

**PHARMACOGNOSTIC STUDY OF FIVE
MEDICINAL PLANTS OF FAMILY SOLANACEAE
FROM DISTRICT PESHAWAR, PAKISTAN**



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**PHARMACOGNOSTIC STUDY OF FIVE MEDICINAL PLANTS OF
FAMILY SOLANACEAE, FROM DISTRICT PESHAWAR,
PAKISTAN**

**The Thesis submitted to Department of Botany, University of Peshawar in the
partial fulfillment of the requirement for the degree of
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In
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In the Name of

Allah,

The Most Merciful and Compassionate the Most
Gracious and Beneficent Whose help and guidance we
always solicit at every step, at every moment.

DEDICATED TO

My Parents who prayed for my
success and teachers showed
me the light of the day.

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ISHFAQ HAMEED

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ABSTRACT
PHARMACOGNOSTIC STUDY OF FIVE MEDICINAL PLANTS OF FAMILY
SOLANACEAE FROM DISTRICT PESHAWAR, PAKISTAN
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The research is a multidimensional including pharmacognostic, phytochemical evaluations and pharmacological activities and was conducted during 2009 – 2011 on *Datura innoxia* Miller, *Solanum surattense* Burm.f., *Solanum nigrum* Linn, *Withania coagulans* (Stocks) Dunal and *Withania somnifera* Linn. The pharmacognostic studies showed that *Datura innoxia* root was slightly pungent, cylindrical and had fibrous fracture while stem was slight green with trichomes. Leaves were alternate, dentate and ovate and fruits were succulent and capsule. *Solanum nigrum* root had uneven fracture while stem was dark green with trichomes. Leaves were sinuate to irregular dentate and ovate and fruits were berry. *Solanum surattense* root had uneven & fibrous fracture while stem was prostrate, dark green, leaves were pinnatifid and elliptic oblong and fruits were berry. *Withania somnifera* root had fibrous fracture while stem was erect, shrubby, leaves were ovate, acute, entire and fruits were berry. *Withania coagulans* root had fibrous fracture while stem was erect, shrubby, leaves were ovate, obtuse, entire and fruits were berry.

The anatomical study of the root exhibited that the roots of these plants had epidermis, casparian strips, tracheae, medullary rays, vascular bundles, while the stem contained epidermis, hypodermis, vascular tissues, pith, medullary rays, endodermis, pericycle, cortical cells were present in all the five plants that were investigated and besides these idioblast cells were only present in the stem of *S. nigrum*. The vein islet number was highest (22.5 – 27.75) in *W. coagulans* and was lowest (11.5 – 16.5) in *D. innoxia*, while the vein termination number was highest (22.5 – 26.75) in *W. somnifera* and was lowest (11.75 – 17.5) in *W. coagulans* and the palisade ratio was highest (27.5 – 34.5) in *W. coagulans* and was lowest (10.75 – 18.5) in *S. nigrum*. The study reported variation in the structure and distribution of stomata in these plants and included types, frequency, density, stomatal index, size of guard cells & stomatal pore and percentage of the open and close stomata. In these five plants the upper epidermises contain anisocytic, anomotetracytic and amphianisocytic and the lower epidermises of these plants contain

paracytic, anomotetracytic, anisocytic and amphianisocytic. The statistical evaluation of the stomata and epidermis included mean, variance, standard deviation, coefficient of variance, standard error and 95% confidence interval for mean.

Alkaloids, saponins, proteins, fats, anthraquinone derivatives, cellulose, calcium oxalates were present in all parts of the plants species while mucilage, starch and tanins were present in some parts while absent in other parts of the same plants. ANOVA showed that ash and moisture contents was non significant between the plant parts and phenological stages. Crude protein was non significant between the plant parts and phenological stages except for *Datura innoxia* parts but not for its phenolglical stages. While crude fats were non significant between the plant parts and phonological stages except for *S. nigrum* and *S. surattense* parts but not for their phenolglical stages. Crude fiber was non significant between the plant parts and phonological stages except for *Datura innoxia* parts but not for its phenolglical stages. And carbohydrates was non significant between the plant parts and phenological stages except for the phenolglical stages of *S. surattense* and *W. coagulans*.

The mineral analysis showed the presence of Cr, Zn, Cu, Mn, Fe, Ca, K, Mg and Na in the roots, stems, leaves, flowers and fruits of the plants in three different phenological stages. Only the micro-minerals were present in traces while the macro-minerals were present high quantities as compared to the micro-minerals.

The methanolic extract of *D. innoxia* exhibited maximum activity against *Escherchia coli* and minimum activity against *Klebsella pneumoni*, *S. nigrum* showed maximum activity against *Pseudomonas aeruginosa* and minimum activity against *Staphylococcus aureus*, *S. surattense* showed maximum activity against *P. aeruginosa* and minimum activity against *Salmonella typhi*, *W. somnifera* exhibited maximum activity against *K. pneumoni* and minimum activity against *S. typhi*, *W. coagulans* exhibited maximum activity against *S. typhi* and minimum activity against *S. aureus*. The methanolic extract of the *D. innoxia*, *S. surattense* and *W. somnifera* showed maximum activity while in case of *S. nigrum* and *W. coagulans* the acetone extract exhibited maximum activity against the bacterial strains. As far as the antifungal activity the methanolic extract of *D. innoxia* exhibited maximum activity against *Aspergillus parasiticus* and low activity against *Fusarium solani*, *S. nigrum* showed maximum

activity against *A. parasiticus* and low activity against *A. flavus* and *Candida glabarata*, *S. surattense* showed maximum activity against *C. glabarata* and low activity against *F. solani*, *W. somnifera* exhibited maximum activity against *Candida albicans* and low activity against *C. glabarata* and *W. coagulans* showed maximum activity against *Aspergillus niger* and low activity against *A. flavus*. Overall the methanolic extract showed maximum activity as compared to the acetone extract. All the plants showed cytotoxicity to some extents. The LD₅₀ of the methanolic extract of *D. innoxia* was 94.86, *S. nigrum* was 93.73, *S. surattense* was 95.18, *W. somnifera* was 93.66 and *W. coagulans* was 94.86. LD₅₀ of the acetone extract of *D. innoxia* was 87.75, *S. nigrum* was 86.78, *S. surattense* was 74.31, *W. somnifera* was 86.56 and *W. coagulans* was 93.66. Both the methanolic and acetone extracts of the all the plants are phytotoxic in nature. But the toxicity varied with the change in concentration. The maximum toxicity (87.27%) was exhibited by the methanolic extract of *D. innoxia* at the concentration of 10 µg/ml and lowest toxicity (54.54%) was showed at the concentration of 1000 µg/ml by *W. coagulans*. The maximum toxicity (92.72%) was exhibited by the acetone extract of *S. nigrum* at the concentration of 10 µg/ml and lowest toxicity (54.54%) was showed at the concentration of 1000 µg/ml by *D. innoxia*, *S. nigrum* and *W. coagulans*. The maximum mortality was exhibited by methanolic extract of *D. innoxia* against *Callobrochus analis* and the minimum mortality was exhibited against *Rhyzopertha dominica*, *S. nigrum* showed maximum mortality against *Trochoderma granarium* and the minimum mortality against *R. dominica*. *S. surattense* exhibited maximum mortality against *Sitophilus oryzae* and the minimum mortality against *T. granarium*, *W. somnifera* showed maximum mortality against *R. dominica* and the minimum mortality against *C. analis* and *W. coagulans* exhibited maximum mortality against *C. analis* and the minimum mortality against *R. dominica*. Generally the methanolic extract showed the maximum mortality against these insects however, there were very few insects against which the acetone extract exhibited maximum mortality viz, *D. innoxia* against *Tribolium castaneum*, *S. nigrum* against *R. dominica*, *S. surattense* against *C. analis*.

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INTRODUCTION

Since time immemorial, humankind has used plants to cure diseases. The earliest mention of the medicinal use of the plants is found in Rig-Veda, which is one of the oldest repositories of human knowledge having been written between 4500 and 1600 B. C. In Ayurveda (2500-1600 B. C) definite properties and use of these drugs are mentioned. Chavaka (1000 B. C) has given remarkable description of materia - medica. The Assyrians, Babylonians and ancient Hebrews were all familiar with the use of these drugs. The Greek were also quite familiar with the use of these drugs as evidenced by the works of Aristotle, Hippocrates, Pythagoras and Carsia (Kumar, 2002).

Human desire for medicine has received the use of medicinal herbs. Medicinal plants continue to be used in medical practice based on a strong traditional belief in herbal medicine due to the limited availability and high cost of modern medicines, medical and pharmaceutical services (Shabbier *et al.*, 2004).

Pakistan has a varied climate and is quite rich in medicinal plants. These medicinal plants have been used by Hakims and in folklore medicines. Eighty percent of the population lives in villages and in rural areas and mostly depend on Unani system of medicines (Soomro *et al.*, 1997).

Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance (Reddy *et al.*, 1999; Venkateshet *et al.*, 2004). Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken.

Morphology and histology makes the first step to get knowledge about the diagnostic features, which are ascertained through the study of the tissue and their arrangement, cell wall and cell content (Youngken, 1950). Microscopy of the powder drug has the advantage of material. This study provides useful references not only for forensic scientists and for other engaged in the valuation of powder drug but also to analysts who are concerned with standardization and authentication of prepared drug material (Jackson & Snowdon, 1992).

Leaves can have a wide range of anatomical and morphological structures that partially isolate stomata from the turbulent atmosphere. Traditionally, such stomatal protection has been considered as an adaptation to dry climates (Raven *et al.*, 2005), with the suggestion that they increase the effective boundary layer, decreasing stomatal conductance and thereby reducing stomatal transpiration (Roth-Nebelsick, 2007). Scotland *et al.*, (2003) opinioned that careful and decisive anatomical studies of fewer morphological characters, in the framework of molecular phylogenies is productive to integrating the powers of morphological data with those of sequence information. Since the leaf epidermal studies are considered important in phylogeny and taxonomy (Taia, 2005), attention of plant taxonomists has been attracted toward the leaf epidermal anatomical studies to resolve the taxonomic problems (Hardin, 1979; Fang & Fan, 1993).

The stomatal and trichome studies provide efficient tool for separation and in some cases linking at least higher level of taxonomic hierarchy (Dilcher, 1974). The stomata allow gases exchange. In green leaves they occur either on both surfaces (amphistomatic leaf) or on only one, either the upper (epistomatic leaf) or more commonly on lower that is hypostomatic leaf (Perveen *et al.*, 2007). Due to inconsistency of stomatal type in different taxa, Pant & Kidwai (1964) and Sen & Hennipman (1981), however, did not feel so sure about the effectiveness of stomata as a tool in taxonomy. Zahur & Parveen (1982) and Siddiqi *et al.*, (1991) have utilized the stomatal characteristics in the study of taxonomic relationships in different families with some success.

Microchemical tests may provide considerable knowledge about the chemical constituents, which are responsible for the medicinal properties. Preliminary screening of

plant samples for alkaloids, saponins, triterpines and steroids is the usual initial step undertaken by a natural product chemist in the quest of novel or bioactive compound (Ahmed, 1995).

The nutrients essential for life are proteins, fat and carbohydrates, all contribute to caloric content of the dietary, minerals including trace elements, vitamins and water. Numerous studies including same in man have demonstrated clearly that life may be sustained by nutrient mixtures in which every component is definable chemically and soluble in water (Underwood, 1994). The quality and quantity of protein in the seed are basic factors important in the selection of plants for nutritive value systematic classification and plant improvement programs (Siddique, 1998).

When the deficiency is prevented or cured, some 20 or 30 trace elements which do not meet these exacting requirements occur more or less constantly in highly variable concentration in living tissues. They include Al, Sb, Hg, Cd, Ge, V, Si, Rb, Ag, Au, Pb, Bi, Ti and others. Such elements are believed to be acquired by the animal body as environmental contaminant and to reflect the contact of the organism with its environment (Goodwin & Mercer, 1998).

Impressive developments in the field of mineral elements have been taken place in the chemical, biochemical and immunological areas of research. Deficiency of trace elements in human subjects can occur under the most practical dietary conditions and in many diseased statuses. In recent years, scientists and nutritionists have started believing in the therapeutic role of metals in human health (Udayakumar & Begum, 2004).

Trace elements play both curative and preventive role in combating diseases. There is a vast scope to exploit the preventive medicinal aspects of various trace elements such as Cu, Cr, etc. Medicinal plants play the most important role in the traditional medicines. In most developing countries most of the flora remain virtually unexplored from the point of view of the medicinal utilizing through traditional Eastern system of medicines strongly upholds the use of these elements for curing many diseases (Kaneez *et al.*, 1998). Mineral elements though usually form a small portion of total composition of most plant materials and of total body weight; they are nevertheless of great

physiological importance particularly in the body metabolism. Their effects are related to concentration and recorded observation range from a deficiency state, to role as biological essential component to imbalance created when excess of one interferes with the function of another, to pharmacological activities (Bamiroet *al.*, 1995).

Globalization interfere with infectious disease control at the national level while microbes move freely around the world, unhindered by borders, human response to infectious diseases are conditioned by jurisdictional boundaries (Stepanovicet *al.*, 2003). According to W. H. O important progress has been made in controlling major infectious diseases. About 43 % of total deaths were occurred in developing countries due to the infectious diseases in recent years. The search for new effective antimicrobial agents is necessary due to the appearance of microbial resistance and occurrence of fatal opportunistic infections. In epidemic areas recurrent of infectious emerges resistance against antimicrobial agents (Carballoet *al.*, 2002).

Over the last few decades, a great interest has developed in searching for antimicrobial drugs from natural plant products. This interest primarily arises from the belief that drugs derived from plants are safe and dependable compared with synthetic drugs that may have adverse effects on host besides their high cost. Natural antimicrobials came from a wide array of sources including plants, animals and microorganisms (Gordon & David, 2001). Researchers have so far discovered approximately over 10,000 biologically active compounds of microbial origin (Shahidiet *al.*, 2004). Recently, many bacterial pathogens are becoming resistant to existing antibiotics due to their indiscriminate use in the treatment of infectious diseases (Davis, 1994; Service, 1995; Shears, 2000). Therefore, there is an exigency to discover new and efficient antimicrobials from other sources such as plants (Cordell, 2000; Karamanet *al.*, 2003; Raghavendraet *al.*, 2005).

There is need to discover fungitoxic compounds in views of the many plants and human fungal diseases. Some of the common plants fungal diseases are potato late blight, tobacco blue mould, hop dawy mildew, dutch elm disease, ergot of rye, cereal rusts, corn blight and actinomyces and histoplasmosis. The human fungal diseases include athlete's foot, aspergillosis and actinomyces, histoplasmosis and coccidiomycosis (Blank

&Rewbell, 1965). It was in the 1970s, however, when the use of systematic fungicides became widespread, that new isolates of numerous fungal plant pathogens appeared that were resistant to a fungicide that had previously been effective. The appearance of pathogen races resistant to chemicals prompted the development of new strategies in controlling plant diseases with fungicides and bactericides. Such strategies include the use of mixture of fungicides, alternating compounds in successive sprays, and spraying with a systematic compound in the early stages of the disease and with a broad spectrum compound in the latter stages of the disease (Agrios, 2000).

Pathogenesis caused by infectious agents begins as soon as the pathogen comes into contact with any portion of the susceptible plant. Most of the bacterial and fungal pathogens come into contact with their hosts accidentally in the form of wind-born spores. Some fungi, many bacteria and most of the viruses are carried to the host plants by insects, nematodes, and other vectors (Mehrotra&Aggarwal, 2005).

Bioactive compounds are often toxic to *Artemiasalina*(leach) shrimp larvae. The eggs of the brine shrimp *Artemiasalina* are readily available as fish food in pet shops (Kivack et al., 2001, Carballo et al., 2002). When placed in artificial seawater, the eggs hatch within 48 hours, providing large numbers of larvae. This is a rapid, inexpensive, general bioassay, which has been developed for screening, fractionation and monitoring of physiologically active natural products (Carballo et al., 2002, Dey&Herborne, 1991).

Members of the Family Lemnaceae are suitable organisms to investigate physiological processes and effects of different chemical substances. *Lemna* plants are miniature aquatic monocot consists of a central oval frond or mother frond with two attached daughter fronds and a filamentous root. Under normal conditions, the plants reproduce exponentially with budding of daughter fronds from pouches on the sides of the mother frond. Using the *Lemna* assay, it is observed that natural antitumour compounds can inhibit *Lemna* growth. In addition, it was also discovered that some substances stimulate frond proliferation, and the assay may be useful to detect new plant growth stimulants. The commercial need for such natural, biodegradable, herbicides and plant growth stimulants may someday be filled with natural products detected by the simple and convenient *Lemna* bioassay (Atta-ur-Rehman, 1991).

Entomologists throughout the world have long been aiming to devise efficient control measures against pest attack on store grains. Synthetic chemical pesticides have been used for many years for this purpose (Salem *et al.*, 2007). Recently interest has increased in developing plant origin insecticides as an alternative to chemically synthesized insecticides, as these are safe, effective, economical convenient to use and can replace the toxic synthetic fumigants (Mohiniet *al.*, 2008; Ayazet *al.*, 2008). Freedom from insect infestation and contamination has become an important consideration in storage of grain and to maintain high quality product (Coolins, 1998). Nearly one thousand species insect have been associated with store products through out the world, of which the majority belong to Coleopteran (60%) and Lepidoptera (8-9%) (Champ, 1981). Pesticides, including residual grain protectants and fumigants are used extensively in grain industry. Resistance to one or more of these materials has occurred in most major pest species. This relentless development of resistance is a serious threat to the future use of this material and consequently, there is an urgent need to develop economically safer and more sound pest control techniques (Tabassumet *al.*, 1997).

The pharmacognostic study includes collection, preservation, macroscopical, anatomical, vein islet number, vein termination number, palisade ratio, stomatal study, microscopic characters of powdered drug, nutritional and elemental analyses, microchemical tests and biological activities.

DESCRIPTION OF INVESTIGATED PLANTS

2.1 *Daturainnoxia* Miller.

(Synonyms: *Daturametelauctt.* non L. sensu Clarke)

Local names: Dathura, Bhatura (Pashto); Downy Datura (English); Nao Yang, Yang Chi Chu (Chinese); Metel De Los Arabes (Spanish); Talon, Punay (Philippines).

Taxonomic Description

Plant up to 130 cm tall, branched, dense pubescent-villous. Leaves broadly ovate, repand to sinuate-dentate, base oblique to cuneate. Calyx 5-lobed, slightly inflated toward the base; acuminate. Corolla broad triangular, acute. Capsule globose, dense spiny, nodding; spines acicular. Seeds reniform, compressed. *Fl. Per.*: May-October (Nasir & Ali, 1985).

Distribution

It is found in Central and Southern America, W. Indies, S. Western USA. Naturalised in the Mediterranean area, it is also found Afghanistan, India, Malaysia and Pakistan. In Pakistan it is from sea level to 1524m (Nasir & Ali, 1985).

Uses of *Daturainnoxia* Miller.

Leaves cure cough and asthma (Hussain *et al.*, 2006), treatment for swollen limbs (Khan *et al.*, 2009a), repellent and vermicide. Fruit is used to heat-up the buffaloes. Grind seeds cures scabies (Ajaib *et al.*, 2010) and are antipyretic and narcotic (Hazrat *et al.*, 2011). Extract of leaves is used for curing toothache, headache and epilepsy.

Due to its poisonous properties the plant is used in the preparation of drugs in Mexico, Africa, India and Australia (Mossa *et al.*, 1987; Chopra *et al.*, 1956; Watt, 1962). Smoke from the burning dried leaves is inhaled as a treatment for asthma (Singh *et al.*, 2002). For softening the boils green leaves of *Daturainnoxia* are used. Seeds and leaves are used for its narcotic action and anodyne. Aqueous extract causes dilation of pupil when locally applied. Internally, leaves juice is useful with curdled milk in gonorrhea and hydrophobia and flower juice is useful for earache (Shinwari & Khan, 2000).

Ramadan *et al.*, (2007) reported that seeds of *Datura innoxia* contained fatty acids and fat-soluble compounds which were linoleic acid, oleic, palmitic and stearic acids, stigmasterol, β -sitosterol, lanosterol, Δ^5 -avenasterol, sitostanol and tocopherol. Vernay *et al.*, (2008) reported the presence of proline contents and alkaloids production, including hyoscyamine and scopolamine in *Datura innoxia*. The aqueous extract of *Datura innoxia* leaves contains chemical compounds which have antidiarrhoeal properties. Due to these properties, the plant is used as antidiarrhoeal agent in the traditional medicine (Abbas & Jaafar, 2010).

2.2 *Solanum nigrum* L.

(Synonym: *Solanum rubrum* Mill.)

Local name: Mako, Kach-Mach, Makoya (Urdu); Kachmachu (Pashto); Black Nightshade, Common Nightshade (English); Ambusalap, Enabeddir (Arabic); Alpkraut, Berstelbeere (German); Ballerina, Morella, Solano (Italian); Kanperun (Sindhi).

Taxonomic Description

Perennial, branches and shoots subglabrous or pubescent. Leaves sinuate to irregularly dentate, glabrous to puberulous-pubescent. Calyx glabrous to pubescent. Corolla much exceeding the calyx in length. Filaments as long or shorter than the anthers, pilose. Stigma capitate. Ovary glabrous. Berry globose to subovoid. Seeds discoid, minutely (Nasir & Ali, 1985).

Distribution

It is cosmopolitan, being absent from the Arctic and subarctic region of the both hemisphere. In Pakistan it is found from sea level to 3450m (Nasir & Ali, 1985).

Uses of *Solanum nigrum* Linn.

The leaves are used in many diseases like including rheumatism and gouty joints, skin diseases, used in the treatment of tuberculosis, said to produce diaphoresis, cure for dropsy, nausea and nervous disorders. The decoction of the berries and flowers are useful in curing cough, erysipelas, cardiopathy, leprosy, haemorrhoids, nephropathy, ophthalmopathy, dropsy and general debility. Decoction of the plant depresses the CNS and reflexes of the spinal cord (Kirtikare *et al.*, 1988; Hussain *et al.*, 1992), narcotic,

antispasmodic, diuretic, and laxative (Evans, 2009). Warmed leaves are applied to painful and swollen testicles. Fresh juice added with the pulp of *Cassia fistula* is used as gargle for diphtheria, tonsillitis and inflammation of the tongue. Extract from leaves is given orally for treating jaundice and inflammation of the liver (Qureshiet *al.*, 2010).Decoction of leaves, 50 ml per day, is used orally to relieve liver and skin diseases. Fruits are edible and are also used to treat eye diseases. Decoction of the seeds, one litre per day, is given orally to mothers after childbirth for reshaping the body. For this, 50 g of seed is boiled in 2 l of water until only 50% water remains (Singh *et al.*, 2002).

Fruit are cooked (Hedrick, 1972; Polunin& Huxley, 1987; Reid, 1977). Used in preserves, jams and pies (Facciola, 1990). A pleasant musky taste (Harrington, 1967). Somewhat like a tomato, but much less pleasant, it improves slightly after a frost. Only the fully ripe fruits should be used, the unripe fruits contain the toxin solanine (Frohne&Pfänder, 1984; Crowe, 1990; Facciola, 1990). The fruit contains about 2.5% protein, 0.6% fat, 5.6% carbohydrate, 1.2% ash (Reid, 1977). The fruit is about 9mm in diameter (Huxley, 1992). Young leaves and new shoots - raw or cooked as a potherb or added to soups (Hedrick, 1972; Harrington, 1967; Polunin& Huxley, 1987; Crowe, 1990; Reid, 1977; Facciola, 1990).

The whole plant is antiperiodic, antiphlogistic, diaphoretic, diuretic, emollient, febrifuge, narcotic, purgative and sedative (Grieve, 1984; Lust, 1983; Singh &Kachroo, 1976; Emboden, 1979; Duke &Ayensu, 1985). It is harvested in the autumn when both flowers and fruit are upon the plant, and is dried for later use (Grieve, 1984). Use with caution (Lust, 1983). The leaves, stems and roots are used externally as a poultice, wash etc in the treatment of cancerous sores, boils, leucoderma and wounds (Duke &Ayensu, 1985; Moerman, 1998). Extracts of the plant are analgesic, antispasmodic, anti-inflammatory and vasodilator (Duke &Ayensu, 1985). The plant has been used in the manufacture of locally analgesic ointments and the juice of the fruit has been used as an analgesic for toothaches (Chiej, 1984).

This species has been found to be effective in removing PCB's from the soil and detoxifying them. The plant is more effective in doing this if it is infected with the bacterial parasite *Agrobacterium tumefaciens*(Anderson, 1997).

Lee & Lim (2006) isolated new glycoprotein (150 KDa) from *Solanumnigrum* which consist carbohydrate content (69.74%) and protein content (30.26%) which contain more than 50% hydrophobic aminoacids such as glycine and proline. An *et al.* (2006b) phytochemically investigated whole plant of *Solanumnigrum* and reported that the plant contains alkaloids, flavonoids, tannins, saponins, glycosides, proteins, carbohydrates, coumarins & phytosterols. It has been found that *Solanumnigrum* contains the substances, such as total alkaloid. Mbagwuet *al.*, (2007a) reported the anatomical interspecific similarities and differences in the roots of *Solanummacrocarpum* and *Solanumnigrum*. The similarities in structures were due to genus features while the differences in root anatomical structures were basis of species separation. Mbagwuet *al.*, (2007b) compared the leaf epidermal features of *Solanummacrocarpum* and *Solanumnigrum*. They stated that upper and lower epidermal cell wall of *S. nigrum* varied in shape i.e. pentagonal, rectangular to hexagonal and in the *S. macrocarpum* they were irregular in shape. Stomata are more abundant at the lower surfaces than upper leaf surfaces in the leaves of *Solanummacrocarpum* and *Solanumnigrum*. Stomata were anomocytic type with stomatal index ranging from 5.70%-33.02% in *S. nigrum* and 10.14% - 32.35% in *S. macrocarpum*. Dhellotet *al.*, (2006) reported that *Solanumnigrum* seeds have high lipid contents. Their protein contents and minerals elements (Mg being prominent) are considerable and *Solanumnigrum* oil is an important source of linoleic acid. Mohy-Ud-Din *et al.*, (2010) reported that the comparison of alkaloidal components among the 5 taxa of *Solanumnigrum*. Qualitative comparison showed significant distances among *Solanumchenopodioides* and *Solanumvillosum* as well as in *Solanumamericanum* and *Solanumnigrum* and indicated them as distinct species. But *Solanumretroflexum* did not show such a marked difference and hence might be regarded as a variety or subspecies of *S. nigrum*.

Akubugwoet *al.*, (2007) reported that the nutritional and phytochemical potential of the leaves and seeds of *Solanumnigrum* L. *Var virginicum*. They indicated that protein content of the leaves and seed as 24.90% and 17.63% respectively. Other findings were ash 10.18% and 8.05%, crude fibre, 6.81% and 6.29 and carbohydrate, 53.51 and 55.85% for the leaves and seed respectively. Mineral analysis revealed the order

Mg>K>Ca>Fe>Na>Mn>Zn in the leaves and Mg>K>Fe>Ca>Na>Mn>Zn in the seeds. Phosphorus and sulphur levels were 75.22 and 8.55 mg/100g in the leaves and 62.50 and 14.48, g/100g in the seeds. Vitamin content indicated the order vit C>vit B₂>Folic acid>Vit E>Vit A in both the leaves and seeds. Phytochemical analysis revealed high oxalate, phenol but low sterol content in the studied plant materials. Cyanide levels were higher in the leaves compared to the seeds. These results suggest that *S. nigrum* L. *Varvirginicum* to be nutritive despite the presence of some anti-nutritive components like oxalate.

2.3 *Solanum surattense* Burm. f.

(Synonyms: *Solanum diffusum* Roxb.; *Solanum jacquini* Willd.; *Solanum xanthocarpum* Schrad. & Wendl.)

Local names: Kundiari, Momoli, Mokri, Bhakkatiya, Kotilla (Urdu); Maragunay (Pushto); Badanjankare (Arabic); Aderayjadenay (Sindhi); Katai, Kataikhuadda, Katelia (Hindi); Batkateya, Mamoli, Katela, Pilak (Punjabi).

Taxonomic Description

Prostrate, diffuse prickly herb. Stem and branches glabrous to stellate pubescent. Leaves elliptic-oblong, sinuate to deeply lobed, lobes unequal, obtuse or acute, often toothed. Flowers cymes. Calyx lobes 5 mm long, acute, prickly. Corolla limb ovate-triangular. Anthers elongated. Berry globose. Seeds discoid. *Fl. Per.*: Mostly throughout the year (Nasir & Ali, 1985).

Distribution

It is found N. Africa, S. and S. E. Asia, Australia, Polynesia and Pakistan. In Pakistan it is from sea level to 1300m (Nasir & Ali, 1985).

Uses of *Solanum surattense* Burm. f.

The fruits are anthelmintic, antipyretic, laxative, anti-inflammatory, anti-asthmatic and aphrodisiac (Kiritikaret *al.*, 1988). Roots cures phlegmatic cough, asthma, chest pain. Fruits cure bronchial asthma, headache and migraine. Juice of the leaves cures joint's pain. Powdered ripened fruits cure cough and asthma (Qureshiet *al.*, 2010). Roots used as expectorant. Seeds are used as blood purifier and improve blood level

(Mananet *al.*, 2007). Fresh stems cure fever, cough and ingestion. It is also grazed by camels. Leaves are cooked as vegetable (Abbasi& Khan, 2010). The berries are used for toothache, demulcent, expectorant. The berries are boiled and the extract is applied to broken organs. The roots are used in cough, asthma, and catarrh. The plant is given in various animal diseases to cattle (Shinwari& Khan, 2000).

It is more commonly used in the diseases like cough, bronchial asthma, worms etc. The fruit is used for seminal ejaculation, alleviate itching, worms, fever and reduce fats. The whole plant including of fruits and roots is used for medicinal purpose. The powder form with oils is mixed well and used externally to alleviate nasal disorders. And also, nasal administration is beneficial in migraine, asthma and headache. The dried fruits are smoked in the form of cigarette and the smoke held up in the mouth cavity for some time ameliorates the dental infections. The fumigation is helpful in piles. The paste applied on swollen and painful joints in arthritis, reduces the pain and swelling effectively. The powder form of the fruits with honey relieves chronic cough in children. Juice extracted from the fruit is used to treat sore throats and the vapours of the burning seeds have been used to relieve toothache (Gunaselviet *al.*, 2010).

The fruits are known for several medicinal uses like anthelmintic, antipyretic, laxative, anti-inflammatory, antiasthmatic and aphrodisiac activities (Kiritikar& Basu, 1994). The stem, flowers and fruits are prescribed for relief in burning sensation in the feet accompanied by vesicular eruptions (Chopra *et al.*, 1956). The hot aqueous extract of dried fruits is used for treating cough, fever and heart diseases (Saaved, 1963). The fruit paste is applied externally to the affected area for treating pimples and swellings (Jain & Puri, 1984). The fruits are reported to contain several steroidal alkaloids like solanacarpine (Gupta & Dutt, 1938), solanacarpidine, solancarpine, solasonine (Saaved, 1963) and solamargine (Siddiquiet *al.*, 1983). Other constituents like caffeic acid (Siddiquiet *al.*, 1983) coumarins like aesculetin and aesculin (Tupkariet *al.*, 1972), steroids carpesterol, diosgenin, campesterol, daucosterol and triterpenes like cycloartanol and cycloartenol were reported from the fruits (Sato & Latham, 1953; Kusanoet *al.*, 1973). The antispasmodic, antitumor, cardiogenic, hypotensive, antianaphylactic and cytotoxic activities were also reported (Gupta *et al.*, 1967; Dharet *al.*, 1968; Smitet *al.*, 1995). S.

xanthocarpumis non toxic and has been reported to be safe for human use (Govindanet *al.*, 2004) was already in use and is clinically safe to consume. The whole plant is useful in vitiated conditions of vata and kapha, helminthiasis, dental caries, inflammations, flatulence, constipation, dyspepsia, anorexia, leprosy, skin diseases, hypertension, fever, cough, asthma, bronchitis, hiccough, lumbago, haemorrhoids and epilepsy. The plant is bitter, acrid, thermogenic, anthelmintic, anti-inflammatory, digestive, carminative, appetizer, stomachic, febrifuge, expectorant, laxative, stimulant, diuretic, rejuvenating, emmenagogue and aphrodisiac (Siddiquiet *al.*, 1983).

A glucoalkaloid termed solanocarpine is found in the fruits. A sterol known ascarpesterol and solanocarpidine are also present. Potassium nitrate, a fatty acid, a resinous and phenolic substance, diosgenin and sitosterol are present. Dry fruits contain traces of isochlorogenic, neochlorogenic, chlorogenic and caffeic acids. Solasodine, solasonine, solamargine and solamargine, quercetin isolated together with apigenin and sitosterol, flavanoids like quercitrin and apigenin glycosides (Gunaselvi *et al.*, 2010).

2.4 *Withaniasomnifera* (L.) Dunal.

(Synonyms: *Physalissomnifera* L.)

Local name: Aksan, Ak, Asgand, Asgandnagori (Punjabi); Kutilal (Pushto); Asgandanagaori (Urdu); Kaknajehindi (Persian); Ashvagandha, Balaji, Balada, Kala (Sanskrit), Kaknajehindi (Arabic); Ashvaganda, Asvagandha (Bengali).

Taxonomic Description

A suffruticose shrub. Branches ascending. Shoots stellate-tomentose. Leaves elliptic-ovate to broadly ovate, acute. Flowers sessile to subsessile, greenish-yellow, stellately hairy, becoming glabrescent and membranous. Corolla triangular, tomentose to the outside. Anthers subincluded. Berry globose. Seeds sub pyriform to reniform. *Fl. Per.*: Mostly throughout the year (Nasir & Ali, 1985).

Distribution

It is found in Carnay Island, Mediterranean, Africa, Iraq, S. Iran, Syria, Turkey, Palestine, Arabia, India and Pakistan. In Pakistan it is found from sea level to 2300m (Nasir& Ali, 1985).

Uses of *Withaniasomnifera* Linn.

It has been used for curing libido, anxiety, inflammation, Parkinson's disease, cognitive and neurological disorders and sedation (Ilayperumaet *al.*, 2002). It is aphrodisiac and rejuvenative (Williamson, 2002), counteracts chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotency, premature ageing, and emaciation, debility, and muscle tension. The leaves are used for the treatment of tumors, inflammation, psoriasis, bronchitis, asthma, ulcer, scabies, insomnia, hypnosis, alcoholism, anathematic and hepatoprotective (Karet *al.*, 2010), an antihelmantic, fever, tuberculosis glands, tumors. Fruits are applied to treat tumors and tubercular glands, carbuncles and ulcers (Kapoor, 2001). The tubers are tonic, useful in inflammations, bronchitis, asthma, consumption and ulcers, aphrodisiac, leucoderma, lumbago arthritis, favors conception (Ahmad, 2007). Kaure *al.*, (2007) reported that medicinal value of *Withaniasomnifera* extends from anti-inflammatory, antiseptic, anti-stress, anti-arthritic, anti-rheumatic, anti-bronchitis, immuno- and appetite- stimulator to promote overall memory, rejuvenation, vitality, endurance, stamina and longevity.

2.5 *Withaniacoagulans* (Stocks) Dunal.

(Synonyms: *Puneeriacoagulans* Stocks)

Local name: Panirband, panir (Sindi); Akri, Kakanaj (Urdu); Khamazora, Shappranga (Pushto); Javzulmizaja, Kaknajehindi (Arabic); Ashvaganda (Bengali); Cheese-maker, Indian Rennet, Vegetable Rennet (English); Khamjaria, Kutilana, Panir (Punjabi).

Taxonomic Description

Branched small shrub. Shoots rigid, stellate-tomentose. Leaves elliptic-ovate to elliptic-lanceolate, obtuse or acute, flowers bisexual, calyx cupular-campanulate. Corolla campanulate, stellate-tomentose outside, yellowish. Stamens included. Stigma subexserted. Male flowers: stamens $\pm$ subexserted. Female flowers: stamens included. Style glabrous. Berry globose. Seeds reniform, minutely rugose-reticulate. *Fl. Per.*: Jan.-April (Nasir & Ali, 1985).

Distribution

It is found in Iran, Afghanistan, India and Pakistan. In Pakistan it is found from sea level to 1700m (Nasir and Ali, 1985).

Uses of *Withaniacoagulans* (Stock) Dunal.

It is useful in curing chronic complaints of liver, asthma and biliousness. The twigs are used to clean teeth and the smoke of the plant is inhaled to relieve toothache. Leaves are used as vegetable and fodder for livestock (Nadkarni, 1976; Kirtikaret *al.*, 1988). Dried fruit powder is used for curing dyspepsia, stomachache and milk coagulant (Hussainet *al.*, 2006), stomach ulcer and poultice (Waziret *al.*, 2007), for purification of blood, gastric trouble and face pimples. Seeds are also chewed for purification of blood (Tareenet *al.*, 2010). Fruits are carminative, depurative, used for dyspepsia, flatulence and strange. The properties are attributed to the pulp and husk of the berry. The berries contain a milk- co-agulating enzyme, esterase, free amino acids, fatty oil, an essential oil and alkaloids (Khare, 2007). The milk- coagulating activity is due to the presence of an enzyme, under optimum conditions (Atal & Sethi 1963).

Different withanolides, withacoagin and coagulan reported from *W. coagulans*. Withaferin A (Steroidal lactones of withanolide series) had been isolated from fruits of *W. coagulans* (Khare, 2007). Prasad *et al.*, (2010) reported total phenolic, tannin, flavonoids and flavonols from fruits of *Withaniacoagulans*. The hydro alcoholic extract of *W. coagulans* berry showed significant anti-inflammatory activity in carragenin induced rat paw oedema model (Rajurkaret *al.*, 2001). Lalsare and Chutervedi (2010) reported

that various extracts of *W. coagulans* fruits have anti-inflammatory activities. Khodaei *et al.*, (2012) reported that the extract of *W. coagulans* has the potential to inhibit thymidine incorporation and antiproliferative activity. The aqueous extract of *W. coagulans* was studied for its anti-cytotoxic effect. The extract showed remarkable DMSO (Dimethyl sulfoxide) inhibitory activity which was induced to produce cytotoxicity and decreased the TNF- α production in chicken Lymphocyte (Chattopadhyay *et al.*, 2007). *W. coagulans* has been studied for possible spasmolytic and calcium channel blocking effects. Potassium chloride induced contractions also the *W. coagulans* crude extract possesses calcium channel blocking activity (Ali *et al.*, 2009). The extracted coagulin from fruits has antidyslipidemic effect on mice (Maurya *et al.*, 2008). Hoda *et al.*, (2010) showed the aqueous and chloroform extracts of the fruits decreased triglyceride, total cholesterol, LDL and VLDL increased the HDL levels. The volatile oil from the fruits showed antibacterial activity against *Staphylococcus aureus* and *Vibrio cholerae* (Choudhary *et al.*, 1995, Khan *et al.*, 1993). Also antibacterial properties have been demonstrated for isolated withanolides from ethanolic extract of the leaves (Gai and Budhiraja 1967). Two withanolides (14,15 β -epoxywithanolide I [(20S,22R) 17 β ,20 β -dihydroxy-14 β , 15 β -epoxy-1-oxo-witha-3,5,24-trienolide] and 17 β -hydroxywithanolide K (20S,22R) 14 α ,17 β ,20 β -trihydroxy-1-oxo-witha-2,5,24-trienolide]) have been isolated from the whole plant. The second compound was found to be active against a number of potentially pathogenic fungi (Choudhary *et al.*, 1995). Extracted coagulin L from fruits also showed significant drop of a fasting blood glucose profile and improved the glucose tolerance of db/db mice (Maurya *et al.*, 2008). Siddiqui *et al.*, (1963) studies showed the extract of *Withania coagulans* is muscular relaxing in experimental animals and is also a hypotensive, respiratory stimulant.

Fruit extracts have antioxidant potential against several diseases such as ageing, arthero-sclerosis etc. which caused due to ROS (Mathure *et al.*, 2011). Dabheiliya *et al.*, (2010) investigation's supports using *W. coagulans* as the diuretic agent in traditional folklore medicine. A steam volatile oil of the petroleum ether extract has been found to possess lethal effect on earth-worms (Gai and Budhiraja, 1967). The aerial parts of *W. coagulans* have anthelmintics in ruminants (Jabbare *et al.*, 2006; Khare *et al.*, 2007).



Fig. 1. *Datura innoxia* Miller.



Fig. 2. *Datura innoxia* Miller. fruit.



Fig. 3. *Solanum nigrum* Linn.



Fig. 4. *Solanum surattense* Burm.f.



Fig. 5. *Withania somnifera* Linn.



Fig. 6. *Withania coagulans* (Stock) Dunal.

REVIEW OF LITERATURE

3.1 Macroscopical Features

Jegade *et al.*, (2005) investigated the macroscopical features of fresh leaf of *Mitracarpus villosus* and indicated the presence of simple leaf with opposite / decussate arrangement, lanceolate shape, cuneate base, entire margin, parallel venation. Yadav *et al.*, (2007) reported that *Chenopodium album* root is cylindrical, having numerous rootlets, unbranched, pale white in colour, fracture fibrous, surface is smooth without striations and is tasteless. Abere *et al.*, (2007) stated that the leaves of *Mitracarpus scaber* are simple with whorled arrangement, an entire margin with lanceolate shape, acute apex and base, with parallel venation. Vidhu *et al.*, (2007) reported that leaf of the *Solanum nigrum* is simple, 2.5 – 8.5 cm long and 2.5 cm wide, ovate, or oblong, sinuate, toothed or lobed, narrow at both ends, petiolate, thin and pubescent. Dorsal surface is green in colour but the ventral surface is slightly pale. Venation is reticulate. The odour is characteristics and the taste is bitter.

Nirmal *et al.*, (2009) while working pharmacognostic features of *Sesbania sesban* qualitative reported yellowish white coloured wood, cylindrical shape and bitter taste. Patil *et al.*, (2009) while investigating the pharmacognostic features of *Ficus bengalensis* reported that it is a fine odorless powder with pale green, with a slightly bitter taste.

Gautam *et al.*, (2010) while standardizaing pharmacognostic features of the *Toona ciliata* bark stated that the dry bark is grey to reddish-brown in colour, 200 mm in length, 20 to 60 mm in width and 2 to 3 mm in thickness outer surface brown coloured, strong odour, bitter taste, rough and hard, double quill and curved curvature. Mazumder *et al.*, (2010) stated macroscopical characters of the stems of *Berberis aristata* included size which was 15 - 20mm, shape was cylindrical, colour was light yellowish, odour was aromatic and taste was woody taste. Nayak & Patel (2010) reported that *Jatropha curcas* leaf was broadly ovate with cordate margin, palmate venation, apex was acute, surface was glabrous, colour was dark green adaxially, light green abaxially, taste was

characteristic, bitter and odour was none. Mahmud *et al.*, (2010) reported the organoleptic features of the *Holoptelea integrifolia*. They noted that it was green colour, odour; slightly aromatic; herbal taste, tingling and burning, external margin of leaf somewhat rough; venation particulate, sinuate; apex acute, base symmetrical, with curved small petiole, subrib alternate, lamina broad; the texture, rough, external marking: internal marking: not prominent; fracture; soft and fibrous; shape; somewhat broad, lanceolate.

Ismail *et al.*, (2011) reported that the bark of *Pistacia integerrima* is grayish-brown in color, thick and hard. The external surface has striations, fractures and is rough. The inner surface is slightly smooth, sticky and yellowish in color, attached with the wood. Nurit-Silva *et al.*, (2011) reported that the leaves of *Solanum torvum* were characteristically ovate to elliptic, with lobed margins, oblique at the base. Muhammad *et al.*, (2012) reported macroscopical features of *Viola betonicifolia*. Stem light green, glabrous or somewhat pubescent. Leaves dark green colour, lanceolate to oblong, margins entire or slightly serrate, linear stipules. Flowers violet, cleistogamous. Sepals 3.5 to 7 mm long, petals are violet 8.5 to 14 mm long, fruits pods, pale brown, many tiny blackish seeds in each pod. The taste of the plant is light spice and order is spice like.

3.2 Anatomical Features

Evans (2009) reported that the powder drug of *Datura stramonium* contains stomata with 3 neighboring cells, calcium oxalate in rosette aggregate; non-glandular hairs, few glandular hairs, tracheae, stem fragments with spiral tracheae, wood-fibers, collenchymatous cells and microcrystals.

Jegade *et al.*, (2005) reported that leaf of *Mitracarpus villosus* had calcium oxalate crystals, lignin and oil globules with palisade ratio of 4 – 7 and stomatal number of 13.5, vascular bundle and trichomes.

Abere *et al.*, (2007) observed in the leaves of *Mitracarpus scaber* thin and wavy anticlinal walls with numerous calcium oxalate crystals. Stomata arrangement was anomocytic with numerous covering trichomes on both surfaces. Perveen *et al.*, (2007) reported that *Melilotus indicus*, *Datura alba* and *Tribulus terrestris* have anomocytic

stomata while *C. procera* and *C. brachycarpa* have paracytic type of stomata. Yadav *et al.*, (2007) reported that the powdered drug of root of *Chenopodium album* had parenchyma cells, fibres, annular vessels, reticulate vessels and starch grains. Aliero *et al.*, (2007a) reported that leaves of *Solanum pseudocapsicum* were characterized by anisocytic stomata, short multicellular glandular trichomes and calcium oxalate crystals. Mbagwu *et al.*, (2007a) reported the anatomical interspecific similarities and differences in the roots of *Solanum macrocarpum* and *Solanum nigrum*. The similarities in structures were due to genus features while the differences in root anatomical structures were basis of species separation. Mbagwu *et al.*, (2007b) compared the leaf epidermal features of *Solanum macrocarpum* and *Solanum nigrum*. They stated that upper and lower epidermal cell wall of *S. nigrum* varied in shape i.e. pentagonal, rectangular to hexagonal and in the *S. macrocarpum* they were irregular in shape. Stomata are more abundant at the lower surfaces than upper leaf surfaces in the leaves of *Solanum macrocarpum* and *Solanum nigrum*. Stomata were anomocytic type with stomatal index ranging from 5.70%-33.02% in *S. nigrum* and 10.14% - 32.35% in *S. macrocarpum*.

Patel & Kumar (2008) reported stomatal number, stomatal index, vein islet number and vein termination of *Neolamarckia cadamba* leaf. Kumar *et al.*, (2008) reported the vein islet number, vein termination number and stomatal index of the *Portulaca oleracea*. Hameed *et al.*, (2008a) reported variation in the structure and distribution of stomata in *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Rheum australe*, *Persicaria maculosa* and *Polygonum plebejum*. The type of stomata, density, frequency, stomatal index, size of stomatal pore, size of guard cells and percentage of the close and open stomata varied among these species.

Patil *et al.*, (2009) stated that the powdered drug of *Ficus bengalensis* had trichomes, fibres, epidermal cells with anticlinal walls, calcium oxalate crystals, and spiral thickenings. Nirmal *et al.*, (2009) while working anatomical features of *Sesbania sesban* reported the presence of uni to biseriate medullary rays, pitted xylem fibers, big vessels in groups, xylem parenchyma, pith, calcium oxalate crystals, starch grains, lignified fibers. Abere *et al.*, (2009) reported the palisade ratio, stomatal number and stomatal index of the upper and lower surfaces, vein islet number and vein termination

number of *Dissotis rotundifolia*. Ahmad *et al.*, (2009) reported seven types of stomata in 36 dicot species. They found that amphianisocytic was the dominant. It was found in 12 species while staurocytic and diacytic were found in 7 and 6 species respectively. In 7 species two and in one species three different types of stomata were found. Putiyanan *et al.*, (2009) investigated that the microscopic characters of Fak khao's leaves and were compared to the leaf powders showing the upper and lower epidermis, trichome, collenchyma, palisade mesophyll, spongy mesophyll, stoma (guard cell) and vascular bundles. Krishnaveni & Sent (2009) reported that stomatal index of upper epidermis is 4.5/mm² and 16/mm² lower epidermis in the leaves of *Argyrea nervosa*.

Mazumder *et al.*, (2010) reported microscopical characters of the stems of *Berberis aristata* which is circular in outline central narrow pith surrounded by wide xylem with medullary rays, multilayered cork, cortex narrow, 2-3 stone cells, lignified fibres, phloem narrow. Xylem consists of vessels, tracheids and parenchyma. Simple starch grains and prismatic crystals of calcium oxalate are present. Daly & Gastaldo (2010) investigated that stomatal frequencies, stomatal densities and stomatal index of *Quercus rubra*. Nayak & Patel (2010) reported that *Jatropha curcas* leaf had single layer of epidermis on both the surface. Upper epidermis is covered by thin cuticle. Trichomes are very rare on both epidermises. Both the epidermis showed anomocytic stomata, a single layer of closely pack palisade cells. Midrib show 5-7 layered thick wall closely pack collenchyma on both surface, spongy mesophyll and vascular bundle. Gautam *et al.*, (2010) determine that the transverse section revealed the presences of periderm, cortex, sclerides, mednllary rays and phloem fiber in *Toona ciliata* bark. Hameed *et al.*, (2010) envisage the anatomical features of *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Rheum australe*, *Polygonum plebeium* and *Persicaria maculosa*. They showed that epidermis, parenchyma, collenchyma, sclerenchyma, endodermis, pericycle, xylem, phloem, pith, mesophyll cells and stone cells varied among these species.

