

Available online at <http://www.harmanpublications.com>

World Journal of Science and Research

Harman Publications. All rights reserved



Research Article

Botany

STUDIES ON PHARMACOGNOCY, PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF *Ocimum sanctum L.*

M. Siya and A. Pauline Fatima Mary

Department of Botany, Kunthavai Naacchiyaar Govt. Arts College For Women (Autonomous)
Thanjavur – 613 007, Tamilnadu

ABSTRACT

Article Info:

Present study to investigate the phytochemical and antimicrobial properties of *Ocimum sanctum*. The phytochemical screening aqueous extract of *Ocimum sanctum* leaf showed that the presence of, steroids, saponins, terpenoids, glycosides, anthraquinone and protein Flavonoids, triterpenoids while tannin, alkaloids, protein, carbohydrate, phlobatannins, phenolics were absent. Methanol extract of *Ocimum sanctum* leaf showed that the presence of alkaloids, steroids, saponins, Flavonoids, triterpenoids, Polyphenol, naphthoquinone and glycosides while tannin, alkaloids, protein, phlobatannins, were absent. Results of the histochemical study revealed that *Ocimum sanctum* powder treated with phloroglucinol and diluted HCl gave red colour indicates lignin, treated with diluted ammonia and H₂SO₄ gave yellow colour indicates flavonoids and treated with Mayer reagent reddish brown colour indicates alkaloids. This study further confirmed the presence of phytochemicals. The results reveal that extract of *Ocimum sanctum* were significantly effective against both bacteria *E. coli*, *St. aureus* and fungi *C. albicans* and *Aspergillus flavus*. The leaves of *Ocimum sanctum* are a newly discovered potential source of natural antimicrobial compounds. The synergistic effect of plant extract against resistant bacteria and fungi leads to new choices for the treatment of infectious diseases.

Received on 10th March 2017

Accepted on 30th Mar. 2017

Online April 2017

Keywords:

Phytochemicals,
Antimicrobial activity,
Ocimum sanctum

Citation M. Siya and A. Pauline Fatima Mary (2017) Studies on Pharmacognocoy, Phytochemical And Antimicrobial Activity Of *Ocimum Sanctum L.*. *World Journal of Science and Research*. 2 (2): 01-12.

INTRODUCTION

Use of plants for treating various ailments of both man and animal is as old practice as man himself. India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society

whether directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine (Bhagwati Uniyal, 2003). In recent times, focus on plant research has increased all over the world and a large body of evidence collected to show immense potential of medicinal plants used in various

traditional systems (Ayurveda, Siddha and Unani) (Dahanukar *et al.*, 2000). Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available supposedly. Owing to the global trend towards improved quality of life there is considerable evidence of an increase in demand for medicinal plant (Kotnis *et al.*, 2004).

Pharmacognosy is the study of medicinal drugs derived from plants or other natural sources. The American Society of Pharmacognosy defines pharmacognosy as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources." It is also defined as the study of crude drugs (Dhami, 2013).

Microbes are truly the most underappreciated living organisms on Planet Earth. Billions of them can fit on a fingernail, and they make up more than half of the living biomass on the planet. The world we live in is one full of microbes. Microbes, whether they are good, bad, or benign, are certainly everywhere. This includes on our body, in our homes, far below the earth's surface and up to the atmosphere, in cold, cool, warm and hot and very hot places, and even in places without oxygen. Our body temperature and wealth of nutrients provide an ideal home for these micro-organisms to thrive. Microorganisms always live in water (directly in aquatic environments, in water inside animals or plants, or in water around soil particles). They can eat all sorts of things, including oil, rocks, dead and living plants and animals (Needham, 2000). There are 4 major types of Microbes: bacteria, fungi, protists and viruses (Lynch and Hobbie, 1988).

The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from natural sources including plants. Plant and plant products play a wide range of antimicrobial properties. Keeping in view, the present study to investigate the phytochemical and antimicrobial properties of *Ocimum sanctum*

MATERIALS AND METHODS

Plant materials:

The leaf of *Ocimum sanctum* were collected in January 2017 from Ganapathy Nagar, Thanjavur, Tamil Nadu, India.

PHARMACOGNOSTICAL STUDY

Anatomical Studies on leaf, root and stem of *Ocimum sanctum*

The fresh leaf, root and stem of *Ocimum sanctum* was collected and free hand sections were taken to obtain a thin section. The thickness of the section was 10-12 micrometers. Anatomical study invariably slides were prepared. The transverse sections of leaf was taken on a glass slide to which are added a few drops of chloral hydrate and was heated for 1-2 min, After placing a cover slip, care should be taken to avoid air bubbles and to see that there is sufficient chloral hydrate under the cover slip. Excess of chloral hydrate outside the cover slip is to be withdrawn using a blotting paper (Chloral hydrate is used to clear the tissues and to bring in clarity of the view) Lignified tissue is to be confirmed by staining. To the powder a few drops of mixture of 1:1 Phloroglucinol + Conc HCl was added and after 3 to 4 minutes observed under microscope. The well known identifying characters were taken Photomicrographs by Sony digital camera under microscope (10 x & 40x) (Wallis, 1989; Dutta, 1971; Evan and Trease, 2009).

Preparation of plant powder

The *Ocimum sanctum* leaves were collected and dried under shade. These dried leaves were mechanically powdered and stored in an airtight container. These powdered materials were used for further analysis.

DETERMINATION OF PHYSICO-CHEMICAL PARAMETERS

Physico-chemical parameters of the powdered drug such as ash value, extractive value, loss on drying and crude fiber content were performed according to the method described in WHO guidelines (WHO, 1998).

4.3 QUALITATIVE ANALYSIS OF PHYTOCHEMICAL OF *Ocimum sanctum*

Preparation of alcoholic extract:

The leaf of *Ocimum sanctum* was first washed well and dust was removed from the leaves. The leaf was dried at room temperature and coarsely powdered. The powder was extracted with aqueous and 70% methanol for 24 hours. The extract was stored in refrigerator until used.

Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

HISTOCHEMICAL ANALYSIS OF *Ocimum sanctum*

The powder of *Ocimum sanctum* was treated with specific chemicals and reagents. The treated plant powder further analysed in light microscope. The *Ocimum sanctum* treated with phloroglucinol and diluted HCl to gave red colour indicates lignin, treated with diluted ammonia and H₂SO₄ gave yellow colour indicates flavonoids and treated with Dragant draft reagent gave brown colour indicates alkaloids. Plant powder treated with ferric chloride to gave Dark blue to black indicates the presence of tannin. Plant powder treated with Conc HCl to gave Dark Black indicates the presence of crystals. Plant powder treated with 5 drops of acetic anhydride and 5 drops of H₂SO₄ to gave Violet to Blue (or) Green colour indicates the presence of steroids. Plant powder treated with Toluidine blue to gave Blue green/Red colour indicates the presence of polyphenol. Plant powder treated with Dinitrophenol hydrazine (few drops) to gave Orange colour indicates the presence of Terpenoids. Plant powder treated with H₂SO₄ (few drops) to gave Yellow colour indicates the presence of Saponin

DETERMINATION OF ANTIMICROBIAL ACTIVITY OF *Ocimum sanctum*

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Staphylococcus aureus* and *Escherichia coli* specie of bacteria were spread on Nutrient agar plates for bacteria and *Candida albicans* was spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room

temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

5. RESULTS

5.1. PHARAMACOGNOSTICAL STUDY OF *Ocimum sanctum*

5.1.1. Transversal section of the leaf

Transverse section of *Ocimum sanctum* through the midrib showed an upper and lower, single- layered epidermis that was externally covered with a thick, striated cuticle, a few epidermal cells on both lower and upper surfaces, parenchymatous cells that were thin-walled and isodiametric to circular Fig- 2. Intracellular spaces were present in ground tissue and the stele was crescent-shaped and composed of bicollateral and open vascular bundles. The xylem consisted mostly of vessels and tracheids, and a strip of cambium was present between the xylem and phloem tissues. The lamina which was dorsiventral with the mesophyll, was seen to be differentiated into a palisade and spongy tissue. The upper and lower epidermis were covered externally with a thick, striated cuticle. Below the upper epidermis were three rows of elongated, closely arranged, palisade parenchyma. Spongy parenchyma tissues were almost radially elongated with intracellular spaces. Central cells were irregular in shape; laticifers and vascular bundles were also present scattered in this region.

5.1.2. Transversal section of the root

Staining reveals different cell types in this light micrograph of a *Ocimum sanctum* root cross section. Sclerenchyma cells of the exodermis and phloem cells stain green. Other cell types stain black. The stele, or vascular tissue, is the area inside endodermis (indicated by a green ring). Root hairs are visible outside the epidermis. (credit: scale-bar data from Matt Russell). The vascular tissue in the root is arranged in the inner portion of the root, which is called the stele ([link]). A layer of cells known as the endodermis separates the stele from the ground tissue in the outer portion of the root. The endodermis is exclusive to roots, and serves as a checkpoint for materials entering the root's vascular system. A waxy substance called suberin is present on the walls of the endodermal cells. This waxy region, known as the Casparian strip, forces water and solutes to cross the plasma membranes of

endodermal cells instead of slipping between the cells (Figure- 3). This ensures that only materials required by the root pass through the endodermis, while toxic substances and pathogens are generally excluded. The outermost cell layer of the root's vascular tissue is the pericycle, an area that can give rise to lateral roots.

5.1.3. Transversal section of the stem

The outline of the stem and root section was almost circular Figure- 4. Section showed the structures as follows. The epidermis was an outermost layer of barrel to rectangular cells. The cells were thickly cuticularised. A few stomata occurred in the epidermis and a few unicellular or multicellular hairs were also present. The cortex was multi-layered and differentiated into (a) collenchyma and (b) parenchyma. A few layered collenchymatous hypodermises follows epidermis. It was 3-5 layered deep. Parenchyma followed collenchymatous hypodermis and was few cells deep. The cells were spherical to oval. The cells might contain a few to many chloroplasts. In endodermis, a distinct endodermis with Casparian strip was present. A prominent starch sheath was present in its place. Pericycle was represented by a few sclerenchymatous cells in the old stem. A large zone of vascular tissue lied just below the starch sheath. Starch sheath was followed by a large amount of conjunctive tissue in which secondary vascular bundles were embedded. Secondary phloem was situated just below the starch sheath. It was found in small groups. Two- layered ring of cambium separated secondary phloem from secondary xylem. Secondary xylem of secondary vascular bundle lied below the cambium. This secondary xylem was embedded in conjunctive tissue that appeared as a complete ring below the primary 6 vascular bundles were called medullary bundles. The central part of the section had large parenchymatous pith. Cambial activity took place in these medullary bundles.

5.2. DETERMINATION OF PHYSICO-CHEMICAL PARAMETERS

The results of physicochemical parameters such as loss on drying, total ash, acid insoluble ash, alcohol soluble extractive and water soluble extractive are shown in (Table - 1)

5.3 QUALITATIVE ANALYSIS OF PHYTOCHEMICAL OF *Ocimum sanctum*

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Ocimum sanctum* investigated and summarized in Table-2. The phytochemical screening aqueous extract of *Ocimum sanctum* leaf showed that the presence of, steroids, saponins, terepenoids, ,

glycosides, anthroquinone and protein Flavonoids, , triterpenoids alkaloids while tannin, alkaloids, protein, carbohydrate, phlobatannins, phenolics were absent. Methanol extract of *Ocimum sanctum* leaf showed that the presence of alkaloids, steroids, saponins, Flavonoids, triterpenoids, Polyphenol, anthriquinone and glycosides while tannin, alkaloids, protein, , phlobatannins, were absent

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Ocimum sanctum* investigated and summarized in Table-2. The phytochemical screening aqueous extract of *Ocimum sanctum* leaf showed that the presence of, steroids, saponins, terepenoids, , glycosides, anthroquinone and protein Flavonoids, , triterpenoids alkaloids while tannin, alkaloids, protein, carbohydrate, phlobatannins, phenolics were absent. Methanol extract of *Ocimum sanctum* leaf showed that the presence of alkaloids, steroids, saponins, Flavonoids, triterpenoids, Polyphenol, anthriquinone and glycosides while tannin (Figure - 5).

