International Journal of Phytopharmacy
Vol. 1 (2), pp.43-49, Nov-Dec 2011

Research Article

COMPERATIVE PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION (LEAF) OF DIFFERENT SPECIES OF OCIMUM

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ABSTRACT

Medicinal properties of *Ocimum* are known for thousand years to various civilizations of the world. This medicinal herb is considered as a sacred plant by the Hindus in the Indian subcontinent. Scientific explorations of traditional belief of medicinal properties of *ocimum* have got momentum mostly after the middle of the 20th century. In the present article, efforts have been made to comperative pharmacognostical and phytochemical evaluation (leaf) of different species of *ocimum*.There are three types of tulsi mentioned in ayurvedic texts – Rama or green leaf tulsi (*O. gratissimum*), Shyama or krishna or purple leaf tulsi (*O. sanctum*) and Vana or wild leaf tulsi (*O.canum*). Although, all three types of Tulsi have their uses in ayurveda, the Rama and Krishna are the most widely used. We work with all three types of Tulsi. Morphological studies of Leaves showed the presence of various diagnostic characters. In the microscopical studies of leaves showed the presence of vascular bundle, trichomes, spongy parenchyma cells etc. Ash value and extractive value was determined for quality standard of drugs. Phytochemical investigation shows the presence of alkaloids, flavanoids, phenol, phytosterol and tannins etc.

Keywords- *Ocimu;, O.gratissimum; O.sanctu; O.canum*; Pharmacognostical

1. INTRODUCTION[1-7]

The *Ocimum* genus, belonging to the *Lamiaceae* family, includes annual and perennial herbal plants, as well as shrubs, from tropical and subtropical zones of Asia, Africa and South America, plants that are widely spread in the world. The complex taxonomy of the genus, determined by interspecific hybridizations and polyploidy, includes 150 species. Whole plant is used as a source of remedy**.** In India two forms of Tulsi are more common - dark or *Shyama* (Krishna) Tulsi and light or Rama Tulsi. The former possesses greater medicinal value and is commonly used for worship. Various other species are also commonly found in India like *O. canum, O. basilicum*, O. *kilimandscharicum, O. ammericanum, O. camphora* and *O. micranthum.*
An impressive array of health promoting, disease preventing and life prolonging properties of Ocimum have been described and documented over five millennia. In the past few decades, many of these benefits have been investigated and verifi ed by modern scientific

research.*Ocimum's* main Ayurvedic guna (quality) as a healing herb is its ability to enhance the energetic resonance between the body and the environment.

"The Elixir of Life", *Ocimum* has been traditionally employed in hundreds of different formulations for the treatment of a wide disorders, including those of the mouth and throat, lungs, heart, blood, liver, kidney, and the digestive, metabolic, reproductive and nervous systems. Tulsi is commonly used to treat coughs, colds and fl u, head and ear aches, rheumatism and arthritis, malaria, fever, allergies, and various skin diseases, to reduce the toxicity of various poisons, including insect and reptile bites, to expel intestinal parasites, repel insects and purify the air.

2. MATERIALS AND METHODS[8-14] :

There are three varieties of Tulsi namely, Rama Tulsi (Ocimum gratissimum), Krishna Tulsi (Ocimum sanctum) and Vana Tulsi (Ocimum canum).leaves were collected from local area of Bhopal Madhya Pradesh India and authenticated at Safia college of Science Bhopal, Madhya Pradesh The voucher specimen **(238-39-40/BOT/SAFIA/11)** was deposited in Department of Pharmacognosy TIT college of Pharmacy, Bhopal, Madhya Pradesh.

2.1 Pharmacognostical study: Macroscopical evaluation– Organoleptic evaluation of drugs refers to the evaluation of drugs by color, odour, size, shape, taste and special features including touch and texture etc. Organoleptic evaluations can be done by means of organs of special sense which includes the above parameters and thereby define some specific characteristics of the material which can be considered as a first step towards establishment of identity and degree of purity.

Microscopical Evaluation: Microscopical parameters observed were,

- Arrangement of tissues in a transverse section.
- Type of epidermal cells, stone cells, testa and endosperm.
- Presence and type of crystalline structures eg. Calcium oxalate, starch etc.
- Presence of oil globules, aleurone grains and trichomes.

All determination was carried out by using Almicro compound microscope (10x, 40x) attached with a camera.

***2.2 Physicochemical studies*:** Physicochemical studies include ash value and extractive value to determine the quality and purity of the powder of plants of *Ocimum genus.*

2.2.1 Determination of ash values: The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material. This values varies with in fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drug.