Hameed & Hussain (2011) reported variation in the structure and distribution of stomata in *Datura innoxia*, *Solanum nigrum*, *Solanum surattense*, *Withania somnifera* and *Withania coagulans*. The type of stomata, density, frequency, stomatal index, size

of stomatal pore, size of guard cells and percentage of the close and open stomata varied among these species. Sher *et al.*, (2011b) reported epidermis, xylem, phloem, endodermis, pericycle, pith, cortex and parenchyma in the root and stem, vein islet number, vein termination number, palisade ratio and stomal index of the upper and lower epidermises in the leaves of *Convolvulus arvensis*. Khan *et al.*, (2011) reported epidermis, cortex, xylem, phloem, pericycle, endodermis, pith and parenchyma in the root and stem, vein islet number (14.56 - 23.57/mm²), vein termination number (44.65 - 47.25/mm²), palisade ratio (15.62 - 17.42/mm²), stomatal index (21.25 - 24.62 & 42.54 - 43.47) of the upper and lower epidermis in the leaves of *Amaranthus viridus*. Nurit-Silva *et al.*, (2011) worked on the anatomy of the leaves of *Solanum torvum*. They reported that leaf was amphistomatic with anisocytic and anomocytic stomata and the epidermal cells had anticlinal walls sinuate on the upper and wavy on the lower surface. The mesophyll was dorsiventral; the midrib had a central bicollateral vascular bundle. The stem showed external phloem, xylem, internal phloem and a parenchymatic pith. The root was axial and striate longitudinally and the secondary xylem was a massive cylinder. Dastagir *et al.*, (2011) while working stomatal morphology of *Alstonia scholaris* and *Bischofia javanica* reported two types of stomata including paracytic and anomocytic stomata were recognized. The leaves of *Alstonia scholaris* were hypostomatic while the leaves of *Bischofia javanica* were amphistomatic. The number of stomata was greater on lower epidermis in *Bischofia javanica* than in *Alstonia scholaris*. Mahmud *et al.*, (2010) reported that transverse section of leaf of *Holoptelea integrifolia* contained parasitic stomata with cuticle, upper epidermis, lower epidermis, upper epidermal trichome, upper palisade zone, spongy parenchyma, pericycle fibre, lower zone spongy parenchyma, xylem vessel and phloem vessel. Muhammad *et al.*, (2012) reported the microscopic study of *Viola betonicifolia* which the presence of epidermis, cortex, spongy parenchyma, anisocytic stomata, vascular bundles, parenchymatous cells, Ca-crystals, xylem vessels, unicellular trichome, cholenchymatous cell of stem, bast fiber, schlerenchyma fiber, palisade parenchyma, single schlerenchyma, lower epidermis with stomata, xylem and phloem of root, cholenchymatous cell, bast fibers, fibers, trichome and parenchymatous cells.

3.3 Chemical Composition

Ferrara *et al.*, (2001) analysed ten commercial tea brands for their mineral composition and their polyphenols and flavonoids contents. This study showed variation in mineral composition, polyphenols and flavonoids and are linked to different origins of the plant. Evans (2009) reported that *Datura stramonium* contains hyoscyamine, daturine, daturic acid, hyoscyamine, atropine scopolamine (hyoscine). Aliero *et al.*, (2005) reported that berries of *Solanum pseudocapsicum* had 25 chemical components. The major components were alkaloids (38.55%), hydrocarbons (22.18%), fatty acid (19.38%), alcohol (13.27%), and carboxylic acids derivatives (3.73%) and mineral like P and N. Dhellot *et al.*, (2006) reported that *Solanum nigrum* seeds have high lipid contents. Their protein contents and minerals elements (Mg being prominent) are considerable and *Solanum nigrum* oil is an important source of linoleic acid.

Magaji *et al.*, (2007) subjected methanolic extracts of the leaves, stem bark and root bark of *Securinega virosa* for preliminary phytochemical analysis and found alkaloids, tannins and saponins. While only the leaves extract contained anthraquinone glycosides. Audu *et al.*, (2007) observed the presence of flavonoids, anthraquinones, carbohydrate, glycoside, saponin steroid and tannin in the ethanol extract of the leave of *Lophira lanceolata* indicated. Abere *et al.*, (2007) reported the presence of lignin, starch, cellulose, mucilage and calcium oxalate crystals, of alkaloids, tannins and saponins in *Mitracarpus scaber*. Aliero *et al.*, (2007b) reported that the volatile components of *Solanum pseudocapsicum* roots had 41 compounds, representing 50% of the oil, were identified. The oil contains fatty acids, terpenoids, and aldehydes, hexadecanoic acid, 2-methoxy-3-isopropylpyrazine, and 15-methylhexadecanoic acid, β -elemene and δ -elemene. Ramadan *et al.*, (2007) reported that fatty acids and fat-soluble compounds in the seeds of *Datura stramonium*, *D. metel*, *D. tatula*, *D. innoxia* and *Hyoscyamus muticus* and *H. niger* which were linoleic acid, oleic, palmitic and stearic acids, stigmasterol, β -sitosterol, lanosterol, Δ^5 -avenasterol, sitostanol and tocopherol. Vernay *et al.*, (2008) reported the presence of proline content and alkaloids production, i.e. scopolamine and hyoscyamine, in *Datura innoxia*.

Patra *et al.*, (2009) showed the availability of alkaloids, steroids, proteins, flavonoids, fats and oils, tannins, mucilage and organic acids in the leaves of in the leaf of *Hygrophila spinosa*. Patil *et al.*, (2009) qualitatively observed that powder, ethanol extract, and aqueous extract of *Ficus bengalensis* had sterols, flavanoids, phenols, tannins, and saponins in large amounts whereas aromatic acids, carbohydrates, triterpenoids, gums, mucilage and volatile oils were absent in the leaf. Janapati *et al.*, (2009) showed the presence of alkaloids, flavonoids, flavanones, tannins, terpenoids, amino acids and carbohydrates in *Holostemma ada*.

Nayak & Patel (2010) observed the presence of alkaloids, flavonoids, saponins, tannins, phenolic compounds, steroids, terpenoids, carbohydrates, and protein & amino acid in *Jatropha curcus* leaves. Mazumder *et al.*, (2010) investigated that the stems of *Berberis aristata* contained starch grains and calcium oxalate crystal, alkaloids, amino acids, flavonoids, phenol, proteins, sterols / terpenes, reducing sugars, non-reducing sugars and tannins.

Shrivastava & Leelavathi (2010) stated that preliminary qualitative chemical test revealed the presence of glycosides, carbohydrates, phytosterols / triterpenoids, saponins, fixed oils & fats and phenolic / tannins in *Catunaregum spinosa*. Preliminary phytochemical analysis of *Ocimum sanctum*, *Hyptis suaveolens*, *Croton tiglium*, *Physalis minima*, *Tephrosia villosa*, *Malachra capitata*, *Cleome viscosa* and *Galphimia glauca* revealed that the presence of alkaloids, flavonoids, tannins, phlobatannin, terpenoid, saponin, in their aqueous leaf extracts (Koche *et al.*, 2010). Hussain *et al.*, (2010c) evaluated *Solanum melongena*, *Trianthema portulacastrum*, *Abelmoschus esculentus*, *Spinacia oleracea*, *Praecitrullus fistulosus*, *Luffa acutangula*, *Cucurbita moschata* and *Cucumis sativus* for their nutritional values. Highest carbohydrate contents were found in *C. Moschata*. In case of protein content, *S. oleracea* and *T. portulacastrum* had higher amount. Mohy-Ud-Din *et al.*, (2010) reported that the comparison of alkaloidal components among the 5 taxa of *Solanum nigrum*. Qualitative comparison showed significant distances among *Solanum chenopodioides* and *Solanum villosum* as well as in *Solanum americanum* and *Solanum nigrum* and indicated them as distinct species. But *Solanum retroflexum* did not show such a marked difference and

hence might be regarded as a variety or subspecies of *S. nigrum*. Araújo *et al.*, (2010) reported alkaloids, tannins and saponins from the *Solanum lycocarpum* fruit. Prasad *et al.*, (2010) reported total phenolic, tannin, flavonoids and flavonols from fruits of *Withania coagulans*. Bamgboye & Adejumo (2010) determined physicochemical properties of oil from Roselle seed such as free fatty acid, 0.435-2.300 and 0.510-3.311 for fine and coarse samples, respectively. These values compared favourably with standard values, indicating that the oil extracted is edible. Ibrahim *et al.*, (2010) examined the ethanolic extracts and essential oils of nutmeg seeds and ginger roots for their physicochemical properties comparatively. Qualitative phytochemical screening of the ethanolic extracts of both species showed the presence of alkaloids, tannins, glycosides, saponins, steroids, flavonoids and terpenoids. Chirikova *et al.*, (2010) revealed that aerial parts of *Scutellaria baicalensis* had organic acids, free carbohydrates, polysaccharides, amino acids, alkaloids, flavonoids, phenolic acids, anthocyanins, tannins, lipids and triterpene compounds.

Kalyan *et al.*, (2011) stated that the ethanol extract of seeds of *Clitoria ternatea* contained sterols, alkaloids, glycosides, saponins, tannins, carbohydrates, proteins, phenolic compounds and flavonoids. Khan *et al.*, (2011) reported alkaloids, mucilage, saponins, anthraquinone derivatives, calcium oxalate, tannins, starch, fats, proteins, cellulose, lignin and cutin in root, stem, leaf and flower of *Amaranthus viridis*. Jain *et al.*, (2011) revealed the occurrence of alkaloids, tannins, cardiac glycosides saponins, moisture content and terpenoids in the leaves of *Zizyphus xylopyrus*. Nasreen & Radha (2011) reported steroids, flavons, alkaloids, carbohydrate, phenol, glycosides, proteins, resins, saponins, tannins, terpenoids, gum, and anthraquinone derivatives in the roots of *Withania somnifera*.

3.4 Proximate Composition

Lee & Lim (2006) isolated new glycoprotein (150 KDa) from *Solanum nigrum* which consist carbohydrate content (69.74%) and protein content (30.26%) which contain more than 50% hydrophobic aminoacids such as glycine and proline. An *et al.*, (2006b) phytochemically investigated that *Solanum nigrum* contained alkaloids, flavonoids, tannins, saponins, glycosides, proteins, carbohydrates, coumarins and phytosterols.

Nzikou *et al.*, (2007) reported that *Solanum nigrum* seeds had oil content, rich in protein and carbohydrate. Ash content was of 7.18%, moisture content was $3.86 \pm 0.97\%$. Akubugwo *et al.*, (2007) reported that the leaves and seeds of *Solanum nigrum* var *virginicum* had protein content of the leaves and seed as 24.90% and 17.63%, ash 10.18% and 8.05%, crude fibre 6.81% and 6.29 and carbohydrate 53.51 and 55.85% for the leaves and seed respectively. Akubugwo *et al.*, (2007) revealed that the order of mineral $Mg > K > Ca > Fe > Na > Mn > Zn$ in the leaves and $Mg > K > Fe > Ca > Na > Mn > Zn$ in the seeds *Solanum nigrum* var *virginicum* and had high oxalate. Bukhsh *et al.*, (2007) determined the nutritional value and trace element content in *Carthamus oxyacantha*, *Eruca sativa* and *Plantago ovata*. It was seen that crude proteins, total proteins in seeds and total carbohydrates in leaves are significantly higher in *E. sativa* as compared to *C. oxyacantha* and *P. ovata*. The amount of total fats is significantly higher in seeds of *C. oxyacantha* as compared to *E. sativa* and *P. ovata*. While the concentration of crude fibers were significantly higher in seeds of *P. ovata* as compared to the seeds and leaves of both *E. sativa* and *C. oxyacantha*. Major trace elements include Cu, Fe, Mg, Mn, Cr, Zn, Mo, P, K, Na and Ca.

Hameed *et al.*, (2008b) reported proximate composition of protein, crude fibers, fats and oils, moistures, ash contents and carbohydrates and different elements like C, O, Na, Mg, Al, Si, S, P, Cl, K, Ca, Ti, Fe and Br in some medicinal plants viz., *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Rheum australe*, *Persicaria maculosa* and *Polygonum plebeium* of the family Polygonaceae is presented. Hussain *et al.*, (2009) stated that crude powders and extracts of different parts of *Piper sarmentosum* had varied levels of moisture, total ash, acid insoluble ash, sulphated ash in water and methanol. Hameed & Dastagir (2009) reported the presence of moisture, ash, crude fiber, proteins, fats and oils, and carbohydrates in proximate percentage in *Rumex hastatus*, *Rumex dentatus* and *Rumex nepalensis*. Bano *et al.*, (2009) determined the protein and sugar in the leaves of *Galium aparine*, *Onobrychis dealbata* and *Polygonum alpinum*. *Onobrychis dealbata* showed the highest sugar and protein content. Sultan *et al.*, (2009) evaluated *Athyrium acrotiochoides*, *Chenopodium album*, *Polygonum amplexicaule*, *Atrimisia maritima*, *Oriosma lispidum*, *Cynoglossum lanceolatum*, *Plantago ovata*, *Hackalia*

macrophylla, *Lespedeza spp.*, *Urtica dioica* for dry matter, ash, crude protein, hemicellulose, and lignin contents.

Sultan *et al.*, (2010) determined the nutritive value of *Indigofera gerardiana*, *Myrsine africana*, *Impatiens bicolor* and *Adhatoda vasica*. Maximum crude protein (14.7%) was observed for *Marisine affricana* while, minimum (15.6%) was noted for *Impatiens bicolor* and *Adhatoda vasica*. Higher ash content (14.7%) was observed for *Myrsine africana*. Folarin & Igbon (2010) reported moisture, ash, crude protein, crude fiber, oils and carbohydrate, Na, Ca, Mg, Fe, Cu and Zn from *Enterolobium cyclocarpum* seed.

Chinedu *et al.*, (2011) reported proximate analysis of fresh fruits *Solanum aethiopicum* and *Solanum macrocarpon*. *S. aethiopicum* had (per 100 g) 89.27 ± 0.12 g moisture, 2.24 ± 0.03 g protein, 0.52 ± 0.04 g fat, 0.87 ± 0.03 g ash, 2.96 ± 0.08 g crude fiber, 4.14 ± 0.11 g carbohydrate and fruits of *S. macrocarpon* contained (per 100 g): 92.50 ± 0.14 g moisture, 1.33 ± 0.05 g protein, 0.17 ± 0.01 g fat, 0.47 ± 0.02 g ash, 1.11 ± 0.03 g crude fiber, 4.42 ± 0.12 g carbohydrate. Auta *et al.*, (2011) reported that heat treatment reduces crude protein, lipid and crude fibre, The results from phytochemical screening indicates that there is no much difference in raw and heat processed samples for 15 min. Heating *Solanum incanum* for 30 min was found to reduce flavonoid composition indicating that flavonoid is thermal sensitive. Bangash *et al.*, (2011) reported that vegetables namely pot purslane, spinach, turnip, garlic, mustard (sarson), radish, bitter gourd, lady finger, bath sponge and brinjal were analyzed for their proximate composition. The maximum content of moisture, carbohydrate, crude protein, crude fat, crude fiber, and ash recorded in these vegetables was (92.50 %, 26.88 %, 5.0%, 0.40%, 1.4% and 1.9%), respectively, with a minimum content (66.80 %, 3.91 %, 0.7%, 0.08%, 0.4% and 0.38%), respectively.

3.5 Mineral Composition

Yusuf *et al.*, (2003) evaluated the levels of Cd, Cu and Ni in *Talinum triangulare*, *Celosia trigyna*, *Corchorus olitorus*, *Venomia amygdalina* and *Telfaria accidentalis*, and the soils in which they were grown. There were statistically significant differences between the levels of Cu and Ni in all the plants as compared to Cd.

Narendhirakannan *et al.* (2005) determined the elemental composition in the leaves of *Murraya koenigii*, *Mentha piperita*, *Ocimum sanctum*, and *Aegle marmelos*. The levels of Cu, Ni, Zn, K, and Na were found to be in trace amounts, whereas Fe, Cr, and V levels were found in marginal levels.

Ozcan (2005) determined major and minor mineral contents of young shoots, flower buds, fruits and seeds of *Capparis ovata*. All parts had amounts of Ca, K, Mg, Na, P, Pb, and Zn. Ba, Cd, Cr, Cu, Li, Ni, Pb, and Se contents were found to be very low.

Aremu *et al.*, (2006) detected Na, K, Ca, Mg, Mn, Cu, Zn, Fe and P in *Anacardium occidentale*. Demirezen *et al.*, (2006) evaluated Cu, Cd, Ni, Pb and Zn in cucumber, tomato, green pepper, lettuce, parsley, onion, bean, eggplant, peppermint, pumpkin and okra and the soils in which they were grown. The results also show that onion and peppermint had the ability to accumulate more Cd and Cu than the other vegetables. Okwu & Josiah (2006) when analyzed the chemical compositions of *Aspilia africana* and *Bryophyllum pinnatum* revealed the presence of alkaloids, saponins, flavonoids, phenols, tannins, ascorbic acid, riboflavin, thiamine and niacin. These herbs are good sources of minerals such as Ca, P, K, Mg, Na, Fe and Zn.

Aliero *et al.*, (2007a) reported Al, K, Na and Si in the leaves of *Solanum pseudocapsicum*. Rehman & Iqbal (2008) evaluated the mineral composition of *Prosopis juliflora*, *Abutilon indicum* and *Senna holosericea* and indicated the presence of Fe, Pb, Cu, Cr and Zn. The level of Cu and Cr was highest in the foliage of *S. holosericea*. The excessive Cu was recorded in foliage of *A. indicum*. The foliage of *S. holosericea* showed highest concentration of Zn.

Bangash *et al.*, (2011) reported that vegetables namely pot purslane, spinach, turnip, garlic, mustard (Sarson), radish, bitter gourd, lady finger, bath sponge and brinjal were analyzed for their mineral contents. Maximum concentration of macro minerals K, Ca, Mg, and Na, observed was (400, 210, 109, and 55) mg/100 g, respectively, with a minimum concentration (26, 15, 17, and 10) mg/100 g, respectively. Maximum concentration of micro minerals Fe, Cu, Zn, Mn and Cr observed was (29, 0.33, 3.05, 1.70, and 0.36) mg/100 g with a minimum concentration (2, 0.05, 0.43, 0.21, and 0.06) mg/100 g, respectively.

3.6 BIOLOGICAL STUDIES

3.6.a Antimicrobial activity

Dabur *et al.*, (2004b) reported 2beta-1'-methylethyl pentanoate from the leaves of *Datura metel*, which showed antifungal activity at 87.5 microg/ml dose. Dabur *et al.*, (2004a) reported methanolic extracts of *Solanum xanthocarpum* and *Datura metel* inhibited the growth of *Aspergillus fumigatus*, *A. flavus* and *A. niger*. Phongpaichit *et al.*, (2004) demonstrated antifungal activities of crude methanol extracts from leaves of *Cassia alata*, *Cassia fistula* and *Cassia tora* for their antifungal activities against *Microsporum gypseum*, *Trichophyton rubrum* and *Penicillium marneffeii*. *Cassia alata* was the most effective against *T. rubrum* and *M. gypseum* whereas *C. fistula* extract was the most potent inhibitor of *P. marneffeii*. Cvetni & Vladimir-Kne (2004) tested the ethanolic extract of grapefruit (*Citrus paradisi*, *Rutaceae*) seed and pulp against 20 bacterial and 10 yeast strains. Ethanolic extract exhibited the strongest antimicrobial activity against *Salmonella enteritidis*.

Eftekhar *et al.*, (2005) reported that methanolic extracts of the aerial parts of the *Datura innoxia* and *Datura stramonium* were inhibitory to gram (+) bacteria. Little or no antibacterial activity was found against *Escherichia coli* and *Pseudomonas aeruginos*.

Koduru *et al.*, (2006) reported the methanolic extracts from fruits and the leaves of *Solanum aculeastrum* showed appreciable activity against bacteria. The water extracts showed the least activity against the bacteria. The methanol and acetone extracts exhibited significant activity against *Aspergillus flavus* and *Pencillium notatum*, and the water extract of the fruit significantly inhibited the growth of *P. notatum*. Latha & Kannabiran (2006) reported that aqueous, methanolic and n-butanol extracts of aerial parts of *Solanum trilobatum* had antimicrobial activity. Presence of tannins, saponins, flavanoides, phenolic compounds, cardiac glycosides and carbohydrates indicates in *S. trilobatum*, is one of the potential medicinal plant for therapeutic use. Bajwa *et al.*, (2006) investigated antifungal potential of *Cicer arietinum*, *Drechslera tetramera* and *Drechslera hawaiiensis* when tested against different concentrations of aqueous extracts of aerial parts of *C. arietinum*, the crude water extract showed most significant antifungal activity even at lower concentration of 5%. Wansi *et al.* (2006) isolated two alkaloid

derivative, oriciacridone A and B from the stem bark of *Oriciopsis glaberrima* (Rutaceae) which exhibited *in vitro* significant antimicrobial activity against a range of micro-organisms. Moolla *et al.*, (2007) studied antimicrobial activities of the extracts obtained from 17 indigenous *Agathosma* species. *Agathosma ovata* (round-leaf) displayed the best activity against *S. aureus* and *B. cereus* with MIC values of 0.16 mg/ml and 0.13 mg/ml, respectively. Satish *et al.*, (2007) tested aqueous extract of fifty-two plants from different families for their antifungal activity against eight species of *Aspergillus*. Of these, aqueous extract of 12 plants including *Datura stramonium* had significant antifungal activity against one or the other *Aspergillus* species tested. *A. flavus* was highly susceptible. Hoque *et al.*, (2007) stated that *Psidium guajava* and *Azadirachta indica* showed higher antimicrobial activity against these against 21 strains except for *V. parahaemolyticus*, *P. aeruginosa*, and *A. hydrophila*. None of the extracts showed antimicrobial activity against *E. coli* and *Salmonella enteritidis*. Mandal *et al.*, (2007) observed that steam distillation extract of *Hyptis suaveolens* leaves exhibited broad-spectrum antimicrobial activity against the tested organisms. It showed highest antifungal and antibacterial activity against *Aspergillus niger* and *Micrococcus luteus*, respectively.

Essien *et al.*, (2008) observed that the essential oils of *Citrus medica* exhibited a wide spectrum of fungitoxicity, inhibiting all 14 tested fungal species. Parekh & Chanda (2008) conducted that the methanolic extract of 9 Indian medicinal plants had antifungal activity against yeasts. *Aspergillus flavus* was the most susceptible fungal strain while *Candida glabrata* was the most resistant one. *Saussurea lappa* showed the best antifungal activity. Segismundo *et al.* (2010) stated *Gouania javanica* leaves when tested for antifungal assay against the fungal strains like *Candida albicans*, *Trichophyton mentagrophytes* and *Aspergillus niger* were significant inhibitory. Hadizadeh *et al.*, (2009) stated that essential oils are one of the most promising candidate groups of natural compounds for the development of safer antifungal agents. Essential oils extracted from five plant species were evaluated against *A. alternata* and to control *Alternaria* rot of stored tomatoes. Nwinyi *et al.*, (2009) screened aqueous and ethanol extracts of *O. gratissimum* and *P. guineense* leaves for antibacterial activity against *Escherichia coli*

and *Staphylococcus aureus*. Both extracts exhibited selective inhibition against the isolates. Ethanol extracts showed more inhibitory effect compared to the aqueous extracts. Kirbaşlar *et al.*, (2009) observed that peel oils of *Citrus* fruits showed strong antimicrobial activity against the test organisms. Lemon and bergamot peel oils have a little higher activity than the other *Citrus* peel oils.

Ruttoh *et al.*, (2009) tested the methanol, hexane, dichloromethane and ethyl acetate extracts of leaves, fruits, roots and stem barks of *Tabernaemontana stapfiana* for antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Microsporum gypseum* and *Trichophyton mentagrophytes*. The extracts of the different plant parts showed varied activities against the tested fungi with the stem and root barks showing significant antifungal activities. Hoskeri *et al.*, (2010) tested bactericidal activity of crude extracts from lichen *Ramalina pacifica* against 20 strains including *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhi*, *Salmonella paratyphi*, *Escherichia coli*, and *Staphylococcus aureus*. The extracts exhibited predominant antibacterial activity against all the multi-resistant strains with significant zone of inhibition. Ali *et al.*, (2010) tested hydroxychavicol, isolated from the chloroform extraction of the aqueous leaf extract of *Piper betle* for its antifungal activity against 124 strains. Hydroxychavicol exhibited inhibitory effect on fungal species of clinical significance. Hussain *et al.*, (2010a) reported the antibacterial and antifungal activities of the crude extract of *Polygonum persicaria*, *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Polygonum plebeium* and *Rheum australe*. It was seen that *C. frundii*, *E. coli*, *E. aerogenes* and *S. aureus* were the most susceptible bacterial species. Among the tested fungal species *F. solani*, *A. flavus* and *A. niger* were more susceptible. Apu *et al.*, (2010) reported that the crude methanolic and its different fractions (*n*-hexane, carbon tetrachloride and chloroform, aqueous) extract of *Dillenia indica* leaves has antimicrobial activities. *n*-hexane, carbon tetrachloride and chloroform fractions showed moderate antimicrobial activity. But the aqueous fraction was found to be insensitive to microbial growth. Hussain *et al.*, (2011b) reported the antibacterial activity of aqueous methanolic extracts of 10 plants against *Pasteurella multocida*, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Corynebacterium bovis*. All the bacteria were susceptible to

different plant extracts. *Lawsonia inermis*, *Embellia ribes* and *Santalum album* showed antibacterial activity against all the tested bacteria. The extract of *Santalum album* showed maximum antibacterial activity of the 10 plant extracts used. *Bacillus cereus* and *Pasteurella multocida* were the most sensitive bacteria against most of the plant extracts.

3.6.b Cytotoxic activity

Khan & Khan (2007) observed that methanol fraction of *Rhazya stricta* showed significant cytotoxicity against brine shrimp lethality with LC_{50} 17.809 μ g/ml, having mortality rate 73.33 % at highest dose. While pet-ether, chloroform and carbon tetrachloride possessed moderate to low cytotoxicity with their LC_{50} values. Tolulop (2007) working on the cytotoxicity of aqueous-methanolic extract of *Hibiscus sabdariffa* found it to be potent against brine shrimps with LC_{50} value of 55.1 ppm. anticancer agents from *Hibiscus sabdariffa*. The present results support the use of this plant as anti cancer agent. Koduru *et al.*, (2007) worked on isolation of two steroid glycosides (tomatidine & solasodine) from *Solanum aculeastrum* and their cytotoxic effects. It was reported that the compounds showed very low apoptotic activity. Saeed *et al.*, (2010) evaluated the cytotoxicity of the aerial parts of the *Polygonatum verticillatum*. Ethyl acetate fraction showed no cytotoxicity. There was no sign of brine shrimp cytotoxicity except in the chloroform fraction (LD_{50} was 1205.07 μ g/mL). Hussain *et al.* (2010b) reported the cytotoxicity of the crude methanolic extracts of *Rumex hastatus*, *R. dentatus*, *R. nepalensis*, *Rheum australe*, *Polygonum persicaria* and *P. plebeium* (Family *Polygonaceae*) was determined against *Artemia salina* at 1000, 100 and 10 mg/ml. *Rumex hastatus*, *Rumex dentatus* and *Rumex nepalensis* showed significant activity at a concentration of 1000 mg/ml against *Artemia salina*. *Rheum australe* showed low activity at 1000 mg/ml and no activity at 100 and 10 mg/ml. At concentration of 10 mg/ml, *Rheum australe* showed no activity. Nisar *et al.*, (2010) reported that crude methanolic extracts of *Impatiens bicolor* and its different fraction namely n-hexane, dichloromethane, ethyl acetate, n-butanol and aqueous were tested *In vitro* for cytotoxic activities. Cytotoxic results observed were also very low as compared to standard used and only dichloromethane fraction showed cytotoxicity at higher dose while all other fractions as well as crude extract exhibited moderate to low activity in killing the tested

brine shrimps. Ngoumfo (2010) chemically investigated the roots and fruits of *Zanthoxylum leprieurii* against *Artemia salina* lethality bioassay and found modest cytotoxicity with LD₅₀ at 13.1microg/mL. Isolated compounds were found to be moderately cytotoxic with IC₅₀ values ranging from 27 to 77mM. Apu *et al.*, (2010) reported that the crude methanolic extract of *Dillenia indica* leaves and its different fractions exhibited cytotoxic activity against *Artemia salina*. Compared to vincristine sulfate, *n*-hexane and chloroform fractions demonstrated a significant cytotoxic activity. Syahmi *et al.*, (2010) reported the brine shrimp lethality 50% lethal concentration (LC₅₀) values of more than 1.0 mg/mL of methanolic extract of *Elaeis guineensis*. Maximum mortalities occurred at 100 mg/mL concentration while the least mortalities happened to be at 0.195 mg/mL concentration. Bulbul *et al.*, (2011) observed cytotoxic activities of the *n*-hexane, chloroform and ethyl acetate extracts of leaves of *Luffa cylindrica* and *Luffa acutangula* with considerable general toxicity towards brine shrimps. Qureshi *et al.*, (2011) reported that the toxicity of plant or microbial material is considered as the presence of antitumor compounds. They studied culture filtrates of 51 fungal isolates, belonging to 15 genera viz., *Alternaria*, *Aspergillus*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Drechslera*, *Fusarium*, *Macrophomina*, *Memmoniella*, *Myrothecium*, *Paecilomyces*, *Penicillium*, *Rhizoctonia*, *Trichoderma* and *Verticillium* (*Pochonia*) isolated from rhizosphere and rhizoplane of cultivated and wild plants showed significant toxicity on brine shrimp.

3.6.c Phytotoxic activity

Javaid *et al.*, (2008) reported the phytotoxic activity of aqueous and methanolic and *n*-hexane extracts of shoots and roots of *Datura metel* against *Phalaris minor*. Aqueous shoot extracts were proved highly toxic to plant biomass where as organic solvent extract concentrations significantly reduced biomass and it was concluded that root and shoot extracts of *D. metel* contain herbicidal constituents. Khan *et al.*, (2008) reported the phytotoxic activity of aqueous extracts of *Eucalyptus camaldulensis* on the germination (%) and seedling growth (fresh and dry weight) against wheat. It was noted that aqueous extracts had inhibitory effect on wheat germination, fresh and dry weight and was found significantly higher than control treatment. Zaidi *et al.*, (2008) studied the

methanolic extract of *Arceuthobium oxycedri* and showed that it is extremely phytotoxic for *Lemna* species.

Javaid *et al.*, (2009) reported that aqueous extracts of *Withania somnifera* and *Datura alba* against *Rumex dentatus*. Aqueous extracts of roots and shoots of both species suppressed in germination and seedling growth of weed species. Application of aqueous extracts caused 68% reduction in germination, 62% in shoot length, 96% in root length and 68% in seedling biomass. Fatima *et al.*, (2009) reported that methanol extracts of leaf and stem of *Rumex dentatus* inhibited root length more than the hexane extracts. The *Rumex dentatus* methanol extract showed presence of alkaloids, saponins, anthraquinones and tannins while flavonoids were also found in both methanol as well as hexane extract. Aliferis *et al.*, (2009) worked on different herbicides like mesotrione, norflurazone, paraquate and pyrenophorol against *Lemna minor* to detect phytotoxicity of these compounds but they did not reported any toxic macroscopic symptoms. Ateeq-ur-Rehman *et al.*, (2009) while working on the methanolic extract of *Thymus serpyllum* (Labiatae) for phytotoxicity against *Lemna minor* reported significant results ($P < 0.05$).

Gilani *et al.*, (2010) screen out 81 medicinal plants of Khyber Pakhtunkhwa, Pakistan in which *Seriphidium kurramense*, *Andrachne cordifolia* and *Rhazya stricta* were found strongly phytotoxic as compared to the other. Hussain *et al.*, (2010b) reported the phytotoxicity of the crude methanolic extracts of *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Rheum australe*, *Polygonum persicaria* and *Polygonum plebeium* was determined against *Lemna minor*. All the plants, except *R. hastatus*, showed significant activity at a concentration of 1000 mg/ml. Moderate activity was shown by *R. australe*, *R. nepalensis* and *P. persicaria* at the concentration of 100 mg/ml. All the plants showed low phytotoxic activity at concentration of 10 mg/ml. Knox *et al.*, (2010) reported that *Cassia occidentalis*, *Rumex dentatus*, *Calotropis procera* and *Withania somnifera* were inhibitory against *Parthenium hysterophorus*. The activity depended on the synergistic effect of 3rd and 9th day aqueous shoot leachates of above mentioned weeds. Nisar *et al.*, (2010) reported that crude methanolic extracts of *Impatiens bicolor* and its different fraction namely n-hexane (A), dichloromethane (B), ethyl acetate (C), n-butanol (D) and aqueous (E) indicated significantly phytotoxicity against *Lemna minor*. Ayatollahi *et al.*, (2010) investigated that the chloroform fraction of *Euphorbia aellenii* showed significant phytotoxic activity. Onocha & Ali (2010) reported that the methanolic extract of *Phyllanthus muellerianus* was significantly phytotoxic at a concentration of 1000 µg/ml

against *Lemna minor*. They showed that the antitumor compound can inhibit the growth of *Lemna minor*.

Hussain *et al.*, (2011a) reported phytotoxicity of natural rain leachates from shoots and root exudates of *Cenchrus ciliaris* and *Bothriochloa pertusa* against *Brassica campestris*, *Lactuca sativa*, *Setaria italica* and *Pennisetum americanum*. The rain leachates and root exudates had differential toxicity against the *Brassica campestris*, *Lactuca sativa*, *Setaria italica* and *Pennisetum americanum*. Low concentration of inhibitors were either non inhibitory or were stimulatory to the germination and / or growth of tested species. Dzoyem *et al.*, (2011) studied *in-vitro* phytotoxic activity of the methanol extract, fractions and isolated compounds from the stem bark of *Diospyros canaliculata* and showed phytotoxicity similar to standard inhibitor, paraquate. Nisar *et al.*, (2011) proved that crude methanolic extract and various fractions of *Zizyphus oxyphylla* stem were strongly phytotoxic at higher doses which was tested against *Lemna minor*. Ahmad *et al.*, (2011) worked on the ethyl acetate fraction of *Zizyphus jujuba* and reported that it is moderately phytotoxic against *Lemna minor* at 1000 µg/ml as compared to the other extract which showed low phytotoxic activity. Sher *et al.*, (2011a) reported the phytotoxicity of *Populus euphratica* against some crop species which invariably retarded the plumule, radical growth, fresh and dry weight of *Sorghum vulgare*, *Setaria italica* and *Triticum aestivum*. The aqueous extracts obtained after 48 h were more inhibitory than 24 h. Leaves were more toxic than bark. It is suggested that the various assayed parts of *Populus euphratica* have strong phytotoxic potential at least against the tested species. Ehsan *et al.*, (2011) and Mubarak *et al.*, (2011) reported the phytotoxic effects of *Papaver pavoninum* against maize, brassica and two cultivars of wheat using aqueous extracts, litter and mulches from whole plant. The plant showed phytotoxic effect against all the tested species.

3.6.d Insecticidal activity

Jbilou *et al.*, (2006) studied the effects of methanolic extracts of *Peganum harmala*, *Ajuga iva*, *Aristolochia baetica* and *Raphanus raphanistrum* against *Tribolium castaneum*. All the plants extracts gave varied response against *Tribolium castaneum*. The development of the insect was also affected by these extracts. Srivastava & Gupta (2007) worked on the effect of different formulations viz., aqueous suspension, aqueous extract and ether extracts of different parts of plant *Solanum surratense* on

Callosobruchus chinensis and reported a significant insecticidal activity of above mention plant against *C. chinensis* and the plant can be used at least partially substituted as against synthetic pesticides. Oigiangbe *et al.*, (2007) checked the effect of the aqueous extracts of the leaf and stem bark of *Alstonia boonei* against *Sesamia calamistis*. The LC₅₀ values were calculated for each extract. Their results suggested that both the leaf and stem bark extracts of *Alstonia boonei* were toxic to *Sesamia calamistis* larvae and can be used in pest control formulations. Khan *et al.*, (2008) worked on the insecticidal activity of the ethanolic extract of *Aconitum leave*, *Trichodesma indicum* and *Sauromatum guttatum*. All the plant extracts showed good insecticidal activity. Arabi *et al.*, (2008) reported insecticidal activity of oil from *Perovskia abrotanoides* against *Sitophilus oryzae* and *Tribolium castaneum*. It was noticed that the essential oil of *P. abrotanoides* can play an important role in stored grain protection and reduce the need for the same, and also the risks associated with the use of synthetic insecticides. Madhi & Rehman (2008) reported the insecticidal potency of *Syzygium aromaticum*, *Piper nigrum*, *Cinnamomum zeylanicum*, *Amomum subulatum*, *Myristica fragnans*, *Nigella sativa*, *Curcuma longa*, *Capsicum frutescens*, *Cuminum cyminum*, *Elettaria cardamomum* and *Cinnamomum tamala*, against *Callosobruchus maculatus*. The results showed that all the spices were effective as protestants of black gram seeds. However, clove and black pepper were most effective. Boussaada *et al.*, (2008) worked on the insecticidal activity of sixteen aromatic plant extracts of Asteraceae family against adults and larvae of *Tribolium confusum*. For all extracts, mortality was higher for larvae than adults. It reached respectively 83%, 77% by using petroleum ether and methanol extracts of *R. acaule*. The results suggested that *M. duriaei* and *R. acaule* may be used in grain storage against insect pests. Yang *et al.*, (2009) isolated garlic essential oil and tested for its insecticidal activity against adult *Tribolium castaneum*. It was found that garlic essential oil had insecticidal activity against adult *Tribolium castaneum*.

Hussain *et al.*, (2010a) reported insecticidal activity of the crude extract of *Polygonum persicaria*, *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Polygonum plebeium* and *Rheum australe* have been studied against *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Callosobrochus analis* and *Trogoderma granarium*. The

crude extracts of *R. dentatus* and *R. nepalensis* show significant insecticidal activity against *S. oryzae*; *P. persicaria* and *P. plebejum* show significant insecticidal activities against *T. castaneum*, respectively. Nisar *et al.* (2010) reported that crude methanolic extracts of *Impatiens bicolor* and its different fraction namely n-hexane (A), dichloromethane (B), ethyl acetate (C), n-butanol (D) and aqueous (E) were tested *In vitro* for their insecticidal activities. Only (A) fraction showed moderate insecticidal activity while (C), (D) and (E) fractions indicated low insecticidal activity. Liu *et al.*, (2011) screened out new agrochemicals from local wild plants, essential oil of *Ostericum sieboldii* flowering aerial parts was found to possess strong insecticidal activity against *Tribolium castaneum* and *Sitophilus zeamais*. The essential oil also showed fumigant toxicity against *S. zeamais* and *T. castaneum* adults with LC₅₀ values of 27.39 mg/L air and 20.92 mg/L air, respectively.

AIMS AND OBJECTIVES

The review of literature on various aspects of macroscopical, anatomical features, chemical and pharmacological activities indicated that some work has been done on these five plants of Family Solanaceae. The aims of the present study were therefore to:

1. To study the macroscopical features of the plants.
2. To study the anatomical aspects.
3. To study the leaf constant parameters like vein islet numbers, vein termination numbers and palisade ratio.
4. To see the morphology and anatomy of stomata.
5. To know the microscopic features of powdered drug.
6. To analyze the proximate and elemental compositions.
7. To know the microchemical parameters.
8. To demonstrate the biological activities like antibacterial, antifungal, cytotoxic, phyto-toxic and insecticidal activities.

Chapter 4

MATERIALS AND METHODS

4.1 Collection and Preservation

Fresh mature specimens of *Datura innoxia* Miller, *Solanum nigrum* Linn, *Solanum surattense* Burm. f, *Withania somnifera* L. and *Withania coagulans* (Stocks) Dunal were collected from Peshawar and its surrounding during 2009 - 2011. The plant materials were washed with water, separated and dried in shade for 15 days. These materials were used subsequently for pharmacognostic, chemical, anatomical, nutritional analysis, elemental analysis and biological activities. The plant material was powdered and meshed through 60nm and were stored in airtight bottles. Macroscopical and anatomical characters studied were done by using from fresh materials.

4.2 MACROSCOPICAL FEATURES

The macroscopical morphological observations of roots, stems, leaves and fruits including colour, odour, taste, size shape, fracture, fracture surface, texture, duration and rootlets, margin, venation, apex, duration, type, phyllotaxis, presence and absence of petiole and stipule were carried out following the standard procedure of (Evans, 2009).

4.3 ANATOMICAL FEATURES

Transverse free hand-sections were made from fresh specimens. Thin sections were stained in safranin for 2-3 minutes. These were gradually dehydrated by pass through 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% alcohol. At each grade the sections were left for 2-5 minutes. The dehydrated sections were then placed into a drop of light green followed by dehydration in absolute alcohol for 2-3 minutes. The sections were finally permanently mounted in canada balsam (Puruis *et al.*, 1966; Johnson, 1940). The size of the cells was determined following Singh (2009).

4.4 Leaf Constant Parameters

4.4.a Vein islet number and vein termination number

The minute areas of the photosynthetic tissue surrounded by ultimate divisions of vascular strands in the leaf are known as vein islet and their number per square mm of the leaf is considered vein-islet number. A vein termination is ultimate free termination of vein-let and their number per square mm is known as vein termination

number (Evans, 2009). These values are constant in range for a species and can be used for identification of a drug or species.

Procedure

Small pieces from the leaves were taken midway from margin to midrib and cleared by boiling in super saturated chloral hydrate solution in a test tube placed in a boiling water bath. Vein islet and vein termination were counted using 10 x 10 magnification. Ten readings from continuous squares were taken for counting the vein-islets and vein termination (Evans, 2009).

4.4.b Palisade cell-ratio

The average number of palisade cells present beneath each upper epidermal cell is called palisade cell ratio. This value is also constant in range for a species. Palisade ratio is not affected by geographical variations, therefore it is a diagnostic feature for characterization, standardization and identification of particular species (Evans, 2009).

Procedure

Leaf pieces were cleared by placing on boiling with super saturated chloral hydrate solution as before. These were examined under microscope using 10 x 10 magnifications. The palisade ratio was determined in two steps. First focused a number of groups of each of four epidermal cells and in a second step change the focus to enable the palisade cells to be visible within the epidermal cells. Palisade cells including those, which are more than half covered by the epidermal cells, also were counted. Palisade ratio of a group was obtained by dividing the resulted value by 4. The range of a number of groups from different particles was recorded (Evans, 2009).

4.5 Stomatal study

The fresh leaves were immersed in water to prevent desiccation. Peels from both upper and lower surfaces were cleaned with distilled water and mounted in canada balsam for examination at 10 x 40 magnification (Chaudhary & Imran, 1997).

The data recorded included number of stomata on both upper and lower epidermises, type of stomata, density, frequency, stomatal index, size of stomatal pore (average length and width having 10 readings), size of guard cells (average length and width having 10 readings) and percentage of open stomata (Wallis, 1985). The statistics evaluation of the stomata and epidermis included mean, standard deviation, variance,

coefficient of variance, standard error and difference of standard error (Choudhary & Kamal, 2004).

4.6 Analysis of powered drug

Microscopic study

The powder material was macerated with chloral hydrate solution. One drop of solution taken on a slide was heated and examined under microscope using 10x10 magnification. Types of tissues were observed under the microscope and sketched (Wallis, 1985).

4.7 MICROCHEMICAL TESTS

Following qualitative microchemical tests were carried out.

4.7.a Detection of alkaloid

Alkaloids were detected following Evans (2009). Dragendorff's reagent was added and the appearance of reddish brown or pinkish purple indicated the presence of alkaloids.

4.7.b Detection of mucilage

A small quantity of powder drug was mixed with few drops of water and allowed to stand, if the powder became gummy then mucilage was present (Evans, 2009).

4.7.c Detection of anthraquinone

A small quantity of powder was macerated with ether, the presence of pink, red, or violet colour, in aqueous layer after shaking indicated the presence of anthraquinone derivations (Evans, 2009).

4.7.d Detection of calcium oxalate

The crystals may be identified as calcium oxalate, if these are soluble in HCl and H₂SO₄ without effervescence (Evans, 2009).

4.7.e Detection of saponin

Powdered material was shaken a test tube with half filled with water any marked frothing indicated saponin (Evans, 2009).

4.7.f Detection of tannins

The presence of bluish black or greenish colour after treating the drug with dilute solution of ferric chloride indicated the presence of tannin (Evans, 2009).

4.7.g Detection of starch

The drug plant material was treated with iodine solution and the appearance of deep blue marked the presence of starch (Evans, 2009).

4.7.h Detection of fat and oil

A small quantity of powdered drug was pressed between filter papers, the appearance of an oily stain indicated the presence of fats and oil (Evans, 2009).

4.7.i Detection of protein

The drug was treated with aqueous solution of picric acid and the appearance of intense yellow precipitate indicated the appearance of proteins (Johnson, 1940).

4.7.j Detection of cellulose

The appearance of blue colour on treating the drug with chloro-zinc iodide solution indicated the presence of cellulose (Evans, 2009).

4.8 PROXIMATE ANALYSES

4.8.a Determination of ash

Ash was determined by following AOAC (2000) method. A clean crucible was heated in a muffle furnace at 660⁰C for an hour, then cooled it in desiccators and weighed it as (W₁). 1 g sample was placed in the crucible (W₂). The sample was charred over the burner with the help a blowpipe. The crucible was then placed in a muffle furnace at 550⁰C for 6 -8 hours. After the complete ignition the furnace was turnoff. The crucible was cooled and weighed (W₃). Percent ash was calculated as follow.

$$\% \text{ Ash} = \frac{\text{Wt. of Ash (W}_3 - \text{W}_1) \times 100}{\text{Wt of sample}}$$

4.8.b Determination of the moisture

Moisture contents were determined by following a standard (AOAC, 2000). A clean petri-dish was weight (W₁) and 1-2 gram of sample was added to it. It was placed partially covered with lid in the oven at 105 °C, for 4-6 hours, until constant weight was obtained. After which it was then removed and placed in desiccator for 30 minutes in order to cool it. After cooling the dish (W₂) was weighted. Percent moisture may be calculated as follow.

Calculations

$$\% \text{ Moisture} = \frac{W1 - W2}{\text{Wt of the sample}} \times 100$$

4.8.c Determination of protein

Crude protein was determined using Macrojeldahl distillation method following AOAC (2000). One g sample was taken in a digestion flask and 8 g of digestion mixture K_2SO_4 : CuSO_4 (8:1) and 12 ml of conc. H_2SO_4 was added. Some piece of pumice stone was also added to avoid bumping of solution. The flask was swirled in order to mix the contents thoroughly. It was then placed on heater under fume hood to start digestion and continued till the mixture becomes clear. After digestion, the heater was turned off and flask was cooled. The digest was then transferred to 100ml flask and volume was made up to the mark by the addition of distilled water. Distillation of the digest was performed in markam still distillation apparatus. 10 ml of digest was taken with the help of pipette and was introduced in the distillation tube through funnel. Then 10 ml of NaOH was gradually added through the same way. Distillation was continued for at least 10 minutes and NH_3 produced was collected as NH_4OH in a conical flask containing 20ml of 4% boric acid solution with few drops of modified methyl red indicator. The tip of condenser was washed in the flask with distilled water. During distillation pink color changed to yellowish due to NH_4OH . The distillate was then titrated against standard 0.05HCl till the appearance of pink color. A blank was also run following the steps as mentioned above. % crude protein was calculated as follow.

$$\% \text{ Crude Protein} = 6.25 \times \% \text{N}$$

$$\% \text{ N} = \frac{(S - B) \times N \times 0.014 \times D \times 100}{\text{Wt.of.sample} \times V}$$

Where

S = Sample titration reading

B = Blank titration reading

N = Normality of HCl

D = Dilution of the sample after digestion

V = Volume taken for distillation

0.014 = Mili-equivalent Wt of Nitrogen

4.8.d Determination of fat or ether extract

Crude fat was determined by ether extract method using Soxhtec (labconco) apparatus following AOAC (2000). One g moisture free sub sample was wrapped in filter paper, placed in thimble and then introduced in the extraction tube. Pre weighed, clean and dry receiving beaker was filled 1/ 3rd with petroleum ether and fitted into the apparatus. Extraction was done up to complete drying of solvent.

The percent of crude fat was determined as follow.

$$\% \text{ Crude Fat} = \frac{(\text{Wt of beaker} + \text{Ether extract}) - (\text{Wt of Beaker}) \times 100}{\text{Wt of Sample}}$$

4.8.e Determination of crude fiber

Crude fiber was determined by acid and alkali digestion method using fiber tec apparatus by following AOAC (2000). The weighed samples were first digested with acid and then with alkali. One gm sample was taken in a clear beaker and 100 ml of 2.5 % HCl was added to it. The mixture was boiled with stirring for about half an hour. It was drained into the beaker. The fiber residue was again digested in 2.5 % NaOH in a similar manner like that of acid digestion. The residue so collected was transferred to a dried crucible to remove the moisture. Then the weight of the dry crucible was noted. The crucible was then kept in furnace for red dull heat till the formation of white and grey ash. The crucible was cooled in desiccator and weighed again. The loss in weight of the dry residue upon ignition was taken as the amount of crude fiber. Percent crude fiber was calculated as follow.

$$\% \text{ Crude Fiber} = \frac{(W1 - W2 \times 100) \times F}{\text{Wt. of Sample}}$$

Where

F = Value of crude fat

W1 = Weight of dry crucible

W2 = Weight of crucible after heating

4.8.f Determination of carbohydrate contents

Carbohydrates were determined by subtracting the weights of protein fats, crude fibers, ash, and moisture contents from 100.

