-	-	-	-	-
Leaf	Extract	57.44±0.45	68.72±1.19	29.54±0.39	12.39±0.19	66.67±0.71
	AuNPs	63.54±0.27	83.39±0.73	42.06±0.91	28.51±0.31	55.27±1.34
Stem	Extract	40.09±0.65	32.67±0.52	9.22±0.22	-	39.44±0.37
	AuNPs	53.54±0.46	49.08±0.33	43.16±0.58	33.56±0.18	52.14±0.48
Root	Extract	43.68±0.52	59.38±0.62	68.90±1.57	47.10±0.31	52.36±1.22
	AuNPs	70.60±0.41	59.47±1.47	40.59±0.60	61.69±0.48	81.51±1.25

4.10.3. Anti-Promastigote Activity of Gold Nanoparticles of Leaf, Stem and Root of *S. thea*

Leishmaniasis is a group of tropical disease and one of the serious health problems worldwide. A disease caused by a number of species of protozoan parasites belonging to genus *Leishmania*. *Leishmania* follows a digenic life cycle, completed in two hosts. The flagellated extracellular promastigote form occurs in sand fly. The non flagellated and intramacrophagic form, amastigote occur in vertebrate host. Though both the amastigote and promastigote forms show differences, they do have similarities in their metabolic machinery and pathways. So the target against the first form could be relevant against the second one. In the current study the effect of various concentrations of AuNPs of leaf, stem and root extract of *S. thea* were evaluated against promastigote. The result shows different lethality rate at

different concentration. The lethality rate is dose dependent, as well as concentration of AuNPs increase the cell viability value decreases as shown in (Table 19). The biggest effect on metabolic activity of promastigote was seen at 150µg/ml for all three parts. There is variation in these parts the effect is also different against promastigotes. The leaf extract AuNPs shows 91.21% lethality at 150µg/ml followed by root extract AuNPs and stem extract AuNPs with 89.17 and 83.17% lethality rate respectively. The difference in effect is due to the accumulation of various secondary metabolites in different parts of the plant. Various researchers investigated the effect of AuNPs against bacterial and leishmanial cells. They concluded that AuNPs bind to sulfur and phosphate containing compounds and because of this property they can damage the bacterial cell wall by impairing sulfur containing protein. AuNPs damage sulfur containing enzymes that phosphorus containing DNA which leads to the inhibition of ATP synthesis. Another antileishmanial mechanism is the release of silver ions (Song *et al.*, 2006). These ions have the capacity of producing ROS, which the leishmania parasites are known to be very susceptible, because these ions form complexes with cysteine group of enzymes and prevent the enzymatic functions of protein. Similarly Butkus *et al.*, (2004) explored for the first time the synergistic effects of silver ions and UV light together on RNA virus they demonstrated that RNA viruses were not affected by UV light only but by silver ions and UV light giving rise to inactivation of viruses together. It is concluded that for the first time plant extract Ag-NPs were determined to possess antileishmanial effects against leishmanial parasites through examining their effects on various cellular parameters of promastigote. The results demonstrate that AuNPs synthesized by plant extract is the future alternative to the current antileishmanial drugs. Further due to budget constraints we were pushed to skip the *in vitro* assay against amastigotes.

Table 21. Anti-Promastigote Activity of AuNPs of Leaf, Stem and Root of *S. thea*

Concentrations (µg/ml)	% inhibition (mean)	IC ₅₀	IC ₇₀	IC ₉₀	Chi-square value
Leaf AuNPs	5	28.15	98.72	605.70	2.106
	10				
	20				
	50				
	100				
	150				
Stem AuNPs	5	32.00	84.13	340.32	6.95
	10				
	20				
	50				
	100				
	150				
Root AuNPs	5	19.81	55.39	244.99	5.22
	10				
	20				
	50				
	100				
	150				
DMSO (0.5%)	-	-	-	-	-