5.4. HISTOCHEMICAL ANALYSIS OF *Ocimum sanctum*

The use of histochemical characters in taxonomic conclusions is now a common practice. Table 3 and figure-6 represents histochemical studies of *Ocimum sanctum* leaf powder. This study further confirmed the presence of phytochemicals in *Ocimum sanctum* leaf.

5.5. DETERMINATION OF ANTIMICROBIAL ACTIVITY OF *Ocimum sanctum*

Extract of *Ocimum sanctum* was screened against *Escherichia coli* and *Staphylococcus aureus* species of bacteria and *Candida albicans* species of fungi were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Ocimum sanctum* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Figure- 8. The inhibitory activities in culture media of the *Ocimum sanctum* reported in Table-4 were comparable with standard

antimicrobial viz. chloromphenical and fluconazole.

6. DISCUSSION

Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments. Plants synthesize an array of chemical compounds that are not involved in their primary metabolism. These 'secondary compounds' instead serve a variety of ecological functions, ultimately to enhance the plants survival during stress. In addition these compounds may be responsible for the beneficial effects of leaf s and vegetables on an array of health related measures. Medicinal plants are assumes greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines (Liu, 2003).

6.1. Pharmacognostical studies on *Ocimum sanctum*

Pharmacognosy may be defined as "an applied science that deals with the biologic, biochemical and economic feature of natural drugs and their constituents." Modern aspects of science include not only the crude drugs but also their natural derivatives. Plant anatomy, in turn, has given rise to the independent science of cytology, which is the study of the cell, a rapidly developing field that plays a great role in the understanding of vital processes in general and of the phenomena of heredity and mutability in particular. Plant Anatomy is the branch of botany concerned with the internal structure of plants. It is closely related to plant physiology, the science of the vital processes which take place in plants.

In present study revealed the T.S. of *Clerodendrum phlomidis* leaf containing, upper and lower epidermis, spongy parenchyma, palisade cells. The upper and lower epidermis are single cell layered with cuticle, the epidermal consist of a single layer of cubical cells with covering trichomes and emergences, which are sparsely distributed and show the presence of an external cuticular layer. The upper epidermal cells are somewhat larger than the lower. The mesophyll consist of a polysade and spongy parenchyma. A single layer of polysade parenchyma only present. Spongy parenchyma with large intercellular space present. The vascular bundle is in the leaf do not show the presence of bundle sheath. Ground tissue of midvein consists of collenchyma and parenchyma tissues. Collenchyma is present as a

group of cells in the adaxial ridge and is three to four layered on the abaxial side, with cells angular thickened. Vascular tissue of midvein consists of an arc shaped vascular bundles, laterally.

Dinesh Kumar *et al* (2014) examined the pharmacognostic evaluation of *Clerodendrum phlomidis* Linn. in terms of organoleptic, fluorescence analysis, macro-microscopy and physicochemical parameters. The characteristic macroscopic study showed that the root consists of 7-15 cm long, 0.2 -3.0 cm thick pieces which are cylindrical, tough and yellowish-brown externally, with hard fracture and slightly astringent taste. The main microscopic characters of the root show exfoliating cork, having 10-15 rows of tangentially elongated, thick-walled cells. Cortex consists of round to oval parenchymatous cells, a few containing rhomboid shaped calcium oxalate crystals. Endodermis consists of 3-4 layers of non-lignified, thick-walled rounded parenchymatous cells followed by a single pericyclic layer. Phloem consists of isodiametric, thin-walled, parenchymatous cells whereas xylem contains lignified pitted vessels. Medullary rays consisting of biseriate layer of lignified and radially elongated parenchymatous cells is narrower in the xylem region during wider in the phloem region. The physicochemical analysis of the root, i.e., total ash, water-soluble ash, sulphated ash are 7.8, 0.9 and 10.3 (% w/w) respectively. Further successive extraction of the root powder with petroleum ether, chloroform, alcohol, water yielded 2.2, 2.4, 12.4 and 9.6 (% w/w) extracts respectively. Fluorescence study imparted characteristic colours to the root powder when observed under visible, short and long wavelength light.

6.2. Physicochemical analysis of *Ocimum sanctum* leaf

According to WHO (1992, 1996a and b) standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion.

The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. The

standardization of crude drugs is important before any work carried out. Therapeutic efficacy of medicinal plants depends upon the quality and quantity of chemical constituents. The misuse of herbal medicine or natural products starts with wrong identification. The physicochemical test is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken (Tatiya *et al.*, 2012).

Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Moisture content of drugs should be at minimal level to discourage the growth of Bacteria, Yeast or Fungi during storage. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not (Tatiya *et al.*, 2012). The results of physicochemical parameters such as loss on drying, total ash, acid insoluble ash, alcohol soluble extractive and water soluble extractive are shown in Table 1.

6.3. Qualitative analysis

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Ocimum sanctum* investigated and summarized in Table-2. The phytochemical screening aqueous extract of *Ocimum sanctum* leaf showed that the presence of, steroids, saponins, terpenoids, glycosides, anthroquinone and protein Flavonoids, triterpenoids alkaloids while tannin, alkaloids, protein, carbohydrate, phlobatannins, phenolics were absent. Methanol extract of *Ocimum sanctum* leaf showed that the presence of alkaloids, steroids, saponins, Flavonoids, triterpenoids, Polyphenol, anthriquinone and glycosides while tannin, alkaloids, protein, phlobatannins, were absent.