4.9 ELEMENTAL COMPOSITION

0.5 gm dried crude powdered plant sample was taken in a beaker and 10 ml concentrated HNO₃ was added and allowed to stand overnight. After 24 hours 4ml perchloric acid was added to each sample. After 25 minutes, the sample was heated till it evaporated to a small volume. The samples were cooled and then diluted with distilled water so that 100ml solution was obtained and filtered. The solutions were marked with names and coded numbers and were analyzed through atomic absorption spectrometer for quantitative detection of the following elements (AOAC, 2000; Sucman *et al.*, 2007). The elemental analysis was done for roots, stems, leaves, flowers and fruits of all the five species at three phenological stages.

4.10 BIOLOGICAL ACTIVITIES

Preparation of Extracts

Shoots of *Datura innoxia* Miller, *Solanum nigrum* Lin, *Solanum surattense* Burm. f, *Withania somnifera* L and *Withania coagulans* (Stocks) Dunal were ground to 60 mesh. Fifty gm of each sample was separately soaked in 250 ml methanol and acetone for 72 hrs. Thereafter, each plant extract was passed through Whatman filter paper No. 1823 for 3 times. It was evaporated in a rotatory evaporator at 40 °C to concentrate the extracts. These extracts were stored at 4 °C prior to use. The methanolic and acetone extracts and the standard drug were dissolved in dimethylsulphoxide (DMSO) at the concentration of 2 mg/ml and 1 mg/ml for antibacterial, 24 mg/ml and 1 mg/ml for antifungal, 10 mg/ml and 1 mg/ml for cytotoxic 30 mg/ml and 1 mg/ml for phytotoxic and 200 mg/ml and 1 mg/ml for insecticidal activities, respectively.

4.10.a Antibacterial activity

The antibacterial study of the crude extracts was carried out by agar well diffusion method following Carron *et al.*, (1987). On the first day a single colony of bacterial culture in nutrient broth was inoculated and incubated at 30 °C for 24 hours. On the second day soft agar tube was taken, melted and cool up to 40 °C. 10 ml of fresh bacterial culture was added. It was well shaken and then poured on to the nutrient agar containing

plate. The plate was rotated to make even distribution of the cultured and allowed to solidify. Well was made by using 7 mm- dia sterile borer. 100 µl of sample was added in respective well plate according to bacterial culture. Other wells supplement with ampicillin serving as positive control. The plates were inoculated at 30 °C for 24 hours. The zones of inhibitions (mm) were recorded next day.

4.10.b Antifungal activity

Antifungal activity of the crude extract was evaluated by agar tube dilution method following Atta-ur-Rehman *et al.*, (2001). The extracts (24 mg) were dissolved in sterile DMSO (1.0 ml), which served as stock solution. Sabouraud dextrox agar (SDA) was dispensed into screw cap tubes, which had been autoclaved at 121 °C for 15 minutes. The non-solidified SDA media was poisoned with stock solution (66.6 µl), giving the final concentration of 400 µg of the extract/ml of SDA. Each tube was inoculated with a 4 mm dia piece of inoculum removed from a seven days old culture of fungi. For non-mycelial growth, an agar surface streak was employed. Inhibition of fungal growth was observed after days of incubation at 28 °C. Mucanazol was used as a positive control.

4.10.c Cytotoxicity

The materials and reagents used for cytotoxicity included test sample *Artemia salina* (shrimps eggs), sea salt (38 g/L of D/W, pH 7.4), hatching tray with perforated partition, lamp to attract brine-shrimp larvae, micro pipette (5, 50, 500 µl), vials tray, 30 vials, organic solvents methanol and acetone. The cytotoxic activity of the crude extracts of the plants was carried out by following the method of Meyer *et al.*, (1982).

Hatching

The hatching tray (a rectangular dish 22x32 cm) was half filled with filtered brine solution and 50mg (eggs of brine shrimp were sprinkled in it). It was incubated at 37°C and after 24h brine shrimp hatched. The plant extracts were applied to see the cytotoxicity of these extracts.

Sample preparation

Test sample was dissolved (10mg) in 1ml of DMSO and from this solution 5, 50 and 500 µl was transferred to vials (3vials/concentration). The concentration was 10, 100 and 1000 µg/ml respectively. After 2 day of hatching and maturation 10 larvae/vials were placed, using a pasteur pipette. The volume was made 5 ml with seawater (38.5g of sea

salt / 1000ml of distilled water). It was incubated at 25 - 27 °C for 24 hours under illumination. Other vials were supplemented with DMSO and etoposoid was used as reference cytotoxic drug which served as negative and positive controls, respectively. The data was analyzed with Probit Analysis program to determine LD₅₀ values (Finney, 1971).

4.10.d Phytotoxicity

Phytotoxic activity of the extracts was carried out against the *Lemna minor* following McLaughlin *et al.*, (1991). The medium was prepared by mixing various constituents in distilled water (1000 ml) and the pH was adjusted (5.5-5.6) by adding KOH pellets. The medium was then autoclaved at 121 °C for 15 minutes. The extracts (30.0 mg) dissolved in methanol (1.0 ml) served as stock solution. Thirty petri plates, three for each concentration, were inoculated with 1000, 100 and 10 µl of the stock solution to give the final concentration of 1000, 100 and 10 ppm, respectively. The solvent was allowed to evaporate overnight under sterile conditions. To each plate, medium (20 ml) and plants (10), each containing a rosette of three fronds of *Lemna minor* were added. Other plates supplement with solvent and reference growth inhibitor (Paraquate), served as a negative control. All plates were kept in the growth cabinet for seven days. The number of fronds per plates were counted and recorded on day seven.

Table 1. Chemical composition of E-medium

S. No	Chemical Name	Concentration g/L
1.	Potassium dihydrogenphosphate (KH_2PO_4)	0.68
2.	Potassium nitrate (KNO_3)	1.515
3.	Calcium nitrate ($\text{Ca}(\text{NO}_2)_4 \cdot 4\text{H}_2\text{O}$)	1.180
4.	Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	0.492
5.	Boric acid (H_3BO_3)	0.00286
6.	Manganous chloride ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$)	0.00362
7.	Ferric chloride ($\text{FeCl}_3 \cdot 4\text{H}_2\text{O}$)	0.00540
8.	Zinc sulfate ($\text{ZnSO}_4 \cdot 5\text{H}_2\text{O}$)	0.00022
9.	Copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)	0.00022
10.	Sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$)	0.00012
11.	Ethylene Diamino Tetra Acetic acid (EDTA)	0.01120

4.10.e Insecticidal activity

The materials and reagents used for insecticidal activity included test insects, volatile organic solvent (methanol), standard insecticide (Permethrin), petri plates (9cm diameter), micropipette (1000 μl), growth chamber, test sample, filter papers, glass vials, brush. The insecticidal activity of the crude extract was carried out by impregnated filter paper method following Naqvi & Parveen (1991).

Preparation of Test sample

The test sample is prepared by mixing 200 mg test sample and 3 ml methanol and acetone.

Rearing technique

The stored grain pests are reared in the laboratory under controlled conditions (temperature and humidity) in plastic bottles containing sterile breeding media. Insects of uniform age and size are used for the experiment.

Procedure

On day one filter paper were cut according to the size of petri plate (9 cm or 90 mm) and was put them in the plate. Then the whole sample was loaded over the filter

paper and these plates were left for 24 hours to evaporate the solvent completely. On the second day (after the evaporation of solvent) put 10 healthy and active insects of same size and age of each species in each plate (test and permethrin was used + ve and DMSO was used as – ve control, respectively) with the help of a clean brush. The plates were incubated at 27 °C for 24 hours. On the third day readings were noted and the percentage inhibition or percentage mortality with the help of the following formula was calculated:

$$\text{Percent mortality} = 100 - \frac{\text{No of insects alive in test}}{\text{No of insects alive in control}} \times 100$$

Chapter 5

RESULTS AND DISCUSSION

5.1 Macroscopical Features

5.1.a Roots

The features of the roots are shown in Table 2. All roots were whitish in colour both in fresh and dry form. The odour in roots of *Solanum nigrum* and *W. coagulans* was indistinct in fresh and dry conditions. In case of *S. surattense*, it was indistinct in fresh and pungent in dry condition. *Datura innoxia* had slightly pungent odour in fresh form and pungent in dry state. Pungent odour was present in *W. somnifera* in fresh form and indistinct in dry state. Nirmalet *et al.*, (2009) reported yellowish white coloured wood, bitter taste and cylindrical shape in *Sesbania sesban* which agrees with the present findings. The roots of all tested species were cylindrical in shape both in fresh and dry conditions. Rootlets were present in all the roots of the investigated plants in fresh and dry state. Direction of root growth was vertically downwards in all the five plants. Fracture was fibrous in *D. innoxia* in fresh and dry conditions, uneven in *S. nigrum*, *S. surattense* and *W. somnifera* in fresh state and fibrous in dry specimen. It was uneven in *W. coagulans* in fresh and dry cases. Root of *Withania somnifera* was straight, unbranched, conical in shape, fracture was short and uneven, with acrid, bitter taste and mucilaginous (Singh & Bhandari, 2007). This agrees with our findings. Texture in fresh and dry form was hairy in *D. innoxia*. *S. nigrum* and *S. surattense*, hair were present in fresh form but were absent in dry form. In *W. coagulans*, they were absent in fresh and dry conditions (Table 2). Yadav *et al.*, (2007) reported that *Chenopodium album* root is cylindrical, having numerous rootlets, unbranched, pale white in colour, fracture fibrous, surface is smooth without striations and is tasteless which is agree with the present findings.

5.1.b Stems

Table 3 illustrated various features of the stems. Colour of the stem was light green in *Datura innoxia* and *Withania somnifera* in fresh and dry state, dark green in

Solanum nigrum in fresh form that turned light green when dried. It was dark green in *Solanum surattense* in fresh and dry conditions. *Withania coagulans* had whitish colour in fresh and dry specimens. Odour was slightly pungent in *Datura innoxia* both in fresh and dry state, indistinct in *Solanum nigrum*, *Solanum surattense* and *Withania somnifera* both in fresh and dry form. It was pleasant in *Withania coagulans* in fresh form and indistinct in dry form. The shape of stem was cylindrical with ridges in *Datura innoxia* and *Solanum nigrum*, cylindrical without ridges in *Solanum surattense*, *Withania somnifera* and *Withania coagulans*. Mazumder *et al.*, (2010) reported macroscopical characters of the stems of *Berberis aristata* which are similar to the present findings. The macroscopical characters included size varying from 15–20mm, shape was cylindrical, colour was light yellowish, odour was aromatic and taste was woody. Phyllotaxis was alternate in all the studied plants. Direction of stem growth was upright in all plants, except *Solanum surattense* which was prostrate. Variation in the stem fracture differed from that of roots. It was uneven and fibrous in all the plants both in fresh and dry state, except *Withania coagulans* which was even. Texture was hairy in *Datura innoxia* and *Withania coagulans* both in fresh and dry form, glabrous in *Solanum surattense* both in fresh and dry form, and hairs were absent in *Solanum nigrum* and *Withania somnifera* both in fresh and dry form (Table 3). Muhammad *et al.*, (2012) while reporting macroscopical features of stem of *Viola betonicifolia* indicated that it was light green, glabrous or somewhat pubescent which agree with the present findings.

5.1.c Leaves

The macroscopy of the leaves is summarized in Table 4. Leaf lengths of *Datura innoxia* were 7–18cm in fresh and 6.9–17.9cm in dry state, *Solanum nigrum* had 2.5–8.5cm in fresh and 2.4–4.4cm in dry form, *Solanum surattense* had 3–8cm in fresh and 2.9–7.9cm in dry condition. Leaf of *Withania somnifera* was 4–10cm in fresh and 3.9–9.9cm in dry state. While *Withania coagulans* leaf was 4–8cm in fresh and 3.9–7.9cm in dry condition. The range of widths of *Datura innoxia* leaf was 4.5–12cm in fresh and 4.4–11.9cm in dry condition, that of *Solanum nigrum* had 2.1–3.2cm in fresh and 2.0–3.1cm in dry state, *Solanum surattense* leaves were 2.5–5.0cm width in fresh and 2.4–4.9cm in dry form. The leaf width of *Withania somnifera* was 2–7cm in fresh and

1.9–6.9cm in dry state and that of *Withania coagulans* was 1.4–3.5cm in fresh and 1.3–3.4cm in dry condition. This reduction in the range length and width in the dry state was due to the loss of the water content from the plant parts. In *Datura innoxia* and *Solanum nigrum* and *Withania somnifera* the upper surface of leaf was dark green while the lower surface was light green in the fresh condition. When dried the surfaces of these plants turned light green. Both the surfaces of *Solanum surattense* were dark green. In *Withania coagulans* both the surfaces were whitish in the fresh and dry forms. Vidhu *et al.*, (2007) reported that leaf of the *Solanum nigrum* is simple, 2.5–8.5 cm long and 2.5 cm wide, ovate, or oblong, sinuate, toothed or lobed, narrow at both ends, petiolate, thin and pubescent. Their findings support the present findings. Dorsal surface was green in colour but the ventral surface is slightly pale. Venation was reticulate. The odour was characteristic and the taste is bitter and this agrees with the present study. Evans (2009) reported that *Datura stramonium* leaf had a slight but unpleasant odour with a bitter taste, and this agrees with the present findings. Incision was present in *Datura innoxia*, *Solanum nigrum*, *Solanum surattense* and was absent in *Withania somnifera* and *Withania coagulans* both in fresh and dry form. Similarly, Abereet *et al.*, (2007) also indicated the presence of simple leaf whorled arrangement, an entire margin with lanceolate shape, acute apex and base, parallel venation in the leaves of *Mitracarpus scaber* and this agrees with the present findings. Composition was simple in all the plants. Venation was reticulate and unicostate in all the studied plants both in fresh and dry form. Nayak & Patel (2010) reported that shape of the leaf of *Jatropha curcas* was broadly ovate, cordate, palmate, acute, glabrous, dark green adaxially, light green abaxially, taste was bitter and this study agrees with the present study. Margin was dentate in *Datura innoxia*, sinuate to irregular dentate in *Solanum nigrum*, pinnatifid in *Solanum surattense*, entire in *Withania somnifera* and *Withania coagulans* both in fresh and dry form. Apex to be found was acute in *Datura innoxia*, *Solanum nigrum* and *Withania somnifera* and obtuse in *Solanum surattense* and *Withania coagulans* both in fresh and dry form. The general outline i.e. the shape of the leaf was ovate in *Datura innoxia*, *Solanum nigrum* and *Withania somnifera*, elliptic-oblong in *Solanum surattense*, and elliptic-ovate in *W. coagulans* both in fresh and dry form (Table 4). Nurit-Silva *et al.*, (2011) reported that the leaves of *Solanum*

torvum were characteristically ovate to elliptic, with lobed margins, oblique at the base and these support the present study.

5.1.dFruits

The characters of fruits of all five investigated species are shown in Table 5. The lengths of *Datura innoxia* fruit was 5–6cm in fresh and 4.9–5.9cm in dry state, 6–9mm in fresh and 5–8mm in dry form of *Solanum nigrum*, *Solanum surattense* had 22–27mm in fresh and 21–26mm in dry condition. The fruit of *Withania somnifera* was 5–6mm in fresh and 4–5mm in dry state and that of *Withania coagulans* was 14–16mm in fresh and 13–15mm dry condition. The range widths of *Datura innoxia* fruit was 4–5cm in fresh and 3.9–4.9cm in dry condition, *Solanum nigrum* had 5–8mm in fresh and 4–7mm in dry state, *Solanum surattense* had 15–20mm in fresh and 14–19mm in dry form. The fruits of *Withania somnifera* had 6–8mm in fresh and 5–7mm in dry state and *Withania coagulans* had 10–12mm in fresh and 9–11mm in dry condition. Evans (2009) reported that fruit of *Datura stramonium* is a thorny capsule about 3-4cm long which showed similarity with that of the *Datura innoxia* fruits and the present study. Fruit of the *Datura metel* is capsule or ellipsoid, nodding, spinous, 4-valved or irregular breaking up near the apex (Singh & Bhandari, 2007) which is agree with the present findings. All the fruits were indehiscense in nature. Odour was pungent in *Datura innoxia*, indistinct in *Solanum nigrum*, *Solanum surattense*, *Withania somnifera* and pleasant in *Withania coagulans*. Taste of the fruits was irritating in *Datura innoxia*, *Solanum surattense* and *Withania coagulans* and indistinct in *Solanum nigrum* and *Withania somnifera* (Table 5). Wallis (1985) reported that the fruits of the *Datura stramonium* are usually about 5 – 10mm, long, conical and covered with bristly emergences; which agree with the present findings.

Table 2. Macroscopical features of the roots of the five medicinal plants of Family Solanaceae.

Species	Parameters													
	Colour		Odour		Shape		Rootlets		Direction of growth		Fracture		Texture	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
<i>Datura innoxia</i> Miller.	Whitish	Whitish	Slightly pungent	Pungent	Cylindrical	Cylindrical	Present	Present	Horizontally downward	Horizontally downward	Fibrous	Fibrous	Hairy	Hairy
<i>Solanum nigrum</i> Linn.	Whitish	Whitish	Indistinct	Indistinct	Cylindrical	Cylindrical	Present	Present	Horizontally downward	Horizontally downward	uneven	Uneven & Fibrous	Hairy	Hairy
<i>Solanum surattense</i> Burm. f.	Whitish	Whitish	Indistinct	Pungent	Cylindrical	Cylindrical	Present	Present	Horizontally downward	Horizontally downward	Uneven & Fibrous	Fibrous	Hairy	Hairy
<i>Withania somnifera</i> Linn.	Whitish	Whitish	Pungent	Indistinct	Cylindrical	Cylindrical	Present	Present	Horizontally downward	Horizontally downward	uneven	Fibrous	Hairy	Hairy
<i>Withania coagulans</i> (Stock) Dunal.	Whitish	Whitish	Indistinct	Indistinct	Cylindrical	Cylindrical	Present	Present	Horizontally downward	Horizontally downward	uneven	uneven	Not hairy	Not hairy

Table 3. Macroscopical features of the stems of the five medicinal plants of Family Solanaceae.

Species	Parameters													
	Colour		Odour		Shape		Phyllotaxis		Direction of growth		Fracture		Texture	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
<i>Datura innoxia</i> Miller.	Light green	Light green	Slightly pungent	Slightly pungent	Cylindrical with ridges	Cylindrical with ridges	Alternate	Alternate	Upright	Upright	Uneven & Fibrous	Uneven & Fibrous	Hairy	Hairy
<i>Solanum nigrum</i> Linn.	Dark green	Light green	Indistinct	Indistinct	Cylindrical with ridges	Cylindrical with ridges	Alternate	Alternate	Upright	Upright	Uneven & uneven	Uneven & Fibrous	Not Hairy	Not Hairy
<i>Solanum surattense</i> Burm. f.	Dark green	Dark green	Indistinct	Indistinct	Cylindrical	Cylindrical	Alternate	Alternate	Prostrate	Prostrate	Uneven & Fibrous	Uneven & Fibrous	Glabrous	Glabrous
<i>Withania somnifera</i> Lin.	Light green	Light green	Indistinct	Indistinct	Cylindrical	Cylindrical	Alternate	Alternate	Upright	Upright	Uneven & fibrous	Fibrous	Not hairy	Not hairy
<i>Withania coagulans</i> (Stock) Dunal.	Whitish	Whitish	Pleasant	Indistinct	Cylindrical	Cylindrical	Alternate	Alternate	Upright	Upright	uneven	uneven	Hairy	Hairy

Table 4. Macroscopical features of the leaves of the five medicinal plants of Family Solanaceae.

Species	Dimension		Colour		Incision		Lamina composition		Margin		Apex		General outline	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
<i>Datura innoxia</i> Miller.	Range = 7-18cm; Range = 4.5-12cm	Range = 6.9-17.9cm; Range = 4.4-11.9cm	US light green; LS dark green	BS light green	Present	Present	Simple	Simple	Dentate	Dentate	Acute	Acute	Ovate	Ovate
<i>Solanum nigrum</i> Linn.	Range = 2.5-8.5cm; Range = 2.1-3.2cm	Range = 2.4-8.4cm; Range = 2.0-3.1cm	US light green; LS dark green	BS light green	Present	Present	Simple	Simple	Sinuate to irregular dentate	Sinuate to irregular dentate	Acute	Acute	Ovate	Ovate
<i>Solanum surattense</i> Burm. f.	Range = 3-8cm; Range = 2.5-5cm	Range = 2.9-7.9cm; Range = 2.4-4.9cm	BS dark green	BS dark green	Present	Present	Simple	Simple	Pinnatifid	Pinnatifid	Obtuse	Obtuse	Elliptic oblong	Elliptic oblong
<i>Withania somnifera</i> Linn.	Range = 4-10cm; Range = 2-7cm	Range = 3.9-9.9cm; Range = 1.9-6.9cm	US light green; LS dark green	BS light green	Nil	Nil	Simple	Simple	Entire	Entire	Acute	Acute	Ovate	Ovate
<i>Withania coagulans</i> (Stock) Dunal.	Range = 4-8cm; Range = 1.4-3.5cm	Range = 3.9-7.9cm; Range = 1.3-3.4cm	Whitish	Whitish	Nil	Nil	Simple	Simple	Entire	Entire	Obtuse	Obtuse	Elliptic ovate	Elliptic ovate

Key: RL = US = Upper surface; LS = Lower surface; BS = Both surfaces

Table 5. Macroscopical features of the fruits of the five medicinal plants of Family Solanaceae.

Species	Dimension		Dehiscence		Odour		Taste	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
<i>Datura innoxia</i> Miller.	Range = 5-6cm; Range = 4-5cm	Range = 4.9-5.9cm; Range = 3.9-4.9cm	Indehiscence	6-valves	Pungent	Pungent	Irritating	Irritating
<i>Solanum nigrum</i> Linn.	Range = 6-9mm; Range = 5-8mm	Range = 5.9-8.9mm; Range = 4.9-7.9mm	Indehiscence	Indehiscence	Indistinct	Indistinct	Indistinct	Indistinct
<i>Solanum surattense</i> Burm. f.	Range = 22-27mm; Range = 15-20mm	Range = 21-26mm; Range = 14-19mm	Indehiscence	Indehiscence	Indistinct	Indistinct	Irritating	Irritating
<i>Withania somnifera</i> Linn.	Range = 5-6mm; Range = 6-8mm	Range = 4-5mm; Range = 5-7mm	Indehiscence	Indehiscence	Indistinct	Indistinct	Indistinct	Indistinct
<i>Withania coagulans</i> (Stock) Dunal.	Range = 14-16mm; Range = 10-12mm	Range = 13-15mm; Range = 9-11mm	Indehiscence	Indehiscence	Pleasant	Pleasant	Irritating	Irritating

5.2 Anatomical Features

5.2.a Root

Table 6 depicts various anatomical features of roots of five investigated species.

a) Epidermis

This epidermis was single layer in *Datura innoxia*, *Solanum surattense* and *Withania somnifera* and two layered in *Solanum nigrum* and *Withania coagulans*. The epidermal cells were spherical in *Datura innoxia* (Fig. 7), rectangular in *Solanum nigrum* (Fig. 8), oval in *Solanum surattense* (Fig. 9) and *Withania somnifera* (Fig. 10) and *Withania coagulans* (Fig. 11). The maximum lengths (12 μ) of the epidermal cells was found in *Datura innoxia*, and the minimum length (4.5 μ) was found in *Solanum surattense* and the maximum widths (9 μ) of the epidermal cells was found in *Datura innoxia*, and the minimum length (3 μ) was found in *Solanum surattense* (Table 6). Many workers have reported feature of epidermal cells from the roots of different plants viz, *Convolvulus arvensis* (Sher *et al.*, 2011b), *Amaranthus viridis* (Khan *et al.*, 2011), *Rumex dentatus*, *Polygonum plebejum*, *Rumex hastatus*, *Rumex nepalensis*, *Rheum australe* and *Polygonum persicaria* (Hameed *et al.*, 2010). All these findings also support the present study.

b) Vascular bundle

The vascular bundle in all the five plants was bicolateral type. Number of vascular bundle is less in *W. coagulans* and *S. surattense* as compared to *D. innoxia*, *S. nigrum* and *W. somnifera*. This is because *D. innoxia* (Fig. 7), *S. nigrum* (Fig. 8) and *W. somnifera* (Fig. 10) grow in mesophytic condition and *S. surattense* (Fig. 9) and *W. coagulans* (Fig. 11) grows in xerophytic condition. The maximum length (28.3 μ) of the xylem cells was recorded in *S. nigrum* and the minimum length (15.5 μ) in *Datura innoxia*. The maximum width (19 μ) was noted in *W. coagulans* and the minimum width (10.5 μ) in *Datura innoxia*. The maximum length (19 μ) of the phloem cells was measured in *S. surattense* and the minimum length (6 μ) in *W. somnifera* and the maximum widths (15 μ) was obtained in *S. surattense* and the minimum width (4 μ) in *W. somnifera* (Table 6). A bicolateral type of the vascular bundles is present in different members of the Solanaceae like *Datura stramonium*, *Withania somnifera* and *Hyoscyamos*

niger(Rangari, 2008; Evans, 2009; Singh & Bhandari, 2000; Youngken, 1950) which agree with the present work.

c) Medullary rays

Medullary rays were present in all the five plant species. The maximum length (19 μ) cells was obtained in *S. nigrum* and the minimum length (3 μ) was found in *W. coagulans* and the maximum widths (13 μ) was shown in *S. nigrum* and the minimum width (6 μ) was exhibited in *W. somnifera* (Table 6). The cells were somewhat rectangular in shape in all the five plants species i.e. *D. innoxia* (Fig. 7), *S. nigrum* (Fig. 8), *S. surattense* (Fig. 9), *W. somnifera* (Fig. 10) and *W. coagulans* (Fig. 11). Medullary rays were reported by various workers like Kadam *et al.* (2012) noted in *Agave americana*, Prasad *et al.* (2010) in *Dalbergia latifolia*. These agree with our work.

d) Tracheae

Tracheae were noticed in *S. nigrum*, *S. surattense*, *W. somnifera* and *W. coagulans* except *Datura innoxia*. The maximum length (86 μ) cells was found in *S. surattense* and the minimum length (19 μ) was obtained in *S. nigrum* and the maximum widths (50.2 μ) was exhibited in *S. surattense* and the minimum width (11 μ) in *S. nigrum* (Table 6). The cells were somewhat spherical in shape in *S. nigrum* (Fig. 8) and spherical – oval in *S. surattense* (Fig. 9), *W. somnifera* (Fig. 10) and *W. coagulans* (Fig. 11). Singh (1965); Singh (1972); Prasad & Chauhan (1975) who concluded similar results.

e) Casparian strips

The strips were present in *D. innoxia*, *S. surattense*, *W. somnifera* and *W. coagulans* except *S. nigrum*. The maximum length (8 μ) cell was obtained in *W. somnifera* and the minimum length (4.7 μ) in *D. innoxia*. The maximum width (4.5 μ) was found in *W. somnifera* and the minimum width (3.1 μ) was exhibited in *D. innoxia* (Table 6). The cells were somewhat rectangular in shape in the investigated species i.e. *D. innoxia* (Fig. 7), *S. surattense* (Fig. 9), *W. somnifera* (Fig. 10) and *W. coagulans* (Fig. 11). Toma *et al.* (2010) for *Inula* species and Perrot & Paris (1971) for *Inula helenium* also provided similar findings.

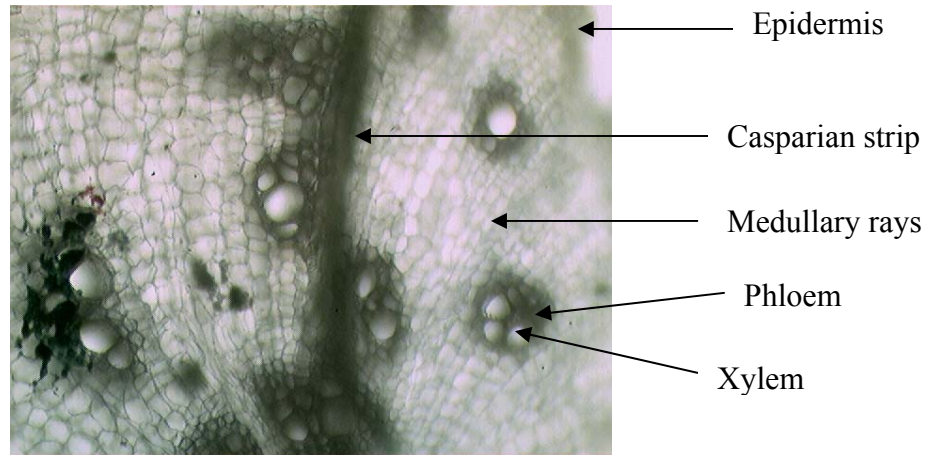


Fig. 7. Transverse section of the root of *Datura innoxia* Miller.

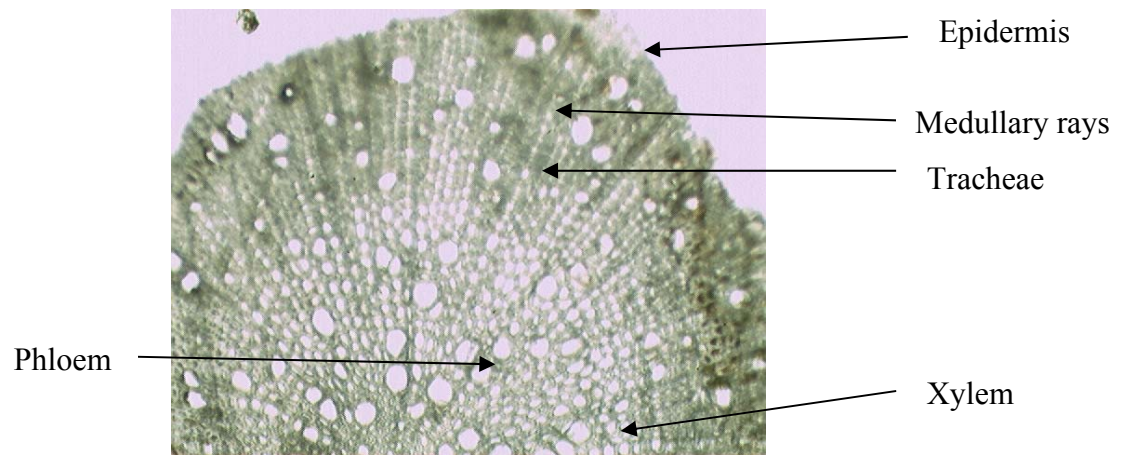


Fig. 8. Transverse section of the root of *Solanum nigrum* Linn.

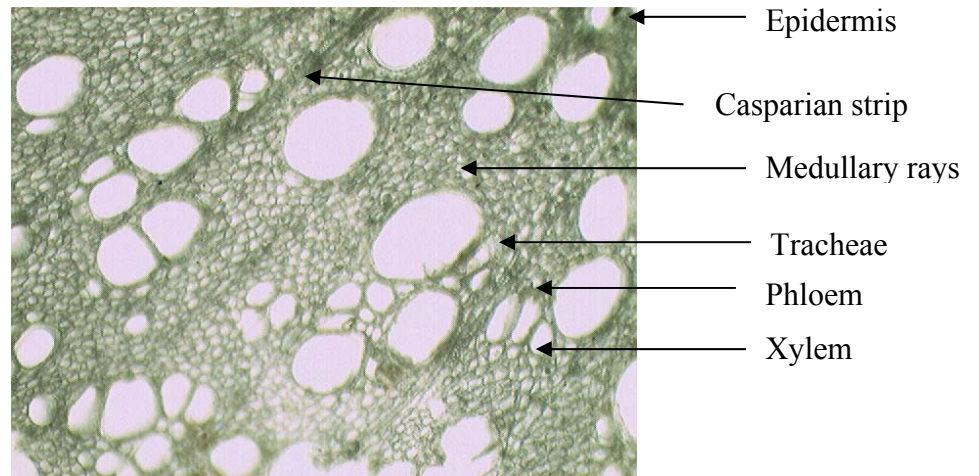


Fig. 9. Transverse section of the root of *Solanum surattense* Burm.f.

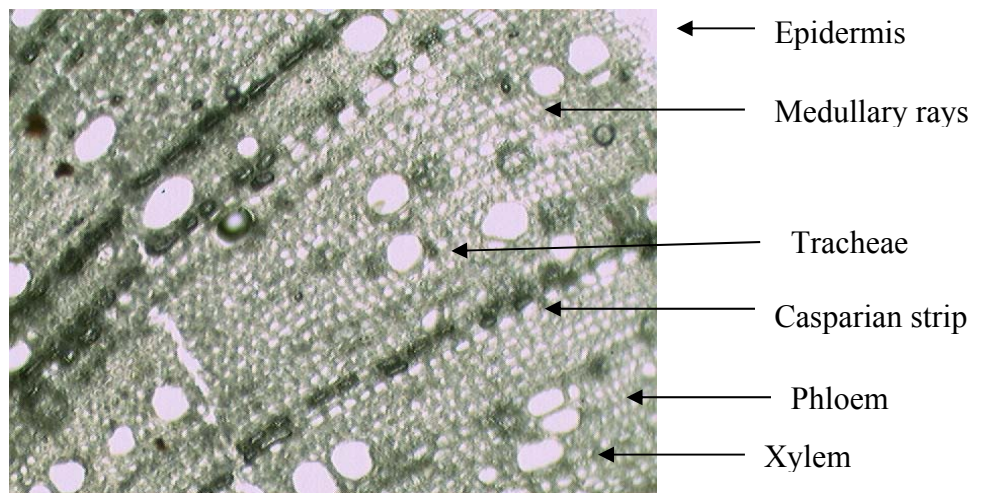


Fig. 10. Transverse section of the root of *Withania somnifera* Linn.

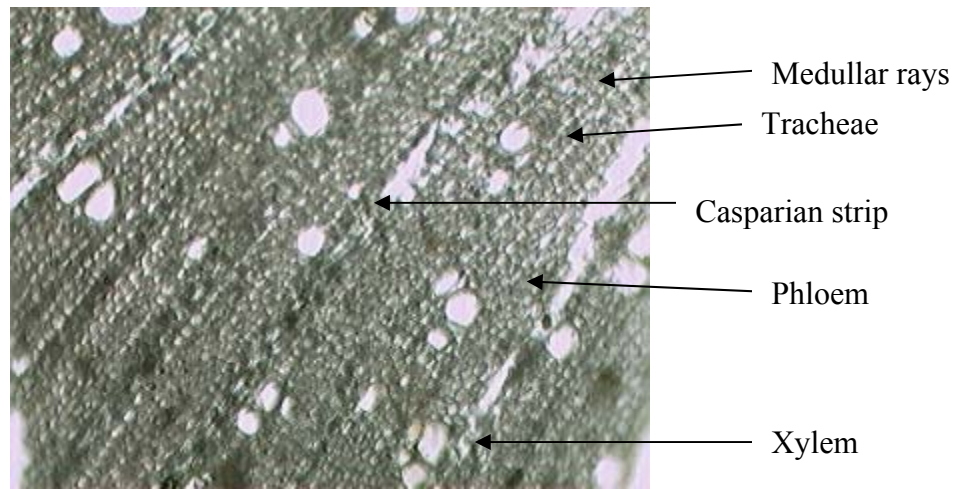


Fig. 11. Transverse section of the root of *Withania coagulans* (Stock) Dunal.

Table 6. Microscopical measurements (μm) of roots of five selected medicinal plants of Family Solanaceae.

Cell type	Dim	<i>Datura innoxia</i> Miller.		<i>Solanum nigrum</i> Linn.		<i>Solanum surattense</i> Bur m.f.		<i>Withania somnifera</i> Linn .		<i>Withania coagulans</i> (Stock) Dunal.	
		L	W	L	W	L	W	L	W	L	W
Epidermis	Min	10	7	5	3	4	2	6	4	6	4
	Max	14	12	9	7	7	4	10	7	9	5
	Mean	12	9	7	6	4.5	3	8.8	5	8	4.9
	Freq value	8	10	6	5	4	3	7	5	8	5
Xylem	Min	14	9	26	15	21	10	12	9	25	17
	Max	18	12	33	19	26	16	18	13	29	21
	Mean	15.5	10.5	28.3	17.6	24.8	14.0	16	11	28	19
	Freq value	14	10	29	18	25	15	15	12	27	19
Tracheae	Min	-	-	17	8	83	47	31	28	30	25
	Max	-	-	22	13	88	55	37	33	35	21
	Mean	-	-	19	11	86	50.2	35	32	33	20
	Freq value	-	-	18	10	86	50	36	29	33	9
Phloem	Min	5	2	4	2	13	10	2	2	5	3
	Max	12	6	9	6	19	15	6	4	8	7
	Mean	9	3	7	5	16	13	4	3	6	5
	Freq value	8	4	8	5	15	12	3	3	5	4
Medullary rays	Min	11	6	17	10	14	8	5	3	2	6
	Max	16	12	23	15	18	12	11	8	4	9
	Mean	15	10	19	13	16	10	8	6	3	8
	Freq value	14	9	18	11	15	9	9	5	3	8
Casparian strip	Min	3	2	-	-	4	2	6	4	5	2
	Max	6	5	-	-	6	4	9	7	8	5
	Mean	4.7	3.1	-	-	4.5	3.5	8	4.5	6.4	3.3
	Freq value	5	3	-	-	4	4	8	4	6	3

Key words: Min = Minimum; Max = Maximum; Freq value = Frequent value; L = Length; W = Width

5.2.b Stem

The anatomical features of stems of the five medicinal plants are illustrated in Table 7.

a) Epidermis

Epidermis in the stem of the studied plant species greatly varied among them. It was single layered and was spherical in shape in *D. innoxia* (Fig. 12). In *S. nigrum* (Fig. 13), *S. surattense* (Fig. 14), *W. somnifera* (Fig. 15) and *Withania coagulans* (Fig. 16), the epidermal cells were spherical-rectangular, oval – rectangular and spherical in shapes respectively. It was single layered in *Solanum surattense* and *Withania somnifera* while in *Withania coagulans* it was 2 layered. The maximum length (13 μ) cell was exhibited in *D. innoxia* and the minimum length (4 μ) was noted in *S. surattense* and the maximum width (8 μ) was obtained in *D. innoxia* and the minimum width (3 μ) in *S. surattense* and *W. coagulans* (Table 7). Many workers have reported variation in the epidermal cells from the different plants including *Inula germanica* and *Inula oculus-christi* (Toma *et al.* 2010), *Convolvulus arvensis* (Sher *et al.*, 2011b) and *Amaranthus viridus* (Khan *et al.*, 2011), which parallel with the present study.

b) Hypodermis

Hypodermis was absent in *W. coagulans*. In rest of the studies plant species it varied in the size, shape and number of layers. It was 3 layered in *Datura innoxia* and *Solanum surattense*. The cells in *Datura innoxia* (Fig. 12) were tetragonal in shape and that in *S. surattense* (Fig. 14) were oval. Hypodermis was 2 layered in *Solanum nigrum* and *W. somnifera*. The cells in *S. nigrum* (Fig. 13) are rectangular in shape and polygonal in shape *W. somnifera* (Fig. 15). The maximum length (22.8 μ) was obtained in *S. surattense* and the minimum length (4.5 μ) in *D. innoxia*. *Solanum surattense* had the maximum width (21.3 μ) and *Datura innoxia* had the minimum width (3 μ) (Table 7). Sanghvi *et al.* (2011) reported 2-3 layers of hypodermis in the stem of *Solanum pseudocapsicum* and this support the present study.

c) Cortex

Cortical regions of stems also showed variation among the plants. The cortex was composed of many layered in *D. innoxia* (Fig. 12), *Solanum surattense* (Fig. 14), *Withania somnifera* (Fig. 15) and *Withania coagulans* (Fig. 16). *Solanum nigrum* (Fig. 13)

had 3-4 layered. It was compactly packed in *Solanum nigrum*. In *Datura innoxia* and *Withania somnifera* there were also no intercellular spaces but they were not as compactly packed as compared to the *S. nigrum*. There were intercellular spaces between the cortical cells in *Solanum surattense* and *Withania coagulans*. But in *Withania coagulans* they were smaller in size as compared to the *Solanum surattense*. In all the plant species the cortex was made of the parenchymatous cells. The maximum length (80.1 μ) cell was noted in *W. coagulans* and the minimum length (43 μ) in *W. somnifera* and the maximum width (50.2 μ) was exhibited in *W. coagulans* and the minimum width (22.8 μ) was shown in *W. somnifera* (Table 7). Mbagwuet *al.* (2007a) also reported variation in the cortical cells in stems of *Solanum nigrum* and *Solanum macrocarpum* that also strengthened the present findings.

d) Pith

Pith in the stem of all the tested species was composed of parenchymatous cells. In *Datura innoxia* (Fig. 12) the pith cells were spherical in shape and closely packed. In *S. surattense* (Fig. 14) and *W. somnifera* (Fig. 15) there were some collenchymatous cells different in their size and shape. In *S. surattense* the cell size was gradually increase in size as move towards the centre. In *S. nigrum* (Fig. 13) these cells polygonal – somewhat rectangular in shape. The cells increased in size as move towards the centre. In *W. somnifera* the pith composed of thin walled parenchymatous cells which were small in size near the vascular tissues and become large in size as move towards the centre. These cells were polygonal in shape in *S. surattense* and *W. somnifera*. As far as in *Withania coagulans* (Fig. 16) the cells were spherical in shape but show somewhat tetragonal in outlines. They are thin parenchymatous cells. The maximum length (97 μ) cell was found in *W. somnifera* and the minimum length (41 μ) was found in *W. coagulans* and the maximum width (80.1 μ) was found in *S. nigrum* and the minimum width (36.9 μ) was found in *W. coagulans* (Table 7). Novello *et al.* (2012) reported that the pith in young stem of *Croton echioides*, is compose by sclerenchymatic sheath which itself consists of brachysclereids which is differed from the present study.

e) Endodermis

Endodermis was absent in *S. nigrum* (Fig. 13). It was single layered in *Solanum surattense* and two layered in *Datura innoxia*, *Withania somnifera* and *Withania*

coagulans. The endodermal cells were spherical in *D. innoxia* (Fig. 12), oval in *W. somnifera* (Fig. 15) and elongated in *Solanum surattense* (Fig. 14) and *W. coagulans* (Fig. 16). The maximum length (18.2 μ) cell was exhibited in *S. surattense* and the minimum length (4.7 μ) was found in *W. coagulans*. The maximum width (14.9 μ) was obtained in *S. surattense* and the minimum width (3.3 μ) was noted in *W. coagulans* (Table 7). Many workers have reported variation in the endodermal cells from other plants (Evans, 2009, Singh & Bhandari, 2000, Wallis, 1985, Hameed *et al.*, 2010, Sher *et al.*, 2011b, Khan *et al.*, 2011) that support the the present findings.

f) Pericycle

Pericycle was absent in *Solanum nigrum* (Fig. 13). It was single layered in *S. surattense* (Fig. 14) and *W. somnifera* (Fig. 15) and two layered in *D. innoxia* (Fig. 12) and *W. coagulans* (Fig. 16). The cells were spherical in *D. innoxia* and *W. somnifera* and oval in *S. surattense* and *W. coagulans*. The maximum length (37 μ) cell was found in *W. coagulans* and the minimum length (6.4 μ) was noticed in *S. surattense*. The maximum width (13.4 μ) was measured in *W. coagulans* and the minimum width (3.3 μ) was obtained in *S. surattense*. The pericycle contained the lignin on their cell walls in *D. innoxia* and *S. surattense*. But the deposition was more in *D. innoxia* than *S. surattense*. In other tested species there were no evidences of lignin in the pericycle. Though there was stratification in pericycle due to lignified walls yet there were no pit channels in this region of these plants (Table 7). Altundag *et al.* (2012) reported that in subspecies *D. romana* subsp. *romana* and *D. romana* subsp. *georgica* single-layered tissue constituted tuber endodermis. Pericycle and endodermis were single layered. which agree with the present study.

g) Vascular tissue

Xylem was spherical in *Datura innoxia* and *Solanum surattense*, oval in *Solanum nigrum*, and elongated *Withania somnifera* and *Withania coagulans*. The maximum length (66 μ) cell was found in *S. nigrum* and the minimum length (35.8 μ) was found in *W. coagulans* and the maximum width (54.1 μ) was found in *S. nigrum* and the minimum width (23.1 μ) was found in *D. innoxia*. As far as phloem was considered it was hexagonal in *D. innoxia* (Fig. 12), spherical in *S. nigrum* (Fig. 13) and *W. somnifera* (Fig. 15), elongated in *S. surattense* (Fig. 14) and *W. coagulans* (Fig. 16). The maximum length

(54.1 μ) cell was found in *D. innoxia* and the minimum length (4.7 μ) was found in *S. nigrum* and the maximum width (42 μ) was found in *D. innoxia* and the minimum width (3 μ) was found in *W. coagulans* (Table 7). Duarte & Debur (2005) reported that the stem had phloem outside and xylem inside, of *Maytenus ilicifolia* which is differed from the present study. Gostin (2011) reported the collateral type of vascular tissue in *Adonis vernalis* which is similar to the present study.

h) Idioblast cells

These cells were absent in all the species except *S. nigrum*. They were located in pith of the stem of *Solanum nigrum*. The plant had tetragonal and monoclinic calcium oxalate crystal. The mean length and width of the idioblast cell was 66 μ and 54.1 μ (Fig. 13; Table 7). Essiett *et al.* (2010) reported calcium oxalate abundant in pith and parenchyma of the cortex region of the stem of *Diodia scandens* which agree with the present study.

i) Medullary rays

These cells were located between the two vascular bundles in these plants. They were 2-3 in *D. innoxia*, 3-21 in *S. surattense*, 6-23 in *W. somnifera* and 6-many layers in *S. nigrum* and *W. coagulans*. The cells were spherical in *S. surattense* (Fig. 14) and *S. nigrum* (Fig. 13), spherical – elongated *D. innoxia* (Fig. 12) and *W. somnifera* (Fig. 15), tetragonal in *W. coagulans* (Fig. 16). The maximum length (8 μ) cell was found in *D. innoxia* and the minimum length (2.8 μ) was found in *S. nigrum* and the maximum width (5.8 μ) was found in *W. somnifera* and the minimum width (1.8 μ) was found in *W. coagulans* (Table 7).

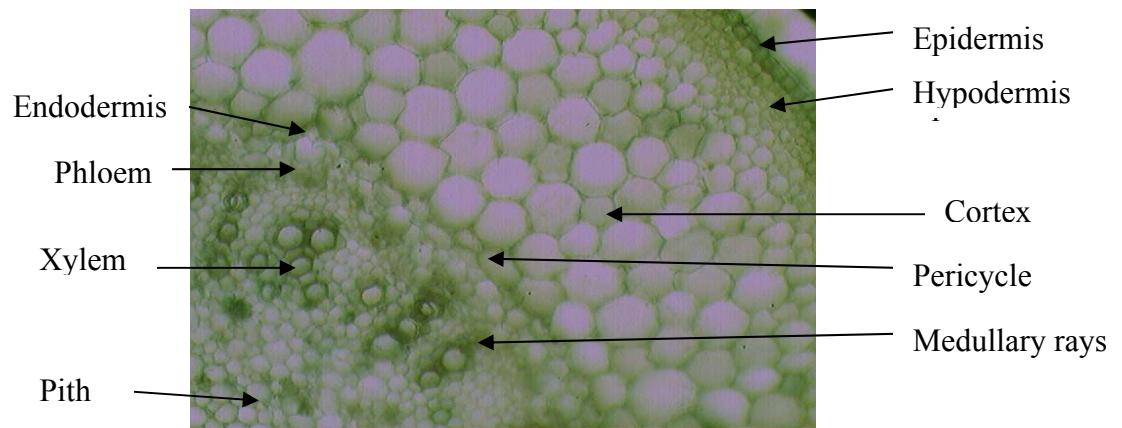


Fig. 12. Transverse section of the stem of *Datura innoxia* Miller.

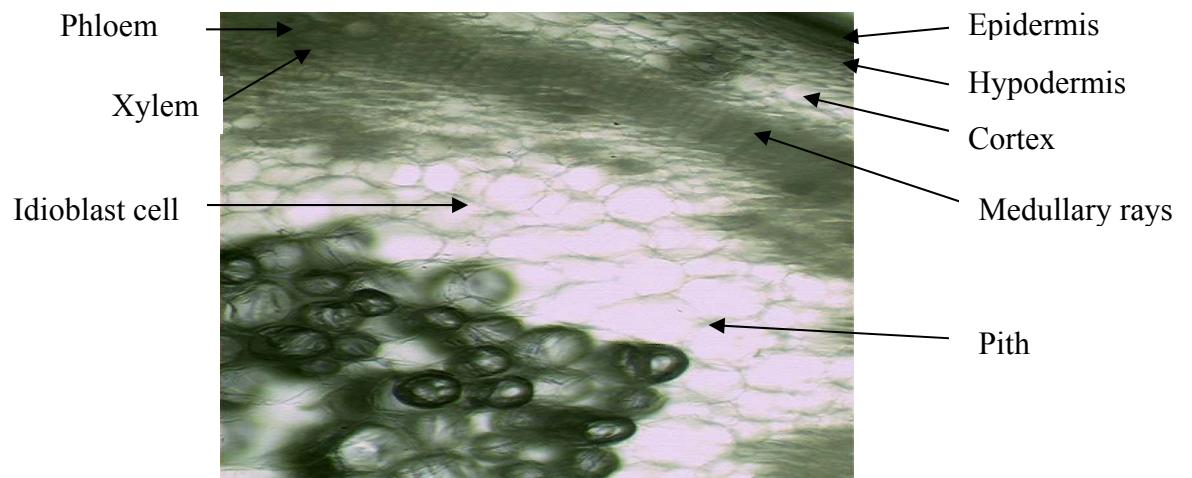


Fig. 13. Transverse section of the stem of *Solanum nigrum* Linn.

