1.1: Introduction

India is considered as hub of medicinal plants. Long before the birth of western allopathic medicines, Ayurveda and other Indian literatures have mentioned the use of herbal plants in treatment of various ailments in humans and animals. Ayurveda is a Sanskrit word. Ayu means life and Veda means knowledge. So it deals with knowledge of life. Thus, Ayurvedic medicine is a system of medicine with historical roots in the Indian subcontinent (Wells, 2009). Ayurveda therapies have varied and evolved over more than two millennia (Meulenbeld, 1999). Therapies are typically based on complex herbal compounds, minerals and metal substances.

Basic principles of ayurvedic philosophy revolve around three important factors of life termed as Dosha's viz. *Kapha*, *Pitta* and *Vata* (Tiwari, 2005). At molecular level, *Kapha* represents nutritious regimens and may be either of dietary origin or nutritious substances at systemic or tissue level. *Pitta* refers to the factors responsible for digestion, absorption and metabolism of nutritional substances at any level of physiological processes and may be regarded as enzymatic activities, etc. *Vata* represents processes like respiration, oxygen (vayu) required for combustion of nutritious substances during metabolic activities to release and mobilize energy. Ayurveda enforces the qualitative and quantitative appropriateness and balance of all these factors to maintain normal physiological fuel homeostasis in order to live a healthy life. Therefore, it emphasizes that any vitiation either due to exogenous or endogenous over consumption or accumulation in these three factors (Tridoshas) creates an imbalance in normal physiological balance and biochemical processes. This disturbance inappropriately activates the inbuilt defence mechanisms, leading to either disproportionate accumulation or depletion of factors required for maintenance of normal physiological and biochemical homeostasis. The absence of appropriate corrective measures and homeostasis leads to disorderliness in normal physiological functions and favors multiple diseases. Hence, as a remedy, Ayurveda recommends multiple herbal preparations with appropriate characteristics to manipulate risk factors (doshas) to bring physiological homeostasis back to state of normal equilibrium. Ayurvedic concept of 'Tridosha theory' which advocates imbalance in different doshas (factors) producing various disorders and diseases, is now increasingly being accepted in modern medicine that an imbalance in physiological homeostasis generates several diseases. It is also being realized that progress of disease depends on

individual susceptibility. This concept of 'altered homeostatic theory' asserts that multiple acquired and genetic factors move the basic homeostatic balance in an action direction, which activates defence mechanism in favor of various diseases. Therefore, therapeutic regimens containing opposite qualities may help to improve and correct the imbalanced physiological homeostasis (Tiwari, 2005).

Modern science recognizes that, life is based on a complex network of reduction and oxidation reactions that are under homeostatic control. Cells or organisms are constantly subjected to the factors that alter this redox balance and results in generation of free-radicals (Nedelcu *et al.*, 2004). Imbalance in oxidant and antioxidant defence in the body is being recognized as the causative and fostering factor for development of various diseases like diabetes, urolithiasis, atherosclerosis, cancer, neurodegenerative diseases and disorders of ageing, etc. All these diseases are now being observed to share common patho-physiological platforms, irrespective of their source of origin (Tiwari *et al.,* 2004 and 2011).

Ayurveda also recognizes *Vata-Vriddhi* (oxidative stress) as the cause of several diseases if corrective measures are not taken up in time (Upadhyaya, 1993). Many therapeutic treatments like metformin, acarbose, paclitaxel, doxorubicine, neurobion etc. are available to treat various diseases like oxidative stress, diabetes, urolithiasis, atherosclerosis, cancer, neurodegenerative diseases and disorders of ageing etc.

In recent years, synthetic (Allopathic) drugs are generally less preferred because of their high cost and side effects, so it is necessary to develop traditional and alternative medicines which are specially based on medicinal plants. Herbal drugs play an important part of traditional medicine. The use of a medicinal herb, alone or in combination with other herbs, can be used as a type of combination therapy owing to the complexity of the phytochemicals and bioactivities found in the plants. Although, the herbal medicine systems are sometimes misinterpreted as being unscientific and anachronistic but, their long term existence and usage has proved, that they are capable of competing with allopathic drugs. There are many medicinal plants which are regularly used as a drug, because of their minimal side effect and cost effectiveness, which provide scientific support to the therapeutic use of the plants in medical field. The ability of plants based medicines is to serve as inhibitors of several enzymes, decrease free radical, promote apoptosis in injured cells, suppress microbial growth, and regulate inflammation and immunocompetence. Medicinal herbs have an

important role in health system all over the world for both humans and animals **(**Pandey *et al.*, 2013). However, it is essential to do further research to understand efficacy, mechanism of action and side effects of plants based natural medicines before it is developed into efficient and safe medicine system.

In the present thesis work screening and chemo-biological evaluation of some medicinal plants against various diseases like oxidative stress, diabetes, urolithiasis and cancer have been attempted (Table 1.1). The following review of literature, therefore, includes botanical and medicinal information about selected plants in the beginning followed by details of the specific diseases for which these plants were evaluated and finally other therapies used for these diseases.

Table: 1.1- Pant details

Collection No.	**Name of Plant**	**Family**	**Parts Collected for Study**	**Common Name**
Agawane 01	*Adhatoda vasica* (L.) Nees	Acanthaceae	Leaves	Adulsa
Agawane 04	*Bacopa Monnieri* (L.) Penn	Scrophulariaceae	Leaves	Brahmi
Agawane 02	*Centella asiatica* (L.) Urb.	Apiaceae	Leaves	Brahma Manduki
Agawane 08	*Chromolaena odorata* (L.) R.M. King & H.Rob	Asteraceae	Leaves	Siam Weed
Agawane 06	*Duranta erecta* (L.)	Verbenaecea	Leaves	Golden Dew Drop
Agawane 03	*Mentha arvensis* (L.)	Lamiaceae	Leaves	Pudina
Agawane 05	*Ocimum tenuiflorum* (L.)	Lamiaceae	Leaves	Krishna Tulsi
Agawane 07	*Sphagneticola trilobata* (L). Pruski	Asteraceae	Leaves	Wedelia

1.2: Plant based medicines

Medicinal plants or medicinal herbs have been identified and used from historic times. Healing by medicinal plants is as old as mankind itself. Plants make many chemical compounds for biological functions. Over 12,000 active compounds are known to science. About 20 to 25 percent of the drugs used in modern medicine have chemicals derived from plants. These chemicals work on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines may be beneficial just like

conventional drugs. However, since a single plant may contain many substances, the effects of taking a plant as medicine can be complex. The chemical content and pharmacological actions of many medicinal plants remain unknown and the possible benefits and safety of many such plants have not been studied in detail. Drug research makes use of ethnobotany to search for pharmacologically active substances in nature, and has in this way discovered hundreds of useful compounds. These include common drugs viz. aspirin, digoxin, quinine, opium and many more. The compounds found in plants are of several types, but most are in four major biochemical classes, the alkaloids, glycosides, polyphenols, and terpenes. Medicinal plants are widely used in societies because they are far cheaper than modern medicines (Lichterman, 2004).

There are many chemical substances derived from plants that are considered as important drugs currently in use in one or more countries in the world. Some of these chemical substances are shown in the Table 1.2 below.

Table: 1.2- Representative chemical substances originated from plants.

Drugs	**Action**	**Plant Source**
Acetyldigoxin	Cardiotonic	*Digitalis lanata*
Adoniside	Cardiotonic	*Adonis vernalis*
Aescin	Anti-inflammatory	*Aesculus hippocastanum*
Atropine	Anticholinergic	*Atropa belladonna*
Benzyl benzoate	Scabicide	*Several plants*
Berberine	Bacillary dysentery	*Berberis vulgaris*
Betulinic acid	Anticancerous	*Betula alba*
Borneol	Antipyretic, analgesic	*Several plants*
Caffeine	CNS stimulant	*Camellia sinensis*
Camphor	Rubefacient	*Cinnamomum camphora*
Camptothecin	Anticancerous	*Camptotheca acuminata*
Curcumin	Choleretic	*Curcuma longa*
Digitalin	Cardiotonic	*Digitalis purpurea*
Digitoxin	Cardiotonic	*Digitalis purpurea*
Digoxin	Cardiotonic	*Digitalis purpurea*
Emetine	Amoebicide, emetic	*Cephaelis ipecacuanha*
Ephedrine	Sympathomimetic, antihistamine	*Ephedra sinica*

Etoposide	Antitumor agent	*Podophyllum peltatum*
Galanthamine	Cholinesterase inhibitor	*Lycoris squamigera*
Gossypol	Male contraceptive	*Gossypium species*
Hesperidin	Capillary fragility	*Citrus species*
Hydrastine	Hemostatic, astringent	*Hydrastis Canadensis*
Hyoscyamine	Anticholinergic	*Hyoscyamus niger*
Irinotecan	Anticancer, antitumor agent	*Camptotheca acuminata*
Kheltin	Bronchodilator	*Ammi visage*
Lanatosides	Cardiotonic	*Digitalis lanata*
Lapachol	Anticancer, antitumor	*Tabebuia sp.*
Menthol	Rubefacient	*Mentha species*
Morphine	Analgesic	*Papaver somniferum*
Nicotine	Insecticide	*Nicotiana tabacum*
Noscapine	Antitussive	*Papaver somniferum*
Palmatine	Antipyretic, detoxicant	*Coptis japonica*
Papain	Proteolytic, mucolytic	*Carica papaya*
Papavarine	Smooth muscle relaxant	*Papaver somniferum*
Quinidine	Antiarrhythmic	*Cinchona ledgeriana*
Quinine	Antimalarial, antipyretic	*Cinchona ledgeriana*
Qulsqualic acid	Anthelmintic	*Quisqualis indica*
Rescinnamine	Antihypertensive, tranquillizer	*Rauvolfia serpentine*
Reserpine	Antihypertensive, tranquillizer	*Rauvolfia serpentine*
Salicin	Analgesic	*Salix alba*
Scopolamine	Sedative	*Datura species*
Silymarin	Antihepatotoxic	*Silybum marianum*
Sparteine	Oxytocic	*Cytisus scoparius*
Taxol	Antitumor agent	*Taxus brevifolia*
Teniposide	Antitumor agent	*Podophyllum peltatum*
Thymol	Antifungal (topical)	*Thymus vulgaris*
Vasicine	Cerebral stimulant	*Vinca minor*
Vinblastine	Antitumor, Antileukemic agent	*Catharanthus roseus*
Vincristine	Antitumor, Antileukemic agent	*Catharanthus roseus*

(Sourced from Veeresham, 2012)

1.3: Medicinal plants known for antioxidant activity

The realization of oxidative stress as the cause of majority of diseases in modern medicine has initiated several global programmes to harness and harvest natural herbal antioxidant-rich resources and boost antioxidant defense by various means. Some plants which are reported for antioxidants activity are as follows.

Plumbago zeylanica is the oldest herb used in Ayurveda and a native to India. It is an evergreen shrub which is an ornamental plant and has been used in traditional medicine from thousands of years. The roots of this plant contain source of an alkaloid called pumbagin which is traditionally used as germicidal and against cancer, liver diseases, fever, body pain and inflammation. Greater amount of phenolic compounds leads to more potent free radical scavenging effect as shown by crude extracts of root of *Plumbago zeylanica* (Kanungo *et al.,* 2012).

Asparagus racemosus is a species of asparagus common throughout India. It grows one to two meters tall and prefers to take root in gravelly, rocky soils high up in piedmont plains. The antioxidant activities of of *A. racemosus* roots were reported by Acharya *et.al*, (2012).

Bacopa monniera, locally known as brahmi in India, has been used for centuries in the Ayurveda. The entire plant is used medicinally; it has been also used as a folk medicine to treat cancer disease. Its antioxidant potential was proved by Kapoor *et al.,* (2009).

Some plants like *Adhatoda vasica, Centella asiatica, Dendrophthoe falcata, Hypericum perforatum, Hypericum patulum, Ocimum killimandscharicum, Sophora japonica* and *Terminalia bellerica* have also been studied and reported for antioxidant activity. All these plants have shown very good antioxidant potential against 1,1-diphenyl-2-picryl-hydrazyl free radicals when compared with standard drug vitamine E. The percentage of inhibition of DPPH free radical was more than 90 % (Jadhav and Bhutani, 2002)

The methanolic root extract of *M. arvensis* L. has shown good antioxidant potential and can be used as promising antioxidants from natural plant sources (Londonkar and Poddar, 2009)

Further, *Sphagneticola trilobata (Wedelia trilobata*) is a member of the family Asteraceae, with yellow flowers borne singly at the end of each stem. Its antioxidant

properties have been assessed by DPPH and 2,2'-azino-bis 3- thylbenzthiazoline-6-sulphonic acid (ABTS) assays. The methanol extract and standard ascorbic acid showed antioxidant activity with IC_{50} value of 90µg/mL and 60µg/mL, respectively in DPPH radical scavenging assay (Chethan *et al.*, 2012).

The antioxidative effects of the methanolic extracts of *Ficus religiosa*, *Chromolaena odorata*, *Cynodon dactylon* and *Tridax procumbens* have also been studied. Among them *T. procumbens* showed the lowest capacity. *T. procumbens* and *C. odorata* have the highest scavenging activity followed by *F. religiosa* and *C. dactylon*. The results of DPPH scavenging activity indicated that *T. procumbens* induced the largest elevation as the concentration of its extract increased, followed by *C. odorata* and *F. religiosa* and *C. dactylon (*Krishanti *et al.*, 2010*).*

The study has also been carried out to evaluate the antioxidant activities of *Ocimum tenuiflorum*. The leaf and stem extracts have been screened for their free radical scavenging properties using butylated hydroxyl toluene and ascorbic acid as standard antioxidant. DPPH free radical scavenging activity was assayed with leaf showing 71% higher activity than stem. Leaf extract showed more (82%) of superoxide anion scavenging activity than that of stem. Hydroxyl radical scavenging activity was assayed where in leaf showed 69.2% higher activity than that of stem. This study revealed that *O. tenuiflorum* has very good antioxidant potential (Balaji *et al*., 2001). Thus, many plants have been studied for antioxidant activities which are the potential candidates to develop into drugs.

1.4: Medicinal plants under study

Based on the literature, availability and reported antioxidant activity, following medicinal plants were selected for *in-vitro* and *in-vivo* screening for their activity against three important diseases, namely antidiabetic, antiurolithiasis and anti breast cancer activities.

1.4.1: *Adhatoda vasica* (L.) Nees

Kingdom: Plantae **Order**- Lamiales **Family:** Acanthaceae **Genus:** *Adhatoda* **Species-** *Adhatoda vasica* **Common name:** Adulsa	

Fig. 1.1: Scientific classification of *Adhatoda vasica*

Adhatoda vasica (L.) Nees (Acanthaceae), commonly known as Adulsa (Fig.1.1), is a small shrub growing in many regions of India and throughout the world and used in traditional ayurveda to treat various diseases. It is well-known for its efficacy in treating respiratory conditions. The leaves of *A. vasica* possess positive effects on respiratory system. *A. vasica* shows antispasmodic effect to treat asthma, chronic bronchitis, and other respiratory conditions. A poultice of the leaves of *A. vasica* can be applied to wounds for their antibacterial and anti-inflammatory properties. It is used in some parts of India to stimulate uterine contractions and speeding childbirth (Sen and Ghosh, 1925). The plant has pungent and astringent taste. It normalizes kapha and pitta and improves the voice. It is useful in ridding coughing, asthma and can be given as a cure in any disease with which these symptoms are associated. It is beneficial to the tuberculosis patient. *A. vasica* has special virtue in stopping bleeding due to the aggravation of *pitta*, through mouth, nose, genitals and urinary systems. The flowers are either white or purple (Wakhloo *et al*., 1979). Inflorescences in axillary spicate cymes, densely flowered; peduncles short; bracts broadly ovate, foliaceous (Doshi *et al*., 1983).

1.4.2: *Bacopa Monnieri* (L.) Penn

Kingdom: Plantae **Order**: Lamiales **Family:** Scrophulariaceae **Genus:** *Bacopa* **Species:** *Bacopa monnieri* (L.) **Common name:** Brahmi	

Fig.1.2: Scientific classification of *Bacopa monnieri*

Bacopa monnieri (L.) (Fig.1.2) belongs to Scrophulariaceae family. It is widely known as Brahmi and considered as 'Medhya rasayanas' in ayurveda meaning brain tonic. *B. monnieri* is a small, creeping herb. It is found in marshy areas near streams and ponds throughout India especially in the North eastern regions. It is found at elevations from sea level to altitudes of 4,400 feet. The leaf and flower bearing stems are 10-30 cm long and arise from creeping stems that form roots at the nodes. Flowers are small, white and purplish in color and appear most of the months in a year. Due to its ability to grow in water it is also called water aquarium plant. Propagation is often achieved through cuttings. The corolla is five lobed, white or pinkish. The fruit is an up to 5 mm capsule, which develops in the persistent calyx (Nadakarni, 1976). Generally brahmi contains brahmine, herpestine, alkaloids and saponins. The saponins are designated as bacoside A, bacoside B, and betulic acid. D-mannitol, stigmastanol, β-sitosterol, and stigma sterol have been isolated, while bacoside A, on acid hydrolysis give three sugars, two of which have been identified as glucose and arabinose, bacoside B also gives arabinose on hydrolysis (Warrier, 1994). Bacopa has been used in ayurvedic treatment for epilepsy and asthma. The Sushruta Samhita defines the properties of the herbs. Brahmi belongs to tikta rasa (bitter), is warming, indicated in kapha and vata. It is also used for ulcers, tumors, ascites, enlarged spleen, inflammations, leprosy, anemia, and gastroenteritis (Rajani, 2004) and also promotes fertility and sustains implantation of the embryo in the uterus (Khare, 2003).

1.4.3: *Centella asiatica* (L.) Urb.

Kingdom: Plantae **Order**- Apiales **Family:** Apiaceae (Umbelliferae) **Genus:** *Centella* **Species:** *Centella asiatica* **Common name:** Brahma manduki	

Fig. 1.3: Scientific classification of *Centella asiatica*

Centella asiatica (L.) Urb. is a stoloniferous perennial herb which belongs to the family Apiaceae (Umbelliferae) and contains 20 different species (Fig.1.3). It is a slender, creeping plant, rooting at the nodes, growing in the damp areas in different

tropical countries. It is found throughout India growing in moist places at high altitude. *C. asiatica* is a tasteless, odourless plant found in and around water. It contains small fan shaped green leaves with white or purple or pink or white flowers and bears small oval fruit. Generally whole plant is used for medicinal purposes (Singh, 2002). The stem of plant is green to reddish green in colour; leaves are borne on pericladial petioles, smooth surface thin and soft. It is widely used as a blood purifier as well as for treating high blood pressure, for memory enhancement and promoting longevity. It is recommended for the treatment of some skin conditions such as leprosy, lupus, varicose ulcers, psoriasis, diarrhoea, fever, amenorrhea and diseases of the female genitourinary tract (Brinkhaus, 2000). The primary active constituents of *C. asiatica* are saponins which include asiaticosides, in which a trisaccharide moiety is linked to the aglycone asiatic acid, madecassoside and madasiatic acid, mainly responsible for the wound healing (Singh, 1969). In Ayurveda, *C. asiatica* is one of the main herbs for revitalizing the nerves and brain cells. During the middle of the twentieth century it has shown positive results in the treatment of leprosy (Baily, 1945), also shown a wound healing, antidepressant, antiepileptic and antioxidant activity.

1.4.4: *Chromoleana odorata* (R.M.King & H.Rob)

Kingdom-Plantae **Order**-Asterales **Family**-Asteraceae **Genus**-*Chromolaena* **Species**- *Chromolaena odorata* **Common name:** Siam Weed	

Fig.1.4: Scientific classification of *Chromolaena odorata*

Chromolaena odorata (R.M.King & H.Rob) (Fig.1.4) previously known as *Eupatorium odorata* is a rapidly growing perennial shrub found in North and South America, tropical Asia, West Africa and parts of Australia. It belongs to Asteraceae family and is commonly known as Siam weed, Christmas bush, devil weed and triffid weed. *C. odorata* is a multi-stemmed shrub, grows around 2.5 m tall in open areas. It

has a soft stem with a woody base. It becomes etiolated and behaves like a creeper, growing on other vegetation. These plants have hairy and glandular leaves which gives, pungent, aromatic odour after crushing. It bears white to pale pink flowers, in panicles of 10 to 35 flowers that form at the ends of branches. They get spread by the wind, but can also cling to fur, clothes and machinery, enabling long distance dispersal. Traditionally, *C. odorata* has been used for the treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dental alveolitis (Le *et al*., 1995). It is also popular for its antispasmodic, antifungal, anti-trypanosomal, anti-hypertensive and antibacterial activities (Phan, *et al*., 2001). The methanolic leaf extract of *C. odorata* is endowed with potent anti-helmintic properties (Panda *et al*., 2010). Presence of the phenolic compunds (protocatechuic, p-hydroxybenzoic, p-coumaric, ferulic and vanillic acids) and complex mixtures of lipophilic flavonoid aglycones demonstrates powerful antioxidants properties to protect cultured skin cells against oxidative damage (Panda *et al*., 2010). The aqueous extract of *C. odorata* leaves has been tested for its ability to alter blood pressure and pulse rates in normal and sub-chronic salt-loaded rats. The extract has shown to lower the systolic, diastolic, pulse and mean arterial pressures of the treated rats, compared to test control in dose dependent manner (Ikewuchi *et al*., 2012).

In another report it is shown that *C. odorata* has good nitric oxide radical scavenging potential and presence of phenolic compounds, flavonoids and tannins might be responsible for the observed scavenging activity (Alisi and Onyeze, 2008). Adedapo, (2016) has further shown that the methanolic leaf extract of *C. odorata* alone can cause marked inhibition of HT-29 cells (colorectal cancer cell lines). It has been stated that plants rich in phenols are of great medicinal value due to their antioxidant potential and the relation between antioxidant activity and presence of phenols in common medicinal plants (Fu *et al*., 2011). *C. odorata* has also been tested against *Staphylococcus aureus; Escherichia coli*, and the leaves and root extracts are most active exhibiting MIC around 500µg/mL (Mihigo *et al*., 2015).

1.4.5: *Duranta erecta* (L.)

Duranta erecta also known as golden dewdrops (Fig.1.5) is a smooth, unarmed shrub, with straggling and drooping branches. Leaves are small, obovate-elliptic, 3 to 6 cm long, with pointed or rounded tips and pointed base; the margins, entire or slightly toothed. Flowers are scentless, borne on one side of the rachis, white or lilac-blue

with two violet stripes, about 1 centimeter long and 1 centimeter wide. Fruit is fleshy, ovoid, orange-yellow and 7 to 8 mm long.

Kingdom: Plantae **Order**- Lamiales **Family:** Verbenaceae **Genus:** *Duranta* **Species:** *Duranta. erecta* **Common name:** Golden Dew Drop	

Fig. 1.5: Scientific classification of *Duranta erecta*

D. erecta is a native of South America to Argentina, Florida, Mexico, Central America, Bermuda, Bahamas and West Indies through India, along the sides of ravines and banks of streams. *D. erecta* has been reported for rich plant chemicals. The plant is known to contain alkaloids, iridoid glycosides, iridoid lailid, acteoside and saponin. Fruit of *D. erecta* are used for febrifuge and treating malaria, intestinal worms. Whole plant is used as insect repellent, treatment of itches, infertility, fever and pneumonia by the tribal and the mainstream in Bangladesh. Various reports have shown the use of infusion of *D. erecta* leaves and fruits as diuretic (Md Rahmatullah *et al*., 2013), which can be used to expel the kidney stone out of the body. Khan *et al*., (2013) revealed the antioxidative potential of *D. erecta* fruits on hydrogen peroxide induced oxidative cell death. *In-vitro* studies showed DPPH and hydrogen peroxide scavenging and reducing potential of the extract. The *D. erecta* extract has shown 76% reduction in the viral titer of Hepatitis A. The activity may be attributed to the aceteoside or lamiide content which shows antioxidant activity (Lobna *et al*., 2007).

1.4.6: *Mentha arvensis* (L.)

Kingdom: Plantae **Order**-Lamiales **Family:** Lamiaceae **Genus:** *Mentha* **Species:** *Mentha arvensis* **Common name:** Pudina	

Fig.1.6: Scientific classification of *Mentha arvensis*

It is known as pudina in Hindi (Khan *et al*., 2008). Basically *Mentha arvensis* (Fig.1.6) is native of Europe and widely distributed throughout India. It is herbaceous perennial plant generally growing up to 10-60 cm tall and leaves are in opposite pairs, simple 2-6 cm long and 1-2 cm broad, hairy with coarsely serrated margin (Londonkar and Poddar, 2009). The flowers are pale purple and some time pink. This plant is broadly distributed all over India and leaves of *M. arvensis* are extensively used in traditional system for various ailments like carminative, digestive, expectorant, inflammation of liver, peptic ulcer, diarrhea, bronchitis cardio tonic, diuretic, dentifrice, jaundice, hepatalgia, and skin diseases (Sola, 1995, Kiritikar and Basu, 1999, Chopra *et al*., 1994, Khare, 2004). *M arvensis* L. has antimicrobial compounds, menthol, isomenthone, menthone, pulegone and carvone. The leaves of *M. arvensis* are medicinally used for stomach problems and allergy (Khan *et al*., 2008) and is also used for the treatment of liver and spleen disease, asthma and jaundice (Nair and Chanda, 2007). Essential oil of *M. arvensis* can be used for skin irritations, itching, burns, inflammations, scabies, and ringworm or to repel mosquitoes. When applied on the skin relieves pain and reduces sensitivity (Khan *et al*., 2008). The *Mentha* plant has been scientifically studied for anti-inflammatory, sedative, hypnotic, hepatoprotective, antioxidant, antibacterial and antifertility activities (Verma *et al*., 2003, Kowti *et al*., 2013, Coutinho *et al*., 2009 and Kanjanapothi *et al*., 1981). This plant has anticandida and radio protective activity against gamma radiation (Ganesh and Manjeshwar, 2002). In Kashmir, the dried powder of aerial parts of mentha mixed with curd is used to cure cough, sore throat, indigestion and constipation; also the leaves are used in diarrhea and asthma (Akhtar *et al*., 2012).

1.4.7: *Ocimum tenuiflorum* (L.)

Kingdom: Plantae **Order**- Lamiales **Family:** Lamiaceae **Genus:** *Ocimum* **Species:** *Ocimum tenuiflorum* **Common name:** Tulsi	

Fig.1.7: Scientific classification of *Ocimum tenuiflorum*

Ocimum tenuiflorum commonly known as Tulsi (Fig.1.7) is being used in Ayurveda for thousands of years for its diverse healing properties. Various parts of *O. tenuiflorum* such as the leaves, stem, flower, root, seeds and even whole plant are used for the treatment of several diseases. The decoction of leaves and flowers are used to treat cough and cold and eye diseases. The poultice prepared from the stem is applied on wound for fast healing. The plant has also been used as an anti-oxidant, anti-inflammatory, immuno-modulatory, anti-infective, anti-stress, antipyretic, anti-diabetic, cardio protective, neuroprotective and hepatoprotective agent. Chemically, the stem and leaves of *O. tenuiflorum* are known to contain a variety of chemical constituents including alkaloids, phenols, tannins and flavonoids (Pattanayak, 2010). These phytoconstituents may provide this herb its medicinal property against various diseases. Pharmacological studies have demonstrated that *O. tenuiflorum* posses protective effect against several diseases, however, the biological effect of *O. tenuiflorum* leaves extract has never been specifically studied for urolithiasis.

1.4.8: *Sphagneticola trilobata* (L.) Pruski

Kingdom: Plantae **Order**- Asterales **Family:** Asteraceae **Genus:** *Sphagneticola* **Species:** *Sphagneticola trilobata* **Common name:** Wedelia	

Fig.1.8: Scientific classification of *Sphagneticola trilobata*

Sphagneticola trilobata belongs to family Asteraceae. It is commonly known as "wedelia", "trailing daisy" and "Singapore daisy" (Fig.1.8). Leaves shortly petiolate, opposite-decussate, ovate, lobed, capitula heterogamous, receptacle convex and ray florates are golden yellow in colour (Hossain, 2005). It has high ecological tolerance and grows like perennial herb with 30 cm in height, rounded stem and fleshy leaf. It is nearly obtained all over the world. Origin of a wadelia is Native to Mexico, Central America, Caribbean and tropical South America. Wedelia is now cultivated as an ornamental ground cover in many areas of the tropics and subtropics. It is a mat-forming perennial herb with fast-growing rounded stems up to 40 cm long or longer

than roots at the nodes and grows upwards when flowering. The stems are coarsely hairy when it is young. The leaves are opposite and simple, the blade obovate to elliptic or ovate, 2 to 9 cm long and 2 to 5 cm wide, acute at the apex and winged and sessile at the base. The leaves are attractive bright shiny green, somewhat fleshy, and irregularly toothed on the margins, commonly with three shallow lobes. The bright yellow daisy-like flowers are borne on the end of terminal and axillary stalks 2 to 9 cm long, with 2 to 4 series of bracts forming the involucres at the base of the flower. Fruits are usually cornered nuts, very small (3-5mm) with corky covering and topped by short scales, mature from green to brown, dispersed by water. Plant seldom sets fertile seed under local conditions. In folk medicine, *S. trilobata* is used to treat backache, muscle cramps, rheumatism, stubborn wounds, sores, swellings and painful arthritic joints. It has antidiabetic and antioxidant activity (Arvigo, 1993).

Based on the available literature as above, biological activities of these eight plants against diabetes, urolithiasis and breast cancer diseases have been tabulated in the following table (Table 1.3).

Table: 1.3: Details of known biological activities of plants under study

Plant name	**Antidiabetic activity**	**Anticancer activity**	**Antiurolithiasis activity**
Adhatoda vasica (L.) Nees	Known	Unknown	Unknown
Bacopa Monnieri (L.) Penn	Known	Known	Unknown
Centella asiatica (L.) Nees	Known	Known	Unknown
Chromolaena odorata (L.) R.M. King & H.Rob	Known	Unknown	Unknown
Duranta erecta (L.)	Unknown	Unknown	Unknown
Mentha arvensis (L.)	Unknown	Unknown	Unknown
Ocimum tenuiflorum (L.)	Known	Known	Unknown
Sphagneticola trilobata (L). Pruski	Known	Known	Unknown

1.5. Details regarding the diseases under study

1.5.1: Diabetes Mellitus

1.5.1.1: Nature of disease: Diabetes mellitus is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease (Joshi *et al.,* 2007 and Kumar *et al.,* 2013). The number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014. The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014 (Wild *et al*., 2004). WHO projects that diabetes will be the 7th leading cause of death in 2030. The prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 and maximum will be in India (Wild *et al.,* 2004).

Diabetes mellitus is a chronic disease that occurs when the pancreas is no longer able to make insulin, or when the body cannot make good use of the insulin it produces. Insulin is a hormone produced by the pancreas that allows glucose from food to enter the cells in the body where it is converted into energy. All carbohydrate foods are broken down into glucose in the blood and insulin channelizes the glucose to get into the cells (Atlas *et al.,* 2000). Not being able to produce insulin or use it effectively leads to increase in blood glucose level, is known as hyperglycemia. Over the long-term, high glucose levels are associated with damage to the body and failure of various organs and tissues. The effect of diabetes has characteristic symptoms such as polyuria, blurring of vision, weight lose. In the most severe forms, keto-acidosis or non-ketosis hyper-osmolar state may develop and may lead to stupor, coma and in absence of effective treatment, death. The chronic effects of diabetes include various complications of retinopathy with potential blindness, nephropathy which may lead to renal failure, neuropathy with risk of foot ulcers and also increased risk of cardiovascular diseases (Atlas *et al.,* 2000).

1.5.1.2: Histroy of Diabetes Mellitus

The initial description of diabetes was reported in the writings of Hindu scholars as early as in 1500 BC. The term "*diabetes*" was probably coined by Apollonius of Memphis which literally meant "siphon- to pass through" as the disease drains more fluid than a person could consume. Later on, the Latin word "*mellitus*" meaning honeyed or sweetened was added because excess sugar was found in the urine and blood samples of the patients (MacCracken *et al.,* 1997). During the 17th century it

was known as the pissing evil. Sushruta, Arataeus and Thomas Willis were the early pioneers of the treatment of diabetes. Greek physicians prescribed exercise preferably on horseback to alleviate polyuria. Some other forms of therapy applied to diabetes include wine, overfeeding to compensate for loss of fluid weight, starvation diet, etc. The sweet taste of urine of diabetics was due to excess of sugar in the urine and blood. In ancient times and medieval ages diabetes was usually a death sentence. Sushruta, an Indian healer identified diabetes and classified it as "Madhumeha" where the word "Madhu" means honey and combined term means sweet urine. The ancient Indians tested for diabetes by looking at whether ants were attracted to a person's urine. The relation of diabetes with glycogen metabolism and the islet cells of pancreas were discovered by Paul Langerhans, a young German medical. In 1916, Sharpey-Shafer suggested that a single chemical was missing from the pancreas and proposed its name as "insulin". The term insulin originates from the word 'Insel' which meant islet or island in German study (Genuth *et al.,* 2003).

1.5.1.3: Types of Diabetes Mellitus: Diabetes mellitus is classified into 3-types:

- Type 1 Diabetes mellitus
- Type 2 Diabetes mellitus
- Gestational Diabetes mellitus

1.5.1.3.1: Type 1 Diabetes Mellitus

This form of diabetes accounts for only 5–10% of those suffering with diabetes. It is also referred as Insulin dependent diabetes mellitus (IDDM) or juvenile diabetes. Type-1 DM is a result of cell-mediated autoimmune destruction of the insulin producing beta-cells of langerhans, so that the insulin production decreases. Markers of the immune destruction of the beta-cell include islet cell autoantibody, autoantibody to insulin, autoantibody to glutamic acid decarboxylase (GAD65), and autoantibody to the tyrosine phosphates IA-2 and IA-2 (Mellitus, *et al.,* 2005). The disease affects people of any age, but usually occurs in children or young adults. People suffering from type-1 DM need insulin dosage on everyday basis in order to control the levels of glucose in their blood. Without insulin, a person with type-1 diabetes can die. Type-1 diabetes often develops suddenly and can produce symptoms such as abnormal thirst and a dry mouth, frequent urination, lack of energy and extreme tiredness, constant hunger, sudden weight loss, slow-healing wounds, recurrent infections and blurred vision etc. People with type-1 diabetes may become

normal through a combination of daily insulin therapy, close monitoring, a healthy diet and regular physical exercise. The number of diabetes patients is increasing. The reasons for this are still unclear but may be due to changes in environmental risk factors, early events in the womb, diet early in life, or viral infections (Mellitus *et al.*, 2005).

1.5.1.3.2: Type 2 Diabetes Mellitus (Post-prandial Hyperglycemia): This form of diabetes accounts for 90–95% of those with diabetes is also referred to as non-insulin dependent diabetes mellitus or post-prandial hyperglycemia. It usually occurs in adults. In T2DM, the body is able to produce insulin but either this is not sufficient or the body is unable to respond to its effects, leading to increased glucose levels in the blood (Fig. 1.9). Many people with type 2 diabetes are unaware of their illness for a long time because symptoms may take years to appear or be recognized, during which time the body is being damaged by excess blood glucose level. They get diagnosed only when complications of diabetes have already developed. There are several important risk factors, these include obesity, poor diet, physical inactivity, advancing age and family history of diabetes.

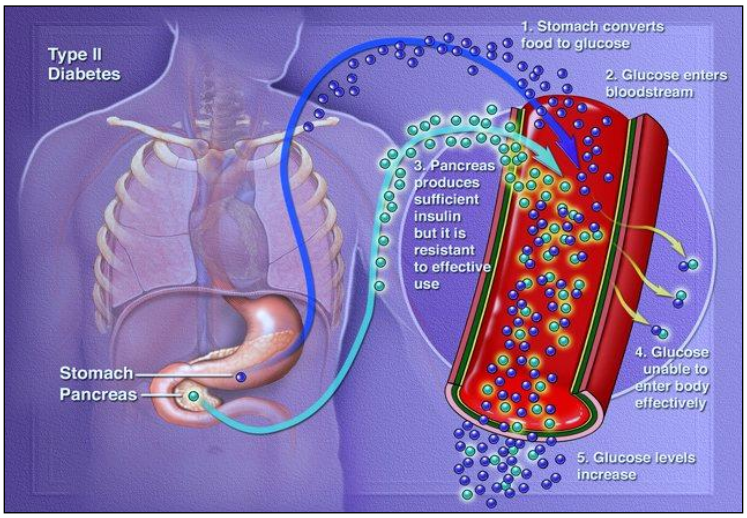


Fig.1.9: Type 2 Diabetes mellitus disease process (Source- Wehbe *et al.*, 2016)

Insulin secretion is improper in these patients and not sufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and pharmacological treatment of hyperglycemia but is seldom restored to normal. This form of diabetes increases with age, obesity and lack of physical activity. This condition can be managed through a healthy diet and increased physical activity or oral medication. The number of people with type 2 diabetes is increasing rapidly

worldwide. This rise is associated with economic development, ageing populations, increasing urbanization, dietary changes, reduced physical activity and lifestyle.

1.5.1.3.3: Gestational Diabetes Mellitus

Women who develop a resistance to insulin and subsequent high blood glucose during pregnancy are said to have gestational diabetes mellitus. Gestational diabetes tends to occur around the 24^{th} week of pregnancy. The condition arises because the action of insulin is blocked, probably by hormones produced by the placenta. This type of diabetes normally develops later in pregnancy when the unborn baby is already well-formed but still growing. Gestational diabetes normally disappears after birth. Babies born to mothers with gestational diabetes also have a higher lifetime risk of obesity and developing type 2 diabetes. Women with gestational diabetes need to be monitored and to control their blood glucose levels to minimize risks to the baby.

1.5.1.4: Pre-Diabetes Mellitus

The Expert Committee (Alberti *et al.,* 1998 and Genuth *et al.,* 2003) has formulated an intermediate group of subjects whose glucose levels, although not meeting criteria for diabetes, are nevertheless too high to be considered normal. People whose blood glucose levels are high but not as high as those in people with diabetes are said to have Impaired Glucose Tolerance (IGT) or Impaired Fasting Glucose (IFG). Impaired glucose tolerance is a pre-diabetic state of hyperglycemia which leads to insulin resistance and increased risk of cardiovascular pathology. IGT may precede T2DM by many years (Thompson *et al*., 2010).