Falodun *et al.* (2006) reported the occurrence of flavonoids, saponins, diterpenes and phorbol estersin in the aqueous and methanol extracts of *Euphorbia heterophylla*. Raghavendra *et al.* (2006) examined the powdered leaf material of different solvent of *Oxalis corniculata* and reported the presence of

phenols, glycosides, carbohydrates, phytosterols and tannins. Awoyinka *et al.* (2007) extracted eight bioactive compounds from dry leaf of *Cnidioscolus aconitifolius* using water and ethanol. Different extracts of *Semecarpus anacardium* were analysed by Mohanta *et al.* (2007) for its phytochemical properties.

Onwukaeme *et al.* (2007) detected reducing sugars, phenols, tannins and flavonoids in *Pycnanthus angolensis*. Uma Devi *et al.* (2007) carried out the phytochemical analysis in *Achyranthes bidentata*. The methanol and acetone extracts of 14 plants belonging to different families were evaluated for phytochemical analysis and this study revealed the presence of tannins, cardiac glycosides, steroids and saponins (Vaghasiya and Chanda, 2007). Ayoola *et al.* (2008) investigated the phytochemical components of four medicinal plants used for the treatment of malaria in Southwestern Nigeria. *Ichnocarpus frutescens* leaf, stem and root were investigated (Mishra *et al.*, 2009) for its phytochemical and phytochemical properties.

6.4. Histochemical studies

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues (Krishnamurthy, 1998). Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major storage compounds such as proteins, lipids, starch, phytin and minerals like calcium, potassium and iron (Krishnan *et al.*, 2001). The importance of histochemistry in solving critical biosystematic problems is as popular as the use of other markers. According to botanical literatures, the use of histochemical characters in taxonomic conclusions is now a common practice. Table 3 and fig.7 represents histochemical studies of *Ocimum sanctum* leaf powder. This study further confirmed the presence of phytochemicals in *Ocimum sanctum* leaf.

6.5. Antimicrobial activity

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects are commonly associated with popular synthetic

antibiotics, such as hypersensitivity, allergic reactions, and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman *et al.*, 2003).

This situation forced scientists to search for new antimicrobial substances with plant origin. Extract of *Ocimum sanctum* was screened against *Escherichia coli* and *Staphylococcus aureus* species of bacteria and *Candida albicans* species of fungi were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Ocimum sanctum* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 8. The inhibitory activities in culture media of the *Ocimum sanctum* reported in Table.4 were comparable with standard antimicrobial viz. chloromphenical and fluconazole.

Ali Rehman *et al.* (2002) proposed that the aqueous and ethanolic extracts of *Azadirachta indica* have antimicrobial activity against *Microsporum canis*, *Aspergillus fumigatus*, *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* by disc diffusion method. There was no zone of inhibition of *Acalypha indica* towards *Aspergillus fumigatus* and *Candida albicans*. The leaves and roots of the aqueous extract of *Azadirachta indica* inhibit the growth of *Microsporum canis*. There was no inhibition zone of inhibition of ethanol and aqueous extract of leaves, seeds roots and stem of *Acalypha indica* against *Staphylococcus aureus* and *Escherichia coli*.

Uma and Sasikumar (2005) stated that different organic and alcoholic extracts of *Calotropis gigantea*, *Justica adhatoda*, *Moringa oleifera* and *Poper betle* have antimicrobial activity against certain bacterial pathogens *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumoniae* and fungal strains of *Aspergillus niger* and *Rhizopus sp.* The plant extracts exhibited broader and moderate activity against all the microbial pathogens at all 100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml concentration.

CONCLUSION

Various pharmacognostic parameters evaluated in this study helps in botanical identification and standardization of *Ocimum sanctum* leaf part in crude form and provide the authentic data for the researchers and scientists involved in carrying out further research on this plant part. The phytochemical screening aqueous extract of *Ocimum sanctum* leaf showed that the presence of, steroids, saponins, terpenoids, glycosides, anthraquinone and protein Flavonoids, triterpenoids while tannin, alkaloids, protein, carbohydrate, phlobatannins, phenolics were absent. Methanol extract of *Ocimum sanctum* leaf showed that the presence of alkaloids, steroids, saponins, Flavonoids, triterpenoids, Polyphenol, ntriquinone and glycosides while tannin, alkaloids, protein, phlobatannins, were absent. Results of the histochemical study revealed that *Ocimum sanctum* powder treated with phloroglucinol and diluted HCl gave red colour indicates lignin, treated with diluted ammonia and H₂SO₄ gave yellow colour indicates flavonoids and treated with Mayer reagent reddish brown colour indicates alkaloids. This study further confirmed the presence of phytochemicals. The results reveal that extract of *Ocimum sanctum* were significantly effective against both bacteria *E. coli*, *St. aureus* and fungi *C. albicans* and *aspergillus flavus*. The leaves of *Ocimum sanctum* are a newly discovered potential source of natural antimicrobial compounds. The synergistic effect of plant extract against resistant bacteria and fungi leads to new choices for the treatment of infectious diseases.

