WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 18, 865-893. Research Article ISSN 2277–7105

PHARMACOGNOSTIC & PHYTOCHEMICAL EVALUATION OF OCIMUM SANCTUM (ROOT) WITH INVITRO ANTIOXIDANT & ANTIARTHRITIC ACTIVITY

Rameshwar Kumar*, Safadar Hasan Qureshi, Dr. Yogesh Shivhare and Dr. Pratyush Jain

Rkdf College of Pharmacy Nh-12, Hoshangabad Road, Bhopal.

Article Received on
31 July 2024,

Revised on 20 August 2024,
Accepted on 09 Sept. 2024

DOI: 10.20959/wjpr202418-33890

***Corresponding Author**
Rameshwar Kumar
Rkdf College of Pharmacy
Nh-12, Hoshangabad Road,
Bhopal.

ABSTRACT

Humans have relied on nature for their easy necessities as being the sources for drug treatments, shelters, meals, fragrances, apparel, flavours, fertilisers and method of transportation. For the large proportions of global's populace medicinal plants retain a dominant role in the healthcare gadget and this is in particular proper in developing international locations, where herbal remedy has non-stop history of lengthy use. The improvement and popularity of medicinal and economic aids of those flora are on rise in each industrialised and growing nation (WHO, 1998). The rules of regular traditional systems of drugs for heaps of years which have been in life have formed from flora. The flowers remain to provide mankind with new drugs. some of the beneficial properties ascribed to plant life have been known to be incorrect and medicinal plant treatment is based totally on the experimental findings of loads to thousands of years. The earliest reports carved on clay pills in cuneiform date from about 2600 BC are from Mesopotamia; many of the substances that have been used were oils of Commiphora species (Myrrh), Cedrus species (Cedar), Glycyrrhiza glabra (Licorice), Papaver somniferum (Poppy juice) and Cupressus sempervirens (Cypress) are nonetheless used today for the treatment of diseases extending from colds and coughs to irritation and parasitic infections (Fakim, 2006). Ocimum sanctum, popularly known as Tulsi, is one of the sacred annual herbs belonging to the genus Ocimum and mint family Lamiaceae. Tulsi is a native of Iran, Afghanistan and India (Zargari, 1990; Mirhaidar, 1990; Volak and Jiri, 1997 and Mann et al. 2000). Tulsi is believed to be the "Queen of Herbs"(Verma, 2016). It is the most therapeutic herb distributed mainly in all

regions of India (Jeba et al. 2011).

KEYWORDS: Ocimum sanctum, Tulsi, Myrrh, Cedar, Cypress, Lamiaceae, drugs, Licorice, flora.

1.1 INTRODUCTION

Humans have relied on nature for their easy necessities as being the sources for drug treatments, shelters, meals, fragrances, apparel, flavours, fertilisers and method of transportation. For the large proportions of global's populace medicinal plants retain a dominant role in the healthcare gadget and this is in particular proper in developing international locations, where herbal remedy has non-stop history of lengthy use. The improvement and popularity of medicinal and economic aids of those flora are on rise in each industrialised and growing nation (WHO, 1998).

The rules of regular traditional systems of drugs for heaps of years which have been in life have formed from flora. The flowers remain to provide mankind with new drugs. some of the beneficial properties ascribed to plant life have been known to be incorrect and medicinal plant treatment is based totally on the experimental findings of loads to thousands of years. The earliest reports carved on clay pills in cuneiform date from about 2600 BC are from Mesopotamia; many of the substances that have been used were oils of Commiphora species (Myrrh), Cedrus species (Cedar), Glycyrrhiza glabra (Licorice), Papaver somniferum (Poppy juice) and Cupressus sempervirens (Cypress) are nonetheless used today for the treatment of diseases extending from colds and coughs to irritation and parasitic infections (Fakim, 2006).

Advantages of herbal medicine

- They have got lengthy records of use and higher affected person tolerance in addition to reputation.
- Medicinal plants have a renewable supply, that is only hope for sustainable elements of cheaper drugs for the sector's growing populace.
- Availability of medicinal flora isn't a hassle mainly in developing international locations like India having wealthy agro-climatic, cultural and ethnic biodiversity.
- Extend and reputedly uneventful use of natural medicines may additionally offer testimony of their protection and efficacy.
- At some stage in the sector, herbal medication has provided a number of the maximum mighty medicines to the huge arsenal of medicine to be had to fashionable medicinal

science, each in crude shape and as a natural chemical upon which current medicines are based (Harrison, 1998).

Natural Remedy: The WHO has currently defined traditional medicine (which include natural drugs) as comprising therapeutic practices that have been in existence, regularly for loads of years, before the improvement and spread of present day medicine and are still in use today. conventional medicine is the synthesis of therapeutic enjoy of generations of practicing physicians of indigenous structures of medicine. traditional preparations contain medicinal flowers, minerals and organic count numbers and so forth. Natural pills constitute only those traditional medicines which typically use medicinal plant preparations for remedy. The earliest recorded proof of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts consist of Rigveda, Atharvaveda, Charak Samhita and Sushruta Samhita. The natural drug treatments / traditional medicaments have therefore been derived from rich traditions of historic civilizations and scientific history (Rastogi et al., 1990).

Bioavailability of Herbal Drugs: The bioavailability of the lively elements of the herb is some other region of huge significance. Before a compound can act systemically it must pass from the gastrointestinal tract into the bloodstream. that is a place in which extraordinarily little is known for natural parts (Galbley et al., 1999).. Compounds, such as berberine and hydrastine inside the popular botanical are basically not absorbed following oral consumption. Studies displaying systemic impact in animals have all involved parenteral administration of those alkaloids. yet goldenseal stays one of the exceptionally-selling herbs, is extensively promoted, and is common by a misinformed public as a nonspecific immune stimulant (Cragg et al., 1997). Cinnabar has been for a long term in conventional medication. The toxic consequences of inorganic mercury are well identified, however due to its insolubility it's been assumed that this compound could not be considerably absorbed from the gastrointestinal tract. However, research of the oral absorption of cinnabar in mice discovered a significant increase in mercury awareness inside the liver and kidney. Concomitant use of cinnabar and capsules containing bromides, sulphates, sulphides, nitrates and iodine may additionally decorate its toxicity by increasing the gastrointestinal absorption (Mittermeier et al., 2005).

Status of Herbal Medicinal Drug in India: India has a wealthy subculture of natural medicine as evident from Ayurveda, which could not have flourished for 2 thousand years