1.5.1.5: Complications of Diabetes Mellitus

Diabetic people are at higher risk of developing a numerous life-threatening health problems. Uncontrolled diabetes can lead to a number of health complications, including hypoglycemia, heart disease, and nerve damage and amputation, and vision problem and therefore, diabetes is an emerging cause of cardiovascular disease, blindness, kidney failure, and lower-limb amputation in most of the developing countries. Maintenance of blood glucose, blood pressure and cholesterol levels close to normal can help delay or prevent diabetes complications.

1.5.1.6: Mechanism of Type 2 Diabetes Mellitus

According to American Diabetic Association, type 2 diabetes is the most common form of diabetes, occurring at increasing frequency with age, commonly associated

with insulin resistance either with relative or absolute insulin deficiency and not generally requiring insulin treatment for survival. In type 2 diabetes, hyperglycemia is always a consequence of insulin resistance, hyperinsulinemia and obesity. Any rise in glucose levels is the net result of glucose influx exceeding glucose outflow from the plasma compartment (Inzucchi *et al.*, 2012) Insulin resistance is responsible for reduced insulin mediated glucose uptake from muscles, increased glucose production from liver and higher free fatty acid mobilization from adipose tissues. The above factors initially culminate into postprandial hyperglycemia, which is later followed by fasting hyperglycemia. Insulin resistance, whether genetic or acquired leads to the development of type 2 diabetes by increasing the requirements for insulin, thus leading to insulin insufficiency in those individuals whose beta cells have limited secretary reserve. The requisite feature of type 2 diabetes is abnormal functioning of islets cells in pancreas. During the early state of diabetes insulin production is close to normal or increased but disproportionately low for the degree of insulin sensitivity, which is typically reduced. However, insulin kinetics, such as the ability of the pancreatic beta cell to release adequate hormone in phase with rising glycemia, are profoundly compromised. This functional islet incompetence is the main quantitative determinant of hyperglycemia and progresses over time (Ferrannini, 1998).

1.5.1.7: Advanced Glycation End Product, oxidative stress and Type 2 Diabetes Mellitus

Advanced glycation end products (AGEs) are heterogeneous entities formed from non-enzymatic glycation of proteins and lipids which get oxidized after contact with reducing sugars. The initial products of this reaction are Schiff base and Amadori product which later undergo molecular rearrangement to lead to generation of AGEs (Fig.1.10). AGEs may fluoresce, produce reactive oxygen species (ROS), and bind to specific cell surface receptors, and form cross-links. AGEs are formed in the *in-vivo* hyperglycemic environment and contribute to the pathophysiology of vascular diseases in diabetes.

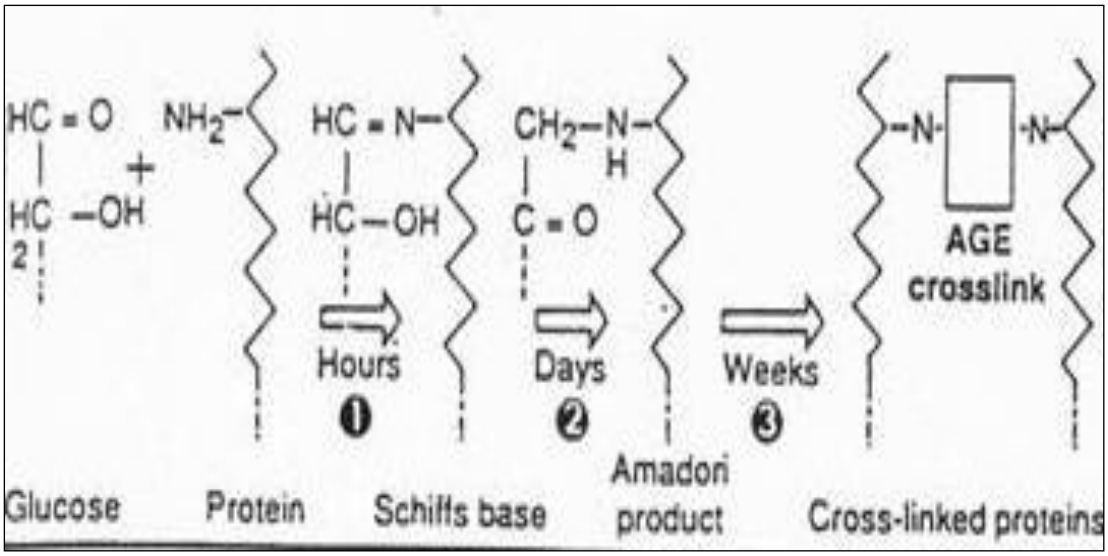


Fig.1.10: Stages of protein glycation

Glycation of protein and formation of AGE is accompanied by increased free radical activity that contributes to the bimolecular damage in diabetes (Schmidt *et al.*, 1994). AGEs act as mediator and can initiate a wide range of abnormal responses in cells and tissues such as the inappropriate expression of growth factors, structural and functional modifications of cell, alterations in growth dynamics, accumulation of extra-cellular matrix and initiation of cell death. Therefore, the need of the hour is to come up with compounds that have the potential to combat AGEs and free radicals in order to protect the diabetic population from severe complications of the disease (Schmidt *et al.*, 1994).

1.5.1.8: Treatment of Type 2 Diabetes Mellitus

Carbohydrates like starch and sucrose are the prime constituents of normal diet and the quickest source of energy as they contribute to 70-80% energy requirement of the body. Enzyme α-amylase found in the saliva and pancreatic juices catalyses the decomposition of starch into oligosaccharides. Cleavage of glucose from oligosaccharides and disaccharides is catalyzed by α-glucosidase. It is a membrane bound enzyme located in the brush border of small intestine. The digestion of carbohydrates begins in the mouth and continues even in the small intestine. The product of carbohydrate digestion is mainly glucose. The glucose produced is absorbed into the blood stream through the walls of intestine and then distributed to different parts of the body through liver. The α-amylase and α-glucosidase are major enzymes involved in the breakdown of complex carbohydrates. Suppressing the

activity of these carbohydrates hydrolyzing enzymes can inhibit the absorption of glucose in the liver and kidney and also maintain blood glucose concentration post meals. Enzyme inhibitors prevent the glucose from the food to enter the blood stream by delaying the breakdown of starch in the small intestine, and therefore can be matched to an impaired insulin response (Prabhakar *et al.*, 2008). Over the counter drugs such as Acarbose and Miglitol prevent T2DM by this mechanism. The herbal drugs exhibit hypoglycemic effect by retarding the rate and speed of glucose absorption from the gut. This includes herbal plants like *Euphorbia prostate*.

1.5.1.9: Medicinal plants known for antidiabetic activity

Many kinds of natural products, such as terpenoids, alkaloids, flavonoids, phenolics, and some others, have shown antidiabetic potential. Among active medicinal herbs, *Momordica charantia* L., *Pterocarpus marsupium* and *Trigonella foenum graecum* L. have been reported as beneficial for treatment of type 2 diabetes (Jung *et al.*, 2006). *Annona squamosa* is commanly known as custard apple plant and it possesses antidiabetic activity in rat. *Annona squamosa* act by promoting insulin secrition from pancreatic β-cell and also increases utilization of glucose in muscle and even inhibits glucose output from the liver (Malviya *et al.*, 2010). *Azardirachta indica* is native to India and is commanly known as neem. Aqueos and alcoholic extract of *azardirachta indica* showed significant hypoglycemic activity at low dose of 0.5 gm and high dose of 2gm (Waheed *et al.*, 2006). *Lantana camara* is a large aromatic shrub found all over India and is known in Ayurveda for its use in various treatments. Leaf juice of *lantana camara* at 1500 mg/kg/day for 14 days, has shown significant hypoglycemic activity in rats.This plant has some hepatotoxic effects also (Shram *et al.*, 1992).

Aqueous leaf extract of *Aegle marmelos* showed antihyperglycemic activity in streptozotocin induced diabetic rats after 14 days treatment either by stimulating utilization of glucose or by direct stimulation of glucose uptake by insulin secretion (Ayodhya *et al.*, 2010).

Psidium guajava is commonly known as Guava. This plant is highly rich in vitamins B1, B2, B6, vitamin C, free sugars (glucose, fructose and sucrose) and carotene. Oral administration as well as intraperitoneal injection of aqueous leaves extract of *P. guajava* to Alloxan-induced rats has shown significant effects on blood glucose, body weight and glucose (Oh *et al.* 2005).

Ficus religiosa is commonly known as peepal in India, belongs to family Moraceae. Decoction prepared from the bark of *Ficus religiosa* has been reported to be used in traditional system of Ayurveda for the treatment of diabetes (Bnouham *et al.*, 2006). The plant is believed to contain several bioactive phytochemials including tannins, saponins, polyphenolic compounds, flavonoids and sterols which may have the ability to impart hypoglycemic effect. Sitosterol-d-glucoside present in the bark of *Ficus religiosa* is believed to elicit hypoglycemic activity in rabbits (Singh *et al.*, 2010).

Eugenia jambolana (black plum or jamun) belongs to the Myrtaceae family. The seeds, leaves, fruits and bark are the most commonly used plant parts. Jamun has been reported to be used in numerous complementary and alternative medicine systems and is also a frontline antidiabetic medication traditionally. The brew prepared by jamun seeds in boiling water has been used in the various traditional systems of medicine in India. The plant is rich in compounds such as anthocyanins, glucoside, ellagic acid, isoquercetin, kaempferol, myricetin and hydrolysable tannins. The seeds also contain alkaloid jambosine and glycoside jamboline, which slows down the conversion of starch into sugar (Ayyanar *et al.*, 2012)

Ocimum tenuiflorum (holy basil or tulsi) belongs to the family Lamiaceae. Every part of the plant is used as a therapeutic agent against several diseases. It has been reported that Eugenol (phenyl propanoid and an active constituent present in *O. tenuiflorum*) is a potent inhibitor of AGEs both by *in-vitro* and *in-vivo* studies. It might inhibit intestinal α-glucosidase and block the conversion of complex carbohydrates to glucose, resulting in lower blood glucose level and subsequent reduction in AGE formation (Singh *et al.* 2016).

Acanthopanax senticosus belongs to Araliaceae family. It has been used as a nutritional supplement, sedative and also demonstrated anti-stress properties. The experimental results revealed that *A. senticosus* suppresses glucose absorption by the inhibition of intestinal glucosidase activity and glucose uptake at a concentration equivalent to that of a commercially available OTC drug. In this regard, it is expected to inhibit the rapid rise in blood glucose level immediately after a meal and to improve impaired glucose tolerance (Watanabe *et al.*, 2010). A list of plants and their therapeutic action in diabetes are enclosed as Table 1.4

Table: 1.4: List of some plants known for antidiabetic activity and their therapeutic action

Therapeutic action	Plant name
Acting like insulin	*Momordicacharantia* and *Panax ginseng*
Increasing insulin secretion from beta cells of pancreas	*Panax ginseng*, *Azadirachtaindica*, *Eugenia jambolana* and *Medicago sativa*
Regeneration of γ-cells of the islets of Langerhans	*Morusbomoysis*, *Pterocarpusmarsupium* and *Gymnemasylvestre*
Reduction absorption of glucose from gastrointestinal tract	*Cyamposistertragonoloba* and *Ocimum sanctum*
Inhibition of aldose reductase activity	*Aralia elata*
Increasing glucose utilization	*Zingiberofficinale*, *Grewiaasiatica* and *Cyamospsistetragonolobus*
Reduction of lactic dehydrogenase	*Lythrumsalicaria*
Inhibition glycogen-metabolizing enzymes	*Allium sativum*
Increasing glyoxalase activity in liver Increasing creatine kinase in tissues	*Trigonellafoenumgraecum*
Inhibition of glucose-6-phosphate	*Bauhinia megalandra*
Oxygen radical scavengers	*Momordicacharantia*
Protection of pancreatic beta cells from degeneration and diminish lipid peroxidation	*Morus alba*

Sourced from Singab *et al.,* 2014

1.5.2: Urolithiasis

1.5.2.1: Nature of disease

Kidney stones are also known as renal calculi, urinary calculi, urinary tract stone disease, nephrolithiasis, urolithiasis and ureterolithiasis depending on where they are located in the urinary system. Stones in the urinary tract are predominantly crystalline with several important physiochemical mechanisms involved in stone formation which include CaOx (calcium oxalate) supersaturation and CaOx crystal nucleation, growth and aggregation. Kidney stones consist of inorganic and organic crystals amalgamated with proteins. Calcium oxalate stones represent up to 80% of analysed stones (Awari *et al.,* 2009) and calcium phosphate accounts for 15-25%, while 10-15% can be mixed stones. The others are struvite 15-30%, cysteine 6-10% and uric

acid stones 2-10% (Fig 1.11 and 1.12). Calcium oxalate stones are of primarily two types, calcium oxalate monohydrate (whewellite) and calcium oxalate dihydrate (weddellite). The occurrence frequency of whewellite is 78% while that of weddellite is 43% (Rao *et al*., 2011).

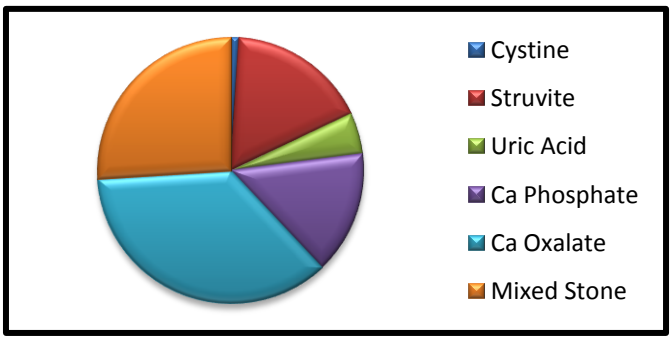


Fig.1.11: Proportion of kidney stone by types

Urolithiasis occurs due to constituents and supersaturation of urine. Urine of most of the people gets supersaturated with respect to calcium oxalate, so in principle all people can form such stones. However, normal urine is less supersaturated with respect to uric acid, cysteine or struvite. Conditions those raise calcium oxalate supersaturation raise the risk of calcium oxalate stones. Supersaturation developes stone by causing ions in solution to combine with one another into a solid phase, a process called nucleation. Calcium and oxalate ions can promote themselves on surfaces of another crystal, such as uric acid and such heterogeneous nuclei may promote calcium oxalate stones. The conditions those raise supersaturation and promote heterogeneous nucleation are main causes of urolithiasis. Their diversity and multiplicity of current treatments complicate clinical management of patients and offer many opportunities for new fruitful research (Khan, 2008). There are many factors that can influence stone forming propensity, such as inhibitors of calcium oxalate crystallization and urothelial surface properties which affect crystal retention. Normaly urinary environment is inhibitory to crystallization. Occasionaly crystals get internalized by the renal epithelial cells and sequestered to lysosomes or externalized into the interstitium which is further handled by the inflammatory cells. Elevated levels of oxalate and calcium oxalate crystals predispose renal cells to increase the synthesis of osteopontin, bikunin, heparan sulphate proteoglycan, monocyte chemoattractant and prostaglandin, which are known mediators of the inflammatory processes and extracellular matrix production. Exposed renal epithelial cells are often injured and undergo apoptosis or necrosis initiating a cascade of events leading to

further crystallization, crystal retention and development of stone. Reactive oxygen species are produced during the interactions between the oxalate crystals and renal cells which are responsible for the various cellular responses (Khan, 2008).

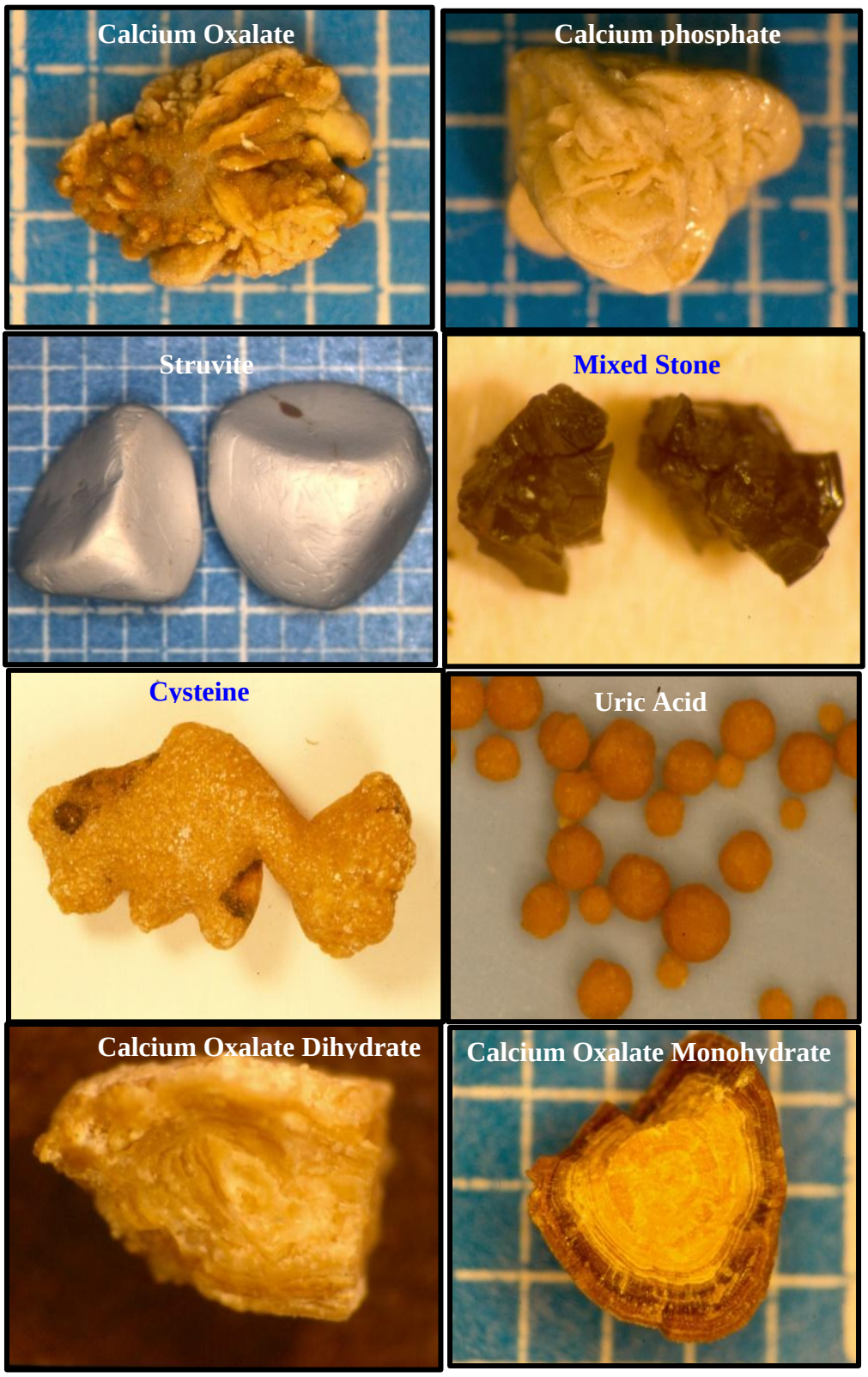


Fig.1.12: Types of kidney stones

Although CaOx renal stones are known to grow attached to renal papillae, and specifically to regions of papillae that contain Randall's plaque (interstitial apatite deposits), the mechanisms of stone overgrowth on plaque needs to be discovered. Urinary calcium and oxalate ions eventually overgrow on the large crystals for formation of attached stone (Table 1.5).

Table 1.5: Mechanism of urolithiasis

Age, Profession, Nutrition, Climate, Inheritance, Sex, Mentality, Constitutions, Race

⬇

Abnormal renal morphology, Disturbed urine flow, Urinary tract infection, Metabolic abnormalities, Genetic factors

⬇

Increased excretion stone forming constituents, Decreased excretion of inhibitors of crystallizations

⬇

Supersaturation

⬇

Abnormal crystalluria, Crystal’s aggregations, Crystal’s growth

⬇

Formation of stone

1.5.2.2: History of urolithiasis

The deposition of stone in urinary tract has been documented in ancient Greek literature and it is known to be one of the oldest diseases to human. Urinary stones have been found in Egyptian mummies dating back as far as 7000 years and the symptoms of the condition were described by Hippocrates who suggested that drinking of muddy river water causes the excretion of sand in urine (Butt *et al.*, 1956). Incidence of urolithiasis has increased over last 100 years, even today millions of people are affected by urolithiasis and there is a serious functional implication associated with formation of kidney stone. Although urinary calculi can be formed in any part of urinary tract but since last two decades it has been shown that the frequency and the occurrence of stone formation have gradually shifted from bladder and ureter (lower urinary tract) to the kidney (upper urinary tract). The epidemiological study has demonstrated that in India, the "stones belt" occupies parts

of Maharashtra, Gujarat, Punjab, Haryana, Delhi, Rajasthan and the less prevalent areas include the South and East Coastal parts (Fig.1.13). 12% of the Indian population is expected to have urinary stones, out of which 19% may end up with renal damage or loss of kidney. The incidence and prevalence are estimated to be 100-300/1,00,000/year and 5-10%, respectively and relapses occur in 19-70% of all the cases (Agarwal *et al.*, 2014).

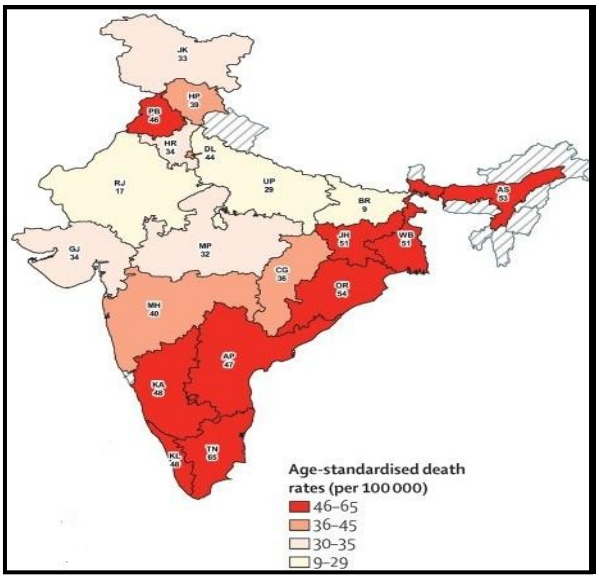


Fig.1.13: Stones belt in India

1.5.2.3: Factors responsible for urolithiasis

There are many major factors which have been linked to the development of urolithiasis. However, some of these risk factors can be altered but some cannot. The risk of becoming a stone former is more than 2.5 times greater in individuals who have a family history of urolithiasis. This increased risk has been linked to genetic, environmental and dietary factors (Curhan, 2007).There are many systemic disorders, which have been linked to kidney stone formation including; primary hyperparathyroidism, renal tubular acidosis and Crohn'sdisease. However, a history of gout increases the likelihood of forming kidney stones; both uric acid and calcium oxalate (Kramer *et al.*, 2003). Moreover, a history of type 2 diabetes mellitus increases the risk of stone formation by 19 to 30%. Some environmental factors have been implicated, for example working in a hot environment, lack of access to water or lower fluid intake all have been shown to predispose to renal stone formation (Atan *et al.*, 2005). Some urinary factors have been suggested as the risk factors for renal stones formation, e.g. hypercalciuria, hyperoxaluria, hyperuricosuria and hypocitraturia (Curhan, 2007).

A correlation between Randall's Plaques and the presence of calcifying nanoparticles (CNP, Nanobacteria) which are similar to snowballs has been reported. These structures were discovered over a decade ago in blood and blood products. They have been detected in numerous pathological calcifications, such as in kidney stones (Ciftcioglu *et al*., 1999), atherosclerotic plaques, bodies of cancer, prostatic stones and in gallbladder. CNP are calcified self-propagating entities, morphologically very similar in mineral composition to spherical bodies observed in Randall's plaques. Due to lack of their genomic evidence, CNP are controversial agents as prions (Ciftcioglu *et al*., 1999). Bacterial urea splitting including; Proteus, Pseudomonas, Klebsiella species and many other bacteria can cause urease induced stone like struvite and carbonate-apatite stones (Griffith, 1979). Some viruses such as Feline Calicivirus (FCV), Feline Syncytium Forming Virus (FeSFV) and Cell Associated Herpes Virus (CAHV) have been reported as causes of urolithiasis. The cytopathic effects observed in human explanted cell cultures paralleled those observed in the feline cell cultures infected with the herpes virus. Further, it has been documented that human populations may carry persistent infections with up to five herpes viruses (Fabricant, 1979).

Some dietary factors have also been linked to kidney stones formation. Nutrients that have been implicated include calcium, animal protein, oxalate, sodium, sucrose, magnesium and potassium (Curhan, 2007).

a) Calcium: High dietary calcium has been strongly suspected of raising the risk of stone disease, a recent cohort study of more than 19,000 male health professionals aged 18 to 75 years, has shown that very low calcium intake can actually predispose to kidney stone formation (Curhan *et al*., 1997 and 2004).

b) Oxalate: One-third of patients with calcium oxalate urolithiasis may have increased absorption of dietary oxalate, but in some cases a deficiency of oxalate degradation by the Bacterium Oxalobacter formigenes in the gut could also be responsible (Holmes *et al.*, 2004).

c) Other nutrients: Several other nutrients have been reported to be implicated in the development of stone formation, including high animal protein intake, high sodium intake, high sucrose intake, low potassium intake and low calcium intake. Furthermore, potassium supplementation decreases calcium excretion and many

potassium-rich foods increase urinary citrate due to their alkali content (Curhan *et al*., 2004). Magnesium makes complexes with oxalate and reduces oxalate absorption in the gastrointestinal tract and decreases calcium oxalate supersaturation in the urine. High vitamin C intake and vitamin B6 deficiency could increase the risk of calcium oxalate stone formation as well. Vitamin B6 deficiency leads to oxalate production, whereas vitamin C can be metabolized to oxalate and both can cause hyperoxuluria (Curhan, 2007 and Curhan *et al*., 1999). Observational studies and a randomized controlled trial in kidney stones have demonstrated that when the urine output is less than 1 L/day the risk of stone formation is higher than those with higher urine output. Some beverages have been shown to increase the risk of stone (Curhan, 2007). Grapefruit juice intake has been associated with 18% higher risk of stone formation. Other studies suggested an increased risk of kidney stone formation with soda consumption (Curhan *et al*., 1998).

1.5.2.4: Treatment of urolithiasis

Potassium nitrate and distilled product of resin from pine trees called turpentine oil were known for their diuretic action and calcium carbonate from pulverized egg shells were ingested to complex with potential stone forming compounds (Dardioti *et al.*, 1997).

Calcium oxalate monohydrate (COM) binding protein (45 kDa) is a promoter of calcium oxalate kidney disease, which is markedly up regulated by oxalate induced oxidative stress. A study was done and found that COM binding protein can serve as a diagnostic marker for CaOx stone formation (Asokan *et al.*, 2004).

Cell membrane fragments derived from renal tubular epithelial cells are common in the human urine. They contain phospholipids such as Sphingomyelin (SM), Phosphatidylethanolamine (PE), Phosphatidylserine (PS), Phosphatidylcholine (PC) and Phophatidylinositol (PI) that can promote nucleation of CaOx crystals. COM crystals can bind within seconds to anionic, sialic acid-containing glycoproteins on the apical surface of kidney epithelial cells in culture, suggesting one mechanism whereby crystals could be retained in the tubule (Calero *et al.*, 2000).

For the removal and treating the kidney stone, technological advancements like Extracorporeal Shock Wave Lithotripsy (ESWL) have made dramatic improvement but still some of the drawbacks of these technologies exist viz; their being very costly for a common man, recurrence of stone formation, renal damage, kidney failure along

with a number of other side effects (Prasad *et al*., 2007). Surgical procedure, lithotripsy and laparoscopy for local calculus disruption are used to remove the calculi. However, these procedures may cause risk of acute renal injury that reduces renal function and stone recurrence is observed in many cases with an approximate recurrence rate of 10% at 1^{st} year, 33% at 5^{th} year, and 50% at 10^{th} year.

Costly treatment regimen and complicated clinical management of patients indicates an important need to develop suitable alternative therapies (Parmar, 2004). Medicinal plants are an important source of drugs because herbal natural products are effective as well as not as costly as their chemical counter parts. Hence, search for new antilithiatic drugs from natural sources becomes very important as herbal drugs are cost effective and cause least side effects. In Ayurveda, many plants having the property of disintegrating and dissolving the stone are referred to as "Pashanbheda". Few medicinal plants and proprietary composite herbal preparations have been reported as an effective therapeutic option for prevention of recurrence of renal calculi with minimal side effects (Prasad *et al*., 2007).

1.5.2.5: Medicinal plants for treatment of urolithiasis

Pashanbheda is a drug mentioned for various ailments but mainly as a diuretic. It is known to have the property of breaking and dissolving the stones. It is widely used as a form of drug. In Sushruta Samhita, decoction of Pashanbhed, Ashmantaka, Satavari, Vrihati, Bhalluka, Varuna (Crataeva nurvula), kulatha, kola and kataka seeds have been described for the patients of Vataja Ashmari, while Kusa, Ashmabhid, Patala, Trikantaka, Sirisha, Punarnava and Silajatu and Meduka flower for Pittaja Ashmari have been mentioned (Havagiray *et al.,* 2010). It has been reported that an extract from the herb *Herniaria hirsuta* L., a plant that traditionally used in Morocco for the treatment of lithiasis, promoted the nucleation of calcium oxalate crystals, increasing their number but decreasing their size. In a follow-up study the authors could demonstrate that *H. hirsuta* could block crystal binding to cultured renal cells (Atmani *et al.,* 2000). The Varuna bark - *Crataeva nurvala* has been extensively studied by a number of investigators in India. The pharmacology division of Central Drug Research Institute, Lucknow, India has carried out detailed pharmacological and chemical studies on this plant. Studies have shown that ethanolic extract has a dose related antiurolithiatic activity in albino rats induced by foreign body insertion method using glass beads (Prasad *et al*., 2007). Similar effects on calcium oxalate

crystallization *in-vitro* have been shown for an aqueous extract from *Phyllantus niruri* L., a plant which is used in traditional Brazilian medicine for the treatment of stone disease. The authors could show that the extract interfered with the CaOx crystallization process by reducing CaOx crystal growth and aggregation. The *in-vitro* effect of *P. niruri* on a model of CaOx crystal endocytosis by Madin-Darby canine kidney cells was studied. The extract exhibited a potent and effective nonconcentration dependent inhibitory effect on the CaOx crystal internalization (Barros *et al.,* 2003).

Takusha *(Alisma orientale*), one of the components of the kampo medicine chorei-to is commonly used in Japan to cure and to prevent recurrent CaOx kidney stones. Studies in rats receiving ethylene glycol to induce CaOx stone formation have shown that Takusha prevented stone formation by inhibiting CaOx aggregation. In cats fed with a diet containing Takusha, a reduction of urine pH was observed as well as reduced struvite crystal formation in cat urine (Shu *et al.,* 2016). A study was intended to determine anti-urolithiatic effect of *Lagenaria siceraria* fruit powder (*LSFP*) against sodium oxalate (NaOx) induced urolithiasis in rats. It was concluded that *LSFP* showed beneficial effect against urolithiasis by decreasing CaOx excretion and preventing crystal deposition in the kidney tubules (Takawale *et al.,* 2012). The studies showed that *Morus alba* prevented the formation of urinary stones and based on histopathological studies and biochemical markers it was concluded that ethanolic extract of *Morus alba* possessed anti nephrolithiatic activity (Maya *et al.,* 2014). A list of representative plants used in treatment of urolithiasis activity is given as Table 1.6.

Table 1.6: List of some plants known for antiurolithiasis and their therapeutic action

Plant name	**Therapeutic action**
Achyranthus Aspera	Prevent renal epithelial damage, Diuretic
Aerva lanata	Decrease crystal ppt
Amni visnaga	Potent diuretic, khellin & visnagin prevent renal epithelial cell damage caused by oxalate & COM
Bergenia ligulata	Decreases calcium
Costus spiralis	Decrease stone size
Cranberry juice	Decrease urinary oxalates
Cynodon dactylon	Increase COD as compare to COM

Dolichos biflorus	Decreases oxalate crystals
Grapefruit juice	Increases urinary excretion
Herniaria hirsute	Decrease crystal size & increase COD, diuretic
Lemonade juice	Increase urinary excretion
Moringa oliefera	Diuretic
Paronychia argentea	Antioxidant activity
Phyllanthus niruri	Antispasmodic & relaxant
Pyracantha crenulated	Diuretic & lowering of urinary stone forming constituents
Quercus salicina	Reduction in oxalate induced renal cell injury
Raphanus sativus	Diuretic
Tribulus terrestris	Decrease oxalate
Trachyspermum ammi	Maintain renal functioning; Reduce renal injury
Vediuppu chunnam	Decrease urinary calcium oxalate, uric acid & Diuretic

(Sourced from Yadav *et al*., 2011)

1.5.3 Cancer

1.5.3.1: Nature of disease

Cancer is an abnormal growth of cells which tend to proliferate in an uncontrolled way and, in some cases leads to metastasis (spread). Cancer is multifactorial syndrome. Benign tumors are not cancer; malignant tumors are cancer. Cancer is not contagious. Cancer is the Latin word for crab. The ancients used the word to mean a malignancy, doubtless because of the crab-like tenacity a malignant tumor sometimes seems to show in grasping the tissues it invades (ACS, Breast Cancer Facts & Figures, 2015). 13 % deaths occur due to cancer. Cancer gets first position among the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2014. The number of new cases is expected to rise by about 70% over the next 2 decades (World Cancer Report, 2014). Cancer producing cells are present in every human body. Proto-oncogene is normal gene present in the cells. It becomes oncogene due to mutation (Todd, *et al*., 1999). Our immune system plays important role to keep control over cancer. When immune system fails to keep control over cancer then more cancer cells are being created than body gets rid of that. Although surgical excision, chemotherapy, and radiotherapy are available, survival is still very poor when the disease is diagnosed at an advanced

stage and better treatments are desperately needed. Thus, novel therapeutic agents, multitargeted to several mechanisms of carcinogenesis are needed.

1.5.3.2: Types of cancer

There are more than 100 types of cancer. Types of cancer are based on the organs or tissues where the cancers form. For example, lung cancer starts in cells of the lung, and brain cancer starts in cells of the brain. Cancers are also described by various types of cells that are formed, such as an epithelial cell or a squamous cell. Here are some categories of cancers that begin in specific types of cells.

Carcinoma

Carcinomas are the most common type of cancer. Carcinomas are formed by epithelial cells which cover the inside and outside surfaces of the body. There are many types of epithelial cells, which often have a column-like shape. Carcinomas that begin in different epithelial cell types have specific names.

Adenocarcinoma is a cancer that forms in epithelial cells and produce fluids or mucus viz. breast, colon, and prostate are adenocarcinomas.

Basal cell carcinoma is a cancer that begins in the lower or basal (base) layer of the epidermis, which is a person's outer layer of skin.

Squamous cell carcinoma means a cancer that forms in squamous cells, which are epithelial cells that lie just beneath the outer surface of the skin.

Transitional cell carcinoma is a cancer that forms in a type of epithelial tissue called transitional epithelium, or urothelium.

Sarcoma- Soft tissue sarcoma forms in soft tissues of the body, including muscle, tendons, fat, blood vessels, lymph vessels, nerves, and tissue around joints. Osteosarcoma is the most common cancer of bone.

Leukemia- Cancers that begin in the blood-forming tissue of the bone marrow are called leukemia.

Lymphoma is cancer that begins in lymphocytes (T cells or B cells). These are white blood cells that are part of the immune system.

Multiple myeloma is cancer that begins in plasma cells, another type of immune cell.

Melanoma is tumor of melanocytes, which are specialized cells that make melanin.

Brain and spinal cord tumors are tumors of central nervous system.

Germ cell tumors begin in the cells that give rise to sperm or eggs.

Neuroendocrine tumors form from cells that release hormones into the blood in response to a signal from the nervous system.

Carcinoid tumors are a type of neuroendocrine tumor. They are slow-growing tumors that are usually found in the gastrointestinal system (rectum and small intestine).

1.5.3.3: Breast cancer

Breast cancer is one of the most common invasive cancers among females worldwide. It accounts for 16% of all female cancers and 22.9% of invasive cancers in women. 18.2% of all cancer deaths worldwide, including both males and females, are from breast cancer (DeSantis *et al*., 2014). There are two main types of breast cancer, ductal carcinoma and lobular carcinoma.

Ductal carcinoma starts in ducts which move milk from the breast to the nipple. Most breast cancers are of this type. Lobular carcinoma starts in the parts of the breast, called lobules, which produce milk.

Breast cancer can be invasive or non invasive. Ductal carcinoma is breast cancer in the lining of the milk ducts that has not yet invaded nearby tissues. It may progress to invasive cancer if untreated. Lobular carcinoma is a marker for an increased risk of invasive cancer in the same or both breasts. Many breast cancers are sensitive to the hormone estrogen. This means that estrogen causes the breast cancer tumor to grow. They are called estrogen receptor-positive cancer or ER-positive cancer. Some women have HER2-positive breast cancer. HER2 refers to a gene that helps cells grow, divide and repair themselves. When cells (including cancer cells) have too many copies of this gene, they grow faster. They have a higher risk that the disease will return (recur) in women who do not have this type. This may be changing with specifically targeted treatments against HER2.

1.5.3.4: Major causes of cancer

Tobacco use is the major cause of about 22% of cancer deaths. Another 10% is due to obesity, poor diet, lack of physical activity and drinking alcohol. Other factors include certain infections, exposure to ionizing radiation and environmental pollutants. In the world nearly 20% of cancers are due to infections such as hepatitis B, hepatitis C and

human papillomavirus (HPV). These factors act, at least partly, by changing the genes of a cell. Typically many genetic changes are required before cancer develops. Approximately 5–10% of cancers are due to inherited genetic defects from a person's parents (De Martel *et al*., 2012). General signs and symptoms of cancer are weight loss, fever, fatigue, pain, blood in urine, skin changes, unusual bleeding or discharge, thickening or lump in the breast, trouble swallowing, cough and hoarseness (Moscow and Cowan, 2011).

1.5.3.5: Usual treatments of cancer

Chemotherapy

Chemotherapy is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs. It encompasses a variety of drugs, which are divided into broad categories such as alkylating agents and antimetabolites. Targeted therapy is a form of chemotherapy that targets specific molecular differences between cancer and normal cells. The first targeted therapies blocked the estrogen receptor molecule, inhibiting the growth of breast cancer. Another common example is the class of Bcr-Abl inhibitors, which are used to treat leukemia. Currently, targeted therapies exist for breast cancer, lymphoma, prostate cancer and melanoma (DeSantis *et al*., 2014).