REFERENCES

- Akoachere JF, Ndip EB, Chenwi LM, Ndip TE, Anong DN. Antibacterial effect of *Zingiber officinale* and *Garcinia kola* on respiratory tract pathogens. *East Afr. Med. J.* 2002;79:588-592.
- Akter S, Tabibul I, Humayun K. A selection of medicinal plants used as blood purifiers by folk medicinal practitioners of Bangladesh. *American-Eurasian J of Sustainable Agriculture.* 2012;6(3):188-194.
- Ali Rehman, Farkhanda Asad, Noureen Aziz Qureshi, Shabnoor Iqbal. Effect of plant feed ingredients (soybean and sunflower meal) on the growth and body composition of *Labeo rohita*. *American Journal of Life Sciences* 2002; 1(3): 125-129
- Alper, J (1998). Effort to combat microbial resistance lags. *ASM News* 64: p440-441.
- Amritpal LV, Kakkar KK, Chakre OI. Glossary of Indian medicinal plants with active

- principles. Publication and Information Directorate, Council of Scientific and Industrial Research, NewDelhi, India. 2008;27.
- Ashis G. (2003) Herbal folk remedies of Bankura and Medinipur districts, West Bengal. Indian Journal of Traditional Knowledge. 2: 393-396.
- Awoyinka OA, Balogun IO and Ogunnow AA.(2007) Phtochemical screening and in vitro bioactivity of *Cnidoscopus aconitifolius* (Euphorbiaceae).J. Med. Plant Res, 1: 63-95.
- Awoyinka, O.A., Balogun I.O. and Ogunnow, A.A. 2007. Phtochemical screening and in vitro bioactivity of *Cnidoscopus aconitifolius* (Euphorbiaceae). J. Med. Plant Res, 1: 63-95.
- Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello, Obaweya K, Ezennia EC and Atangbayila TO. (2008) Phytochemical screening and antioxidant activities of some selected medicinal plants used for *Malaria therapy* in Southern Nigeria. *Tropical, J. Pharmaceutial. Res*, 7: 1019-1024.
- Baby Joseph and Vrundha M. Nair.. Ethanopharmacological and Phytochemical Aspects of *Ocimum sanctum* Linn- The Elixir of Life. British Journal of Pharmaceutical Research, 3(2): 273-292, 2013
- Bhagwati Uniyal. (2003) Utilization of medicinal plants by the rural women of Kulu, Himachal pradesh, Indian journal of Traditional knowledge, 2(4): pp 366-370.
- Bhatt N, Chawla SL, Rao MV. Contraception evaluation of seed extracts of *Abrus precatorius* L. in male albino rats (*Mus musculus*). J Herb Med Toxicol. 2007;1:45-48.
- Bohlin L., Goransson U., Backlund A. (2007). Modern pharmacognosy: Connecting biology and chemistry. Pure and Applied Chemistry 79, 763-774.
- Bolivara, P., Paredesa, C.C., Hernándezb, L.R., Juárezc, Z.N., Sánchez-Arreolab, E., Av-Gaya, Y., Bacha, H., 2011. Antimicrobial, anti-in.ammatory, antiparasitic and cytotoxic activities of *Galium mexicanum*. Journal of Ethnopharmacology 137, p141–147.
- Bowersox., and John., (1999). Experimental Staph Vaccine Broadly Protective in Animal Studies, NIH. Retrieved on 2007.
- Bramowisz, M., (1990). The choice of antimicrobial drugs med. Left. Drugs Therapies. 32, p41-48
- breathing fish, *Clarias batrachus* and in non-air breathing one *Ctenopharyngodon idellus*. In: International Congress on the Biology of Fish, University of British Columbia, Vancouver, Canada, July 21-26, pp.109-118.
- Britto J, Gracelin H. *Datura metel* – a plant with potential as antibacterial agent. International J. Applied Biol. And Pharmaceutical Technol. 2011;2:429-433
- Chakraborty A, Chowdhury BK, Bhattacharya P. Clausenol and clausenine-two carbazole alkaloids from *Clausena anisata*. Phytochemistry.1995;40:295-8.
- Corner BE, Magee PT (1997) *Candida* pathogenesis: Unravelling the threads of infection. Curr Biol 7:R691–R694
- Curran, J.P., and Al-Salihi, F.L.,(1980). Neonatal staphylococcal scalded skin syndrome: massive outbreak due to an unusual phage type. Pediatrics. 66 (2), 285-90
- Dahanukar SA, Thatte UM. Current status of ayurveda in phytomedicine. Phytomedicine. 1997;4:359-68.
- Dahanukar. (2000) Will the ‘good fairies’ please prove to us that vitamin E lessens human degenerative disease? Free Radical Research, 27: 511-532.
- Davis RH, Agnew PS, Shapiro E. Antiarthritic activity of anthraquinones found in aloe vera for podiatric medicine. J. The American Podiatric Medical Assoc. 1986;76(2):1-8.
- Dey BB, Choudhary MA. Effect of plant development stage and some micronutrients on eugenol content in *O. sanctum* L. determination of eugenol by Folin-Ciocalteu reagent. Indian Perfumer. 1980;24:199–203.
- Dhami, N. (2013). “Trends in Pharmacognosy: A modern science of natural medicines”. Journal of Herbal Medicine. 3 (4): 123–131.
- Dinesh Kumar., Ajay Kumar and Om Prakash., (2014). Pharmacognostic investigation of *Clerodendrum phlomidis* L. root. Journal of Tropical Life Science., 4(2):96-100.
- Doern, G.V., (1995). Susceptability test for fastidious bacteria. Manual of clinical microbiology, 6 th edition. Murray, P.R., Boron, E.J., Pfaller, M.A., Tenover, F.C., and Yolken, R. American society for microbiology, Washington DC. 1342-1349
- Dorman HJD, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Applied Microbiol.2000;88:308-316.
- Dorman HJD, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J. Applied Microbiol.2000;88:308-316.