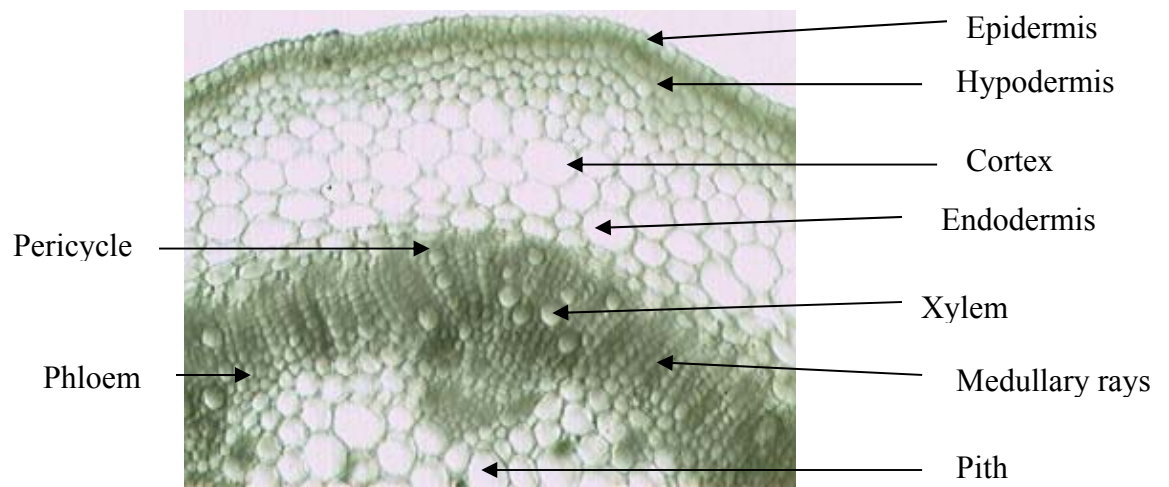


Fig. 14. Transverse section of the stem of *Solanum surattense* Burm.f.

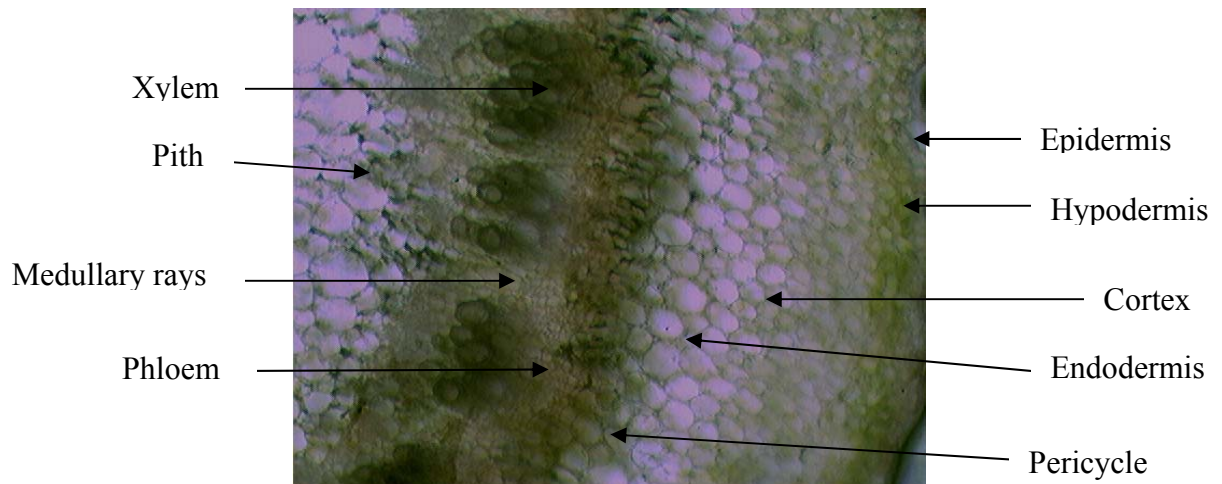


Fig. 15. Transverse section of the stem of *Withania somnifera* Linn.

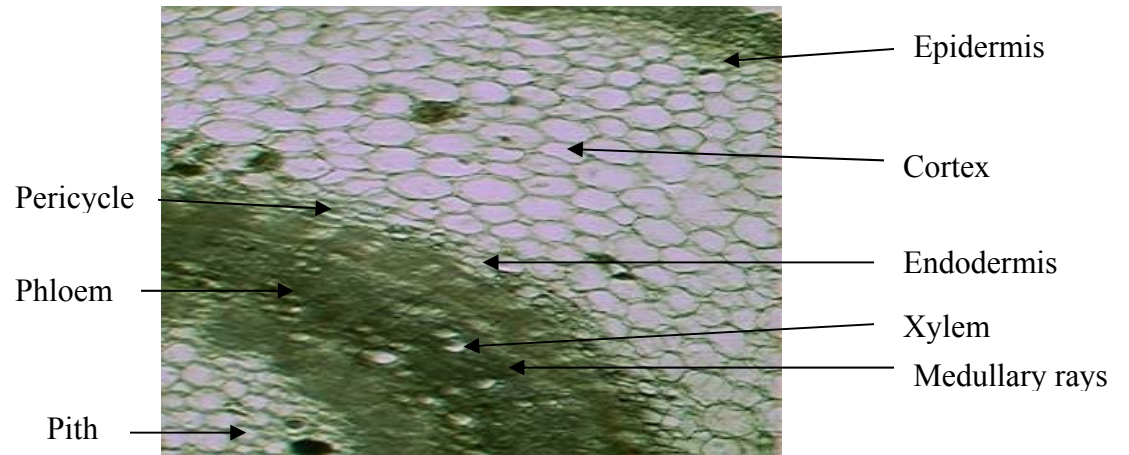


Fig. 16. Transverse section of the stem of *Withania coagulans* (Stock) Dunal.

Table 7. Microscopic measurements (μm) of the stems of the some selected medicinal plants of Family Solanaceae.

Cell type	Dim	<i>Datura innoxia</i> Miller.		<i>Solanum nigrum</i> Linn.		<i>Solanum surattense</i> Burm.f.		<i>Withania somnifera</i> Linn.		<i>Withania coagulans</i> (Stock) Dunal.	
		L	W	L	W	L	W	L	W	L	W
Epidermis	Min	13	9	6	4	4	2	8	6	3	2
	Max	18	13	9	7	7	5	12	9	6	5
	Mean	13	8.3	8	6.1	4.5	3.1	9.1	6.8	4.7	3.3
	Freq value	13	8	8	6	4	3	8	6	5	3
Hypodermal cells	Min	4	2	6	4	21	19	3	2	-	-
	Max	7	4	9	5	26	23	6	5	-	-
	Mean	4.5	3	8	4.9	22.8	21.3	4.7	3.1	-	-
	Freq value	4	3	8	5	22	21	5	3	-	-
Cortex	Min	58	39	52	38	50	46	43	21	78	47
	Max	65	45	59	46	62	53	48	26	82	55
	Mean	62.8	41.7	54.1	40	55.8	49	43	22.8	80.1	50.2
	Freq value	63	42	55	42	57	47	43	22	80	50
Xylem	Min	39	20	64	52	36	24	34	22	33	25
	Max	43	25	70	59	39	25	37	26	39	28
	Mean	38.1	23.1	66	54.1	38	24.9	36.1	23.6	35.8	25.9
	Freq value	38	23	66	55	38	25	36	23	35	25
Phloem	Min	52	38	3	4	4	2	9	5	6	3
	Max	59	44	6	7	5	5	13	10	9	5
	Mean	54.1	42	4.7	4.5	4.9	3.1	11	6.8	8	3
	Freq value	55	40	5	4	5	3	11	6	8	3
Pith	Min	44	37	95	78	58	39	95	60	38	31
	Max	50	41	99	82	65	45	101	72	45	37
	Mean	46	40.2	96.7	80.1	62.8	41.7	97	66.4	41	36.9
	Freq value	46	40	96	80	63	42	96	66	40	35
Medullary rays	Min	6	3	5	2	3	2	4	4	3	2
	Max	9	6	8	6	7	5	5	6	5	5
	Mean	8	4.7	2.8	4.1	3.1	2.9	3.5	5.8	4.8	1.8
	Freq value	8	5	3	5	3	4	4	5	4	2
Endodermis	Min	13	11	-	-	16	14	11	6	4	2
	Max	19	16	-	-	20	16	14	9	7	5
	Mean	15.8	13.8	-	-	18.2	14.9	12.3	8.1	4.7	3.3
	Freq value	15	14	-	-	18	15	12	8	4	3
Pericycle	Min	24	10	-	-	5	2	13	9	19	7
	Max	28	18	-	-	8	5	16	12	57	17
	Mean	25.5	13.1	-	-	6.4	3.3	14.7	10.7	37	13.4
	Freq value	25	12	-	-	6	3	15	11	35	15
Idioblast cells	Min	-	-	64	52	-	-	-	-	-	-
	Max	-	-	70	59	-	-	-	-	-	-
	Mean	-	-	66	54.1	-	-	-	-	-	-
	Freq value	-	-	66	55	-	-	-	-	-	-

Key words: Min = Minimum; Max = Maximum; Freq value = Frequent value; L = Length; W = Width

5.2.cLeaf

a) Epidermis

In *Datura innoxia*(Fig. 17; Fig. 19; Fig. 21; Fig. 22), *Solanum nigrum*(Fig. 23; Fig. 25), *Solanum surattense* epidermises were thin, composed of epidermal cells, trichomes, stomata and subsidiary cells. The epidermal cells were compactly arranged and variously shaped (*Datura innoxia*), wavy (*Solanum surattense*), containing small amount of granulated chloroplast. In *Datura innoxia*, *Solanum nigrum*, *Solanum surattense*, the epidermal cells were generally greater in number as compared to stomata. In *Datura innoxia* the epidermal cells were mostly polygonal shaped with slightly straight walls, some of the cells also show some waviness in their walls. In *Solanum nigrum* and *Withania somnifera*(Fig. 26; Fig. 27) the epidermal cells were irregular in shape, with wavy outlines; the waviness was deep showing sinousities (lower) and the upper epidermal cells are polygonal in shape, with wavy outlines; the waviness was not so deep. In *Solanum surattense* the epidermal cells were either polygonal or irregular (lower) and elongated, polygonal, or irregular in shape (upper), with wavy outlines; the waviness was not so deep. The maximum length (21μ) of the upper epidermis was noted in *Withania somnifera* and the maximum widths (6μ) in *Datura innoxia* and *Withania coagulans*. The maximum length (18μ) of the lower epidermis was exhibited in *Withania somnifera* and the maximum widths (6μ) in *Datura innoxia* and *Withania coagulans*(Fig. 31; Table 8). Mbagwu *et al.* (2007) reported the comparative leaf epidermal studies of *Solanum macrocarpon* and *Solanum nigrum*. The upper and lower epidermal cell wall of *Solanum nigrum* varied in shape i.e. pentagonal, rectangular to hexagonal and in the *S. macrocarpon* they are irregular in shape that support the present findings.

b) Vascular bundle

In all the five plant species the transverse sections show bicollateral vascular bundle with intraaxillary phloem towards the centre and outer phloem which is the basic characteristics of the Solanaceae family. In *Datura innoxia*(Fig.20), *Solanum nigrum*(Fig. 24), *Solanum surattense*, *Withania somnifera* (Fig.30) and *Withania coagulans* (Fig. 31) the total number of the xylem cells was 45 – 55, 35 – 42, 30 – 38, 40 – 65 and 25 – 36, respectively. The xylem forms the curved arc like structure. The xylem contained large vessels. The maximum length (63.8μ) of the xylem was recorded in *Datura innoxia* and

the minimum (8.5 μ) in *Solanum nigrum*. The maximum width (17.1 μ) was exhibited in *Datura innoxia* and the minimum (5.2 μ) in *Solanum nigrum*. Phloem was scattered in the ground tissue in all the five plant species. The phloem region of *Datura innoxia* was devoid of bast fibers and in other plant these bast fibers were present in the phloem region. The maximum length (66 μ) of the phloem was obtained in *Datura innoxia* and the minimum (4.7 μ) in *Solanum nigrum*. The maximum width (54.1 μ) was noted in *Datura innoxia* and the minimum (3.3 μ) in *Solanum nigrum* (Table 8). Rangari (2008) reported the bicollateral vascular tissues in the leaf of *Datura stramonium*. Similarly Evans (2009) also reported such type of tissues from *Datura stramonium* and *Hyoscyamus niger* and *Atropa belladonna* which agree with the present study.

c) Collenchyma

Subepidermal collenchyma was present in both upper and lower epidermis which was 2 layered in *Datura innoxia* (Fig. 19), 3 layers in *Solanum nigrum*, 3-4 layers in *Solanum surattense* and 3- 5 layers in *Withania somnifera* and was absent in *Withania coagulans* on both epidermises. The maximum length (95 μ) of the cell was exhibited in *Datura innoxia* and the minimum (38 μ) in *Solanum nigrum*. The maximum width (66 μ) of the cell was obtained in *Datura innoxia* and the minimum (26 μ) in *Solanum nigrum* (Table 8). Geetha *et al.* (2006) reported that there is a broad zone of collenchymatous cells beneath the leaf epidermis of *Achyranthes rubrofusca*. Putiyananet *al.* (2009) reported microscopic characters of transverse section of Fak khao's leaves and were compared to the leaf powders showing the upper and lower epidermis, trichome, collenchyma, palisade mesophyll, spongy mesophyll, stoma (guard cell), vascular bundles, who also concluded similar results.

d) Mesophyll cells

The mesophyll is divided in to palisade and spongy tissue. Palisade tissue cells are large, elongated, arranged in a single layer in *D. innoxia* (Fig. 18), *Solanum nigrum* (Fig. 23; Fig. 24) and *Withania somnifera* and 2 layers in *Solanum surattense* and *Withania coagulans* (Figs. 31; 32). These cells had no intercellualr spaces and were compactly packed. The maximum lengths (97 μ) was noticed in *Solanum nigrum* and the minimum was (50.2 μ) in *Datura innoxia*. The maximum widths (80.1 μ) was exhibited in *Withania coagulans* and the minimum was (41.7 μ) in *Datura innoxia*. The spongy tissue is made

of cells which were spherical in *Datura innoxia*, rounded or irregular in *Solanum nigrum*, polygonal in *Solanum surattense* and *Withania somnifera* and somewhat rectangular in *Withania coagulans* in shape and arranged in 3-5 layers in *S. nigrum*, a single layer in *Datura innoxia*, 2 layers in *Solanum surattense* and *Withania somnifera* and 3 layers in *Withania coagulans*. There are large intercellular spaces among these cells in *Solanum nigrum* and in other these intercellular spaces were absent. The maximum length (43μ) of the cell was showed by *Withania coagulans* and the minimum (16μ) in *Solanum nigrum*. The width (25.9μ) of the cell was recorded by *Datura innoxia* and the minimum (5.8μ) in *Solanum nigrum* (Table 8). Geetha *et al.* (2006) reported that mesophyll tissue is not well differentiated into palisade. Spongy parenchymatous cells with 4 or 5 layers are seen in the adaxial part which occurs in the vertical rows, the cells are oblong and resemble the palisade in the leaf of *Achyranthes rubrofusca*, which is differed from the present study.

e) Idioblast cells

Some cells of the palisade and spongy tissues contain dark groups of crystals (tetragonal, raphides and monoclinic) in *Solanum nigrum*, (monoclinic, rosette and sphaerocrystal) in *Withania somnifera* and in other plants these crystals were absent. The mean length of the idioblast cells in *Solanum nigrum* and *Withania somnifera* was 4.5μ , 8.2μ and width 3μ and 4.9μ , respectively (Table 8). Evans (2009) reported that *Datura stramonium* contained microcrystal which is differed from our results, also support the present study.

f) Sclerenchyma

The sclerenchymatous cells were also present in all the five species. They were rounded in *Datura innoxia*, spherical in *Withania somnifera* and *Withania coagulans*, and somewhat polygonal in *Solanum nigrum* and *Solanum surattense*. The maximum lengths (62.8μ) was observed in *Datura innoxia* and the minimum (36.1μ) was noticed in *Withania somnifera*. The maximum widths (54.1μ) was recorded in *Solanum surattense* and the minimum was (25.9μ) in *Withania somnifera* which is similar to the present study.

g) Pith

It was present in the center. It was composed of parenchymatous cells. The cells were spherical in *Solanum nigrum*, *Withania somnifera* and *Datura innoxia* and were rounded in *Solanum surattense* and *Withania coagulans*. The maximum length (19 μ) of the cell was showed in *Datura innoxia* and the minimum (6.4 μ) in *Withania coagulans*. The maximum width (10 μ) of the cell was exhibited in *Datura innoxia* and the minimum (3.3 μ) in *Withania coagulans*(Table 8). Hameed *et al.* (2010) reported pith cells from *Rumex dentatus*, *Rumex hastatus*, *Rumex nepalensis*, *Rheum austral*, *Polygonum plebejum* and *Polygonum persicaria*, also support the present study.

h) Trichomes

The trichomes in *Datura innoxia*(Figs. 17, 21, 22) were uniseriate, peltate, glandular, each hair was up to 302 μ in lower and 277 μ in the upper epidermis long. The hairs consisted of simple or compound foot, body differentiated into 2-3 in lower and 2-celled in upper epidermis celled stalk and unicellular head. The cells of the stalk were thick walled, tapering towards the apex, the upper most cell of the stalk was slightly curved, so that head lie somewhat obliquely to the stalk. Head was thin walled possessing denser contents in both epidermis trichomes. In the upper epidermis of *S. nigrum* hairs were simple, conical and non-glandular; each hair was up to 234 μ long. Youngken (1950) reported that *Atropa belladonna* leaves possessed glandular trichomes, long and short, with unicellular and multicellular heads. Foot simple, body was uniseriate and three or more celled. The apical cells was conical, the hair was somewhat curved with thick and straight; having denser contents. Evans (2009) reported that *Datura stramonium* contained glandular hairs and clothing trichomes. In the lower epidermis of *Solanum nigrum* the trichomes were of two type namely simple conical non-glandular hairs and globose glandular hairs. In simple conical type the hairs were 2-4 celled, the emergent portion of the hair was being straight or curved. The apical cell was conical in each case, thin walled with thin contents. Each hair was up to 201 μ long. While in globose types the hair consists of a simple foot, unicellular stalk and 5 celled head. The cells of the stalk were broader at the base, with thin contents, whereas the head was rounded, with dense contents. Geetha *et al.*, (2006) reported dense trichomes on both adaxial and abaxial surfaces of the leaf of *Achyranthes rubrofusca*. These trichomes are multicellular,

uniseriate and unbranched. Trichomes were absent in both epidermises of *Solanum surattense*. In lower epidermis of *Withania somnifera* (Figs. 26, 27, 28) the trichomes were multicellular branched and non-glandular. Each hair was up to 145 μ long, whereas the lateral branches were up to 84 μ long. Foot was simple, the body consists of branched emergent portion; the lateral branches were unicellular, the branches were pointed at the apices and were somewhat curved. The cells contents were present even at maturity. The cell walls were thick and smooth. In upper epidermis of *Withania somnifera* the trichomes were of two types viz, simple branched non-glandular and simple conical glandular. In the former type each hair was up to 179 μ long, with simple foot and branched emergent portion; the branches were unicellular, thin walled and somewhat curved, the apices of the branches were pointed, whereas in the later type each hair was up to 243 μ long, with simple foot, the body consists of stalk and a head. The stalk was 5-8 celled tapering towards the tip. The head was somewhat tri-angular in shape and unicellular. The cell walls were thick and smooth. Similarly different types of trichomes were reported by Rashid *et al.*, (1987) from *Solanum nigrum*, *Datura stramonium*, *Withania somnifera*, *Datura metel* and *Atropa acuminata* who concluded similar results.

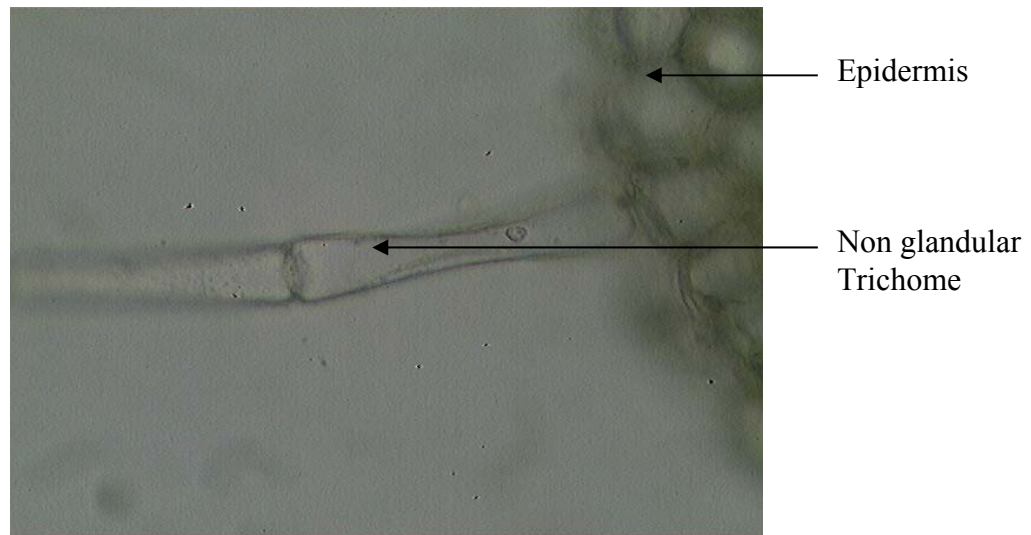


Fig. 17. A portion of leaf of *Datura innoxia* Miller.

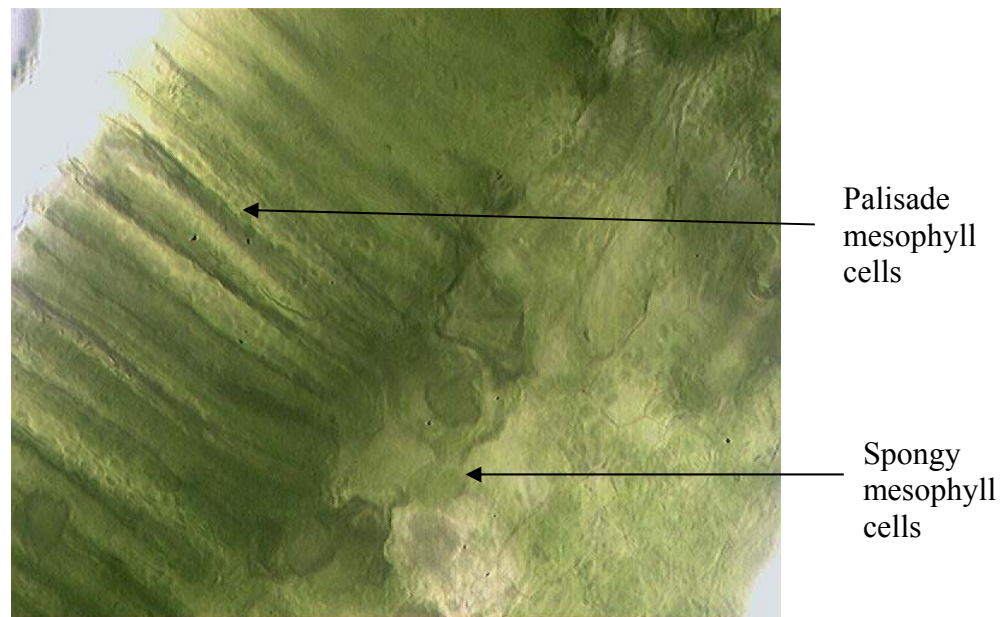


Fig. 18. A portion of leaf of *Datura innoxia* Miller.

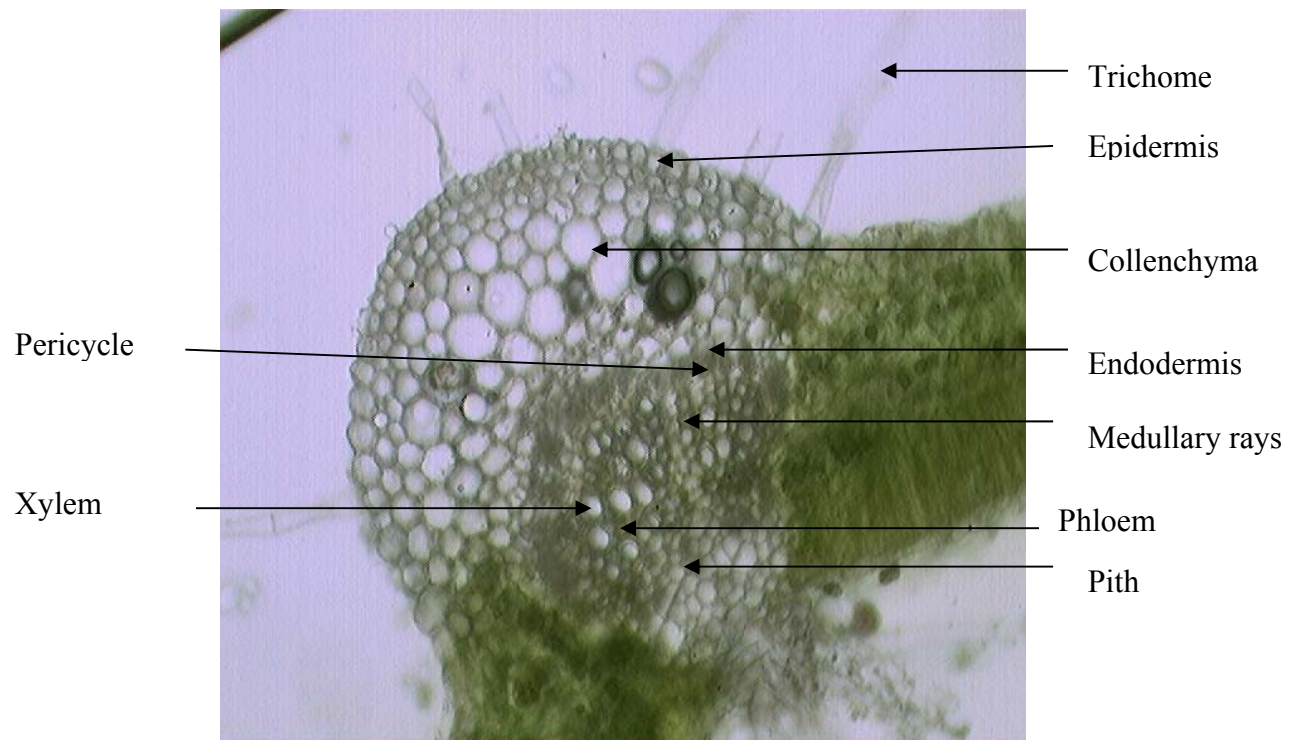


Fig. 19. A portion of leaf of *Datura innoxia* Miller.

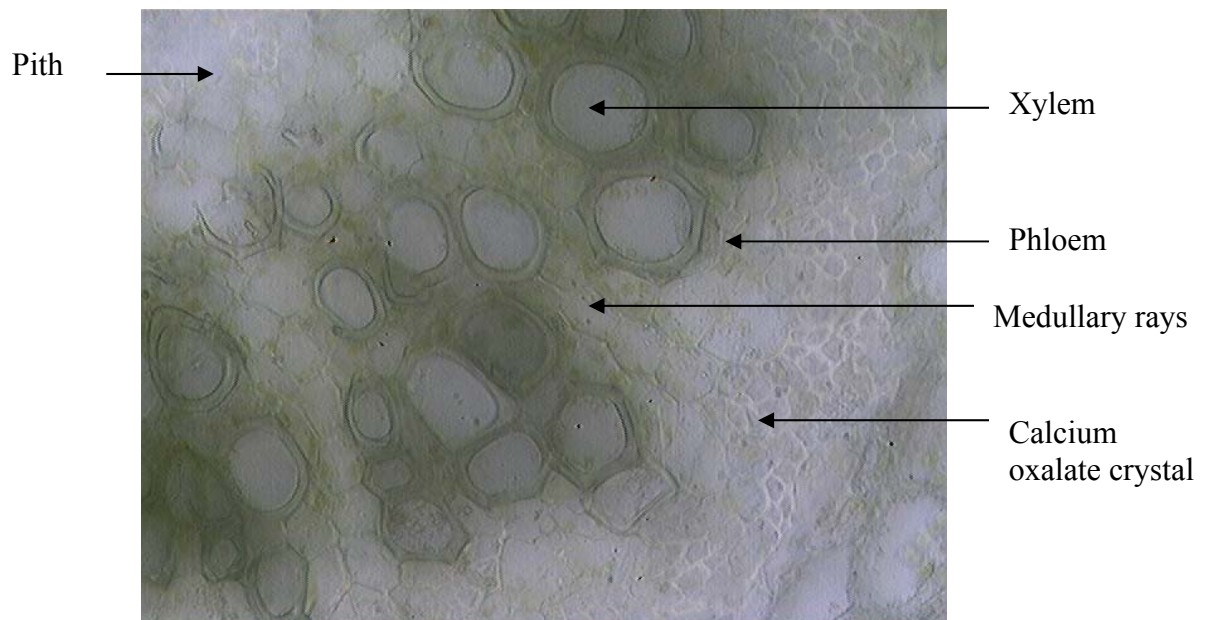


Fig. 20. A portion of leaf of *Datura innoxia* Miller.

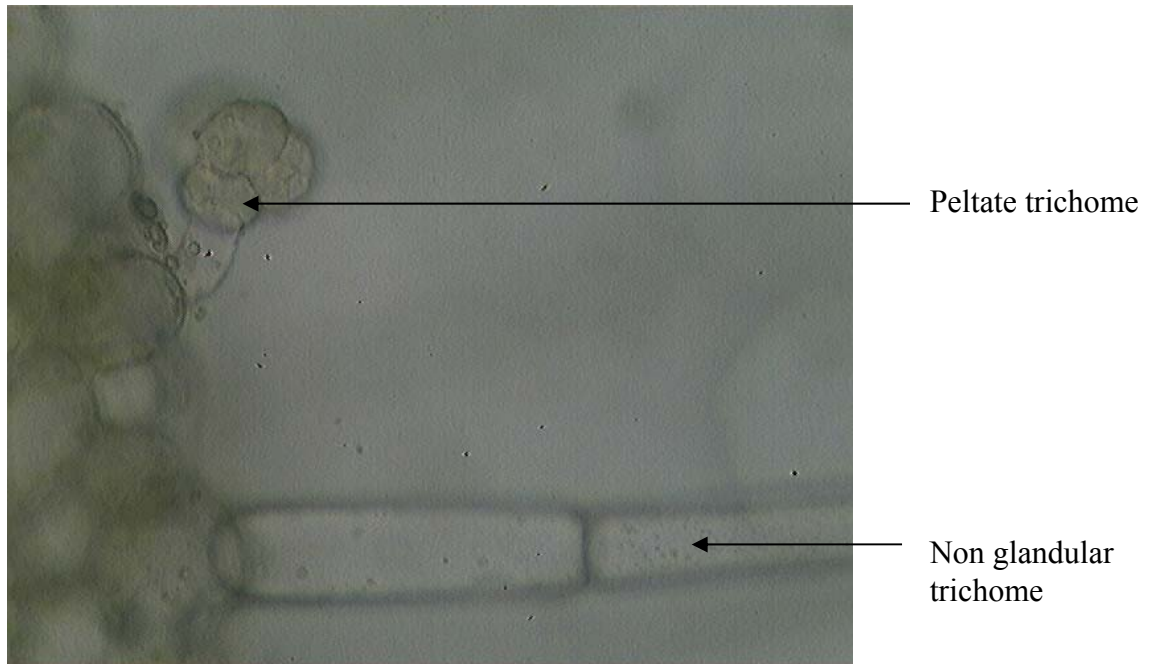


Fig. 21. Two different trichomes on the leaf of *Datura innoxia* Miller.

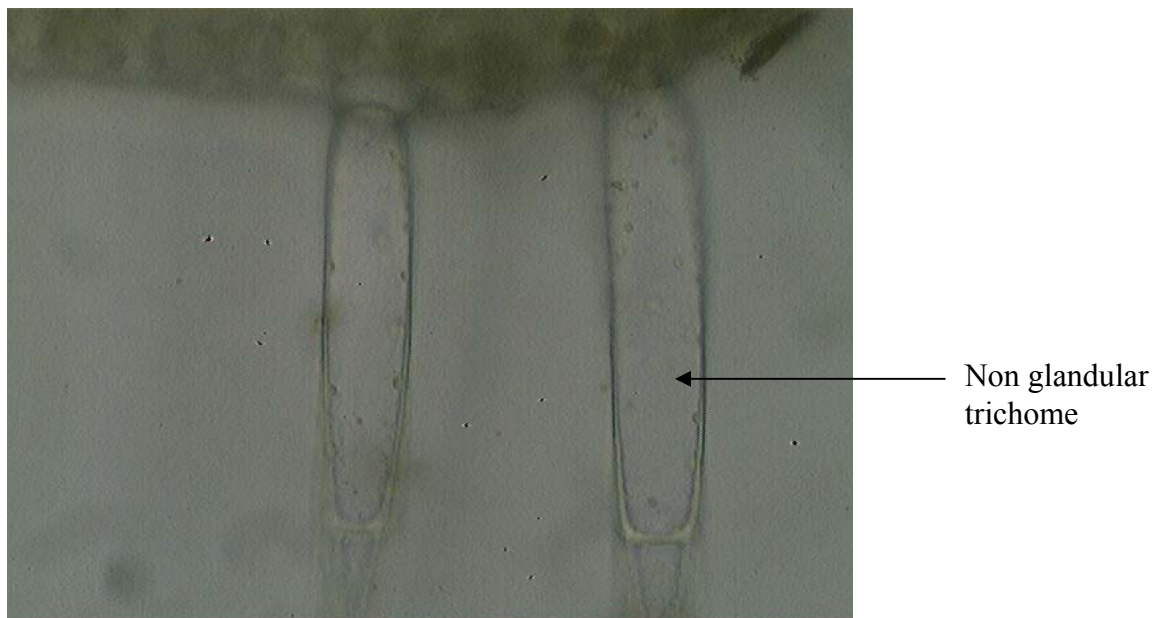


Fig. 22. Trichomes on the leaf of *Datura innoxia* Miller.

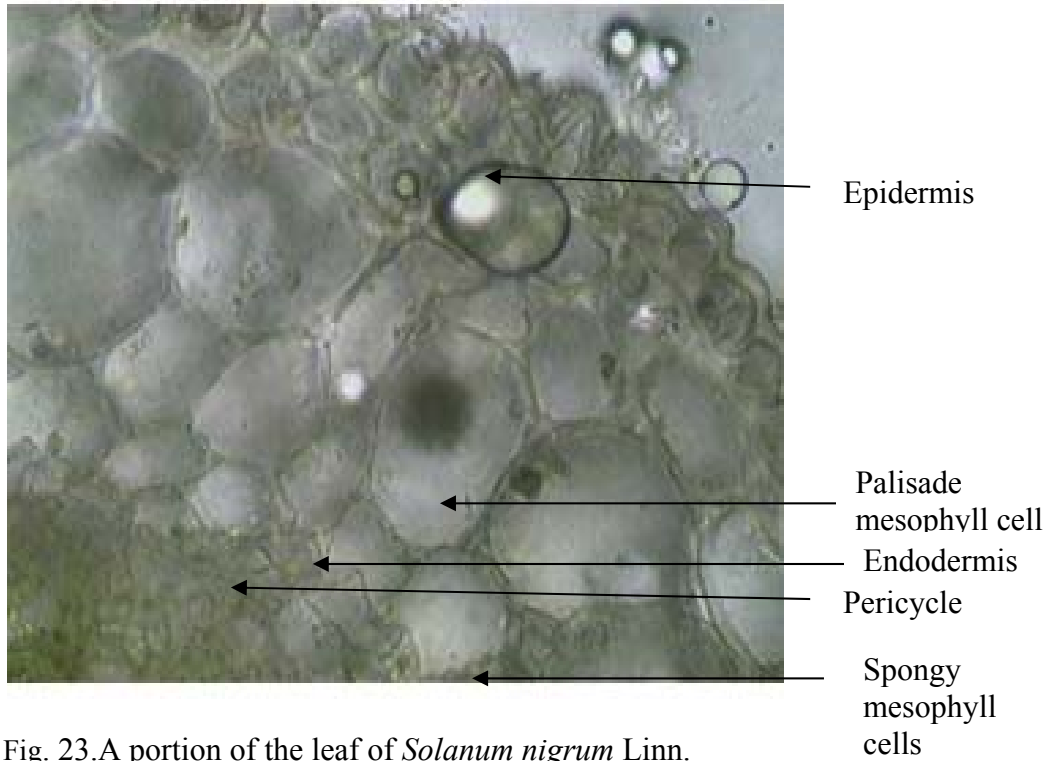


Fig. 23. A portion of the leaf of *Solanum nigrum* Linn.

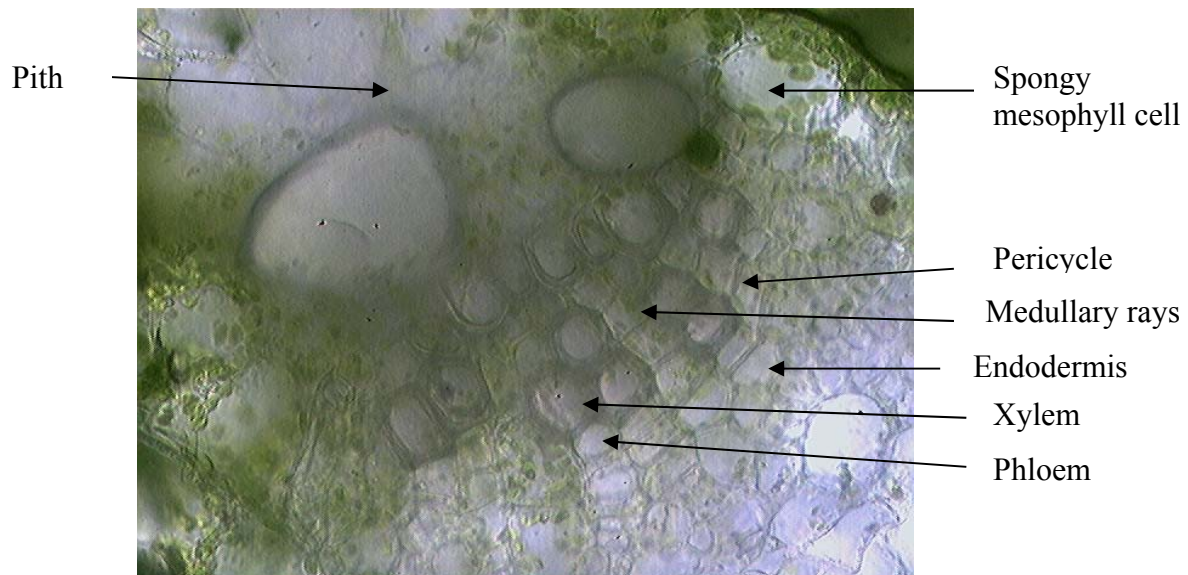


Fig. 24. A portion of the leaf of *Solanum nigrum* Linn.

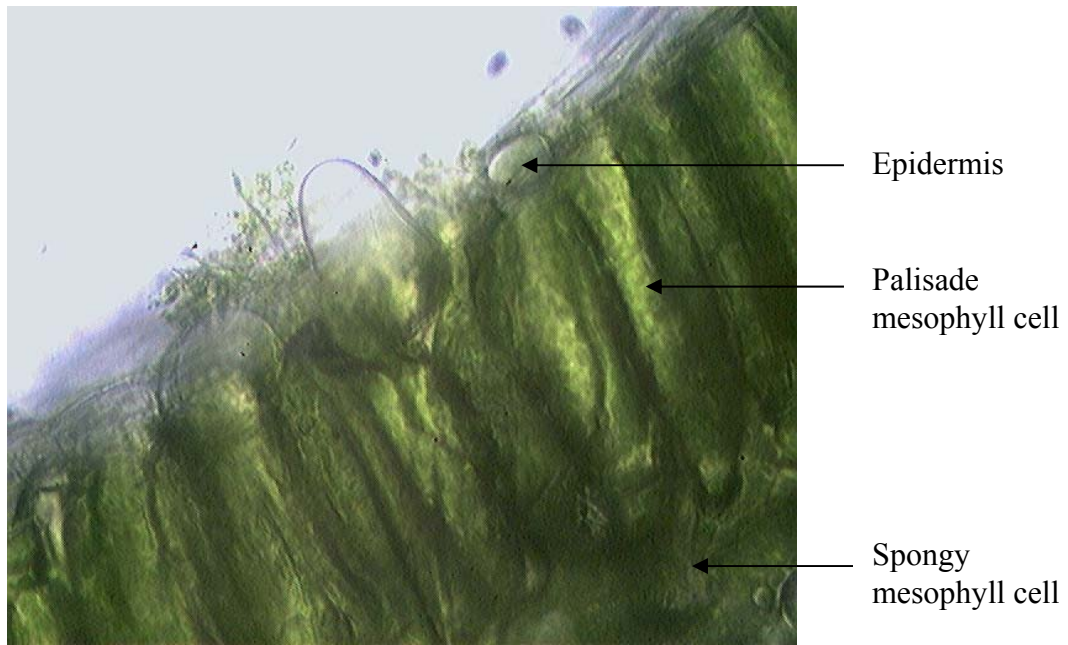


Fig. 25. A portion of the leaf of *Solanum nigrum* Linn.



Fig. 26. A portion of the leaf of *Withania somnifera* Linn showing trichome.



Fig. 27. A portion of the leaf of *Withania somnifera* Linn. showing trichome.



Fig. 28. A portion of the leaf of *Withania somnifera* Linn. showing trichome.



Fig. 29. A portion of leaf of *Withania somnifera* Linn. Showing stomata.

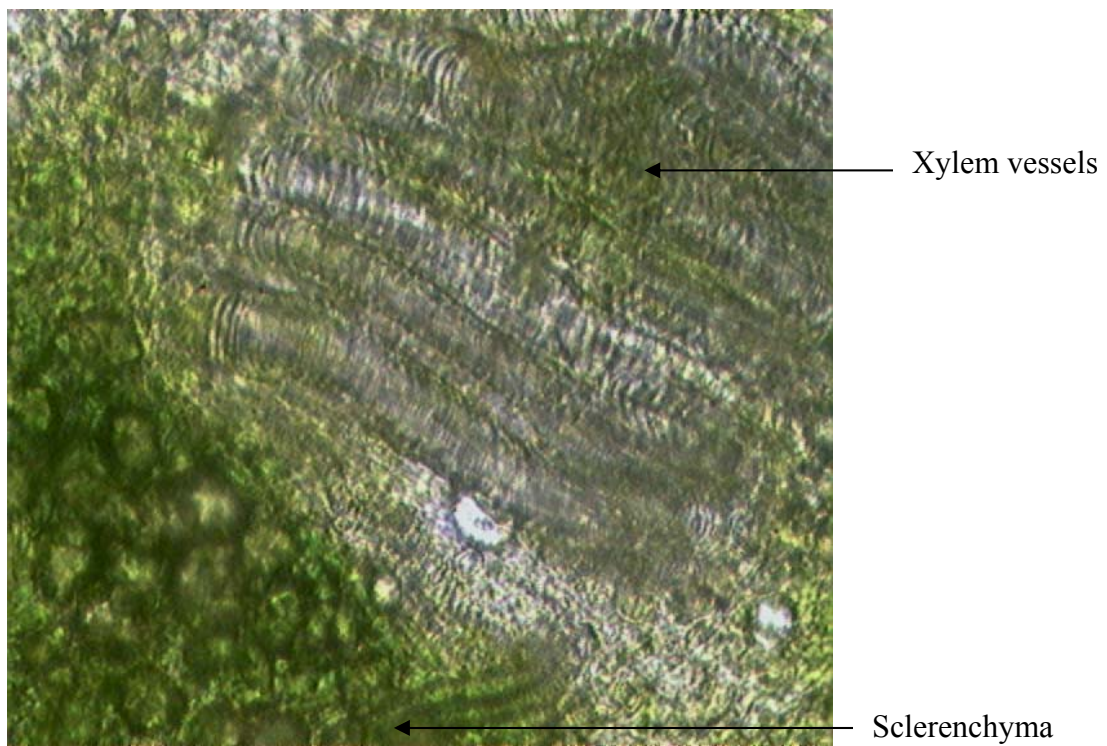


Fig.30. A portion of leaf of *Withania somnifera* Linn.

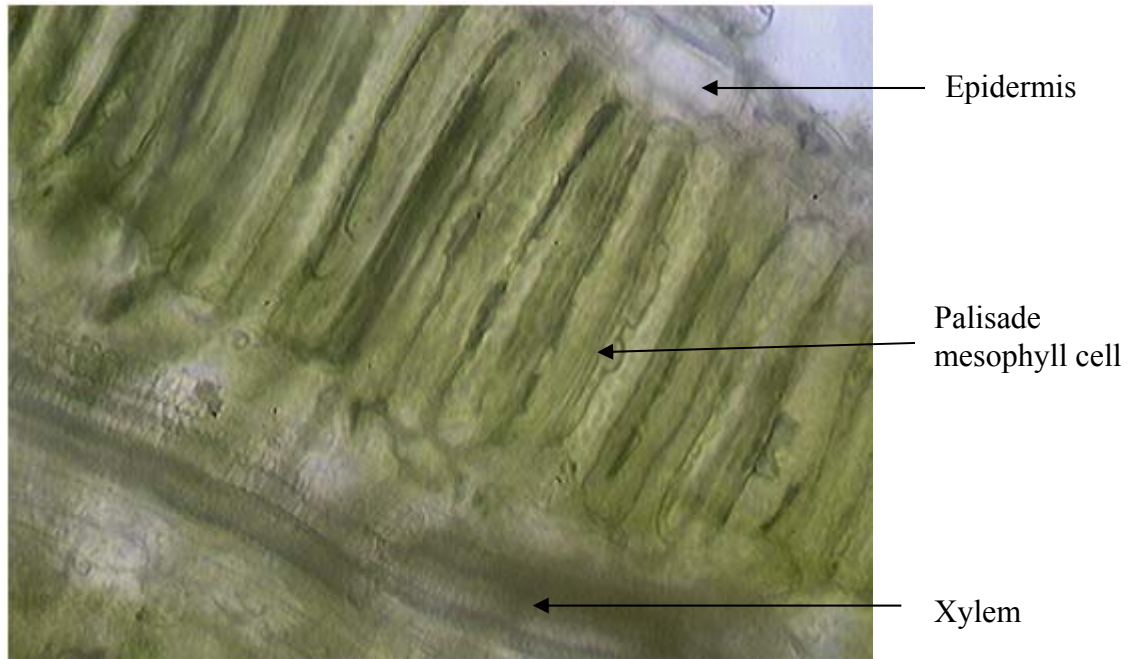


Fig. 31. A portion of leaf of *Withania coagulans* (Stocks) Dunal.

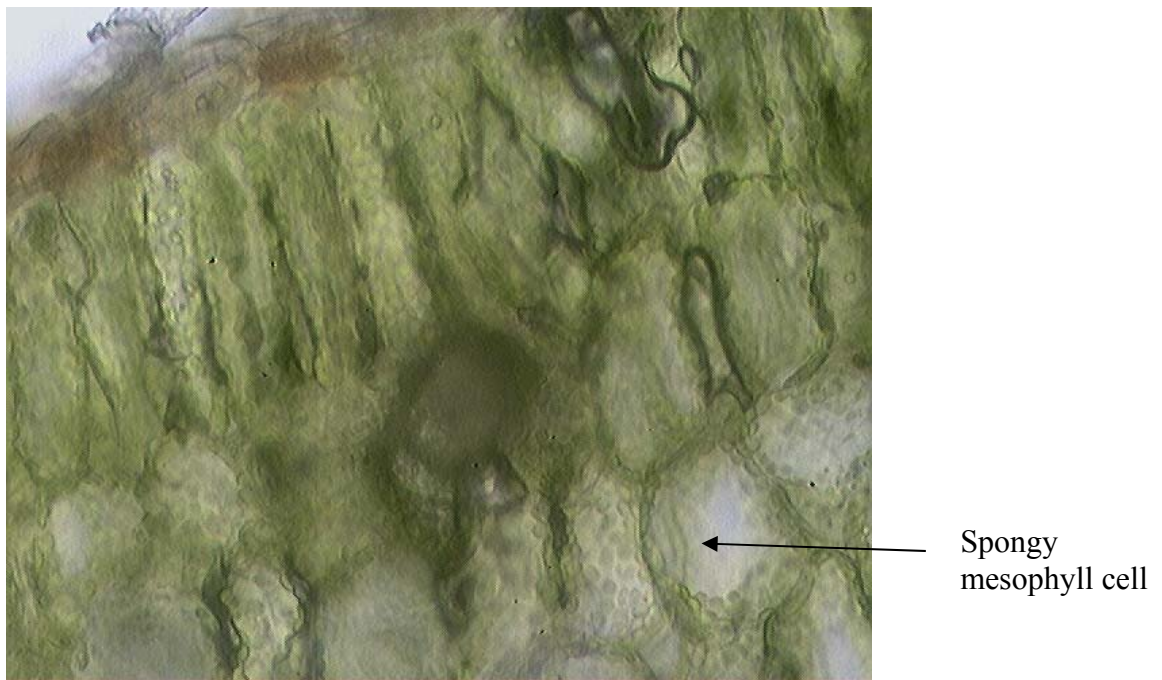


Fig. 32. A portion of leaf of *Withania coagulans* (Stocks) Dunal.

Table 8. Microscopic measurements (μm) of the leaves of the some selected medicinal plants of Family Solanaceae.