Radiation

Radiation therapy means the use of ionizing radiation in an attempt to either cure or improve symptoms. It works by damaging the DNA of cancerous tissue, killing it. Radiation beams are aimed from multiple exposure angles to intersect at the tumor, providing a much larger dose there than in the surrounding, healthy tissue. Radiation helps in addition to surgery and or chemotherapy to cure the cancer. In case of some cancer, such as early head and neck cancer, it may be used alone. In painful bone metastasis, it has been found to be effective in about 70% of patients (DeSantis *et al*., 2014). Side effects of chemotherapy and radiotherapy are vomiting and nausea, dehydration, mouth ulcer with pain, loss of appetite, gastric ulcer, loss of body hairs, skin infection, severe weakness, irregular menses, severely low immunity, anaemia, renal toxicity, cardiac problem, and hepatic insufficiency (Chun *et al*., 2007).

Surgery

Surgery is the primary method of treatment for most isolated, solid cancers and may play a role in palliation and prolongation of survival. In localized cancer, surgery

attempts to remove entire mass along with, in certain cases, the lymph nodes in the area (DeSantis *et al*., 2014).

Immunotherapy

Immunotherapy aims to get the body's immune system to fight the tumor. Local immunotherapy injects a treatment into an affected area, to cause inflammation that causes a tumor to shrink. Immunotherapy can also be considered non-specific if it improves cancer-fighting abilities by stimulating the entire immune system and it can be considered targeted if the treatment specifically tells the immune system to destroy cancer cells. Bone marrow transplantation can also be considered immunotherapy because the donor's immune cells will often attack the tumor or cancer cells that are present in the host (Fry and Mackall, 2013).

Hormone therapy

Several cancers are linked to some types of hormones, most notably breast and prostate cancer. Hormone therapy is designed to alter hormone production in the body so that cancer cells stop growing or are killed completely. Breast cancer hormone therapies mainly focus on reducing estrogen levels and prostate cancer hormone therapies often focus on reducing testosterone levels. Some leukemia and lymphoma cases can be treated with the hormone cortisone (Dent *et al.,* 2011).

Gene therapy

The aim of gene therapy is to replace damaged genes with ones that work to address a root cause of cancer damage to DNA. Researchers are trying to replace the damaged gene that signals cells to stop dividing (p53 gene) with a copy of a working gene. Other gene based therapies mainly focus on damaging cancer cell DNA to the point where the cell commits suicide. Gene therapy is comparatively young field and has not yet resulted in any successful treatments (Zeimet *et al.,* 2000).

Homeopathy

Homeopathy is a therapeutic treatment of using preparations of substances whose effects when administered to healthy subjects correspond to the manifestation of the disorder in the individual patient. This was developed by Samuel Hahnemann (1755–1843) about 200 years ago. Homeopathy treatment is in practice to treat cancer but there is no evidence at all that homeopathic remedies can change the natural history of any cancer (Swayne, 2000).

Gerson therapy

Gerson used his therapy as a treatment for headaches and tuberculosis. In 1928, he started to use it as a supposed treatment for cancer. Gerson therapy is based on the belief that disease is caused due to accumulation of unspecified toxins and it attempts to treat the disease by having patients consume a predominantly vegetarian diet including hourly glasses of organic juice and various dietary supplements. Animal proteins are deleted from the diet under the unproven premise that tumors develop as a result of pancreatic enzyme deficiency. Patients receive enemas of coffee, castor oil and sometimes hydrogen peroxide or ozone. The protocol calls for patients to consume fresh vegetables each day, including raw carrots or apples and green-leaf juice (Weitzman, 1998).

Proteolytic enzyme therapy

John Beard first proposed that pancreatic proteolytic enzymes have the body's main defense against cancer in 1906. Beard focused on high dose porcine-based pancreatic enzyme and eating a holistic diet to create an internal environment in which the body can more thoroughly heal itself. Physicians recommend taking 5 gm of proteolytic enzymes 3 times daily on an empty stomach between meals to reduce inflammation. The studies proved that the patients who have pancreatic cancer, those who chose gemcitabine based chemotherapy survived more than three times as long and had better quality of life than those who chose proteolytic enzyme treatment (John *et al.* 2010).

Chelation therapy

It is a medical procedure that involves administration of chelating agents to remove heavy metals from body. Chelation therapy has a long history of use in clinical toxicology and remains in use for very specific medical treatments, although it is administered under very careful medical supervision due to various risks. Medical evidence does not support the effect of chelation therapy for any purpose other than the treatment of heavy metal poisoning. The American Cancer Society says that in chelation therapy scientific evidence does not support claims that it is effective for treating other conditions such as cancer (Buss *et al.*, 2003).

Probiotic foods

Probiotics are microorganisms which promote a natural balance in one's intestinal microflora. Probiotics can improve digestive function and mineral absorption in

healing leaky gut, which all contribute to cancer prevention. The consumption of probiotics products containing lactic acid bacteria was associated to reduce breast cancer risk in some studies. The modulation of the host's immune mechanism by use of probiotics is various animal models of cancer. Further assays in human are very important before the medical community can accept the addition of probiotic containing lactic acid bacteria as supplements for cancer patients. (Felix *et al.,* 2014)

Sunshine and vitamin D

Vitamin D is known to be essential to the immune system for activations of T-cells. But the sun provides more than just vitamin D. It increases levels of nitric oxide in human body, links to a dilation of blood vessels, less strokes and heart attacks and increases serotonin levels. It cuts depression, encourages melatonin production. Melatonin is an important antioxidant and anti-inflammatory agent.The publication in the American Journal of Clinical Nutrition, is truly ground breaking as it evaluated nearly 1,200 postmenopausal women for four years and tracked how a 1400-1500 milligram supplement of calcium compared to a calcium supplement plus 1,100 IU vitamin D3 is involved in preventing cancer (Lappe *et al.,* 2007).

Herbal medicine

There are many natural solutions that people have successfully implemented for centuries. Research suggests that natural treatments not only prevent, but also treat cancer and its side effects. It is important to remember that various articles discuss the success clinical researchers have experienced in using many natural treatments to prevent, stop and reverse the growth of various cancer lines in animal and human cell studies. Natural cancer treatments are interested in killing cancer cells, or reverting them to normal cells, than in shrinking tumors. Natural product treatments play a dominant role in pharmaceutical care. Herbal drugs used to treat cancer may inhibit the growth of tumour, destroy infected cells and allow healthy cells to grow. They are often helpful for body to adjust the disruption of its natural protective mechanisms. Various plant species have been used or recommended in various parts of the world for the treatment of cancer or other conditions such as warts and tumours. This is especially obvious in the case of antitumor drugs like paclitaxel (Taxol), vincristine (Oncovin), vinorelbine (Navelbine) and camptothecin (Hycamtin).Treatment of Ayurveda is based on rejuvenate cell theory, which works at DNA level. It means cancerous cell will rejuvenate into normal healthy cell (Agarwal, 2008). The ability of

plants based natural medicines to serve as inhibitors of carcinogen, decreases free radical, suppresses cell division and promotes apoptosis. A tentative list of plants with anticancer activities is given as Table 1.7.

The consumption of berry fruits, including strawberries, has been suggested to have beneficial effects against oxidative stress mediated diseases. Berries contain multiple phenolic compounds and secondary metabolites that contribute to their biological properties. This study investigates the anticancer activity of the methanolic extract of strawberry (MESB) fruits in leukaemia (CEM) and breast cancer (T47D) cell lines *ex-vivo*, and its cancer therapeutic and chemopreventive potential in mice models (Somasagara *et al*., 2012). Genistein is a small, biologically active flavonoid that is found in high amounts in soyabean. This important compound possesses a wide variety of biological activities, but it is best known for its ability to inhibit cancer progression. Genistein has emerged as an important inhibitor of cancer metastasis. Consumption of genistein in the diet has been linked to decreased rates of metastatic cancer in a number of population-based studies. Extensive investigations have been performed to determine the molecular mechanisms underlying genistein's antimetastatic activity, with results indicating that this molecule has significant inhibitory activity at nearly every step of the metastatic cascade (Janet *et al*., 2001).

Tribulus terrestris is a flowering plant in the family Zygophyllaceae, native to warm temperate and tropical regions. *T. terrestris* has long been a constituent in tonics in Indian Ayurveda practice, where it is known by Gokharu. Its antioxidant property is well known. *T. terrestris* has been shown to enhance sexual behavior in an animal model. Svetla *et al.*, (2013) studied antitumor activity of *Tribulus terrestris* L. on human breast cancer cells human breast cancer (MCF7) and normal (MCF10A) cell lines. The results showed that total extract of the herb has a marked dose-dependent inhibitory effect on viability of MCF7 cells. Cell viability of MCF10A was moderately decreased without visible dose dependent effect. Bhushan *et al.*, (2011) has mentioned about acetyl-11-keto-beta-boswellic acid (AKBA) taken from the gum resin of the *boswellia serrata* known as salai guggal or Indian frankincense. Traditionally, this substance has been used in ayurvedic medicine to treat inflammatory conditions. A triterpenediol (TPD) comprising of isomeric mixture of 3alpha, 24-dihydroxyurs-12-ene and 3alpha, 24-dihydroxyolean-12-ene from *Boswellia serrata* induces apoptosis in cancer cells. Anticancer effects of

pomegranate extracts in human breast cancer cells *in-vitro* and also chemopreventive activity of pomegranate fermented juice polyphenols in a mouse mammary organ culture were reported by Lansky and Newman, (2007). Further, an Indian study, in 2011, looked at *Selaginella bryopteris*, a traditional Indian herb referred to as Sanjeevani. It was found that compounds taken from this herb stopped the growth of cancer cells in the laboratory (Mishra *et al.,* 2011). *Vernonia amygdalina* (VA) is a woody shrub reported to have not only diverse therapeutic effects but also anti-cancer properties. The study aimed to investigate the mechanisms of action of VA that underlie its anti-cancer effects in human breast cancer cell lines (MCF-7 and MDA-MB-231 cells). Results from MTT assay revealed that VA inhibits the proliferation of MCF-7 and MDA-MB-231, in a time- and dose-dependent manner. The mechanism of this growth inhibition involved the stimulation of cell-type specific G1/S phase cell cycle arrest in only MCF-7 cells and not in MDA-MB-231 cells. The growth arrest was associated with increased levels of p53 and p21 and a concomitant decrease in the levels of cyclin D1 and cyclin E, it was shown that VA causes cell cycle arrest through a p53-independent pathway as tested by the wild type p53 inhibitor, pifithrin-α. Furthermore, this study revealed that VA induces apoptosis in the two cell lines, as indicated by the increase in Annexin V-positive cells and sub-G1 population, and that this VA-induced apoptosis occurred through both extrinsic and intrinsic apoptotic pathways (Wong *et al.,* 2013).

The crude extract of *A. planci starfish* has potent cytotoxic as well as apoptotic effects on human breast cancer MCF-7 cell lines. *A. planci starfish* crude extract could be a promising chemotherapeutic agent to be used more effectively than tamoxifen in the treatment of breast cancer (Ambedkar and Shan, 2014). *Alpinia scabra*, is an aromatic, perennial and rhizomatous herb from the family Zingiberaceae. It is a wild species which grows largely on mountains at moderate elevations in Peninsular Malaysia. The cytotoxic potential of *A. scabra* extracts from different parts of the plant was studied. Results revealed the cytotoxic effect of *A. scabra* on cancer cells. Cell death induced by the cytotoxic extracts and fractions may be due to apoptosis which were characterized by apoptotic morphological changes, DNA fragmentations and DAPI nuclear staining (Reddy *et al.,* 2013).

Curcuma longa Linn. (Turmeric) is used extensively in Indian cuisine and now proved to be useful in treating various types of cancers, diabetic wounds and biliary

disorders etc. The anticancer activity of turmeric was evaluated prophylactically and therapeutically against the MNU induced breast tumors. The anticancer activity was evaluated by using latency period, tumor incidence, tumor burden, tumor volume, tumor growth inhibition, histology and hematological parameters. Oral administration of turmeric showed anticancer activity in a dose dependent manner and it was more in pre-induction treatment than in-post induction treatment groups. Topical application of turmeric was found to be more effective in pre-induction treatment and topical treatment was more effective when compared to oral treatment. Chemo-preventive role of turmeric was more effective compared to therapeutic role of turmeric (Annapurna *et al.*, 2011).

Table 1.7: List of plants known for anticancer activity

Plant Name	Part Used	Family
Alium cepa	Bulb	Liliaceae
Aloe barbadensis	Leaves	Liliaceae
Androgarphis paniculata	Dried leaves	Acanthacae
Bauhinia variegata	Root	Caesalpinaceae
Butea monosperma	Bark	Fabaceae
Cajanus cajan	Leaves	Fabaceae
Calotrophis gigantea	Whole plant	Asclepiadaceae
Cassia auriculata	Root	Caesalpinaceae
Curcuma longa	Rhizome	Zingibaracae
Catharanthus roseus	Whole plant	Apocynacae
Cassia senna	Leaves	Caesalpinaceae
Citrus medica	Root	Rutaceae
Daucus carota	Root	Apiaceae
Jatropha curc	Leaves	Euphorbiaceae
Morinda citrifolia	fruit	Rubiaceae
Mimosa pudica	Whole plant	Mimosaceae
Ochrosia elliptica	Trunk Bark	Apocynacae
Podophyllum peltatum	Dried Rhizome	Berberidacae
Semecarpus anacardium	fruit	Anacardiaceae
Terminalia arjuna	Bark	Combertacae
Vaccinium stamineum	fruit	Ericaceae
Zingiber officinalis	Rhizome	Zingibaracae

(Sourced from Umadevi *et al*., 2013)

1.6: Genesis of thesis.

India is well known for medicinal plants. There is long history of use of herbal medicine for treatments of various diseases. Allopathic drugs are costly and sometime have side effects while herbal drugs comparatively economic and have fewer/no side effects. As, many plants were used for many centuries and sometimes as regular constituents of the diet, it is assumed that they do not have many side effects. Synthetic drugs and drugs from plant extracts have their own importance and now are studied extensively. There is need to screen and explore various herbal plants for treatment of various diseases by systematic and advanced study approach. The Biochemical Sciences Division of CSIR-National Chemical Laboratory has various resources to screen herbal plants for its medicinal properties. The present study was, therefore, undertaken to generate more such Ayurvedic plants resources efficiently using conventional and highly advance technologies.

1.7: Organization of thesis

The thesis is organized in five chapters and the content of each chapter is as follows.

Chapter 1: Introduction and review of literature (Current chapter)

Chapter 2: This chapter describes the materials used and methodology adapted to carry out various experiments required to prove the hypothesis of this work.

Chapter 3: It includes *in-vitro* and *iv-vivo* results of various experiments carried out during this research work. It includes studies like identification of various plants, their phytochemical and molecular analysis and antioxidant, antidiabetic, antiurolithiasis and anti breast cancer activities.

Chapter 4: In this chapter there is discussion of results of this research work and previous work done by other researchers on similar line, conclusions and future prospects.

Chapter 5: This includes details of the literature referred in the present studies

1.8: Publications from this thesis work

a) From antidiabetic data

Sachin B. Agawane, Vidya S. Gupta, Mahesh J. Kulkarni, Asish K. Bhattacharya and Santosh S. Koratkar (2017), Chemo-biological evaluation of antidiabetic activity of

mentha arvensis l. and it's role in inhibitions of advanced glycation end products, Journal of Ayurveda and Integrative Medicine, **(In Press).**

b) From antiurolithiasis data

Sachin B. Agawane, Vidya S. Gupta, Mahesh J. Kulkarni, Asish K. Bhattacharya, Santosh S. Koratkar and Vamkudoth Koteswara Rao (2017), Patho-physiological evaluation of *Duranta erecta* for the treatment of urolithiasis, Journal of Ayurveda and Integrative Medicine, **(In Press).**

c) From anti breast cancer data

Sachin B. Agawane, Vidya S. Gupta, Mahesh J. Kulkarni and Asish K. Bhattacharya (2018), Prevention and treatment of breast cancer by use of *Chromolena odorata* leaves in *in-vitro* and *in-vivo* animal model, **(Manuscript under review in Advances in Pharmacological Sciences Journal).**

2.1: Collection of plants from various locations

Plants were collected from various locations fromWestern Maharashtra as given in Table 2.1

Table: 2.1- Plant material collection, locations and codes

Sr. No.	Plant Names	Locations of collection	Codes	Sr. No.	Plant Names	Locations of collection	Codes
1	*Adhatoda vasica*	Solapur	AS	5	*Duranta erecta*	Solapur	DS
		Kolhapur	AK			Kolhapur	DK
		Sangali	ASG			Sangali	DSG
		Satara	AST			Satara	DST
		Pune	AP			Pune	DP
2	*Bacopa monnieri*	Solapur	BS	6	*Mentha arvensis*	Solapur	MS
		Kolhapur	BK			Kolhapur	MK
		Sangali	BSG			Sangali	MSG
		Satara	BST			Satara	MST
		Pune	BP			Pune	MP
3	*Centella asiatica*	Solapur	CAS	7	*Ocimum tenuiflorum*	Solapur	OS
		Kolhapur	CAK			Kolhapur	OK
		Sangali	CASG			Sangali	OSG
		Satara	CAST			Satara	OST
		Pune	CAP			Pune	OP
4	*Chromolena odorata*	Solapur	CS	8	*Sphagneticola trilobata*	Solapur	SS
		Kolhapur	CK			Kolhapur	SK
		Sangali	CSG			Sangali	SSG
		Satara	CST			Satara	SST
		Pune	CP			Pune	SP

2.2: Preparation of Herbarium and authentication of plants

The dried specimens of each plant were fixed into herbarium sheet (Fig. 2.1) and submitted to the Botanical Survey of India, Western Circle, Pune for identification and authentication. The plants were then used for all the further analysis.

2.3: Preparation of methanolic extracts of collected plants samples

The fresh leaves of each plant were washed properly with 5 ppm potassium permanganate ($KMnO_4$) water and then washed under running tap water. They were shade dried at room temperature for 4-5 days. Dried leaves were grinded to obtain fine powder. The collected powder weighing 500 gm was subjected to separating funnel for extraction using 100% methanol solvent (Fig 2.2A). The mixture was kept in separating funnel for 48 hours at room temperature. After 48 hours mixture

was filtered into a beaker. The methanolic extract was concentrated using rotary evaporator (Heidolph, USA). It evaporated the solvent at reduced pressure and temperature (Fig 2.2B). The extract was further concentrated on high vacuum pump (Soni and Sosa, 2013). Extracts of all the accessions of all the eight species were prepared as detailed above (Fig 2.2C).

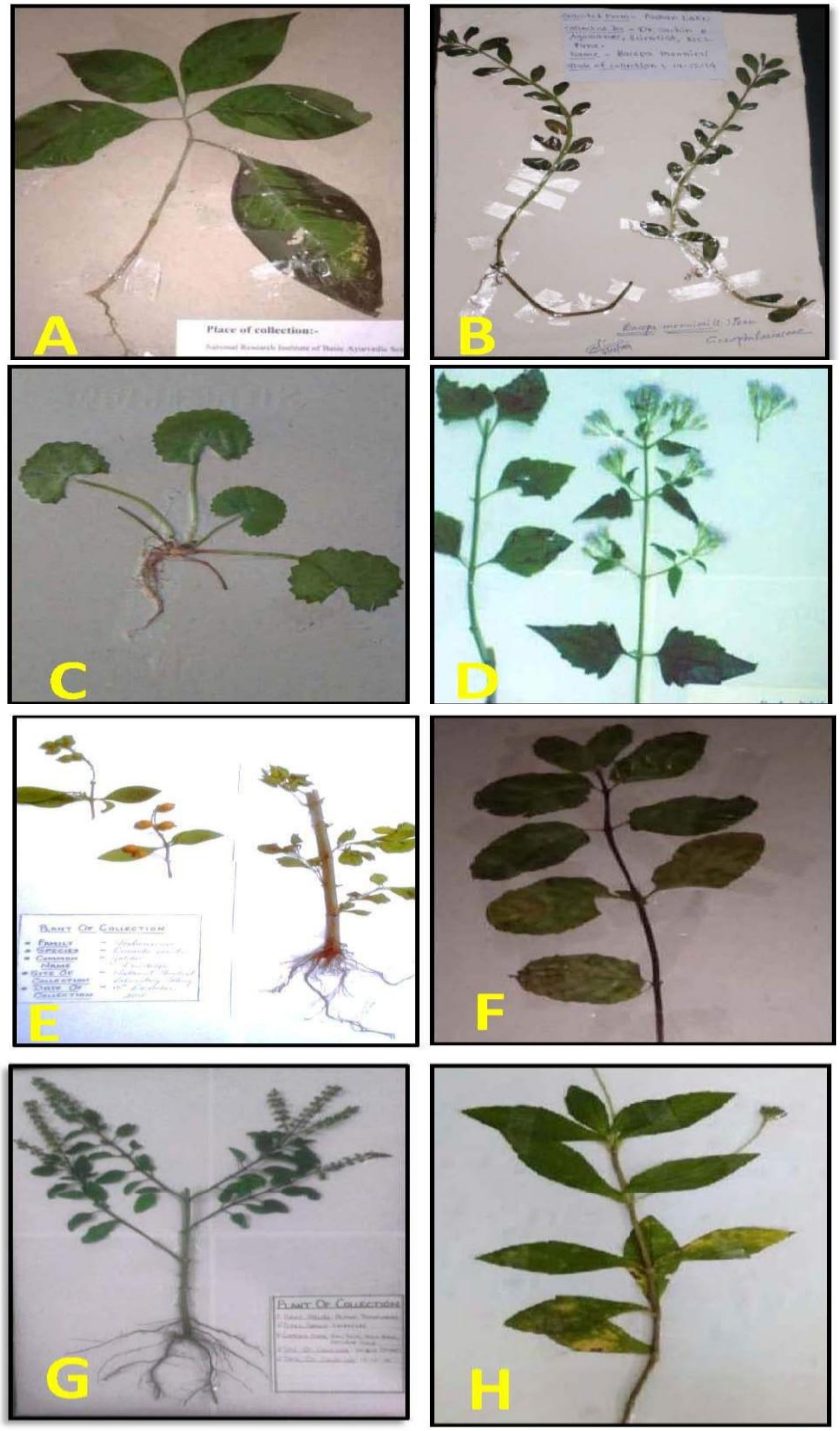


Fig.2.1. Herbaria of A: *A. vasica*, B: *B. monnieri*, C: *C. asiatica*, D: *C. odorata*, E: *D. erecta*, F: *M. arvensis*, G: *O. tenuiflorum*, H: *S.trilobata*

Fig.2.2-A: Plant extraction, B: Evaporation and C: Plant extracts stored in RB flasks

2.4: Preliminary phytochemical screening

Detection and qualitative estimation of phytochemicals of all the plant extracts was carried out by method suggested by Tiwari *et al*., (2011) and detailed as below.

Detection of alkaloids: 20 mg of each extracts was dissolved in diluted HCL and filtered.

Mayer's test: Mayer's reagent was prepared by dissolving 1.36 gm of $HgCl_2$ and 5.0 gm of KI in 100 mL of water. Filtrate was treated with 5 mL of Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicated the presence of alkaloids.

Wagner's test: Wagner's reagent was prepared by dissolving 2.0 gm of iodine with 6.0 gm of KI in 100 mL of water. Filtrate was treated with 5 mL of Wagner's reagent

(Iodine in Potassium Iodide). Formation of brown/reddish precipitate showed the presence of alkaloids.

Dragendroff's test: Preparation of Dragendroff's reagent: **Solution A:** 10 mL D/W was added to 0.5 gm of $Bi(NO_3)_3.5H_2O$. Then 10 mL conc. H_2SO_4 was added to the solution and mixed thoroughly. **Solution B:** 4 gm of KI was completely dissolved in 10 mL of water. Solution A and B were mixed to form orange colored Dragendroff's reagent. Filtrate was treated with 5 mL of Dragendroff's reagent. Formation of red precipitate depicted the presence of alkaloids.

Detection of saponins

Froth test: 0.5 mg of extract was diluted with distilled water to 20 mL and was shaken in a 50 mL falcon tube for 15 minutes. Formation of 1cm layer of foam indicated the presence of saponins.

Foam test: 0.5 gm of extract was shaken vigorously with 5 mg of $NaHCO_3$ and 2 mL of water. Honeycomb like froth showed the presence of saponins.

Detection of phytosterols

Salkowski's test: 1gm of each extract was treated with 2 mL chloroform and filtered. The filtrate was treated with 3 mL of conc. H_2SO_4, shaken and allowed to stand. Appearance of reddish-brown color at the interface depicted the presence of sterols.

Detection of phenols

Ferric chloride Test: To test the presence of phenols, extract was warmed in water followed by addition of 2 mL of $FeCl_2$. Formation of green blue color indicated the presence of phenols.

Lead acetate: 2 mL of lead acetate was added to 10 mg of each of the extract and observed the formation of precipitate which indicated the presence of phenols.

Detection of flavonoids: 10 mg of each extract was heated with 10 mL of ethyl acetate in a boiling water bath for 3 min. Then the mixture was filtered and filtrate was used for the following test. a) 5 mL diluted ammonia was added to 2 mL of filtrate followed by addition of conc. H_2SO_4. Disappearance of yellow color on standing indicated the presence of flavonoids.

b) 5 mL filtrate was treated with few drops of 1% $AlCl_3$. Formation of yellow color depicted the presence of flavonoids.

Alkaline reagent test: 2 mg of each extract was dissolved in water and filtered. The filtrate was treated with 10% NaOH solution. Formation of intense yellow color which disappeared on addition of dil. HCL indicated the presence of flavonoids.

Detection of tannins: 0.5 gm of each extract was boiled with 5 mL of 45% ethanol for 5 min. The mixture was cooled and filtered. The filtrate was used for the following test.

Ferric chloride test: Few drops of $FeCl_3$ solution was added to 1 mL of filtrate. Formation of greenish blackish color showed the presence of tannins.

Lead acetate test: 3 mL of 10% w/v lead acetate was added to 5 mL filtrate. A creamy gelatinous precipitate indicated positive for tannins.

Detection of terpenoids: 0.5 gm of of each extract was dissolved in water. 1 mL of acetic acid was added followed by conc. H_2SO_4 to the extract. A change in color from pink to violet confirmed the presence of terpenoids.

Detection of carbohydrates: To 200 µL of each plant extract filtrate, 2 mL DNSA (3, 5-Dinitro salicylic acid) reagent was added and the solution was boiled for 20 second on flame. Formation of brick red color due to reduction of DNSA to ANSA (3-amino, 5-nitro salicylic acid) and presence of reducing sugars confirmed presence of carbohydrates.

Test for proteins

Xanthoprotein test: To 2 mL of each extract, 3 drops of HNO_3 were added by the side of the test tube. Presence of yellow colouration indicated the presence of proteins and free amino acids.

Tests for glycosides: 2 mL of each extract was subjected to the following tests

Keller–Killiani test

1 mL of glacial acetic acid containing traces of $FeCl_3$ and 1 mL of concentrated H_2SO_4 were added to the extract carefully. A reddish brown colour was formed at the junction of the 2 layers and the upper layer turned bluish green indicating the presence of glycosides.

2.5: DNA extraction

Leaf samples of individual plants at various locations were collected as detailed in Table 2.1 and stored at –80°C till further use. DNA of three plant species which

showed biological activity under present study was extracted from individual accession by using modified CTAB method (Murray and Thompson, 1980). The quality and quantity of DNA were checked visually on 0.8% agarose gel stained with GelRed (Biotium, USA) comparing with standard DNA as well as spectrophotometrically using Nanodrop 1000 spectrophotometer (Thermo Scientific, USA).

2.6: PCR amplification and gel electrophoresis

A set of 65 ISSR primers, procured from University of British Columbia (Vancouver, Canada) were used for amplification of all the plant DNAs. PCR reaction was carried out as detailed by Deshpande *et al.,* (2001). Amplification of 10 ng DNA was performed in 25 µL reaction volume containing dNTP, PCR buffer, Taq polymerase and ISSR primers. PCR was performed on Verity thermal cycler (Applied Biosystems, USA) using following cycling conditions: initial denaturation at 94°C for 5 min; followed by 45 cycles of denaturation at 94°C for 0.30 min, annealing at 50°C for 0.45 min and extension at 72°C for 2 hr, with a final extension at 72°C for 5 min. The amplified products were separated on 1.5% agarose gel in 0.5X TAE buffer and bands were detected by GelRed staining. Reproducibility of the amplification was confirmed by repeating each experiment thrice.

After screening of 65 ISSR primers with five accessions of each plant species, final amplifications of all the plant samples were performed using only those ISSR primers selected from the primary screening.

2.7: *In-vitro* analysis for antidiabetic activity

2.7.1: DPPH free radical scavenging activity (Antioxidant activity)

The free radical scavenging activity of each plant extract was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay using ascorbic acid as standard (Rao *et al*., 2009). DPPH is a well-known free radical usually used as a reagent to evaluate free radical scavenging activity of antioxidants. DPPH is a free radical accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The DPPH solution was prepared by dissolving 25 mg DPPH in 100 mL methanol and stored at 20 °C till further use. The plant extracts and the standard were prepared at various concentrations ranging from 25µg/mL to 1000 µg/mL in methanol. To 50 µL of various concentrations of plant extract and standard, 150 µl of the above prepared DPPH solution was added. The reaction mixture was mixed well and incubated in the

dark for 30 min at room temperature. The absorbance was measured at 517 nm on a microplate reader (BIORAD xMark microplate spectrophotometer). The blank was prepared using methanol. The scavenging activity was calculated using the following formula based on the percentage of DPPH• radical scavenged,

$$\text{Percentage Inhibition (\%)} = \frac{(\text{Blank}_{(A517)} - \text{Sample}_{(A517)})}{\text{Blank}_{(A517)}} \times 100$$

2.7.2: α-Amylase inhibition assay

DNSA: DNSA reacts with reducing sugars to form 3-amino 5-nitrosalicylic acid which absorbs light strongly at 540 nm, however cannot bind to non-reducing sugars (e.g. starch, sucrose) (Fig.2.3). During incubation of sample at 37 ºC enzyme α-Amylase acts upon substrate to give products i.e. reducing sugar. Then DNSA was added and reaction mixture was boiled which was responsible for heat inactivation of enzyme and for efficient binding of reducing sugars to DNSA to give 3-amino 5-nitrosalicylic acid that shows maximum absorbance at 540 nm.

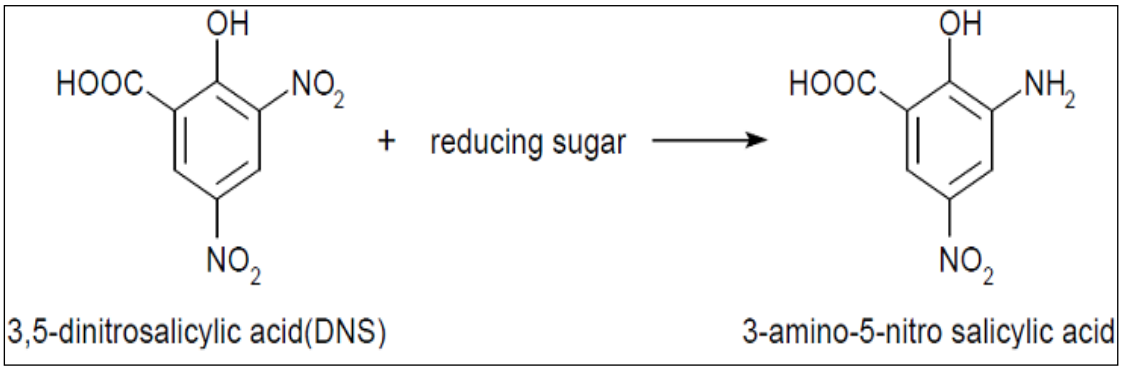


Fig.2.3: Conversion of DNS to ANSA

Triplicate reactions were carried out containing 20 mM sodium phosphate buffer (pH 7.0) and porcine pancreatic alpha amylase (Sigma-Aldrich, USA) to which 150 µL of starch (0.25%) was added. After 15 min of incubation at 37°C, reaction was stopped by adding 500 µL of Dinitrosalicylic acid (DNSA) reagent and reaction tube was incubated in boiling water bath for 5 min. Absorbance was measured at 540 nm. 1 amylase unit was defined as the amount of enzyme required to release 1 µM maltose/min at 37 °C from substrate (starch) under the given assay conditions (Bhide *et al*., 2015).

Amylase inhibitory (AI) activity was performed in a similar way at pH 7.0 except that enzyme and inhibitor were incubated for 15 min at 37 °C before incubating with the substrate. Amylase inhibitors viz various plant leaves extracts and Acarbose

(Standard) were tested in a concentration range of 2 to 10 µg/µL. Amylase inhibitory activity was calculated by measuring the reduction in liberated reducing sugars from starch compared to the standard amylase activity mentioned above (Bhide *et al*., 2015). AI activity was expressed as percentage of inhibition using the following formula,

$$\text{Percentage Inhibition (\%)} = \frac{(\text{Control}_{(A\ 540)} - \text{Sample}_{(A540)})}{\text{Control}_{(A\ 504)}} \times 100$$

2.7.3: α-Glucosidase inhibition assay

2.7.3.1: Isolation of α- glucosidase from rat intestine

Isolation of α- glucosidase from rat intestine was performed as per method suggested by Chougale *et al.,* (2009). One male Wistar rat was sacrificed by cervical dislocation and intestine was collected in Phosphate Buffered Saline (PBS) pH 7.0. The rat intestine was repeatedly flushed with PBS and epithelial layer (mucosal tissue) was collected by scraping the luminal surface firmly with a spatula (Fig.2.4 and 2.5). The mucosal layer was homogenized in PBS in a mortar and pestle. Then the intestine was homogenized in a Sonicator for 10 min with 40% amplitude and on/off cycle of 10 sec. The homogenized intestine was centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant contained the enzyme. The aqueous layer was subjected to overnight dialysis against the same buffer to get rid of impurities. A 12 kD dialysis bag was filled with enzyme, clipped on both the sides and suspended in 50 mM PBS on a magnetic stirrer. Next day the enzyme was collected and checked for its activity with 5 mM *p*-nitrophenyl α -D-glucopyranoside (PNPG) as substrate. One unit (U) of enzyme activity of α- glucosidase is the amount of enzyme that catalyzes the hydrolysis of 1.0 µmole substrate per minute at pH 7.0

Fig.2.4: Washing of rat intestine with PBS

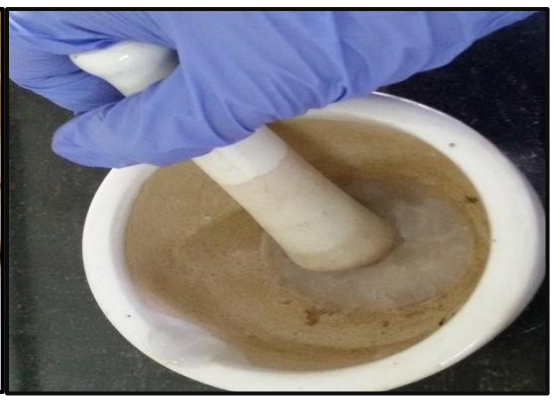


Fig.2.5: Trituration of the intestine

2.7.3.2: Inhibition assay: α-glucosidase inhibition assay was performed as described earlier (Elya *et al*., 2012). The α-glucosidase (*Saccharomyces cerevisiae*, Sigma-Aldrich, USA) was the source of enzyme and *p*-nitrophenyl- α -D-glucopyranoside (PNPG) (Sigma-Aldrich, Switzerland) was used as the substrate for the assay. To 100 µL plant exract in varying concentration (2 to 10 mg/mL), 50 µL of 5 mM PNPG and 50 µL α-glucosidase (1.0 U/mL) were added, mixed and incubated at 37 °C for 30 min. Reaction was terminated by addition of 1 mL of 200 mM Na_2CO_3. Amount of *p*-nitrophenol released was measured using a microplate reader (BIORAD xMark microplate spectrophotometer, USA) at 405 nm by measuring the quantity of *p*-nitrophenol released from PNPG. Acarbose was used as positive control of α-glucosidase inhibition. Percent inhibition of α-glucosidase was calculated by using the formula,

$$\text{Percentage Inhibition (\%)} = \frac{(\text{Control}_{(A405)} - \text{Sample}_{(A405)})}{\text{Control}_{(A405)}} \times 100$$

2.7.3.3: BSA-AGE fluorescence assay

Stock solutions of varying concentration of various plant extracts were prepared in 30% DMSO and vortexed for 15 min. The reaction for BSA-AGE inhibition assay was set up as described earlier with slight modifications (Singh *et al*., 2016). BSA glycation reactions were conducted by incubating 1 mL of 50 mg/mL BSA in 0.1M phosphate buffer (pH7.4) and 0.5 M dextrose monohydrate containing 0.01% sodium azide as bacteriostat at 37°C for 7 days with various concentration (0.5, 1, 10 solutions of varying concentrations of various plant extracts were prepared in 30% DMSO and, 25, 50 mg/mL) of various plant extracts. DMSO (30%) was used as solvent (vehicle control). The BSA glycation was monitored at 370/440 nm by using spectrofluorometer (PROMEGA GLOMAX spectrofluorometer, USA). Percent inhibition of glycation was calculated by using given formula,

$$\text{Percent Inhibition (\%)} = \frac{C - T}{T} \times 100$$

Where, C is the relative fluorescence intensity of glycated BSA in absence of an inhibitor and T is the relative fluorescence intensity of glycated BSA in presence of an inhibitor.