- Doshi JC, Vaidya AB, Antarkar DS. A twostageclinical trial of *Phyllanthus amarus* in
- Dua VK, Nagpal BN, Sharma VP. Repellent action of neem cream against mosquitoes. Indian J Malariol. 1994;32:47-53.
- Dua VK, Nagpal BN, Sharma VP. Repellent action of neem cream against mosquitoes. Indian J Malariol. 1995;32:47-53.
- Dubey R, Dubey K, Sridhar C, Jayaveera KN. Sperm immobilization activity of aqueous, methanolic and saponins extract of bark of *Ziziphus mauritiana*. DerPharmacia Sinica. 2011;2(2):11-16.
- Dutta NK and Roy RN., Bhagwager S., Chavan R. (1971) Preliminary pharmacological studies on extracts of roots of *Asparagus racemosus* satavari, Willd. (Liliaceae). Journal of Research Indian Medicine. 6: 132-138.
- Eisenberg, DM., Kessler RC., Foster C., Norlock FE., Calkins DR, and Delbanco TL. (1993). Unconventional medicine in the United States: prevalence, costs and patterns of use. N. Engl. J. Med. 328: p246–252
- Eisenberg, DM., Kessler RC., Foster C., Norlock FE., Calkins DR, and Delbanco TL. (1993). Unconventional medicine in the United States: prevalence, costs and patterns of use. N. Engl. J. Med. 328: p246–252
- Evans WC and Trease GE.,(2009).Pharmacognosy.11th edn. Brailliar Tiridel can.Macmillian Publishers. U.S.Environmental protection Agency, Draft Criteria document for carbon tetrachloride, criteria and standards Division,office of Drinking Water, Washington,DC.
- Falodun A, Okunroba L.O and Uzoamaka N. (2006) Phytochemical screening and anti-inflammatory evaluation of methanolic and aqueous extracts of *Euphorbia heterophylla* L. (Euphorbiaceae). Afr. J. Biotechnol, 5: 529-531.
- Fotadar, U., Zaveloff, P., and Terracio, L., (2005). Growth of *Escherichia coli* at elevated temperatures. J. Basic Microbiol. 45 (5), p403–4
- Ganesan S, Suresh N, Kesaven L. Ethnomedicinal survey of lower Palani Hills of Tamilnadu. I J Trad Knowledge.2004;3(3):299-304.]
- Ganesan S, Suresh N, Kesaven L. Ethnomedicinal survey of lower Palani Hills of Tamilnadu. I J Trad Knowledge.2004;3(3):299-304.]
- Gupta I, Parihar A, Malhotra P, Effects of *Boswellia serrata* gum resin in patients with ulcerative colitis. Eur J. Med Res. 1997;2:37-43.
- Ham A, Sancheti G, Shrivastava A, Pawar S. Traditional medicines of Gonds and Bharias-26- Herbal medicines for Dental Diseases. Indian J. Pharmacol. 2006;38: 142-144.
- Harborne JB.,(1984).Phytochemical methods. A Guide to Modern Technique of Plant Analysis. London: Chapman and Hall.
- Harborne,JB.,(1973).Phytochemical methods, London. Chapman and Hall Ltd.pp.49-188.
- Jadhav D. Plant sources used for the treatment of different types of fevers by Bhil Tribe of Ratlam district, Madhya Pradesh, India. J Econ Taxon Bot.2006;30(4):909-911.
- Javadzadeh HR, Davoudi A, Davoudi F, Valizadegan G, Goodarzi H, Mahmoodi S. Citrullus colocynthis as the cause of acute rectorrhagia. Case Reports in Emergency Medicine. 2013;Article ID 652192:1-5. DOI: 10.1155/2013/652192
- Joshi, P.K., Bose, M., 2002. Toxicity of cadmium: a comparative study in the air
- Joy PP, Thomas J, Mathew SS and Baby.(2002). Medicinal plants, Aromatic and Medicinal Plants. Research Station., 2-9.
- Junaid A, Abdul M, Maheshwar PS. Somatic embryos in *Catharanthus roseus*: A scanning electron microscopic study electron microscopic study. Not Sci Biol. 2014;6(2):167-172.
- Karaman L, Sahin F, Gulluce M, Ogutcu H, Sngul M, Adiguzel A. (2003) Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J Ethnopharmacol.; 85: 231-235.
- Kotkar HM, Mendki PS, Sadan SV, Jha SR, Upasani SM, Maheshwari VL. Antimicrobial and pesticidal activity of partially purified flavonoids of *Annona squamosa*. Pest. Manag. Sci. 2002;58:33-37.
- Kotnis P, Patel SN, Menon and Sane RT. (2004) Reneprotective effect of *Hemisdesmus indicus*, a herbal drug used in gentomicin-induced renal toxicity. Nephrology.(Carlton), 3: 142-152.
- Kripa KG, Chamundeeswari D, Thanka J. Reddy CUM effect of hydro alcoholic extract of aerial parts of *Leucas aspera* (Willd.) link on inflammatory markers in complete freund's adjuvant inducedarthritic rats. International J. Green Pharmacy. 2010;4(4):281-287.
- Krishnamurthy KV. (1998) Methods in plant histochemistry, S. Viswanathan, Chennai.
- Krishnan S, Ebenezer GAI and Dayanandan P. (2001) Histochemical localization of storage

