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Preliminary Phytochemical Screening of Indigenous Medicinal Plants *Ocimum tenuiflorum, Ocimum basilicum,* and *Ocimum gratissimum*

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ABSTRACT

The *Ocimum* species has been suggested to possess antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic and diaphoretic actions. This study focuses on the preliminary phytochemical screening of three species belonging to the genus *Ocimum* namely, *O. tenuiflorum, O. basilicum* and *O. gratissimum*. Six solvents (distilled water, petroleum ether, acetone, chloroform, ethanol, methanol) were used for the solvent extraction. The extracts were screened for phytochemicals. Among the tested samples, aqueous extracts showed the presence of bioactive compounds such as alkaloids, flavonoids, phenols, tannins, steroids and terpenoids. Among the tested samples a higher concentration of phytochemicals was found in *O. tenuiflorum*. Our study revealed the presence of various secondary metabolites in *Ocimum* species with pharmacological importance.

***Key words*:** Phytochemicals, Secondary metabolites, Medicinal plants, *Ocimum tenuiflorum, Ocimum basilicum, Ocimum gratissimum*

Medicinal plants are of great importance to the health of an individual and a community. The utilisation of herbaceous plant has achieved a predominant role in healthcare context all over the world. Moreover, herbs utilised in the traditional system of medicine like Ayurveda, traditional chinese medicine, folk medicine etc. are obvious rival for substances with novel structures, along with mechanisms of action [1]. Population rise, inadequate supply of drugs, cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. The drugs derived from natural resources have a significant contribution in the traditional and modern system of medicines. Relevant steps have been taken by the World Health Organization (WHO) to carry out the research with the aim of finding new and effective medicinal agents from plants [2]. The vast and versatile pharmacological effects of medicinal plants are basically dependent on their phytochemical constituents. Plants produce diverse bioactive molecules like flavonoids, alkaloids, tannins, steroids etc. that can act as defence against a wide range of pathogens. These molecules are effective substitution for chemicals used in pharmacology since they have no side effects. The phytochemical constituents such as alkaloids, steroids, flavanoids, tannins, phenols and several other aromatic compounds of plants serve a defence mechanism against predation by many microorganisms, insects and other herbivores. Secondary metabolites have shown to possess various biological effects, which provide the scientific base for the use of herbs in the traditional medicine [3-4]. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability.

Ocimum sanctum Linn. commonly known as holy basil (tulsi) is a perennial herbaceous plant, belongs to the Lamiaecae family. It is a popular home remedy for many ailments such as wound, bronchitis, liver diseases, catarrhal fever, otalgia, lumbago, hiccough, ophthalmia, gastric disorders, genitourinary disorders, skin diseases, various forms of poisoning and psychosomatic stress disorders [5]. *Ocimum basilicum* L., commonly known as sweet basil with green or purplish branches is known for its peculiar strong odours. The plant *Ocimum gratissimum*, also known as Tea bush, is one of those plants widely known and used for both medicinal and

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nutritional purposes. It is a perennial plant that is widely distributed in the tropics of Africa and Asia, and it is the most abundant of the genus *Ocimum* [6].

It was reported that the aqueous extract of *O. sanctum* shows immunomodulatory activity by stimulating antibody production thereby enhancing the production of immune cells and haemoglobin [7]. Leaves of this plant are used for antipyretic, analgesic, antioxidant, antibacterial, antifungal, anti-inflammatory, and anticancer activity. Physicochemical, phytochemical, antioxidant, and anticancer studies of *O. tenuiflorum* revealed the presence of phytosterols and triterpenoids, alkaloids, tannins, flavonoids, sugar, protein, amino acid, fat, and oils. The antioxidant activity reveals that the fixed oil possesses the potent antioxidant activity [2].

The administration of hydroalcoholic extract of *O. basilicum* leaves potently protects the myocardium against isoproterenol induced infarction and suggest that the cardioprotective effect of the extract could be related to its antioxidant activities [8]. A comparative study on the antioxidant properties of *Ocimum* species namely *O. basilicum* and *O. sanctum* was carried out and it was concluded that *O. basilicum* had more activity than the later [9]. *O. basilicum* and many other herbs belonging to the genus *Ocimum* are used as treatment for the diseases related to the central nervous system. Protection of central nervous system against oxidative damages of electromagnetic field by using *O. basilicum* has been reported [6]. Study on the phytochemical analysis antibacterial activity of *O. gratissimum* found out the ability of ethanol to extract more of the essential oils and secondary plant metabolites which are believed to exert antibacterial activity on the test organisms [10]. It is considered as an Ayurvedic herb of Southeast Asia with a long history of the traditional use. The phytochemical compounds of *Ocimum* are highly complex containing many nutrients and other biologically active compounds. The phytochemicals present in them are oleanolic acid, ursolic acid, eugenol, rosmarinic acid, apigenin, myretenal, luteolin, β-sitosterol, and carnosic acid [11]. In recent years, attention has been turned to researchers to discover new alternative sources of anti-inflammatory agents, especially of plant origin. *Ocimum sanctum* (tulsi) has served humanity as a source of medicinal agents since its inception [12]. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability [13]. During the last two decades, *Ocimum* has demonstrated various pre-clinical activities in animal models *in vitro* testing [14]. The present study aims to investigate the phytochemical constituents present in common species of *Ocimum* namely, *Ocimum sanctum*, *Ocimum basilicum* and *Ocimum gratissimum*.