4.10.4. Cytotoxic Activity of Gold Nanoparticles

The discovery of new and efficient drugs against various cell diseases like cancer and skin disease etc are made by screening of active compounds from various parts of plants (Amara *et al.*, 2008). For detection of general toxicity of active compound derived from various parts of the plant, brine shrimps (*Artimia salina*) bioassay has been used for the last 30 years (Persoone and Wallis, 1987). In the present study the cytotoxic activity of crude ethanolic extract of leaf, stem and root mediated AuNPs of *S. thea* were evaluated. The percent mortality of crude extract was significant at 30µg/ml while the lowest mortality rate were recorded at 5µg/ml. The results showed that the lethality of shrimps was dose dependent with LD₅₀, LD₇₀ and LD₉₀ values. The comparative mortality rate of leaf crude extract and leaf extract mediated AuNPs revealed that AuNPs showed more mortality at 30µg/ml as compared to crude leaf extract. The leaf extract mediated AuNPs showed 73.3% mortality while crude leaf extract showed 40% death rate. Similar results were also recorded for stem and root crude extract and stem and root mediated AuNPs. Stem extract mediated AuNPs showed 70% mortality while root extract mediated AuNPs showed 66.7% mortality at

30µg/ml. Comparatively crude stem and root extract showed low lethality at 30µg/ml. The LD₅₀, LD₇₀ and LD₉₀ values for leaf extract mediated AuNPs are 15.35, 29.16 and 73.73 respectively with regression line $Y=2.77 + 1.88 X$ and chi-square value 0.72 (0.95). Similarly stem extract mediated AuNPs showed 19.24, 40.15 and 116.33 LD₅₀, LD₇₀ and LD₉₀ values respectively while root extract mediated AuNPs showed 21.25, 39.62 and 97.52 LD₅₀, LD₇₀ and LD₉₀ values respectively with $Y= 2.43+1.94X$ regression line and 7.42 (0.11) chi-square value as shown in (Table. 20). The results revealed that the effect of AgNPs is more toxic as compared to crude drug. Different scientists studied the effect of AgNPs against brain shrimps. Joshi *et al.*, (2012) reported the anticancer potential of chloroquine gold nanoparticles against MCF-7 breast cancer cells. Similarly Geetha *et al.*, (2013) disclosed the biosynthesizing capacity of *Couroupita guianensis* extract gold nanoparticles. The NPs were further investigated by disclosing the applications like MTT assay, DNA fragmentation, apoptosis by DAPI staining, and comet assay for DNA damage. From the above discussion it is concluded that AuNPs of *S. thea* have great effect against brine shrimps larva, as the response of shrimps larva is similar to that of common body cell, so we can use it as anti cancer and also for cell growth control to prevent cancer and tumor formation.

Table 22. Cytotoxic activity of Gold Nanoparticles from Leaves, Stem and Root of *S. thea*

Extract Conc. (µg/ml)	T. No. of Larvae	No. of survivors	% mortality	LD ₅₀	LD ₇₀	LD ₉₀	Regression Line	χ ² (p)
	Leaf ethanolic extract							
0	30	30	0.00	48.058	93.12	242.35	Y=1.93 +1.82 X	4.47 (0.34)
5	30	28	6.7					
10	30	27	10.0					
15	30	26	13.4					
20	30	24	20.0					
25	30	20	33.4					
30	30	18	40.0					
Gold Nanoparticles of Leaf Extract								

0	30	30	0.0	15.35	29.16	73.73	Y=2.77 +1.88 X	0.72 (0.95)
5	30	24	20.0					
10	30	20	33.3					
15	30	15	50.0					
20	30	13	56.7					
25	30	10	66.7					
30	30	08	73.3					
Root ethanolic extract								
0	30	30	0.00	69.75	182.56	733.86	Y=2.69 +1.25 X	2.39 (0.66)
5	30	27	10					
10	30	26	13.3					
15	30	25	16.6					
20	30	23	23.3					
25	30	21	30.0					
30	30	19	36.6					
Gold Nanoparticles of Root Extract								
0	30	30	0.0	21.25	39.62	97.52	Y= 2.43+1.94X	7.42 (0.11)
5	30	25	16.7					
10	30	23	23.3					
15	30	22	26.7					
20	30	14	53.3					
25	30	13	56.7					
30	30	10	66.7					
Stem ethanolic extract								
0	30	30	0.00	59.56	116.47	307.20	Y= 1.81+1.80X	2.17 (0.70)
5	30	29	3.33					
10	30	27	10.00					
15	30	27	10.00					
20	30	24	20.00					
25	30	23	23.33					
30	30	20	33.33					
Gold Nanoparticles of Stem Extract								
0	30	30	0.0	19.24	40.15	116.33	Y= 2.89+1.64X	8.25 (0.08)
5	30	23	23.3					
10	30	21	30.0					
15	30	21	30.0					
20	30	16	46.7					
25	30	11	63.3					
30	30	09	70.0					