- components in Caryopsis of rice (*Oryza sativa* L.) *Curr Sci*; 80: 567-571.
- Kumar AM. Ethnomedicinal plants as anti-inflammatory and analgesic agents. *Research Signpost*. 2010;3(2):267-293.
- Lev E. Ethno-diversity within current ethnopharmacology as part of Israeli traditional medicine: A review. *J. Ethnobiol. And Ethnomedicine*. 2006;2(4).
- Liu C.Z., Murch S.J., El-Demerdash M. (2003) Regeneration of Egyptian medicinal plant *Artemisia judaica* L. *Plant Cell Rep*. 21(6): 525-530
- Lynch, J.M and J.E Hobbie. (1988). *Micro-organisms in action: concepts and application in microbial ecology*. Blackwell Scientific Publications.
- Mayo JL. (1998) Black cohosh and chaste berry: herbs valued by women for centuries. *Clinical Nutrition Insight* 6: 1-4.
- Mazumdar UK, Gupta M, Maiti S. Effect of petroleum ether extract from *Hygrophila spinosa* on hematological parameters and hepatorenal functions in mice. *Indian J Exp Biol*. 1996;34:1201-1203.
- Mishra A, Dusmanta kumar P, Mishra Mases Ranjan, Susil K and Arnabaditya M. (2009) phytochemical screening of *Ichnocarpus frutescens* plant parts. *Inter J. Pharmacogony and Phytochemical Res*, 1: 25-31.
- Mohanta TK, Patrea JK, Rath SK, Pal DK and Thantoi HN. (2007) Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Samacapus anacardium* L.F. *Sci. Res. Essay*, 2: 486-490.
- Mpiana PT, Tshibangu DST, Shehonde OM, Ngbolua KN. In vitro antidrepanocytary activity (antisickle cell anemia) of some congolese plants. *Phytomedicine*. 2007;14(2-3):192-195.
- Mundewadi A, Female infertility. *J. Ayurvedic Herbal Treatment*. 2009;3:121-125.
- Nair R, Chanda S. Activity of some medicinal plants against certain pathogenic bacterial strains. *Indian J. Ethnopharmacol*. 2005;96:461-509.
- Nair R, Chanda S. Activity of some medicinal plants against certain pathogenic bacterial strains. *Indian J. Ethnopharmacol*. 2005;96:461-509.
- Narayana, Subhose V. *Bull Indian Inst hist med Hyderabad*. *Indian J. Pharmacology*. 2005;35:21-35.
- Needham, C et al. (2000). *Intimate Strangers: Unseen Life on Earth*. ASM Press, Washington D.C.
- Nguyen H, Lemberkovics E, Tarr K, Mathe II, Petri G. A comparative study on formation of flavonoid, tannin, polyphenol contents in ontogenesis of *Ocimum basilicum* L. *Acta Agronomica Hungarica*. 1993;42:41-50.
- Norr H, Wanger H. New constituents from *Ocimum sanctum*. *Planta Med*. 1992;58:574.
- Ogston (1984). Bacterial infections in surgical patients. Prospects, goals and means in modern research. *Pharm. Wkly. (Sci)* 4, 93-103
- Onwukaeme, D.N. Ikuegbvweha, T.B. and Asonye, C.C. (2007). Evaluation of phytochemical constituents, antibacterial activities and effects of exudates of *pycanthus angolensis* Wedl. (Myrsicaceace) on conneal ulcers in rabbits. *Trop. J. Pharm. Res*. 6: 725-730.
- Owoyele BV, Soladoye AO. Analgesic and anti-inflammatory activity of ethanolic extract of *Chromolaena odorata* leaves. *Recent Progr Med Plants*. 2007;18:397-406.
- Panghal M, Vivek K, Jay P. In vitro antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. *J. Clinical Oncology*. 2011;18:3083-51.
- Parmar S, Gangwal A, Sheth N. *Solanum xanthocarpum* (Yellow Berried NightShade): A review. *Der Pharmacia Lettre*. 2010;2:373-383.
- Pattewar SV. *Kalancho epinnata*: Phytochemical and pharmacological profile. *International J. Phytopharmacy*. 2012;2:1-8.
- Perea S, Patterson TF. Antifungal resistance in pathogenic fungi. *Clin Infec Dis* 2002; 35:1073-80.
- Pradhan D, Panda PK, Tripathy G, Wound healing activity of aqueous and methanolic bark extracts of *Vernonia arborea* Buch.-Ham. in Wistar rats, *Natural Product Radiance*, 8(1), 2009, 6-11.
- Premanathan M, Nakashima H, Kathiresan K. *In vitro* anti-human immunodeficiency
- Raghavendra MP, Sathish S and Raveesha KA. (2006) Phytochemical analysis and antibacterial activity of *Oxalis corniculata*-A medicinal plant. *My Sci*, 1: 72-78.
- Ray, G., Batra, S., Shkula, N.K., Deo, S., Raina, V., Asok, S., Husain, S.A. (1996). Lipid peroxidatio, free radicals production and antioxidant status in breast cancer. *Breast Cancer Res Treat* 59,163-170.
- Robert, K., Egon, S., Daniela, B., Florian, D., Christoph, W., G'unter, J.K., Emil, C.R., (2001). Role of candida in antibiotic-associated diarrhoea. *Journal of Infectious Diseases* 184, 1065-1069.