Cell type	Dim	<i>Datura innoxia</i>		<i>Solanum nigrum</i>		<i>Solanum surattense</i>		<i>Withania somnifera</i>		<i>Withania coagulans</i>	
		L	W	L	W	L	W	L	W	L	W
Upper epidermis	Min	5	4	7	4	5	2	9	4	8	5
	Max	12	8	13	9	10	6	14	7	12	9
	Mean	9	6	8.5	7	6	4	11	5	10	6
	Freq value	8	5	9	6	4	4	10	6	10	7
Idioblast cells	Min	-	-	4	2	-	-	6	4	-	-
	Max	-	-	7	4	-	-	10	6	-	-
	Mean	-	-	4.5	3	-	-	8.2	4.9	-	-
	Freq value	-	-	4	3	-	-	8	5	-	-
Pith	Min	14	9	6	4	11	6	6	4	5	2
	Max	21	12	10	6	14	9	9	7	8	5
	Mean	19	10	8.2	4.9	12.3	8.1	8	4.5	6.4	3.3
	Freq value	18	10	8	5	12	8	8	4	6	3
Xylem	Min	52	8	7	4	12	6	15	10	14	8
	Max	66	21	12	7	16	11	22	16	19	13
	Mean	63.8	17.1	8.5	5.2	15	9.5	18.6	14	17	12
	Freq value	58	18	8	6	14	8	19	12	16	11
Phloem	Min	64	52	6	4	11	6	4	2	13	9
	Max	70	59	9	7	14	9	7	5	16	12
	Mean	66	54.1	8	6.1	12.3	8.1	4.7	3.3	14.7	10.7
	Freq value	66	55	8	6	12	8	4	3	15	11
Lower epidermis	Min	64	52	12	4	30	12	52	39	24	10
	Max	70	59	25	10	52	25	59	45	28	18
	Mean	66	54.1	16.9	5.8	43.8	15.8	54.1	41.7	25.5	13.1
	Freq value	66	55	15	5	45	15	55	42	25	12
Collenchyma	Min	88	65	33	24	58	39	52	35	-	-
	Max	100	79	42	30	65	45	59	43	-	-
	Mean	95	66	38	26	62.8	41.7	54.1	38.7	-	-
	Freq value	96	70	40	29	63	42	55	42	-	-
Palisade cells	Min	47	39	95	60	52	38	64	47	95	78
	Max	55	45	101	72	59	44	70	55	99	82
	Mean	50.2	41.7	97	66.4	54.1	42	66	50.2	96.7	80.1
	Freq value	50	42	96	66	55	40	66	50	96	80
Spongy mesophyll cells	Min	33	25	12	4	15	12	21	20	43	19
	Max	40	28	25	10	21	16	26	25	48	23
	Mean	36.1	25.9	16.9	5.8	19	13	22.8	21.3	43	22.5
	Freq value	35	25	15	5	18	14	22	22	43	21
Sclerenchyma	Min	58	38	34	21	55	52	33	25	47	39
	Max	65	46	42	28	62	59	40	28	55	45
	Mean	62.8	40.7	37	27	58.8	54.1	36.1	25.9	50.2	41.7
	Freq value	63	44	38	27	60	55	35	25	50	42

Key words: Min = Minimum; Max = Maximum; Freq value = Frequent value

5.3 Constant parameters of leaf

Quantitative microscopical study gives valuable information regarding specific leaf constants such as vein islet, vein termination number and palisade ratio. Vein islet number of *D. innoxia* was $11.5 - 16.5/\text{mm}^2$, *Solanum nigrum* was $8.75 - 19.25/\text{mm}^2$, *Solanum surattense* was $19.5 - 23.75/\text{mm}^2$, *Withania somnifera* was $20.5 - 26.75/\text{mm}^2$ and that of *Withania coagulans* was $22.5 - 27.75/\text{mm}^2$. The vein termination number of *Datura innoxia* was $17.5 - 20.75/\text{mm}^2$, *Solanum nigrum* was $18.75 - 23.75/\text{mm}^2$, *Solanum surattense* was $21.5 - 26.75/\text{mm}^2$, *Withania somnifera* was $22.5 - 26.75/\text{mm}^2$ and that of *Withania coagulans* was $11.75 - 17.5/\text{mm}^2$. The palisade ratio of the *Datura innoxia* was $19.5 - 28.75/\text{mm}^2$, *Solanum nigrum* was $10.75 - 18.5/\text{mm}^2$, *Solanum surattense* was $23.75 - 32.75/\text{mm}^2$, *Withania somnifera* was $26.5 - 34.8/\text{mm}^2$, and that of the *Withania coagulans* is $27.5 - 34.5/\text{mm}^2$ (Table 9). Khan *et al.*, (2011) studied *Amaranthus viridis* for the various parameters for pharmacognostical standards and reported vein islet, vein termination number and palisade ratio of the leaf. Abere *et al.* (2009) reported these parameters for the leaf of *Dissotis rotundifolia*. Putiyananet *et al.*, (2009) studied the vein islet, vein termination number and palisade ratio of Fak khao's leaves. Sethuramani *et al.*, (2010) reported the vein islet number, vein termination number and palisade ratio of the leaf of *Morus alba* which agree with the present findings.

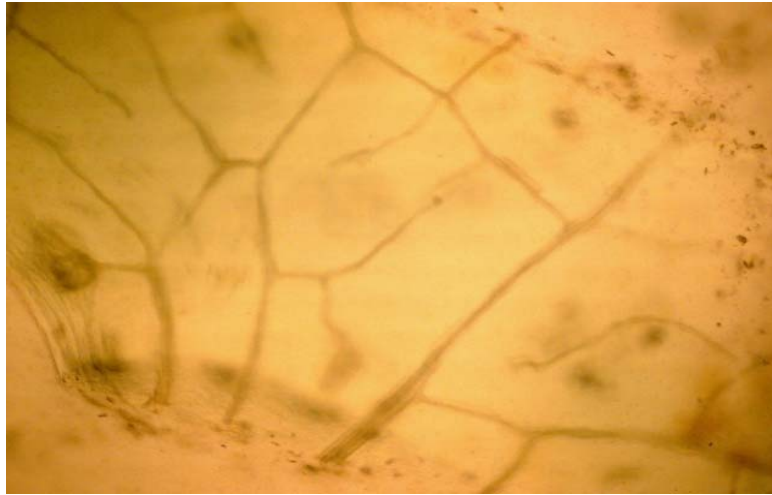


Fig.33. A leaf portion of *Datura innoxia* showing vein islet and vein termination number

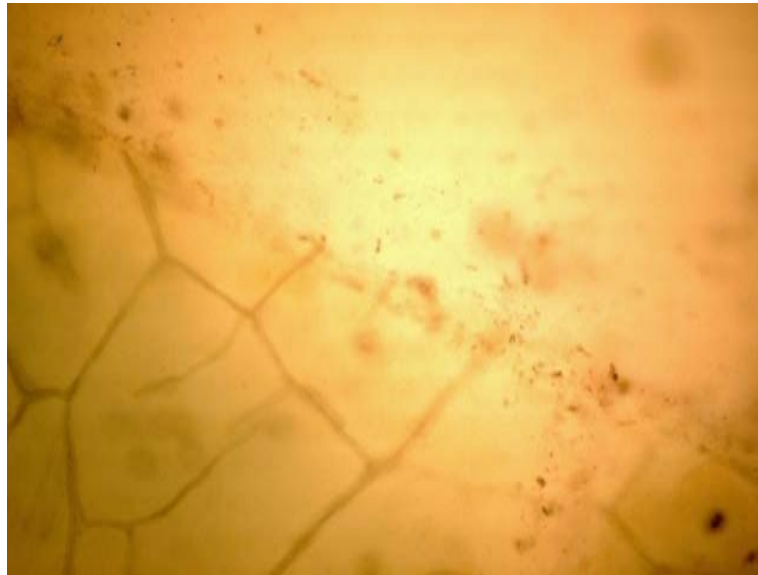


Fig.34. A leaf portion of *Solanum nigrum* showing vein islet and vein termination number



Fig.35. A leaf portion of *Solanum surattense* showing vein islet and vein termination number

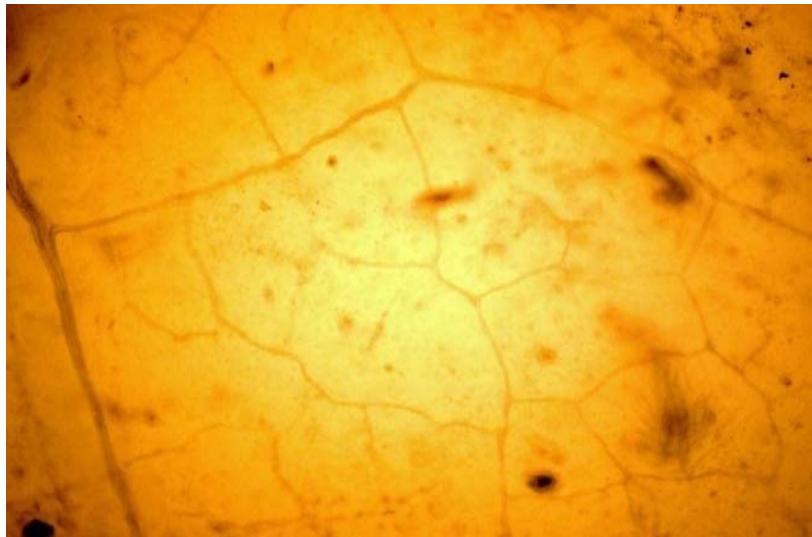


Fig.36. A leaf portion of *Withania somnifera* showing vein islet and vein termination number

Table 9. Leaf constant parameters of five selected medicinal plants of Family Solanaceae

S. No	Plant species	Vein islet number	Vein termination Number	Palisade ratio
1	<i>Datura innoxia</i> Miller.	11.5 - 16.5	17.5 - 20.75	19.5 – 28.75
2	<i>Solanum nigrum</i> Linn.	8.75 - 19.25	18.75 - 23.75	10.75 – 18.5
3	<i>Solanum surattense</i> Burm.f.	19.5 - 23.75	21.5 - 26.75	23.75 – 32.75
4	<i>Withania somnifera</i> Linn.	20.5 - 26.75	22.5 - 26.75	26.5 – 34.8
5	<i>Withania coagulans</i> (Stock) Dunal.	22.5 - 27.75	11.75 - 17.5	27.5 – 34.5

5.4 Stomatal Features

Different stomatal parameters are shown in Table 9 and 10.

a). Stomatal types

The upper and lower epidermal layers of *Datura innoxia* respectively contained anisocytic (Fig. 40) and anisocytic (Fig. 37) stomata. The upper epidermis of *Solanum nigrum* had anisocytic (Fig. 41) stomata while the lower epidermis showed anisocytic (Fig. 38) stomata. Both upper and lower epidermis of *S. surattense* exhibited anisocytic (Fig. 43) and anisocytic stomata respectively. Aliero *et al.*, (2007a) reported that the leaves of *Solanum pseudocapsicum* had anisocytic stomata, which were more abundant on the abaxial surfaces and this agrees with the present findings. Upper epidermis of *Withania somnifera* maintained anisocytic while the lower epidermis had both anisocytic and anisocytic stomata (Fig. 39). Anisocytic stomata were present in upper epidermis of the *W. coagulans* lower epidermis contains paracytic (Fig. 42) and anisocytic stomata. Dastagir *et al.*, (2011) while working on stomatal morphology of *Alstonia scholaris* and *Bischofia javanica* reported two types of stomata including paracytic and anisocytic stomata which agree with the present study. The leaves of *Alstonia scholaris* were hypostomatic while the leaves of *Bischofia javanica* were amphistomatic which is different from the present study. The number of stomata was greater on lower epidermis in *Bischofia javanica* than in *Alstonia scholaris*. Density of the anisocytic was high in both upper and lower epidermises in *Solanum nigrum* and it was low for anisocytic of upper epidermis in *Withania coagulans* and paracytic stomata of lower epidermis in *Withania coagulans*. Similarly frequency of the anisocytic was high in both upper and lower epidermises in *Solanum nigrum* and it was low for anisocytic of upper epidermis in *Withania coagulans* and paracytic stomata of lower epidermis in *Withania coagulans* (Table 10). Daly & Gastaldo (2010) investigated that stomatal frequencies, stomatal densities and stomatal index of *Quercus rubra* which agree with the similar observation in the present study.

b) Stomatal index

There was wide range of variation in the stomatal indices. Although, stomatal indices have been given considerable importance for comparison in different taxa, yet it

does not seem to be of any significance in the present study. This is because their values vary inconsistently in different taxa. The stomatal index of the upper epidermis was maximum (43.88) in *Solanum nigrum* and was lowest (32.25) in *Withania coagulans*. Stomatal index of the lower epidermis was highest 45.8 in *Solanum nigrum* and lowest 21.3 in *Withania coagulans* (Table 10). Mishra *et al.*, (2008) reported stomatal index (upper epidermis) is 30.12 and stomatal index (lower epidermis) is 36.31 in the leaves of *Tridax procumbens*. Krishnaveni & Sent (2009) reported stomatal index (upper epidermis) is 4.5/mm² and stomatal index (lower epidermis) is 16/mm² in the leaves of *Argyrea nervosa*. Similar variation was recorded in the present case.

c) Mean length and width of stomatal pore

Mean length of the stomatal pore of the upper epidermis was highest 53.2µ in *W. coagulans* and lowest 6µ in *Datura innoxia*. Mean length of the stomatal pore of the lower epidermis was highest 36.1µ in *Solanum surattense* and lowest 6.3µ in *Datura innoxia*. Mean width of the stomatal pore of the upper epidermis was maximum 33.6µ in *Withania coagulans* and least 3.2µ in *Solanum nigrum*. Mean width of the stomatal pore of the lower epidermis was highest 16.6µ in *Solanum surattense* and was lowest 2.8µ in *Datura innoxia* (Table 10). These results are in agreement with those of the Hameed *et al.*, (2008a) who reported similar findings for *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Polygonum plebejum* and *Polygonum persicaria*. Hameed & Hussian (2011) also reported similar findings for *Datura innoxia*, *Solanum nigrum*, *Solanum surattense*, *Withania somnifera* and *Withania coagulans* and this also are in line with the current findings.

d) Percentage of open

Percentage of the open stomata of the upper epidermis was highest (52.76) in *Withania somnifera* and lowest (35.03) in *Solanum surattense*. The lower epidermis showed highest (52.54) in *Withania somnifera* and lowest (24.42) in *Solanum nigrum* (Table 10). These results are in agreement with those of the Hameed *et al.*, (2008a) for various members of Polygonaceae and for Solanaceae (Hameed & Hussian, 2011).

e) Statistical Comparison

The mean number of the stomata of the upper epidermis was highest 70.3 in *D. innoxia* and was lowest 12 in *Withania coagulans*. The mean number of the stomata of the lower epidermis was highest 77.8 in *Solanum nigrum* and was lowest 18.5 in *Withania coagulans*. Mbagwu *et al.*, (2007a) reported that stomata are more abundant at the lower surfaces than upper leaf surfaces in the leaves of *Solanum macrocarpon* and *Solanum nigrum*. Stomata were anomocytic type with stomatal index ranging from 5.70%-33.02% in *Solanum nigrum* and 10.14% - 32.35% in *Solanum macrocarpon*. The standard deviation of the stomata of the upper epidermis was highest 8.52 in *Solanum nigrum* and was lowest 2.68 in *Withania coagulans*. The standard deviation of the stomata of the lower epidermis was highest 7.2 in *Solanum nigrum* and was lowest 2.24 in *Solanum surattense*. The variance of the stomata of the upper epidermis was highest 72.61 in *Solanum nigrum* and was lowest 7.2 in *Withania coagulans*. The variance of the stomata of the lower epidermis was highest 51.96 in *Solanum nigrum* and was lowest 5.44 in *Datura innoxia*. The coefficient of variance of the stomata of the upper epidermis was highest 22.36 in *Withania coagulans* and was lowest 7.95 in *Datura innoxia*. The coefficient of variance of the stomata of the lower epidermis was highest 23.96 in *W. coagulans* and was lowest 9.11 in *Datura innoxia*. The standard error of the stomata of the upper epidermis was highest 2.69 in *Solanum nigrum* and was lowest 0.84 in *Withania coagulans*. The standard error of the stomata of the lower epidermis was highest 2.27 in *S. nigrum* and was lowest 0.7 in *S. surattense* (Table 11). Hameed *et al.*, (2008a) reported variation in the structure and distribution of stomata in some members of Polygonaceae. The type of stomata, density, frequency, stomatal index, size of stomatal pore, size of guard cells and percentage of the close and open stomata and the statistical evaluation of the stomata and epidermis included mean, standard deviation, and variance, coefficient of variance, standard error and difference of standard error, which agree with the present study.

The result of the statistical analysis was shown in Table 11. The mean number of the epidermal cells of the upper epidermis was highest (89.9) in *S. nigrum* and was lowest (25.2) in *Withania coagulans*. The mean number of the epidermal cells of the lower epidermis was highest (92.1) in *Solanum nigrum* and was lowest (32.6) in *Datura*

innoxia. The standard deviation of the epidermal cells of the upper epidermis was highest (6.44) in *Withania somnifera* and was lowest (2.7) in *Solanum surattense*. The standard deviation of the epidermal cells of the lower epidermis was highest (7.89) in *Solanum nigrum* and was lowest (3.23) in *Datura innoxia*. The variance of the epidermal cells of the upper epidermis was highest (41.49) in *Withania somnifera* and was lowest (7.29) in *Solanum surattense*. The variance of the epidermal cells of the lower epidermis was highest (62.29) in *S. nigrum* and was lowest (9.4) in *Solanum surattense*. The coefficient of variance of the epidermal cells of the upper epidermis was highest (14.94) in *Withania somnifera* and was lowest (3.86) in *Solanum surattense*. The coefficient of variance of the epidermal cells of the lower epidermis was highest (9.91) in *Datura innoxia* and was lowest (5.74) in *Withania coagulans*. The standard error of the epidermal cells of the upper epidermis was highest (2.03) in *Withania somnifera* and was lowest (0.85) in *Solanum surattense*. The standard error of the epidermal cells of the lower epidermis was highest (2.49) in *Solanum nigrum* and was lowest (0.96) in *S. surattense*. Hameed & Hussain (2011) reported variation in the structure and distribution of stomata in some solanaceae plants. The type of stomata, density, frequency, stomatal index, size of stomatal pore, size of guard cells and percentage of the close and open stomata varied among these species and suggested a taxonomic uniformity of stomatal types. These studies support and strengthened the present study.



Fig.37. Anisocytic stoma of *Datura innoxia*



Fig.38. Anisocytic stoma of *Solanum nigrum*

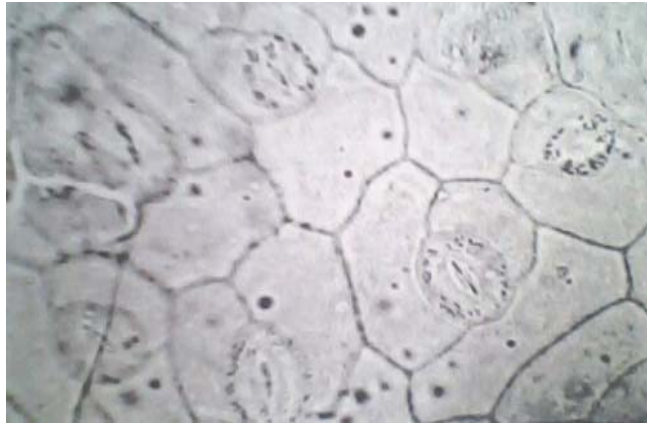


Fig. 39. Anisocytic stoma of *Withania somnifera*

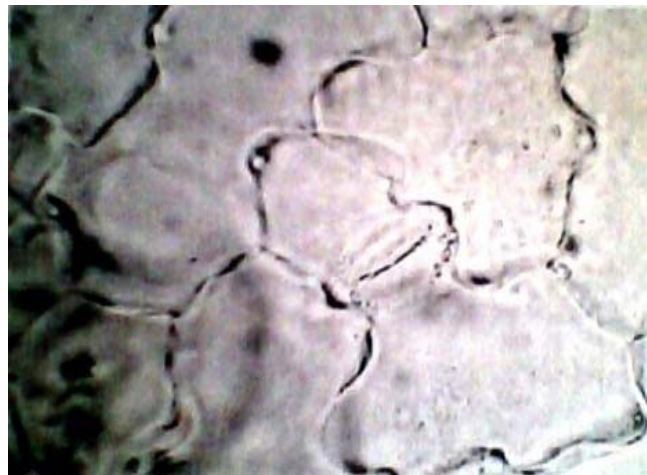


Fig.40. Anomotetracytic stoma of *Datura innoxia*



Fig.41.Anomotetracytic stoma of *Solanum nigrum*

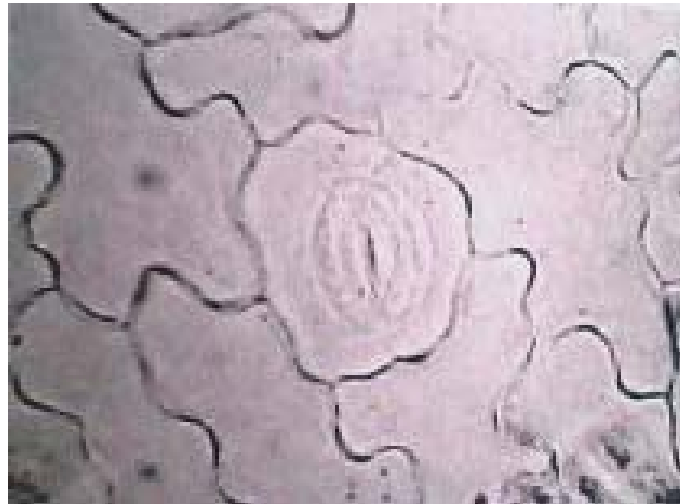


Fig.42. Paracytic stoma of *Withania coagulans*

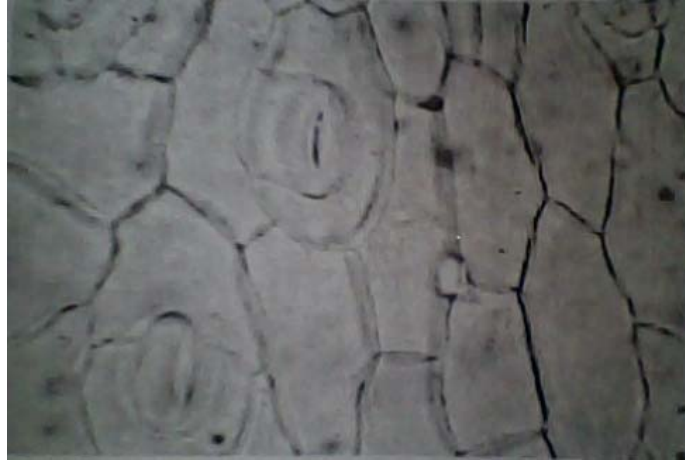


Fig. 43.Amphianisocytic stoma of *Solanum surattense*

Table 10.Stomatal study of five medicinal plants of Family Solanaceae.

Name of species	Type		Density		Frequency		Index		Size of stomatal pores				Size of guard cells				%age of open and close stomata			
	U	Lo	U	Lo	U	Lo	U	Lo	U	Lo	U	Lo	U	Lo	U	Lo	U		Lo	
									L(μ)	W(μ)	L(μ)	W(μ)	L(μ)	W(μ)	L(μ)	W(μ)	O	C	O	C
<i>Datura innoxia</i> Miller.	Anomotetracytic & anisocytic	Anisocytic; anomotetracytic	30.5; 28.5	9.5; 16.1	3050; 2850	950; 1610	40.29	44	6	3.7	6.3	2.8	15.2	4.1	13.8	2.4	37.83	62.16	39.84	60.15
<i>Solanum nigrum</i> Linn.	Anomotetracytic	Anisocytic	70.3	77.8	7030	7780	43.88	45.8	8.1	3.2	28.8	14.6	17.3	6.5	35	10.3	51.06	48.93	24.42	75.57
<i>Solanum surattense</i> Burm. f.	Amphianisocytic ; anomotetracytic	Amphianisocytic ; anomotetracytic	19.5; 21.6	13.5; 11.1	1950; 2160	1350; 1110	39.44	38.7	15.8	7.4	36.1	16.6	24.7	15	50.4	35.3	35.03	64.96	37.39	62.6
<i>Withania somnifera</i> Linn.	Anomotetracytic	Anomotetracytic; anisocytic	27.1	35.6; 21.3	2710	3560; 2130	38.6	40.7	13.7	7.1	14	8.2	12.5	5.6	24.8	13.5	52.76	47.23	52.54	47.45
<i>Withania coagulans</i> (Stock) Dunal.	Anomotetracytic	Paracytic; Anisocytic	12	7;11.5	1200	700; 1150	32.25	21.3	53.2	33.6	19	8.5	56.1	38.3	38.5	17.3	48.33	51.66	48.64	51.35

Key: U= Upper, Lo = Lower, L = Length, W = Width, O = Open, C = Close

Table 11.Statistical evaluation of stomata and epidermis of the five medicinal plants of Family Solanaceae.

Plant Species	Stomata												Epidermis											
	M		S.D		V		C.V		S.E		C.I.M		M		S.D		V		C.V		S.E		C.I.M	
	U	L	U	L	U	L	U	L	U	L	U	L	U	L	U	L	U	L	U	L	U	L	U	L
<i>Datura innoxia</i> Miller.	59.2	25.6	4.7	2.33	22.16	5.44	7.95	9.11	1.48	0.73	55.83-62.56	23.93-27.26	87.7	32.6	5.1	3.23	26.01	10.44	5.81	9.91	1.61	1.02	84.05-91.34	30.28-34.91
<i>Solanum nigrum</i> Linn.	70.3	77.8	8.52	7.2	72.61	51.96	12.12	9.26	2.69	2.27	63.63-76.96	72.64-82.95	89.9	92.1	5.14	7.89	26.49	62.29	5.72	8.56	1.62	2.49	86.21-93.58	86.45-97.74
<i>Solanum surattense</i> Burm. f.	41.1	24.6	7.04	2.24	49.69	5.04	17.15	9.12	2.22	0.7	36.05-46.14	22.99-26.20	69.9	39	2.7	3.06	7.29	9.4	3.86	7.86	0.85	0.96	67.96-71.83	36.80-41.19
<i>Withania somnifera</i> Linn.	27.1	56.9	3.58	5.99	12.87	35.89	13.23	10.52	1.13	1.89	24.53-29.66	52.61-61.18	43.1	83	6.44	6.44	41.49	41.6	14.94	7.77	2.03	2.03	38.49-47.70	78.38-87.61
<i>Withania coagulans</i> (Stock) Dunal.	12	18.5	2.68	4.43	7.2	19.65	22.36	23.96	0.84	1.4	10.08-13.91	15.32-21.67	25.2	68.2	3.73	3.91	13.96	15.36	14.82	5.74	1.17	1.23	22.52-27.87	65.39-71.00

Key: U= Upper, L = Lower, M = Mean, S.D = Standard Deviation, V = Variance, C.V = Co-efficient of Variance, S.E = Standard Error, CIM = Confidence Interval for Mean

5.5 Powdered Drug Composition

The powdered crude drugs can be identified based on the form, the presence or absence of different cell types based, cytomorphological characters, including parenchyma, collenchyma, fibers, stone cells, vessels, trichomes, secretory cells and epidermal cells. Cell inclusion characteristics are of considerable value in the identification of unorganized crude drugs, e.g. ergastic contents as aleurone grains, cluster crystals and prisms of calcium oxalate, silica and starch granules (Jackson & Snowdon, 1992). *Datura innoxia* leaves was light green coloured, bitter in taste, and contained epidermal cells, collenchyma, sclerenchyma, palisade and spongy mesophyll cells, xylem, phloem, stomata, simple multicellular trichome and glandular trichome (Fig.44). *Solanum nigrum* leaves had astringent taste, light green colour, and contained epidermis, palisade and spongy mesophyll cells, sclerenchyma, stomata, pith, collenchyma, calcium oxalate, simple multicellular trichome and glandular trichome, xylem and phloem (Fig.45). The root of *Solanum surattense* had acrid taste, whitish in colour and contained epidermal cells, medullary rays, cortex, endodermal cells, hypodermal cells, pericycle, xylem and phloem (Fig.46). The stem of this plant had epidermis, cortex, endodermis, pericycle, xylem, phloem, pith, hypodermis and medullary rays (Fig.47). The leaves of *Withania somnifera* was bitter in taste, light green in colour. It also had epidermal cells, collenchyma, sclerenchyma, palisade and spongy mesophyll cells, xylem and phloem, stomata, spiral and reticulate trachea (Fig.48). The stem of the *Withania coagulans* was distinguished by the presence of epidermal cells, pith, cortex, endodermal cells, medullary rays, collenchyma, pericycle, xylem and phloem (Fig.49) and the leaves of this plant showed epidermis, xylem, phloem, palisade and spongy mesophyll cells (Fig. 50). Patil *et al.*, (2009) stated that the powdered drug of *Ficus bengalensis* had trichomes, fibres, epidermal cells with anticlinal walls, calcium oxalate crystals, and spiral thickenings. Nirmalet *et al.*, (2009) reported the presence of uni to biseriate medullary rays, pitted xylem fibers, big vessels in groups, xylem parenchyma, pith, calcium oxalate crystals, starch grains, lignified fibers in the powdered drug of *Sesbania sesban*. Yadav *et al.*, (2007) reported that the powdered drug of root of *Chenopodium album* had parenchyma cells, fibres, annular vessels, reticulate vessels and starch grains. Youngken (1950) while studied the root of *Atropa belladonna* reported

medullary rays, parenchyma, reticulate tracheae, starch grains, microcrystals. Evans (2009) reported epidermal cells, stomata, palisade parenchyma, spongy parenchyma, rosette aggregate of calcium oxalate and non glandular trichomes in the leaves of *Datura stramonium* which agree with the present work.

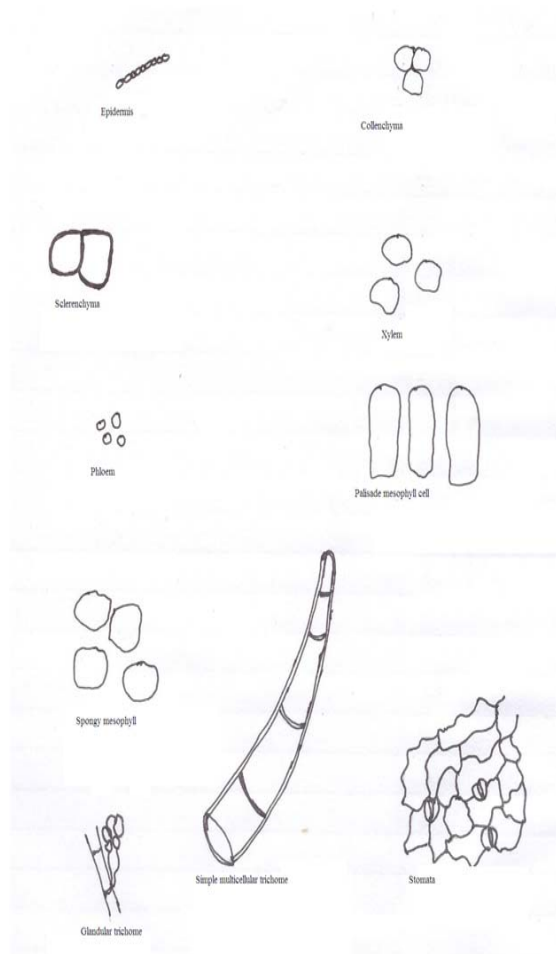


Fig. 44. Constituent of powder drug of leaf of *Datura innoxia* Miller.

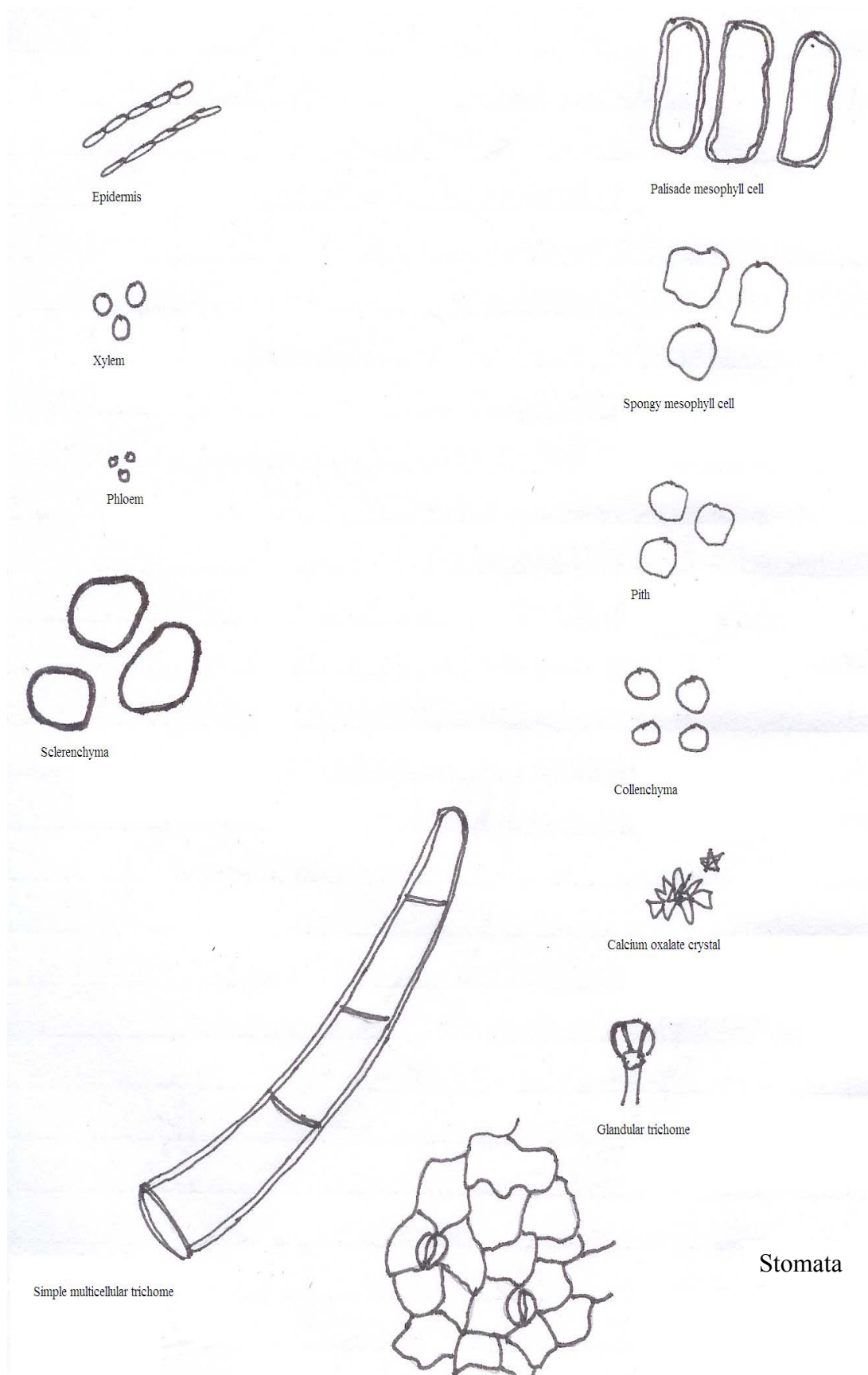
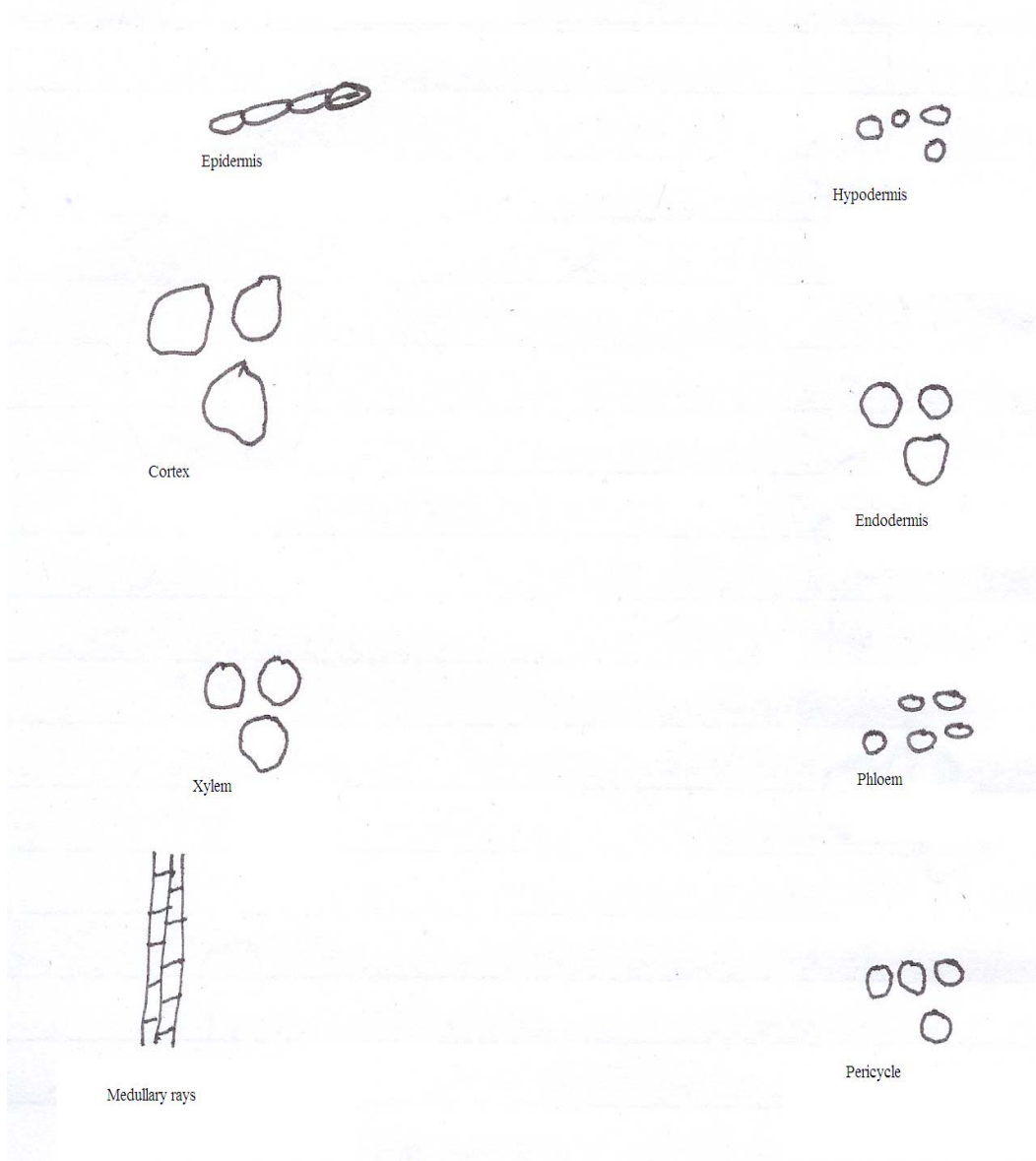


Fig. 45. Constituent of powder drug of leaf of *Solanum nigrum* Linn.



¹ Fig. 46. Constituent of powder drug of root of *Solanum surattense* Burm.

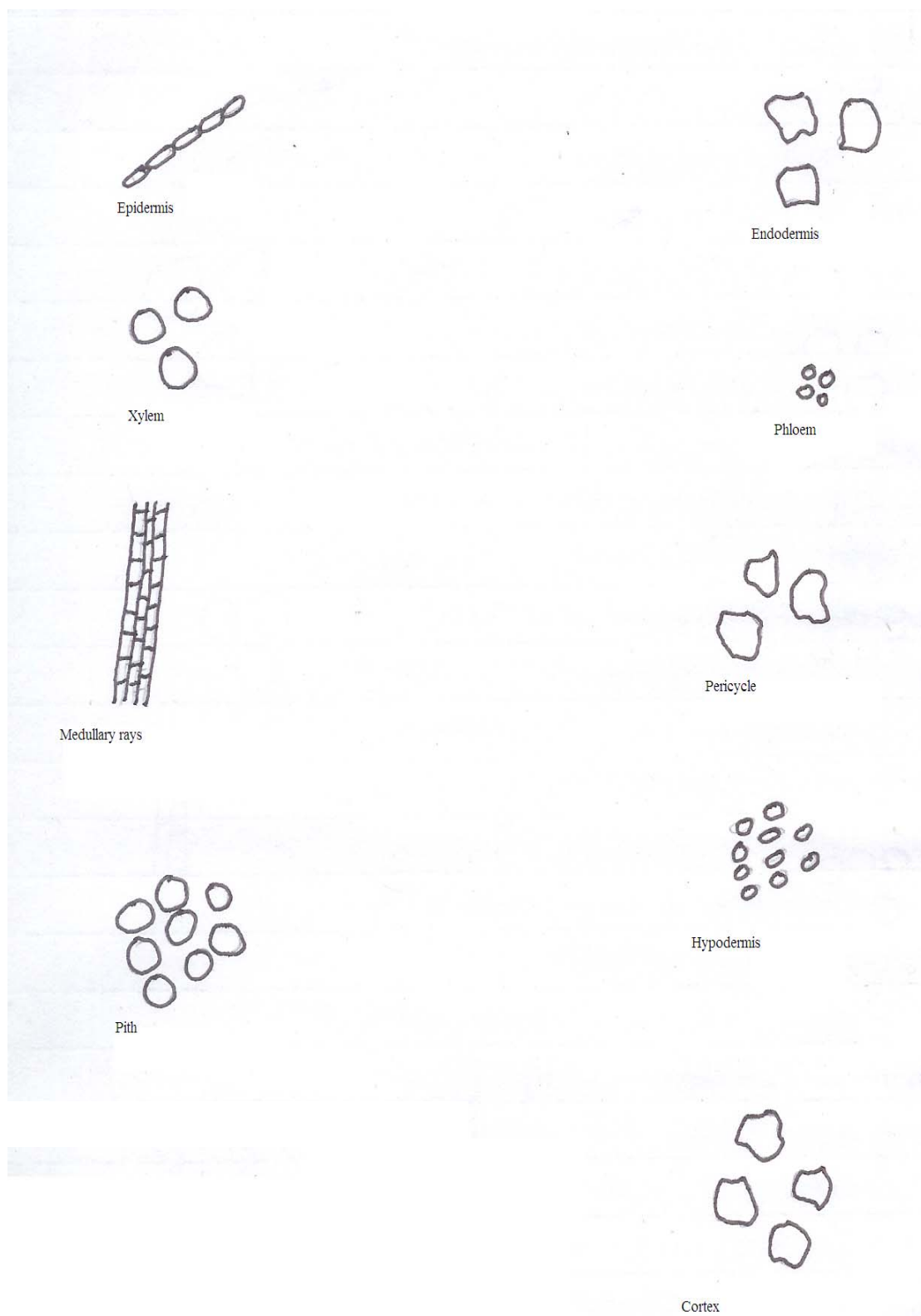


Fig. 47. Constituent of powder drug of stem of *Solanum surattense* Burm.f.

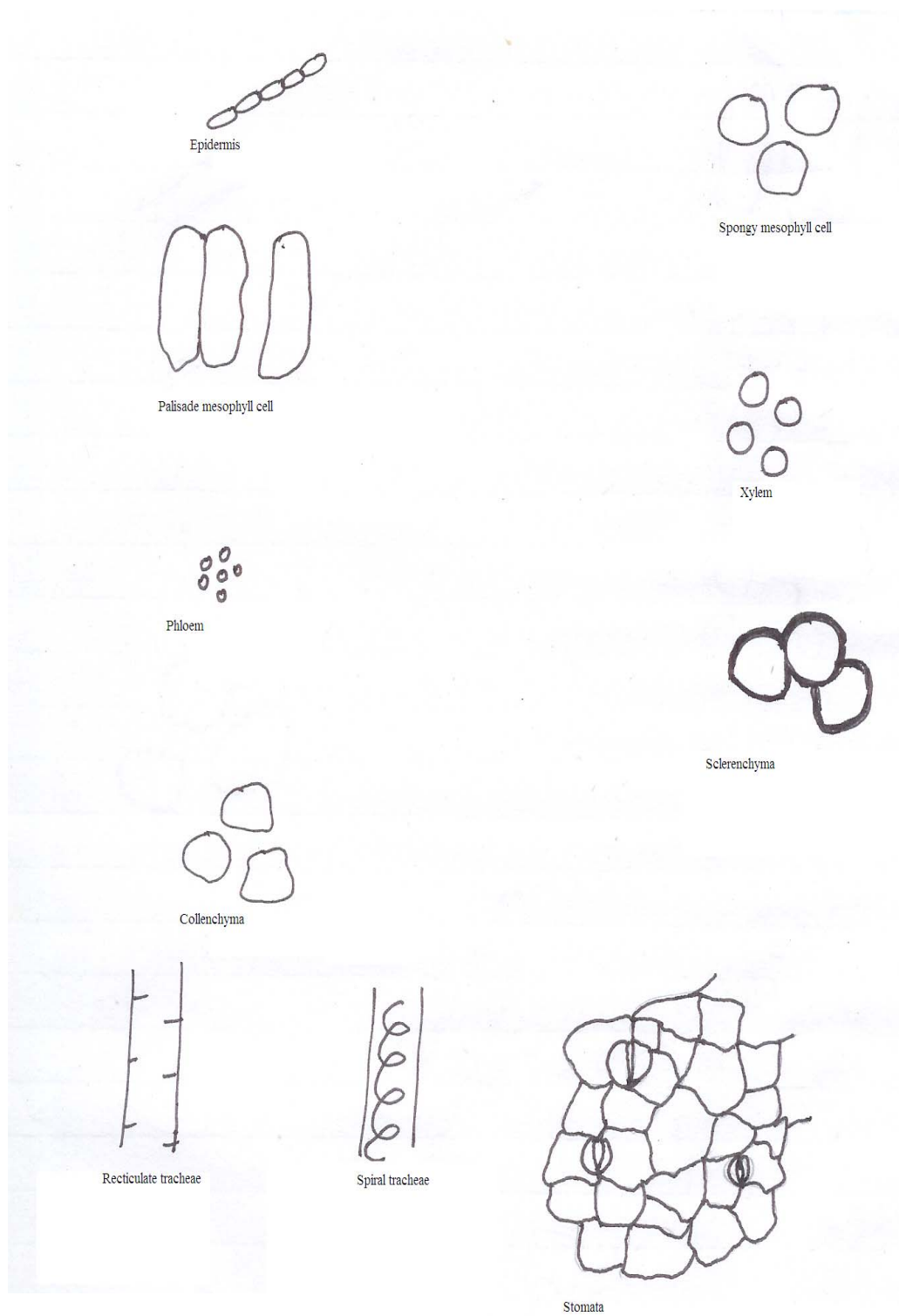


Fig. 48. Constituent of powder drug of leaf of *Withania*

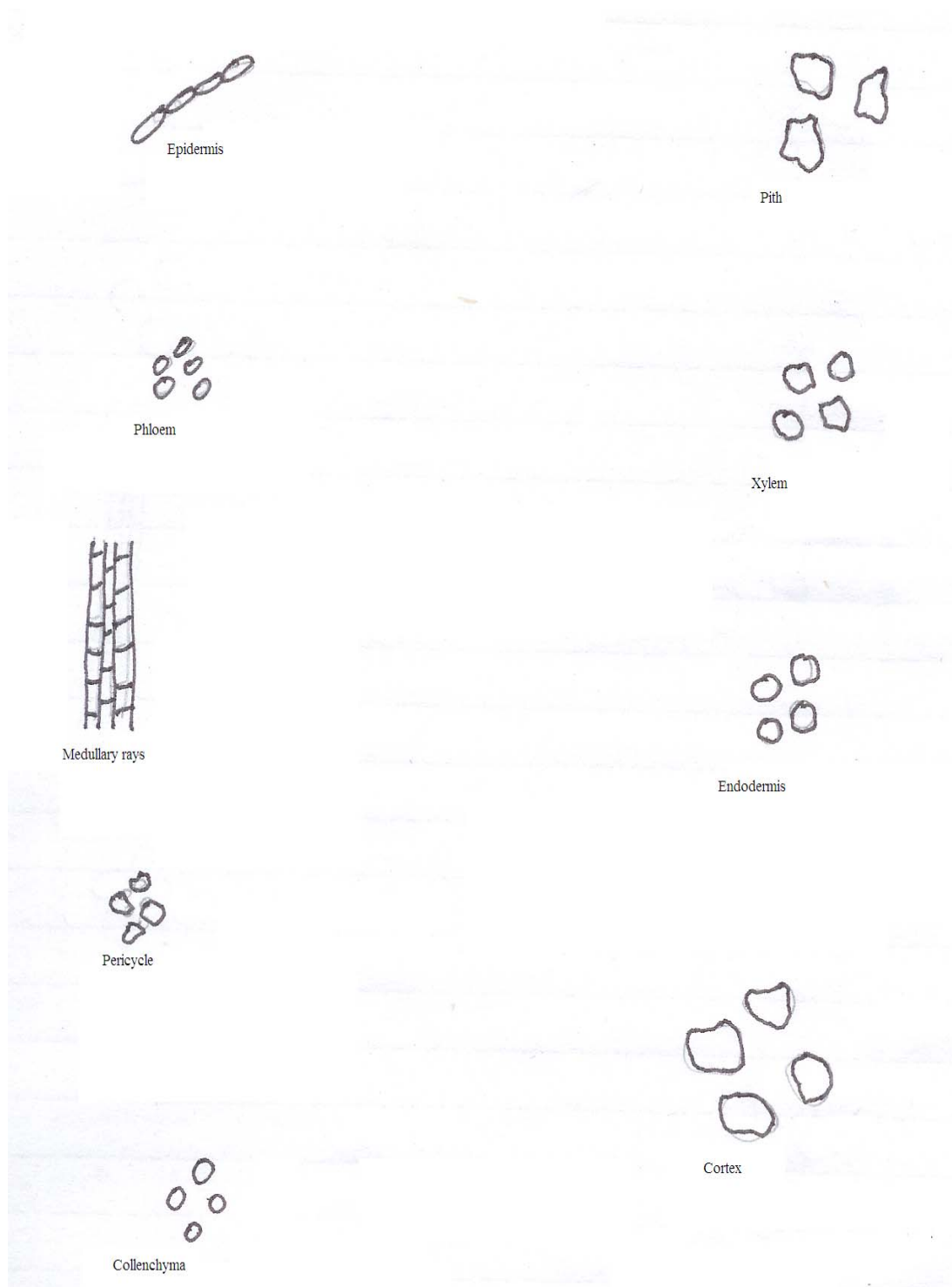


Fig. 49. Constituent of powder drug of stem of *Withania coagulans* (Stock) Dunal.