Total Ash: To determine the total ash place about 2-4 gm of ground air dried drug, accurately weight in a previously ignited and and tered crucible of platinum or silica. Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600^{0}C until it is white, indicating the absence of carbon. Cool in a dessicator and weight.Calculate the percentage of ash with reference to air-dried drug.

Acid Insoluble Ash: To determine the acid insoluble ash boil the ash with 25 of dilute HCL for 5 minutes, collect the insoluble matter in a Gooch crucible or an ashless filter paper, wash with hot water , ignite, cool in a dessicator and weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

Water Soluble Ash: To determine water soluble ash total ash boil with the 25 ml of water for 5 minutes. Collect insoluble ash in a sintered glass crucible or an ashless filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450^{0}C cool and weight. Calculate the percentage of water soluble ash with reference to the air dried drug.

2.2.2 Determination of solvent extractive values: This method determines the amount of active constituents in a given amount of medicinal Plant material when extracted with solvents. For determination of solvent extractive values 5gm of the air dried , coarsely powdered macerated with 100 ml of water close flask for 24 hours , shaking frequently during first 6 hours and allowing stand for 18 hours. Thereafter, filter rapidly taking precautions against loss of solvent; evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, at 105^0 C and weight. The percentage of solvent soluble extractive with reference to air dried drug has to be calculated.

2.2.3 Extraction of plant material: The collected, cleaned leaves of ocimum were used for the extraction process. 200g of powder of Leaves were macerated with Pet. ether, Acetone, Methanol, and Water shaking frequently during first 6 hours and allowing stand for 18 hours. The extracts were filtered through what Mann filter paper to remove any impurities if present. The extracts were concentrated by vacuum distillation to reduce the volume 1/10. The concentrated extracts were transferred to 100 ml beaker and to removing solvent were evaporated on the water bath. The dried extracts were packed and labeled in air tight container for the further studies.

***2.2.4 Phytochemical screening*[8-14]:** Screening was carried out on all the *Ocimum* extracts to determine the active principles or secondary plant constituents. Two (2) milliliters of each extract was measured into a test tube for each of the tests. Tests were carried out for carbohydrates, reducing sugars, tannins, polyphenols, lipids, flavonoids, ketones, alkaloids, steroids and triterpenes.

Various chemical tests in order to determine the secondary plant constituents presents by employing the use of various methods as follows

Test for reducing sugars: To 2 ml of the extract, 5ml of a mixture (1:1) of Fehling's solution IA and Fehling's solution II (B) was added and the mixture boiled in a water bath for five minutes. A brick-red precipitate indicated the presence of free reducing sugars.

Test for the presence of anthraquinones: 0.5g of the extract was shaken with 10 ml of benzene, filtered and 5 ml of 10 percent Ammonia solution added to the filtrate. The mixture was shaken; the presence of a pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of anthraquinones.

Test for saponins: 0.5g of the extract was dissolved in a 10 ml of distilled water in a test-tube, the test tube was stopperred with a cork and shaken vigorously for 30 seconds and then allowed to stand for 45 minutes. The appearance of frothing which persists on warming indicated the presence of saponins.

Test for flavonoids: To a portion of the dissolved extract, a few drops of 10 % ferric chloride solution were added. A green or blue colour indicated the presence of phenolic nucleus.

Test for steroids/terpenes: 0.5g of the extract was dissolved and 2 ml of acetic anhydride and cooled well in ice. Sulphuric acid was then carefully added. A colour change from violet to blue to green indicated the presence of a steroidal nucleus.

Test for tannins: 0.5 g of the extract was dissolved in 5 ml of water followed by a few drops of 10 % ferric chloride. A blue-black, green, or blue-green precipitate would indicate the presence of tannins.

Test for alkaloids: 0.5g of ethanol extract was stirred with 5ml of 1 percent aqueous hydrochloric acid on a stem bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated with Dragendorff's reagent. Turbidity or precipitation with either of these reagents would indicate the presence of alkaloids in the extracts.

Test for resins: 10ml of petroleum ether extract was obtained in a test-tube, the same amount of cupper acetate solution was added and the mixture was shaken vigorously and allowed to separate, a green colour indicates the presence of resin.

Test for proteins and amino acid: Amino acids and proteins when boiled with 0.2% solution of ninhydrin (Indane 1, 2, 3 trione hydrate), violet colour appears.

3. RESULT AND DISCUSSION:

3.1 Macroscopical evaluation: Organoleptic evaluation of drugs refers to the evaluation of drugs by color, odour, size, shape, taste and special features including touch and texture etc. Macroscopical evaluation of *O.gratissimum, O.sanctum, O.canum* shown in table 1.