2.8: *In -vitro* analysis for antiurolithiasis activity

2.8.1: Nucleation assay: This assay was chosen for the study of oxalate crystallization because of its satisfactory results simplicity and reproducibility. In order to assess the inhibiting capacity of various plant extracts this assay was used, which included the study of crystallization in the form of turbid solution with and without plant extracts. Calcium chloride and sodium oxalate solutions were prepared at the final concentrations of 9mMol/L and 3mMol/L, respectively, in a buffer containing 0.15 M NaCl and 0.05 M Tris at pH 6.5. 250 µL of calcium chloride solution was mixed with 100 mL of extracts at different concentrations. Crystallization took place upon adding 250 mL of sodium oxalate solution. The temperature was maintained at 37ºC. Spectrophotometer (Systronics digital spectrophotometer 166, India) was used to monitor the OD of the solution after 30 minutes at 620 nm (Atmani *et al*., 2000). The growth of the crystals was expected due to the following reaction, $CaCl_2 + Na_2C_2O_4 \rightarrow CaC_2O_4 + 2NaCl$

The percentage of inhibition was calculated with the help of following formula.

$$\text{Percentage inhibition} = \frac{Abs_{Ctrl} - Abs_{Test}}{Abs_{Ctrl}} \times 100$$

Abs_{Ctrl} represents the turbidity without plant extract, while Abs_{Test} represents the turbidity after addition of plant extract.

2.8.2: Synthetic urine assay

Preparation of synthetic urine: Synthetic urine assay was performed for the study of oxalate crystallization in order to assess the inhibiting capacity of various plant extracts, which included the study of crystallization with and without addition of plant extracts. Synthetic urine was prepared by adding NaCl (9 gm) in solution A composed of $N_2C_2O_4$ (2 mM/L) and Solution B of $CaCl_2$ $2H_2O$ (10 mM/L). Equal volumes of 50 mL of solutions A and B each were mixed and stirred at constant temperature (37°C) in capped vessels to give final artificial urine. Mixture agitations were maintained to prevent sedimentation.

2.8.3: Simulation of the sedimentary crystal formation:

The crystal developed was monitored by a simple light microscope in a sample drop after mixing the solution A and B. A series of experiments were conducted to study physiological concentrations 25, 50, 75 and 100 µg/mL of each extract.

Haemocytometer counting chamber was used to observe and count the number of crystals in a drop of sample under microscope after 30 minutes of mixing, for all the concentrations (Beghalia *et al*., 2008). The percentage of inhibition was calculated with the help of following formula.

$$\text{Percentage inhibition} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

Where Control- represents the number of calcium oxalate monohydrate crystals without inhibitor and Test- represents the number of calcium oxalate monohydrate crystals after addition of inhibitor.

Activity of methanolic extract of *D. erecta* was studied by agar well diffusion method. The 100 µl ($1x10^{-5}$) stock culture was inoculated onto 25 mL nutrient broth and incubated at 37°C for 48 hr. At the end of the incubation period, 10µL bacterial suspension was spread using L-shaped spreader onto precooled NAM agar plates and 10µL test compound was added to the respective wells (6 mm). Streptomycin (100µg) dissolved in 1000µL DMSO was used as the standard antibiotic and incubated at above mentioned conditions. At the end of the incubation period, the zone of inhibition (mm) around the well was calculated by using HiAntibiotic Zone Scale-CTM (Himedia, India).

2.9: *In-vitro* analysis (Cell lines tests) for anti breast cancer activity

2.9.1: MTT cell proliferation assay

Sample preparation for MTT assay: The crude extract of *C. odorata* and its 12 fractions were dissolved and diluted in DMSO (Dimethyle sulfoxide) to prepare different dilutions ranging from 50 µg/mL to 0.39 µg/mL using double dilution method (Mosmann,1983).

Cell culture

MCF-7 (Breast cancer) and 4T1 (Mouse mammary carcinoma) cell lines were obtained from the National Center for Cell Science (NCCS), Pune, India and maintained in T25 flasks with 10 % (v/v) fetal bovine serum (FBS) containing Dulbecco's Modified Eagle Medium (DMEM) and Roswell Park Memorial Institute medium (RPMI). Cells were maintained at 37 ºC under 5% CO2 and 95% air in a humidified atmosphere.

2.9.2: Cytotoxicity assay

All the compounds were tested for their cytotoxicity by using modified MTT assay as described previously (Mosmann,1983) on two cancer cell lines MCF-7 (Human Breast cancer) and 4T1 (Mouse mammary tumor). 80% confluent cells were trypsinized and centrifuged at 1500 RPM for 5 minutes.Cell pellets were then taken and seeded at the density of 1×10^5 cells/mL in a 96 well plate. Countess cell counter was used to measure no. of cells per µl. The plates were incubated overnight in CO_2 incubator (37ºC under 5% CO_2 and 95% air in a humidified atmosphere). Next day, cells were treated with different concentration of test compounds (50-0.39 µg/mL) and incubated for additional 48 hr (MCF-7 and 4T1). Post incubation, cell media were replaced with MTT (0.5 mg/mL)-PBS mediums and incubated for 2-4 hr to form reduced MTT or Formazan crystals. This reduced MTT or Formazan crystals were solubilized by addition of acidified isopropanol. The optical density was read on a microplate reader (Spectramax plus 384 plate reader, Molecular Devices Inc, USA) at 570 nm filter against a blank prepared from cell-free wells. Absorbance given by cells treated with the vehicle alone was taken as 100% cell growth. IC50 and MIC values were calculated from graphs, using Origin Pro software. The percent cytotoxicity in the presence of test fractions was calculated by given formula.

$$\text{Percent cytotoxicity} = \frac{[\text{absorbance of control} - \text{absorbance of a compound}]}{[\text{absorbance of control} - \text{absorbance of blank})]} \times 100$$

Whereas DMSO treated culture cells were control and culture medium without cells was blank.

2.9.3: Detection of apoptosis

Apoptosis was determined using Fluorescent dye Annexin V FITC and Propadium Iodide assay (Maslinska, 2003). Apoptosis plays a key role in the regulation of normal tissue homeostasis and participates in the elimination of abnormal cells. Most of the antitumor drugs kill the cancer cells by stimulating the apoptotic pathway. 2×10^4 cells/mL were seeded in a 96 well plate and allowed to adhere overnight in CO_2 incubator. Next day, cells were treated with the sample at two different concentration i.e 16 µg/mL and 32 µg/mL for 24 hr. Untreated cells were kept as negative control, whereas, cells treated with Doxorubicin (2nM) were kept as positive control. 1X Annexin binding buffer was prepared (Hepes 10mM, NaCl 140 mM, $CaCl_2$ 2.5 mM, DW 100 mL, pH 7.4). Post incubation, cells were washed with 1X

PBS followed by 1X Annexin binding buffer Cells were stained with 1X Annexin binding buffer containing 5 µl of Annexin V and 10 µg/mL propidium iodide and incubated for 15-20 minutes in dark and observed under fluorescence microscope.

2.10: *In-vivo* animal experiment for evaluation of antidiabetic potential of *Mentha arvensis* L.

Postprandial non-insulin dependent anti-hyperglycemic activity of methanolic leaves extract of *Mentha arvensis* L. was determined by postprandial glycemic test.

2.10.1: Animals used

Healthy wistar rats were obtained from National Institutional of Bioscience (CPCSEA Registration No. 1091/abc/07/CPCSEA) Pune, Maharashtra and used for this study upon approval by the Institutional Animal Ethics Committee of Symbiosis School of Biomedical Science (CPCSEA Reg. No.1710/PO/a/13/CPCSEA). The study approval number is SSBS/IAEC/3/4.3.2015. Animals were maintained under standard laboratory conditions at Symbiosis School of Biomedical Science (SSBS), Pune. Animal welfare guidelines were observed during the maintenance and experimentation period. For this study 18 male wistar rats were divided into three groups each containing six male rats. All the animals were kept for overnight fasting. Next day in the morning, blood was collected from the retro-orbital plexus and blood glucose level (‘0’ hr) was estimated by auto blood analyzer (Bayer Express Plus, Germany). All the groups of animals (A, B and C groups) were given soluble potato starch 2 gm/kg body weight to induce diabetes mellitus.

2.10.2: Treatments

Group A (Diabetes mellitus control): Diabetes mellitus rats received only distilled water followed by starch.

Group B (Standard compound): Diabetic rats received standard antidiabetic drug Acarbose (50 mg/kg) 15 minutes before starch feeding.

Group C (Sample treated): Diabetic rats treated with methanolic leaves extract of *Mentha arvensis* (L.) (50 mg/kg) 15 minutes before starch feeding.

Blood was collected at the intervals of 0^{th}, 30^{th}, 60^{th}, 90^{th} and 120^{th} minutes. Blood glucose levels were measured as described by Tiwari *et al*., (2011). All the data related to the animal study were analyzed by standard statistical methods. Degree of significance $P<0.05$ between the groups of animals was determined by Two-way

ANOVA followed Tukey's multiple comparison test and applied to compare difference between animal study groups.

2.11: *In-vivo* animal experiment for evaluation of antiurolithiasis activity of *Duranta erecta*

2.11.1: Animals used

Antiurolithiasis activity of methanolic leaves extract of *Duranta erecta* was determined against sodium oxalate in wistar rats. For this study male wistar rats weighing around 150-200 gm and 6 weeks old were obtained from National Institute of Bioscience, Pune, after approval from Institutional Animal Ethical Committee (IAEC approval No.SSBS/IAEC/04-2016) of Symbiosis School of Biomedical Sciences (SSBS), Pune. Rats were housed in standard polyvinyl cages in the animal house facility of SSBS, Pune. They were fed with a balanced diet of standard pellet and maintained under standard laboratory conditions providing 22-24^{o}C temperature, 60-70 % relative humidity, standard light-dark cycle (12 hours light, 12 hours dark) and water was provided adlibitum. Rats were divided into four groups (A-D) with 6 animals in each group. Groups A, B, C and D were served normal control; disease control, standard drug treated and *D. erecta* extract treated animals, respectively. Groups B, C and D received 70 mg/kg Sodium oxalate via intraperitoneal injection (IP) for 7 days. Group C then received 50 mg/kg of standard drug (Cystone) (suspended in 2% gum acacia) orally and Group D received 50 mg/kg of *D. erecta* extract orally.

2.11.2: Serum parameters

On day 7 of the treatment, blood was withdrawn by retro-orbital puncture from each rat. The serum was separated by centrifugation at 10,000 rpm at 4°C using cryocentrifuge machine for the estimation of serum parameter (creatinine, BUN, SGOT and SGPT). Blood related parameters were measured using auto blood analyzer instrument (Bayer Express Plus, Germany).

2.11.3: Urine parameters

Urine color, transparency, pH and urine oxalate were checked and microscopic examination was done to observe calcium oxalate crystallization.

2.11.4: Histopathology

After 7th day of the treatment period the animals were sacrificed by ethical method. Both kidneys were dissected and one kidney from each rat was placed in 10% formalin

solution. The kidney tissues were trimmed longitudinally and routinely processed. Tissue processing was done to dehydrate in ascending grades of alcohol, clearing in xylene and embedded in paraffin wax. Paraffin wax embedded tissue blocks were sectioned at 5µm thickness with the rotary microtome. All the slides of kidneys were stained with Hematoxylin & Eosin (H & E) stain. The prepared slides were examined under microscope.

2.12: *In-vivo* efficacy of *Chromolaena odorata* against breast cancer

2.12.1: Animals used

Healthy wistar rats were obtained from in-house source of APT Research Foundation (CPCSEA Registration No. 1091/abc/07/CPCSEA) Pune, Maharashtra and used for this study upon approval by the Institutional Animal Ethics Committee. The study approval number is RP 14/1718. Animals were maintained under standard laboratory conditions. 11-12 weeks old female wistar rats, six in each group were used.

2.12.2: Study design

DMBA (7, 12–dimethylbenz(a) anthracene) 7.5 mg/kg was dissolved in 1 ml of vehicle (0.5 ml of sunflower oil + 0.5 ml of saline) and injected by subcutaneous route beneath the mammary gland at right side, once a week for four weeks (Fig. 2.6). The animals were housed till development of tumor. Beginning at 28 days after last DMBA injection, the animals were observed twice weekly to detect the presence and location of mammary tumors. Plant extracts were administered at 50 mg/kg b.w. dose to respective groups.

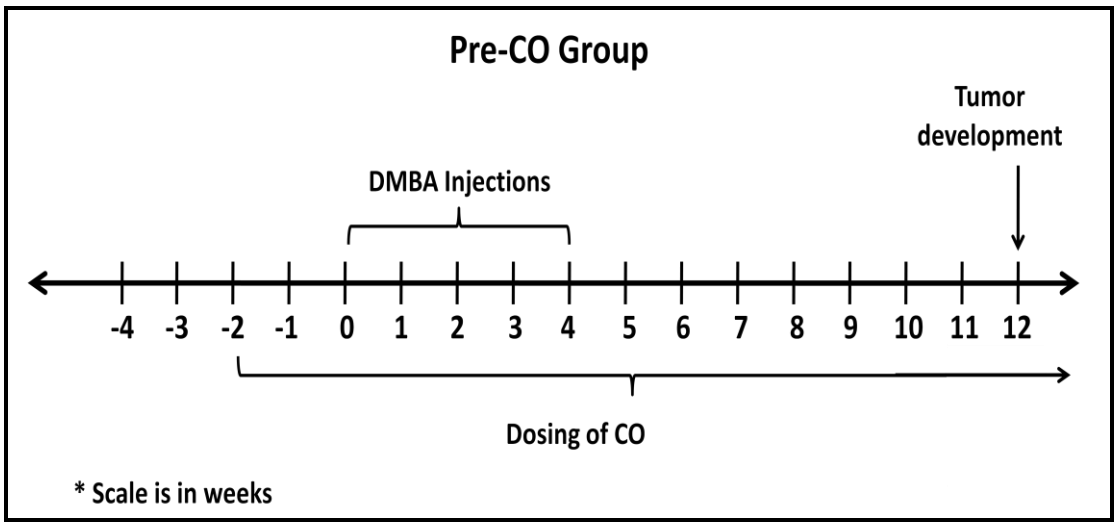


Fig.2.6: Schematic representation of tumor development in wistar rat.

Table: 2.2: The study groups involved in anti breast cancer study of *C. odorata*

Group name	**Specification (n=6)**
NC	Normal Control
DC	Disease Control (DMBA)
STD-A	DMBA + Ayurvedic Standard Drug (Carsocare 50 mg/kg)
STD-D	DMBA + Doxorubicin 4 mg/ kg i.p once a week for 4 weeks
Pre-CO	Pretreatment with plant extract CO (50 mg/kg orally) from 2 weeks prior to DMBA injections
Post-CO	DMBA + CO Extract (50 mg/kg orally) for 4 weeks after tumor formation

*STD-A: Standard Marketed Ayurvedic Drug Carsocare, STD-D: Standard drug Doxorubicin

As indicated in (Table 2.2) Group 5 (Pre-CO) received dose of plant extracts from 2 weeks prior to DMBA injections. Whereas Group 6 (Post-CO) received dose of respective plant extracts after tumor formation. After 28 days of DMBA injection (4 times once in a week), the animals were frequently observed by palpitation to detect the presence and location of mammary gland tumors. Animals were randomized after detection of tumors (n=6 per group).

2.12.3: Parameters under study

During the experimental parameters like body weight, tumor volumes (using digital Vernier caliper) were recorded on weekly basis. At the end of the study all the animals were sacrificed. Blood was analyzed for hematology and serum was used for estimations of biomarkers like ALP, LDH, Glucose, Total Protein, Urea, Creatinine, SGPT, SGOT, Lipid Profile and GGT. The homogenates of liver and mammary gland were analyzed for Catalase, Lipid peroxidation and reduced glutathione (GSH) content.

2.12.4: Histopathology

At the end of the study animals were dissected and the tumor, uterus, liver, lung and kidneys were fixed in 10% formalin for monitoring adverse effect or efficacy after the treatment. The tissues were then processed by undergoing a series of steps that include fixation, dehydration, clearing, wax impregnation, embedding & subsequent

sectioning on the microtome as per standard method described by Ragavan *et al.*, 2006.

2.12.5: Statistical analysis: The results were analyzed for statistical significance by ANOVA followed by Dunnett's test. GraphPad PRISM® Version 6.01 (GraphPad software, Inc., California, USA) was also used for statistical analysis.

2.12.6: Column chromatography

C. odorata methanolic leaf extract was run on 60-120 mesh silica gel (Spectrochem, India). A clean glass column was filled with slurry of silica gel and petroleum ether up to height of 15 cm. The column was repeatedly flushed with petroleum ether to set the bed of the column. The extract was dissolved in DCM and concentrated on rotary evaporator along with small amount of silica to obtain a free flowing powder. The powder was loaded on the top silica bed of the glass column and elution was started with petroleum ether. Successive elutions from 0.5- 10% ethyl acetate/petroleum ether (200 mL each) were performed through column. Each fraction was collected separately and concentrated on rotary evaporator.

2.12.7: Gas chromatography/Gas chromatography mass spectrometry (GC/GCMS)

The fractions obtained from column chromatography of *C. odorata* methanolic leaves extract were submitted for GC-MS analyses. The Gas chromatography (GC) analysis was carried out on a 5977A GC chromatograph fitted with HP-5 MS column (30 m x 0.25 mm, 0.25 μm) and interfaced with a mass spectrometer 5977C (both Agilent Technologies, USA). The GC analytical conditions were as follows: carrier gas He (99.999% purity; 1 mL/min), injector temperature 280°C, column temperature programmed from 100 °C (4 min hold) to 300 °C (16 min hold) at 10 °C/min. Samples were injected by splitless mode. The volume injected and the inlet pressure was 1.0 μL and 72.553 kPa, respectively; and the total running time was 21 minutes. The MS conditions were as follows: ionisation voltage 70 eV; emission current 34 mA; acquisitions scan mass range of 50 – 600 amu at a sampling rate of 2.0 scan per second.

2.12.8: Liquid Chromatography-Mass Spectroscopy (LC-MS)

To prepare 1 mL of the sample 1mg of the analyte was taken. 1 mL of the solvent was added and vortexed it for 2 minutes, then centrifuged for 5 minutes at 10,000 rpm and

took 20µl of the supernatant in separate clean LC-MS vials and at last added 980 µl of solvent to the vials. The instrument used for liquid chromatography analysis was Accela 1200 and the mass spectrometer was Q exactive orbitrap (Thermo Fisher Scientific, USA). Conditions were as follows: Mobile phase - A- Acetonitrile (0.1 % formic acid) B- methanol (0.1 % formic acid). Both the solvents were LC-MS grade. Samples were injected by splitless mode. The volume injected was 1.5µl. The total running time was 10 minutes with a flow rate 350 µl/min, gradient was 90 % acetonitrile throughout for 10 minutes. Column used was Hypersil gold (50 mm x 2.1 mm, 1.9 µm). The MS conditions were as follows; polarity was positive, sheath gas fow rate 45 µl /min, auxillary gas flow rate 12 µl/min; acquisitions scan mass range of 100 – 1000 amu., capillary voltage - 4.2 kv, capillary temperature – 320 ºC and drying gas temperature - 350 ºC. The software used for analysis of the data obtained from LC-MS was "X-Caliber".

2.12.9: Thin Layer Chromatography

Thin layer chromatography is a technique used to separate non volatile mixtures. It was performed on a thin sheet of aluminum coated with silica that was used as the stationary phase and after the mixture was applied on the plate it was kept in solvent or solvent mixture that was mobile phase, which was drawn up the plate via capillary action. Because different analytes (constituent compounds) ascend the TLC plate a different rates, separation was achivied. The spots were visualized by projecting the sheet under ultraviolet light. This technique was used throughout column chromatography to monitor the process by spotting the compounds present in the mixture. A few mL of solvent was collected time to time and was hecked by TLC to see the eluted compound which ultimately helped in fractionating the crude extract of *C. odorata*.

2.13: Single dose acute oral toxicity study of *M. arvensis, D. erecta and C. odorata*

Single dose oral toxicity study of *M. arvensis, D. erecta* and *C. odorata* (Plants which showed medicinal properties viz. anti breast cancer, antiurolithiasis and antidiabetic respectively) was carried out to determine acute oral toxicity of *M. arvensis, D. erecta* and *C. odorata* in wistar rats as per OECD guidelines. Wistar rats were divided in 8 different groups as given in Table.2.3

Table: 2.3: The study groups involved in single dose acute oral toxicity study of *M. arvensis, D. erecta and C. odorata*

Group Name	**Sex**	**Number of animals**	**Treatments**
A	Male	6	Normal control
B	Female	6	Normal control
C	Male	6	Received *C. odorata* @ 2000 mg/kg b.w. orally on day 1st.
D	Female	6	Received *C. odorata* @ 2000 mg/kg b.w. orally on day 1st.
E	Male	6	Received *D. erecta* @ 2000 mg/kg b.w. orally on day 1st.
F	Female	6	Received *D. erecta* @ 2000 mg/kg b.w. orally on day 1st.
G	Male	6	Received *M.* arvensis @ 2000 mg/kg b.w. orally on day 1st.
H	Female	6	Received *M.* arvensis @ 2000 mg/kg b.w. orally on day 1st.

Healthy wistar rats were obtained from National Institutional of Bioscience (CPCSEA Registration No. 1091/abc/07/CPCSEA) Pune, Maharashtra and used for this study upon approval by the Institutional Animal Ethics Committee of Symbiosis School of Biomedical Science (CPCSEA Reg. No.1710/PO/a/13/CPCSEA). Animals were maintained under standard laboratory conditions at Symbiosis School of Biomedical Science, Pune. Animal welfare guidelines were observed during the quarantine and experimentation period of 14 days. Following parameters were tested. Body weights and feed intakes were estimated on day 1st, 7th and 14th. Hematological parameters i.e. HB, RBC, WBC, PLT and serum parameters viz. SGOT, SGPT, creatinine, BUN were analysed on day 14th. All the animals were sacrificed on day 14th day by use of carbon dioxide euthanasia and organs were collected in 10% formalin for histopathological examination. Histopathology of vital organs organs viz. liver, kidneys, heart, lung and spleen was carried out. The results were analyzed for statistical significance by ANOVA followed by Dunnett's test. GraphPad PRISM® Version 6.01 (GraphPad software, Inc., California, USA) was also used for statistical analysis.

3.1a: Analysis of collected plant material

Plant specimens were identified and authenticated as given below by the Western Regional Centre of Botanical Survey of India (BSI), Pune (Table 3.1). The herbaria of all the collected samples have been deposited with BSI, Pune. The leaves collected were dried as given in chapter 2 and yield of dry powder obtained was in the range of 38 % to 60 % (Table 3.2). The methanolic extracts of all the plants were characterized for presence of various phytoconstituents as detailed in Table 3.3a and 3.3b. All the eight plants showed presence of all the phytoconstituents at various concentrations. Representative pictures of individual tests for identification of constituents are given in Fig. 3.1. These were also analysed by NMR as given in Fig. 3.2a. The peaks of NMR revealed that all plant extracts were the mixtures of several molecules.

Table 3.1: Plant specimen's identification and authentication

Sr. No.	Plant Code	Date of Certification	Authenticated as
1	Agawane 01	01.12.2014	*Adhatoda vasica* (L.) Nees
2	Agawane 04	16.12.2014	*Bacopa monnieri* (L.) Penn
3	Agawane 02	01.12.2014	*Centella asiatica* (L.) Urb.
4	Agawane 08	08.01.2016	*Chromolaena odorata* (L.) R.M.King & H.Rob
5	Agawane 06	21.12.2015	*Duranta erceta* (L.)
6	Agawane 03	01.12.2014	*Mentha arvensis* (L.)
7	Agawane 05	21.01.2015	*Ocimum tenuiflorum* (L.)
8	Agawane 07	21.07.2017	*Sphagneticola trilobata* (L). Pruski

Table 3.2: Yield of dried methanolic extract of leaves of various plants

Sr. No.	Plant Name	Maximum yield (%)
1	*Adhatoda vasica* (L.) Nees	41 %
2	*Bacopa monnieri* (L.) Penn	48 %
3	*Centella asiatica* (L.) Urb.	58 %
4	*Chromolaena odorata* (L.) R.M. King & H.Rob	42 %
5	*Duranta erecta* (L.)	60%
6	*Mentha arvensis* (L.)	45 %
7	*Ocimum tenuiflorum* (L.)	52 %
8	*Sphagneticola trilobata* (L). Pruski	38%

3.1b: ISSR analysis

Total 65 ISSR primers were initially screened for the analysis of genetic similarity within five different accessions of the same plant species viz. *C.odorata, D. erecta* and *M. arvensis* because extracts of these three plants showed medicinal properties viz. anti braest cancer, antiurolithiasis and antidiabetic, respectively which were further characterized in the present work. Out of 65 primers five primers showed amplification with these three plants. It was observed that most of the primers showed monomorphism. Hence best three primers were selected for the analysis in each plant species. Most of the major bands in accession of each species co-migrated. Thus, various loci obtained with these primers were monomorphic in nature and indicated genetic uniformity within five different accessions of the same plant species (Fig. 3.2b).

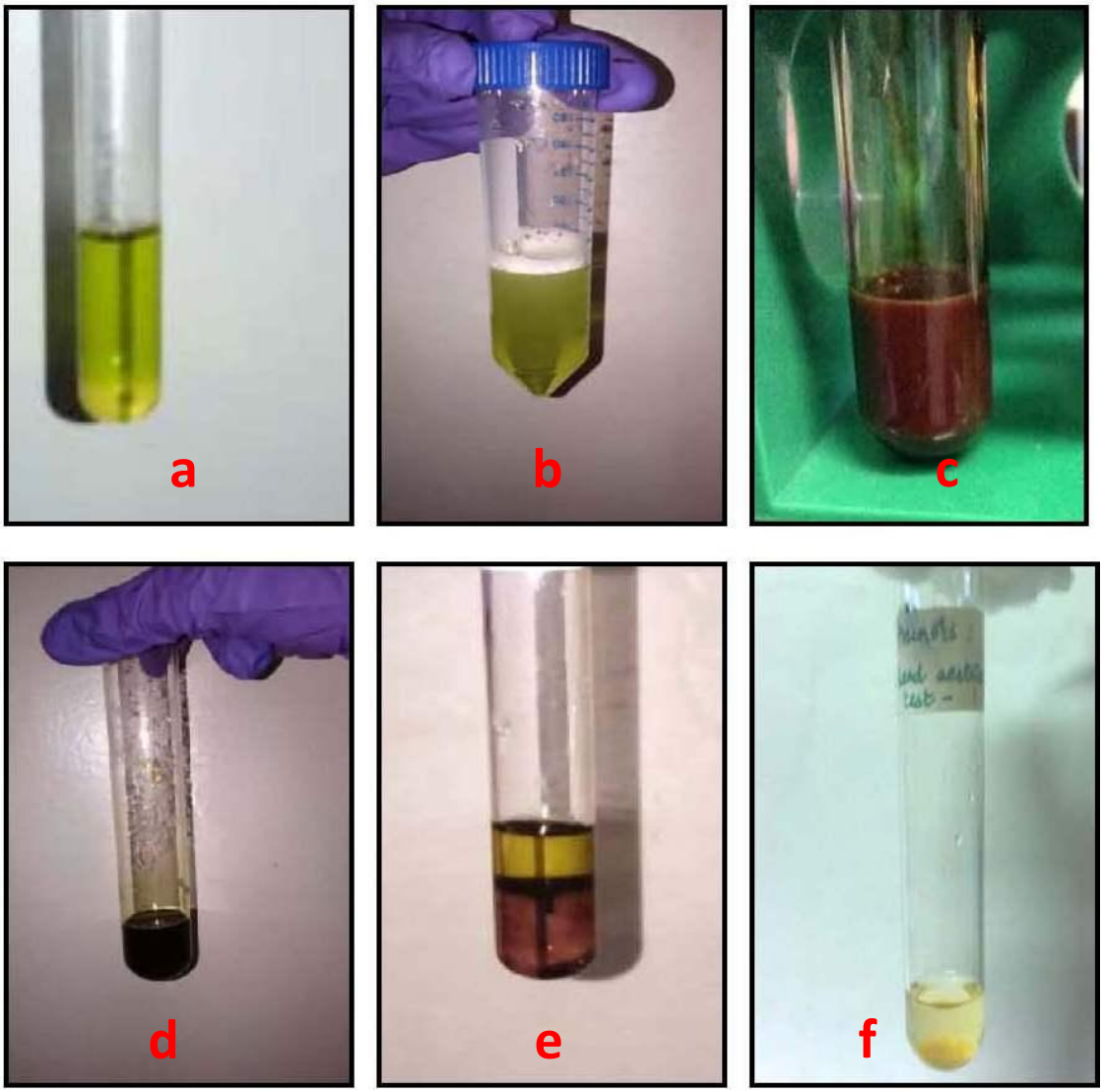


Fig.3.1: Test for phytochemical analysis, a: Flavonids, b: Saponin, c: Alkaloids, d: Tannins, e: Terpenoid and f: Phenols

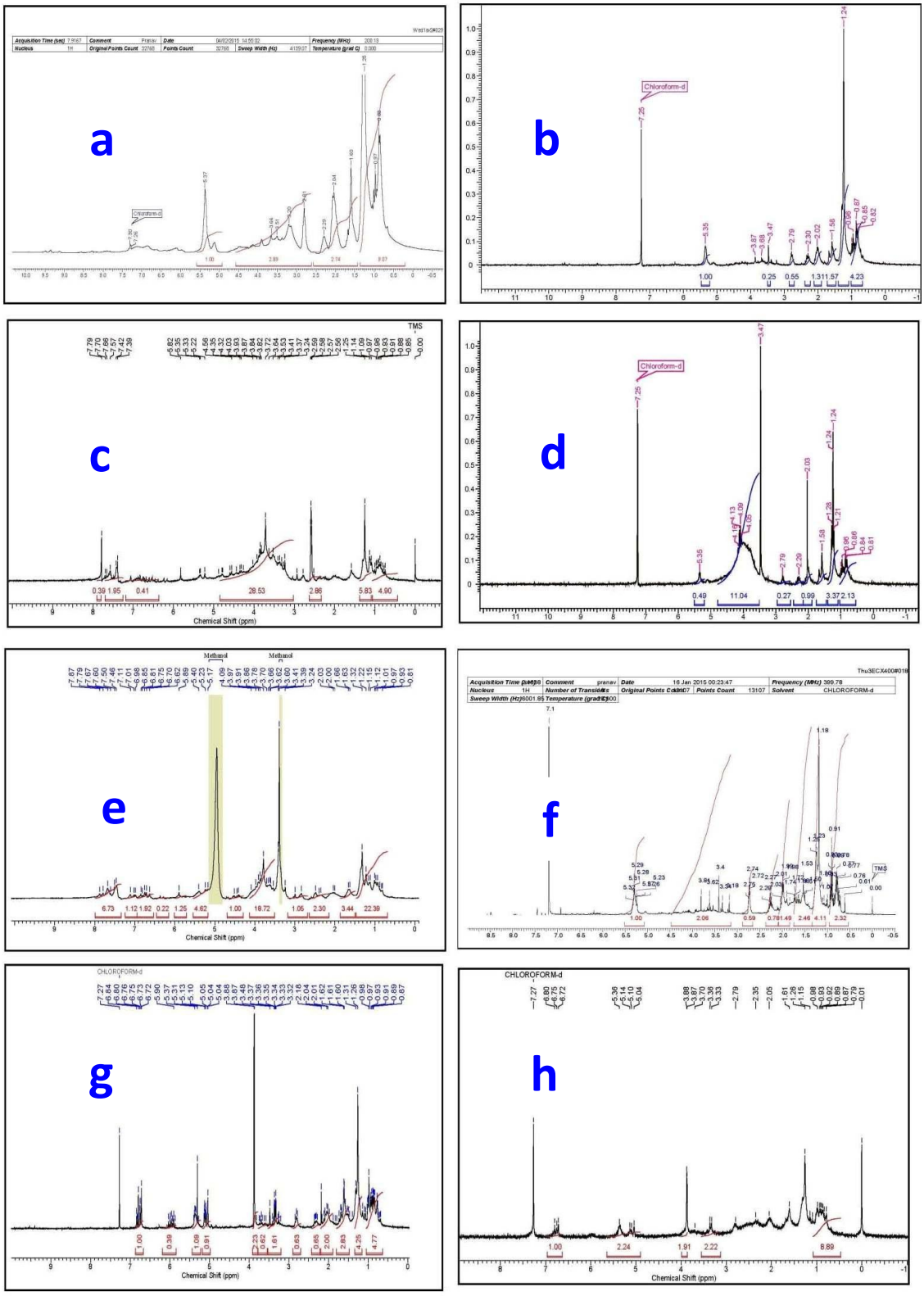


Fig. 3.2a: NMR of plant extracts- a: *A. vasica*, b: *B. monnieri*, c: *C. asiatica*, d: *C. odorata*, e: *D. erecta*, f: *M. arvensis*, g: *O. tenuiflorum*, h: *S.trilobata*

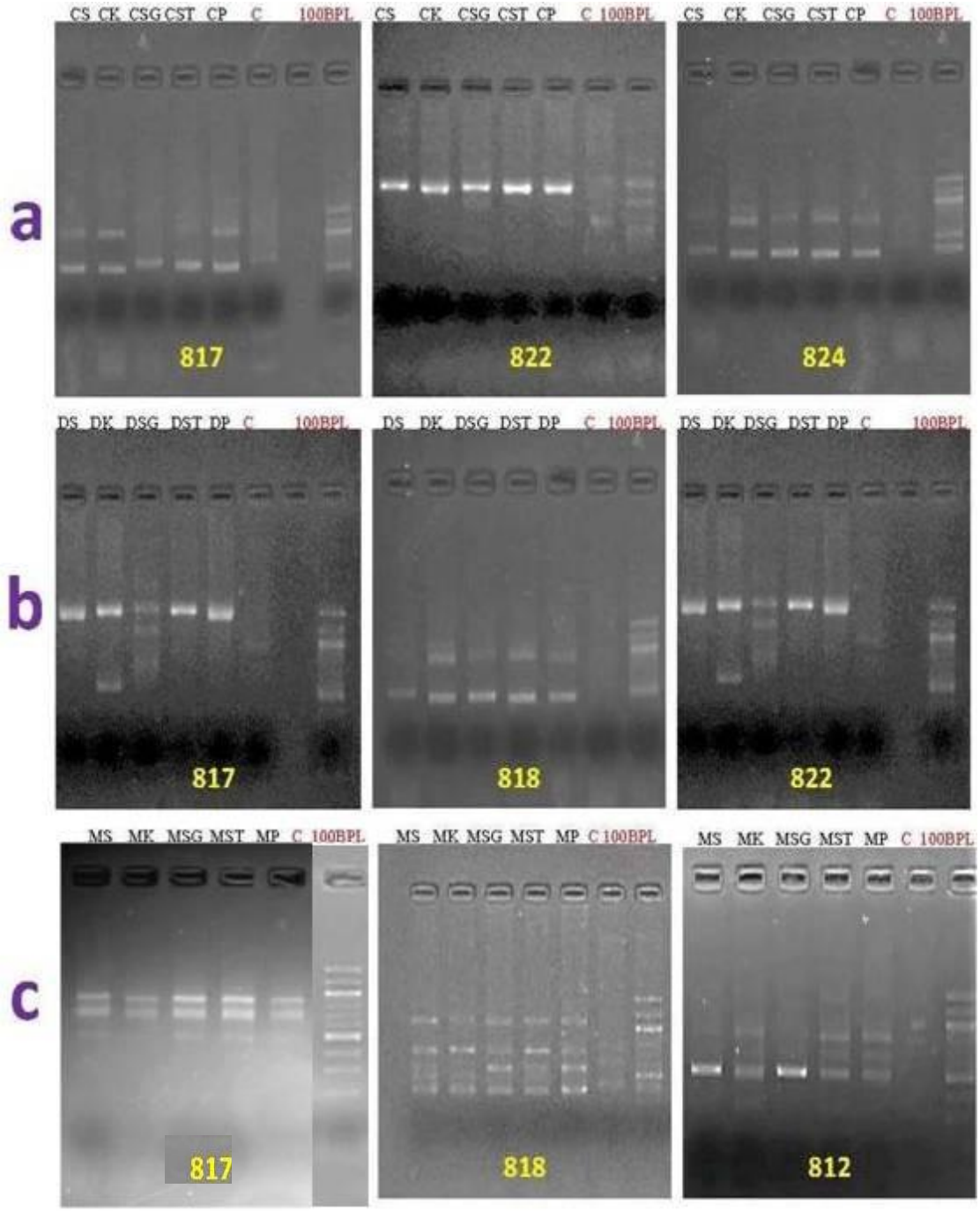


Fig. 3.2b: DNA fingerprinting of five accessions of a) *C. odorata*, b) *D. erecta* and c) *M. arvensis*. Primers used in each plant species are shown in respective banding pattern.

Table 3.3a: Qualitative determination of phytoconstituents

Bioactive Compounds	**Tests**	***Adhatoda vasica***	***Bacopa monnieri***	***Centella asiatica***	***Chromolena odorata***
Alkaloids	Mayer's	+ ve	+ ve	+ ve	+ ve
	Wagner's	+ ve	+ ve	+ ve	+ ve
	Dragendroff	+ ve	+ ve	+ ve	+ ve
Phenolic compounds	$FeCl_3$	+ ve	+ ve	+ ve	+ ve
	Lead acetate	+ ve	+ ve	+ ve	+ ve
Protein	Xanthoprotein	+ ve	+ ve	+ ve	+ ve
	Biuret	+ ve	+ ve	+ ve	+ ve
Carbohydrates	Molish Test	+ ve	+ ve	+ ve	+ ve
	Fehling's	+ ve	+ ve	+ ve	+ ve
Tannins	$FeCl_3$	+ ve	+ ve	+ ve	+ ve
	Lead acetate	+ ve	+ ve	+ ve	+ ve
Flavonoids	Ammonium	+ ve	+ ve	+ ve	+ ve
	$AlCl_3$	+ ve	+ ve	+ ve	+ ve
Terpenoids	Salkowski	+ ve	+ ve	+ ve	+ ve
Saponins	Frothing	+ ve	+ ve	+ ve	+ ve
Glycosides	Keller–Killiani		+ ve	+ ve	+ ve
Sterols	Hersche's Son's	+ ve	+ ve	+ ve	+ ve

Table 3.3b: Qualitative determination of phytoconstituents

Bioactive Compounds	**Tests**	***Duranta erecta***	***Mentha arvensis***	***Ocimum teniaflorum***	***Sphagneticola trilobata***
Alkaloid	Mayer's	+ ve	+ ve	+ ve	+ ve
	Wagner's	+ ve	+ ve	+ ve	+ ve
	Dragendroff	+ ve	+ ve	+ ve	+ ve
Phenolic	$FeCl_3$	+ ve	+ ve	+ ve	+ ve
	Lead acetate	+ ve	+ ve	+ ve	+ ve
Protein	Xanthoprotein	+ ve	+ ve	+ ve	+ ve
	Biuret	+ ve	+ ve	+ ve	+ ve
Carbohydrates	Molish Test	+ ve	+ ve	+ ve	+ ve
	Fehling's	+ ve	+ ve	+ ve	+ ve
Tannins	$FeCl_3$	+ ve	+ ve	+ ve	+ ve
	Lead acetate	+ ve	+ ve	+ ve	+ ve
Flavonoids	Ammonium	+ ve	+ ve	+ ve	+ ve
	$AlCl_3$	+ ve	+ ve	+ ve	+ ve
Terpenoids	Salkowski	+ ve	+ ve	+ ve	+ ve
Saponins	Frothing	+ ve	+ ve	+ ve	+ ve
Glycosides	Keller–Killiani	+ ve	+ ve	+ ve	+ ve
Sterols	Hersche's Son's	+ ve	+ ve	+ ve	+ ve

3.2: *In-vitro* analysis of methanolic extracts of collected plants for antioxidant activity

All the eight plants were tested for their antioxidant activity based on their DPPH free radical scavenging ability (Fig.3.3).