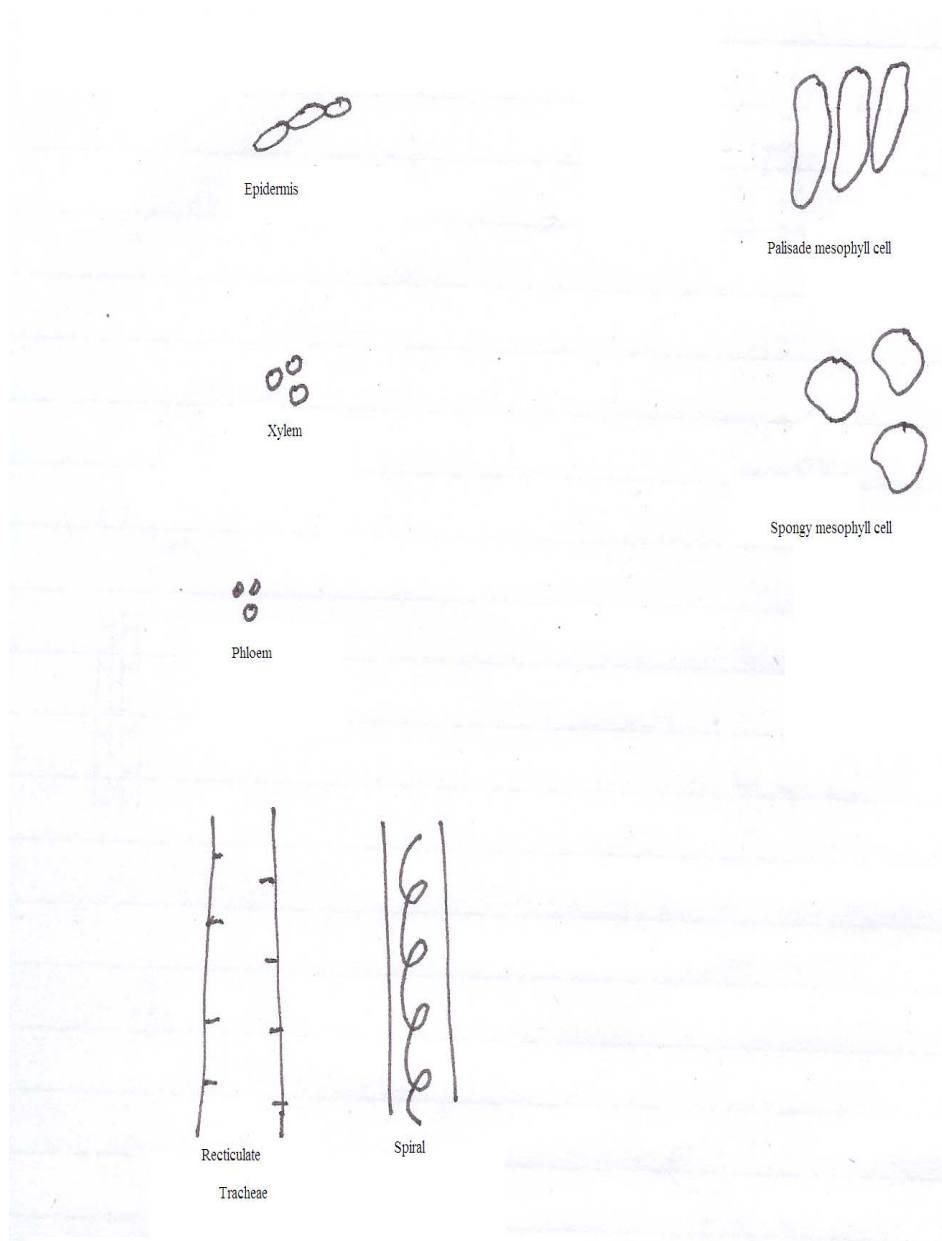


Fig. 50. Constituent of powder drug of leaf of *Withania coagulans* (Stock) Dunal.

5.6 Micro-chemical tests

Microchemical tests may provide considerable knowledge about the chemical constituents, which are responsible for the medicinal properties. Preliminary screening of plant samples for alkaloids, saponins, triterpenes and steroids is the usual initial step undertaken by a natural product chemist in the quest of novel or bioactive compound (Ahmed, 1995). Alkaloids were present in all parts of the *Datura innoxia*. It was absent from root and leaf and was present in stem and fruit of the *Solanum nigrum*. It was present in root, leaf and fruit and was absent from the stem of the *Solanum surattense*. It was absent from the leaf and was present in root, stem and fruit of the *Withania somnifera*. It was present in leaf and fruit and was absent from the root and stem of the *Withania coagulans*. Vernayet *et al.*, (2008) reported the presence of proline content and alkaloids production, i.e. scopolamine and hyoscyamine, in *Datura innoxia*. Mucilage was absent from all parts of the *Datura innoxia*, *Solanum surattense* and *Withania coagulans*. It was absent from the stem and was present in root, leaf and fruit of the *Solanum nigrum*. It was present in all parts of the *Withania somnifera*. Patra *et al.*, (2009) showed the availability of mucilage in the leaves of in the leaf of *Hygrophila spinosa* (Acanthaceae). Anthraquinone derivatives were absent from the stem and fruit and were present in root and leaf of the *Datura innoxia*. It was present in stem and leaf and was absent from root and fruit of the *Solanum nigrum*. It was absent from the root and leaf and was present in stem and fruit of the *Solanum surattense* and *Withania coagulans*. And it was present in all parts of the *Withania somnifera*. Audu *et al.*, (2007) reported the phytochemical screening of the ethanol extract of the leave of *Lophira lanceolata* indicated the presence of anthraquinones in this plant species. Saponins were absent from the leaf and were present in the root, stem and fruit of the *Datura innoxia*. It was absent from the fruit and was present in root, stem and leaf of the *Solanum nigrum* and *Withania somnifera*. It present in the root, lea and fruit and was absent from the stem of the *Soalnum surattense*. It was absent from the stem and fruit and was present in the root and leaf of the *Withania coagulans*. Shrivastava *et al.*, (2010) carried out physicochemical and preliminary phytochemical investigation of *Catunaregum spinosa* Thunb and the presence of saponins in this species. Starch was absent from the fruit and was present in root, stem and leaf of the *Datura innoxia*. It was present in root, stem and fruit and was absent from the leaf of the *Solanum nigrum*. It was present in root and fruit and was absent from the stem and leaf of the *Withania somnifera*. It was present in all the parts of *Solanum surattense* and *Withania coagulans*. Tannins were present in stem and

was absent from root, leaf and fruit of the *Datura innoxia*, *Solanum nigrum* and *Withania coagulans*. It was absent from the leaf and fruit and was present in the root and stem of the *Solanum surattense* and *Withania somnifera* (Table 12). Patra *et al.* (2009) showed the availability of tannins in the leaves of in the leaf of *Hygrophila spinosa* (Acanthaceae). Audu *et al.*, (2007) reported the phytochemical screening of the ethanol extract of the leave of *Lophira lanceolata* and indicated the presence of tannin. Proteins, fats and oils, calcium oxalate and cellulose was present in all parts of the all the five plant species. Alieroet *al.* (2007b) reported that the volatile components of *Solanum pseudocapsicum* roots by GC-MS. A total of 41 compounds, representing 50% of the oil, were identified. The oil contains fatty acids up-to (26.8%), terpenoids (7.6%), and aldehydes (5.3%), hexadecanoic acid (24.1%), 2-methoxy-3-isopropylpyrazine (2.8%), and 15-methylhexadecanoic acid (2.1%), β -elemene and δ -elemene. Shrivastava and Leelavathi (2010) carried out physicochemical and preliminary phytochemical investigation of *Catunaregum spinosa* Thunb. Different physicochemical parameters such as, ash value, extractive value, foaming index, hemolytic index, total tannin content and microbial count were carried out. Preliminary qualitative chemical test for different extract shows the presence of glycosides, carbohydrates, phytosterols/triterpenoids, saponins, fixed oils & fats and phenolic/tannins.

Table 12. Micro chemical screening tests of the different parts of the five selected medicinal plants of Solanaceae

Plant names	Plant parts	Alk	Muc	Anth	Sap	Star	Pro	Fat	Tan	Cao	Cell
<i>Datura innoxia</i> Miller.	Root	++	-	+	+++	+	+	++	-	+	+
	Stem	+++	-	+	++	+	++	++	+	+	+
	Leaf	++	-	++	++	+	+++	+++	-	+	+
	Fruit	+++	-	+++	++	-	++	++	-	+	+
<i>Solanum nigrum</i> Linn.	Root	+	+	+	++	+	++	++	-	+	+
	Stem	++	+	+	+	+	++	++	+	+	+
	Leaf	++	++	+	++	-	++	+	-	+	+
	Fruit	+++	+++	++	++	+	+	++	-	+	+
<i>Solanum surattense</i> Burm.f.	Root	+++	-	+	++	+	++	+++	+	+	+
	Stem	++	-	+	++	+	+++	++	+	+	+
	Leaf	++	-	++	++	+	++	++	-	+	+
	Fruit	+++	-	+++	++	+	++	++	-	+	+
<i>Withania somnifera</i> Linn.	Root	++	++	+	+	+	++	+	+	+	+
	Stem	+	+	++	+	-	++	++	+	+	+
	Leaf	++	++	+	++	-	++	++	-	+	+
	Fruit	+++	+++	++	++	+	++	+	-	+	+
<i>Withania coagulans</i> (Stock) Dunal.	Root	++	-	+	++	+	+++	++	-	+	+
	Stem	+	-	+	++	+	++	++	+	+	+
	Leaf	++	-	+	+	+	++	++	-	+	+
	Fruit	+++	-	+++	+++	+	++	+++	-	+	+

Key: Alk = Alkaloid ; Muc = Mucilage ; Anth = Anthraquinone derivatives ; Sap = Saponins ; Star = Starch ; Pro = Protein ; Fat = Fats ; Tan = Tannins ; Cao = Calcium oxalate ; Cell = Cellulose, +++ = Highly significant, ++ = Significant, + = Present, - = Absent

5.7 Proximate analyses

The results of proximate analysis are shown in Table 13 and 14.

5.7.a Ash contents

When organic matter are decomposed at high temperature (500 - 600 °C), the remaining residue is called the ash. This residue consists of oxides, and salts containing anions such as PO_4^{-3} , Cl^{-1} , SO_4^{-2} and halides and cations such as Na, K, Ca, Mg, Fe and Mn, which are important constituents of animal diet. The ash content varied from 2.08 – 9.37% in vegetative stage to 2.94 – 14.00% in reproductive stage and 1.92 – 7.00% in post reproductive stage. The overall highest (9.37%) ash content was recorded in leaf followed by (9.27%) stem of *Withania coagulans* and (6.93%) root of *Withania somnifera* in vegetative stage. At reproductive stage, it was highest (14.00%) in stem followed by (10.00%) root of *Withania somnifera* and leaf of *Solanum surattense* (9.00%). These results are in contradiction with those of Hussain & Durrani (2008, 2009) but are in line with those of Sultan *et al.*, (2007 & 2008a). In post reproductive stage it was highest (7.00%) in roots followed by (6.00%) leaf of *Datura innoxia* and (5.20%) fruit of *Solanum surattense* and stem of *Withania coagulans*. The results revealed that the ash content increased from vegetative stage to reproductive and decreased in the post reproductive stage (Table 13). ANOVA showed that ash contents was non significant between the plant parts and phenological stages (Table 14). Hameed *et al.*, (2008b) reported highest contents of ash in *R. australe*, *R. hastatus*, *R. dentatus*, *P. maculosa* and *P. plebejum*, which differ from the present results. Hussain *et al.*, (2010c) also reported that ash contents progressively decline with maturity of some plants and this is in the line with the present study.

5.7.b Moisture contents

The moisture content among the analyzed plants varied from 10 – 44.89% in the vegetative stage, 7.08 – 48% in the reproductive stage, 4.44 – 33.3 % in the post reproductive stage (Table 12). The overall highest (44.89%) moisture content was recorded in stem of *Withania coagulans* at vegetative stage, followed by stem (32%) and leaf (28%) of *Solanum surattense*. Reproductive stage showed the highest (48.00%) value in leaf of *Solanum nigrum*, followed by stem (42.00%) of *Withania somnifera* and stem (32.00%) of *Datura innoxia* and *Withania coagulans*. It was highest (33.33%) in fruit of

Datura innoxia in post reproductive stage followed by stem (22.91%) of *Solanum nigrum* and leaf (19.23%) of *Datura innoxia*. The results revealed that the moisture content increased from vegetative stage to reproductive and decreased at the post reproductive stage (Table 13). ANOVA showed non significant differences between the plant parts and phenological stages (Table 14). The results revealed that moisture contents not only varied among the species but also between the different phenological states of the plants. Hussain *et al.*, (2010c) also stated that moisture content vary in different species. Das *et al.*, (2009) and Hanif *et al.*, (2006) concluded that green leafy vegetables had higher moisture content. Our results parallel with them. Saidu & Jideobi (2009) also recorded highest moisture contents at reproductive stages+ in water leaf. Hussain *et al.*, (2009) found high moisture content in *Allium sativum* (67.66 %) and *Valeriana officinalis* (6.82 %) which are higher than in the present findings. The moisture contents in the present case are much less than that of the cited results. Adnan *et al.*, (2010) also provided high moisture in *Bupleurum falcatum*, *Forsskalea tenacissima*, *Lavendula angustifolia*, *Valeriana officinalis* and *Otostegia limbata* and this agrees with our results.

5.7.cCrude protein (CP)

It refers to all the nitrogenous compounds present in plants except that of alkaloids. It is an important constituent of the food as it is related to the vitamins, calcium, phosphorus and digestibility (Ganskopp & Bohnert, 2003; Hussain & Durrani, 2009). The crude protein contents varied from 2.12 – 6.31% in vegetative stage, 2.39 – 6.45% in reproductive stage and 2.36 – 6.45% in post reproductive stage. The overall composition of crude protein was recorded highest (6.31%) leaf of *Datura innoxia*, followed by (6.26%) root of *Withania somnifera* and (6.14%) root of *Solanum nigrum* in vegetative stage. In reproductive stage it was highest in root (6.45%) of *Withania coagulans* followed by leaf (6.21%) of *Withania somnifera* and leaf (5.68%) of *Datura innoxia*. At post reproductive stage it was highest in root (6.45%) of *Solanum nigrum* followed by stem (5.68%) of *Solanum surattense* and root (5.64%) of *Withania somnifera*. The results revealed that the crude protein content increased from vegetative stage to reproductive and decreased in the post reproductive stage (Table 13). ANOVA showed non significant differences between the plant parts and phenological stages, except for *Datura innoxia* parts (Table 14). Hanif *et al.*, (2006) estimated protein 0.9 % to

2.1 % in the selected vegetables. Plants growing at higher altitudes experiences quite different climate and had higher content of CP than plants of plains. Cheema *et al.*, (2011) reported high concentration of CP in leaves of *Morus alba* which is a best source of protein in ruminant feeding. They also described that different level of CP is due to differences of protein accumulation. Yao *et al.*, (2000) said that *Morus alba* is a best source of protein for ruminants. Adenipekun & Oyetunji (2010) observed little differences between *Vigna unguiculata* (23%) and *Arachis hypogea* (24%). Hussain *et al.* (2010c) also found that *Sonchus asper* and *Melia azadirachta* had the highest concentration of proteins. Our results differ from above mentioned workers. Hussain *et al.*, (2009) reported 6.4 % in ginger, James *et al.* (2010) said that protein contents within parts of the same plants which is not the case in the present study. Shah *et al.*, (2009) stated that protein rich plants had 23% -33%, our all investigated plants had moderate level of proteins. The results also differ from those of Hameed *et al.*, (2008b), Adnan *et al.*, (2010) and Hussain *et al.*, (2010c) in this respect.

5.7d Crude fats and oils

The part of a complex organic material that is soluble in ether consists chiefly of fats and fatty acids. It is a measure of the fat or oil (lipid) of plant which may be used as medicinal or feed. Fats and oils are extremely rich sources of energy. Because of impedance of microbial fermentation, ruminant diets should be limited to about 4% fat. The crude fats varied from 6.50 – 11.34% in vegetative stage, 3.50 – 37.05% in reproductive stage and 3.00 – 26.00 % in post reproductive stage (Table 13). The overall composition of oil was recorded highest (11.34%) stem of *Datura innoxia* followed by (11.11%) stem of *Withania coagulans* and (10.30%) root of *Datura innoxia* in vegetative stage. In reproductive stage it was highest (37.05%) root of *Solanum nigrum* followed by (18.27%) flower of *Datura innoxia* and (17.00%) leaf of *Solanum surattense*. In post reproductive stage, it was highest (26.00%) in the leaves of *Datura innoxia*, followed by stem (20.50%) of *Datura innoxia* and root of *Solanum nigrum* and stem (17.00%) of *Withania somnifera*. The results revealed that the crude fats increased from vegetative stage to reproductive and decreased in the post reproductive stage (Table 13). ANOVA showed non significant differences between the plant parts and phenological stages except for *Solanum nigrum* and *Solanum surattense* parts (Table 14). Our results are in

line with Hussain & Durrani (2009), Coskun *et al.*, (2004), Cherney & Cherney (2005). Ayuba *et al.*, (2011) reported crude lipid content was 6% in root and 15.52% in the seed of *Datura innoxia* that agree with the present study.

5.7.eCrude fibers

The crude fiber is the organic residue remaining after digesting with acid and base. The compounds removed are predominantly protein, sugar, starch, lipids and portions of both the structural carbohydrates and lignins. It is an important constituent of balance diet that decreases blood cholesterol level, heart risks, colon cancer and diabetes (Ishida *et al.*, 2000). The RDA values of fibers for children are 19-25% and for lactating mother is 29 %. In the present study the crude fibers varied from 14 – 44% in vegetative stages, 6 – 30% among reproductive stages and 8 – 28% in post reproductive stages. The overall composition of crude fiber was recorded highest (44%) root of *Withania somnifera* followed by (38%) stem of *Solanum nigrum* and (34%) leaf of *Withania coagulans* in the vegetative stages. In reproductive stages it was highest (30%) flower of *Withania coagulans* followed by (26%) stem of *Datura innoxia* and (24%) flower of *Solanum nigrum*. In post reproductive stages it was highest (28.00%) leaves of *S. nigrum* followed by (16%) fruits of *S. nigrum* and (14.00%) leaves of *Datura innoxia* and fruits of *Withania somnifera*. The results revealed that the crude fibers decreased from vegetative stage to reproductive and post reproductive stage (Table 13). ANOVA showed non significant differences between the plant parts and phenological stages except for *Datura innoxia* parts but not for the phenological stages (Table 14). Belewu & Babalola (2009) stated that crude fibers can be used for useful purposes if treated with microorganisms. Hussain *et al.*, (2010c) estimated fibers varied from 9.5 % to 12.12% in selected medicinal plants. This range is similar to the results in the present case. Hameed & Dastagir (2009) studied the presence of moisture, ash, crude fiber, proteins, fats and oils, and carbohydrates in proximate percentage in *R. hastatus*, *R. dentatus* and *Rumex nepalensis* (Family Polygonaceae) which agree with present study. Aberoumand (2012) reported that *Solanum indicum* contained 8.00% crude fiber that differed from the present study.

5.7.fCarbohydrate contents

Carbohydrate is a group of organic compounds that includes sugars, starches, cellulose, and gums. It serves as a major energy source in the diet of animals. These compounds are produced in the photosynthetic plants and contain only carbon, hydrogen and oxygen usually in the ratio 1:2:1. Carbohydrates perform numerous important roles in animals. Polysaccharides serve for the storage of energy (e.g. starch and glycogen) and as structural components (e.g. cellulose in plants and chitin in animals). In the present case the carbohydrate contents varied from 12.08 – 51.43% in vegetative stages, 8.38 – 67.17% in reproductive stages and 29.32 – 75.71% in post reproductive stages. The overall composition of carbohydrate was recorded highest (51.43%) leaves of *S. nigrum* followed by (51.32%) stem of *Datura innoxia* and (50.04%) leaf of *Withania somnifera* in the vegetative stage. In reproductive stage it was highest (67.17%) leaves of *Withania coagulans* followed by (66.94%) flowers of *Withania somnifera* and (65.05%) roots of *Solanum surattense*. In post reproductive stage it was highest (75.71%) leaves of *S. nigrum* followed by (66.71%) roots of *Solanum surattense* and (65.31%) leaves of *Withania coagulans*. The results revealed that the crude carbohydrates increased from vegetative stage to reproductive and decreased in the post reproductive stage (Table 13). ANOVA showed that carbohydrates were non significant between the plant parts and phenological stages except for the phenological stages of *Solanum surattense* and *Withania coagulans* (Table 14). Lee & Lim (2006) isolated new glycoprotein (150 KDa) from *Solanum nigrum* which consist of carbohydrate content (69.74%) and protein content (30.26%). Audu *et al.* (2007) reported carbohydrate from leaves of *Lophira lanceolata*. Hameed and Dastagir (2009) reported carbohydrates contents in *R. hastatus*, *Rumex dentatus* and *R. nepalensis*. Folarin and Igbon (2010) reported carbohydrate from *Enterolobium cyclocarpum* seed. Aberoumand (2012) reported that *Solanum indicum* contained 40.67% carbohydrate which is similar to the present study.

Table 13. Proximate analyses of five selected medicinal plants of Family Solanaceae

Plant name	Plant parts	Ash	Moisture	Protein	Fat	Fiber	Carbohydrate
Vegetative stage							
<i>Datura innoxia</i> Miller.	Root	4.95	22	3.47	10.30	18	41.28
	Stem	2.08	10	5.26	11.34	20	51.32
	Leaf	4.95	18.75	6.31	8.76	22	39.23
<i>Solanum nigrum</i> Linn.	Root	6.12	20.40	6.14	6.56	30	30.78
	Stem	6.06	20.83	2.56	7.77	38	24.78
	Leaf	4.08	20.40	2.95	7.14	14	51.43
<i>Solanum surattense</i> Burm.f.	Root	5.94	20.40	3.12	7.10	18	45.44
	Stem	6.18	32	5.46	6.5	12	37.86
	Leaf	6	28.57	4.52	10.10	26	24.81
<i>Withania somnifera</i> Linn.	Root	6.93	15.68	6.26	6.89	44	20.24
	Stem	5	14.58	2.98	6.63	22	48.81
	Leaf	5.82	12.5	5.64	9.64	16	50.04
<i>Withania Coagulans</i> (Stock) Dunal.	Root	4.04	25.91	3.98	7.57	28	30.59
	Stem	9.27	44.89	4.65	11.11	18	12.08
	Leaf	9.37	22.24	2.12	10	34	22.27
Reproductive stage							
<i>Datura Innoxia</i> Miller.	Root	2.94	13.87	5.41	7.44	20	50.34
	Stem	5	32	5.23	11.61	26	20.16
	Leaf	7	26	5.68	9.23	22	30.09
	Flower	5.76	16	4.95	18.27	14	41.02
<i>Solanum nigrum</i> Linn.	Root	4	12	4.26	37.05	16	26.69
	Stem	3.09	14	3.26	10.05	6	63.60
	Leaf	9	48	5.62	13	16	8.38
	Flower	4	18	4.89	5.5	24	43.61
<i>Solanum Surattense</i> Burm.f	Root	7	8	3.45	4.5	12	65.05
	Stem	6	22	2.39	5.5	7.84	56.27
	Leaf	7	16	5.64	17	9.80	44.56
	Flower	8	20	2.95	4	20	45.05
<i>Withania Somnifera</i> Linn.	Root	10	14	4.61	10	12	49.39
	Stem	14	42	5.23	8.5	15.68	14.59
	Leaf	8	17.5	6.21	4	6	58.29
	Flower	4	13.95	5.61	3.5	6	66.94
<i>Withania Coagulans</i> (Stock) Dunal.	Root	8	34	6.45	7.5	12	32.05
	Stem	5	32	5.42	8	8	41.58
	Leaf	4	7.08	3.25	8.5	10	67.17
	Flower	5	28	5.49	7.5	30	24.01
Post reproductive stage							
<i>Datura innoxia</i> Miller.	Root	7	15.68	5.23	8.5	12	51.59
	Stem	4.08	12.5	2.68	20.5	8	52.24
	Leaf	6	19.23	5.45	26	14	29.32
	Fruit	4.12	33.33	2.36	9.5	10	40.69
<i>Solanum nigrum</i> Linn.	Root	3.12	10.41	6.45	20.5	8	51.52
	Stem	4.16	22.91	2.36	7.5	12	51.07
	Leaf	2.04	4.44	4.54	10	28	50.98
	Fruit	4.12	10.20	4.65	5	16	60.03
<i>Solanum surattense</i> Burm.f.	Root	3.12	6.55	5.62	8	10	66.71
	Stem	4.12	16	5.68	9	8	57.20
	Leaf	3.96	12.5	3.64	8.5	14	57.40
	Fruit	5.20	12.24	3.58	6.5	12	60.48
<i>Withania somnifera</i> Linn.	Root	3.09	14.58	5.64	3	11.66	62.03
	Stem	4.08	16.32	4.95	17	8.33	49.32
	Leaf	3.15	14.58	2.68	6.5	12	61.09
	Fruit	4.16	14.28	4.56	4.5	14	58.50
<i>Withania coagulans</i> (Stock) Dunal.	Root	1.92	8.16	2.95	5.5	5.76	75.71
	Stem	5.20	31.25	3.64	5	10	44.91
	Leaf	3.26	13.72	2.95	3	11.76	65.31
	Fruit	4.21	14	4.65	5	12	60.14

Table 14. Statistical analysis (ANOVA) for proximate analysis among the plant parts and phenological stages of the some selected medicinal plants of Family Solanaceae

	<i>Datura innoxia</i> Miller.	<i>Solanum nigrum</i> Linn.	<i>Solanum surattense</i> Burm.f.	<i>Withania somnifera</i> Linn.	<i>Withania coagulans</i> (Stock) Dunal.
	Ash content				
Plant parts	0.130270NS	0.288382NS	0.338288NS	0.068095NS	0.235311NS
Phenological stage	0.364625NS	0.721509NS	0.397702NS	0.087825NS	0.6591NS
	Moisture content				
Plant parts	0.519266NS	0.26437NS	0.128435NS	0.088781NS	0.07033NS
Phenological stage	0.593882NS	0.522759NS	0.520353NS	0.282527NS	0.650638NS
	Crude protein				
Plant parts	0.026988S	0.138019NS	0.067412NS	0.207269NS	0.2988816NS
Phenological stage	0.469551NS	0.534062NS	0.602314NS	0.657334NS	0.348643NS
	Fats & oils				
Plant parts	0.291864NS	0.02985S	0.024317S	0.07034NS	0.129946NS
Phenological stage	0.374125NS	0.155732NS	0.691154NS	0.810751NS	0.447674NS
	Crude fiber				
Plant parts	0.027996S	0.593498NS	0.401169NS	0.172415NS	0.670117NS
Phenological stage	0.202819NS	0.876411NS	0.899164NS	0.387861NS	0.601484NS
	Carbohydrates				
Plant parts	0.17334NS	0.52711NS	0.079569NS	0.469174NS	0.127234NS
Phenological stage	0.767304NS	0.41873NS	0.009246HS	0.449111NS	0.002195HS

Key: HS = Highly significant, S = Significant, NS = Non significant

5.8 Mineral Composition

The results of mineral compositions are shown in the Table 15 - 18.

5.8.1 Micro-elements

5.8.1a). Chromium (Cr)

The chromium contents varied from 0.020 – 0.065 ppm in vegetative stages, 0.048 – 0.286 ppm in reproductive stage and 0.286 – 0.373 ppm in post reproductive stage (Table 14). The highest chromium contents (0.065 ppm) were recorded in leaves of *Datura innoxia* and in the root of *Withania somnifera*. They were followed by stem (0.060 ppm) and leaves (0.057 ppm) of *Withania coagulans* in the vegetative stage. At reproductive stage, Cr was highest (0.286 ppm) in flowers of *Withania coagulans*, followed by leaves (0.115 ppm) of *Withania coagulans* and flowers (0.110 ppm) of *Withania somnifera*. The post reproductive stage showed highest (0.373 ppm) in fruits of *Withania somnifera*, followed by leaves (0.368 ppm) and fruits of *Withania coagulans* and roots and stems (0.363 ppm) of *Withania coagulans*. The results revealed that the chromium increased from vegetative to reproductive stage and then decreased in the post reproductive stage (Table 15). ANOVA indicated non significant differences among the plant parts. However, the differences were highly significant among the phenological stages of *Datura innoxia*, *Solanum nigrum*, *Solanum surattense* and *Withania somnifera* and significant only *Withania coagulans* (Table 16). Narendhirakannan *et al.* (2005) determined the elemental composition in the leaves of *Murraya koenigii*, *Mentha piperitae*, *Ocimum sanctum*, and *Aegle marmelos* and found marginal levels Cr. Ozcan *et al.*, (2005) determined major and minor mineral contents of young shoots, flower buds, caperberries (fruit), and seeds of *Capparis ovate* and obtained very low Cr contents. This is in the line with the present study. Rehman & Iqbal (2008) evaluated the accumulation of Cr was determined in the foliage of naturally growing plants of *Prosopis juliflora*, *Abutilon indicum* and *Senna holosericea*. The level of chromium was highest in the foliage of *S. holosericea*.

5.8.1.b). Zinc (Zn)

Zn is found in all the living organisms as trace element. There are about 200 enzymes dependent for its activity on Zn. Human body on the average has 2–4 g Zn. It is an essential element for metabolism of RNA and DNA. The permissible limit of Zn is 50

ppm in medicinal plants (Khuda *et al.*, 2012). The zinc contents in the present study varied from 0.078 – 0.628 ppm in vegetative stage, 0.025 – 0.172 ppm in reproductive stage and 0.030 – 0.314 ppm in post reproductive stage among the tested plants (Table 14). The highest (0.628 ppm) Zn contents was recorded in the stems of *Solanum surattense*, followed by stem of *Withania somnifera* (0.245 ppm) and roots (0.221 ppm) of *Solanum surattense* in the vegetative stage. In reproductive stages, it was highest (0.172 ppm) in flowers of *Withania somnifera*, followed by flowers (0.144 ppm) of *Withania coagulans* and roots (0.109 ppm) of *Withania coagulans*. At post reproductive stages, the maximum (0.314 ppm) contents were obtained in roots of *Withania coagulans*, followed by stems (0.188 ppm) of *Withania coagulans* and roots (0.155 ppm) of *Withania somnifera*. The results revealed that the zinc contents decreased from vegetative stage to reproductive but increased in the post reproductive stages (Table 15). ANOVA showed that zinc contents were non significant between the plant parts and phenological stages of all tested plants (Table 16). Rehman & Iqbal (2008) evaluated the accumulation of Zn in the foliage of naturally growing plants of *Prosopis juliflora*, *Abutilon indicum* and *Senna holosericea*. The foliage of *S. holosericea* showed highest concentration of zinc. Okwu & Josiah (2006) stated that *Aspilia africana* and *Bryophyllum pinnatum* were good sources of Zn. Demirezen *et al.*, (2006) evaluated zinc contents of various vegetables and reported the concentrations of Zn in investigated vegetables were within the recommended international standards within the permissible limit and this present the results.

5.8.1.c). Copper (Cu)

Copper is essential trace element, which found in mono and divalent forms in human, animal and plant body. The permissible limit of Cu is 10 ppm in plants (Khuda *et al.*, 2012). In the present study the copper contents varied from 0.033 – 0.278 ppm in vegetative stages, 0.062 – 0.161 ppm in reproductive stages and 0.116 – 0.213 ppm in post reproductive stages among the plants (Table 14). The highest recorded (0.278 ppm) was stems in *Withania coagulans* followed by roots (0.125 ppm) of *Withania somnifera* and leaves (0.095 ppm) of *Withania coagulans* in the vegetative stage. Reproductive stages, it was highest (0.161 ppm) in flowers of *Solanum surattense* that was approached by flowers (0.144 ppm) of *Withania coagulans* and stems (0.142 ppm) of *Solanum nigrum*

and roots of *Withania coagulans*. Copper was highest (0.213 ppm) in roots of *S. nigrum* in post reproductive stage followed by stems (0.207 ppm) of *W. coagulans* and leaves (0.202 ppm) of *Withania somnifera*. The results revealed that the copper contents decreased from vegetative stage to reproductive and then increased at the post reproductive stages (Table 15). The differences in Copper were non significant between the plant parts and phenological stages except for *Solanum nigrum* phenological stages (Table 16). Demirezenet *et al.*, (2006) reported copper contents of various vegetables within the recommended international standards. The results also show that onion (0.97µg/g) and peppermint (76.5µg/g) have the ability to accumulate Cu better than the other vegetables. Narendhirakannan *et al.*, (2005) found Cu to be in trace amounts using atomic absorption spectroscopy in *Murraya koenigii*, *Mentha piperitae*, *Ocimum sanctum*, and *Aegle marmelos*. Yusuf *et al.*, (2003) reported significant variation of copper in *Talinum triangulare*, *Celosia trigyna*, *Corchorus olitorus*, *Venomia amygdalina* and *Telfaria accidentalis*, and the soils in which they were grown. *Corchorus olitorus* (bush okra) had the better ability to accumulate other than copper.

5.8.1.d). Manganese (Mn)

The manganese contents varied from 0.144 – 19.63 ppm in vegetative stages, 0.031 – 3.079 ppm in reproductive stages and 0.026 – 0.583 ppm in post reproductive stages (Table 14). The highest manganese content (19.63 ppm) was recorded in leaves of *Datura innoxia* that was approached by roots (3.364 ppm) of *Datura innoxia* and stems (1.203 ppm) of *Solanum nigrum* in the vegetative stage. At reproductive stages, it was highest (3.079 ppm) in leaves of *Datura innoxia* followed by stems (1.781 ppm) of *Solanum surattense* and roots (1.293 ppm) of *S. surattense*. At post reproductive stage, Mn was maximum (0.583 ppm) in stems of *Solanum surattense* followed by fruits (0.541 ppm) of *W. somnifera* and fruits (0.521 ppm) of *Withania coagulans*. The results revealed that the manganese decreased from vegetative stage to reproductive and post reproductive stage (Table 15). ANOVA showed non significant differences among the plant parts and phenological stages of all plants except for *Solanum surattense* parts (Table 16). Garg *et al.*, (2007) reported that *Nordostachys jatamansi* was enriched specially in Co, Cr, Cu, Na, Mn, Fe, Rb and Zn. Said *et al.*, (1996) reported Cu, Mg, Zn, Fe, Cr and Mn in *Rheum emodi*. Hameed *et al.*, (2008b) reported C, O, Na, Mg, Al, Si, S, P, Cl, K, Ca, Ti, Fe and

Br and Mn was absent from *Rumex hastatus*, *R. dentatus*, *R. nepalensis*, *Rheum australe*, *P. plebejum* and *P. maculosa*. Hussain and Durrani (2008) reported K, P, Cu, Mn, Fe and Zn in the three phenological stage of the grasses and shrubs and stated the concentration of the Cu was higher in the grasses while the concentration of the Mn was higher in the shrubs, all of the above donot agree with the present study.

5.8.2 Macro-elements

5.8.2.a). Iron (Fe)

The iron varied from 1.797 – 36.39 ppm in vegetative stage, 0.379 – 17.66 ppm in reproductive stage and 0.253 – 7.253 ppm in post reproductive stage. The overall composition of iron was recorded highest (36.39 ppm) leaves of *Datura innoxia* followed by (11.93 ppm) stems of *Withania somnifera* and (11.06 ppm) stems of *Withania coagulans* in the vegetative stages. At reproductive stages, the maximum iron contents (17.66 ppm) was in leaves of *Datura innoxia* that was approached by roots (15.76 ppm) of *Solanum surattense* stems (6.779 ppm) of *Datura innoxia*. At post reproductive stage it was highest (7.253 ppm) in fruits of *Datura innoxia*, followed by flowers (6.128 ppm) of *Withania somnifera* and stems (2.782 ppm) of *Datura innoxia*. The results revealed that the iron decreased from vegetative stage to reproductive and post reproductive stage (Table 17). ANOVA showed non significant differences among the plant parts and phenological stages of all plants (Table 18). Folarin and Igbon (2010) reported moisture, ash, crude protein, crude fiber, oils and carbohydrate, Na, Ca, Mg, Fe, Cu and Zn from *Enterolobium cyclocarpum* seed. James *et al.*, (2010) analyzed that *Saba florida* had highest iron content in seeds followed by leaves. Hussian *et al.*, (2010c) reported highest level of iron in *Trianthema potulacastrum*. Rehman & Iqbal (2008) discovered that plants growing in polluted areas can accumulate iron in their leaves. Adnan *et al.*, (2010) observed more iron level in plants of humid region than sub humid areas. They reported highest level of iron in *Valeriana officinalis* (2787 ppm). They explained that various level of iron in plants in different habitats was due to variable soil and physical conditions. This supports our results. Hameed *et al.*, (2008b) also reported iron content in *Polygonum plebium*, *Rumex hastatus* and petiole of *Rumex nephalensis*. Khan *et al.*, (2006) discussed that iron content was higher in forage of grazing pastures. Similarly,

Kabata – Pendias & Pendias (1992) had the view that conditions of soil and climate affect the absorption of iron keeping physiological state of plants.

5.8.2.b). Calcium (Ca)

The calcium varied from 1.749 – 2.510 ppm in vegetative stage, 1.844 – 5.823 ppm in reproductive stage and 0.039 – 3.548 ppm in post reproductive stage. The overall composition of calcium was recorded highest (2.510 ppm) in stems of *Solanum surattense* followed by roots (2.371 ppm) of *Datura innoxia* and stems (2.116 ppm) of *Withania somnifera* in the vegetative stage. At reproductive stages it was highest (5.823 ppm) in leaves of *Withania coagulans* followed by flowers (5.705 ppm) of *Withania coagulans* and stems (3.398 ppm) of *Datura innoxia*. At post reproductive stages, it was highest stems (3.548 ppm) of *Datura innoxia* followed by leaves (2.981 ppm) of *Datura innoxia* and roots (2.535 ppm) of *Datura innoxia*. The results revealed that the calcium increased from vegetative stage to reproductive and then decreased at post reproductive stage (Table 17). ANOVA showed that calcium was only significant *Datura innoxia* parts and for rest of the plants parts i.e. *Solanum nigrum*, *Solanum surattense*, *Withania somnifera* and *Withania coagulans* parts it was non significant and for the phenological stages it was non significant for *Datura innoxia* and significant for all other plants (Table 18). Zafar *et al.*, (2010) described that Ca contents were present invariably in plants. Hammed *et al.* (2008b) also investigated Ca in *Polygonum plebeium*, *Rumex hastatus*, *Rumex dentatus* and *Rumex nepalensis*. It ranged from 0.99 to 7.68 ppm. Hanif *et al.* (2006) found Ca high in spinach (76 ppm) while recorded low in potato (8 ppm). This range of Ca content agreed with our findings. Hussain *et al.* (2009) found higher concentration of Ca in *Hypericum perforatum* (192 ppm). James *et al.* (2010) also reported higher Ca level in *Saba florida*. Bano *et al.* (2009) determined Ca in *Chrysopogon aucheri* and *Cymbopogon jwarancusa*. Khan *et al.* (2009) also reported seasonal effect on Ca in plants. Hussain *et al.* (2010c) stated that plants provide 25 % of Ca in food. They had higher concentration among all vegetables in *Trianthema portulacstrum*.

5.8.2.c). Potassium (K)

The potassium varied from 4.961 – 9.710 ppm in vegetative stage, 4.985 – 6.351 ppm in reproductive stage and 0.014 – 5.127 ppm in post reproductive stage. The overall

concentration of potassium was recorded highest (9.710 ppm) in roots of *Datura innoxia*, followed by stems (8.707 ppm) of *Withania somnifera* and stems (7.927 ppm) of *S. surattense* in the vegetative stage. In reproductive stage it was highest (6.351 ppm) in stems of *S. nigrum* that was approached by (6.056 ppm) flower of *D. innoxia* and (5.923 ppm) stems of *S. surattense*. In post reproductive stage maximum (5.127 ppm) concentration was in stems of *Datura innoxia* which was followed by roots (5.020 ppm) of *Datura innoxia* and fruits (4.717 ppm) of *D. innoxia*. The results indicated that the potassium contents decreased from vegetative stage to reproductive and post reproductive stage (Table 17). Potassium contents showed non significant differences in all the plant parts and phenological stages. *Solanum nigrum*, *S. surattense* and *W. coagulans* showed significant differences (Table 18). Hameed *et al.* (2008b) stated that potassium was absent in the flowers of *Rumex hastatus*. They stated that K varied 1.04 ppm to 6.57 ppm in Polygonaceae. Their findings support our results. Saidu & Jideobi (2009) and Zafar *et al.* (2010) also supported our findings. Sultan *et al.* (2008) stated that potassium content in free grazing lands was higher at early bloom than at maturity. Minson (1990) said that K content is lowered in grasses than herbs. Potassium are important to activate enzymes which affect the plant growth, development and structure (Sultan *et al.* 2008; Sultan *et al.* 2007; Hussain & Durrani, 2007; Khan *et al.* 2007).

5.8.2.d). Magnesium (Mg)

The magnesium contents fluctuated from 8.35 – 19.28 ppm in vegetative stage, 4.67 – 25.34 ppm in reproductive stage and 14.42 – 29.87 ppm in post reproductive stages. The overall concentration of magnesium was highest (19.28 ppm) in stems of *Solanum surattense*, followed by stems (16.76 ppm) of *Solanum nigrum* and stems (16.53 ppm) of *W. somnifera* in the vegetative stage. It was highest in flowers (25.34 ppm) of *Withania somnifera*, followed by leaves (20.38 ppm) of *S. surattense* and flowers (20.26 ppm) of *Solanum nigrum* at reproductive stage. In post reproductive stage, Mg was highest in flowers (29.87 ppm) of *Datura innoxia* which was followed by leaves (25.64 ppm) of *Datura innoxia* and leaves (19.22 ppm) of *W. coagulans*. The results revealed that the magnesium increased from vegetative stage to reproductive and post reproductive stage (Table 17). ANOVA showed that sodium was non significant all the plant parts for the phenological stages and it was significant for *S. surattense* and

W.coagulans for the plant parts and non significant for *Datura innoxia*, *S. nigrum* and *W. somnifera* plant parts (Table 18). Akubugwo *et al.* (2007) revealed that the order of mineral Mg>K>Ca>Fe>Na>Mn>Zn in the leaves and Mg>K>Fe>Ca>Na>Mn>Zn in the seeds of *Solanum nigrum* var *virginicum*. Bangash *et al.*, (2011) reported that vegetables had maximum concentration of macro minerals K, Ca, Mg, and Na, observed was (400, 210, 109, and 55) mg/100 g, respectively, with a minimum concentration (26, 15, 17, and 10) mg/100 g, respectively. Availability of Ca and Mg in soil affect the intake of Mg by the plants (Skerman & Riveros, 1990; Rahim *et al.*, 2008). Georgievskii (1982) revealed equal amount of Mg in leaves and stem. Its uptake was generally low at low temperature and water logged soil. The grazing pastures had usually higher contents of Mg (ARC, 1980; Islam *et al.*, 2003; Khan *et al.*, 2006). The tested species had higher level of Mg than recommended values and therefore these forages are good for lactating cattle, goat and sheep. Dua & Care (1995) stated that availability of Mg to cattle is affected by other dietary components like K, N, Ca.

5.8.2.e). Sodium (Na)

The sodium varied from 28.62 – 47.67 ppm in vegetative stage, 46.39 – 119.3 ppm in reproductive stage and 0.161 – 109.00 ppm in post reproductive stage. The overall composition of sodium was recorded highest (47.67 ppm) in leaves of *W. coagulans* followed by (45.32 ppm) roots of *W. coagulans* and (43.67 ppm) stems of *Withania coagulans* in the vegetative stages. At reproductive stages, it was highest (119.3 ppm) flowers of *W. somnifera* followed by (98.50 ppm) leaves of *W. somnifera* and (89.21 ppm) flowers of *S. nigrum*. At post reproductive stage, it was highest (109.0 ppm) roots of *Datura innoxia* followed by (96.41 ppm) leaves of *D. innoxia* and (62.80 ppm) stems of *Datura innoxia*. The results revealed that the sodium increased from vegetative stage to reproductive and then decreased at post reproductive stage (Table 17). ANOVA showed non significant differences all the plant parts and for the phenological stages it was non significant for *Datura innoxia*, significant for *S. nigrum*, highly significant for *S. surattense* and *W. coagulans* and *W. somnifera* (Table 18). It is associated with body fluid and regulates acid base balance. It is a major electrolyte of blood and help in hydration (Ayoola, 2010; Gbolahan, 2001). Its intake is related with hypertension in human. James *et al.* (2010) determined highest level of sodium in all parts of *Saba*

florida. It has been proved significant level of sodium in *Dalbergia sisso*(Hussain *et al.*, 2010c). Adnan *et al.* (2010) identified low level of sodium in *Bupleurum falcatum*. They also mentioned that *Otostegia lambata* show much increase in sodium concentration. Hanif *et al.* (2006) found maximum amount of sodium in reddish (63.9 ppm) while low level found in bottle gourd (1.7 ppm) which agrees with the present study.

Table 15. Micro-elements concentrations of the five medicinal plants of Family Solanaceae at different phenological stages.

Species	Parts	Cr (ppm)	Zn (ppm)	Cu (ppm)	Mn (pp)
Vegetative stage					
<i>Datura innoxia</i> Miller.	Root	0.038	0.126	0.056	3.364
	Stem	0.022	0.129	0.066	0.260
	Leaf	0.065	0.146	0.075	19.63
<i>Solanum nigrum</i> Linn.	Root	0.020	0.203	0.033	0.144
	Stem	0.031	0.091	0.053	1.203
	Leaf	0.036	0.109	0.084	0.420
<i>Solanum surattense</i> Burm.f.	Root	0.035	0.221	0.088	0.570
	Stem	0.033	0.628	0.068	0.706
	Leaf	0.045	0.092	0.053	0.321
<i>Withania somnifera</i> Linn.	Root	0.065	0.099	0.125	0.715
	Stem	0.049	0.245	0.087	0.378
	Leaf	0.040	0.086	0.091	0.729
<i>Withania coagulans</i> (Stock) Dunal.	Root	0.036	0.078	0.053	0.151
	Stem	0.060	0.096	0.278	0.349
	Leaf	0.057	0.135	0.095	0.409
Reproductive stage					
<i>Datura innoxia</i> Miller.	Root	0.068	0.078	0.063	0.279
	Stem	0.056	0.059	0.062	0.246
	Leaf	0.058	0.098	0.067	3.079
	Flower	0.058	0.054	0.082	1.103
<i>Solanum nigrum</i> Linn.	Root	0.048	0.025	0.065	0.041
	Stem	0.054	0.102	0.142	0.349
	Leaf	0.057	0.043	0.064	1.145
	Flower	0.064	0.038	0.075	0.343
<i>Solanum surattense</i> Burm.f.	Root	0.091	0.097	0.112	1.293
	Stem	0.067	0.039	0.076	1.781
	Leaf	0.100	0.088	0.116	0.617
	Flower	0.077	0.073	0.161	0.144
<i>Withania somnifera</i> Linn.	Root	0.090	0.041	0.089	0.031
	Stem	0.101	0.075	0.112	0.241
	Leaf	0.096	0.054	0.110	0.974
	Flower	0.110	0.172	0.128	0.173
<i>Withania coagulans</i> (Stock) Dunal.	Root	0.107	0.109	0.142	0.100
	Stem	0.106	0.063	0.141	0.126
	Leaf	0.115	0.058	0.136	0.201
	Flower	0.286	0.144	0.144	0.220
Post reproductive stage					
<i>Datura innoxia</i> Miller.	Root	0.286	0.052	0.139	0.123
	Stem	0.300	0.045	0.134	0.236
	Leaf	0.293	0.055	0.116	0.383
	Fruit	0.319	0.044	0.142	0.399
<i>Solanum nigrum</i> Linn.	Root	0.303	0.112	0.213	0.361
	Stem	0.309	0.092	0.178	0.264
	Leaf	0.318	0.140	0.159	0.137
	Fruit	0.319	0.042	0.131	0.035
<i>Solanum surattense</i> Burm.f.	Root	0.326	0.030	0.122	0.026
	Stem	0.331	0.110	0.157	0.583
	Leaf	0.341	0.062	0.179	0.263
	Fruit	0.341	0.040	0.144	0.060
<i>Withania somnifera</i> Linn.	Root	0.347	0.155	0.194	0.307
	Stem	0.354	0.116	0.170	0.209
	Leaf	0.356	0.071	0.202	0.350
	Fruit	0.373	0.096	0.181	0.541
<i>Withania coagulans</i> (Stock) Dunal.	Root	0.363	0.314	0.152	0.125
	Stem	0.363	0.188	0.207	0.140
	Leaf	0.368	0.140	0.180	0.352
	Fruit	0.368	0.097	0.178	0.521

Table 16. ANOVA results for micro-elements concentration among the plant parts and phenological stages.