3.2 Microscopical evaluation: Microscopy of *O.Gratissimum, O.Sanctum, O.Canum* shown in fig 1, 2 and 3 respectively.

Ash values: The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material. This values varies with in fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drug. The Ash values (total ash, acid insoluble ash and water soluble ash) of *O.*gratissimum*, O.sanctum, O.canum* shown in Table -2.

Extractive values: An extractive value determines the amount of active constituents in a given amount of medicinal Plant material when extracted with solvents. The extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituent. The composition of these phyto-constituents in that particular solvent depends upon the nature of the drug and solvent used. Extractive values of *O.gratissimum, O.sanctum, O.canum* is determined in Methanol, Water, Acetone and Pet. Ether shown in table 3.

Phytochemical screening: The various extract of plant *O.gratissimum, O.sanctum, O.canum* were subjected to phytochemical screening which reveal the presence of various pharmacological active compounds such as alkaloids, flavonoids, saponin, tannins, phenolic compound and amino acids. Phytochemicals in methanolic, acetone pet. ether and aqueous leaf extract of *O. gratissimum O. sanctum, O.canum* shown in Table 4,5 and 6 respectively.

4. ACKNOWLEDGEMENTS:

I wish to extend my sincere thanks to my beloved **Dr. B.K Dubey** Director of TIT College of Pharmacy Bhopal and my beloved guide **Mr. Amit Joshi** professor, TIT College of Pharmacy Bhopal for providing unceasing encouragement, precious and erudite suggestions and directions, constant and untiring guidance along with the freedom of work that he gave me. To work under the guidance of such persons has been a great and inexplicablie experience.

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Table 1. . Macroscopical evaluation of *O.gratissimum, O.sanctum, O.canum*.

Parameters	*Ocimum gratissimum l.*	*Ocimum Sanctum l.*	*Ocimum canum l.*
Colour	Light green	Green to purple	Green
Odour	Aromatic smell	Aromatic smell	Aromatic smell
Taste	Pungent taste	Warm pungent taste	Sharp pungent taste
Size and Shape	2.5-5 cm. long and 1.6-3.2 cm broad, oval, pointed and sharp.	2.5-5 cm. long and 1.5-3.2 cm broad, elliptical, oblong.	2.5-6 cm. long, 1-2.5 cm broad Lanceolate to oblong lanceolate, scattered.

Table 2. Ash Values Of *O.Gratissimum, O.Sanctum, O.Canum.*

Plant	Total Ash	Acid Insoluble	Water Soluble
Ocimum	8.6%	0.3%	3.7%
Ocimum	8.8%	0.4%	3.8%
Ocimum canum	8.7%	0.4%	3.6%

Table 3. Extractive Values of *O.Gratissimum, O.Sanctum, O.Canum,* in Following Solvents

Plant	Water	Methanol	Acetone	Petroleum
Ocimum gratissimum	4.8	6 %	3.7 %	3.5 %
Ocimum sanctum	4.2 %	4 %	3.6%	3.8%
Ocimum canum	4.5 %	3.8 %	3.5 %	3.6 %

Table 4. Phytochemicals in Methanolic, Acetone Pet. Ether and Aqueous Leaf Extract of *O. Gratissimum.*

Phytochemicals	Methanol	Acetone	Water	Pet. Ether
Alkaloids	+	+	+	+
Saponins	-	-	+	-
Tannins	-	+	+	-
Steroids	+	+	-	+
Flavonoids	+	+	+	-
Glycosides	-	-	-	-
Reducing sugar	+	+	+	-
Carbohydrates	+	+	+	-
Amino acid	+	+	+	+

Table 5. Phytochemicals in Methanolic, Acetone Pet. Ether and Aqueous Leaf Extract of *O. Sanctum.*

Phytochemicals	Methanol	Acetone	Water	Pet. Ether
Alkaloids	+	+	+	+
Saponins	-	+	+	-
Tannins	-	+	+	-
Steroids	+	+	-	+
Flavonoids	+	-	+	-
Glycosides	-	-	-	-
Reducing sugar	+	+	+	-
Carbohydrates	-	+	+	-
Amino acid	+	+	+	+

Table 6. Phytochemicals in Methanolic, Acetone Pet. Ether and Aqueous Leaf Extract of *O. Canum*.