4.10.5. Antioxidant activity of AuNPs of Leaf, Stem and Root extract of *S. thea*

Antioxidant bioactive compounds from plant sources are commercially promoted as nutraceutical and have been shown to reduce the incidence of diseases (Hermans *et al.*, 2007). 1, 1- diphenyl 2-picrylhydrazyl (DPPH) radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract. DPPH is a stable compound and it can be reduced by accepting electron. In the present investigation the reducing activity of AgNPs of leaf, stem and root extracts of *S. thea* were quantified spectrophotometrically by changing the color of DPPH from purple to yellow. In present

investigation the antioxidant potential of AuNPs of leaf, stem and root extracts of *S. thea* were evaluated which showed that scavenging effect increase with increasing concentration of AuNPs. Maximum scavenging activity was recorded at 300µg/ml while minimum scavenging activity was recorded at 50µg/ml. The results indicate that as compared to crude extract of all three parts of *S. thea*, synthesized AuNPs of leaf, stem and root extracts showed 81 % and 91% scavenging potential respectively at 300µg/ml (Fig.46). Leaf and root extract mediated AuNPs showed significant antioxidant potential as compared to stem extract mediated AuNPs at 518nm. UV-visible spectroscopy showed that increase in the concentration of test sample decrease the absorbance. The antioxidant potency is due to the presence of phytochemicals in different parts of the plant. The reduction of gold ion into gold nano sized particle is due to the presence of phenols and flavonoids in plant extract. These compounds are naturally present in plant and are strong antioxidants and have high reducing capacity (Pietta, 2000). The redox properties of phenols and flavonoids allow them to act as a reducing agent, singlet oxygen quenchers and hydrogen donors. The results revealed that AuNPs of root and leaf extract are highly antioxidant as compared to stem because flavonoids and phenol contents are mostly stored in these parts and capped on the surface of gold and reduce it.

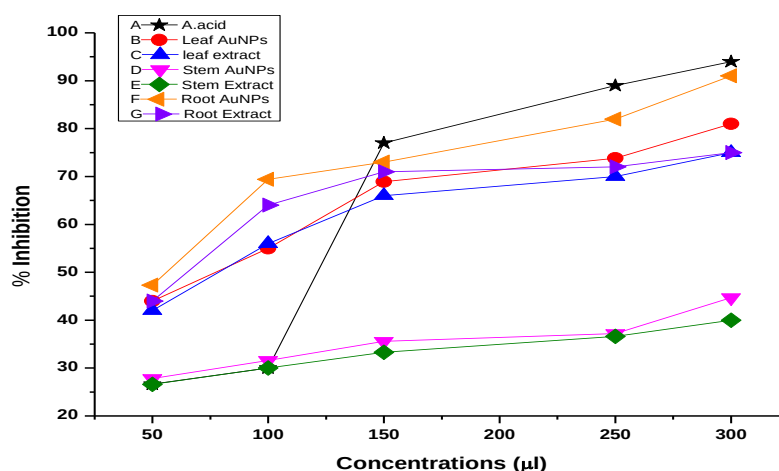


Figure 45. Antioxidant activity of AuNPs of Leaf, Stem and Root extract of *S. thea*. (A) Ascorbic acid showed maximum percent inhibition. (B, C) Leaf extract mediated AuNPs showed more inhibition as compared to crude extracts. (D,E) AuNPs of stem extract showed more antioxidant potential as compared to crude stem extract. (F, G) Similarly AuNPs of root extract showed more percent inhibition as compared to crude extract.