with non clinical foundation. Ayurveda which literally means know-how (Veda) of life (Ayur) had its origin in Atharvaveda (Circa 1500-1000 BC). Charak Samhita and Sushruta Samhita are the 2 maximum well-known treatises of Ayurveda; numerous others have been compiled over the centuries inclusive of Bela Samhita, Kashyap Samhita, Agnivesh. Tantra, Vagbhata's Ashtang hridaya (600), Madhava Nidan (700 ad) (Kaul, 1997). Vegetable products ruled Indian Materia Medica which made large use of bark, leaves, flower, fruit, root, tubers and juices. The idea of rasa, vipaka, virya and prabhava formed the premise of Ayurveda pharmacology, which made no clear distinction between diet and drug, as each have been crucial aspect of remedy harak, Sushruta and Vagbhata defined seven hundred natural capsules with their houses and medical effects (Singh, 2015). primarily based on medical effects 50 classes of drug have been described–consisting of appetisers, digestive stimulant, laxatives, anti-diarrhea, anti- haemorrhoid, antiemetic, antipyretic, antipruritic, antiasthmatic, anti-epileptic, anti-helminthic, haemopoietic, haemostatic, analgesic, sedative, promoter of life (Rasyana), promoter of electricity, complexion, voice, semen and sperm, breast milk secretion, fracture and wound healing, destroyer of kidney stones and many others (Mittermeier et al., 2005). The advent of western remedy within the eighteenth century was a setback to the practice of Ayurveda, which suffered significant forget about on the hands of the colonial administration. After the first fulfilment of reserpine, an substantial quantity of characterization of medicinal plants became done in many laboratories and college Departments, but the final results changed into discouraging because the effort become disorganised, thin unfold and unfocused (Hosseinzadeh et al., 2015). Molecular pharmacology now provides a new interface between Ayurveda and contemporary medication. using contemporary techniques, numerous categories of Ayurvedic capsules could provide novel molecular probes. it's far now possible to discover the mechanism of action of Ayurvedic capsules in terms of the modern-day idea of molecular pharmacology. a few putting instance, of Ayurvedic capsules that are understood in terms of nowadays molecular pharmacology: Sarpagangha (Rauwolfia serpentina) Reserpine uniquely save you presynaptic neuronal vesicular uptake of biogenic amines (dopamine, serotonin and nor-epinephrine). Mainmool (Coleus forskohlii Briq) Forskolin immediately stimulates adenylate cyclase and cyclic AMP, with inotropic and Lusitropic impact on heart muscle. Sallaki (Boswellia serrata) Boswelic acid inhibits five-lipo-oxygenase and leukotriene B4 resulting in anticomplement effects. Shirish (Albizia lebbeck) prevents mast cell degranulation, much like sodium cromoglycate. Aturagupta (Mucona pruriens) consists of L-DOPA Ashwagandha (Withania somnifera) GABA-A receptor agonist. Katuka (Picrorhiza kurua) anti-oxidant

movement identical to a tocopherol, effect on glutathione metabolism in liver and mind indexed 15 crude Ayurvedic pills, that have obtained assist for his or her healing claims. some of Rasyana dravyas had been shown to increase phagocytosis, activate macrophages and enhance resistance to microbial invasion. capsules like Asparagus racemosus, Tinospora cordifolia and Ocimum sanctum antagonise the effect of strain(Clark et al., 1996). Emblica officinalis L., Curcuma longa L., Mangifera indica L., Momordica charantia L., Santalum album L., Swertia chirata BuchHam, Winthania somnifera (L.) have properly defined antioxidant properties and justify their use in conventional medicinal drug inside the beyond in addition to the prevailing Use of the natural medicinal drug in jaundice, probably viral hepatitis, has been known in India technology the Vedic times. about one hundred seventy phyto-elements remoted from 110 plant life belonging to fifty five families have been mentioned up to now to possess liver defensive activities. it's far expected that about 6000 business herbal formulations are bought world over as hepatoprotective pills, of them about 40 patent Polyherbal formulations representing a variety of combinations of 93 Indian herbs from fourty four families are available within the Indian market (WHO, 1993).

Current and future status of Indian herbal medicines: India is sitting on a gold mine of well- recorded and properly practised information of conventional herbal remedy. India is one of the 12-mega biodiversity centres having over 45,000 plant species. Its variety is unmatched due to the presence of sixteen special agro-climatic zones, 10 vegetative zones and 15 biotic provinces. The u. s. has 15,000–18,000 flowering flora, 23,000 fungi, 2500 algae, 1600 lichens, 1800 bryophytes and 30 million microorganisms (Hamburger et al., 1991). India also has equivalent to three/4 of its land one of a kind financial sector inside the ocean harbouring a large variety of plants and fauna, lots of them with therapeutic homes. about 1500 flowers with medicinal uses are stated in ancient texts and round 800 vegetation had been used in conventional medication. however, unlike China, India has no longer been capable of capitalising this herbal wealth via selling its use in the developed international despite their renewed interest in natural drugs (Singh et al., 1981).

Nature has many useful herbs and plant life for humans. inside the twentieth century peoples inside the global on the whole trusted conventional medication for their fitness issues or illnesses because of their safety. Nature has supplied a complete storehouse of treatments to cure the ailment of mankind. approximately 80% of the sector’s populace depends thoroughly or partly on traditional remedy for its number one health care desires (Kunwar et al., 2005).

Consistent with a survey of the sector fitness organisation the practitioners of traditional structures of medicine deal with about eighty% of humans in India, eighty five% in Burma and 90% in Bangladesh (Siddiqui, 1993). A majority of the world's populace in developing nations nonetheless relies on herbal drugs to meet its health wishes. nowadays, focus on natural research has expanded globally and proof gathered from distinctive studies surveys indicates the giant potential of medicinal plants. the attention paid by means of health government to the usage of herbal drug treatments has improved notably, due to the fact natural tablets are regularly the handiest remedy available in much less evolved regions. nowadays massive number of medication are in use are derived from flowers, like morphine from Papaver somniferum, ashwagandha from Withania somnifera, ephedrine from Ephedra vulgaris, atropine from Atropa belladonna, reserpine from Rauwolfia serpentina etc (Gurib, 2006).

The crucial therapeutic benefits of medicinal vegetation are: they may be safe, cost-efficient, effective and clean availability. Even though the conventional medicinal practitioners in the Indian subcontinent extensively use this medicinal plant for control of numerous disorder situations from historic times (Prakash et al., 2005).

Ocimum is an important image of hindu spiritual subculture. Tulasi (sanskrit:-surasa) has been used for heaps of years in ayurveda for its numerous recuperation houses. it is noted within the Charaka Samhita, an historical ayurvedic text. Marked via its robust aroma and astringent flavour, it is seemed in ayurveda as a form of "elixir of lifestyles" and believed to promote sturdiness (Puri et al., 2002).

Tulsi, the queen of herbs, the legendary ‘incomparable one’ of India, is one of the holiest and most cherished of the numerous recovery and healthy giving herbs of the orient. Tulsi, is famed for its non secular and non secular sanctity, in addition to for its important position within the traditional ayurvedic and unani system of holistic fitness and natural medication. Ocimum has been claimed to possess numerous medicinal properties. Traditionally, clean juice or decoction of OS leaves are used to promote health and in remedy of various problems as advocated in ayurveda, the Indian machine of drugs (Warrier, 1995). plant life are potent biochemists and had been additives of phytomedicine on account that instances immemorial; man is able to attain from them a wondrous assortment of business chemical compounds. A wealthy history of knowledge on preventive and healing medicines came to be had in ancient scholastic work protected in the Atharvaveda (an Indian spiritual book),

Ayurveda (Indian traditional machine of drugs) and so on. An estimate suggests that about 13000 plant species worldwide are regarded to have been used as drugs. Plant-based totally herbal components can be derived from any part of the plant like bark, leaves, flora, roots, end result, seeds and so on (Gordon et al., 2001) that is any part of the plant might also include active components. The useful medicinal consequences of plant substances normally end result from the mixtures of secondary products given in the plant. The medicinal moves of vegetation are precise to particular plant species or businesses are consistent with this idea as the combination of secondary merchandise in a specific plant is taxonomically wonderful (Wink, 2000). The systematic screening of plant species with the reason of coming across new bioactive compounds is an ordinary activity in many laboratories. The studies on the medicinal flowers need to be extended with the identity of the lively concepts within the plants. clinical examination of the remedies should lead to standardisation and excellent manipulation of the products to ensure their safety. it's miles after such assessment that they may be authorised to be used in primary health care. Such research sports may also result in the development of recent drugs as within the beyond. conventional antiasthmatic compounds including sodium cromolyn and sodium cromoglycate are a number of the examples of the lead organised from the analogs of the clearly occurring furanochromone khelline (Visammin). Exploration of the chemical ingredients of the flowers and pharmacological screening will therefore provide us the premise for developing new lifestyles-saving drugs (Cor et al., 1970).