Species	Phenological stages	Plant parts
	Chromium (Cr)	
<i>Datura innoxia</i> Miller.	0.004816HS	0.397355NS
<i>Solanum nigrum</i> Linn.	0.004114HS	0.46395NS
<i>Solanum surattense</i> Burm.f.	0.004864HS	0.383271NS
<i>Withania somnifera</i> Linn.	0.007355HS	0.459448NS
<i>Withania coagulans</i> (Stock) Dunal.	0.035745S	0.901351NS
	Zinc (Zn)	
<i>Datura innoxia</i> Miller.	0.245936NS	0.051438NS
<i>Solanum nigrum</i> Linn.	0.4031NS	0.078334NS
<i>Solanum surattense</i> Burm.f.	0.29676NS	0.323041NS
<i>Withania somnifera</i> Linn.	0.909845NS	0.483682NS
<i>Withania coagulans</i> (Stock) Dunal.	0.204481NS	0.257129NS
	Copper (Cu)	
<i>Datura innoxia</i> Miller.	0.087442NS	0.356177NS
<i>Solanum nigrum</i> Linn.	0.032824S	0.157441NS
<i>Solanum surattense</i> Burm.f.	0.170231NS	0.53225NS
<i>Withania somnifera</i> Linn.	0.112147NS	0.251145NS
<i>Withania coagulans</i> (Stock) Dunal.	0.504266NS	0.156031NS
	Manganese (Mn)	
<i>Datura innoxia</i> Miller.	0.315092NS	0.288391NS
<i>Solanum nigrum</i> Linn.	0.608882NS	0.251184NS
<i>Solanum surattense</i> Burm.f.	0.051244NS	0.025047S
<i>Withania somnifera</i> Linn.	0.87249NS	0.174938NS
<i>Withania coagulans</i> (Stock) Dunal.	0.686318NS	0.52993NS

Key: HS = Highly significant, S = Significant, NS = Non significant

Table 17. Macroelements concentrations of the five medicinal plants of Family Solanaceae at different phenological stages.

Species	Parts	Fe (ppm)	Ca (ppm)	K (ppm)	Mg (ppm)	Na (ppm)
		Vegetative stage				
<i>Datura innoxia</i> Miller.	Root	5.486	2.371	9.710	10.01	29.54
	Stem	2.880	1.807	7.211	15.46	28.62
	Leaf	36.39	1.871	6.452	14.32	29.31
<i>Solanum nigrum</i> Linn.	Root	3.324	1.768	5.193	12.29	33.46
	Stem	1.995	2.034	5.743	16.76	41.45
	Leaf	6.514	1.765	7.594	15.36	42.05
<i>Solanum surattense</i> Burm.f.	Root	3.208	1.749	6.227	14.19	36.74
	Stem	1.797	2.510	7.927	19.28	36.20
	Leaf	2.987	1.931	7.322	15.68	36.40
<i>Withania somnifera</i> Linn.	Root	7.586	1.835	7.595	11.15	42.54
	Stem	11.93	2.116	8.707	16.53	41.91
	Leaf	2.827	2.010	7.174	9.25	39.68
<i>Withania coagulans</i> (Stock) Dunal.	Root	2.070	1.791	4.961	15.28	45.32
	Stem	11.06	1.913	5.102	8.35	43.67
	Leaf	2.899	1.953	6.936	8.65	47.67
Reproductive stage						
<i>Datura innoxia</i> Miller.	Root	2.489	2.139	5.438	12.37	46.39
	Stem	6.779	3.398	4.985	14.25	53.51
	Leaf	2.441	1.844	5.493	8.64	49.20
	Flower	0.464	1.865	6.056	6.66	58.81
<i>Solanum nigrum</i> Linn.	Root	0.600	2.459	5.852	14.92	50.69
	Stem	0.657	1.846	6.351	15.64	46.47
	Leaf	0.514	2.393	5.250	19.25	73.37
	Flower	3.242	2.079	5.248	20.26	89.21
<i>Solanum surattense</i> Burm.f.	Root	15.76	2.259	5.268	6.37	52.15
	Stem	1.787	2.186	5.923	19.52	69.47
	Leaf	17.66	1.958	5.869	20.38	79.76
	Flower	1.191	2.511	5.576	10.23	85.35
<i>Withania somnifera</i> Linn.	Root	0.379	2.363	5.768	9.34	67.34
	Stem	3.767	2.694	5.798	5.45	82.94
	Leaf	0.470	2.497	5.273	12.93	98.50
	Flower	1.048	2.669	5.892	25.34	119.3
<i>Withania coagulans</i> (Stock) Dunal.	Root	0.620	2.645	5.349	10.87	70.88
	Stem	3.951	2.759	5.446	10.89	74.66
	Leaf	1.506	5.823	5.554	12.94	65.69
	Flower	1.657	5.705	5.783	4.67	81.20
Post reproductive stage						
<i>Datura innoxia</i> Miller.	Root	0.409	2.535	5.020	15.97	109.0
	Stem	2.782	3.548	5.127	15.36	62.80
	Leaf	0.359	2.981	4.467	25.64	96.41
	Fruit	7.253	0.061	4.717	29.87	31.01
<i>Solanum nigrum</i> Linn.	Root	0.821	0.079	0.796	16.72	30.84
	Stem	0.832	0.097	0.738	16.75	0.253
	Leaf	1.277	0.041	0.697	16.92	0.206
	Fruit	0.258	0.040	0.231	14.86	0.225
<i>Solanum surattense</i> Burm.f.	Root	0.253	0.039	0.014	14.42	0.268
	Stem	1.738	0.048	0.272	16.98	0.259
	Leaf	2.202	0.039	0.812	15.40	0.161
	Fruit	0.529	0.079	0.422	14.87	0.215
<i>Withania somnifera</i> Linn.	Root	1.065	0.084	1.183	16.76	0.274
	Stem	1.518	0.149	1.536	17.50	0.209
	Leaf	1.500	0.122	0.615	18.31	0.270
	Fruit	6.128	0.134	1.413	18.13	0.169
<i>Withania coagulans</i> (Stock) Dunal.	Root	0.571	0.054	0.422	17.84	3.601
	Stem	1.812	0.231	1.441	16.00	0.206
	Leaf	2.091	0.058	0.305	19.22	0.223
	Fruit	1.150	0.539	0.663	17.15	0.207

Table 18. ANOVA results for macro-elements concentrations among the plant parts and phenological stages.

Species	Phenological stages	Plant parts
	Iron (Fe)	
<i>Datura innoxia</i> Miller.	0.478075NS	0.547634NS
<i>Solanum nigrum</i> Linn.	0.326472NS	0.517031NS
<i>Solanum surattense</i> Burm.f.	0.112368NS	0.231195NS
<i>Withania somnifera</i> Linn.	0.320964NS	0.424722NS
<i>Withania coagulans</i> (Stock) Dunal.	0.346754NS	0.101042NS
	Calcium (Ca)	
<i>Datura innoxia</i> Miller.	0.304299NS	0.003655S
<i>Solanum nigrum</i> Linn.	0.012524S	0.13072NS
<i>Solanum surattense</i> Burm.f.	0.022001S	0.213979NS
<i>Withania somnifera</i> Linn.	0.012546S	0.157579NS
<i>Withania coagulans</i> (Stock) Dunal.	0.027938S	0.460172NS
	Potassium (K)	
<i>Datura innoxia</i> Miller.	0.888453NS	0.14047NS
<i>Solanum nigrum</i> Linn.	0.02754S	0.097226NS
<i>Solanum surattense</i> Burm.f.	0.032746S	0.149137NS
<i>Withania somnifera</i> Linn.	0.077105NS	0.174293NS
<i>Withania coagulans</i> (Stock) Dunal.	0.038953S	0.16215NS
	Magnesium (Mg)	
<i>Datura innoxia</i> Miller.	0.171927NS	0.299961NS
<i>Solanum nigrum</i> Linn.	0.476039NS	0.166991NS
<i>Solanum surattense</i> Burm.f.	0.791444NS	0.037128S
<i>Withania somnifera</i> Linn.	0.544009NS	0.819366NS
<i>Withania coagulans</i> (Stock) Dunal.	0.060225NS	0.029694S
	Sodium (Na)	
<i>Datura innoxia</i> Miller.	0.074638NS	0.13708NS
<i>Solanum nigrum</i> Linn.	0.037947S	0.309103NS
<i>Solanum surattense</i> Burm.f.	0.007795HS	0.263812NS
<i>Withania somnifera</i> Linn.	0.008662HS	0.327767NS
<i>Withania coagulans</i> (Stock) Dunal.	0.007455HS	0.188942NS

Key: HS = Highly significant, S = Significant, NS = Non significant

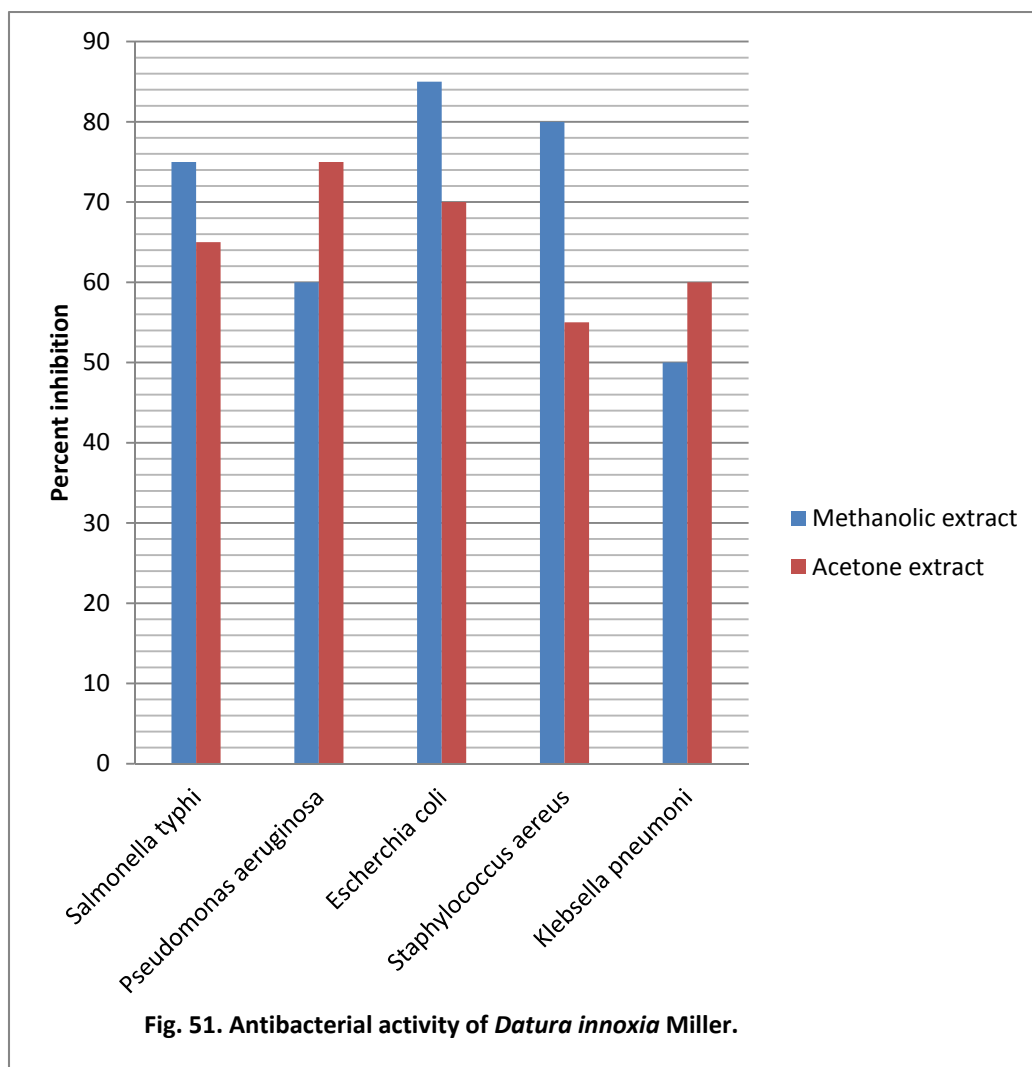
5.9 Pharmacological Activities

5.9.1 Antibacterial activity

The results for antibacterial activities are shown in Table 19. The methanolic extract of *Datura innoxia* exhibited maximum activity against *Escherichia coli* and minimum against *Klebsella pneumonia* (Fig. 51), *Solanum nigrum* showed maximum activity against *Pseudomonas aeruginosa* and minimum activity against *Staphylococcus aureus* (Fig. 52). *Solanum surattense* exhibited maximum activity against *Pseudomonas aeruginosa* and minimum against *Salmonella typhi* (Fig. 53). *Withania somnifera* had maximum activity against *Klebsella pneumonia* and minimum activity against *Salmonella typhi* (Fig. 54). *Withania coagulans* exhibited maximum activity against *Salmonella typhi* and minimum activity against *Staphylococcus aureus* (Fig. 55; Table 19).

The methanolic extract of the *D. innoxia*, *S. surattense* and *W. somnifera* showed maximum activity while in case of *S. nigrum* and *Withania coagulans* the acetone extract was more powerful against the bacterial strains. Mahmood *et al.* (2012) reported that leaves of *Datura innoxia*, *Withania somnifera* and *Solanum surattense* indicated significant antibacterial activities against *Bacillus subtilis* and *Staphylococcus aureus*, *Vibrio cholera*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Agrobacterium tumefaciens*, *Escherichia coli*. This agrees with the present findings. Sarnthima & Khammuang (2012) reported that *Solanum stramonifolium* seed had maximum activity against *Staphylococcus aureus*, *Bacillus licheniformis*, *B. subtilis* and *Xanthomonas* sp. and gram negative bacteria including *Pseudomonas aeruginosa* and *Salmonella typhi*. These antimicrobial activities are due to the phytochemical agents such as withanolides present in these plants (El Bouzidi *et al.* 2011). *Datura innoxia* has atropine, scopolamine, essential oils, saponins, flavonoids, phenols, cardiac glycosides, tannins, coumarins, carboxylic acid (Ayuba *et al.* 2011). The presence of various chemical compounds greatly affects antibacterial activities of plants. The parts and the time of collection and environmental conditions are important in such studies. Jakabova *et al.* (2012) reported that *D. innoxia* had the highest concentrations of scopolamine in different organs (leaves, flowers, stems, seeds) whereas *D. metel* accumulated the lowest scopolamine levels. Hyoscyamine was the highest in *D. stramonium* var. *tatula*, and the lowest in *D. innoxia*. Qamaruddin *et al.* (2012) reported that withaferin A was isolated

from *W. somnifera* in concentration of 10µg/ml that inhibited the growth of various Gram-positive bacteria, acid-fast and aerobic bacilli, and pathogenic fungi. It was active against *Micrococcus pyogenes* var *aureus* partially inhibited the activity of *Bacillus subtilis* glucose-6-phosphatedehydrogenase. This antibiotic activity of withaferin A is due to the presence of the unsaturated lactone-ring (Anonymous, 1982.). Okwu & Igara (2009) isolated 5, 7dimethyl 6– hydroxy 3, phenyl 3 a - amine b - yne sitosterol from *Datura metel* leaves and subjected it for antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabis*, *Solmonella typhi*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Escherichia coli*. The compound exhibited the activity against all the pathogens except *Escherichia coli*.



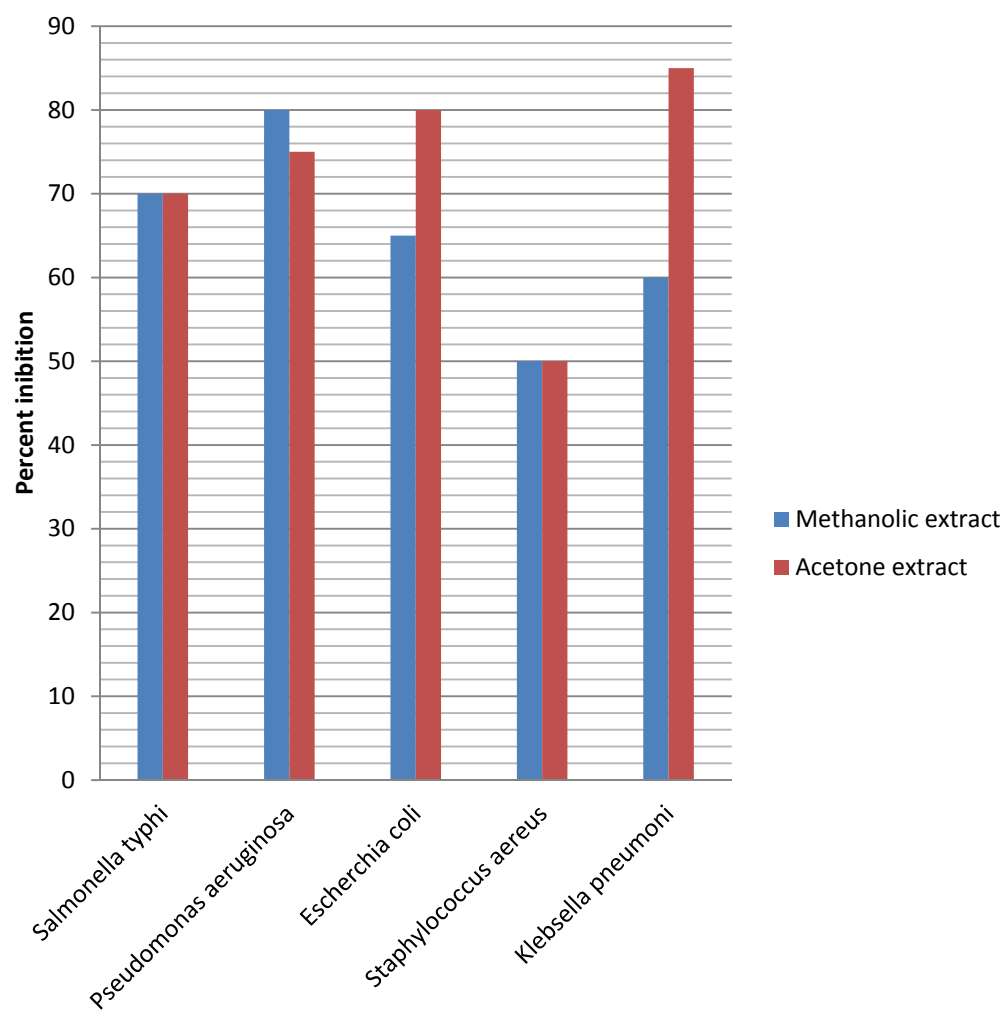
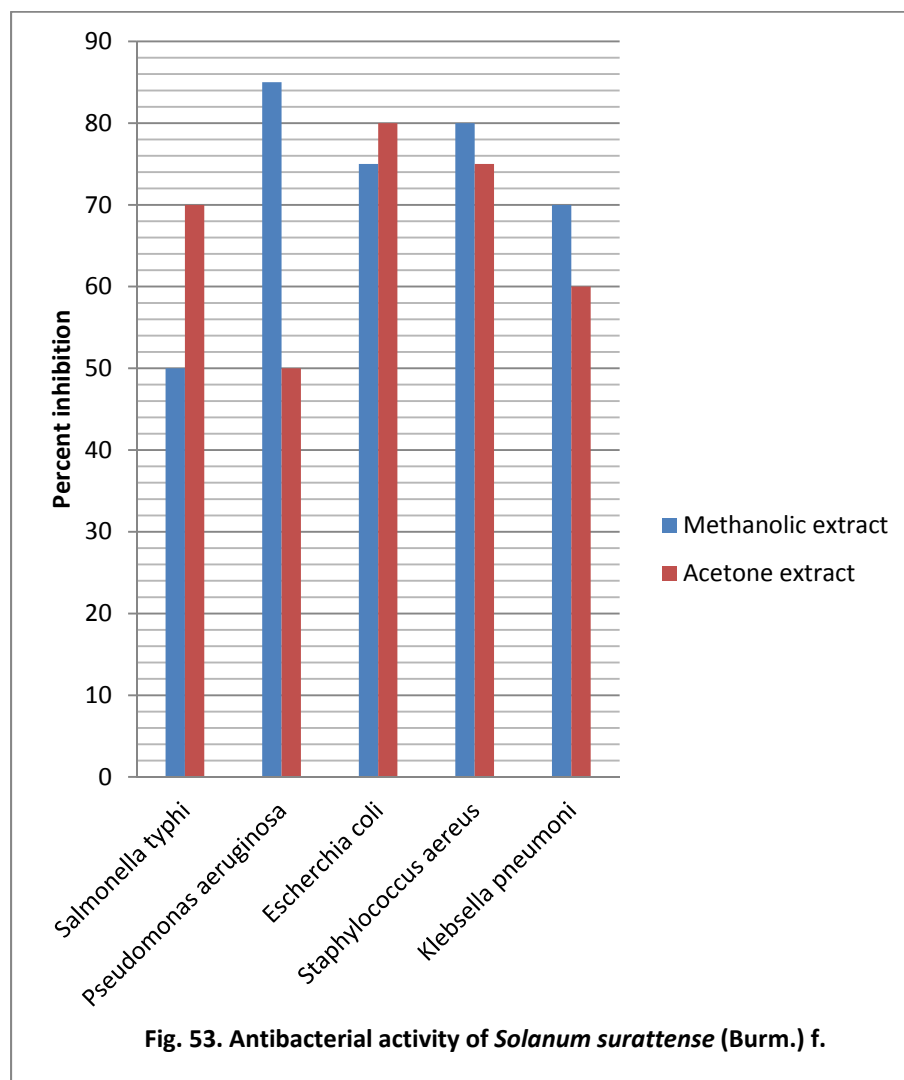


Fig. 52. Antibacterial activity of *Solanum nigrum* Linn.



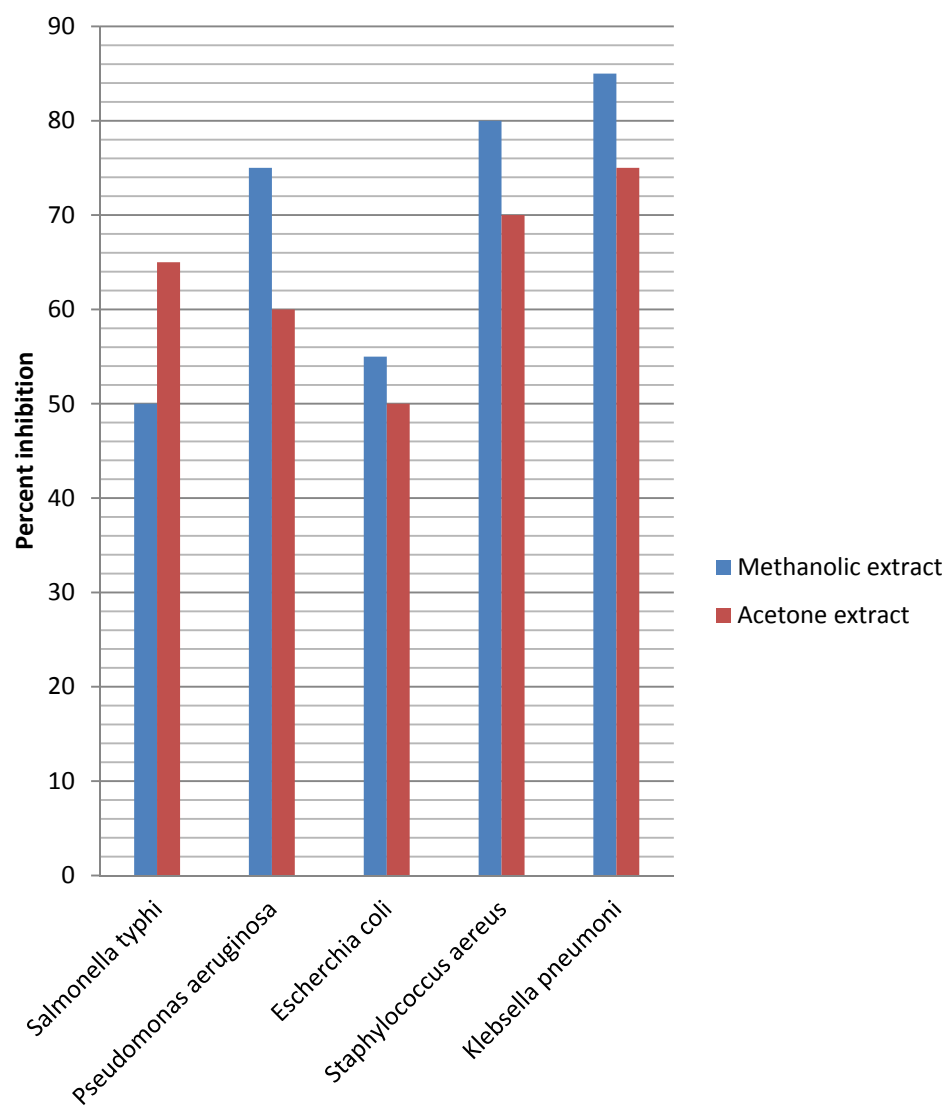


Fig. 54. Antibacterial activity of *Withania somnifera* Linn.

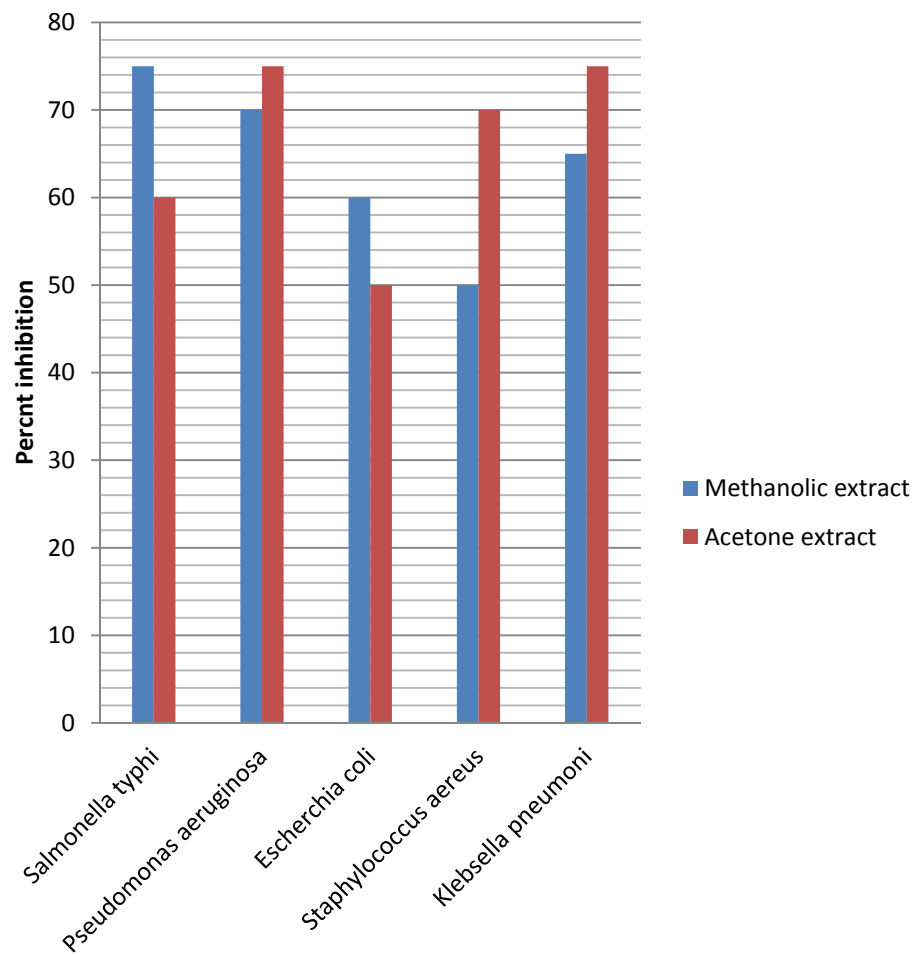


Fig. 55. Antibacterial activity of *Withania coagulans* (Stock) Dunal.

Table 19. Antibacterial activity of five selected medicinal plants of Family Solanaceae

Extract	Bacteria	Methanolic extract		Acetone extract	
		Zone of inhibition of sample (mm)	% inhibition	Zone of inhibition of sample (mm)	% inhibition
Control (mm)		20	-	20	-
<i>Datura innoxia</i> Miller.	<i>Salmonella typhi</i>	15	75	13	65
	<i>Pseudomonas aeruginosa</i>	12	60	15	75
	<i>Escherchia coli</i>	17	85	14	70
	<i>Staphylococcus aureus</i>	16	80	11	55
	<i>Klebsella pneumoni</i>	10	50	12	60
<i>Solanum nigrum</i> Linn.	<i>Salmonella typhi</i>	14	70	14	70
	<i>Pseudomonas aeruginosa</i>	16	80	15	75
	<i>Escherchia coli</i>	13	65	16	80
	<i>Staphylococcus aureus</i>	10	50	10	50
	<i>Klebsella pneumoni</i>	12	60	17	85
<i>Solanum surattense</i> Burm.f.	<i>Salmonella typhi</i>	10	50	14	70
	<i>Pseudomonas aeruginosa</i>	17	85	10	50
	<i>Escherchia coli</i>	15	75	16	80
	<i>Staphylococcus aureus</i>	16	80	15	75
	<i>Klebsella pneumoni</i>	14	70	12	60
<i>Withania somnifera</i> Linn.	<i>Salmonella typhi</i>	10	50	13	65
	<i>Pseudomonas aeruginosa</i>	15	75	12	60
	<i>Escherchia coli</i>	11	55	10	50
	<i>Staphylococcus aureus</i>	16	80	14	70
	<i>Klebsella pneumoni</i>	17	85	15	75
<i>Withania coagulans</i> (Stock) Dunal.	<i>Salmonella typhi</i>	15	75	12	60
	<i>Pseudomonas aeruginosa</i>	14	70	15	75
	<i>Escherchia coli</i>	12	60	10	50
	<i>Staphylococcus aureus</i>	10	50	14	70
	<i>Klebsella pneumoni</i>	13	65	15	75

5.9.2 Antifungal activity

The results for the antifungal activities are shown in Table 20. The methanolic extract of *D. innoxia* exhibited maximum activity against *Aspergillus parasiticus* and low against *Fusarium solani* (Fig. 56), *Solanum nigrum* showed maximum activity against *Aspergillus parasiticus* and low activity against *Aspergillus flavus* and *Candida glabarata* (Fig. 57), *Solanum surattense* revealed maximum activity against *Candida glabarata* and low against *Fusarium solani* (Fig. 58). *Withania somnifera* exhibited maximum activity against *Candida albicans* and low activity against *Candida glabarata* (Fig. 59) and *W. coagulans* showed maximum activity against *Aspergillus niger* and low activity against *Aspergillus flavus* (Fig. 60).

On the whole the methanolic extract showed maximum activity as compared to the acetone extract (Table 20). However, the antifungal activity of the tested plants depended upon the concentration of the compounds, dose of the extracts, pathogen types, type of the solvent. In this study the acetone extract of *D. innoxia* had a maximum activity against *A. flavus* and *S. nigrum* against *C. albicans*. While rest of the acetone extracts from five tested plants had low activity against the tested fungal pathogens. Similar study was conducted by David *et al.* (2010), who also reported the differential activity of solvents of *Solanum surattense* against fungal pathogens. They stated that ethanol seed extracts had high antifungal activity against *C. albicans*, *Aspergillus niger*, and *Aspergillus flavus* while the methanol seed extracts indicated high antifungal activity against *A. fumigates* and *Rhizopus oryzae*. The aqueous seed extracts were more active against *C. albicans*. It did not show antifungal activity against *Aspergillus niger* and *A. flavus*. Sridhar *et al.* (2011) reported antifungal activity of ethanol, methanol and ethyl acetate extract of leaf, seed and roots of *Solanum nigrum* against fungal strains such as *Penicillium notatum*, *Aspergillus niger*, *Fusarium oxysporium* and *Trichoderma viridae*. The above organic solvent extracts of seeds were exhibited strong antifungal activity against all the tested fungal strains compared to leaf and root extracts. They stated that the crude extracts of the plant contained various secondary compounds such as alkaloids, flavonoids, steroids, tanins and phenols which were responsible for this activity which agrees with present study. Mehrotra *et al.* (2011) reported that alkaloids and a mixture of essential oil and phenolic compounds which are responsible for the antimicrobial

activities of *W. somnifera*. Jain & Varshney (2011) reported that the methanolic and aqueous root extracts of *W. somnifera* can be used as a natural drug for the treatment of several infectious diseases caused by *Candida albicans* which also support the present findings. Shamim *et al.* (2004) reported strong antifungal activity of *Solanum nigrum* against *Aspergillus fumigates*, *Aspergillus glaucus*, *Aspergillus terreus*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton verrucosum*, *Trichophyton violaceum*. *Solanum abutiloides* highly resistant to soil-borne pathogens such as *Fusarium oxysporum* f. sp. *melongenae*, *Verticillium dahliae*, and *Ralstonia solanacearum* because of the presence of 3- β -acetoxysolavetivone, a new sesquiterpenoid which inhibits the growth of these fungi (Yokose *et al.*, 2004). Mahmood *et al.* (2012) reported that leaves of *Datura innoxia*, *Withania somnifera* and *Solanum surrattense* considerable biological activities against *Bacillus subtilis* and *Staphylococcus aureus*, *Vibrio cholera*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Agrobacterium tumefaciens*, *Escherichia coli*, *Aspergillus niger* and *Aspergillus fumigates*. Lalitha *et al.* (2010) reported the antimicrobial activity of aqueous and petroleum ether, benzene, chloroform, methanol and ethanol extracts of leaves of *S. torvum* against *Pyricularia oryzae*, *Alternaria alternata*, *Bipolaris oryzae*, *Tricoconis padwickii*, *Dreschlera tetramera*, *D. halodes*, *Curvularia lunata*, *F. oxysporum*, *F. moniliformae*, *F. solani* and *Xanthomonas oryzae*. Highly significant antifungal activity was observed in methanolic and ethanolic extract. The percentage inhibition of the test pathogenic fungi in methanolic extract was 100% (*P. oryzae*), 74.42 (*A. alternata*), 65.68 (*B. oryzae*), 87.62 (*C. lunata*), 100% (*T. padwickii*), 63.33 (*D. halodes*), 60.31 (*D. tetramera*), 76.01 (*F. moniliformae*), 59.21 (*F. oxysporum*), 43.91% (*F. solani*) which is differed from the present study.

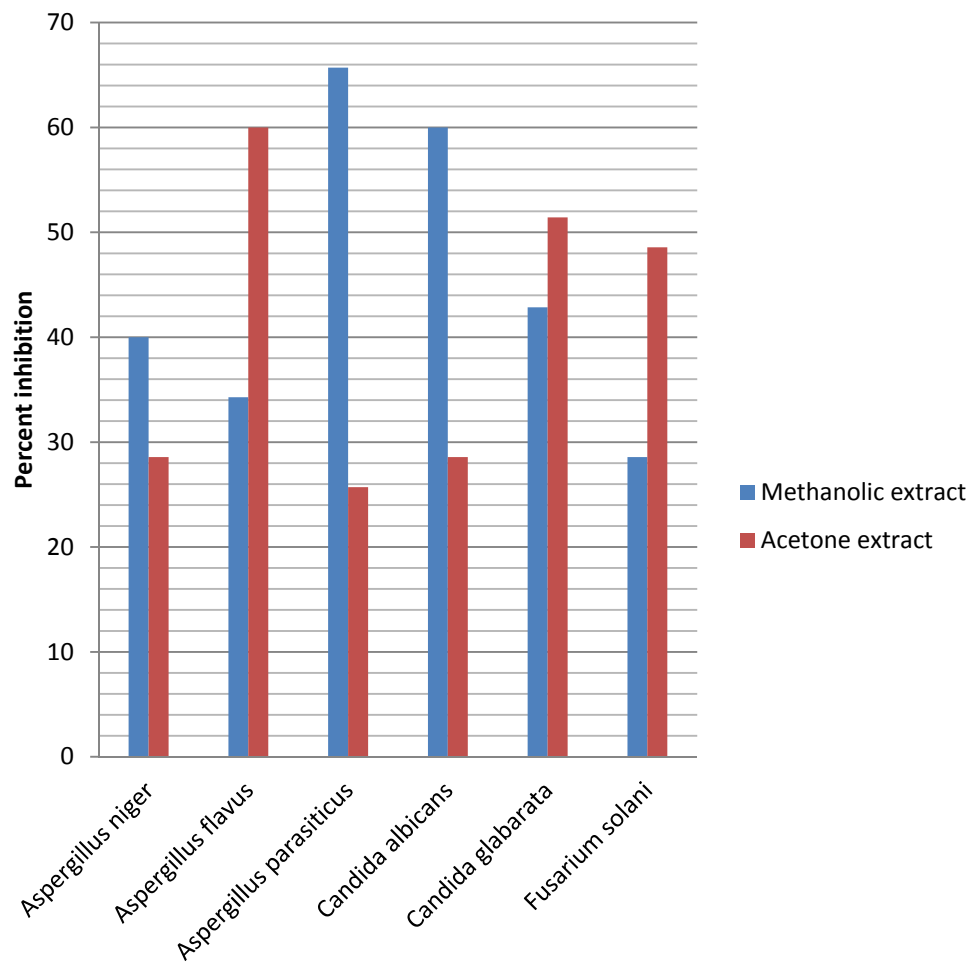
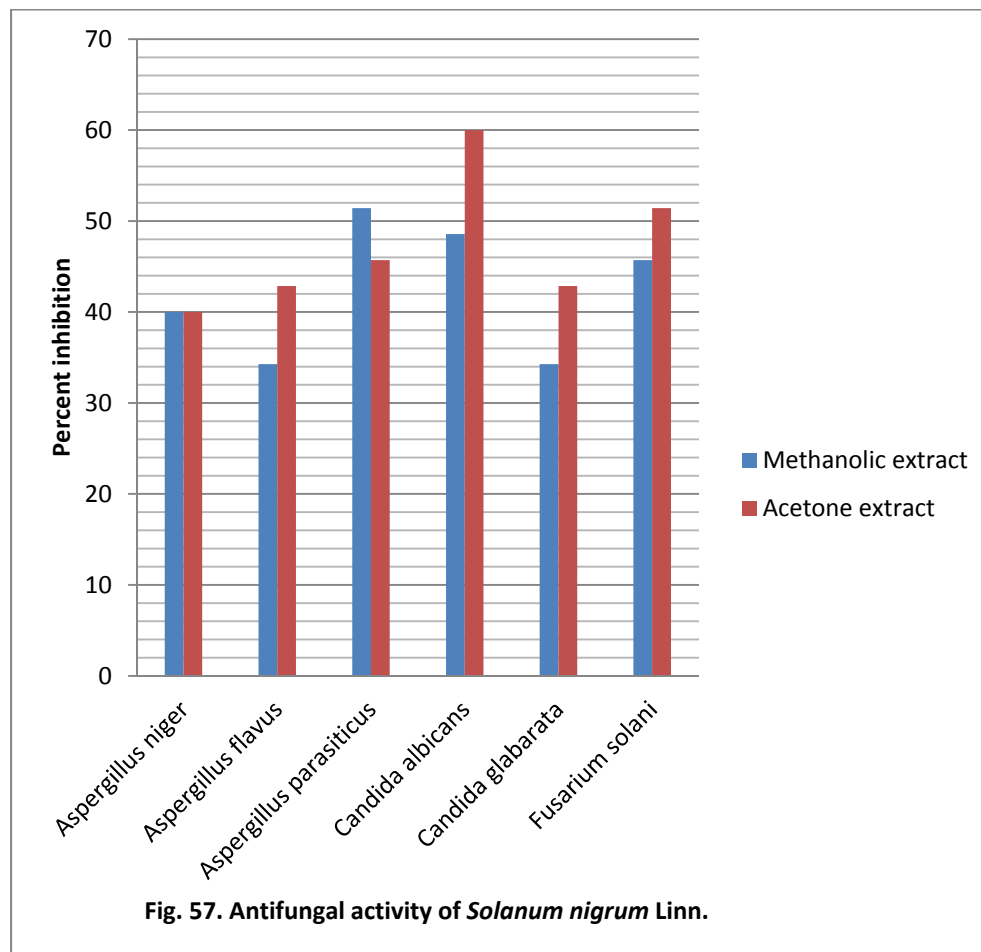


Fig. 56. Antifungal activity of *Datura innoxia* Miller.



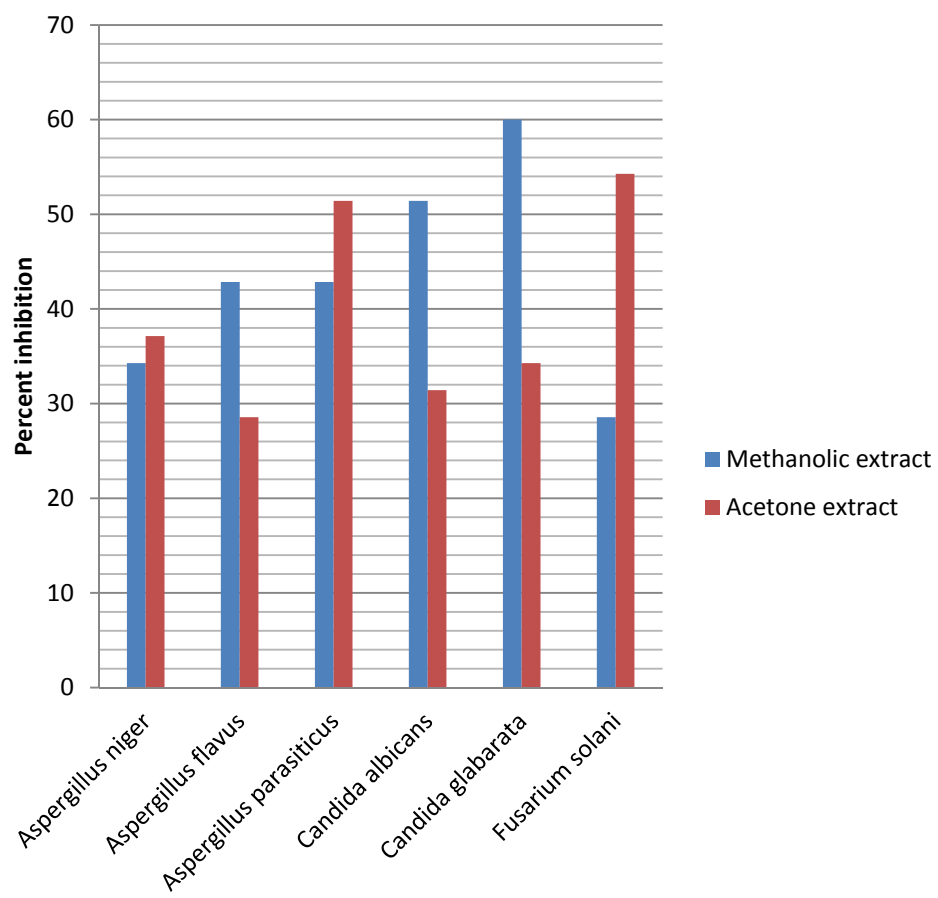


Fig. 58. Antifungal activity of *Solanum surattense* (Burm.) f.

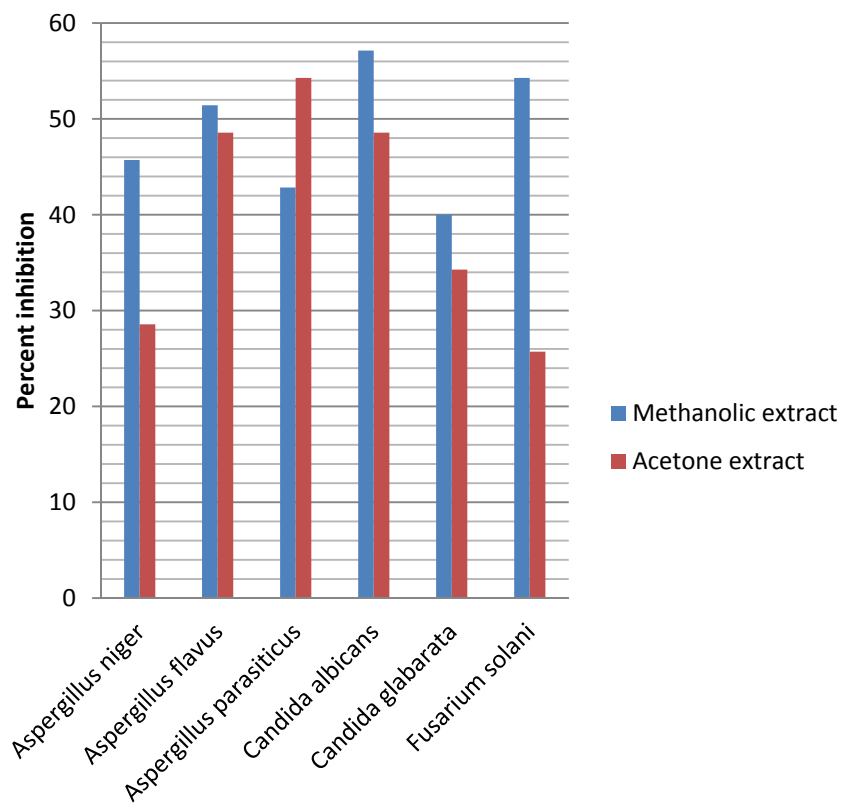


Fig. 59. Antifungal activity of *Withania somnifera* Linn.

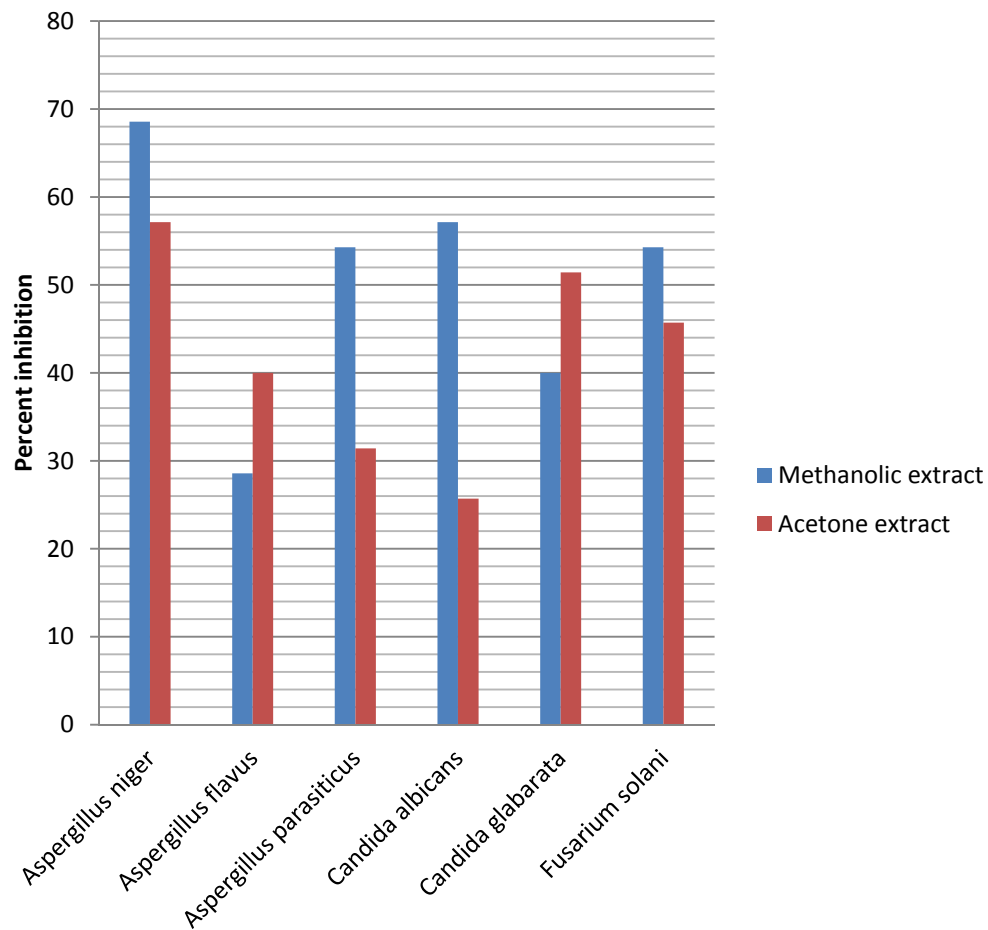


Fig. 60. Antifungal activity of *Withania coagulans* (Stock) Dunal.