MATERIALS AND METHODS

Ocimum tenuiflorum

O. tenuiflorum also known as Ocimum sanctum L. (Tulsi) is an erect, much branched sub-shrub 30-60 cm tall, with simple opposite green or purple leaves that are strongly scented and hairy stems. Leaves have petiole and are ovate, up to 5 cm long, usually somewhat toothed. Flowers are purplish in elongate racemes in close whorls [15].

Ocimum basilicum

It is an erect aromatic nearly glabrous branching herb 62-90 centimetre in height with hairy stem, green or purplish branches and leaves, simple opposite, strongly scented. Flowers are white or pale purple in colour found as branch racemes. Fruits are eclipsed nutlets, black in colour.

Ocimum gratissimum

It is an aromatic, perennial herb, 1-3 m tall; stem erect, round-quadrangular, much branched, glabrous or pubescent, woody at the base, often with epidermis peeling in strips. Leaves opposite petiole 2-4.5 cm long, slender, pubescent Inflorescence a verticillaster, Fruit consisting of 4, dry, 1-seeded nutlets enclosed in the persistent calyx [16].

Drying of the sample

Fresh leaves and stems of the plant material were washed under running tap-water and distilled water for 2-3 times. Then the materials were shade dried and ground to fine powder. The powder was stored in an air tight bottle and kept at 4°C for further use.

Preparation of plant extract

5 gm of the powdered sample was suspended in 50 ml of solvents. Distilled water, ethanol, acetone, methanol, petroleum ether and chloroform were used for extraction. It was kept for 24 hours with occasional shaking and were centrifuged at 3000 rpm for 15 minutes. The supernatant was then stored in labelled screw capped bottles and was used for preliminary phytochemical screening.

Preliminary phtochemical screening

Preliminary phytochemical screening was done by standard methods [17-19].

Detection of alkaloids

Extract was dissolved individually in diluted hydrochloric acid and filtered

Mayer's test: Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of yellow coloured precipitate indicate the presence of persists 10 minutes, indicate the presence of alkaloids.

Wagner's test: Extract is treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/red precipitate, indicate presence of alkaloids.

Detection of carbohydrates

Molish's test: To 2 ml of the filtrate, two drops of alcoholic solution of alpha Naphthol (20% ethyl alcohol) were added, the mixture is shaken well, and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A reddish violet ring indicates the presence of carbohydrates.

Fehling's test: 1 ml of the filtrate was boiled on a water bath with 1 ml of each Fehling's solution A and B. Brick red precipitate indicated the presence of sugar.

Detection of flavanoid

Alkaline reagent test: The extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which become colourless on addition of dilute hydrochloric acid, indicate the presence of flavonoids.

Lead acetate test: The extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicate the presence of flavonoids.

Ferric chloride test: 1 ml of the extract were treated with 1 ml of ferric chloride. Formation of brown colour precipitate indicates the presence of flavonoids.

Ammonia test: A small piece of filter paper is dipped to about 1 ml of the extract and is exposed to ammonia vapour. Formation of yellow spot-on filter paper indicate the presence of flavonoids.

Detection of aminoacids and proteins

Xanthoproteic test: The extract was treated with few drops of concentrated Nitric acid. Formation of yellow colour indicates the presence of protein.

Ninhydrin test: To the extract, 0.25% w/v ninhydrin reagents was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Biuret test: To 2 ml of the extract, add 1 ml of the 10% sodium hydroxide, mix well. Add few drops of 0.01 % copper sulphate solution. Formation of violet or pink colour confirms the presence of protein.

Detection of anthraquinone

Borntranger's test: To 1 ml of the extract, add 1m1 of 10% ferric chloride and 5m1 of concentrated Hydrochloric acid. Boil in water bath for few minutes and filter it. Filtrate with 1 ml of diethyl ether and concentrated ammonia shows appearance of pink or deep red colour.