Pharmacognosy is basically divided into traditional and modern pharmacognosy. traditional pharmacognostical examination is based totally on macroscopic, microscopic and quantitative microscopy. Macroscopic characters include shape, length, shade and texture of the drug in crude or powdered form. Microscopic characters include the anatomical details of drug generating plant as seen in transverse and longitudinal sections, maceration look at and the scale size of various sort of cells. Quantitative microscopy includes the vein islet quantity, palisade ratio, stomatal quantity and stomatal indices and so is limited to leaf drug most effective. The modern-day pharmacognosy utilises characteristics of analytical, phytochemical and positive bodily consistent values over the traditional technology of taxonomy in plant systematics. most of the botanical, chemical, bodily and microbial techniques hired in pharmacognosy are relevant to the evaluation of medication and therefore, used by public analysts, forensic scientists and high-quality manipulate chemists associated with industries.

It is estimated that of 1500 plant species in Ayurveda, 1200 plant species in Siddha had been used for drug practise (Jain, 1987). Although the Indian conventional systems of drugs are time- tested and practised successfully from time immemorial, there's loss of standardisation with regard to identity of crude drugs, strategies of training and first-class of finished merchandise. Tyler et al. (1981) defined that in a huge experience, pharmacognosy embraces understanding of the records, distribution, cultivation, series, choice, coaching, trade, identity, evaluation, preservation and use of medicine and financial substances that have an effect on the health of fellows and other animals.

Pharmacognosy consists of the take a look at of the proper horticulture, harvesting and makes use of of the raw medicinals found in nature. Its scope consists of the identity or authentication of crude capsules (the use of macroscopical, microscopical, radiological or chemical strategies), and their bio pharmacological and medical opinions. throughout earlier investigations research were carried out on ethnobotanical and pharmacognostical characterization of medicinal plants (Kumar, 2000, Sharma, and Kumar, 2012). despite the fact that nowadays pharmacognosy is still taught in a small range of college pharmacy schools within the US and inside the uk, this problem continues to be compulsory in the pharmacy curricula in all universities of continental Europe.

In conventional structures of medication, specific elements (leaves, stem, flower, root, seeds or even whole plant) of O. sanctum were recommended for the remedy of bronchitis, bronchial allergies, malaria, diarrhoea, dysentery, skin diseases, arthritis, continual fever and bug chew (Prakash and Gupta, 2005). In traditional systems of medication, exceptional elements (leaves, stem, flower, root, seeds or even complete plant) of O. sanctum have been advocated for the remedy of bronchitis, bronchial asthma, malaria, diarrhoea, dysentery, skin illnesses, arthritis, continual fever and bug chunk (Prakash and Gupta, 2005) Scientifically, the Ocimum species have proved to own diverse biological activities inclusive of antibacterial (Obinna et al., 2009; Parag et al., 2010) antifungal (Bansod and Rai 2005; Singh, 2010) antioxidant (Ramesh and Satakopan 2010; Hanif et atl. 2011), and anti-diabetic (Chandra et al., 2008). The quantity of medicinal formulations advanced by using Vaidyas has a nice correlation with the quantity of illnesses to be examined and Ocimum sanctum is used in treating fourteen sort of diseases (Kala, 2005). Ansari et al. (2006) have studied the behaviour of powdered drug of O. tenuiflorum with unique chemical reagents and fluorescence characteristics of drug from 4 species of Ocimum with different chemical

reagents beneath daytime and UV light.

Standardisation of herbal tablets is the most suitable at the moment while global hobby in herbal medicinal drug has received momentum. Morphological and anatomical characters play a critical role in crude drug standardisation. Morphological characters involve size, association, venation, texture, floor characters, markings and hardness of the plant substances. inside the present research Ocimum sanctum (Root) had been selected for pharmacognostical research due to their medicinal importance.

2. METHODOLOGY

2.1 COLLECTION OF PLANT

The root part of the plant Ocimum sanctum (Root) will be collected from the region of Samastipur, Bihar, India.

2.2 MATERIALS REQUIRED

2.2.1 Apparatus required

List of apparatus required are given in table 4.1

Table 4.1 Apparatus required.

S.No.	Name of Glasswares	Glassware Type
1	Beaker	Borosil
2	Conical flask	Borosil
3	Pipette	Borosil
4	Glass rod	Borosil
5	Volumetric flask	Borosil
6	Petri dish	Borosil
7	Measuring cylinder	Borosil
8	Round bottom flask	Borosil
9	Test tubes	Borosil
10	Separating funnel	Borosil
11	Funnel	Borosil
12	Watch glass	Borosil
13	Condenser	Borosil
14	Glass slides	Borosil
15	TLC chamber	Borosil

2.2.2 Chemicals required

Table 4.2: Chemicals required.

S.No.	Name of Chemicals	Manufacture
1	Petroleum ether	HIMEDIA
2	Chloroform	HIMEDIA
3	Ethanol	LOBA
4	Silica Gel G	LOBA
5	Sulphuric acid 98%	LOBA
6	Glacial acetic acid	LOBA
7	Hydrochloric acid	CDH
8	Nitric acid	CDH
9	Sodium hydroxide	CDH
10	Dragendorff's reagent	CDH
11	Wagner's reagent	HIMEDIA
12	Hager's reagent	HIMEDIA
13	Mayer's reagent	HIMEDIA
14	Million's reagent	HIMEDIA
15	Molish reagent	HIMEDIA
16	Picric acid	CDH
17	Ferric chloride	CDH
18	Fehling's solution A and B	HIMEDIA
19	Acetic anhydride	CDH
20	Picric acid aqueous solution	CDH
21	Potassium dichromate	LOBA
22	Lead acetate	CDH
23	Biureate reagent	HIMEDIA
24	Barfoed's reagent	HIMEDIA
25	Phenol	CDH
26	Sudan III	LOBA
27	Ruthenium red	LOBA
28	Phloroglucinol	HIMEDIA

2.2.3 Equipments required

Table 4.3: Equipments Required.

S. No.	Name of Equipments	Manufacture
1	Soxhlet apparatus	Perfit, India
2	Electric water bath	Inco. Ambala, India
3	Analytical balance	Shimaduz
4	Hot air oven	Narang scientific works Pvt. Ltd.

2.3 METHODOLOGY

2.3.1 Macroscopy: Macroscopic evaluation was done by identifying the colour, odour, taste, shape, surface characteristics, texture and fracture characteristics. In the present study, root parts of the plant were studied for colour, odour, taste, shape, surface characteristics etc (WHO, 2011).

2.3.2 Microscopy

2.3.2.1 Transverse Section (TS) of the plant part: Transverse section is obtained by cutting along the radial plane of a cylindrical portion of the root and perpendicular to the long axis. This section when prepared and observed under the microscopy reveals the radial arrangement of tissues and shows concentric layers (Trease and Evans, 2007).

2.3.2.2 Preparation of slides: Firstly, selected the clean glass slide and placed the fine powder of the drug on the glass slide and tapped the slide such that uniform powder is distributed on the surface of the slide. Observed the glass slide by using different reagents for the internal study of the powdered drugs with the help of microscopes (Trease and Evans, 2007; Wallis, 1985).

2.3.2.3 Reagents used for powder microscopy (Trease and Evans, 2007; WHO, 2001; Khandelwal, 2007).

- **Chloral hydrate solution**: It is used as a clearing and bleaching agent to dissolve starch, protein, chlorophyll, resins etc. It does not dissolve calcium oxalate crystals.
- **Phloroglucinol:HCl (1:1)**: It stains all lignified walls pink or red.
- **Picric acid solution**: It is used to stain aleurone grains.
- **Ruthenium red**: It stains gums, pectin and mucilage.
- **Iodine water**: Iodine colours the starch blue and imparts a yellow colour to aleurone grains.
- **Hydrochloric acid**: It dissolves cell contents including calcium oxalate.
- **Sudan III**: Few drops of Sudan III to powdered slide and allow for a few min then suberized or cuticular cell walls are stained orange-red to red.
- **Acetic acid**: It is useful for the analysis of calcium oxalate present in drug preparations.
- **Phenols**: It is used as a clearing agent. It causes good penetration and also look starch particles transparent.