4.10.6. Analgesic Activity of Gold Nanoparticles

Pain is officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain is a disabling accompaniment of many medicinal conditions and pain control is one of the most important therapeutic priorities (Rang *et al.*, 2003). Analgesic is drug used to treat or reduce pain but some synthetic compounds have been developed and are associated with serious adverse effects such as gastrointestinal bleeding, ulceration, additive potential, drowsiness, respiratory distress, nausea etc (Mate *et al.*, 2008). Therefore there is a need to search a bioactive compound from natural products especially from medicinal plants used for alternative analgesics with little or no side effects. In current ethanolic extract of leaf of *S. thea* and its derived AuNPs were screened out for pain relieving potential in swiss albino mice using hot plate method. After administration of leaf extract and leaf extract mediated AuNPs the mean latency time (seconds) at different interval (0, 30, 60 and 90 Minutes) were recorded. After 30 min of saline administration, the Control group (Group I) showed mean latency time of 2.37 ± 0.11 seconds, whereas the Group II received Diclofenac sodium (standard) showed a significant average latency time of $7.11^{**} \pm 0.22$ seconds compared to the control condition. Both leaf extract and its derived AuNPs showed significant and dose dependent analgesic property as compared to control condition. After 30 min of drug administration maximum mean latency time $5.98^{**} \pm 0.41$ Sec was observed for leaf derived AuNPs at a dose of 300mg/kg of body weight followed by $5.29^{**} \pm 0.29$ Sec at 200mg/kg and $4.44^{*} \pm 0.20$ Sec at 100 mg/kg of body weight. Leaf extract mediated AuNPs showed 62.45%, 74.40% and 84.11% analgesia at 100, 200 and 300mg/kg respectively as compared to standard Diclofenac sodium. Significant results were observed after 60 min drugs administration for all doses of ethanolic leaf extract and its derived AuNPs in comparison to normal saline doses. Maximum latency time $8.92^{**} \pm 0.32$ was observed for Diclofenac sodium followed by $77.15^{**} \pm 0.39$ sec. at 300mg/kg, $6.77^{**} \pm 0.30$ sec at 200mg/kg representing 80.16% and 75.90% analgesia to the absolute Diclofenac sodium respectively as shown in (Table. 21). Similar response of mice in term of paw flick latency time was observed after 90 minutes of drugs administration. Generally synthesized gold nanoparticles using leaf extract showed more efficacy of analgesic effect as compared to ethanolic crude leaf extract. Many plants containing flavonoids have been shown to have analgesic, diuretic and anti-inflammatory actions (Okuda, 1962). Leaf extract of *S. thea* also contain flavonoids and phenols and have strong analgesic potential (Shah *et al.*, 2013).

Table 23. Analgesic potential of leaf extract of *S. thea* and its AuNPs

Dose (mg/kg,b.w)	Mean Latency time (seconds)±SEM						
	IFT (0 min)	30 min	60 min	90 min			
	Normal saline						
10 ml/kg	2.46±0.17	2.37±0.11	2.44±0.20	2.39±0.14			
	Diclofenac sodium						
10	2.43±0.13	7.11**±0.22	100.00	8.92**±0.32	100.00	12.09**±0.57	100.00
	Leaf extract						
100	2.34±0.18	4.21*±0.27	59.21	4.98**±0.17	55.83	5.87**±0.30	48.55
200	2.27±0.09	5.12**±0.27	72.01	6.16**±0.25	69.06	6.91**±0.29	57.15
300	2.22±0.14	5.75**±0.22	80.87	6.79 **±0.36	76.12	7.81**±0.31	64.60
	AuNPs from leaf extract						
100	2.28±0.21	4.44*±0.20	62.45	5.17**±0.19	57.96	6.90**±0.29	57.07
200	2.37±0.17	5.29**±0.29	74.40	6.77**±0.30	75.90	7.57**±0.38	62.61
300	2.37±0.19	5.98**±0.41	75.32	7.15 **±0.39	80.16	8.81**±0.29	72.87

4.10.7. Analgesic potential of Stem Extract of *S. thea* and its AuNPs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain relieving. However long term administration may induce gastro-intestinal ulcers, bleeding and renal disorders. Therefore researchers searched all over the world new analgesic drugs which lacking those effects. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects such as pain relieving (Gupta *et al.*, 2006). In present investigation analgesic property of crude stem extract and its mediated AuNPs were evaluated. After administration of stem extract and stem extract mediated AuNPs the mean latency time (seconds) at different interval (0. 30, 60 and 90 Minutes) were recorded. After