Detection of glycoside

Extract was hydrolyzed with dilute hydrochloric acid and then subjected to test for glycosides.

Modified Borntranger's test: Extract were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose colour in ammoniacal layer detect the presence of glycosides.

Detection of cardiac glycosides

Keller-kiliani test: 5m1 of the extract is treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of deoxy sugar, a characteristic feature of cardenolides. A violet ring may appear below the brown ring. While in the acetic acid layer, a greenish ring may form.

3,5- dinitro benzoic acid test: To 1 ml of the extract, add few drops of NAOH followed by 2% solution of 3,5 dinitro benzoic acid. Formation of pink colour indicates the presence of cardiac glycosides.

Detection of saponins

Sodium bicarbonate test: To few ml of the extract, few drops of sodium bicarbonate were added and shaken well. Formation of honeycomb like frothing indicates the positive test for saponins.

Detection of steroids

Salkowski's test: To 0.5 ml of the extract, 2 ml chloroform was added. Concentrated sulphuric acid was carefully added to form a reddish-brown colouration at the interface. This indicates the presence of steroids.

Liebermann Burchard test: Extract were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added. Formation of brown ring at the junction indicate the presence of phyto steroids.

Detetion of phenols

Ferric chloride test: Extract were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Lead acetate test: The extract was dissolved in distilled water and 3 ml of 10% Lead acetate solution was added. A bulky white precipitate indicates the presence of phenol compounds.

Detection of tannins

Potassium dichromate test: The extract was added to potassium dichromate solution, formation of a precipitate shows presence of tannins.

Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicate the presence of tannins.

Detection of terpenoids

Salkowski test: 5m1 of each extract was made mixed in 2 ml of chloroform and concentrate sulphuric acid was carefully added to form a layer. A reddish-brown colouration in the interface will be formed.

Copper acetate test: The extract was dissolved in water and treated with 2-3 drops of copper acetate solution. Formation of emerald green colour indicates the presence of terpenoid.

RESULTS AND DISCUSSION

Extracts of *O. tenuiflorum, O. basilicum* and *O. gratissimum* were prepared using distilled water, petroleum ether, chloroform, acetone, methanol and ethanol. Phytochemical screening of *O. tenuiflorum* showed the presence of alkaloids in distilled water, petroleum ether, methanol and ethanol. Carbohydrates, anthraquinones, glycosides and terpenoids were present in all the tested samples of *O. tenuiflorum.* Presence of flavonoids, phenols and tannins was showed only in aqueous extracts.

In a study by Borah and Biswas, they concluded that leaves of *Ocimum sanctum* contain water-soluble phenolic compounds and various other constituents, such as eugenol, methyl eugenol and caryophyllene that may act as an immunostimulant [4]. Parveen *et al*. [20] also observed the positive result for the presence of alkaloid, terpenoids and carbohydrates in aqueous and methanolic extract of *O. sanctum* plant. Tannins have been present in aqueous extract which can

be employed medicinally as antidiarrheal, haemostatic and anti-hemorrhoidal compounds. Its presence in the plant may suggest the medicinal value because tannins because of its potential antiviral, antibacterial and antiparasitic effects [21].

Table 1 Phytochemical constituents of different extracts of *Ocimum tenuiflorum*

S. No.	Chemical compound	Distilled water	Petroleum ether	Chloroform	Acetone	Methanol	Ethanol
1	Alkaloid	+	+	-	-	+	-
2	Carbohydrate	+	+	+	+	+	+
3	Anthraquinones	+	+	+	+	+	+
4	Cardiac Glycoside	+	+	+	+	+	+
5	Glycosides	+	+	+	+	+	+
6	Phenol	+	-	-	-	-	-
7	Flavonoid	+	-	-	-	-	-
8	Amino acid and proteins	+	+	-	+	-	-
9	Steroids	+	+	-	-	-	-
10	Saponins	-	-	-	-	-	-
11	Tannis	+	-	-	-	-	-
12	Terpenoids	+	+	+	+	+	+

Chloroform, ethanol and acetone extract does not showed the presence of alkaloids phenols and flavonoids [22]. Various effects like immunomodulatory, hyperglycaemic, hypolipidemic, anti-inflammatory, hepatoprotective, antimutagenic, antimicrobial, antifungal, antioxidant, lipid peroxidation, insect repellence, antiviral, antierythmic, depigmenting and antitoxic effects of *O. tenuiflorum* has been reported from all over [6]. Presence of alkaloids, anthraquinones, carbohydrates and terpenoids was detected in all extracts of *Ocimum basilicum.* Chloroform extracts did not show any distinctive activity here.