2.3.3 Physicochemical parameters

2.3.3.1 Determination of ash values (WHO, 2011; Indian Pharmacopoeia, 2010)

2.3.3.1.1 Determination of total ash value: Weighed and ignited crucible in an oven upto constant weight occurred. Add 3 g powdered drug into it and put a covered lid on it and put into a muffle furnace at 500-600^{o}C for 6 hrs. Cooled in a desiccator. Weighed the obtained ash and calculated the total ash with reference to the air dried sample of the drug.

2.3.3.1.2 Determination of acid-insoluble ash value: Weighed and ignited crucible in an oven up to constant weight occurred. Add 3 g powdered drug into it and put a covered lid on it and put it into a muffle furnace at 500-600^{o}C for 6 hrs. Cooled in a desiccator. Weighed the obtained ash and calculated the total ash with reference to the air dried sample of the drug. Used 25 ml of dilute hydrochloric acid and washed the ash in the beaker and put the mixture on the water bath upto boiling the mixture. Filtered the mixture and washed the residue with hot water 2 times. Put the filter paper and place it into the crucible and further into the muffle furnace at 500-600^{o}C for 6 hrs. Cooled in a desiccator. Weighed the obtained residue and calculated the acid-insoluble ash with reference to the air dried sample of the drug.

4.4.3.1.3 Determination of water soluble ash value: Weighed and ignited crucible in an oven up to constant weight occurred. Add 3 g powdered drug into it and put a covered lid on it and put into a muffle furnace at 500-600^{o}C for 6 hrs. Cooled in a desiccator. Weighed the obtained ash and calculated the total ash with reference to the air dried sample of the drug. Used 25 ml of water and washed the ash in the beaker and put the mixture on the water bath upto boiling the mixture. Filtered the mixture and washed the residue with hot water 2 times. Put the filter paper and place it into the crucible and further into the muffle furnace at 500-600^{o}C for 6 hrs. Cooled in a desiccator. Weighed the obtained residue and calculated the water soluble ash with reference to the air dried sample of the drug.

2.3.3.2 Determination of extractive values (WHO, 2011)

2.3.3.2.1 Determination of alcohol soluble extractive values: Weighed 10 g of powdered drug and put into 250 ml conical flask. Filled 90% alcohol upto 100 ml marked and cork the flask. Keep the conical flask on a magnetic stirrer for 6 hr. Filtered the mixture and collected 25 ml filtrate into a porcelain dish. Evaporated to dryness on ware bath and complete drying in an oven at 100^{o}C. Cooled in a desiccator. Calculated the percentage w/w of extractive value with reference to the air dried drug.

2.3.3.2.2 Determination of water soluble extractive values: Weighed 10 g of powdered drug and put into 250 ml conical flask. Filled water utpo 100 ml mark and cork the flask. Kept a side the conical flask for 24 hr with shaking frequently. Filtered the mixture and collected 25 ml filtrate into porcelain dish. Evaporated upto dryness on ware bath and complete drying in an oven at 100^{o}C. Cooled in a desiccators. Calculated the percentage w/w of extractive value with reference to the air dried drug.

2.3.3.3 Determination of foreign organic matter: 100 g of drug was weighed and spreaded the sample on a white tile without overlapping. Inspected the sample with the naked eye or by lens (10x or above). Separated the foreign organic matter. After completed separation, weighed the foreign organic matter and calculated the percentage w/w present in the sample (Mukherjee, 2002).

2.3.3.4 Determination of Loss on drying: Weighed 2 g powdered durg and kept into a porcelain dish. Dry in the oven at 100^{o}C or 105^{o}C. Cooled in a desiccator and watched. The loss in weight is usually recorded as moisture (Khandelwal, 2007; Indian Pharmacopoeia, 2010).

2.3.3.5 Fluorescence analysis: The powdered drugs are mixed with the different different solvents to make pasty materials and then put on the glass slides. Then further, see the glass slide in the UV light (Visible light, Long UV light, Short UV light) for the investigation of the fluorescence produced by the extract mixed with solvents (Mukherjee, 2002).

2.3.4 Preliminary phytochemical analysis (Trease and Evans, 2007; Khandelwal, 2007).

The preliminary phytochemical screening was carried out by using the extract which are prepared by using the different solvents for the different types of chemical constituents as per the methods described. The extract was used to perform the preliminary phytochemical screening for the detection of alkaloids, glycosides, carbohydrates, flavonoids, tannins and phenolic compounds, proteins, amino acids, lipids, fats and oils, gums, mucilages etc.

2.3.4.1 Test for alkaloids: For alkaloids test, sample mixture was treated with dilute hydrochloric acid.

i) Mayer's test: To the test sample, add 2 ml of Mayer's reagent (Potassium mercuric iodide solution) then it gives cream colour precipitate.

ii) Dragendorff's test: To the test sample, add 2 ml of Dragendorff's reagent (Potassium bismuth iodide solution) then it give reddish brown precipitate.

iii) Wagner's test: To the test sample, add 2 ml of Wagner;s reagent (Solution of Iodine in Potassium Iodide) then it give orange brown precipitate.

iv) Hager's test: To the test sample, add 2 ml of Hager's reagent then it give yellow precipitate.

4.4.4.2 Test for flavonoids

i) Shinoda test (Magnesium hydrochloride test): To the test solution, added a few fragments of magnesium ribbon and added 2 ml conc. HCl drop wise then pink scarlet crimson red or occasionally green to blue colour appears after few minutes.

ii) Ferric chloride test: To the test solution, added few drops of $FeCl_3$ solution then intense green colour is formed.

iii) Zinc hydrochloride test: To the test solution, zinc dust was added and 2 ml conc. HCl then red colour is formed.

iv) Alkaline reagent test: To the test solution, NaOH solution was added dropwise then yellow colour is formed then further added dilute acid dropwise then colourless liquid is formed.

v) Tollen's reagent test: To the test solution, 2 ml Tollen's reagent was added then reduced reagent to give silver mirror appearance.

vi) Fehling's reagent test: To the test solution, 2 ml Fehling's reagent was added then red precipitate is formed.

4.3.4.3 Test for tannins and phenolic compounds

i) Ferric chloride test - To the test solution, added few drops of neutral 5% $FeCl_3$ solution. A dark green colour indicated the presence of phenolic compounds.

ii) Lead acetate test - To the test solution, added few drops of 10% lead acetate solution then white precipitate indicated the presence of phenolic compounds.

iii) Gelatin test - To the test solution added a few drops of 10% Gelatin solution then white precipitate indicated the presence of phenolic compounds.

4.3.4.4 Test for glycosides

a) For cardiac glycoside

i) Keller-killani test - To the test solution, 2 ml acetic acid containing in $FeCl_3$ in H_2SO_4 was added then reddish brown colour changing to blue colour is formed.

ii) Legal's test - To the test solution, 2 ml pyridine and 2 ml alkaline sodium nitroprusside solution was added then blood red colour is formed.

iii) Baljet's test - To the test solution, 2 ml picric acid solution was added then orange colour was formed.

b) For anthraquinone glycosides

i) Borntrager's test – Boiled the sample with H_2SO_4 (1ml) in the test tube for 5 min then

filter and cool and shaken with equal vol. of chloroform. Separate chloroform layer and shake it with half of its vol. of dilute ammonia then rose pink to red colour is formed.

ii) Modified Borntrager's test – Boiled the sample with H_2SO_4 (1ml) in the test tube for 5 min and treat with (5%) aqueous $FeCL_3$ solution and shaken it with equal vol. of chloroform. Separate chloroform layer and shake it with half of its vol. of dilute ammonia then rose pink to red colour is formed.

c) For saponin/steroidal glycosides

i) Foam test - Sample (2ml) in test tube is shaken well then stable foam is formed.

ii) Haemolysis test - Sample (2ml) in test tube and blood (2ml) in normal saline was added and mix well then centrifuge and note the red supernatant.

iii) Libermann's-Burchard test - To the test solution, 2 ml acetic anhydride and 2 ml conc. Sulphuric acid was added then bluish - green colour was formed.

iv) Salkowski's test - To the test solution, 2 ml chloroform and 2 ml conc. sulphuric acid was added and shaken well then red colour is formed (in chloroform layer) and greenish colour is formed (in acidic layer).