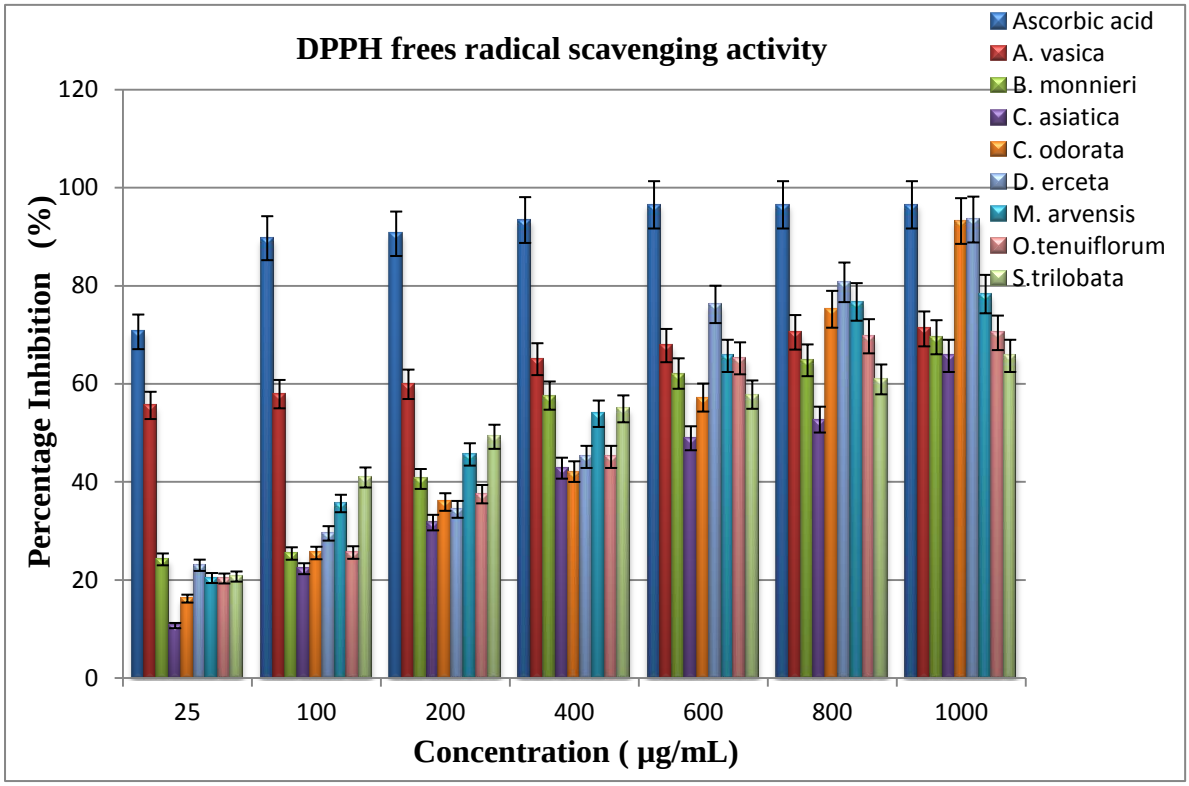


Fig. 3.3: DPPH free radical scavenging activity of *A. vasica, B. monnieri, C. asiatica, C. odorata, D. erecta, M. arvensis, O. tenuiflorum* and *S. trilobata*

DPPH free radical scavenging activity of *A. vasica*, *B. monnieri*, *C. asiatica*, *C. odorata*, *D. erecta M. arvensis*, *O. tenuiflorum* and *S. trilobata* was 71.2±1.74%, 69.5±1.69%, 65.7±1.64%, 93.2±2.03%, 93.5±2.01%, 78.3±1.93%, 70.4±1.81% and 65.6±1.83%, respectively at the concentration of 1000 µg/mL (Highest concentration). The standard compound (ascorbic acid) showed 96.5±1.98% of DPPH free radical scavenging activity at the same concentration. Two way ANOVA followed by Bonferroni's multiple comparison test between the test and standard at different concentrations suggested significant interaction at $p<0.05$. The highest antioxidant activity was shown by *D. erecta* (93.5%) and the lowest by *S. trilobata (*65.6%). Among all these eight plants, three plants namely *C. odorata*, *D. erecta* and *M. arvensis* were selected for further studies due to their high percentage of inhibition (above 75%).

3.3: *In-vitro* analysis of methanolic extracts of collected plants for anidiabetic activity

3.3.1: α-amylase inhibitory activity

Three plants namely *C. odorata*, *D. erecta* and *M. arvensis* were tested for α-amylase inhibitory activity (Fig.3.4). Percentage of α-amylase inhibition of *C. odorata*, *D. erecta* and *M. arvensis* at the highest concentration (100 mg/mL) was 45.6±1.39%, 41.2±1.51% and 59.4±1.64%, respectively. The standard compound acarbose showed 94.8±1.75% of α-amylase inhibition at the same concentration. Two way ANOVA followed by Bonferroni's multiple comparison test between the test and the standard compound acarbose at different concentrations suggested significant interaction at $p<0.05$.

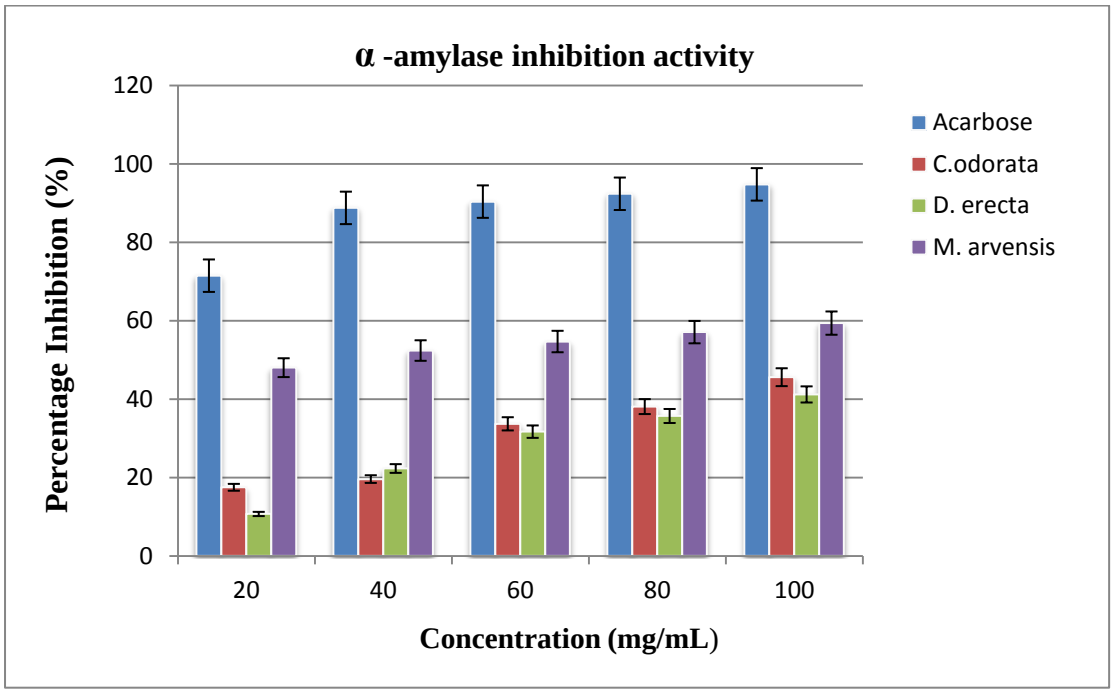


Fig. 3.4: α -amylase inhibition activity of *A. vasica*, *C. odorata* and *M. arvensis*

3.3.2: α -glucosidase inhibitory activity

α-glucosidase inhibitory potential of *C. odorata*, *D. erecta* and *M. arvensis* at the highest concentration (100 mg/mL) was 41.6±1.45%, 43.9±1.53% and 63.3±1.68% respectively (Fig.3.5). The standard compound acarbose showed 93.6±1.79% of α-amylase inhibition at the same concentration. Two way ANOVA followed by Bonferroni's multiple comparison test between the test and the standard at different concentrations suggested significant interaction at $p<0.05$.

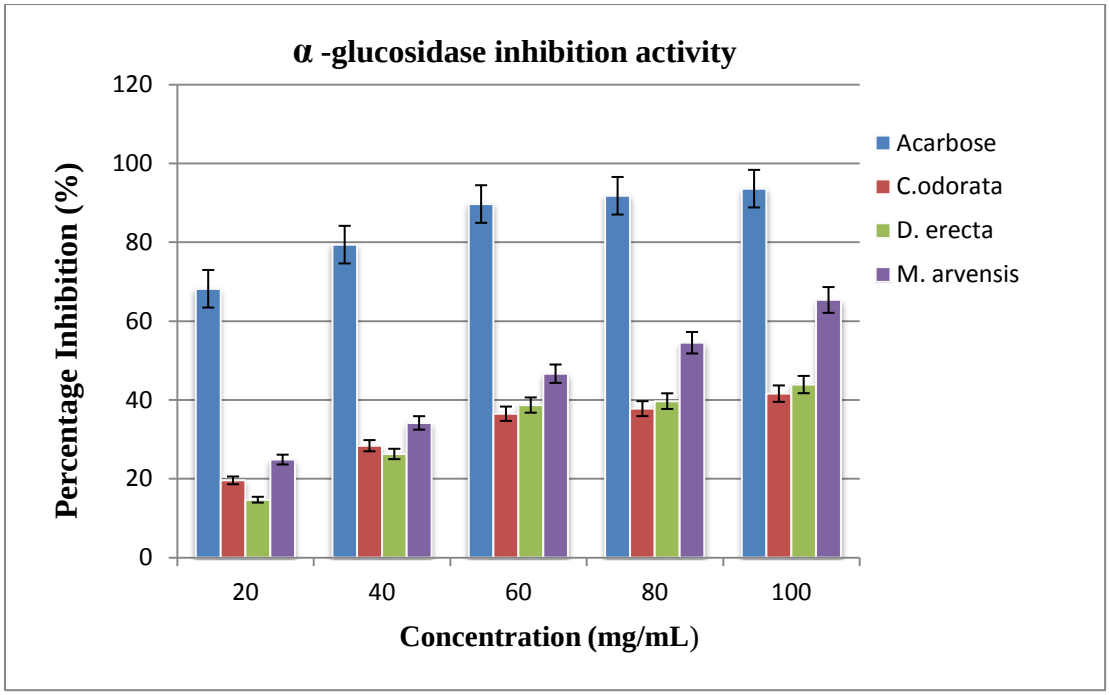


Fig. 3.5: α - glucosidase inhibition activity of *A. vasica*, *C. odorata* and *M. arvensis*.

Among all the three plants *M. arvensis* showed the best α-amylase and α-glucosidase inhibitory acivity hence was selected for further studies.

3.3.3: BSA-AGE fluorescence assay

Effect of *M. arvensis* extract on glucose mediated glycation revealed concentration dependent (2.5 to 15 mg/mL) increase in anti glycation activity. The highest concentration (15 mg/mL) showed maximum glycation inhibition of 94% (Fig.3.6a and 3.6b).

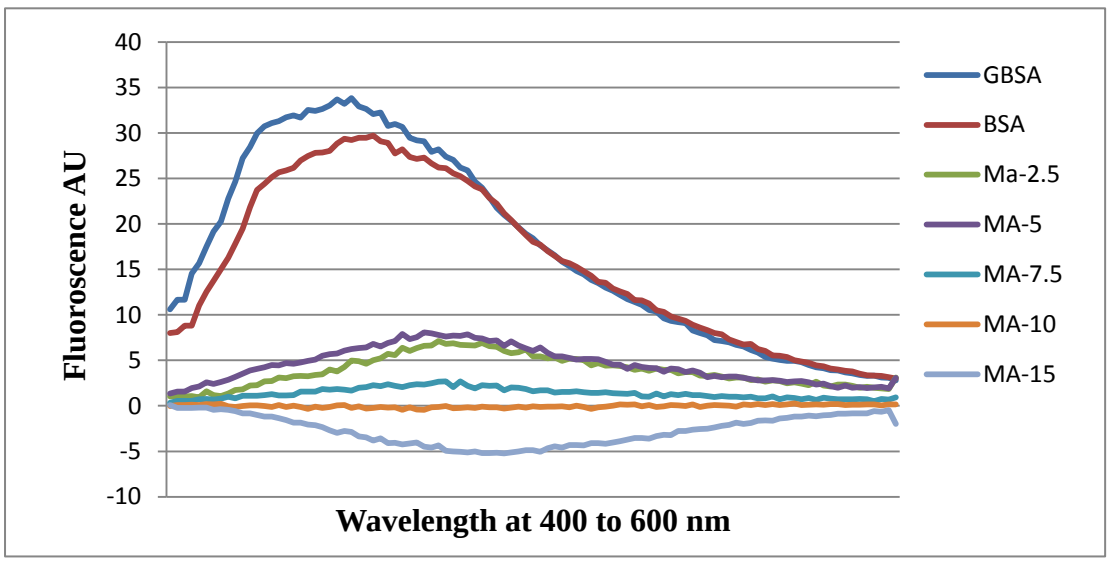


Fig.3.6a: AGE fluorescence spectra of BSA, GBSA and *M. arvensis*

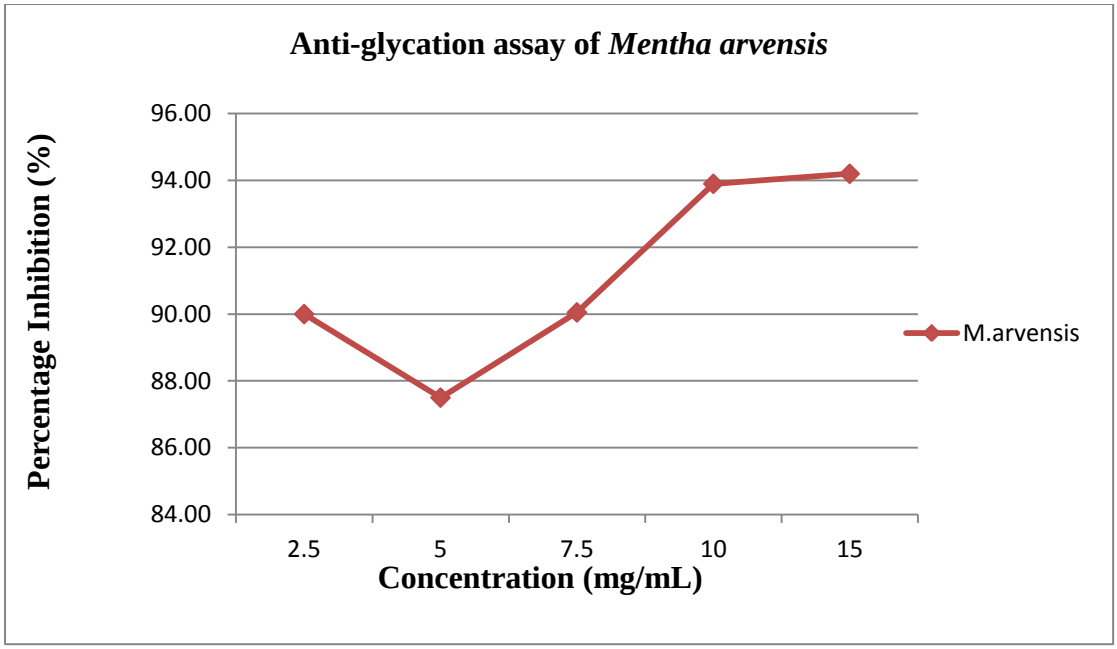


Fig.3.6b: Percentage inhibition of AGE by *M. arvensis*

Glycation inhibition potential (94%) of *M. arvensis* might be due to higher content of polyphenolics. It has been already studied that polyphenolics block the formation of AGEs and other glycated proteins. Glycation reaction involves a series of non-enzymatic reactions between the carbonyl group on reducing sugars and the amino group on proteins to form advanced glycation end product (AGE's), which are involved in the pathogenesis of diabetes mellitus and aging-related complications. In case of postprandial hyperglycemia there is high blood glucose level and due to increase in blood glucose levels, there is increase in glycation reaction, which can alter protein conformation and impair function by altering enzyme activity, altering immunogenicity, modifying protein half life and causing cross linking of structural proteins. Furthermore, due to good *in-vitro* performance of *M. arvensis*, its antidiabetic evaluation was carried out in animal model by inducing post prandial hyperglycemia in Wistar rats.

3.3.4: *In-vivo* anti-hyperglycemic activity of *Mentha arvensis* L. extract

Anti-hyperglycemic activity of methanolic extract of *M. arvensis* in rats revealed reduction of blood glucose levels at different time point's 0, 30, 60, 90 and 120 minutes (Fig. 3.7). Two ways ANOVA analysis followed by Tukey's multiple comparison tests was applied to find difference between the groups of animals at $p < 0.05$ when compared to the control. *M. arvensis* significantly reduced the blood

glucose levels when compared to the diabetic control and had equally potent antidiabetic potential when compared with acarbose.

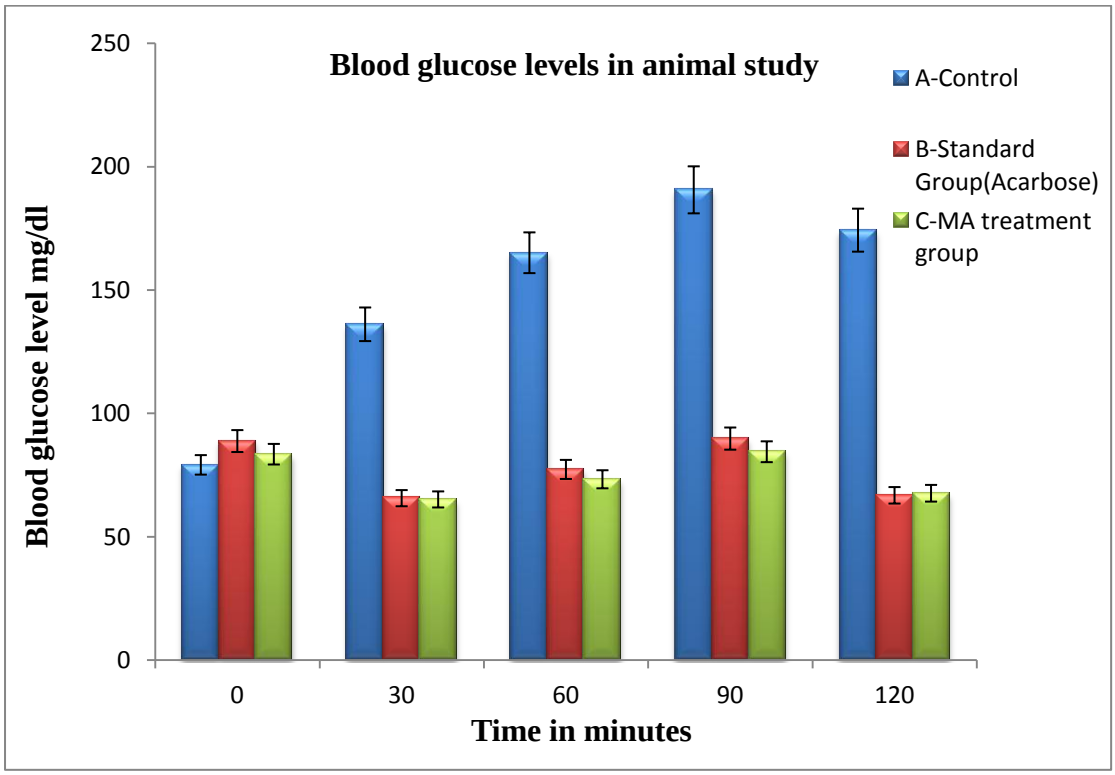


Fig. 3.7: Anti-hyperglycemic activity of *Mentha arvensis* L. extract in rats.

3.4: Analysis of methanolic extracts of collected plants for antiurolithiasis activity

3.4.1: Evaluation of calcium oxalate crystals inhibition activity by use of nucleation assay

Calcium oxalate crystals inhibition by *A. vasica, B. monnieri, C. asiatica, C. odorata, D. erecta M. arvensis, O. tenuiflorum* and *S. trilobata* was 37.8±1.46%, 30.7±1.49%, 35.1±1.45%, 33.5±1.41%, 51.9±1.69%, 38.6±1.62%, 31.5±1.43% and 32.5±1.42%, respectively at the concentration of 100 mg/mL (Fig. 3.8). The standard compound (Cystone) showed 93.5±1.96% calcium oxalate crystals inhibition at the same concentration.

3.4.2: Evaluation of calcium oxalate monohydrate crystals inhibition activity by use of synthetic urine assay

Calcium oxalate monohydrate crystals inhibition activity of *A. vasica, B. monnieri, C. asiatica, C. odorata, D. erecta M. arvensis, O. tenuiflorum* and *S. trilobata* was

36.8±1.47%, 31.5±1.49%, 46.9±1.51%, 43.9±1.53%, 51.9±1.68%, 48.9±1.61%, 36.7±1.48% and 49.7±1.47% respectively at the concentration of 100 mg/mL (Fig. 3.9). The standard compound (Cystone) showed 94.7±1.98% calcium oxalate monohydrate crystals inhibition at the same concentration. Among all the eight plants, *D. erecta* showed the highest calcium oxalate and calcium oxalate monohydrate crystals inhibition (above 50 %) hence selected for further *in-vivo* animal study.

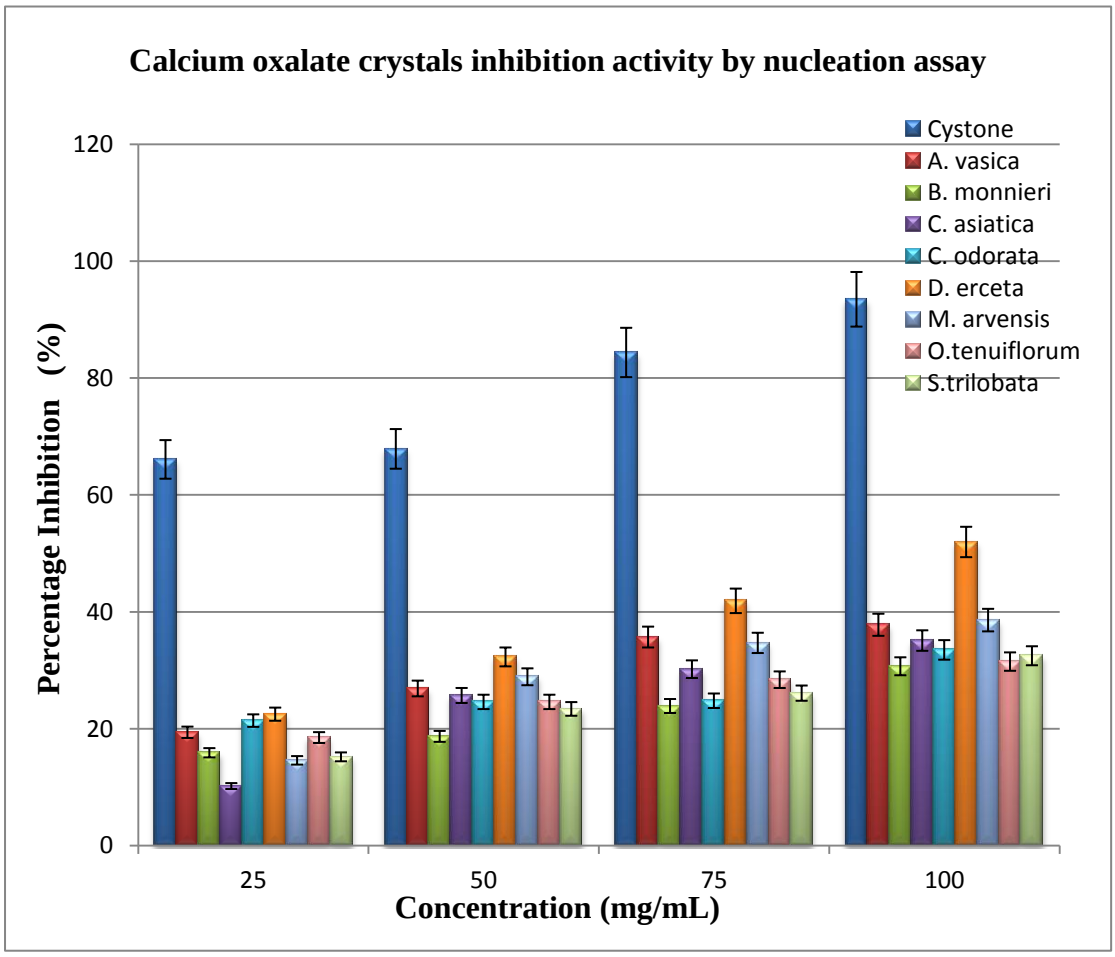


Fig. 3.8: Nucleation assay for evaluation of calcium oxalate crystals inhibition by *A. vasica, B. monnieri, C. asiatica, C. odorata, D. erecta M. arvensis, O. tenuiflorum* and *S. trilobata.*

3.4.3: *In-vivo* antiurolithiasis activity of *Duranta erecta* extract

In-vivo experiment of *D. erecta* for antiurolithiatic activity was performed in wistar rat model. During animal study, serum parameters were obsereved in all the animals.

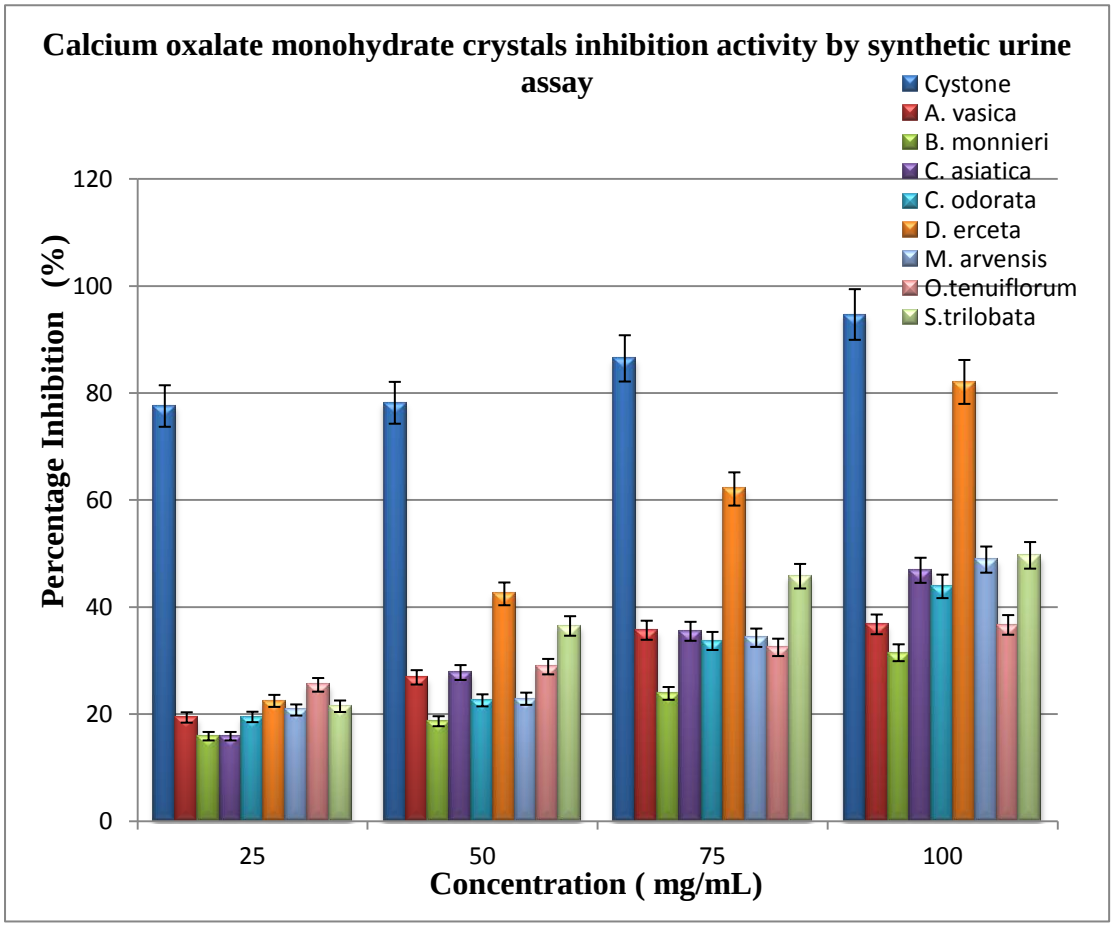


Fig. 3.9: Synthetic urine assay for evaluation of calcium oxalate monohydrate crystals inhibition by *A. vasica, B. monnieri, C. asiatica, C. odorata, D. erecta M. arvensis, O. tenuiflorum* and *S. trilobata.*

3.4.3.1: Serum anyalysis: The values of serum parameters expressed as mean S.E.M., (n=6). Data were analysed by one-way ANOVA followed by Dunnett's test and P<0.05, as compared with the vehicle group. Serum creatinine level was eleveted to 2.49±0.14 mg/dL in group B (Urolithiasis disease control animals), but the value significantly reduced to 1.73±0.22 mg/dL and 1.33±0.14 mg/dL in group C (animals treated with standard drug Cystone 50 mg/kg) and group D (animals treated with *D. erecta* extract 50 mg/kg), respectively (Fig.3.10a). Elevated BUN levels were detected as 29.15±1.83 in group B, however the values were significantly reduced to 20.4±1.59 mg/dL and 19.3±2.89 mg/dL in group C and group D, respectively (Fig.3.10b). Elevated SGOT levels were detected as 41.26±1.30 unit/L in group B while group C and D were in normal range i.e. 17.75±1.97 and 20.4 ±1.59, respectively (Fig.3.10c). The values of SGPT in group B, C and D were 39.3±0.9, 17.93±2.23 and 21.81 ±1.88,

respectively (Fig.3.10d). *In-vivo* evaluation of group B revealed an elevated levels of serum creatinine and BUN which indicated a marked renal damage; however in group A (vehicle group), C (Cystone treated) and D (*D. erecta* treated) serum creatinine and BUN were in normal range.

3.4.3.2: Urine analysis

Urine color was detected pale yellow in all the animals of all the groups. Transparency of urine in Group B animals (Disease Control) was found opaque while the transparency of Group A, C and D treated was clear (Fig.3.11).The pH of urine of group A, B, C and D was 6.8, 6.13, 6.3 and 6.68, respectively. The urine oxalate was observed to be 1.68, 2.05, 0.5 and 0.8 for groups A, B, C and D, respectively. Group A didn't show any granular debris nor crystals of calcium oxalate. The group B urine samples depicted higher amount of granular debris and calcium oxalate crystals. The crystals varied from large to medium size compared to Cystone and *D. erecta* treated group.

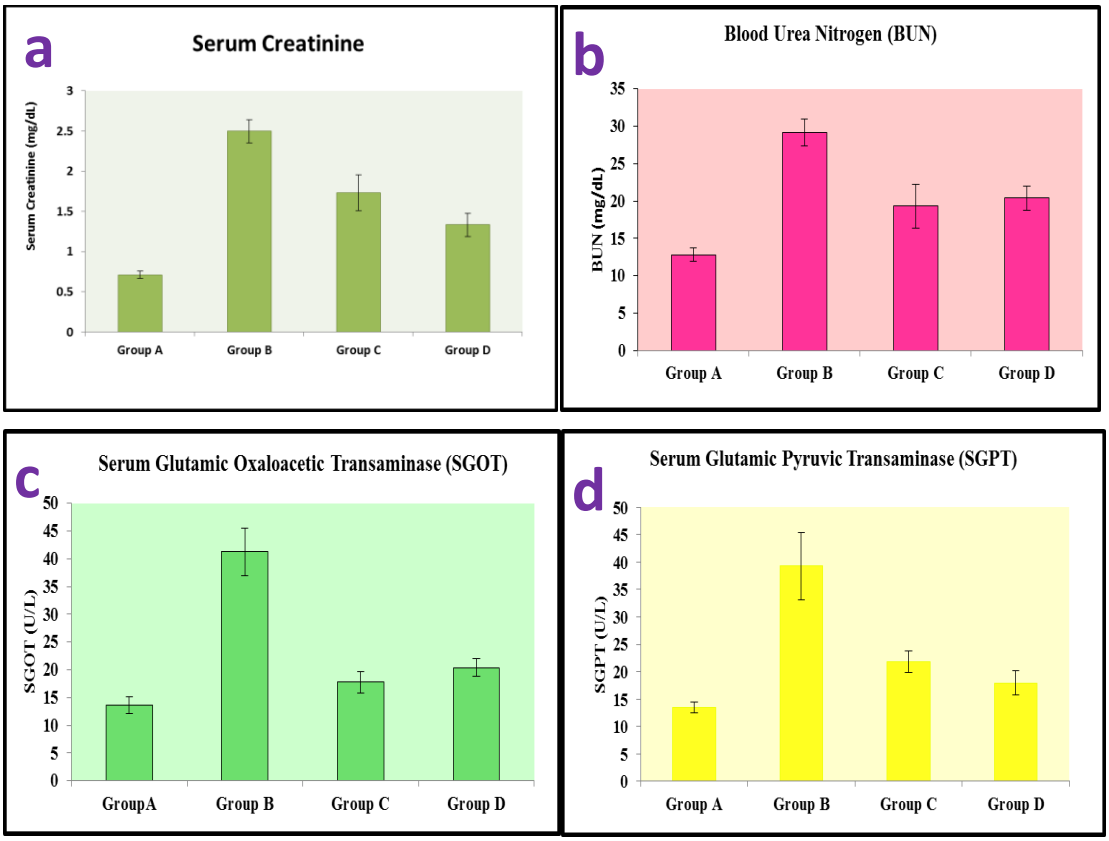


Fig.3.10: a) Serum Creatinine b) BUN c) SGOT and d) SGPT

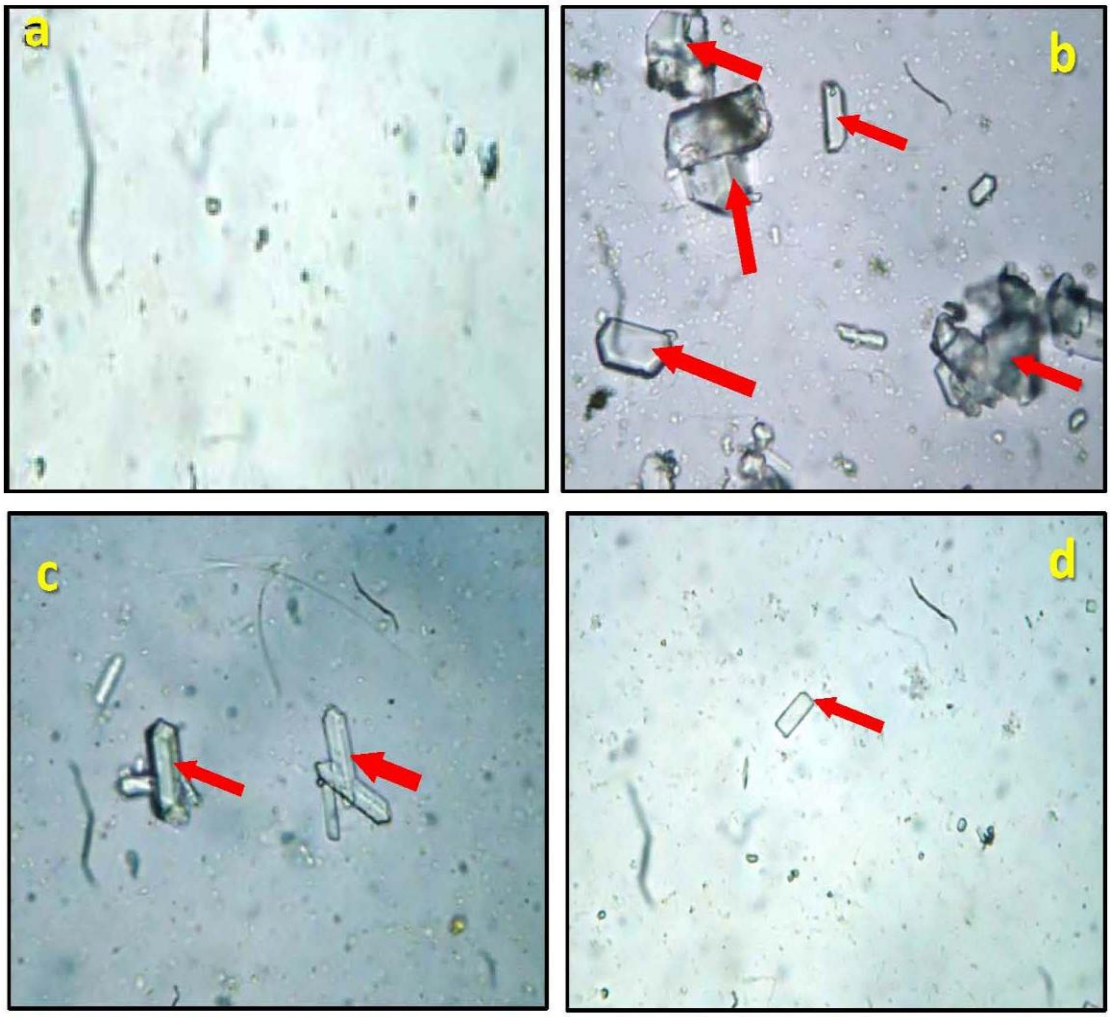


Fig. 3.11: Microscopic examination of urine of groups a, b, c and d animals. Arrows show presence of urinary crystals.