Warsi and Sholichah noted the advantage of using ethanol 70% is the compounds from less polar to polar so that it can be withdrawn from sample, including flavonoids compounds [23]. In our study absolute alcohol was used for solvent extraction but active compounds were unable to extract completely and majority of them like flavonoids, tannins, phenols, saponins and steroids were absent in the sample.

Reports from workers in this area indicated the presence of flavonoids [22]. This is contrary to what was obtained in this work. In most cases, tannins are usually associated with flavonoids, which are their monomeric precursors. The positive result for tannins and flavonoids was only obtained in aqueous extracts. This difference might be attributed to one or both of two factors. The other solvents (Petroleum ether, chloroform, acetone, ethanol and methanol) used in this work might not be one that can extract all the phytochemicals. Even if some were extracted, the levels might have been too low to give positive results in qualitative tests for the compounds. Another factor is geographical [24].

Table 2 Phytochemical constituents of different extracts of *Ocimum basilicum*

S. No.	Chemical compound	Distilled water	Petroleum ether	Chloroform	Acetone	Methanol	Ethanol
1	Alkaloid	+	+	+	+	+	+
2	Carbohydrate	+	+	+	+	+	+
3	Anthraquinones	+	+	+	+	+	+
4	Cardiac Glycoside	-	+	+	-	+	-
5	Glycosides	+	+	+	+	+	+
6	Phenol	-	-	-	+	+	-
7	Flavonoid	+	-	-	-	-	-
8	Amino acid & proteins	-	+	-	+	-	-
9	Steroids	+	+	-	-	-	-
10	Saponins	-	-	-	-	-	-
11	Tannis	+	-	-	-	-	-
12	Terpenoids	+	+	+	+	+	+

The result of phytochemical screening of *Ocimum grattissimum* showed the presence of alkaloids, terpenoids, glycosides and tannins in distilled water extract. All other samples except the aqueous extract showed negative results for phenols, flavonoids and steroids. Aqueous and ethanol extracts showed that the plant leaves contain tannins, flavonoids, terpenoids alkaloids phlobatannins, tannins saponins, steroids and glycosides. HPLC analysis on the same ascertains that *Ocimum gratissimum* contains all the pharmaceutically important phytochemical constituents [18].

The antioxidant, phytochemical and nutritional properties of acetone, methanol and aqueous extracts of the leaves of *Ocimum gratissimum* (Linn) were investigated by Etinosa *et al*. [25], to evaluate the therapeutic and nutritional potential of the leaves of this plant. Investigations on the phytochemical screening of *Ocimum gratissimum* leaf extract revealed the presence of tannins, saponins, steroid, alkaloids, terpenoids, flavonoids, phenols and cardiac-glycosides. These compounds are known to be biologically active and therefore aid the antioxidant activities of *Ocimum gratissimum*. Study on

the aqueous leaf extracts of *Ocimum gratissimum* possessed antidiabetic properties which suggest the presence of biologically active components which may be worth further investigation and elucidation [26]. The beneficial health effects of *Ocimum* oil compounds have also been proven by many randomized, crossover, controlled, human studies on biomarkers of health performed in the last decades. Several preclinical studies suggest that such beneficial effects may be mainly ascribed to the phenolic compounds [27].

Table 3 Phytochemical constituents of different extracts of *Ocimum gratissimum*

S. No.	Chemical compound	Distilled water	Petroleum ether	Chloroform	Acetone	Methanol	Ethanol
1	Alkaloid	+	-	-	+	+	-
2	Carbohydrate	+	+	+	+	+	+
3	Anthraquinones	+	+	+	+	+	+
4	Cardiac Glycoside	+	-	-	+	+	-
5	Glycosides	+	+	+	+	+	+
6	Phenol	-	-	-	-	-	-
7	Flavonoid	+	-	-	-	-	-
8	Amino acid & proteins	-	-	-	-	-	-
9	Steroids	+	-	-	-	-	-
10	Saponins	-	-	-	-	-	-
11	Tannins	+	-	-	-	-	-
12	Terpenoids	+	+	+	+	+	+

CONCLUSION

The analysis of phytochemicals of *O. tenuiflorum*, *O. basilicum* and *O. gratissimum* in the present study has proved that in all the *Ocimum* species the presence of the phytochemicals which are known as biologically active compounds such as phenols, flavonoids, saponins, tannins, alkaloids A higher concentration of phytochemicals is found in aqueous extract of all species. The constituents of *Ocimum* plants have shown a greater advantage in the treatment of various diseases. The plant extract might also be a potential source for drugs design as the plant leaves are used conventionally for curing of many infectious diseases.

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