4.3.4.5 Test for proteins/amino acids

i) Millon's test - To the test solution added about 2 ml of Millon's reagent then white precipitates appeared which turns red upon gentle heating.

ii) Biuret test - To the test solution added 4% NaOH solution and added a few drops of 1% $CuSO_4$ solution then violet colour indicated the presence of proteins.

iii) Ninhydrin test - Amino acid and proteins when boiled with a few drops of 5% solution of Ninhydrin then violet colour appears.

iv) Warming test - Sample in test tube is heated in water bath then proteins get coagulated.

v) Hydrolysis test - Sample is hydrolysed with HCL or sulphuric acid then further carried out in Ninhydrin test for protein.

vi) Heller's test: To the test solution, 2 ml conc. HNO_3 was added then white precipitate appeared at the junction of two liquids.

4.3.4.6 Test for carbohydrates

i) Molisch's test - Treat 1ml of test solution with a few drops of alcoholic α-naphthol. Add 0.2ml of concentrated sulphuric acid slowly along the sides of the test tube, a purple to violet colour ring appears at the junction.

ii) Fehling's test - Equal volume of Fehling's A (Copper sulphate in distilled water) and

Fehling's B (Potassium tartrate and sodium hydroxide in distilled water) reagents are mixed and few drop of sample is added and boiled, a brick red precipitate of cuprous oxide forms, if reducing sugar are present.

iii) Barfoed's reagent test - To the test solution, 2 ml Barfoed's reagent was added then red cupric oxide is formed (for monosaccharides) and heated for 10 min (for disaccharides).

iv) Benedict's reagent test - To the test solution, 2 ml Benedict's reagent was added and heat for 5 min then green, yellow or red colour appears depending upon reducing sugars present in sample.

4.3.4.7 Test for oil and fats - A small quantity of the extract was pressed in between two filter papers. The oil stain on the filter paper indicates the presence of oils and fats.

2.3.5 Thin layer chromatography profiling: Thin layer chromatography (TLC) plates are washed with water and dried in an oven. TLC plates were prepared by using the pouring technique. The slurry was made by using the silica gel G taken in a beaker and sufficient amount of distilled water to make pasty material. The TLC plates were rotated in hand back and forth to make a uniform layer of slurry over the TLC plates. Firstly, the TLC plates are dried at normal room temperature and then dried in an oven at 110°C for 30 minutes for the activation of TLC plates. After activation, the TLC plates were used for examination of different compounds present in the extract by using the different solvents (Mukherjee, 2011).

2.4 Preparation of Root Part Extract of Ocimum sanctum (Root) with

2.4.1 Alcoholic and Water Solvents

Weigh 100 gm of dried drug in shaded (dried crushed dried root) and put into a thimble made up of filter paper. Placed the thimble containing drug into Soxhlet apparatus and solvents were added slowly slowly onto it which was passed through the thimble and collected into a round bottom flask. Here, 3 cycles were done and assembly was operated for 6 hours and temperature not exceeding not more than 60°C. Successive extraction was done by using solvents in the order: Petroleum ether, chloroform, ethyl acetate, ethanol and water. After completing the extraction process, the heating was stopped and the mixture of the liquid was collected and placed into a china dish. Evaporated the mixture upto dryness into a water bath. Cooled properly and stored in a well closed container and kept at 4°C. The extracts obtained after successive extraction are further used for performing physicochemical and preliminary phytochemical screening and TLC fingerprinting analysis of the root of Ocimum sanctum (Root) (Ghangale, 2011).

2.4.2 DPPH Free Radical Scavenging Assay

i. Different concentrations of extract solution (0.03125, 0.0625, 0.125, 0.25, 0.5 and 1.0mg/ml) were prepared as well as control (methanol without extract sample).

ii. Different concentrations of extract solution were subjected to the uniform mixing.

iii. 5.0ml of methanolic solution of DPPH reagent was added individually to each of the different concentrations of the extract solution.

iv. The mixture samples were then subjected to a vortex for a few minutes.

v. The mixture samples were incubated at room temperature in the dark for around 30 minutes.

vi. The absorbance for each concentration mixture sample was measured at 517 nm against a blank by using UV-Visible spectrophotometer.

vii. The percentage of DPPH scavenging activity was calculated using the following equation.

$$\% \text{ Scavenging of test sample} = \frac{(\text{control absorbance} - \text{test absorbance})}{\text{control absorbance}} \times 100$$

viii. All the procedure was repeated for BHT solution (standard) of different concentration (similar to the previous concentration).

ix. The 50% inhibitory concentration value (IC50) for both extracts were calculated and it is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals. The data for both extract sample and standard were obtained and tabulated (Khan et al., 2017; Reglinski et al., 2015).

Total phenolic contents analysis: For the total phenolic contents analysis, Folin- Ciocalteau (FC) assay was applied to Ocimum sanctum ethanolic Soxhlet extract to find out the total phenolic content of this extract. In this analysis, the standard used was Gallic acid and the measurement was done at λ max 765nm. Gallic acid standard calibration curve was generated as shown in Graph 1 and the regression value was 0.9884. From the absorbance obtained, the amount of the phenolic content is measured as Gallic acid equivalent (GAE) using the FC method. The total phenolic content obtained at the concentration of 1.0mg/ml was found to be the highest when compared to other lower concentration extract solutions. As a result, the higher the concentration of the stock solution used for this analysis, the higher the Gallic acid equivalent (Sreelatha et al., 2009; Vijayalakshmi et al., 2007; Sailaja et al., 2010; Bandita et al., 2013; Mohan et al., 2011; Pisoschi et al., 2011; Warsi et al., 2017; Batta et al., 1971).

Materials Required Chemicals

- Calcium chloride was produced from Merck
- Magnesium sulfate, Potassium dihydrogen orthophosphate, Sodium hydrogen orthophosphate, Ammonium chloride, Sodium chloride, Uracil, Bacteriological agar, Peptone, Sodium hydroxide, and Cholesterol were produced from SRL.
- Absolute ethanol was produced from Ureca Consumers co. op. stores.
- Double distilled water and distilled water
- Sodium hypo chloride solution for Axanizing solution preparation.

Glasswares and Plasticwares

Conical flask, measuring cylinders, disposable serological pipettes, beakers, glass spreader, glass slide, coverslip, per dish (9 mm), falcon tube, 96 well plate, Eppendorf tube, Funnel, Round Bottom Flask.

Miscellaneous

Food wrap (cellophane sheet), aluminum foil, spatula, parafilm, needle (worm picker), cotton, micropipette, tissue paper, butter paper, filter pape micropipette, micropipette tips, dispenser, pair of scissors, distilled water, spirit lamp.

Biological Materials

Caenorhabditis elegans: wild type: N2 strain: BZ- 555 strain Animals were received as a gift from DR. Amir Nazir, Sr. Scientist and Head of Functional Genomics and Molecular Toxicology Laboratory, Division of Toxicology, the council of Scientific and Industrial Research (CSIR) -Central Drug Research Institute (CDRI), Lucknow. Escherichia coli: OP 50 (uracil deficient) strain Krishna Tulsi plant (purple- colored leaves) which was grown in the garden area between IFS and IBS department of GFSU.