3.4.3.3: Histopathology

H&E staining of kidney tissues (Fig.3.12) from rats of vehicle group showed normal histology with regular size tubules with single epithelial lining along the margin and did not reveal any lesion of pathological significance. The kidney sections derived from Group B when observed microscopically showed polymorphic irregular crystals deposited in the tubules which led to dilation of the proximal tubules and also interstitial inflammation that might be due to calium oxalate crystals. Group B animals also showed degeneration of epithelial lining, focal mild degeneration of tubules of medulla, focal mild tubular atrophy of cortex and multifocal minimal intertubular lymphocytic infiltration. Treatment with the Cystone and *D. erecta* reduced the number and size of calcium oxalate crystals deposited in different parts of the renal tubules and also saved the tubules and calyxes from damaging through an

antioxidant and nephroprotective property of *D. erecta* extract. Cystone treated group C rats did not reveal any lesion of pathological significance suggesting attenuation of lesions, showing normal histology. *D. erecta* treated group D showed lesions of less severity with less distribution suggesting decrease of adverse effect caused due to calcium oxalate.

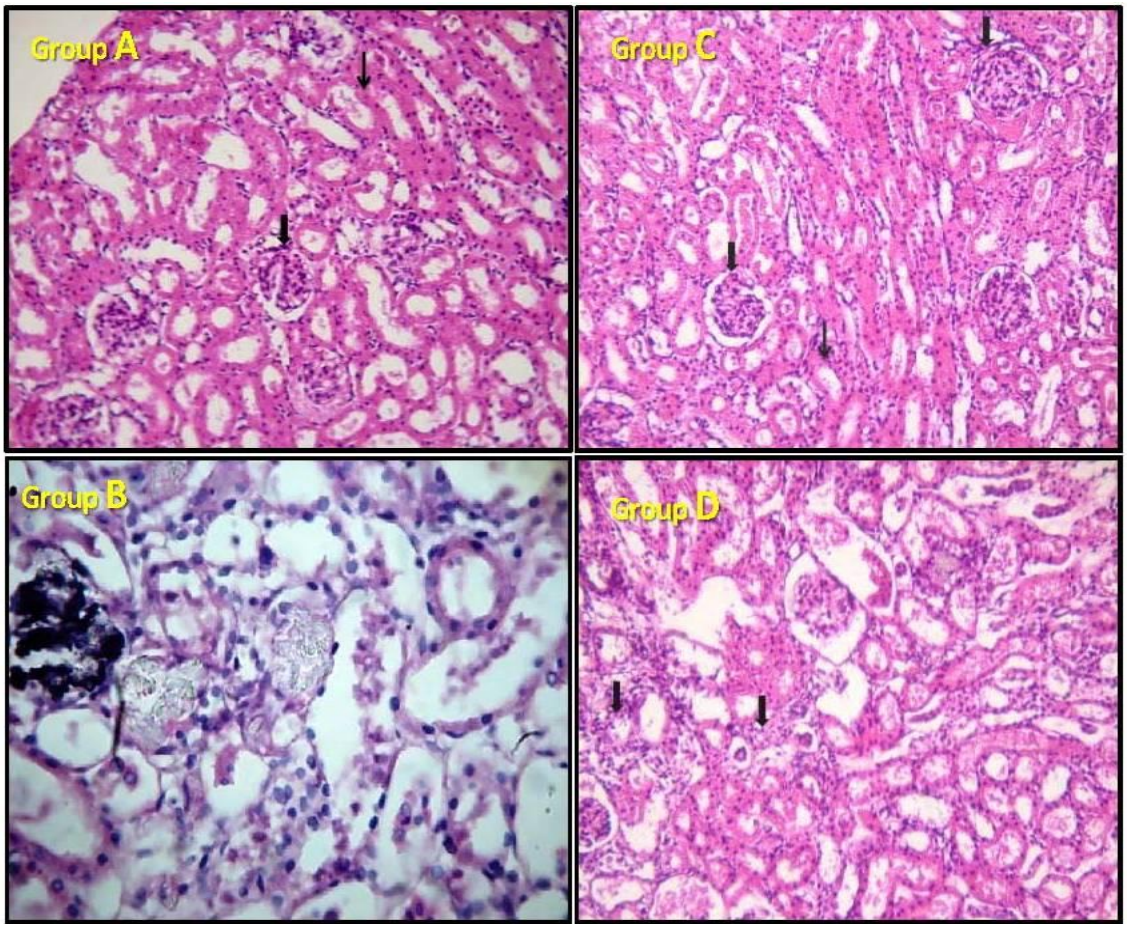


Fig.3.12: Histopathological examination of kidney tissues.

3.4.4: Antimicrobial activity of *Duranta erecta* extract

The antimicrobial activity of methanolic extract of *D. erecta* was tested against both Gram positive and Gram negative bacteria. The highest zone of inhibition was recorded with *Escherichia coli* (25 mm) and *Pseudomonas desmolyticum* (17 mm). However, zone of inhibition of Gram positive bacteria; *Bacillus subtilis* and *Staphylococcus aureus* was recorded to be 17 mm and 16 mm, respectively as compared with the standard antibiotic streptomycin (Fig. 3.13).

Gram negative (*E.coli*)	Gram negative (*P. desmolyticum*)	Gram positive (*B.subtilis*)	Gram positive (*S.aureus*)

Fig.3.13: Antimicrobial activity *D. erecta* against gram-positive and gram negative bacteria

3.5: Analysis of methanolic extracts of collected plants for anti breast cancer activity

3.5.1: Cytotoxicity and MTT assay

In-vitro analysis of *A. vasica, C. odorata, D. erecta* and *M. arvensis* at different concentrations was performed by use of cell line assays. It involved MTT assay, which was the cell viability assay that gave an idea about how effectively the concerned extracts killed the cancer cells. Breast cancer cells of both mice (4T1) and human (MCF-7) were used to test anti breast cancer activity of the plant extracts. The technique involved incubation of known number of cancer cells in the 96 well plates with plant extracts. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) was reduced by dehydrogenase enzyme present in metabollicaly active cells resulting in purple formazan crystals that were solubilized by isopropanol and quantified speactrophotometrically. The cytotoxicity of *A. vasica D. erecta* and *M. arvensis* against MCF-7 human breast cancer cell lines at various concentrations indicated that inhibition of MCF-7 by these three plants was less than 45% (Fig. 3.14a). It suggested that they have no anticancer activity against MCF-7 human breast cancer cell lines. The inhibition of 4T1 mouse mammary tumor by *A. vasica D. erecta* and *M. arvensis* was less than 45, 45 and 58 %, respectively. It showed that *A. vasica* and *D. erecta* have no anticancer activity and *M. arvensis,* has very less anticancer activity against 4T1 mouse mammary tumor cell lines (Fig. 3.14b). The MTT assay of Crude extract of *C. odorata* showed 82.67% and 86.49% of growth inhibition on MCF-7 and 4T1 cell lines respectively (Fig. 3.15). The cytotoxicity of crude extract of *C. odorata* on HUVEC normal cell lines was less than 35%. It was non-toxic for HUVEC normal cell lines (Fig. 3.15). The IC50 values of Crude extract of *C. odorata* for MCF-7 and 4T1 cell lines were 15.63 and 15.95 µg/mL respectively.

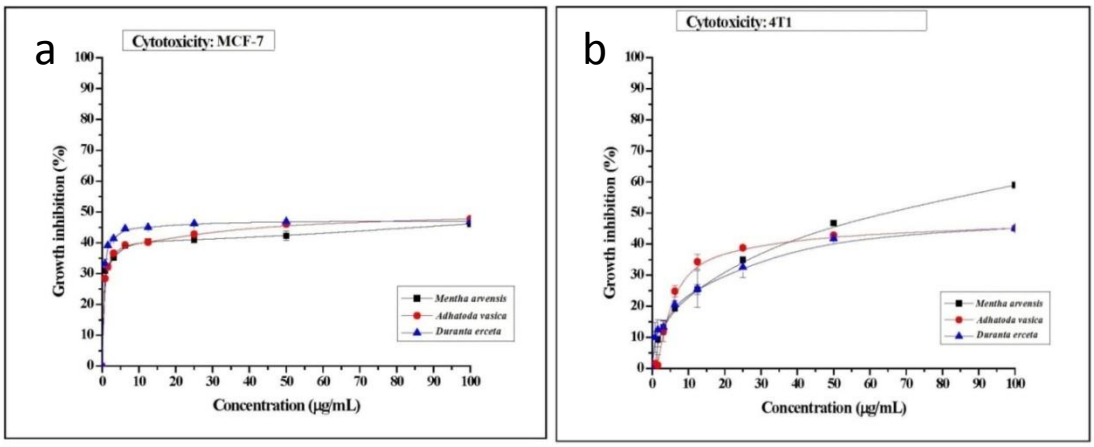


Fig.3.14 a&b: a) Cytotoxicity of *A. vasica D. erecta* and *M. arvensis* against MCF-7 human breast cancer cell line at various concentrations. b) Cytotoxicity *A. vasica D. erecta* and *M. arvensis* against 4T1 mouse mammary tumor cell line at various concentrations.

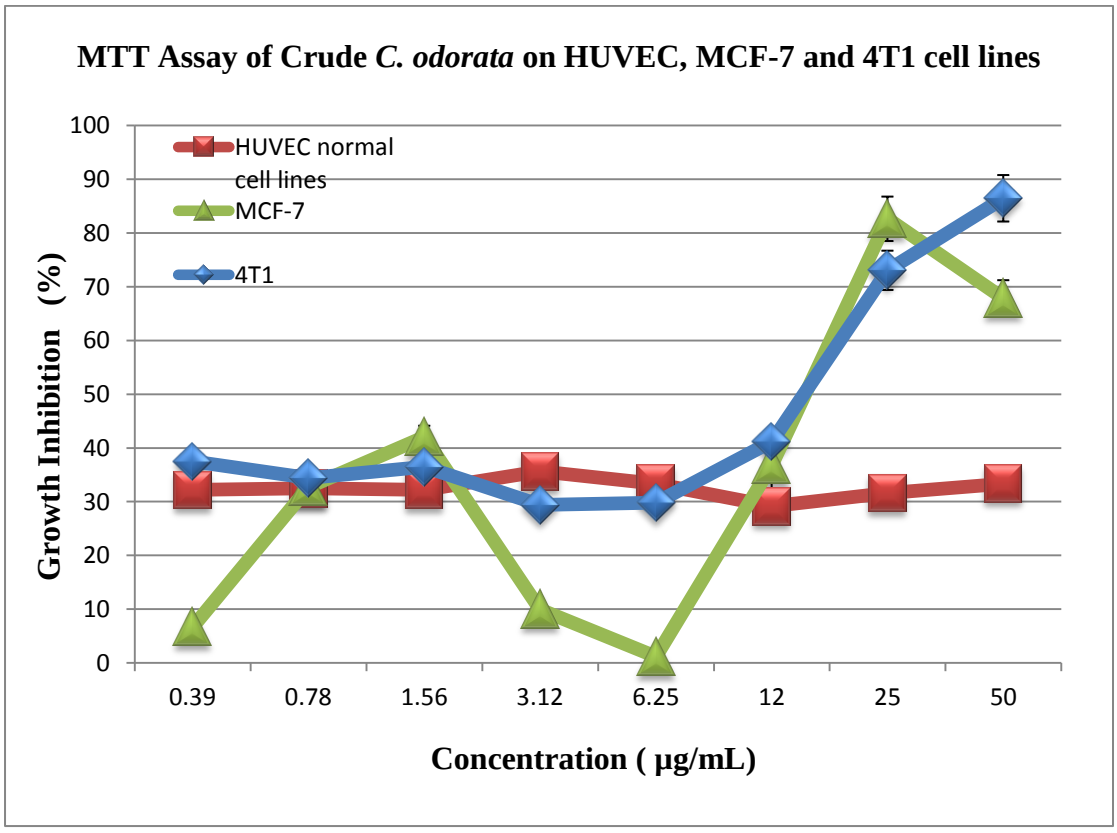


Fig.3.15: MTT assay of crude extract of *C. odorata* on HUVEC, MCF-7 and 4T1 cell lines

3.5.2: Apoptosis test of crude extract of *Chromolena odorata*

The crude extract of *C. odorata* was screened for *in-vitro* apoptosis test. Test for apoptosis was done by treating the cells that has been dozed with the extract with Annexin dye. Annexin V protein has high affinity for phosphatidylserine (PS). During apoptosis, PS was translocated from the inner to the outer leaflet of the plasma

membrane, where it was available for annexin V binding. A fluorescent conjugate of Annexin V was used to detect apoptotic cells by fluorescence microscopy. Apoptotic cells were characterized by DNA fragmentation and consequently loss of nuclear DNA content. Use of a flurochrome such as PI that binds but cannot passively traverse into cells that posses an intact plasma membrane. PI uptake versus exclusion was used to discriminate dead cells, in which plasma membrane became permeable. Both the dyes were compatible to flow Cytometry. Since IC50 was known for the crude extract, that concentration was dozed to MCF7 and both the dyes were used to infer if there was apoptosis and was successfully observed (Fig. 3.16). Thus, methanolic extract of *C.odorata* induced apoptosis in MCF7 cells i.e. breast cancer cells and proved to be anti cancer in nature. This prompted us to carry out fractionation of crude extract of *C. odorata.*

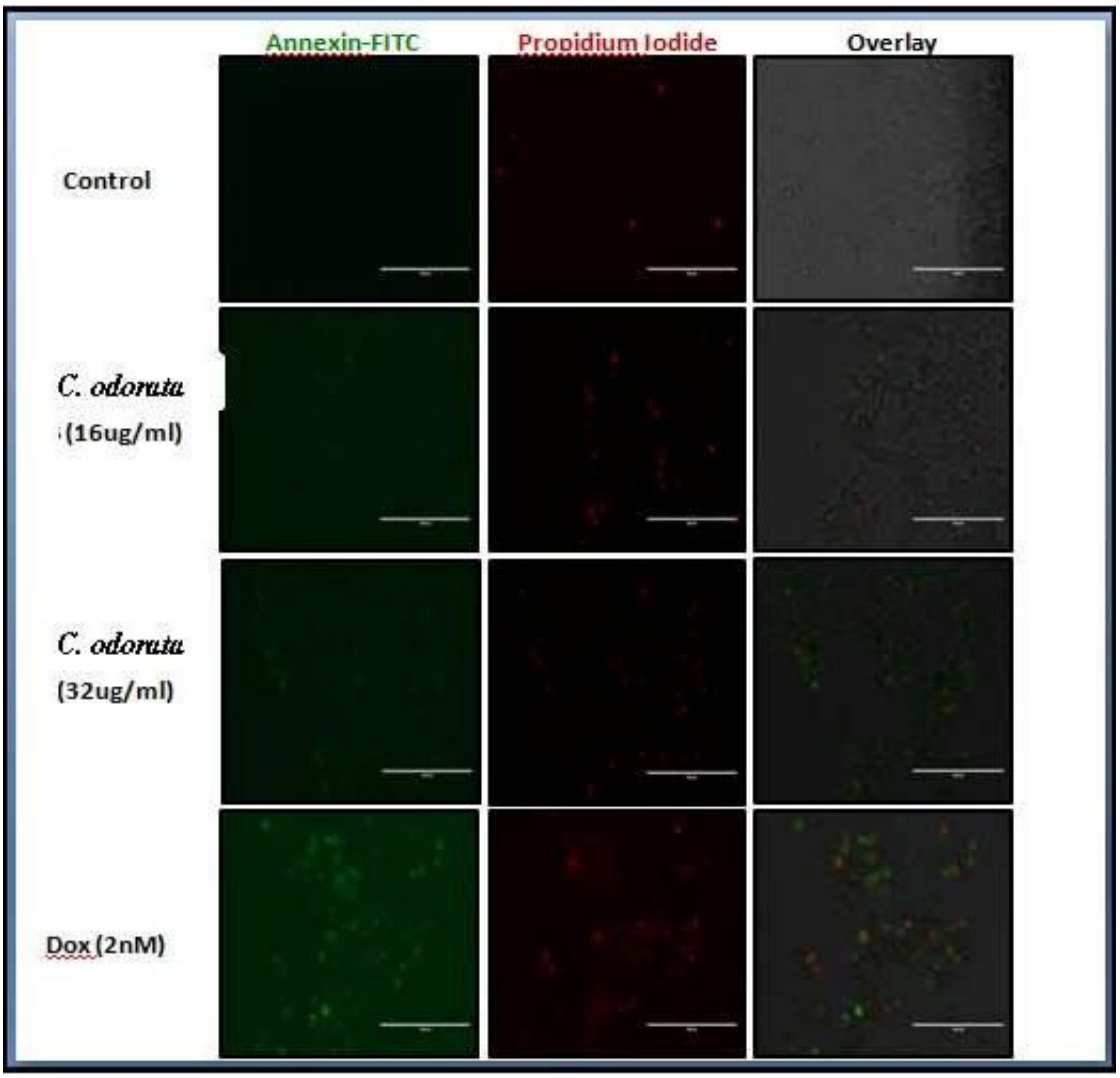


Fig.3.16. Apoptosis of MCF7 cells by crude extract of *C.odorata*

3.5.3: Column Chromatography, Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

The fractionation of crude extract of *C. odorata* was performed by using column chromatography method. Six fractions were obtained from crude extract of *C. odorata*. The characterization of these six fractions and crude extract of *C. odorata* was carried out by gas-chromatography-mass spectrometry (GC-MS) method (Fig.3.17a and 3.17b). The GC-MS analysis of *C. odorata* crude extract and its fractions revealed the presence of several compounds of biological significance like hexadecanoic acid, α-amyrin, lupeol (triterpenoids) etc. (Table 3.4). These fractions were in turn mixtures of many other compounds, So these fractions were as such used for further investigation of their anti cancer activities.

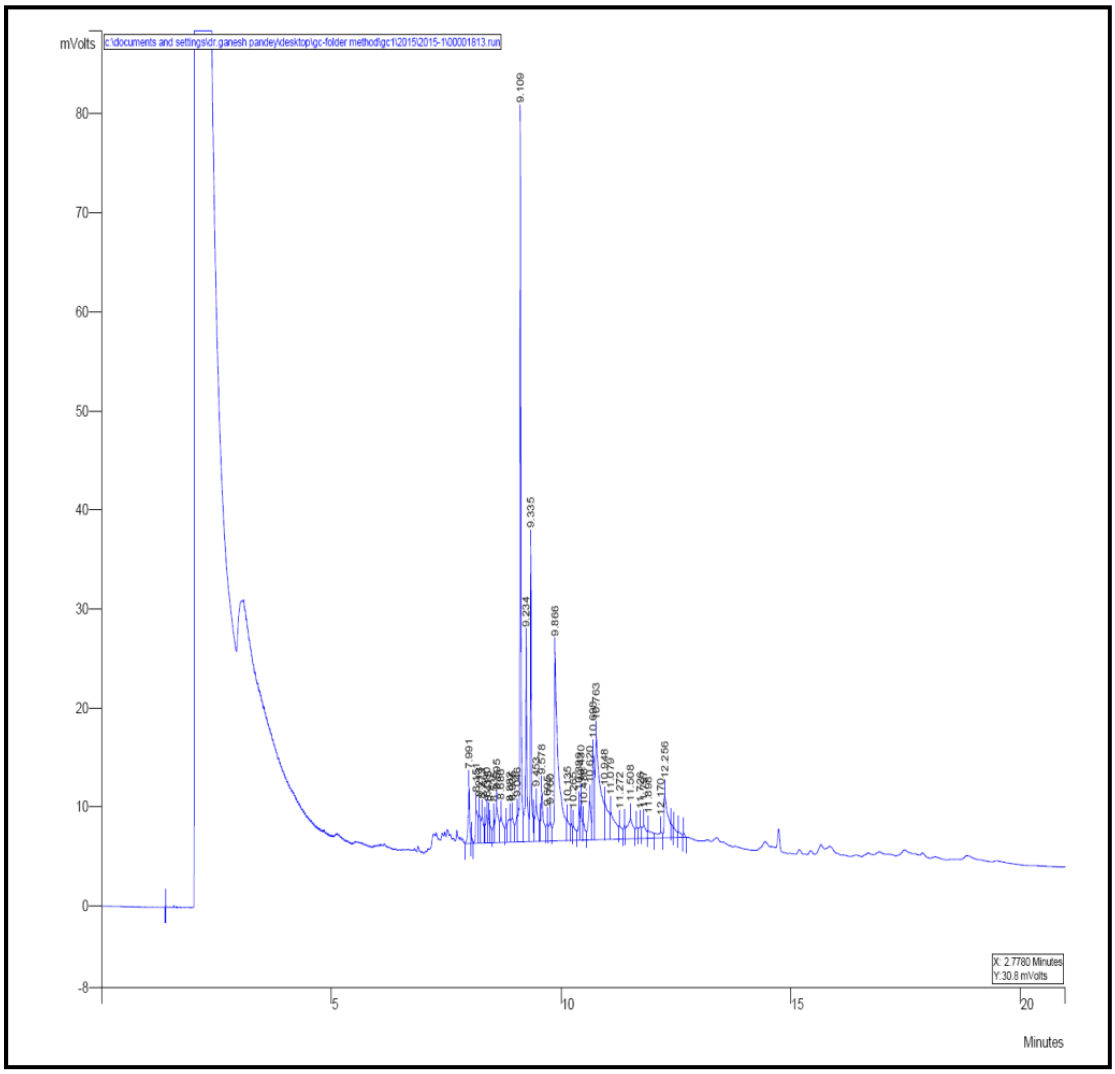


Fig.3.17a. GC-MS analysis of crude extract of *C. odorata*

Table 3.4: Chemical compounds present in derived fractions

Fraction Number	Compound Names	Molecular Formula	Molecular Mass
1	Androstan-17-one, 3-(formyloxy)-11-hydroxy-, (3α,5α,11β)	$C_{20}H_{30}O_4$	334.21
	Androst-11-en-17-one, 3-formyloxy-, (3α,5α)-	$C_{20}H_{28}O_3$	316.20
	γ-Sitosterol	$C_{29}H_{50}O$	414.38
	β-Sitosterol	$C_{29}H_{50}O$	414.38
	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.24
2	Glafenine	$C_{19}H_{17}C_lN_2O_4$	372
	Phenol, 2,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206
	Phytol	$C_{20}H_{40}$	296.53
	Stigmasterol	$C_{29}H_{48}O$	412
3	9,12-octadecadienoic acid(Z,Z)	$C_{18}H_{32}O2$	280
	Caryophyllene oxide	$C_{15}H_{24}O$	270
	Lupeol or α-Amyrin	$C_{30}H_{50}O$	426
	Acacetin (flavone)	$C_{16}H_{12}O_5$	284.26
	Isosakurnaetni	$C_{16}H_{14}O_5$	286.27
4	Tetradecane	$C_{14}H_{30}$	198.39
	Hexadecane	$C_{16}H_{34}$	226.44
	γ-Selinene	$C_{15}H_{24}$	204.35
	Heptadecane, 3-methyl-	$C_{18}H_{38}$	254.49
5	1-eicosanol	$C_{20}H_{42}O$	298.55
	Calmenene	$C_{15}H_{22}$	202.33
	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256.24
	9-octadecanone	$C_{18}H_{36}O$	268.47
6	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	268.47
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.531
	tert-Hexadecanethiol	$C_{16}H_{34}S$	258.511
	Octadecanone	$C_{18}H_{38}$	254.49

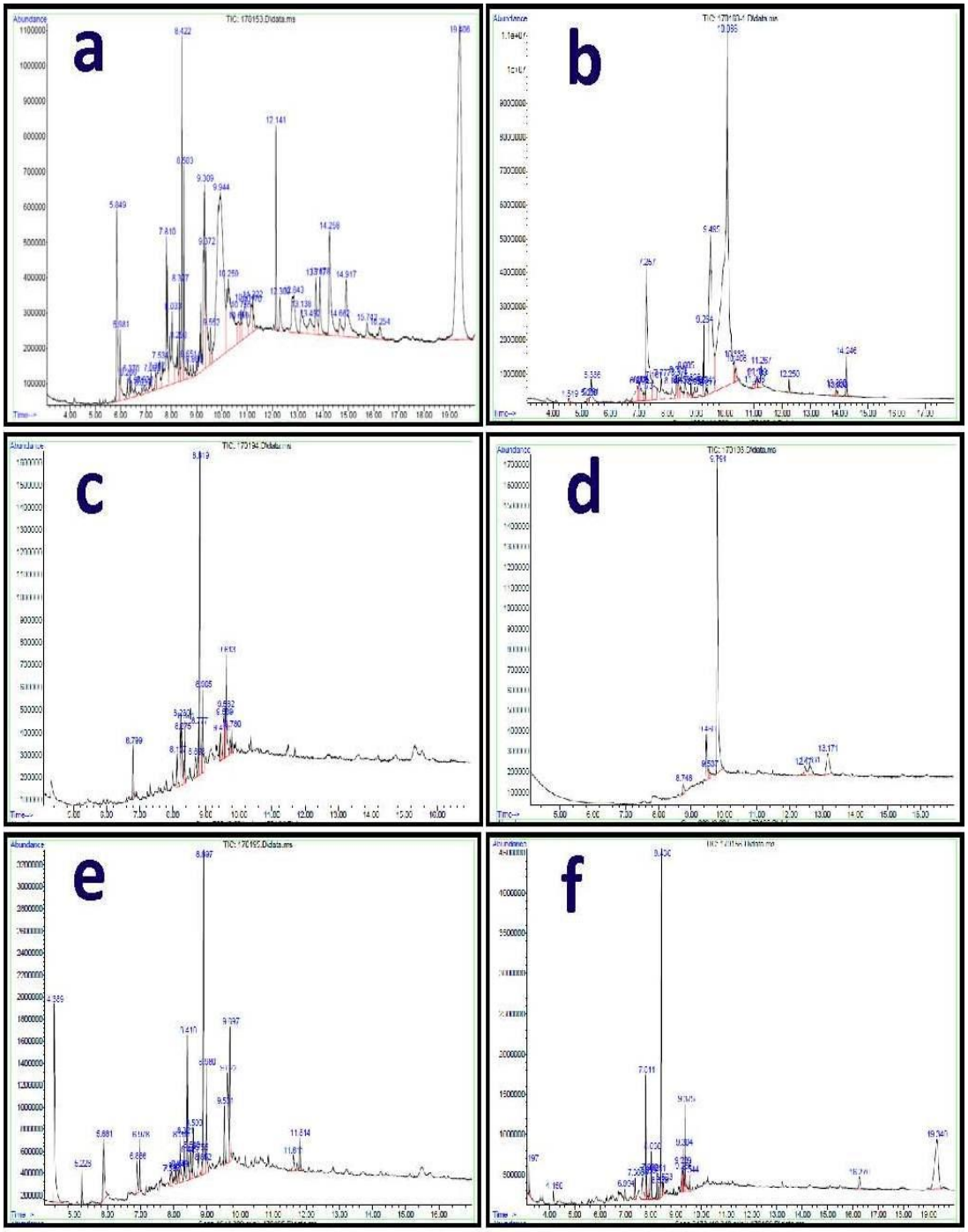


Fig.3.17b. GC-MS analysis of a) fraction-1, b) fraction-2, c) fraction-3, d) fraction-4, e) fraction-5 and f) fraction-6

3.5.4: LC-MS analysis of crude extract of *C. odorata* and its fractions

The chromatograms (Fig.3.18a and Fig.3.18b) showed peaks of chemical compounds that have been indified by the mass analyser after being separated by liquid chromatography technique. By the help of xcaliber software, compounds that are known to be present were analyzed. A few well known flavanoids, chalcones and

other metabolites were confirmed to be present in the *C. odorata* crude extract and its fractions viz. 2'-hydroxy-4, 4',5',6'-tetramethoxy chalcone, 5, 7- dihydroxy- 4'-methoxy flavanone/ acacetin and 5, 7, 3', 4'-tetrahydroxy flavanone/Eriodictyol.

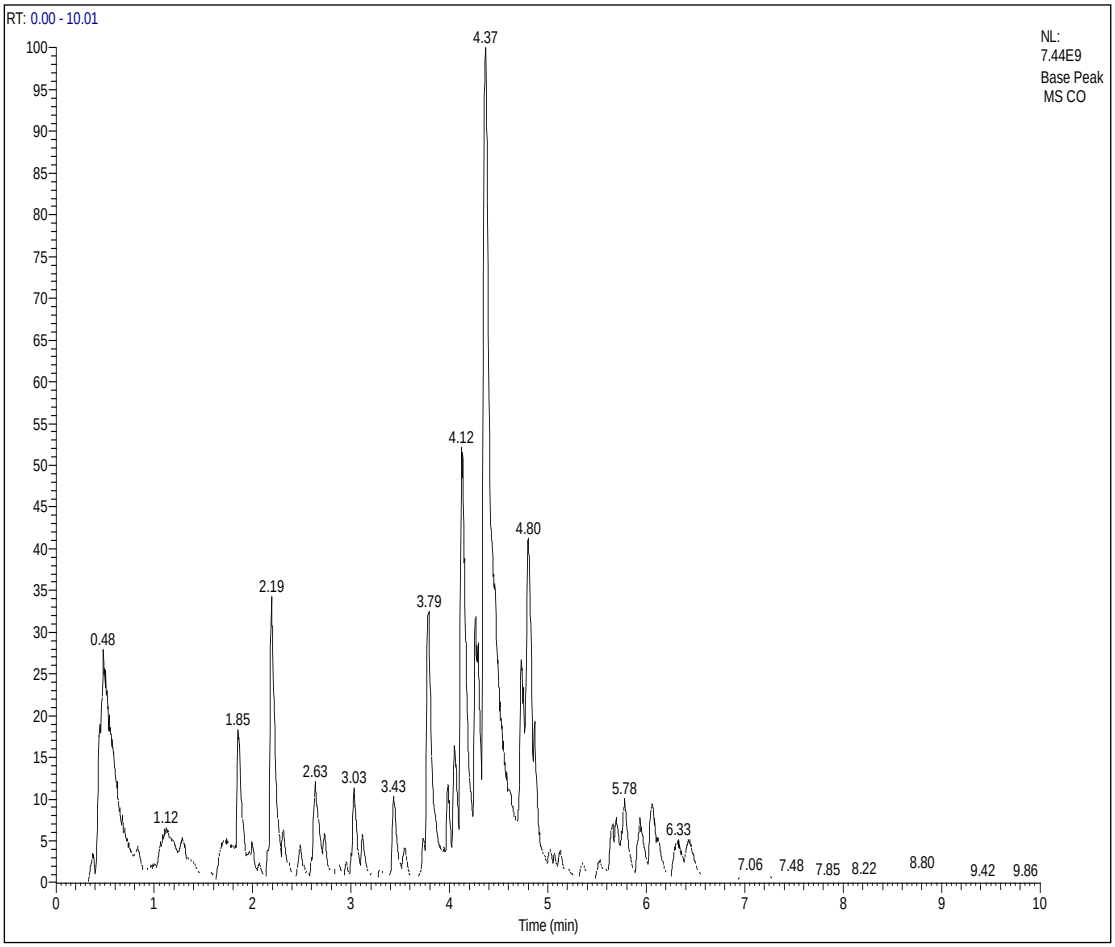


Fig.3.18a. LC-MS analysis of crude extract of *C. odorata*

3.5.5: Anticancer activity of fractions of *C. odorata*

The MTT assay of all the six fractions of *C. odorata* was performed against MCF-7 and 4T1 cell lines to confirm specific fractions indicating anti breast cancer activity. However the MTT assay of all the GC-MS purified fractions of *C. odorata* showed less than 45% of growth inhibition on MCF-7 and 4T1 cell lines. None of the fractions had activity against breast cancer cell lines (Fig.3.19a and 3.19b).

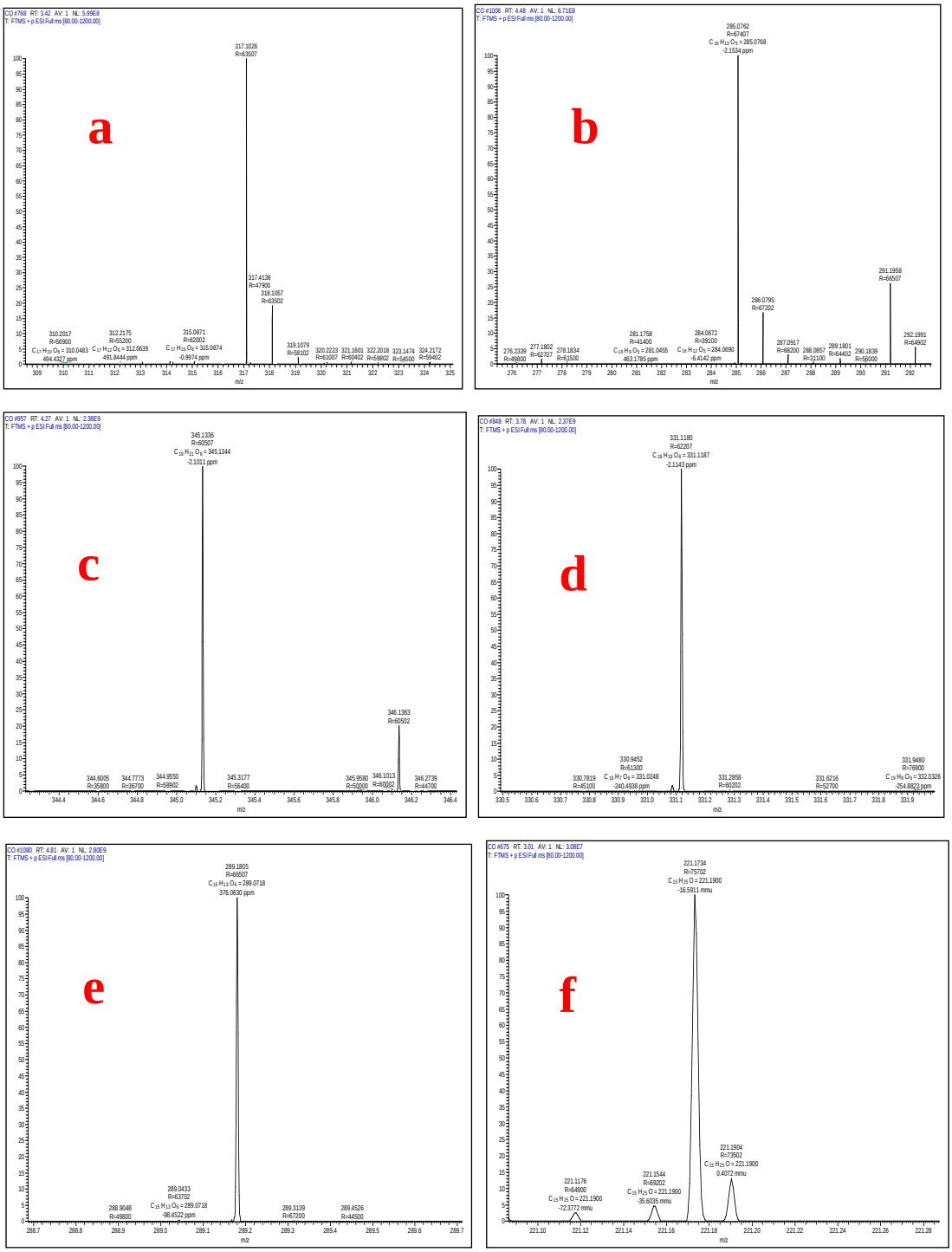


Fig.3.18b. LC-MS analysis of a) fraction-1, b) fraction-2, c) fraction-3, d) fraction-4, e) fraction-5 and f) fraction-6

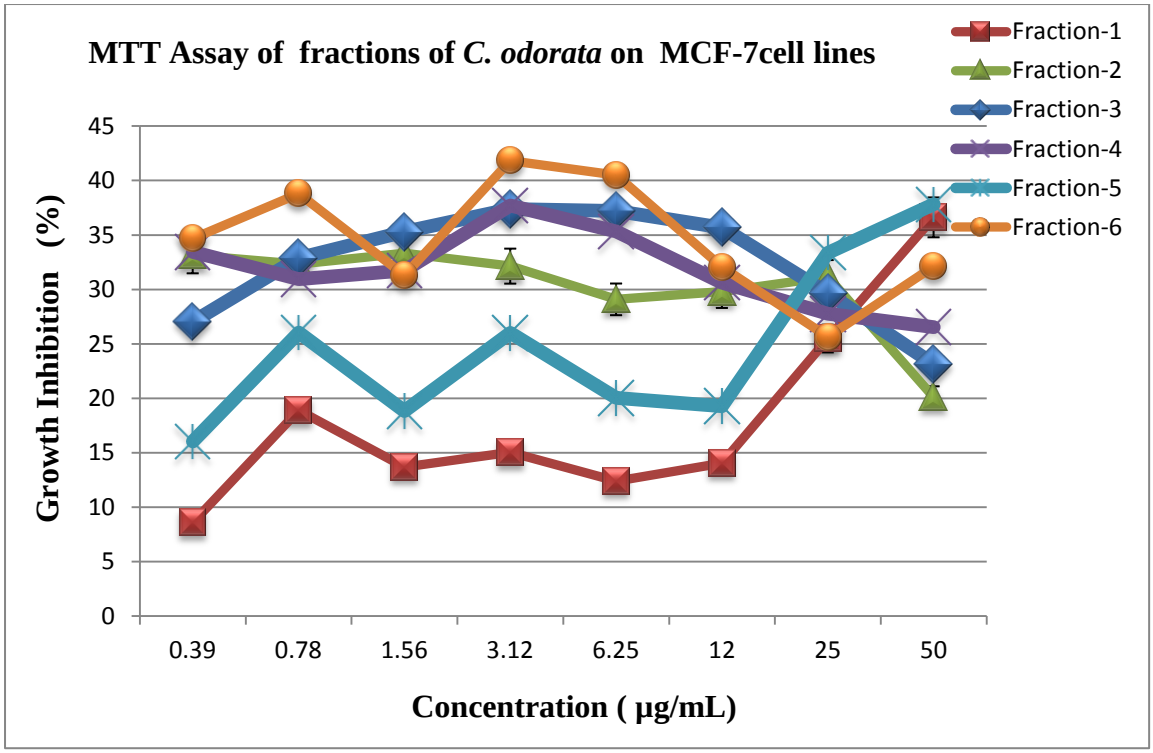


Fig.3.19a: MTT assay of fractions of *C. odorata* on MCF-7cell lines

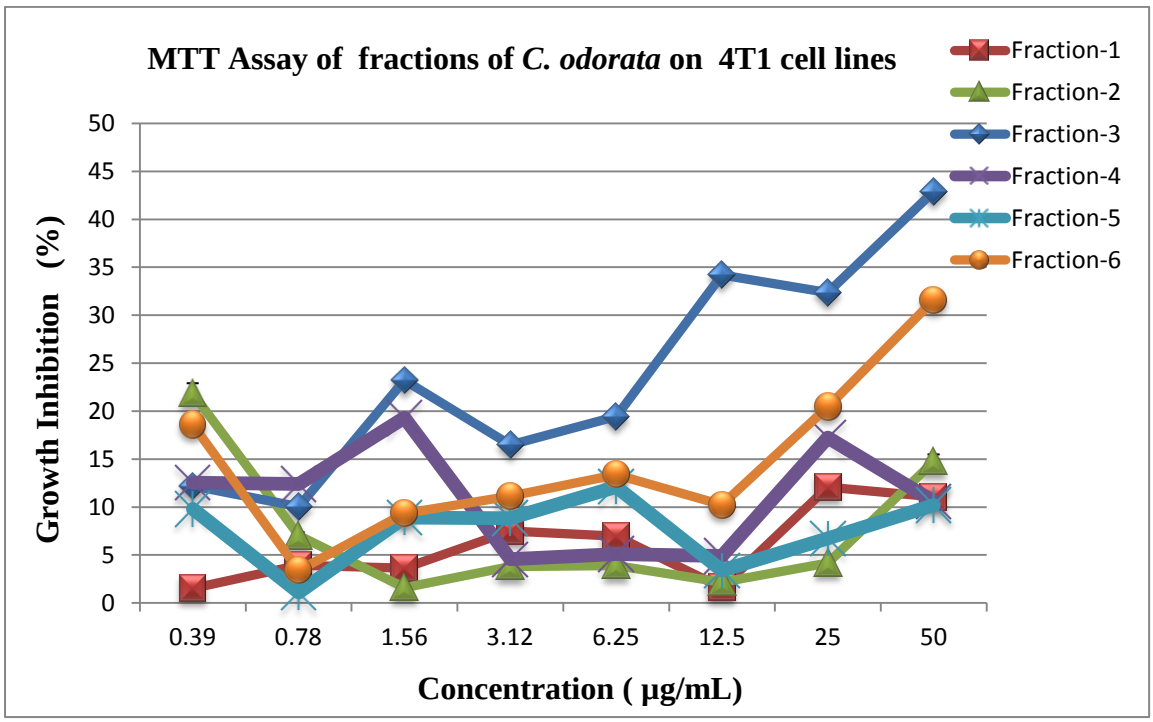


Fig.3.19b: MTT assay of fractions of *C. odorata* on 4T1cell lines

3.6: Evaluation of anti breast cancer activity of *Chomolena odorata* in rat model

As shown in Chapter 2, six groups of rats were analysed for various parameters to study anticancer activity of *C. odorata* in rat model.