30 min of saline administration, the Control group (Group I) showed mean latency time of 2.37 ± 0.11 seconds, whereas the Group II received Diclofenac sodium (standard) showed a significant average latency time of $7.11^{**} \pm 0.22$ seconds compared to the control condition. Stem extract mediated AuNPs showed significant effect $5.87^{**} \pm 0.20$ sec at a dose of 300 mg/kg with 82.56% analgesia as compared to Diclofenac sodium. As compared to crude stem extract AuNPs showed significant effects at 200 and 100mg/kg with 62.03 and 57.52% analgesia respectively. After 60 minutes oral administration of stem extract mediated AuNPs and crude stem extract showed significant results. AuNPs at 300mg/kg showed $6.92^{**} \pm 0.38$ second latency rate for 60 minutes with 77.58% analgesia as compared to standard Diclofenac sodium, but as the concentration of doses reduced latency rate also reduced. Mean latency of AuNPs after 60 minutes at 200 mg/kg and 100 mg/kg showed $7.33^{**} \pm 0.27$ second and $4.91^{**} \pm 0.24$ second producing 82.17% and 55.04% respectively as compared to standard Diclofenac sodium. After 90 minutes the mice analgesic responses were observed as $12.09^{**} \pm 0.57$ second for declophenac sodium which is then followed by AuNPs of stem extract with $8.29^{**} \pm 0.35$ second at 200mg/kg body weight. As compared to crude root extracts at 300mg/kg AuNPs showed more analgesia with 65.18% as given in (Table 22). The current investigations showed that as compared to crude root extract AuNPs showed more analgesia. The significant pain reduction of both plant extract and AuNPs might be due to the presence of analgesic principles acting with prostaglandin pathways. It was found that the analgesic property of AuNPs is due to the presence of analgesic constituents present in stem extract. Stem extract of *S. thea* consist of alkaloids and flavonoids and these secondary metabolites have strong analgesic potent. Flavonoids are also known for triggering prostaglandins responsible for peripheral nociceptive perception (Fernanda *et al.*, 2002). So our present findings are in consistency with the previous findings.

Table 24. Analgesic potential of Stem Extract of *S. thea* and its AuNPs

Dose (mg/kg,b.w)	Mean Latency time (seconds)±SEM						
	IFT (0 min)	30 min	60 min	90 min			
	Normal saline						
10 ml/kg	2.46±0.17	2.37±0.11	2.44±0.20	2.39±0.14			
	Diclophenac sodium						
10	2.43±0.13	7.11**±0.22	100.00	8.92**±0.32	100.00	12.09**±0.57	100.00
	Stem extract						
100	2.33±0.11	3.89*±0.10	54.71	4.14*±0.18	46.41	5.31**±0.20	43.92
200	2.28±0.21	4.50**±0.28	63.29	5.17**±0.22	57.96	6.73**±0.30	55.67
300	2.37±0.08	5.18**±0.25	72.86	6.22 **±0.27	69.73	7.32**±0.31	60.55
	AuNPs from Stem extract						
100	2.25±0.27	4.09*±0.17	57.52	4.91**±0.24	55.04	5.53**±0.18	45.74
200	2.41±0.18	4.41**±0.51	62.03	7.33**±0.27	82.17	8.29**±0.35	68.57
300	2.30±0.14	5.87**±0.20	82.56	6.92**±0.38	77.58	7.88**±0.41	65.18

4.10.8. Analgesic potential of Root Extract of *S. thea* and its AuNPs

Analgesics are defined as the substances which decreases pain sensation by increasing pain threshold to external stimuli. Steroidal and non steroidal chemical is used as a analgesic. The uses of this steroidal drug as analgesic becoming highly controversial due to their multiple side effects. Therefore a need arises for the development of new analgesic drugs from natural sources with more powerful effects and less side effects as a substitute for chemical agents. Therefore natural plants are used as analgesic agents because plants are easily available and safe to handle. In our present investigation ethanolic root extract and its AuNPs is used for analgesic activity. After 30 minutes of administration of root extract mediated AuNPs showed latency rate 5.89**±0.19 and 5.22**±0.17 seconds at a dose of 300 mg/kg and 200 mg/kg followed by ethanolic root extract at a dose of 300mg/kg with 5.13**±0.22 seconds as compared to standard dichlophenac sodium. Significant result was recorded after 90 minutes of oral administration of crude root extract and its AuNPs. At 300mg/kg AuNPs showed 78.91% while crude root extract showed 70.06% analgesia followed by AuNPs with 7.84**±0.29 seconds analgesia at a dose of 200 mg/kg as compared to Diclofenac sodium with 12.09**±0.57 second latency rate as given in (Table 23). The analgesic nature of plant extract is due to the presence of alkaloids and flavonoids because flavonoids are responsible for peripheral nociceptive perception. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Ferdous *et al.*, 2008). The significant pain reduction of both the root

extract and AuNPs might be due to the presence of analgesic principles acting with the prostaglandin pathways.