Instruments

- Rotary Evaporator
- Stereo microscope- Micros Austria: MCX51LED, MC50LED
- BOD incubator- Thermo Scientific
- Water purification system- Lablink Xtrapure Plus
- Digital Autoclave- PSI
- Digital pH meter- LAqua Ph meter by Horiba scientific

- Laminar fume hood with UV light source
- Electronic weighing balance- Rentech
- Orbital Incubator Shake Spire
- Vortex Mixer- Lab net

Protocols

Preparation of Ngm Agar Plates (For 500 Ml) (Nematode Growth Media)

- The size of conical flask was twice to that the amount of media required.
- Following were weighed and added in the conical flask.

1. NaCl – 1.5 gram
2. Peptone – 1.25 gram
3. Agar – 8.5 gram
4. Distilled water – 487.5 ml

- The above mixture was then autoclaved (121^{0}C for 15 minutes)
- Following things are added to it

1. Freshly prepared Cholesterol – 5 mg/ml in 100% ethanol (C_2H_5OH)
2. $CaCl_2$ - 1.47 gm in 10ml distilled water (1 M solution)
3. $MgSO_4$ - 1.2 gram in 10 ml distilled water (1 M solution)
4. KH_2PO_4 - 3.4 gram in 25 ml distilled water (1 M solution, pH should be 6) [0.5 ml each of i, ii, iii, and 12.5 ml of iv was later added to the conical flask]

- Except for Cholesterol, the rest of all solutions are needed to be autoclaved.
- The fuming hood was decontaminated using UV light and then the methanol lamp was lit.
- After autoclave, they were taken out and kept in the fume hood.
- Meanwhile, fresh plates were taken out and kept in the hood and marked with a date.
- Using a micropipette and tips, the required amount of solutions is added to the conical flask and mixed properly.
- Lids of the fresh plates were opened and using a serological pipette (25 ml) fitted in a dispenser (pipette controller), 18-22 ml of agar solution was poured into each plate.
- The plates were allowed to cool for an hour, after which the lids were closed and a stock of 5 was made and wrapped into cellophane sheetsfollowed by aluminum foil and was kept in the fridge.

Preparation of M Buffer Solution

- Reagents required for 1 Liter:

1. Distilled water – 600ml
2. KH_2PO_4 - 3g
3. Na_2HPO_4 - 6g
4. NaCl – 5g
5. $MgSO_4$ (1 M) - 1ml (0.12 g in 1 ml)

- Above reagents are mixed well and made up to 1 liter (After adding each, add 100ml of distilled water each time to ensure even mixing and finally made up to 1 liter).
- Transfer 500 ml of it to another reagent bottle and autoclave both, labeled with name and date on it.
- Cool to room temperature and store it in the fridge.

Preparation of Minimum Essential Media (mem) for E. Coli

- 60 ml of M9 was taken in a sterile measuring cylinder and was autoclaved.
- To this following are added:

1. 1.5 mL of OP 50 culture
2. 0.75 mL/750 µL of NH_4Cl
3. 0.75 mL Glucose (20% in distilled water
4. 15 µL of Uracil (0.2 % in autoclaved distilled water).

- Volume was made up to 75 ml by adding more M9, and to avoid contamination, all the mixing are carried out in a fume hood.
- The final solution was transferred in a sterile conical flask and closed with a cotton plug and was kept in an incubator shaker at 37^0C overnight till turbidity was observed.
- The next day, if sufficient turbidity is not found then again 15 µL of Uracil, can be added and kept in an incubator shaker for more than 2-3 hours at 37^0C.
- The culture is stored in BOD.

Seeding of Plates

- Requirements

1. Fresh NGM plates
2. MEM with E. Coli
3. Spreader

4. Micropipette with tips
5. Methanol fueled lamps

- The laminar hood is sterilized
- Plates are taken out from the fridge and kept at room temperature
- Meanwhile, the incubator is turned on and set at 37^0C to attain require temperature
- Plates are now placed in the incubator in the up and down manner (30 minutes each manner) and then the plates are placed in the fume hood
- Methanol lamp is lit and E. Coli is poured (0.5 ml or 500 µl) onto the NGM plates with the help of a micropipette
- It is then spread on the plates using a sterilized spreader (with continuous cooling and heating)
- Plates are then dried at room temperature and then labeled
- The lids are closed and placed in the incubator overnight (12-18 hours) without sealing
- The next day, the plates are taken out from the incubator and placed in a fume hood
- If chunking is required, the plates are chunked with respective strain
- If not required, then the plates are sealed using parafilm, made to the stock of 5, and wrapped with aluminum foil
- The stock is placed in the fridge for further use

Culturing of Worms

To perform culturing, the worms are needed to be transferred from a well- populated plate of worms to a fresh seeded plate. For transferring worms, a process called “chunking” is done, i.e., a chunk of agar from a well- populated plate of worms are taken using a sterile spatula and placed inverted on a fresh seeded plate so that worms could crawl out of chunk and spread on the bacterial lawn of the new plate.

Chunking involves the following steps

- Freshly seeded plates are taken out from the fridge and placed at room temperature in the hood.
- Meanwhile, the incubator is turned on (to attain a temperature of 37^0C)
- Plates were now placed in the incubator in an up and down manner for 1 hour (30 minutes in each manner)
- Chunk plates of required strain are taken out from BOD and observed under a microscope for live animals and the portion is marked and placed in a fume hood

- Fresh seeded placed are taken out of the incubator and placed in a fume hood
- Methanol lamp is lit and the flat portion of a spatula is heated on it and allowed to cool
- The marked portion of the worm plate is cut and the chunk is placed in a downward direction on a freshly seeded plate
- The lid is now closed, sealed with parafilm, and placed in BOD

Transferring a single worm was done by a worm -picking. A worm-picker is made by flattening and folding the tip of a needle or syringe using a pestle to make it blunt, as sharp edges may damage the worms and poke a hole in the plate. To avoid contamination, the picker was sterilized by flaming. For picking worms, the worms are identified under a stereomicroscope and swiped off the plate with the picker, and transferred on a fresh plate by gently placing the picker on a fresh plate.

RESULTS

5.1 MACROSCOPIC CHARACTERS OF OCIMUM SANCTUM (ROOT)

Results of macroscopic studies are shown in Table 5.1

Plate 5.1 Ocimum sanctum (Root).

Table 5.1: Macroscopic characters of O. sanctum (Root)

S.No.	Characters	Observations
1	Colour	Light brown or Dark brown
2	Odour	Strong aromatic
3	Taste	Characteristics
4	Diameter	2-2.5 cm
5	Shape	Irregular shape with Tap root system

5.3 PHYSICO-CHEMICAL PARAMETERS OF O. SANCTUM (ROOT)

5.3.1 Ash value

Table 5.2 Ash values of O. sanctum (Root).

S.No.	Ash value	Yield (%w/w)
1	Total ash value	4.65 ± 0.090
2	Acid-insoluble ash value	1.05 ± 0.015
3	Water soluble ash value	2.34 ± 0.017

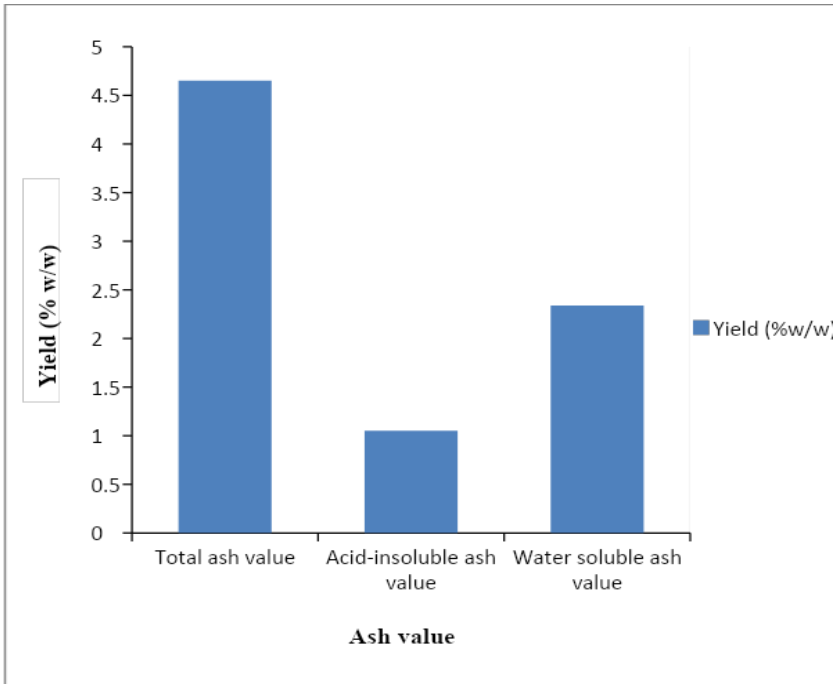


Figure 5.4 Histogram representing different ash value of O. sanctum (Root).