Table 20. Antifungal activity of five selected medicinal plants of Family Solanaceae

Plant name	Name of fungus	Methanolic extract		Acetone extract	
		Linear growth of sample (mm)	% inhibition	Linear growth of sample (mm)	% inhibition
Control (mm)		35	-	35	-
<i>Datura innoxia</i> Miller.	<i>Aspergillus niger</i>	14	40	10	28.57
	<i>Aspergillus flavus</i>	12	34.28	21	60
	<i>Aspergillus parasiticus</i>	23	65.71	9	25.71
	<i>Candida albicans</i>	21	60	10	28.57
	<i>Candida glabarata</i>	15	42.85	18	51.42
	<i>Fusarium solani</i>	10	28.57	17	48.57
<i>Solanum nigrum</i> Linn.	<i>Aspergillus niger</i>	14	40	14	40
	<i>Aspergillus flavus</i>	12	34.28	15	42.85
	<i>Aspergillus parasiticus</i>	18	51.42	16	45.71
	<i>Candida albicans</i>	17	48.57	21	60
	<i>Candida glabarata</i>	12	34.28	15	42.85
	<i>Fusarium solani</i>	16	45.71	18	51.42
<i>Solanum surattense</i> Burm.f.	<i>Aspergillus niger</i>	12	34.28	13	37.14
	<i>Aspergillus flavus</i>	15	42.85	10	28.57
	<i>Aspergillus parasiticus</i>	15	42.85	18	51.42
	<i>Candida albicans</i>	18	51.42	11	31.42
	<i>Candida glabarata</i>	21	60	12	34.28
	<i>Fusarium solani</i>	10	28.57	19	54.28
<i>Withania somnifera</i> Linn.	<i>Aspergillus niger</i>	16	45.71	10	28.57
	<i>Aspergillus flavus</i>	18	51.42	17	48.57
	<i>Aspergillus parasiticus</i>	15	42.85	19	54.28
	<i>Candida albicans</i>	20	57.14	17	48.57
	<i>Candida glabarata</i>	14	40	12	34.28
	<i>Fusarium solani</i>	19	54.28	9	25.71
<i>Withania coagulans</i> (Stock) Dunal.	<i>Aspergillus niger</i>	24	68.57	20	57.14
	<i>Aspergillus flavus</i>	10	28.57	14	40
	<i>Aspergillus parasiticus</i>	19	54.28	11	31.42
	<i>Candida albicans</i>	20	57.14	9	25.71
	<i>Candida glabarata</i>	14	40	18	51.42
	<i>Fusarium solani</i>	19	54.28	16	45.71

5.9.3 Cytotoxic activity

Cytotoxic drugs are known to be highly toxic to cells, mainly through their action on cell reproduction. Many have proved to be carcinogenic, mutagenic or teratogenic. These drugs are increasingly being used in a variety of diseases e.g. for the treatment of cancer, rheumatoid arthritis, multiple sclerosis and auto-immune disorders. Generally, cytotoxic materials are identified by a purple symbol that depicts a cell in late telophase (Edgar-Hughes *et al.*, 2012). The methanolic extract of the *Datura innoxia* showed 2.176 intercept value, 0.126 slope, 0.909 R square, and LD₅₀ was 94.86. Similarly, the methanolic extract of *S. nigrum* showed intercept value 2.151, slope 0.139, R square 0.823 and LD₅₀ 93.73. The methanolic extract of the *Solanum surattense* showed that intercept value 2.109, slope 0.159, R square 0.812 and LD₅₀ 95.18. While the methanolic extract of the *Withania somnifera* showed that intercept value 2.190, slope 0.119, R square 0.838 and LD₅₀ 93.66. And the methanolic extract of the *Withania coagulans* showed that intercept value 2.176, slope 0.126, R square 0.909 and LD₅₀ 94.86. The acetonc extract of the *Datura innoxia* showed that intercept value 2.142, slope 0.145, R square 0.852, and LD₅₀ was 87.75. Similarly, the acetonc extract of the *Solanum nigrum* exhibited that intercept value of 2.155, slope 0.139, R square 0.786 and LD₅₀ 86.78. The acetonc extract of the *S. surattense* showed that intercept value 2.177, slope 0.132, R square 0.862 and LD₅₀ 74.31. While the acetonc extract of the *Withania somnifera* showed that intercept value 2.181, slope 0.126, R square 0.871 and LD₅₀ 86.56. And the acetonc extract of the *Withania coagulans* showed that intercept value 2.190, slope 0.119, R square 0.838 and LD₅₀ 93.66 (Table 21). Son *et al.* (2003) reported that the ethanolic extract of *Solanum nigrum* has cytotoxic effects on MCF-7 cells. Vijayan *et al.* (2004) reported the total alkaloid fractions of the methanolic extracts of the leaves, ripe fruits, roots, seeds and stem of *Solanum pseudocapsicum* were subjected to *in-vitro* cytotoxicity. All the five fractions exhibited potent activity. The total alkaloid fraction of leaves was found to be the most potent. Lim *et al.* (2004) and Heo *et al.* (2004) reported that a glycoprotein isolated from *Solanum nigrum* has a cytotoxic effect against MCF-7 and HT-29 cells, even at low concentrations. Hussain *et al.* (2010b) reported the positive cytotoxicity of the crude extracts of *Rumex hastatus*, *R. dentatus*, *Rumex nepalensis*,

Rheum australe, *Polygonum persicaria* and *Polygonum plebejum* against *Artemia salina*. The solanaceous plants contained cytotoxic compounds which had been reported by so many workers in different plants e.g. saponin from *Solanum surattense* (Lu *et al.*, 2011), glycoprotein isolated from *S. nigrum* might have shown anti-cancer abilities by blocking the anti-apoptotic pathway of NF-kappaB, activating caspase cascades reaction and increasing the production of nitric oxide (An *et al.* 2006a), solanolidide, solanolidide, yamogenin and neochlorogenin from *Solanum torvum* had found *in vitro* cytotoxic against a panel of human cancer cell lines (Lu *et al.*, 2009), Pan *et al.* (2007) isolated ten withanolides from *D. metel* flowers and demonstrated that four were cytotoxic at 10 μ M or lower against three human cancer cell lines. Withanolide D and 17 α -hydroxywithanolide D were isolated from the stems, roots, and leaves of *Tubocapsicum anomalum* exhibited cytotoxicity against Hep G2, Hep 3B, A-549, MDA-MB-231, MCF-7, and MRC-5 cell lines (Hsieh *et al.*, 2007) which agree with the present study.

Table 21. Cytotoxicity of some selected medicinal plants of Family Solanaceae

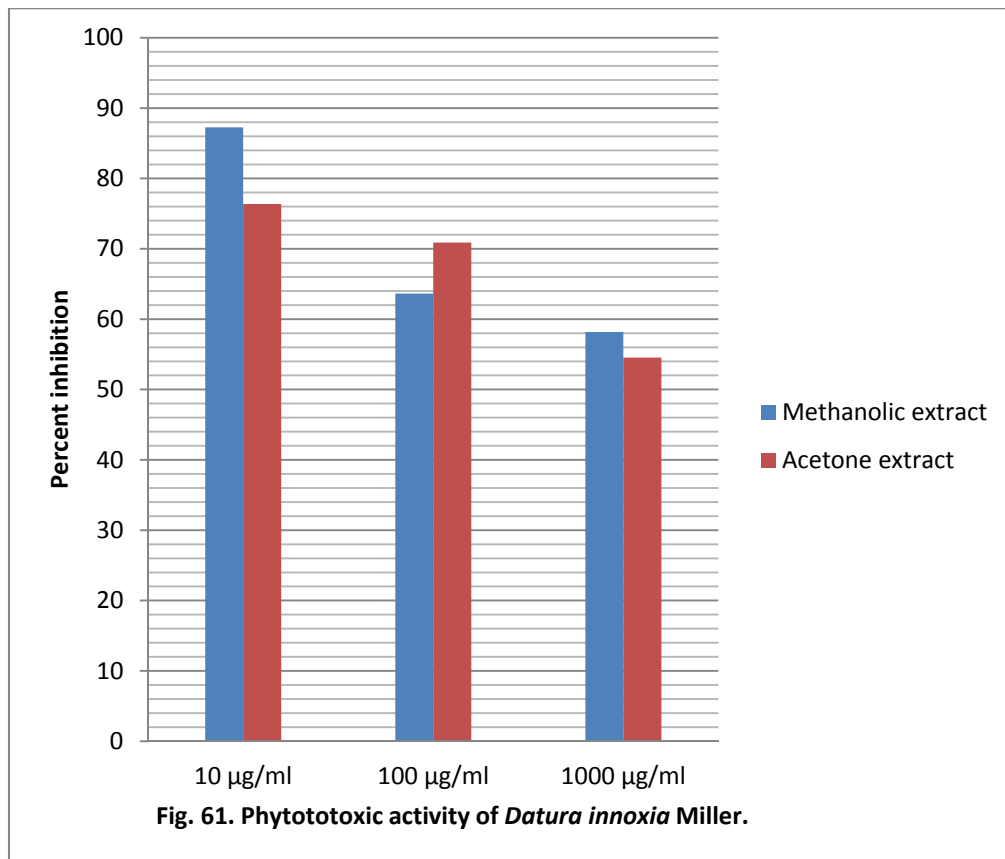
Plant name	Dose	Log 10 (Dose)	% to Probit	Inter cept	Slope	R square	LD ₅₀
<i>Datura innoxia</i> Miller.	10	1	2.279	2.176	0.126	0.909	94.86
	100	2	2.473				
	1000	3	2.530				
<i>Solanum nigrum</i> Linn.	10	1	2.252	2.151	0.139	0.823	93.73
	100	2	2.502				
	1000	3	2.530				
<i>Solanum surattense</i> Burm.f.	10	1	2.224	2.109	0.159	0.812	95.18
	100	2	2.516				
	1000	3	2.543				
<i>Withania somnifera</i> Linn.	10	1	2.279	2.190	0.119	0.838	93.66
	100	2	2.488				
	1000	3	2.516				
<i>Withania coagulans</i> (Stock) Dunal.	10	1	2.279	2.176	0.126	0.909	94.86
	100	2	2.473				
	1000	3	2.530				
<i>Datura innoxia</i> Miller.	10	1	2.252	2.142	0.145	0.852	87.75
	100	2	2.502				
	1000	3	2.543				
<i>Solanum nigrum</i> Linn.	10	1	2.252	2.155	0.139	0.786	86.78
	100	2	2.516				
	1000	3	2.530				
<i>Solanum surattense</i> Burm.f.	10	1	2.279	2.177	0.132	0.862	74.31
	100	2	2.502				
	1000	3	2.543				
<i>Withania somnifera</i> Linn.	10	1	2.279	2.181	0.126	0.871	86.56
	100	2	2.488				
	1000	3	2.530				
<i>Withania coagulans</i> (Stock) Dunal.	10	1	2.279	2.190	0.119	0.838	93.66
	100	2	2.488				
	1000	3	2.516				

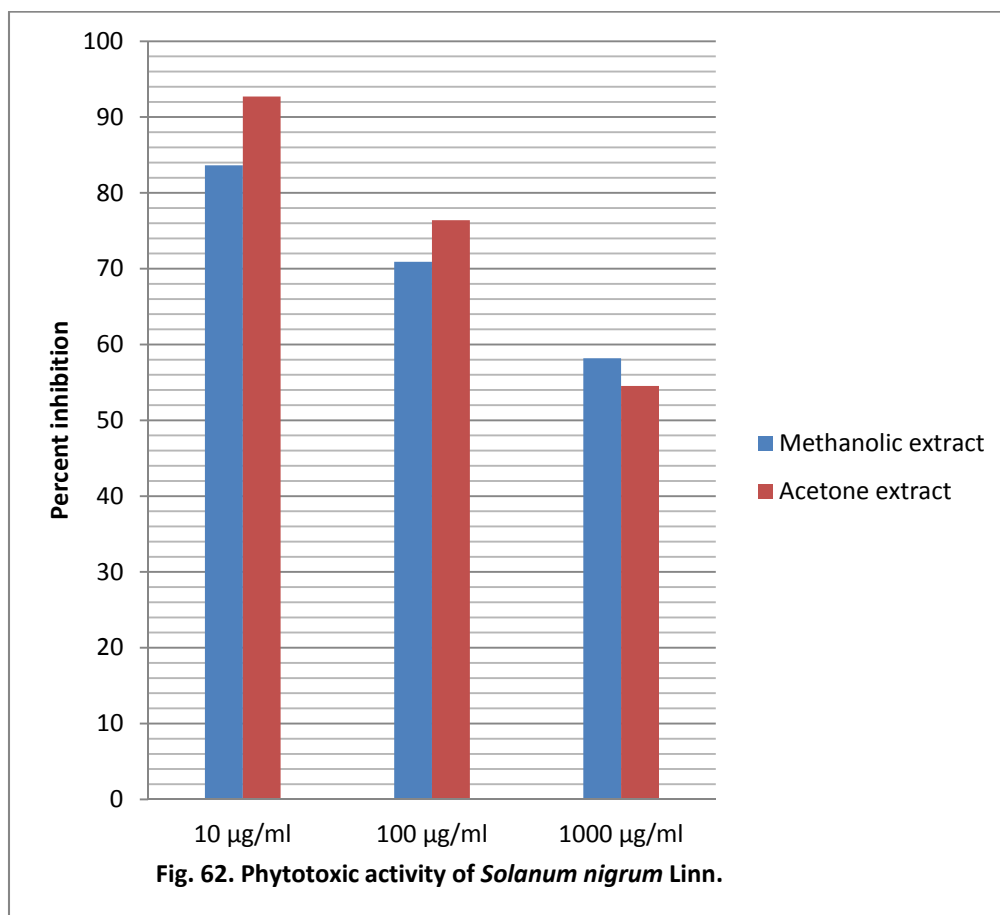
Standard was etoposoid. The data was analyzed through Probit Analysis for the calculation of LD₅₀.

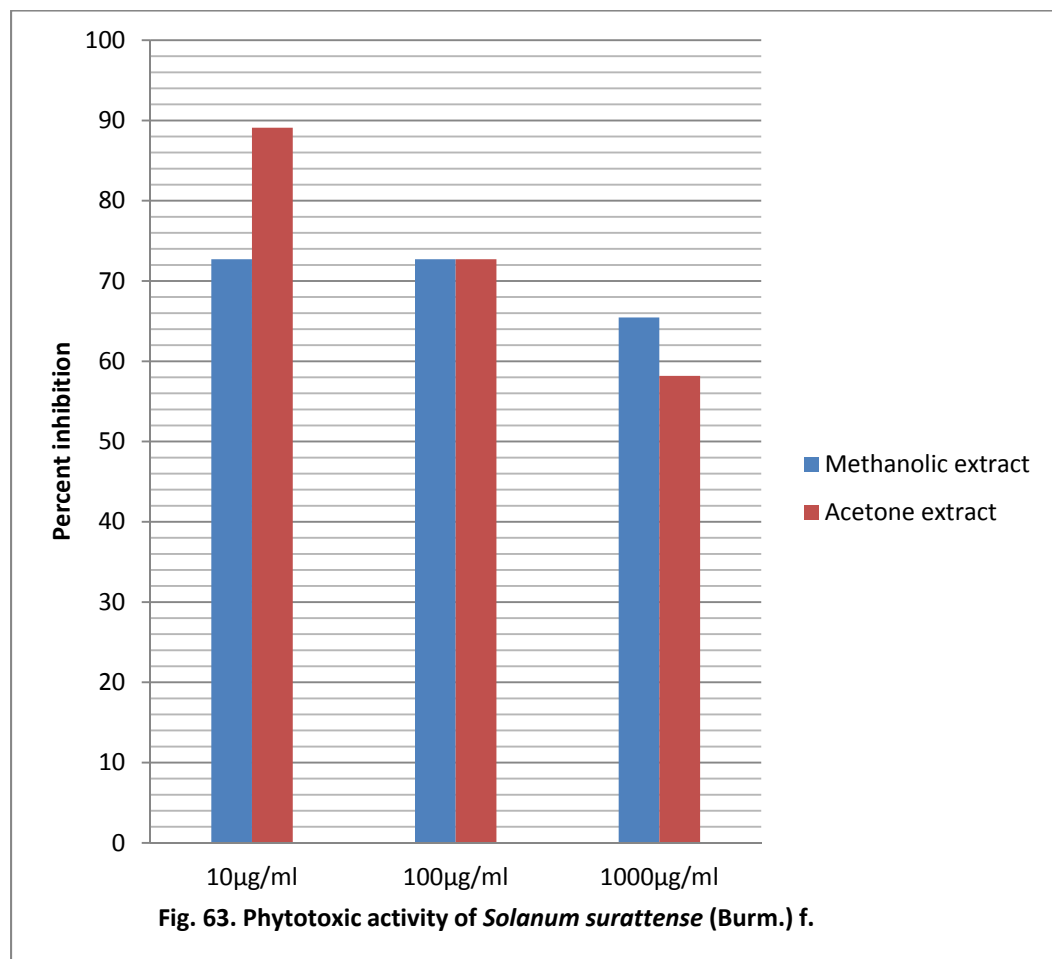
5.9.4 Phytotoxic activity

In all countries including Pakistan, there is great reduction in crop yield due to weeds. The extent of losses caused by weeds was found to be more as compared to the insects and other diseases but their effects are usually ignored. Weeds reduce productivity, because of competition for available natural resources such as sunlight, water and minerals etc. Also weeds might provide habitat for insects which damage the crops by eating them or spreading diseases. Weeds control through synthetic drugs has caused various human health problems and soil water pollution (Barkatullah *et al.*, 2011). So weeds control through harmless means is indispensable, to increase yield of various crops and to protect environments. Phytotoxicity of the methanolic extract of *Datura innoxia* and *Withania coagulans* showed significant activity at 10 µg/ml, good activity at 100µg/ml and moderate activity at 1000 µg/ml. *S. nigrum*, *Solanum surattense* and *Withania somnifera* extract showed significant activity at 10 and 100 µg/ml and moderate activity 1000 µg/ml. At the concentration of 10µg/ml % inhibition was high in *Datura innoxia* and was low in *Withania somnifera*. At the concentration of 100µg/ml % inhibition was high in *Withania somnifera* and was low in *Datura innoxia* and *Withania coagulans*. At the concentration of 1000 µg/ml % inhibition was high in *Solanum surattense* and was low in *Datura innoxia* and *Solanum nigrum*. The acetonic extracts of all the five plants showed significant activity at 10 and 100µg/ml while the extracts of all these plants showed moderate activity at 1000 µg/ml. However, at 10µg/ml the percent inhibition was high in *Solanum nigrum* and low in *Withania somnifera* and at 100 µg/ml concentration percent inhibition was high in *Withania coagulans* and was low in *Datura innoxia*. At the concentration of 1000 µg/ml percent inhibition was high in *Solanum surattense* and was low in *Datura innoxia* and *Solanum nigrum* (Table 22). Knox *et al.* (2010) reported the phytotoxic activity of *Cassia occidentalis*, *Rumex dentatus*, *Calotropis procera* and *Withania somnifera* against *Parthenium hysterophorus*. Javaid *et al.* (2009) reported that phytotoxic activity of aqueous extracts of two *Withania somnifera* and *Datura albawas* evaluated against *Rumex dentatus*. Aqueous extracts of root and shoot of both test species resulted in pronounced suppression in germination as well as seedling growth of target weed species. Application of aqueous extracts caused 68% reduction in germination, 62% in shoot length, 96% in root length and 68% in

seedling biomass. Hussain *et al.* (2010b) reported the phytotoxicity of the *R. hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Rheum australe*, *Polygonum persicaria* and *Polygonum plebeium* of the family Polygonaceae against *Lemna minor*. Moderate activity was shown by *Rumex nepalensis*, *Rheum austral* and *Polygonum persicaria* at the concentration of 100 µg/ml. All the plants showed low activity at the concentration of 10 µg/ml. And all the plants except *R. hastatus* showed high activity at the concentration of 1000 µg/ml which agree with the present study.







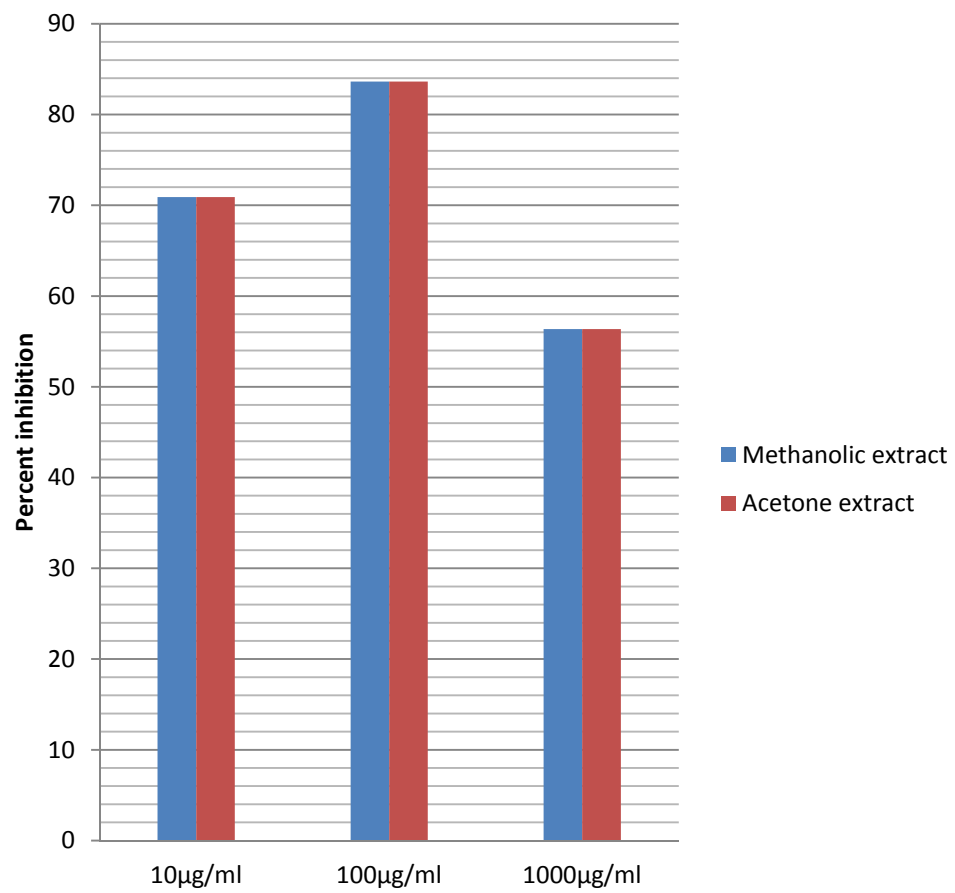


Fig. 64. Phytotoxic activity of *Withania somnifera* Linn.

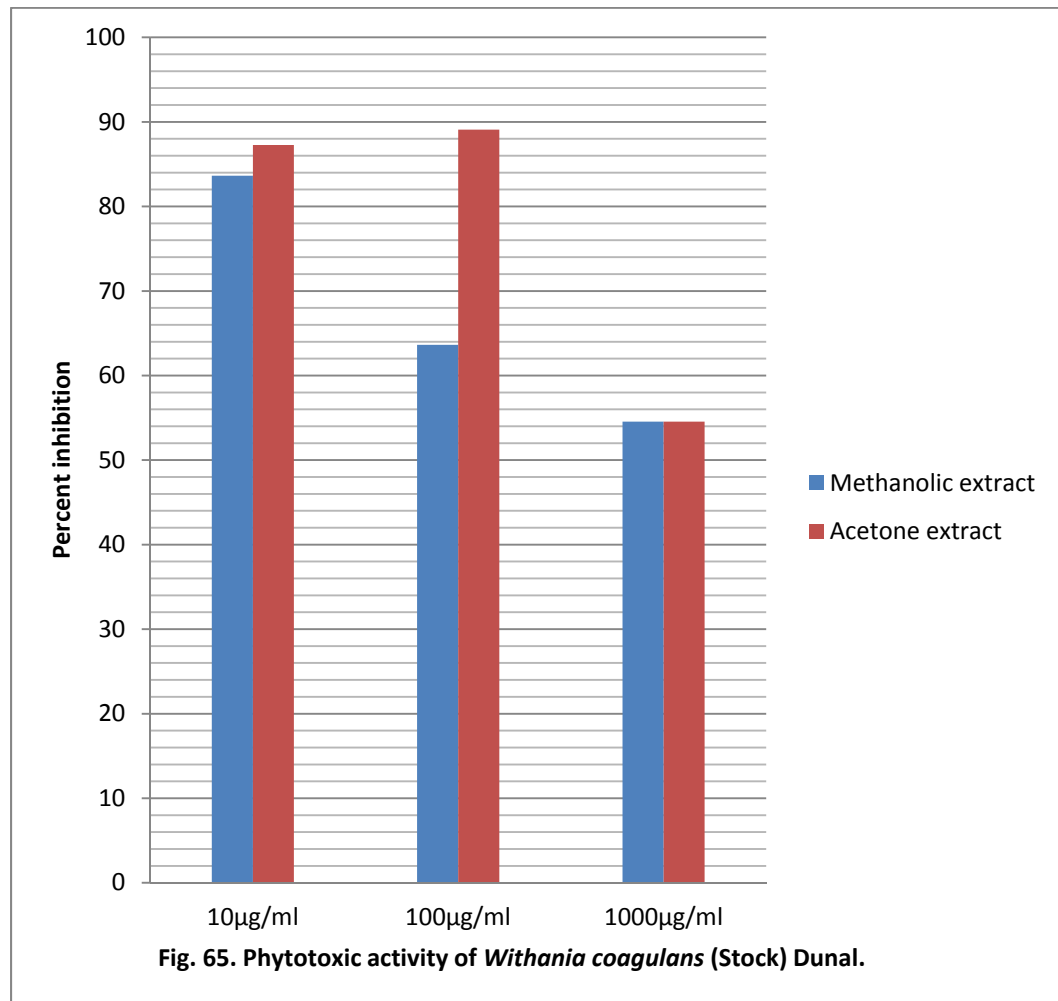


Table 22. Phytotoxicity of five selected medicinal plants of Family Solanaceae against *Lemna minor* on % fronds inhibition

Plant name	% inhibition					
-ve Control	55					
+ve Control	30					
	Methanolic extract			Acetone extract		
	10 µg/ml	100 µg/ml	1000 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml
<i>Datura innoxia</i> Miller.	87.27	63.63	58.18	76.36	70.90	54.54
<i>Solanum nigrum</i> Linn.	83.63	70.90	58.18	92.72	76.36	54.54
<i>Solanum surattense</i> Burm.f.	72.72	72.72	65.45	89.09	72.72	58.18
<i>Withania somnifera</i> Linn.	70.90	83.63	56.36	70.90	83.63	56.36
<i>Withania coagulans</i> (Stock) Dunal.	83.63	63.63	54.54	87.27	89.09	54.54

Criteria:

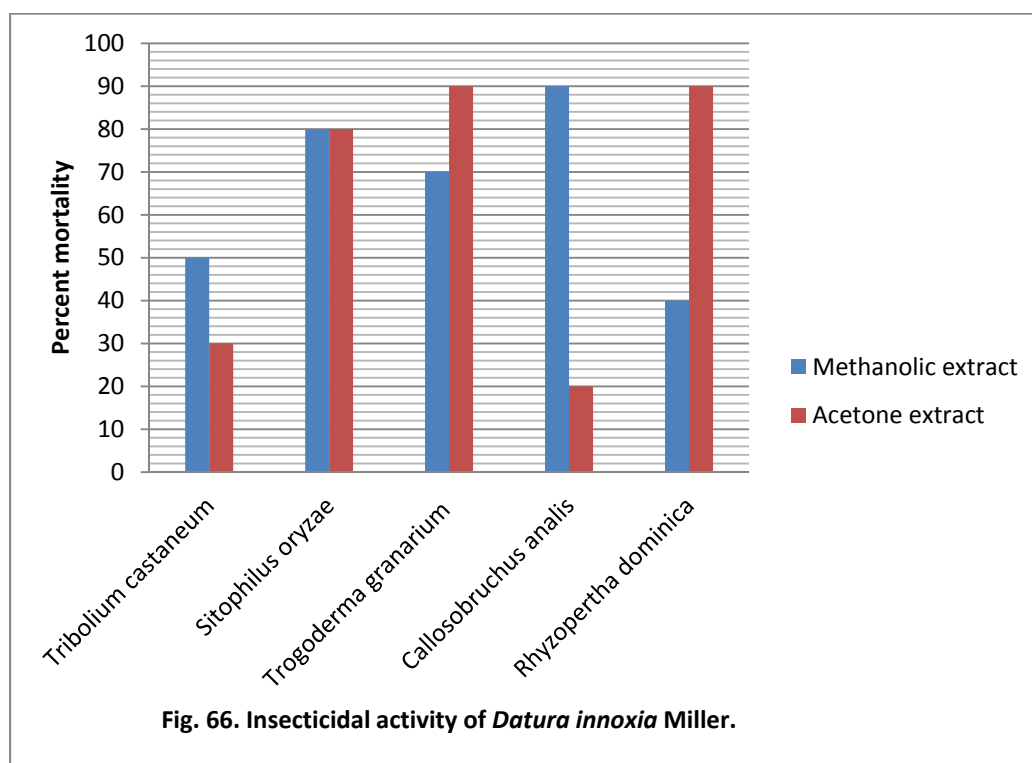
0-39% inhibition	Low activity
40-59% inhibition	Moderate activity
60-69% inhibition	Good activity
Above 70%	Significant activity

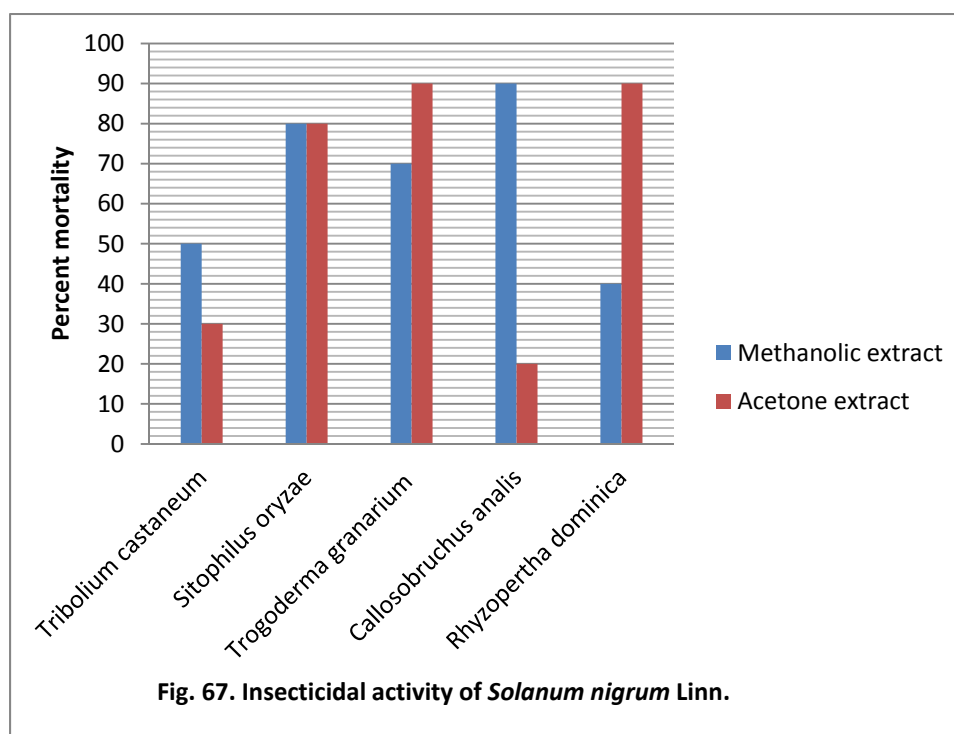
5.9.5 Insecticidal activity

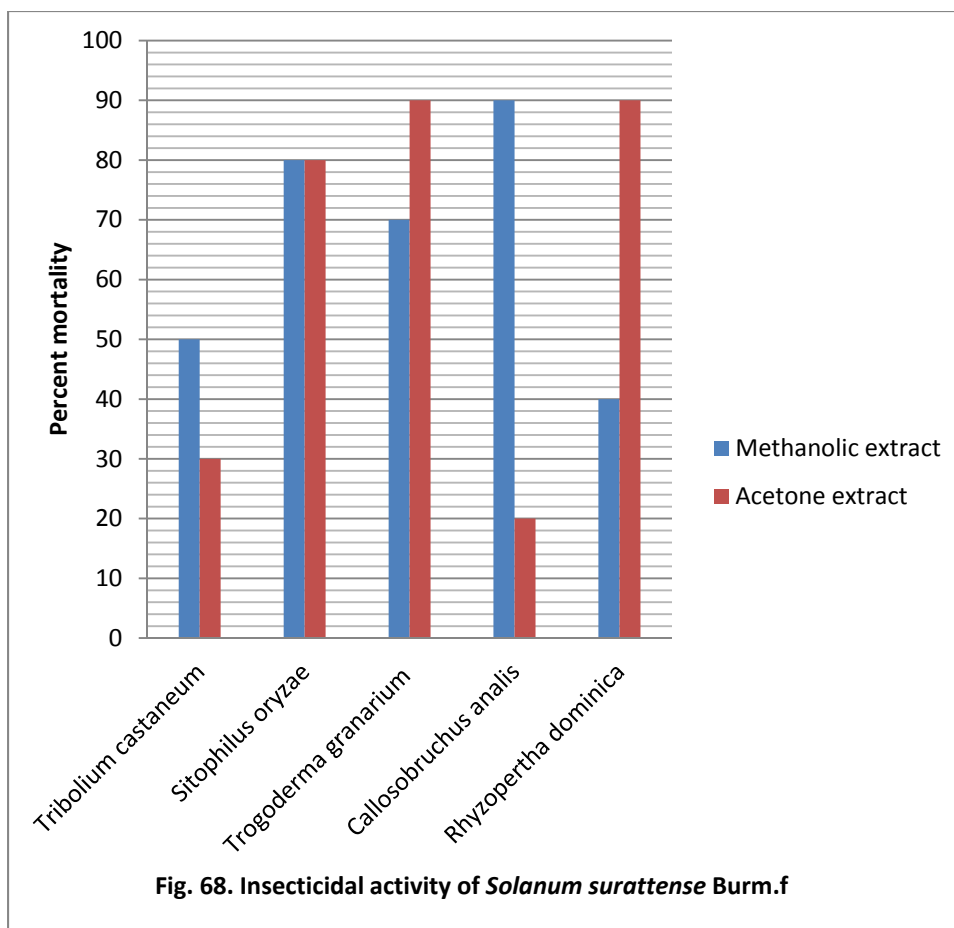
Chemicals largely used as pesticides in crop protection could be environmental pollutants that might have undesirable effects on animals and human beings. Therefore, the development of bioinsecticides has been focused as a viable pest control strategy in the recent years (Khambay *et al.*, 2002; Gonzalez *et al.*, 1999; Meena *et al.*, 1999; Hashim & Devi, 2003; EL-Kamali, 2009). The present study showed that the maximum mortality was exhibited by methanolic extract of *Datura innoxia* against *Callosobruchus analis* and the minimum mortality occurred in against *Rhyzopertha dominica*(Fig. 66). *Solanum nigrum* had maximum mortality against *Trochoderma granarium* and the minimum against *Rhyzopertha dominica*(Fig. 67). *Solanum surattense* revealed maximum mortality against *Sitophilus oryzae* and the least against *Trochoderma granarium*(Fig. 68). *Withania somnifera* caused maximum mortality in *Rhyzopertha dominica* and minimum in *Callosobruchus analis*(Fig. 69). *Withania coagulans* produced maximum mortality in *Callosobruchus analis* and the least in *Rhyzopertha dominica*(Fig. 70; Table 23).

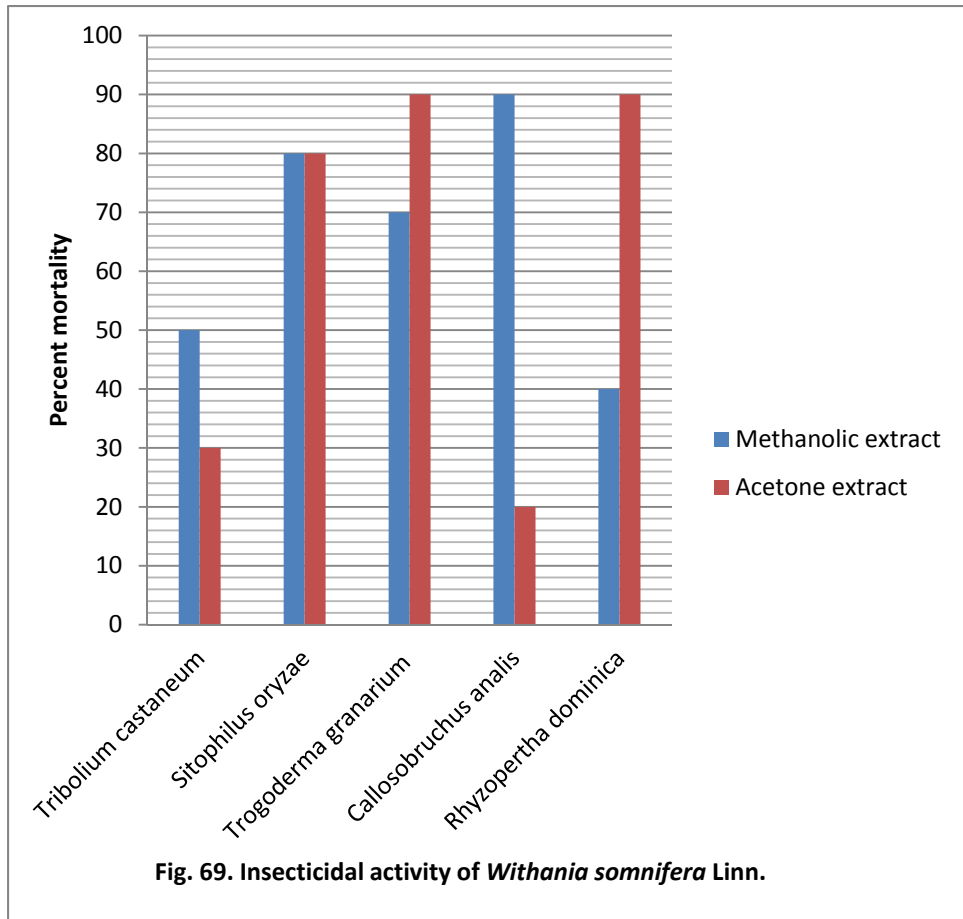
The methanolic extract generally caused the maximum mortality against the tested insects. However, in few insects the acetone extract provide more fatal i.e. *D. innoxia* against *Tribolium castaneum*, *S. nigrum* against *Rhyzopertha dominica*, *S. surattense* against *Callosobruchus analis* (Table 23). Our results agree with Boussaada *et al.* (2008), who worked on the insecticidal activity of sixteen aromatic plant extracts of family Asteraceae against adults and larvae of *Tribolium confusum*. For all extracts, mortality was higher for larvae than adults. It reached respectively 83%, 77% by using petroleum ether and methanol extracts of *R. acaule*. Their results suggested that *M. duriaei* and *R. acaule* may be used in grain storage against insect pests. Similarly, Srivastava & Gupta (2007) worked on the effect of different formulations viz., aqueous suspension, aqueous extract and ether extracts of different parts of plant *Solanum surratense* on *Callosobruchus chinensis* and reported a significant insecticidal activity of above mention plant against *C. chinensis* and the plant can be used at least partially substituted as against synthetic pesticides. Arabi *et al.* (2008) isolated the essential oil from *Perovskia abrotanoides* and reported its insecticidal activity against *Sitophilus oryzae* and *Tribolium castaneum*. It was noticed that the essential oil of *P. abrotanoides* can play an important role in stored grain protection and reduce the need for the same, and also the risks associated with the use of synthetic insecticides. Nisar *et al.* (2011)

reported that the crude methanolic extracts as well as different fraction namely n-hexane, dichloromethane, ethyl acetate, n-butanol, and aqueous of *Impatiens bicolor* were evaluated against different insects viz., *Tribolium castaneum*, *Callosobruchus analis* and *Rhyzopertha dominica*. Only n-hexane fraction showed moderate insecticidal activity and all other fractions showed very low mortality against tested insect species. Nisaret *et al.*, (2010) reported the insecticidal activity of *Impatiens bicolor* and its different fractions in which MQ-2 and MQ-5 showed moderate activity (40% mortality), while MQ-1, MQ-3, MQ-4, and MQ-6 exhibited non-significant activity (20% mortality) against *Tribolium castaneum*. Oil sub-fractions MQ-2 and MQ-3 remained inactive against *Callosobruchus analis* and *Rhyzoperthz dominica*. MQ-4 and MQ- 5 exhibited 20% mortality against *Rhyzoperthz dominica*, similarly MQ-1 and MQ-5 also showed 20% killing against *Callosobruchus analis* which was considered non significant. Hussain *et al.* (2010a) reported insecticidal activity of the crude extract of *Polygonum persicaria*, *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Polygonum plebeium* and *Rheum australe* against *Tribolium castaneum*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Callosobruchus analis* and *Trogoderma granarium*. The crude extracts of *Rumex dentatus* and *Rumex nepalensis* show significant insecticidal activity against *Sitophilus oryzae*; *Polygonum persicaria* and *Polygonum plebejum* show significant insecticidal activities against *Tribolium castaneum*, our results agree with the all the workers given above.









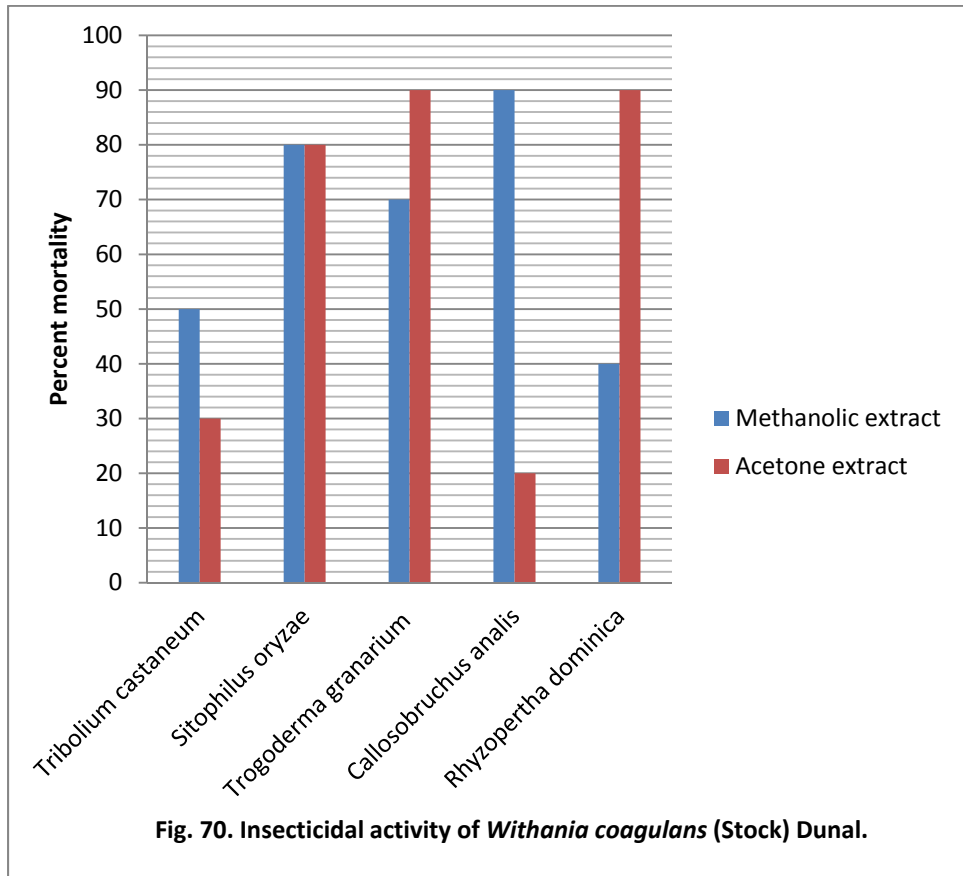


Table 23. Insecticidal activity of five selected medicinal plants of Family Solanaceae

Plant name	Name of insect	% mortality	
		Methanolic extract	Acetone extract
-ve Control		0	0
+ve Control		100	100
<i>Datura innoxia</i> Miller.	<i>Tribolium castaneum</i>	70	90
	<i>Sitophilus oryzae</i>	50	20
	<i>Trogoderma granarium</i>	60	40
	<i>Callosobruchus analis</i>	80	90
	<i>Rhyzopertha dominica</i>	20	30
<i>Solanum nigrum</i> Linn.	<i>Tribolium castaneum</i>	60	50
	<i>Sitophilus oryzae</i>	50	80
	<i>Trogoderma granarium</i>	90	40
	<i>Callosobruchus analis</i>	80	20
	<i>Rhyzopertha dominica</i>	10	90
<i>Solanum surattense</i> Burm.f.	<i>Tribolium castaneum</i>	60	50
	<i>Sitophilus oryzae</i>	90	80
	<i>Trogoderma granarium</i>	20	30
	<i>Callosobruchus analis</i>	80	90
	<i>Rhyzopertha dominica</i>	50	50
<i>Withania somnifera</i> Linn.	<i>Tribolium castaneum</i>	40	80
	<i>Sitophilus oryzae</i>	80	20
	<i>Trogoderma granarium</i>	70	40
	<i>Callosobruchus analis</i>	30	90
	<i>Rhyzopertha dominica</i>	90	20
<i>Withania coagulans</i> (Stock) Dunal.	<i>Tribolium castaneum</i>	50	30
	<i>Sitophilus oryzae</i>	80	80
	<i>Trogoderma granarium</i>	70	90
	<i>Callosobruchus analis</i>	90	20
	<i>Rhyzopertha dominica</i>	40	90

CONCLUSIONS

From the pharmacognostic study of these plants the following conclusions were made:

Daturainnoxia root was slightly pungent, cylindrical and had fibrous fracture while stem was slight green with trichomes, leaves were alternate, dentate and ovate and fruits were succulent and capsule. *Solanumnigrum* root had uneven fracture while stem was dark green with trichomes, leaves were sinuate to irregular dentate and ovate and fruits were berry. *Solanumsurattense* root had uneven & fibrous fracture while stem was prostrate, dark green and leaves were pinnatifid and elliptic oblong and fruits were berry. *Withaniasomnifera* root had fibrous fracture while stem was erect, shrubby, leaves were ovate, acute, entire and fruits were berry. *Withaniacoagulans* root had fibrous fracture while stem was erect, shrubby, leaves were ovate, obtuse, entire and fruits were berry.

The anatomical study of the plants revealed the presence of epidermis, casparian strips, tracheae, medullary rays, vascular bundles, hypodermis, pith, endodermis, pericycle and cortical cells were present in all the five plants that were investigated and besides these idioblast cells were only present in the stem of *S. nigrum*. The leaf anatomy showed the vein islet number, vein termination number and the palisade ratio. Different types of stomata have been reported in the present plants just like anomotetracytic, anisocytic, amphianisocytic and paracytic.

Micro-chemical screening revealed the presence of alkaloids, saponins, proteins, fats, anthraquinone derivatives, cellulose, calcium oxalates were present in all parts of the plants species while mucilage, starch and tanins were present in some parts while absent in other parts of the same plants. Proximate composition showed the presence of ash, moisture content, crude fiber, crude protein, fats and oils, and carbohydrate content at three different phenological stages. The mineral analysis showed the presence of Cr, Zn, Cu, Mn, Fe, Ca, K, Mg and Na in the roots, stems, leaves, flowers and fruits of the plants in three different phenological stages. Only the micro-minerals were present in traces while the macro-minerals were present high quantities as compared to the micro-minerals.

From the pharmacological point of view the plants have great potential. The plants contained such compounds which are responsible not only for antimicrobial activity but also for brine shrimp cytotoxicity. The plant also showed phytotoxicity

against *Lemna minor* which means that the plants had weedicidal properties. The insecticidal properties of these plants were also noted. Generally the methanolic extract showed the maximum mortality against these insects however, the acetone extract exhibited the maximum mortality against few insects viz, *D. innoxia* against *Tribolium castaneum*, *S. nigrum* against *R. dominica*, *S. surattense* against *C. analis*.

The findings are of importance in the establishment of diagnostic indices for the identification, classification and standardization of the plants. Further trials on screening of these plants and their constituents for various pharmacological potentials might be fruitful in terms of advance, natural and economic multipurpose drugs. This may bring a revolution in the pharmaceutical industries and as a result the economic status of the country will also be raised.

SUGGESTIONS AND RECOMMENDATIONS

After completing the whole study, I have certain suggestions and recommendations, which can help enhancing the medicinal values of the plants under study and also to facilitate all the research students to carry out their work appropriately.

1. The morpho-anatomical studies showed various diagnostic features of the Family Solanaceae. Further studies should be done for the identification and authentication of the plants to prevent admixture and adulteration and to separate the original drug from adulterant.
2. The stomatal studies of the plants revealed that further studies on the mentioned plants should be done to know about the environmental conditions where the plants contained the stomatal cluster or not.
3. The elemental analysis showed the presence of various elements in the studied plants. Further studies should be done for their role in disease controlling not only in plants but also in plants as well as in humans.
4. The nutritional analysis (ash, moisture, fats, fiber, protein and carbohydrate) showed the investigated plants contained these compounds and further studies should be done for the isolation of these compounds for usage in nutraceutical as food supplement.
5. The micro-chemical screening of the studied plants showed that various chemical compounds were found in the plants. Further studies should be done for on these plants so that these compounds should be isolated for further pharmacological potentials.
6. The study of antimicrobial activities showed that these plants showed the presence of antimicrobial compounds. Further studies should be done for the isolation of these compounds and the isolated compounds should be used in the preparation of antibiotics against the investigated pathogens.
7. The plants are cytotoxic as indicated by the results. Further studies should be done for the isolation of these compounds and should be used against various types of cancer.
8. The plants are phytotoxic in nature which is shown by the present findings. Further studies should be carried out for the isolation of phytotoxic

compounds should be used for controlling weeds and develop new weedicides of botanical origin.

The plants contained insecticidal compounds because the crude extracts of the plants showed insecticidal activity against the various insects. Further studies should be done for the isolation of these compounds and these compounds should be further used in the preparation of pesticides against these insects.

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