- Rossato SC, Leituo-filho H, Gegossi A. Ethnomedicinal knowledge of the atlantic forest coast (Brazil). *Economic Botany*. 1999;53:387-395.
- Saha S, Goswami G. Study of anti ulcer activity of *Ficus religiosa* L. on experimentally induced gastric ulcers in rats. *Asian Pac J Trop Med* 2012; 791-793.
- Sakharkar PR, Patil AT. Antimicrobial activity of *Cassia alata*. *Indian J. Pharmaceutical Sciences*. 1998;60:311-12.
- Santhya B, Thomas S, Isabel W, Shenbagarathai R. Ethnomedicinal plants used by the Valaiyan community of Piranmalai hills (Reserved Forest), Tamilnadu, Indian- A pilot study. *Afr J Trad CAM*. 2006;3(1):101-114.
- Sarkar M, Gangopadhyay P, Basak B, Chakrabarty K, Banerji J, Adhikary P. The reversible antifertility effect of *Piper betle* Linn. on Swiss albino male mice. *Contraception. J. Life Sci*. 2000;62(5):271-274.
- Satyavati GV. (1982) Some traditional medical systems and practices of global importance. *Indian Journal of Medical Research*, 76: 1-5.
- Sharma P, Tomar L, Bachwani M, Bansal V. Review on neem (*Azadirachta indica*): Thousand problem one solution. *Int. Res. J. of Pharmacy*. 2011;2:97-102.
- Sharma P, Tomar L, Bachwani M, Bansal V. Review on neem (*Azadirachta indica*): Thousand problem one solution. *Int. Res. J. of Pharmacy*. 2011;2:97-102.
- Sharma V, Joshi BD. Traditional uses of medicinal plants in Almora district of Kumaon Himalaya, Uttarakhand. In: *Biodiversity and Environmental management* (Joshi, B. D., Tripathi, C.P.M. & Joshi, P.C. Eds). APH Publishing Corporation- New Delhi. 2008;167173.
- Singh R, Khan NU, Singhall KC. In vitro antifilarial activity of *Sencio nudicaulis* Buch. Ham. Effect on *Setaria cervi* (Nematoda Filarioidea). *Indian J Physiol Pharmacol*. 1996;40:2316.
- Singh V, Munish J, Jyoti G, Pawan K. Antibacterial activity of medicinal plants against extended spectrum beta lactamase producing bacteria causing urinary tract infection. *Int. J. Drug Res. Tech*. 2012;2(3):263-267.
- Sivaranjani R, Ramakrishnan K. Traditional uses of medicinal plants in treating skin disease in Nagapattinam District of Tamilnadu, India. *International Research Jof Pharmacy*. 2012;3(5):201-204
- Skaltsa H, Philianos S, Singh M. Phytochemical study of the leaves of *Ocimum sanctum*. *Fitoterapia*. 1987;8:286.
- Skaltsa H, Tzakou O, Singh M. Polyphenols of *Ocimum sanctum* from Suriname. *Pharmaceut. Bio*. 1999;37:92-94.
- Sofowara A. (1993) *Medicinal plants and Traditional medicine in Africa*. Spectrum Books Ltd, Ibadan, Nigeria. pp. **191-289**.
- Spandana U, Shaik LA, Nirmala T, Santhi M, Sipai Babu SD. A review on *Tinospora cordifolia*. *International J Current Pharmaceutical Review and Research*. 2004;4(2):61-68.
- Subramoniam A, Pushpangadan P, Rajasekharan S, Latha PG. Antipyretic activity of TBR-002, A herbal formulation. *Ancient Sci. Life*. 2005;15:7-14.
- Sukumar E, Hamsaveni GR, Bhima RR. Pharmacological actions of cerpegin, a novel pyridine alkaloid from *Ceropegia juncea*. *Fitoterapia. International J of Medicinal Plant*. 1995;66:403-406.
- Suresh B, Dhanasekaran S, Elango K. Anti-pyretic activity of some plants in female albino rats: A preliminary report. *Ancient Sci. Life*. 2005;14:253-257.
- Tatiya A, Surana S, Bhavsar S, Patil D and Patil Y. (2012) Pharmacognostic and preliminary phytochemical investigation of *Eulophia herbacea* Lindl. Tubers (Orchidaceae). *Asian Pac J Trop Disease*, 2(1):50-55.
- Thongrakard V, Tencommao T. Modulatory effects of Thai medicinal plant extract on proinflammatory cytokines-induced apoptosis in human keratinocyte HaCaT cells. *African J. Biotechnol*. 2012;9:4999-5003.
- Trease GE, Evans WC. (1989). *Pharmacognsy*. 11th edn. Brailliar Tiridel. Macmillian Publishers. U.S. Environmental protection Agency, Draft Criteria document for carbon tetrachloride, criteria and standards Division, office of Drinking Water, Washington, DC.
- Tripathy S, Pradhan D, Anjana M. Antiinflammatory and antiarthritic potential of *Ammania baccifera* Linn. *International J. Pharma and Bio Sci*. 2010;1(3):1-7.
- Uma and Saikumar P. (2005) Pharmacognostic Studies on *Rumex vesicarius*. *Asian Journal of Plant Science and Research*, 1 (1):102-115.
- Uma Devi, P., Murugan, S., Suja, S., Selvi, S., Chinnaswamy, P. and E. Vijayanand. (2007). Antibacterial, in vitro lipid peroxidation and phytochemical observation

- on *Achyranthes bidentata* Blume. Pak. J. Nutrition, 6: 447-451.
- Umar D, Mishra SK, Tandan SK, Tripathi HC. Possible mechanism of anthelmintic action of palasonin on *Ascaridia galli*. Indian J Pharmacol. 1995;27:161-8.
- Upadhyay SN. Therapeutic potential of immunomodulatory agents from plant products. In: SN Upadhyay Ed Immunomodulation, New Delhi, NarosaValecha N, Biaswas S, Badoni V. Antimalarial activity of *Artemisia japonica*, *Artemisia maritima* and *Artemisia nilegarica*. Indian J Pharmacol. 1997;26:144-6.
- Vaghasiya, Y. and Chanda, S.V. (2007). Screening of methanol and acetone extracts of fourteen Indian medicinal plants for antimicrobial activity. Turk J. boil, 31: 243-248.
- Valecha N, Biaswas S, Badoni V. Antimalarial activity of *Artemisia japonica*, *Artemisia maritima* and *Artemisia nilegarica*. Indian J Pharmacol. 1996.;26:144-6.
- Venugopal PV, Venugopal TV. Antidermatophytic activity of neem(*Azadirachta indica*) leaves *in vitro*. IndianJ Pharmacol. 1994;26:141-3.
- Wallis TE., (1989).Practical Pharmacognosy, Sixth edition.178-186.
- WHO, IUCN and WWF.(1993) Guidelines of the conservation of medicinal plants, (IUCN Gland Switzerland).
- WHO.(1996a) Quality assurance of pharmaceuticals: A compendium of guidelines and related materials, good manufacturing practices and inspection. World Health Organization, Geneva. 2.
- WHO.,(1998).Quality control methods for medicinal plant materials. Geneva: World Health Organization.
- Yav, Gorina EV, Saprykina EA, Gereng. Evaluation of hepatoprotective activity of water soluble polysaccharide fraction of *Stellaria media* L. Bulletin of Experimental Biol. and Medicine. 2013;154:645-648.