5.3.2 Extractive values

Table 5.3: Extractive values of O. sanctum (Root).

S.No.	Extractive value	Methods	
		Cold maceration Yield (%w/w)	Hot maceration Yield (%w/w)
1	Alcohol soluble	2.12 ± 0.0438	3.14 ± 0.0201
2	Water soluble	3.3 ± 0.0447	4.10 ± 0.0156
3	Petroleum ether	0.34 ± 0.1520	1.89 ± 0.0347
4	Chloroform	1.05 ± 0.0093	2.10 ± 0.0067

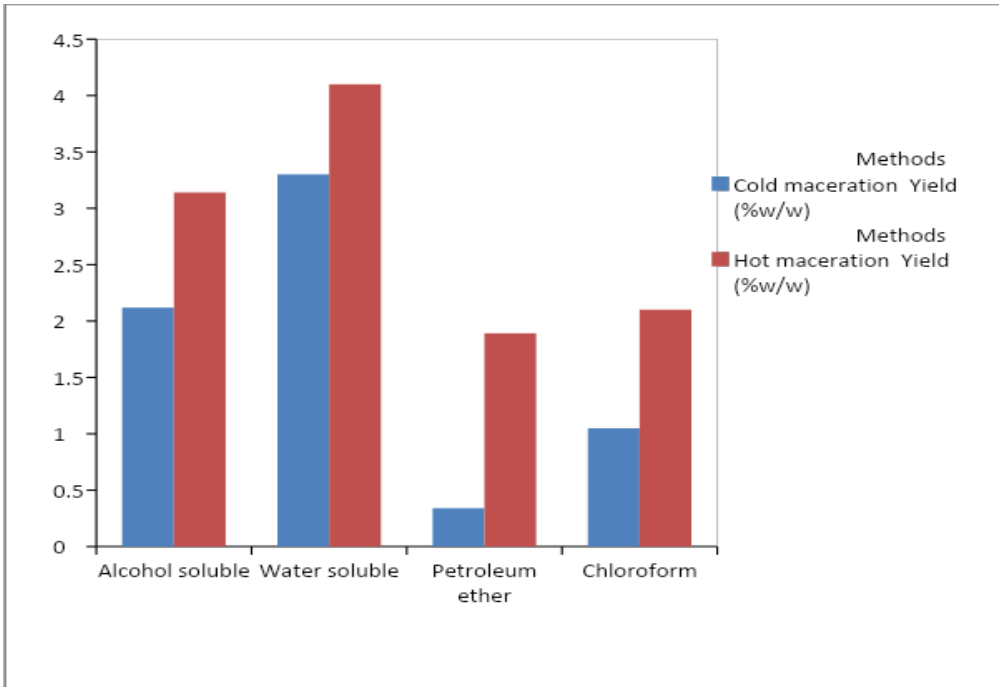


Figure 5.5 Histogram representing different extractive value of O. sanctum (Root).

5.3.3 Loss on drying - This helps to determine the moisture content present in the crude drug. The loss on drying was calculated as 8.9 % w/w.

5.3.4 Foreign organic matter - This helps to determine the adulteration present in the crude drug. The foreign organic matter was found as 1.3 % w/w.

5.3.5 Fluorescence Analysis

5.3.5.1 Fluorescence analysis of powder of O. sanctum (Root) treated with different reagents.

Table 5.4 Fluorescence analysis of powder of O. sanctum (Root).

S.No.	Reagents	Visible light	Short UV light	Long UV light
1	Powder as such	Light brown	Brown	Blackish brown
2	50 % H_2SO_4	Brown	Brownish black	Dark brown
3	95 % Ethanol	Green	Green	Orange
4	Conc. H_2SO_4	Dark brown	Dark green	Brownish orange
5	Cocn. HCL	Pale brown	Dark brown	Balckish brown
6	5 % KOH	Brown	Brownish black	Black
7	5 % $FeCL_3$	Dark brown	Brown	Brownish violet
8	50 % HNO_3	Yellowish brown	Black	Blackish orange
9	5 % NaOH	Yellow	Blackish brown	Black
10	1N HCL	Brownish black	Dark brown	Dark brown

5.3.5.2 Fluorescence analysis of extracts of O. sanctum (Root) with solvents

Table 5.5 Fluorescence analysis of different extracts of O. sanctum (Root)

S.No.	Extracts	Visible light	Short UV light	Long UV light	Consistency
1	Petroleum ether	Brownish green	Blackish green	Blackish	Sticky semi- solid
2	Chloroform	Dark voilet	Black	Blackish green	Slightly solid
3	Ethanol	Blackish green	Black	Black	Sticky semi- solid
4	Water	Dark brown	Brown	Blackish	Solid

5.4 YIELD OF DIFFERENT EXTRACTS USING SUCCESSIVE SOXHLET EXTRACTION OF O. SANCTUM (ROOT)

Table 5.6: Yield of different extracts of O. sanctum (Root)

S.No.	Extracts	Yield (% w/w)
1	Petroleum ether	0.93
2	Chloroform	1.69
3	Ethanol	2.81
4	Water	3.91

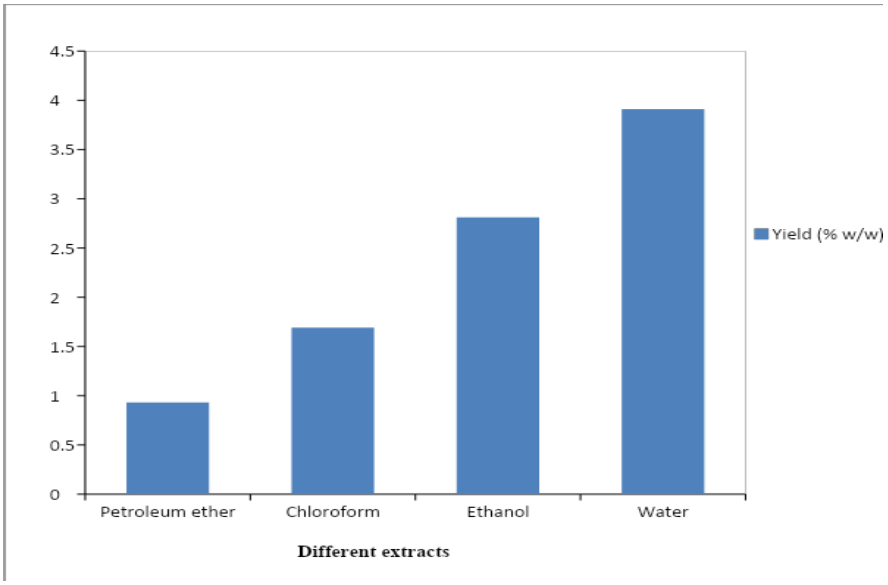


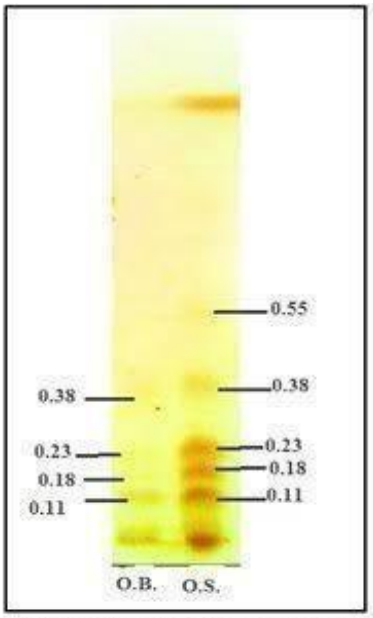
Figure 5.6: Histogram represents the % yield of different extracts of O. sanctum (Root)

5.5 THIN LAYER CHROMATOGRAPHY FINGERPRINTING PROFILING OF DIFFERENT EXTRACTS OF O. SANCTUM (ROOT)

Results of TLC fingerprinting profile of different extracts of O. sanctum (Root) are presented in table 5.7.