Table 25. Analgesic potential of Root Extract of *S. thea* and its AuNPs

Dose (mg/kg,b.w)	Mean Latency time (seconds)±SEM						
	IFT (0 min)	30 min	60 min	90 min			
	Normal saline						
10 ml/kg	2.46±0.17	2.37±0.11	2.44±0.20	2.39±0.14			
	Diclofenac sodium						
10	2.43±0.13	7.11**±0.22	100.00	8.92**±0.32	100.00	12.09**±0.57	100.00
	Root extract						
100	2.39±0.09	4.11*±0.14	57.81	4.67**±0.21	52.35	6.19**±0.32	51.20
200	2.46±0.18	4.84**±0.21	68.07	5.62**±0.19	63.00	7.13**±0.20	58.97
300	2.28±0.12	5.13**±0.22	72.15	5.98**±0.32	67.04	8.47**±0.38	70.06
	AuNPs from Root extract						
100	2.35±0.09	4.19*±0.22	58.93	5.63**±0.27	63.12	6.94**±0.23	57.40
200	2.41±0.13	5.22**±0.17	73.42	5.88**±0.21	65.92	7.84**±0.29	64.85
300	2.29±0.14	5.89**±0.19	82.84	6.22**±0.42	69.73	9.54**±0.28	78.91

4.10.9. Larvicidal activity of Leaf, Stem and Root crude extract of *S. thea* and its AuNPs

Mosquitoes are important vectors of human diseases. It kills million of people every year especially in tropic areas. Control of mosquito's population is the only option to reduce mosquito causing diseases like malaria, filariasis, dengue and chikungunya. Different chemicals are used for mosquitoes control but it cause environmental pollution. So in the present research leaf crude extract and AuNPs of *S. thea* leaf were evaluated for larvicidal activity. The results indicate that synthesized AuNPs of leaf extract showed highest mortality at 10µg/ml which is 70.0% as compared to crude leaf extract which is 63.3%. The lowest mortality rate at 5µg/ml for AuNPs is 23.3% and 16.6% for crude leaf extract. The results indicate that the activity is dose dependent for AuNPs with LD₅₀, LD₇₀ and LD₉₀ values are 18.5, 42.06 and 136.6 respectively. While for crude extract LD₅₀, LD₇₀ and LD₉₀ values are 24.3, 50.7 and 136.9 respectively (Table 24). The larvicidal activity of stem and root extracts of *S. thea* and its AuNPs were also carried out using fourth instars larva of *Aedes aegypti*. The comparative study revealed that the lethal effect of AuNPs is more as compared to crude extract. The crude ethanolic stem extract showed 40% mortality while AuNPs showed 46.6

percent at 30 µg/ml as shown in (Table. 24) with $Y=1.20+3.05X$ of regression line and 3.80(0.03) of *chi*-square value. The percent inhibition rate of AuNPs of root extract also showed significant inhibition rate as compared to crude root extract. The LD₅₀, LD₇₀ and LD₉₀ values for root ethanolic extract are 66.6, 176.03 and 716.86 while for AuNPs of root extract it is 37.1, 116.05 and 603.58 respectively. The previous knowledge about the effect of silver nanoparticles and their larvicidal efficacy was studied by different researchers, like Ramos *et al.*, (2012) showed that the effects of latex from *C. procera* is highly toxic to the egg hatching and larvae of *A. aegypti*, Chandrashekhar *et al.*, (2012) studied the effect of silver nanoparticles of *Plumeria rubra* latex against *A. aegypti* and reported that the synthesized AuNPs were more toxic than crude extract of *Plumeria rubra*, similarly Soni and Prakash, (2012) synthesized gold nanoparticles (AuNPs) from a fungus *Aspergillus niger*. The larvicidal potential of these NPs was evaluated against three mosquito species *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* and reported that the fungal NPs have larvicidal potential.