3.6.1: Haematological parameters

Haematological observations have shown that in Post-CO group there was significant ($p<0.05$) decrease in the WBC count as compared to Disease control. No statistically significant differences were observed in RBC count, platlet count and haemoglobin content of study groups (Fig.3.20).

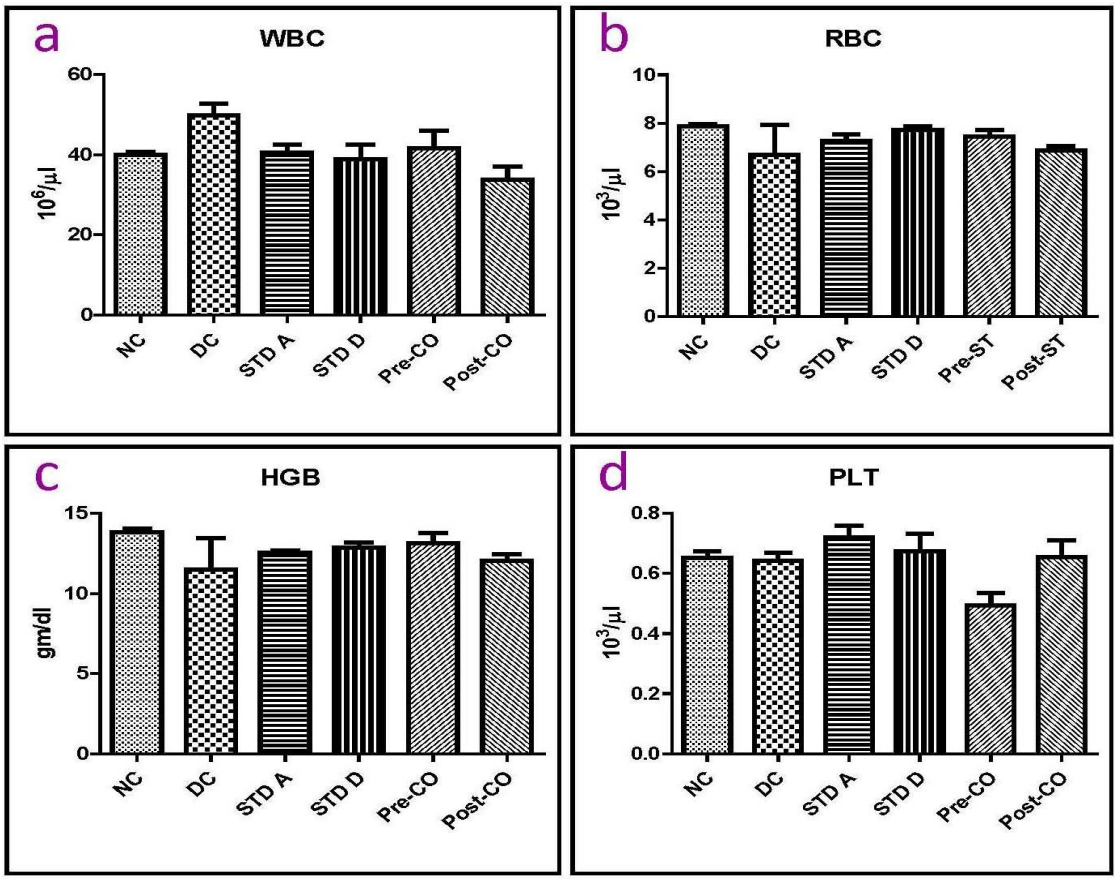


Fig.3.20: Hematological parameters a)WBC, b)RBC, c)HGB and d) Platlets

3.6.2: Oxidative and anti-oxidant markers

After dissection liver and tumor tissues of all the animals were studied for various oxidative and anti-oxidant markers such as LPO, catalase and GSH (Fig. 3.21).The catalase activity in liver tissues was found to be non-significantly altered in all the test groups. The catalase activity in tumor tissue was found to be decreased in Disease control group as compared to Normal control. It was significantly ($p<0.01$) increased in Pre-CO group as compared to Disease control animals. Although the LPO levels increased in Disease control animals, they were significantly lower in Pre-CO group as compared to Disease control animals. In tumor tissues the LPO levels were found to be significantly increased ($p<0.001$) in Disease control animals as compared to Normal control group. These levels were significantly lower in STD-D ($p<0.001$),

Pre-CO ($p<0.001$) and Post-CO ($p<0.001$) groups as compared to Disease control animals. The GSH activity was not significantly altered in liver tissue but there was decrease in GSH levels in tumor tissue of Disease control animals as compared to Normal control. As compared to Disease control animals the GSH activity was found to be significantly increased in STD-D ($p<0.01$), Pre-CO ($p<0.001$) and Post-CO ($p<0.05$) groups. Based on the above study, it could be said that Pre-CO group showed one week of delay in tumor progression as compared to disease control group.

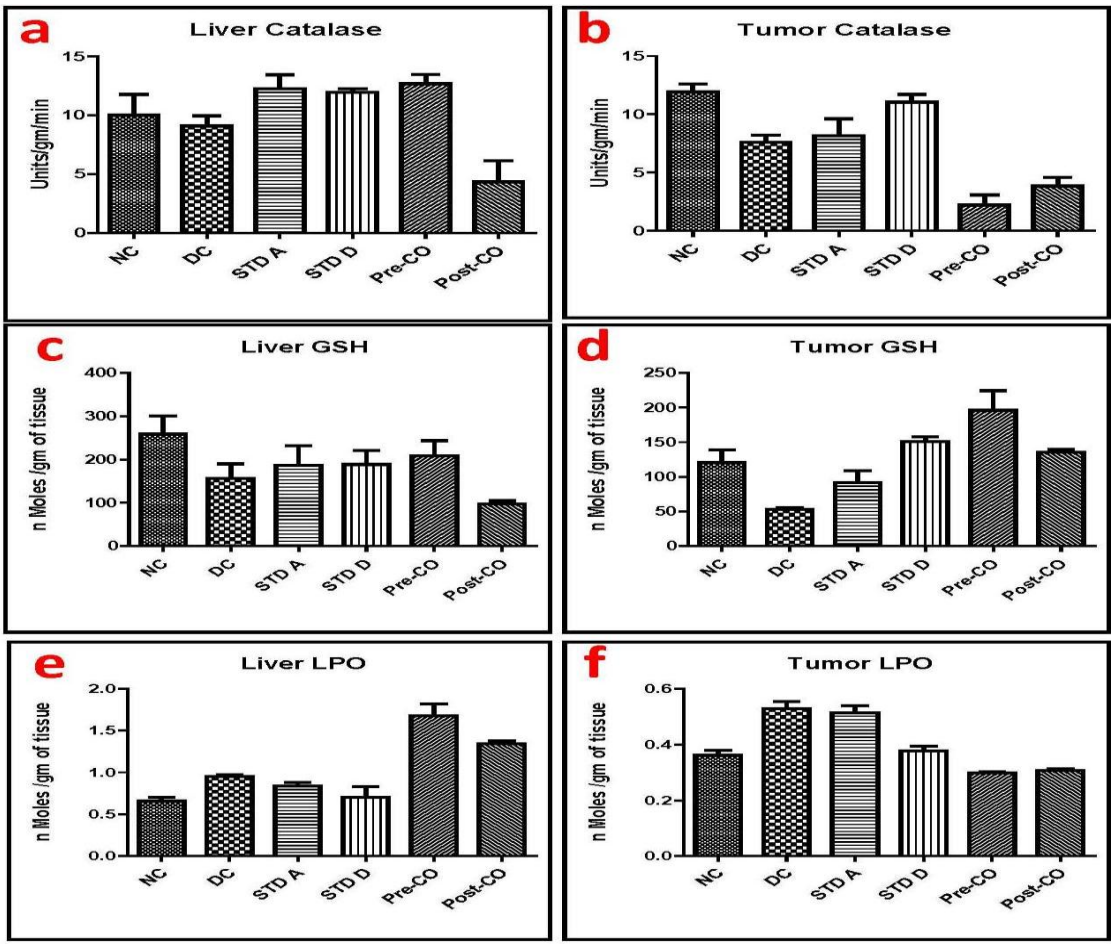


Fig.3.21: Oxidative and anti-oxidant markers a)Liver Catalase, b) Tumor Catalase, c) Liver GSH, d) Tumor GSH, e) Liver LPO and f) Tumor LPO

3.6.3: Tumor volume

There was significant ($P<0.05$) decrease in tumor volume of STD-D group as compared to disease control. Pre-CO and Post-CO groups also showed significant decrease in Tumor volume ($P<0.05$) as compared to Disease control. The tumor volume of Pre-CO group was very low as compared to other groups (Fig. 3.22).

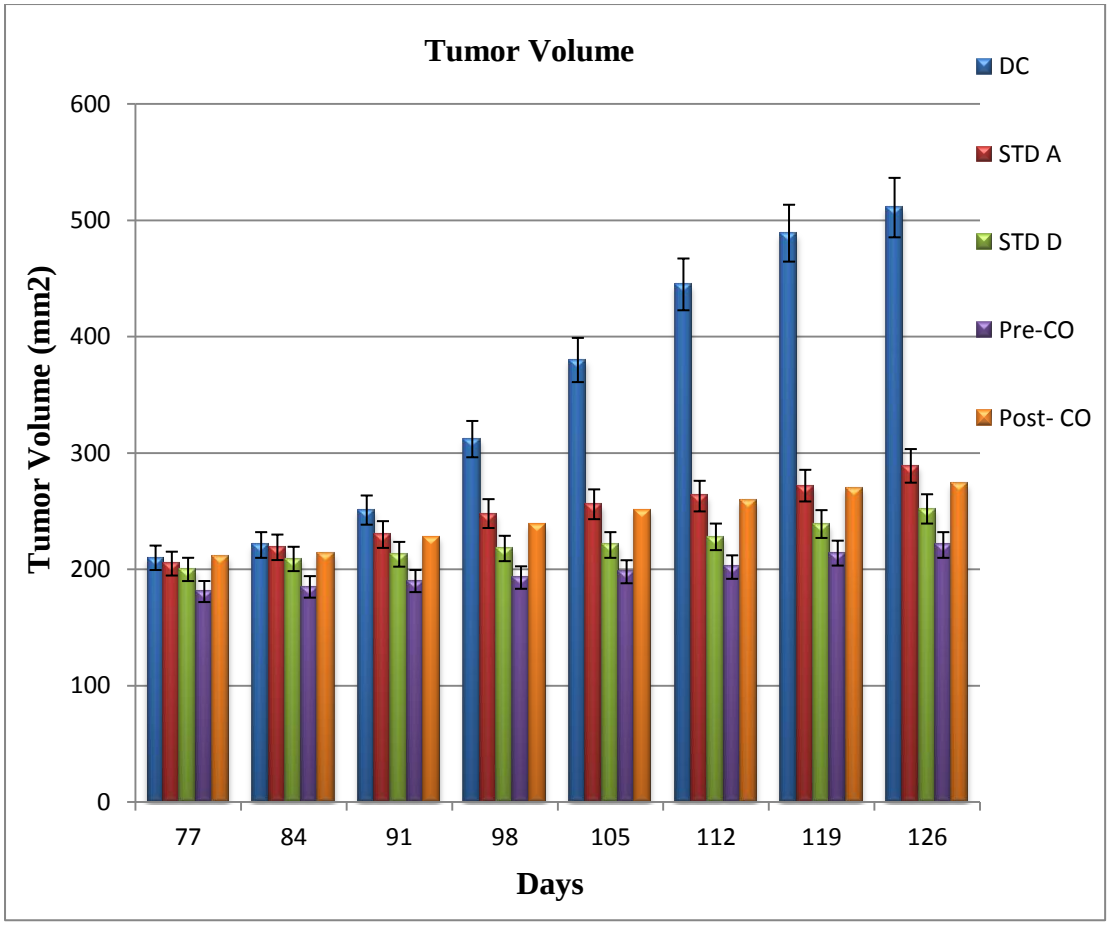


Fig. 3.22: Tumor volume of various groups

3.6.4: Serum parameters

Serum analysis was carried out for various parameters (Fig. 3.23). In case of SGOT, there was no statistically significant change observed in study groups. Although the difference was not significant, SGPT levels in Disease control group were found to be increased as compared to Normal control group and the levels decreased in all the test groups as compared to Disease control group. Similarly, LDH content was found to be decreased in Disease control animals as compared to Normal control and it increased in test groups as compared to Disease control, but the difference was not statistically significant. In case of cholesterol, creatinine, Total protein, and HDL there was no statistically significant difference observed in all the test groups as compared to Disease control group. Whereas, ALP levels were found to be increased in Disease control group as compared to Normal control and the levels were found to be decreased in STD-D, Pre-CO and Post-CO groups as compared to Disease control, but the difference was not statistically significant.

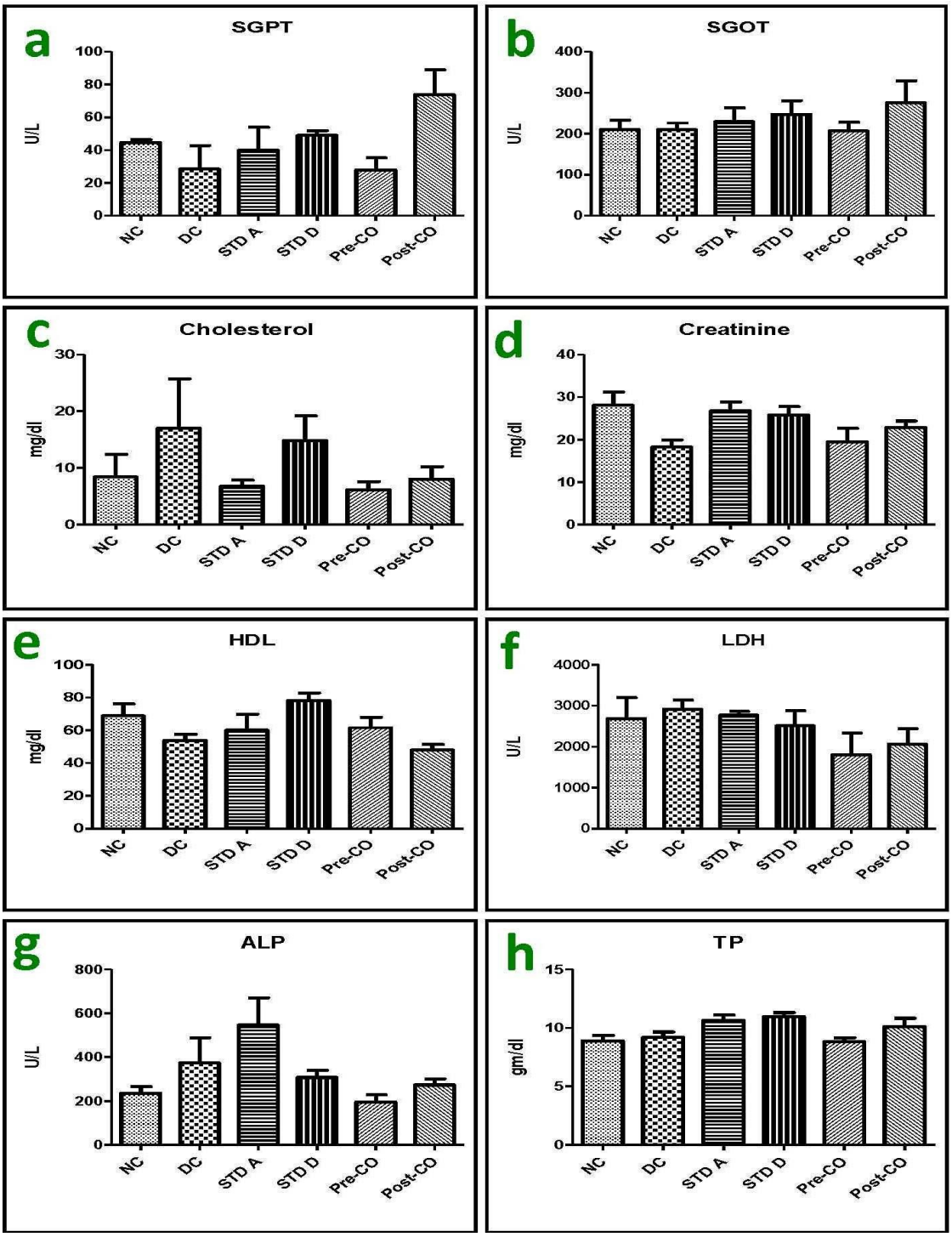


Fig.3.23: Serum parameters a) SGPT, b) SGOT, c) Cholesterol, d) Creatinine e) HDL, f) LDL, g) ALP and h) Total Protein

3.6.5: Relative organ weights

In case of relative organ weight data there was no statistically significant difference observed in the weights of adrenals, hearts, kidneys, livesr, spleens, lungs, ovaries, brains, pancreas and uterus of any of groups as shown in Fig. 3.24.

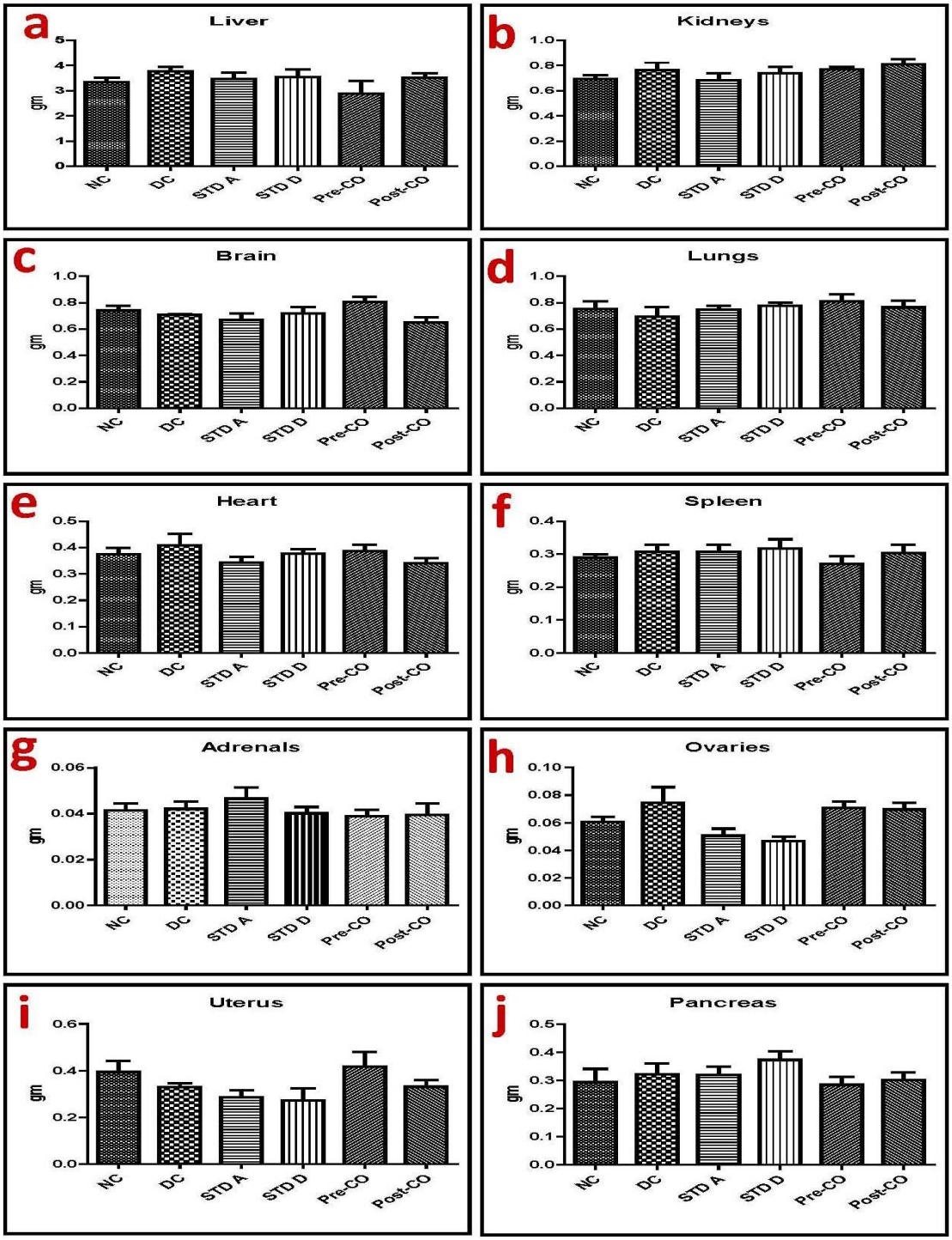


Fig.3.24: Relative organ weight a)Liver, b) Kidney, c) Brain, d) Lung, e) Heart, f) Spleen g) Adrenal, h)Ovaries, i) Uterus and j) Pancrease

3.6.6: Histopathology

The liver tissue sections from the groups including Normal Control, Standard- A, Standard- D, Pre-CO and Post-CO showed normal histomorphology of hepatic parenchyma with normal cellular and vascular details (Fig.3.25a). The hepatocytes were arranged in chord like manner around the central vein. Throughout the section,

hepatocytes showed normal cellular morphology with intact nucleus and cell borders. There was absence of any inflammatory or metabolic or neoplastic cellular changes. However, tissue sections of liver from group Disease control (DC) showed minimal to mild pathological changes with focal areas of cellular swelling, granular cytoplasmic changes with enlarged nucleus and degenerative changes of hepatocytes and focal congested portal and central vein. The kidney tissue sections from the groups including Normal Control, Standard- A, Standard- D, Pre-CO and Post-CO showed normal histomorphology of renal parenchyma with normal cellular details of glomeruli and renal tubules (Fig.3.25a). The renal tubules appeared intact with normal epithelium and borders. There was absence of any degenerative or inflammatory or neoplastic cellular changes. However, tissue sections of kidneys from group Disease Control (DC) showed minimal and focal pathological changes with occasional degenerative features of renal tubules and interstitial haemorrhages in the renal tissue. The lung tissue sections from the groups including Normal Control, Standard- A, Standard- D, Pre-CO and Post-CO showed normal histomorphological features of lung parenchyma with normal bronchi and alveolar tissue with focal congested vessels only (Fig. 3.25a). There was absence of any inflammatory or neoplastic cellular changes. However, tissue sections of lung from group Disease Control (DC) showed focal and minimal pathological changes with focal congestion and haemorrhages in lung parenchyma and focal consolidation of alveolar tissue with occasional infiltration of mononuclear cells in the lumen of alveoli. The Uterine tissue sections from all the groups including Normal Control, Disease Control, Standard- A, Standard- D, Pre-CO and Post-CO showed normal histomorphology of uterine endometrium, myometrium and glandular tissue and uterine epithelium (Fig. 3.25b). However, focal congestion and dilation of blood vessels in the basement membrane of uterine tissue was observed in few sections of Disease Control group.The mammary gland tissue section from Group Normal Control showed normal skin, subcutaneous tissue and glandular epithelium and adipose tissue of normal histomorphology (Fig. 3.25b). The mammary gland tissue sections from Group Disease Control showed marked development of tumour tissue mass with severe proliferative changes of neoplastic cells throughout the section. The glandular tissue of mammary gland showed classical features of tumours viz. fibrosarcoma and adenocarcinoma. The fibrous tissue showed large basophilic nuclei in the spindle shaped cells with dense and compact arrangement occupying the subcutaneous tissue space upto skin surface.

The mammary gland tissue sections from Group Standard – (A) showed presence of development of tumour tissue mass with proliferative changes of neoplastic cells throughout the section. The glandular tissue of mammary gland showed classical features of tumours viz. fibrosarcoma and adenocarcinoma. Certain foci of tumour tissue showed marked degenerative and liquefactive changes in the glandular tumour tissue suggestive of death of neoplastic tissue and were converted into homogenous eosinophilic cellular debris. Focal cystic areas and edematous foci were also observed. The mammary gland tissue sections from Group Standard–(D) showed marked degenerative and liquefactive changes in the glandular as well as fibrous tumour tissue suggestive of death of neoplastic tissue and were converted into homogenous eosinophilic cellular debris. Very few glandular and fibrous remnant cellular tissues from the tumour mass were observed in the sections observed.The mammary gland tissue sections from Group PRE-CO showed presence of development of tumour tissue mass with proliferative changes of neoplastic cells throughout the section. The glandular tissue of mammary gland showed classical features of tumours viz. fibrosarcoma and adenocarcinoma. Diffuse foci of tumour tissue showed marked degenerative and liquefactive changes in the glandular tumour tissue suggestive of death of neoplastic cells and were converted into homogenous eosinophilic cellular debris. However, few glandular tumour tissue mass with fibrous proliferation was observed in the sections. There was moderate destruction of tumour cells in group PRE-CO.The mammary gland tissue sections from Group POST-CO showed presence of development of tumour tissue mass with proliferative changes of neoplastic cells throughout the section. The glandular tissue of mammary gland showed classical features of tumours viz. fibrosarcoma and adenocarcinoma. Few foci of tumour tissue showed marked degenerative and liquefactive changes in the glandular tumour tissue suggestive of death of neoplastic cells and were converted into homogenous eosinophilic cellular debris. However, few glandular tumour tissue mass was observed in the sections.

There was incomplete destruction of proliferating neoplastic tissue. There was mild destruction of tumour cells in the group POST-CO. It can be concluded that animals in groups treated with PRE-CO showed marked reduction in presence of neoplastic cells and controlled the tumour cell growth as like in the STD –D. The STD –D was found to be better than STD- A with respect to higher destruction of tumour cells and resulted into marked cellular decrease in proliferative neoplastic cells in the tumour

mass. Similarly, the group PRE-CO was found to be better than POST-CO with respect to higher destruction of tumour cells and resulted into marked cellular decrease in proliferative neoplastic cells in the tumour mass.

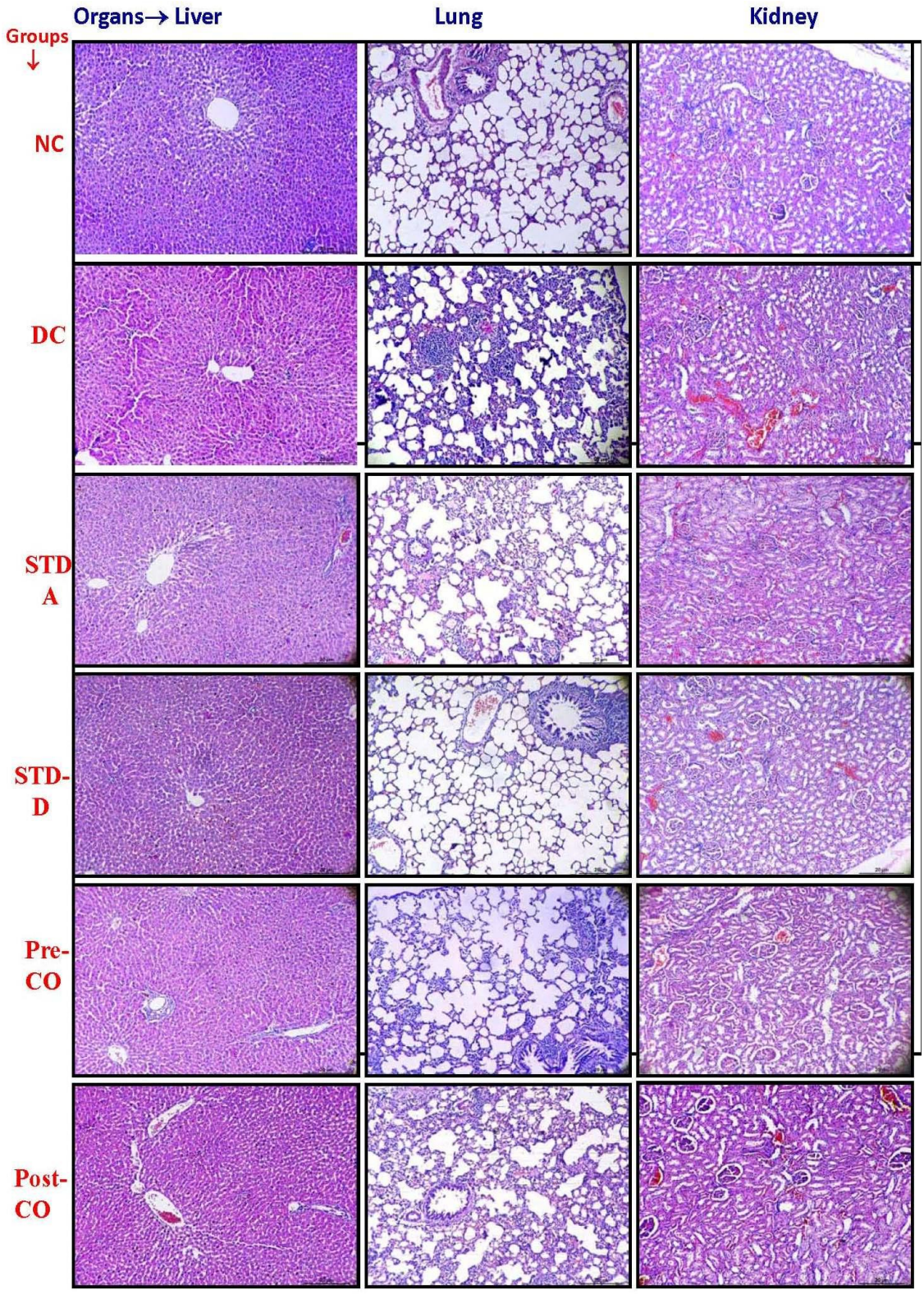


Fig.3.25a**:** Histopathology of liver, lung and kidney

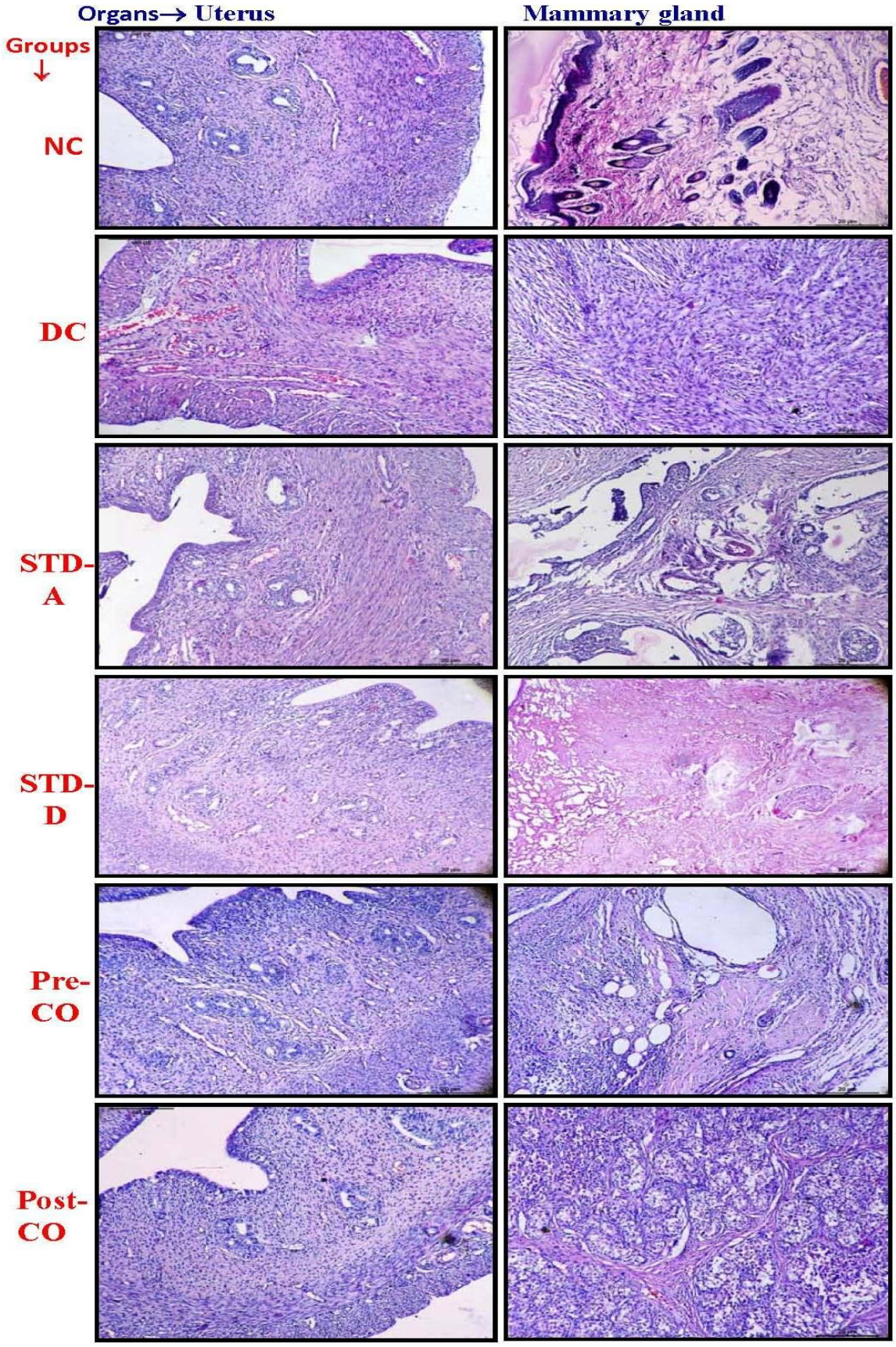


Fig.3.25b: Histopathology of uterus and mammary gland

3.6.7: Tumor weights

There was a significant ($P<0.05$) decrease in tumor weight of Pre-CO, STD-A and STD-D groups observed in Disease control. The Post-CO group showed decrease in tumor weight as compared to Disease control but the difference was not statistically significant (Fig. 3.26).

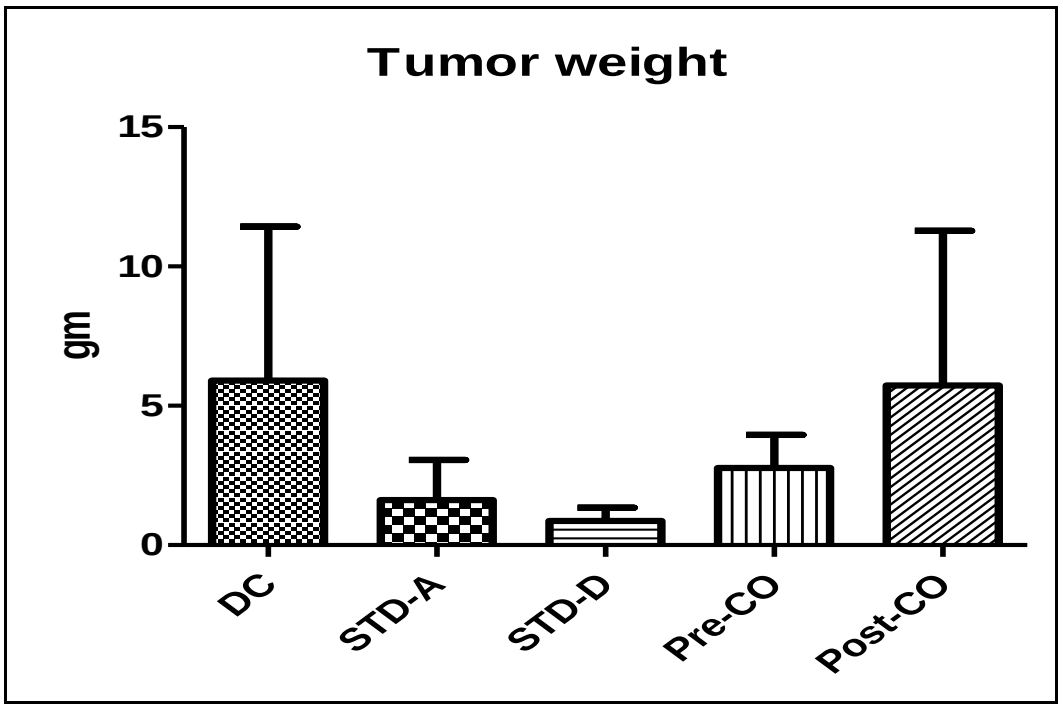


Fig.3.26: Tumor weights of various groups

3.7: Single dose acute oral toxicity study

This study was carried out on male and female Wistar rats as per OECD guidelines. The various parameters studied during this experiment are as follows.