Table 5.7 TLC fingerprinting profile of different extracts of O. sanctum (Root)

S.No.	Extracts	Solvent System	No. of Spots	Spots position	Spot colour	Rf Value
1	Chlorofor m extract	Benzene: Ethyl acetate (8:2)	4	1 2 3 4	Light yellow Yellow Light green Light blue	0.41 0.50 0.72 0.81
2	Ethanol extract	Benzene: Ethyl acetate (8:2)	6	1 2 3 4 5 6	Light yellow Yellow Yellowish green Yellow Bluish green Blue	0.05 0.27 0.39 0.64 0.69 0.80



: *Ocimum basillicum* and O.S*: Ocimum sanctum*)

CONCLUSION

The extensive literature survey revealed that Ocimum sanctum is an important medicinal plant with a diverse pharmacological spectrum. It is remarkably evident that the Tulsi leaves and its juice effectively reduce many diseases including digestive disorders, respiratory disorders, kidney related problems, Cardiovascular disorders and Cancer. Ocimum sanctum known as the "elixir of life" had proved its efficiency in treating a variety of diseases. The scientific research on Ocimum sanctum suggests a huge biological potential of this plant. It is strongly believed that detailed information as presented in this review on the phytochemical and various biological functions of the extracts might provide detailed evidence for the use of this plant in different medicines.

The present study was an attempt to establish diagnostic characteristics of O. Sanctum (Root).

Finding of this study can be employed as suitable quality control measures to ensure the quality, safety, and efficacy of this herbal drug material. The various parameters studied are useful to identify and authenticate the traditionally important medicinal plant O. Sanctum. WHO emphasised to conduct such studies which ultimately are helpful in the preparation of herbal monographs and pharmacopoeia standards.

The results of present study lead to a conclusion that should be further investigated for isolation and purification of medicinally important phytoconstituents. The pharmacognostic studies may be employed as supplement information in respect of identification parameters in the way of acceptability and quality control of this plant. The phytochemical studies indicate that it is a useful plant to investigate for phytochemical and biological assays.

REFERENCES

1. WHO, Regulatory situation of herbal medicines, Geneva, Switzerland. A worldwide review, 1998; 1-5.
2. Fakim, A.G., Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular aspects of medicine, 2006; 27: 1-93.
3. Harrison, P., Herbal medicine takes roots in Germany. Canadian Medical Association Journal, 1998; 10: 637-639.
4. Rastogi, P. R. and Meharotra, B. N., In Compendium of Indian Medicinal Plants. Vol. I, 339; III: 194. PID, CSIR, New Delhi, India, 1990.
5. Galbley, S. and Thiericke, R., Drug Discovery from Nature, Series: Springer Desktop Editions in Chemistry, Springer, Berlin, 1999.
6. Cragg, G.M., Newman, D. J. and Snader, K. M., Natural products in drug discovery and development. Journal of Natural Products, 1997; 60: 52- 60.
7. Mittermeier, R. A., Gil, R. P., Hoffman, M., Pilgrim, J., Brooks, T., Mittermeier, C. G., Lamoreux, J. and Fonseca, G. A. B., Hotspots revisited: Earth's biologically richest and most endangered terrestrial ecoregions. Pp 392. Boston: University of Chicago Press, 2005.
8. Kaul, M. K., Medicinal plants of Kashmir and Ladakh: temperate and cold arid Himalaya. Indus Publishing, New Delhi, 1997.
9. Singh R. Medicinal Plants: A Review. Journal of Plant Sciences. Special Issue: Medicinal Plants, 2015; 3(1-1): 50-55.
10. Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A. and Armand, R., The application of

Medicinal Plants in Traditional and Modern Medicine: A Review of Thymus vulgaris. International Journal of Clinical Medicine, 2015; 6: 635-642.

11. Clark, A.M., Natural Products as a Source for New Drugs. Pharmaceutical Research, 1996; 13: 1133-1141.
12. WHO, Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines. Manila, 1993.
13. Singh, P. and Singh, C. L., Chemical investigations of Clerodendraon fragrans. Journal of Indian Chemical Society, 1981l; 58: 626-627.
14. Hamburger, M. and Hostettmann, K., Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry, 1991; 30: 3864- 3874.
15. Kunwar RM, Adhikari N. Ethnomedicine of dolpa district Nepal: The plant their vernacular names and uses. Lyonia, 2005; 43-9.
16. Siddiqui HH. Safety of herbal drugs-an overview. Drugs News Views, 1993; 7: 10.
17. Gurib FA. Medicinal plants: Traditions of yesterday and drugs tomorrow. Mol. Aspects Med, 2006; 27: 1-93.
18. Prakash P, Gupta N. Therapeutic uses of Ocimum sanctum Linn. Tulsi with a note on eugenol and its pharmacological actions :A short review. Indian J Physiol Pharmacol, 2005; 49: 125-31.
19. Krishnaiah D, Sukla AR, Sikand K, Dhawan V. Effect of herbal polyphenols on atherogenic transcriptome. Mol Cell Biochem, 2009; 278: 177-84.
20. Sobli SN. Pushpangadan P. Studies in the genus Ocimum: cytogenetics, breeding and production of new strains of economic importance. In: Atal CK, Kapur BM (eds) cultivation and utilisation of aromatic plants. Regional Laboratory Council of Scientific and Industrial Research, Jammu-Tawi, India, 1982; 457-472.
21. Farnsworth NR. Bunyapraphatsara N. Thai medicinal plants. Medical Plant Information Center, Bangkok, 1992; 180-2.
22. Marja PK, Anu IH, Heikki JV, Jussi-Pekka R, Kalevi P, Tytti SK et al. Antioxidant activity of plant extracts containing phenolic compounds. J AgricFood Chem, 1999; 47(10): 395462.
23. Lee KJ, Min DB. Comp Rev Food Sci Food Safety, 2004; 3: 21-7.
24. Puri, Rasayana HS. Ayurvedic herbs for longevity and rejuvenation. CRC Press. USA, 2002; 272-280.
25. Warrier PK. In: Indian medicinal plants. Longman O, editor. New Delhi: CBS publication, 1995; 168.

26. Singh S, Majumdar DK, Rehan HMS. Evaluation of anti- inflammatory potential of Ocimum sanctum (holy basil) and its possible mechanism of action. J Ethnopharmacol, 1996; 54: 19-26.
27. WHO. The World Health Report. Mental Health: New Understanding New Hope; WHO: Geneva, Switzerland, 2011.
28. Trease and Evans, Pharmacognosy, 16th edition, First printed in 2007, published by Sunders ELSEVIER, 565-568.
29. Wallis T.E., Textbook of Pharmacognosy, 1st edition, 1985, published by CBS publishers & Distributors Pvt. Ltd., 578-580.