Table 26.A. Larvicidal Activity of Leaf Root Extracts Mediated Gold Nanoparticles

Extract Conc. (µg/ml)	T. No. of Larvae	No. of survivors	% mortality	LD ₅₀	LD ₇₀	LD ₉₀	Regression Line	χ ² (p)
Leaf Ethanolic Extract								
0	30	30	0	24.3	50.7	136.9	Y=1.64+2.72	3.76 (0.05)
5	30	25	16.6					
10	30	23	23.3					
15	30	20	33.3					
20	30	18	40.0					
25	30	15	50.0					
30	30	11	63.3					
Gold Nanoparticles of Leaf Extract								
0	30	30	0	18.5	42.06	136.6	Y=1.48+3.12X	4.04 (0.04)
5	30	23	23.3					
10	30	20	33.3					
15	30	18	40.0					
20	30	16	46.6					
25	30	13	56.6					
30	30	09	70.0					

Table 27.B. Larvicidal Activity of Stem Extracts Mediated Gold Nanoparticles

Extract Conc. (µg/ml)	T. No. of Larvae	No. of survivors	% mortality	LD ₅₀	LD ₇₀	LD ₉₀	Regression Line	$\chi^2(p)$
Stem Ethanolic Extract								
0	30	30	0	47.6	112.9	393.5	$Y=1.39+2.65X$	3.58 (0.04)
5	30	27	10.0					

10	30	25	16.6					
15	30	24	20.0					
20	30	21	30.0					
25	30	19	36.6					
30	30	18	40.0					
Gold Nanoparticles of Stem Extract								
0	30	30	0	41.74	113.94	486.64	Y=1.20+3.05X	3.80 (0.03)
5	30	25	16.6					
10	30	24	20.0					
15	30	22	26.6					
20	30	21	30.0					
25	30	17	43.3					
30	30	16	46.6					

Table 28.C. Larvicidal Activity of Root Extracts Mediated Gold Nanoparticles

Extract Conc. (µg/ml)	T. No. of Larvae	No. of survivors	% mortality	LD ₅₀	LD ₇₀	LD ₉₀	Regression Line	χ ² (p)
Root Ethanolic Extract								
0	30	30	0	66.6	176.03	716.86	Y=1.24+2.73X	3.54 (0.03)
5	30	27	10					
10	30	26	13.3					
15	30	24	20.0					
20	30	23	23.3					
25	30	21	30.0					
30	30	19	36.6					
Gold Nanoparticles of Root Extract								
0	30	30	0	37.1	116.05	603.58	Y=1.05+3.33X	4.00 (0.03)
5	30	24	20					
10	30	22	26.6					
15	30	21	30.0					
20	30	19	36.6					
25	30	17	43.3					
30	30	15	50.0					

CONCLUSIONS

From the current study following conclusions have been derived.

- The macromorphological evaluation of the leaf, stem and root of *S. thea* were useful in species identification and separation from closely allied species.
- Leaf surface parameters like Vein islet & vein termination number, stomatal index and palisade ratio of adaxial and abaxial surfaces were determined which could be used as a marker for microscopical identification and standardization of drugs.
- Florescence analysis of powdered drugs with different chemical reagents in visible light and UV light imparts different fluorescence which provide basis for standardization of drug.
- Preliminary phytochemical analysis showed the presence of carbohydrates, proteins, fats, alkaloids, flavonoids, terpenoids, glycosides, saponins, phytosterol, anthocyanins in one or other parts of the tested plant.
- Elemental analysis showed the presence of different alkali metals and alkaline earth metals in selected plant parts.
- The synthesized nanoparticles were analysed by different characterization which revealed that the nanoparticles are nano in size and crystalline in nature.
- Both silver and gold nanoparticles showed significant antimicrobial activity as compared to crude extract.
- The antileishmanial potential of silver and gold nanoparticles revealed that the synthesized nanoparticles have an alternative potential of antileishmanial drugs.
- The cytotoxic and antioxidant bioassays displayed the effect of silver and gold nanoparticles for the control of cell growth and reduce production of Cancerian cells.
- The presence of flavonoids and phenols in *S. thea* enhanced its analgesic efficacy. The synthesized nanoparticles of leaf, stem and root of *S. thea* showed analgesic property.

➤ The larvicidal effects were also significant of synthesized nanoparticles, because of its nanosized structure can easily passed from biological membrane and disturb protein production in a cell.

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