3.7.1: Body weights and feed intake

All the animals of all the groups (A, B, C, D, E, F, G and H) were weighed and feed intake was estimated on 1st, 7th and 14th day. Body weights of all animals were in normal range viz. 146 to 170±3.40 gm during the experiment (Fig.3.27). The feed intake of all the animals was also in normal range viz. 20 to 30 ±2.30 gm during the experiment (Fig.3.28).

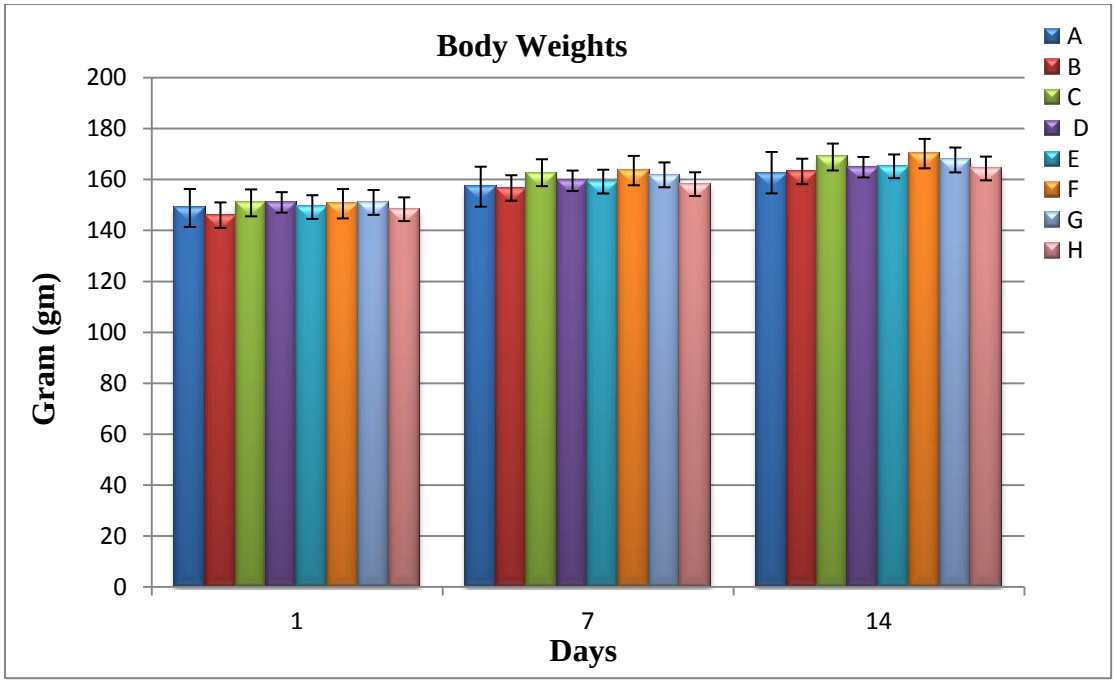


Fig.3.27: Body wights of rats

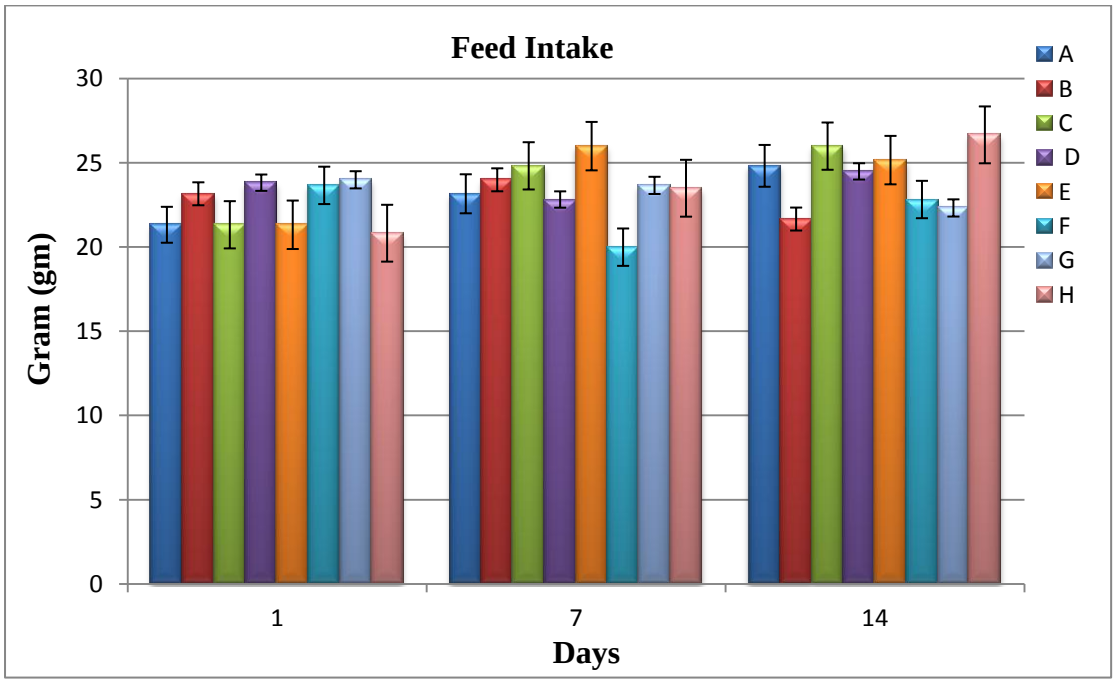


Fig.3.28: Feed intake of rats

3.7.2: Serum parameters: SGOT, SGPT, Serum Creatinine and BUN levels of all the animals of all the groups were estimated on the last day of experiment viz. 14th day. They were in normal range. SGOT levels (U/L) were 83±3.42, 75.33±2.99, 80.5±3.04, 90.67±3.41, 84.17±3.32, 83.5±2.98, 87.83±3.13 and 86.5±3.16 of group A, B, C, D, E, F, G and H, respectively (Fig.3.29a). SGPT levels (U/L) were

31.18±2.31, 25.07±1.98, 29.13±2.03, 32.12±2.02, 34.83±2.12, 33.45±2.33, 32.95±2.40 and 30.62±2.14 of group A, B, C, D, E, F, G and H, respectively(Fig.3.29 b). Serum Creatinine levels (mg/dl) were 0.38±0.02, 0.37±0.03, 0.40±0.02, 0.45±0.02, 0.40±0.03, 0.42±0.02, 0.42±0.03, and 0.40±0.03 of group A, B, C, D, E, F, G and H, respectively (Fig.3.29c). BUN levels (mg/dl) were 13.14±1.83, 15.07±1.72, 14.03±1.69, 12.11±1.70, 14.13±1.76, 12.04±1.75, 11.95±1.73 and 13.62±1.68 of group A, B, C, D, E, F, G and H, respectively (Fig.3.29d).

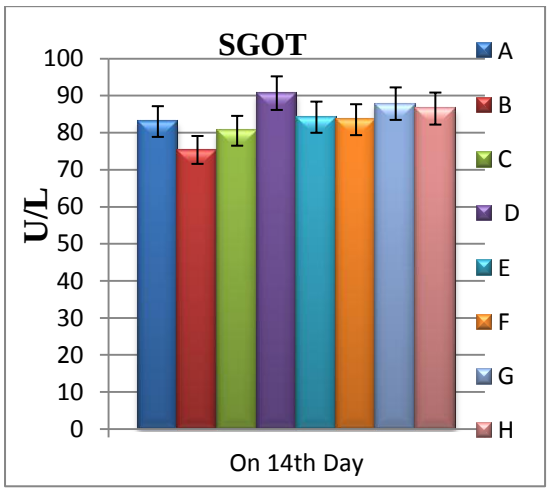


Fig.3.29 a: SGOT levels

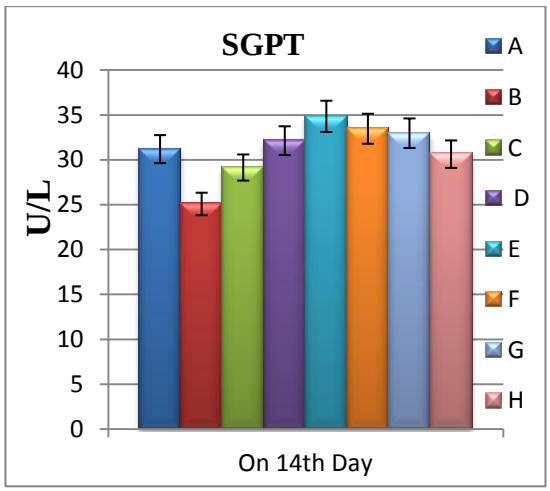


Fig.3.29 b: SGPT levels

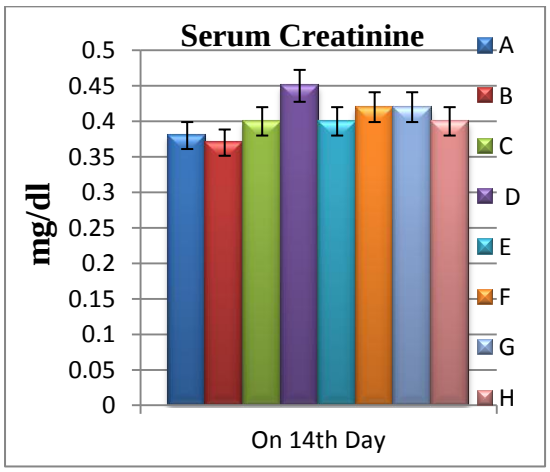


Fig.3.29 c: Serum creatinine levels

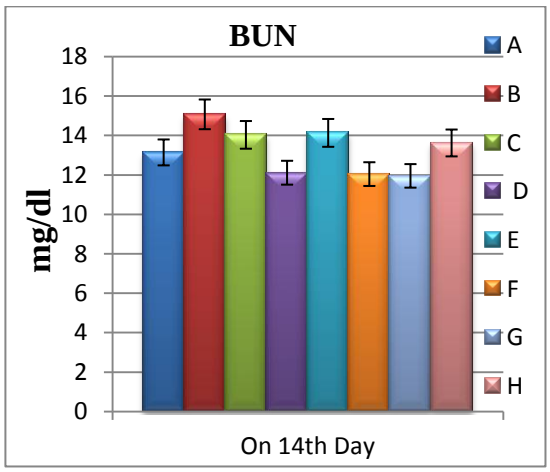


Fig.3.29 d: BUN levels

3.7.3: Haematological parameters: Haemoglobin, RBC, WBC and Platlet levels of all the animals of all the groups were estimated on last day of experiment viz. 14th day. They were in normal range. Haemoglobin levels (mg/dl) were 14.65±0.26, 12.60±0.16, 14.37±0.27, 14.82±0.19, 13.92±0.23, 15.02±0.25, 13.93±0.18 and 14.95±0.23 of group A, B, C, D, E, F, G and H, respectively (Fig.3.30a). RBC levels (10^6/µl) were 7.52±0.21, 6.46, 7.86±0.22, 7.26±0.19, 7.60±0.23, 7.14±0.22, 6.29±0.25

and 6.95±0.16 of group A, B, C, D, E, F, G and H, respectively (Fig.3.30b). WBC levels (10^3/µl) were 9.28±0.63, 8.92±0.65, 10.68±0.53, 8.07±0.49, 10.17±0.53, 8.79±0.61, 9.85±0.57 and 8.18±0.49 of group A, B, C, D, E, F, G and H, respectively (Fig.3.30c). Platlets levels (10^3/µl) were 552.5±27.51, 534.5±29.45, 571.17±26.50, 591.17±25.55, 526.22±23.33, 579.67±26.61, 564.19±27.30 and 522.83±27.11 of group A, B, C, D, E, F, G and H, respectively (Fig.3.30d).

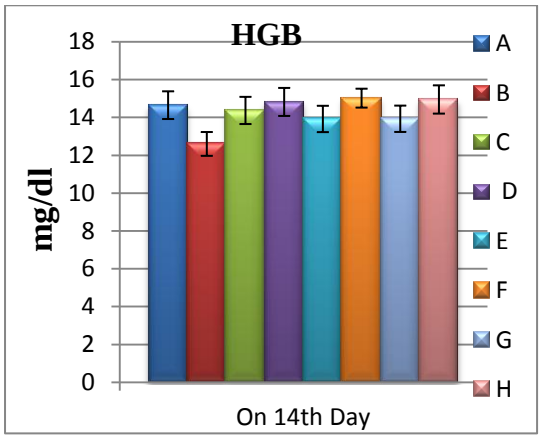


Fig.3.30 a: Haemoglobin levels

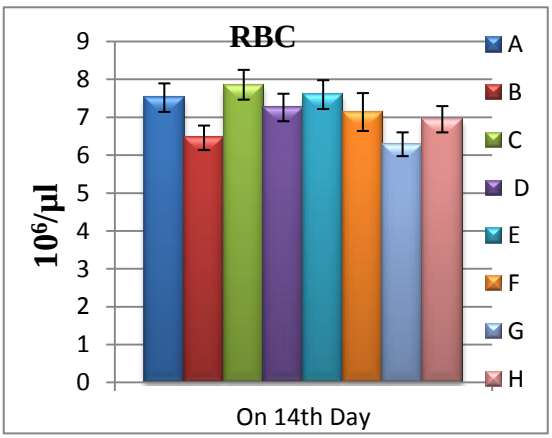


Fig.3.30 b: RBC levels

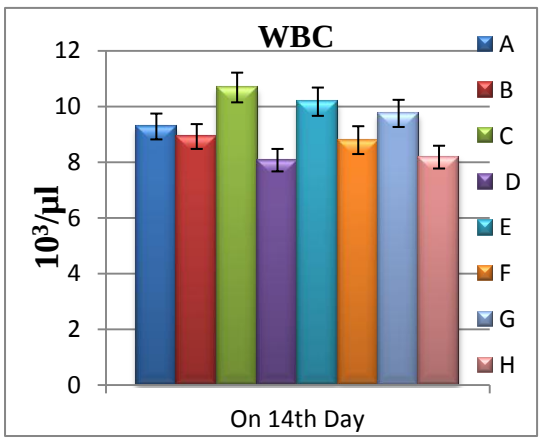


Fig.3.30 c: WBC

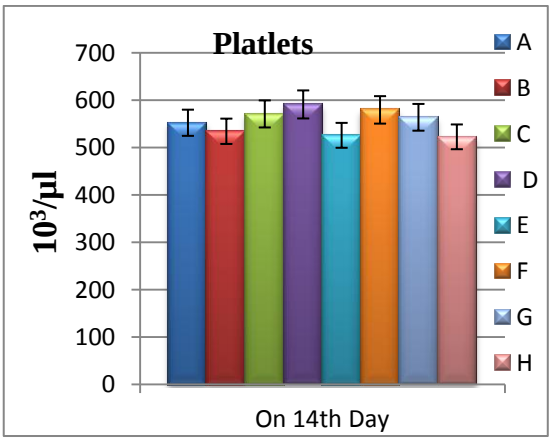
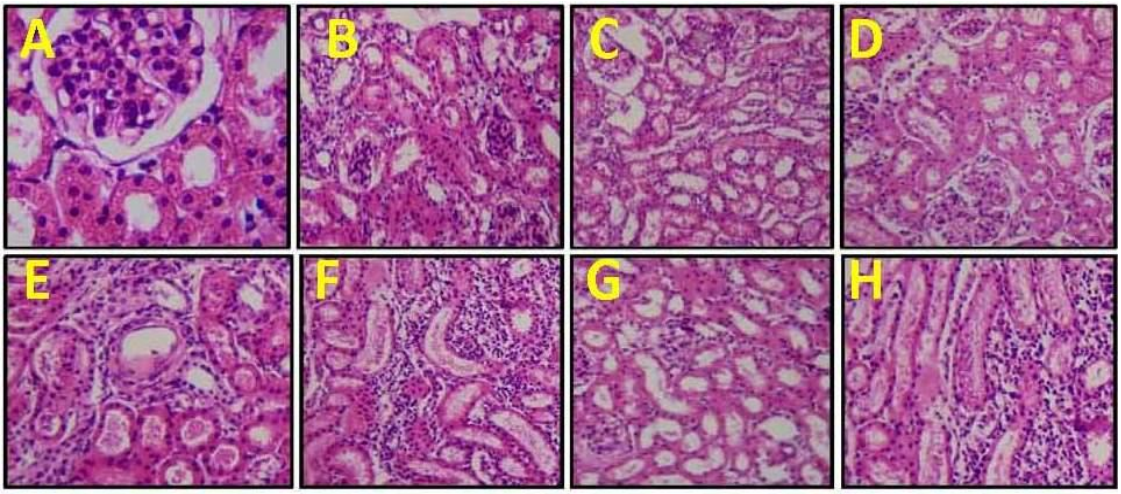


Fig.3.30 d: Platlets levels

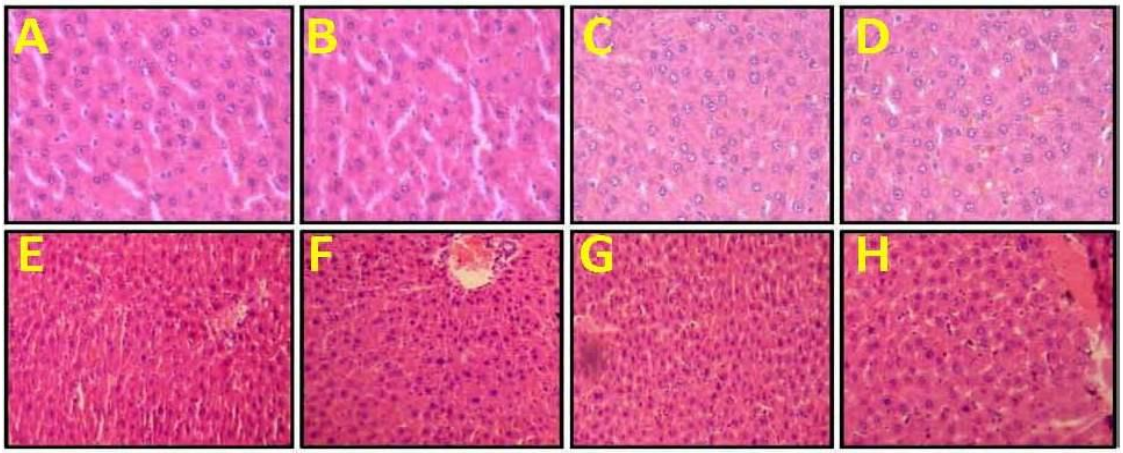
3.7.4: Histopathology

Histopathological examination of kidney, liver, heart, spleen and lung was performed. The tissue sections from all the organs of all the groups showed normal histomorphology with normal cellular details. There was absence of any degenerative or inflammatory or abnormal cellular changes. Histopathology reveled that there was no toxicity of *C. odorata, D. erecta* and *M. arvensis.*

Kidney



Liver



Spleen

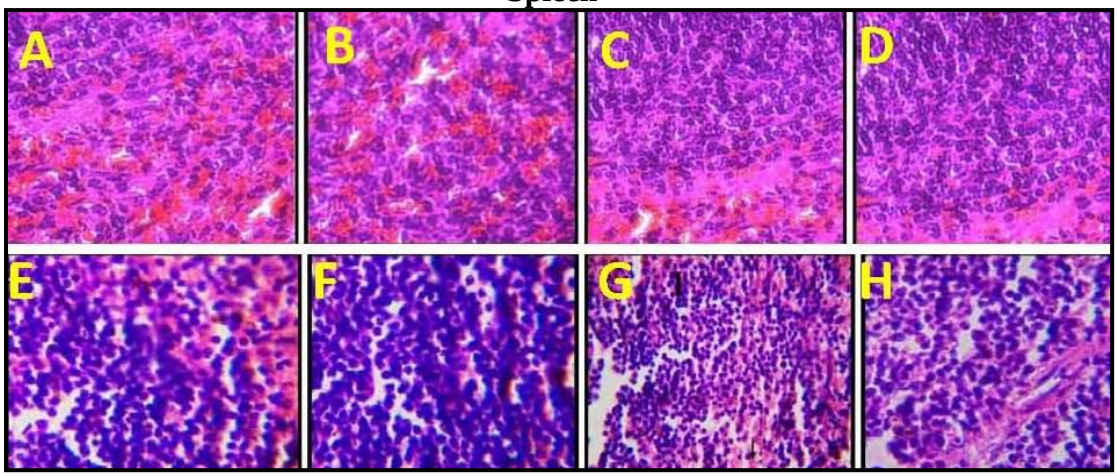


Fig.3.31a: Histopathology of kidney, liver, spleen of groups A, B, C, D, E, F, G & H

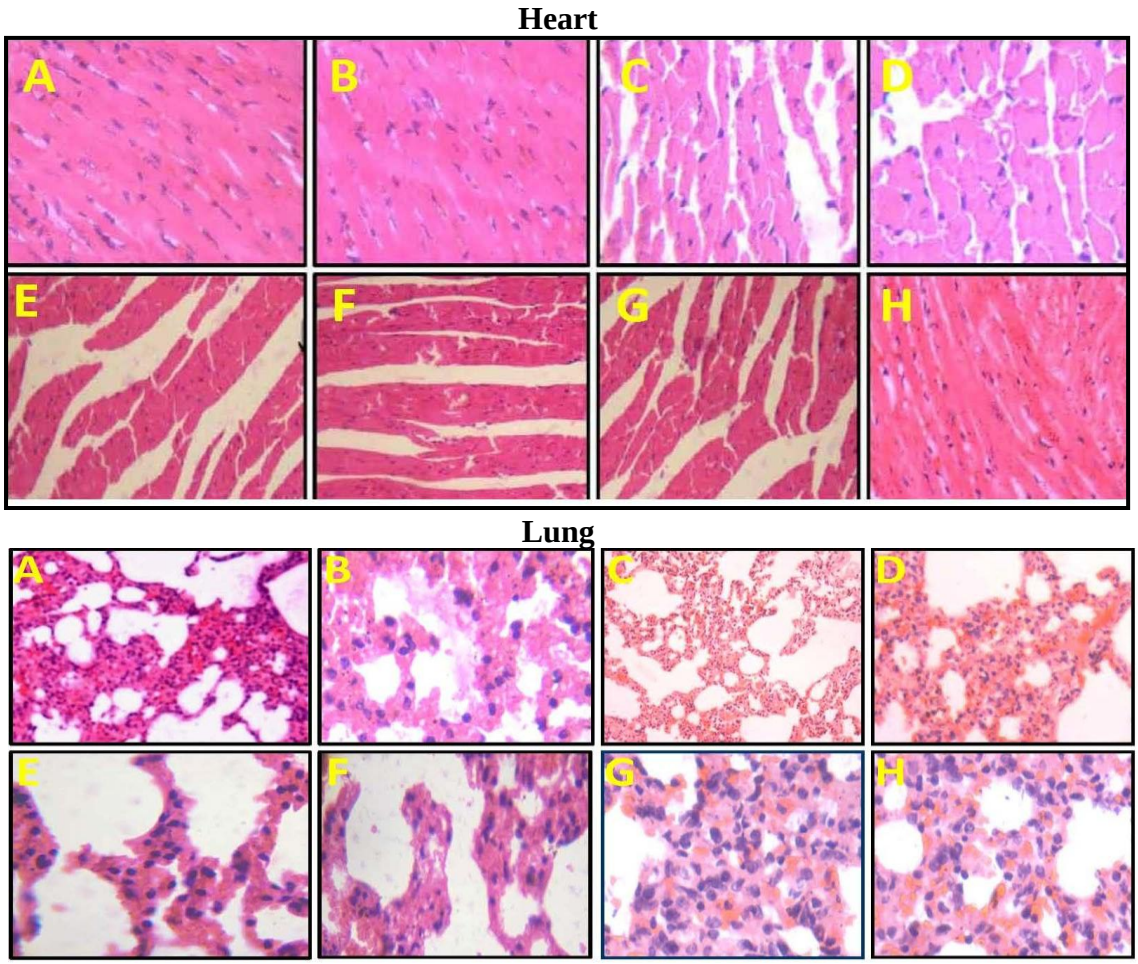


Fig.3.31b: Histopathology heart and lung of groups A, B, C, D, E, F, G & H

4.1: Antioxidant activity of plants under study

The preliminary phytochemical analysis of extract of *Adhatoda vasica* (L.) Nees, *Bacopa Monnieri* (L.) Penn, *Centella asiatica* (L.) Urb., *Chromolaena odorata* (L.) R.M. King & H. Rob, *Duranta erceta* (L.), *Mentha arvensis* (L.), *Ocimum tenuiflorum* (L.) and *Sphagneticola trilobata* (L). Pruski, revealed the presence of high amount of phenolic compounds along with other phyto-constituents like proteins, carbohydrates, tannins, flavonoids, terpenoids, saponins, glycosides, alkaloids and sterols. NMR analysis of these plant extracts confirmed presence of variety of mixtures of phytochemicals.

The methanolic extracts of all these eight plants showed very good antioxidant activity. The highest antioxidant activity was shown by *D. erecta* (93.5%) and the lowest by *S. trilobata (*65.6%). The antioxidant activity may be due to the presence of phenolic compounds in the extracts. Phenols are very important plant constituents. They show high scavenging ability of free radicals due to their hydroxyl group. The high correlation between the content of phenolic compounds in plant extracts which leads to antioxidant activity has been well studied (Borneo *et al.*, 2008). Plants based antioxidants play an important role in neutralizing free radicals and protect important biological molecules from being damaged by free radicals. Antioxidants significantly prevent oxidation of cell content like proteins, lipids, carbohydrates and DNA (Borneo *et al.*, 2008). Among all these eight plants, three plants namely *C. odorata, D. erecta* and *M. arvensis* were selected for further studies due to their high percentage of free radical inhibition (above 75%).

4.2: Antidiabetic activity of *M. arvensis*

There are many therapeutic approaches, which may prove to be beneficial for treatment of type II diabetes mellitus (postprandial hyperglycemia). This can be done by reducing the absorption of glucose through the inhibition of two key enzymes linked to type II diabetes mellitus (PPHG) in the digestive tract. It has been studied that the inhibition of carbohydrate hydrolyzing enzymes, like α -amylase and α-glucosidase are the therapeutic approaches to treat the type 2 diabetes mellitus (Shobana et al., 2009). *In-vitro* and *in-vivo* evaluation of *M. arvensis, C. odorata* and *D. erecta* for antidiabetic activity revealed that only *M. arvensis* had *in-vitro* antidiabetic activity. α-amylase and α-glucosidase inhibition potential of *M. arvensis* was 59.4% and 68%, respectively at the highest concentration (100 mg/mL) as

compared to standard compound acarbose which exhibited 94.8% of α-amylase and 93.6% of α-glucosidase inhibition at the same concentration. Inhibitors of α-amylase and α-glucosidase enzymes delay carbohydrate digestion in the body and overall carbohydrate digestion time causing a significant decrease in the rate of glucose absorption by blunting the postprandial plasma glucose level. In Type2 Diabetes Mellitus (Post Prandial Hyperglycemia), inhibition of α-amylase and α-glucosidase therapy is beneficial to delay absorption of glucose after a meal. These enzymes play a role in the conversion of carbohydrates into glucose. By inhibiting α-glucosidase, glucose levels in the blood can be returned within normal limits (Elya *et al*., 2011). The glycation reaction involves a series of non-enzymatic reactions between the carbonyl group on reducing sugars and the amino group on proteins to form advanced glycation end product (AGE's), which are involved in the pathogenesis of diabetes mellitus and aging-related complications. In case of postprandial hyperglycemia there is high blood glucose level that leads to increase in glycation reaction, which can alter protein conformation and impair function by altering enzyme activity, altering immunogenicity, modifying protein half life and causing cross linking of structural proteins. Due to good α-amylase and α-glucosidase inhibition potential of *M. arvensis,* it was further tested for its glycation inhibition potential. The antiglycation activity of extract of *M. arvensis* was determined using AGE fluorescence assay where in it significantly inhibited the protein glycation. The glycation inhibition potential of *M. arvensis* was 94%. This might be due to higher content of polyphenolics. It has been already studied that polyphenolics block the formation of AGEs and other glycated proteins. Furthermore, due to good *in-vitro* performance of *M. arvensis,* its antidiabetic evaluation was carried out in animal model by inducing post prandial hyperglycemia in Wistar rats. Anti-hyperglycemic activity of methanolic extract of *M. arvensis* in rats revealed reduction of blood glucose levels at different time point's 0, 30, 60, 90 and 120 minutes. The *M. arvensis* significantly reduced the blood glucose levels when compared to the diabetic control and had equally potent antidiabetic potential when compared with acarbose. The present study revealed that the methanolic extract of *Mentha arvensis* L. significantly inhibited the α-glucosidase and in moderate α- amylase enzymes might be due to higher content of polyphenolics. Similar observations have been done in case of *Morinda lucida* Benth Leaf (Kazeem *et al*., 2013) and *Tinospora cordifolia* (Chougale *et al*., 2009). However, further studies are needed to isolate the active principles in these plants which could be

responsible for this activity. It is also needed to see synergistic effect of the phytochemical constituents of various plant extracts against type 2 diabetes.

4.3: Antiurolithiasis activity of *D. erecta*

In-vitro and *in-vivo* evaluation of methanolic extracts of leaves of *Adhatoda vasica, Bacopa Monnieri, Centella asiatica, Chromolaena odorata, Duranta erecta, Mentha arvensis, Ocimum tenuiflorum* and *Sphagneticola trilobata* for antiurolithiatic potential was performed. Various *in-vitro* parameters of urolithiasis namely nucleation and synthetic urine analysis were performed. The results of nucleation and synthetic urine assay showed that only *D. erecta* had antiurolithiasis potential. The *D. erecta* extract inhibited calcium oxalate crystal formation by 51.9% and calcium oxalate monohydrate crystal formation by 82.1% at a concentration of 100 mg/mL as compared to cystone which exhibited more than 85% of inhibition. *In-vivo* experiment of *D. erecta* for antiurolithiasis activity was performed in Wistar rat model. During animal study, serum creatinine, BUN, SGPT and SGOT levels were significantly elevated in urolithiasis disease control animals, but these levels were in normal range in animals treated with standard drug Cystone and animals treated with *D. erecta* extract. The elevated levels of serum creatinine and BUN indicated a marked renal damage. The renal damage caused due to urolithiasis is associated with liver damages which were confirmed by SGOT and SGPT test. In this study Cystone was used as a standard drug. There was an increase in urinary oxalate after sodium oxalate administration while a decrease in oxalate levels was observed in treatment groups. This effect may be due to the inhibition of formation of oxalate by the Cystone and *D. erecta* treatments. The kidney sections derived from disease control group when observed microscopically showed polymorphic irregular crystals deposited in the tubules which led to dilation of the proximal tubules and also interstitial inflammation that might be due to oxalate crystals. Treatment with Cystone and *D. erecta* reduced the number and size of calcium oxalate crystals deposited in different parts of the renal tubules and also saved the tubules and calyxes from damaging through an antioxidant and nephroprotective property of *D. erecta* extract. Urine of disease control group showed abnormalities like turbidity, presence of granular debris and calcium oxalate crystals but that of treatment groups was normal compared to disease control group. The histopathology of kidney tissues from of disease control group showed degeneration of epithelial lining, focal mild degeneration of tubules of

medulla, focal mild tubular atrophy of cortex and multifocal minimal intertubular lymphocytic infiltration. Cystone treated group C rats did not reveal any lesion of pathological significance suggesting attenuation of lesions, showing normal histology. The *D. erecta* treated group D showed lesions of less severity with less distribution suggesting decrement of adverse effect caused due to calcium oxalate.

Based on this analysis, it can be concluded that *D. erecta* treat calcium oxalate crystals deposition in the kidneys by preventing hyperoxaluria-induced peroxidative damage to the renal tubular membrane surface (lipid peroxidation), which in turn might prevent calcium oxalate crystals attachment and subsequent development of kidney stones. The antimicrobial test of methanolic extract of *D. erecta* revealed very good antimicrobial activity against both Gram positive and Gram negative bacteria. Damage of urinary tract leads to bacterial attack and a stone nucleus may develop, leading to a full stone in the urinary tract. At this point, some extracts that show antimicrobial properties can be considered antilithogenic by protecting urinary tract from bacterial infection. The plant extracts which have antimicrobial activity can act as better antiurolithiasis medicine (Bouabdelli *et al.*, 2012).

Similar approaches have been done in case of *Launaea procumbens* leaves (Makasana *et al*., 2014), *Melia azedarach* (Dharmalingam *et al*., 2014*)* and Pergularia daemia (Vyas *et al*., 2011). However, further studies are needed to isolate the active principles in these plants and to see synergistic effect of the phytochemical constituents of various plant extracts against urolithiasis.

4.4: Anti breast cancer activity of *C. odorata*

The *in-vitro* anti breast cancer analysis involved MTT assay, it is the cell viability assay that gives an idea of about how effectively the concerned compounds kills the cells. In this case we used breast cancer cells of both mice (4TI) and human (MCF7), thus, MTT assay on cancer cells indicate anti cancer activity of the compounds. The MTT assay of crude extract of *C. odorata* showed more than 80% of growth inhibition on MCF-7 and 4T1 cell lines. The IC50 values was 15.63µg/mL and 15.95µg/mL, respectively; that means at these concentrations of the plant extract, 50% population of the cells was inhibited to grow and only 50% of the original population of cells was left viable, referring that the extract effectively inhibited growth of cancer cells. The *A. vasica* and *D. erecta* showed no anticancer activity and *M. arvensis*, had very less anticancer activity. The cytotoxicity of crude extract of *C. odorata* on

HUVEC normal cell lines was less than 35% indicating it was non-toxic for HUVEC normal cell lines. Interestingly, the MTT assay of all fractions of *C. odorata* showed less than 45% of growth inhibition on MCF-7 and 4T1 cell lines. None of fractions showed activity against breast cancer cell lines. These fractions were in turn mixtures of many other compounds. The GC-MS analysis of *C. odorata* crude extract and its fractions revealed the presence of several compounds of biological significance like hexadecanoic acid, α-amyrin, lupeol (triterpenoids) and phytol etc. The hexadecanoic acid and α-amyrin are known for antioxidant activity, lupeol (triterpenoid) is reported having a novel anti-inflammatory, hepato-protective and anti-cancer activity and phytol is known to have antibacterial activities (Abubkar *et al.*, 2016). A few well known flavanoids, chalcones and other metabolites were confirmed to be present in the crude of *C. odorata* viz. 2'-hydroxy-4, 4',5',6'-tetramethoxy chalcone, 5, 7-dihydroxy- 4'-methoxy flavanone/ acacetin and 5, 7, 3', 4'-tetrahydroxy flavanone/Eriodictyol. The flavanone/Eriodictyol, are known as anti cancer against (NCI-H187) lung cancer as reported by Suksamrarn *et al.,* 2004.

Here, the *in-vitro* tests proved the whole extract to be anti-cancer in nature, whereas the fractions were not active in the inhibition of growth of cancer cells. There could be many possible reasons for this, one being synergic effect of the constituents of the plant i.e. all the constituent compounds of the plant *C. odorata* showed activity only when they were present together complementing their activities and not when they were separated due to loss of some of the constituent compounds in that particular fraction (Williamson, 2001). The combination of the various plant extracts may improve the anticancer activity than individual plant extract. Specific and selective apoptosis induction in targeted chemotherapy is limited due to multidrug resistance and severe side effects (Au *et al.,* 1997 and Lee *et al.,* 2012). Several herbal plant extracts are rich sources of bioactive molecules that inhibit, reverse, or retard tumorigenesis (Pinmai *et al*., 2008 and Tang *et al*., 2010). Additionally, some research on herbal synergistic effect indicates that the whole herb produces a better effect than any single isolated active ingredient (Wagner and Ulrich, 2009 and Machana *et al.* 2012). This study has enhanced the scope of novel formulations by combinations of various Ayurvedic plants for prevention and treatment of breast cancer.

4.5: Single dose acute oral toxicity study of *C. odorata, D. erecta* and *M. arvensis*

This study was conducted to evaluate toxicity effect of crude extract of *C. odorata, D. erecta* and *M. arvensis*. This study revealed that *C. odorata*, *D. erecta* and *M. arvensis* are safe for the consumption. The physiological, hematological, serological and histopathological investigations during this study showed that *C. odorata, D. erecta* and *M. arvensis* are non toxic. This study confirmed the safety aspect of *D. erecta* and *M. arvensis*.

4.6. Conclusion and Future prospects

Ayurveda as an ancient science of life, has a long history, and its basic principles are valid even today. Many medicinal plants are effective for prevention and treatment of various diseases due to the presence of bioactive molecules. The need for scientific evaluation of Ayurveda has been recognized for a long time. This work advocates scientific evaluation and use of herbal medicine for prevention and treatment of various diseases like diabetes, urolithiasis and breast cancer. It can be concluded that out of 8 selected medicinal plants viz. *Adhatoda vasica, Bacopa Monnieri, Centella asiatica, Chromolaena odorata, Duranta erecta, Mentha arvensis, Ocimum tenuiflorum* and *Sphagneticola trilobata* the *M. arvensis* extract may become very good option to the available drugs in the market for prevention and treatment of diabetes mellitus (Post prandial hyperglycemia), the *D. erecta* leaves extract can be used as potential antiurolithiasis agent for kidney stone prevention and removal while the leaves extract of *C. odorata* may emerge as very good herbal treatment against breast cancer prevention and treatment in women. Ayurveda is a holistic system and ayurvedic medicine should be used in ayurvedic way because individual molecule or fraction of plant sometime may not be effective but become effective in presence with other molecules due to synergetic action.

However, there is a need to explore more natural product chemistry and chemical biology for further fractionations, isolation of various bioactive molecules and its chemo-biological evaluation for treatment of various diseases. There is need to test synergetic activity of various plants extracts together against various diseases. This part of the thesis summarizes the preliminary work presented in the thesis and emphasizes on possible further research in this area.

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