Phytochemicals	Methanol	Acetone	Water	Pet. Ether
Alkaloids	+	+	+	+
Saponins	+	-	+	-
Tannins	-	+	+	+
Steroids	+	+	-	+
Flavonoids	+	-	+	-
Glycosides	-	-	-	-
Reducing sugar	+	+	+	-
Carbohydrates	+	+	+	-
Amino acid	+	+	+	+

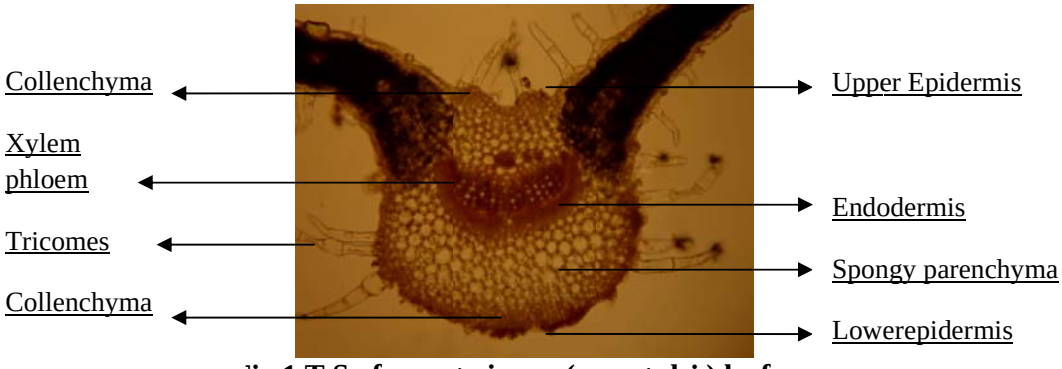

Fig.1 T.S of *o.gratssimum* (ram tulsi) leaf

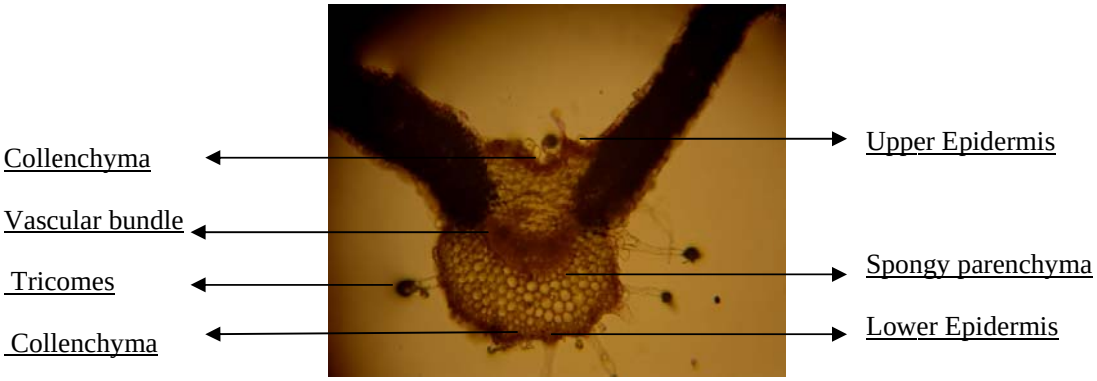

Fig.2 T.S of *o.sanctum* (shyam tulsi) leaf

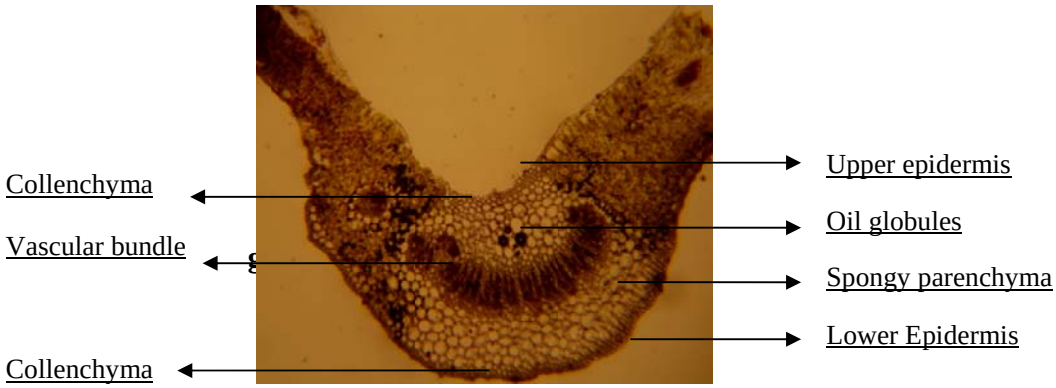

Fig.3 T.S of *o.canum* (vana tulsi) leaf