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Research Article

Phytochemical analysis of medicinal herb (*ocimum sanctum*)

Abstract

It is an aromatic plant. Plants have served human kind as sources of medicinal agents since its earliest beginnings. In fact natural product once served as the source of all drugs. The main chemical constituents of Tulsi are: Oleanolic acid, Ursolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalool, and β -caryophyllene, have been used extensively for many years in food products, perfumery, and dental and oral products and plant extract continues the numerous searches for more effective drugs of plant origin which are less toxic and available for low socio-economic population in the treatment of diseases caused by pathogenic bacteria. Recent studies suggest that Tulsi may be a COX-2 inhibitor, like many modern painkillers, due to its high concentration of eugenol. The present study was to evaluate the phytochemical screening of aqueous extracts of leaves of *Ocimum*. Study has been shown that this medicinal herbs can be used as pharmaceutical adjuvants in the formulation of various dosage form.

Introduction

Ocimum Sanctum also known as Tulsi family of the *ocimum sanctum* is laminaceae. *Ocimum sanctum* are produced in India and Southeast Asia, India is the largest sources of medicinal plant in whole world. Herbs have been provided therapeutic potential to the health of individual. The demand of this plant are increasing day by day for medicinal purpose [1]. There are approximately 35,000 medicinal plants which are used for the therapeutic effect according to Ayurveda and siddha and unani and other traditional system. In which *ocimum sanctum* is one of the most important for medicinal purpose. It is employed in the treatment of various disease such as antimicrobial infection, antifungal, anticancer, arthritis, chronic fever, antifertility, eye disease, hepatoprotective, antispasmodic, and analgesic, antiemetic. Cardio protective [2]. This medicinal herb have also been shown to reduce blood glucose levels, making it an effective treatment of diabetes [3]. There are many chemical constituent present in *ocimum sanctum* such as, oleanolic acid, rosmarinic acid, ursolic acid eugenol, , linalool, carvacrol, β elemene, β caryophyllene, germacrene. *Ocimum sanctum* is considered to have diuretic, stimulant property [4]. Volatile oil, fixed oil also obtained from the leaves of medicinal herbs [5]. Monoterpene are obtained from the the volatile oils such as, camphene, myrcene, sabinene, in which some mono terpene produced oxygen such as linalool, borneol [6]. Phytochemical analysis of this medicinal herb can identify the nature of compounds present in the extract of *ocimum sanctum*. It is also for identify the bioactive compound and their effect. They are commonly helpful as model for the synthetic of new medicine [7].

Material and Methods

Chemicals- Mayer reagent, Wagner's reagent, Lead ethanoate, Alkaline reagent, Ferric chloride, Molisch's reagent, Alkaline reagent, Barford's reagent, Iodine solution, Ninhydrin solution, sodium hydroxide, all chemicals were used to find out the presence of phytochemical constituents which were obtained from the research lab of Galgotias university.

Plant Material- Fresh Leaves of selected medicinal herb *Ocimum sanctum* (Tulsi) was harvested from the herbal garden of Galgotias university, Greater Noida in the month of December, 2018. The collected leaves were thoroughly washed with tap water to avoid dusts and other unwanted materials accumulated on the leaves from their natural environment. The dust free leaves were shade, dried at room temperature. After 4-5 days for obtaining aqueous extract, the properly dried leaves were then grinding into the fine powder by using the grinding machine than the powder material of tulsi leaves were weighed properly. The fine powder of tulsi leaves was stored in a clean and tightly closed container for extraction.

Kingdom	Plantae
Subkingdom	Tracheobionta
Class	Magnoliopsida
Order	Lamiales
Division	Magnoliophyta
Super division	Spermatophyte Seed plants

Genus	<i>Ocimum</i> L.
Species	<i>O. tenuiflorum</i>
Family	lamiaceae

Preparation of aqueous extract of *Ocimum sanctum* (leaves)- The extract of leaves were obtained in sufficient quantity by using distilled water. In this process firstly 20 g powdered leaves of *ocimum sanctum* were placed in 200 ml of beaker and 100 ml of distilled was poured into beaker after addition of water kept for overnight at the room temperature approximately 22 hrs for thorough mixing and also complete elucidation of active materials to dissolve in the respective solvent then, extract was filtered by using muslin cloth followed by Whatman no 1 filter paper then the green colour filtrate was obtained, after done this process filtrate was dried. Finally, the residues were collected and used for the experiment (Figure 1) [8].

Aqueous extract of *ocimum sanctum* leaves which is used in the various treatment. Fresh juice of Tulsi leaves is employ in karma. This technique helps to ease headache and diseases of head and neck. Tulsi leaves act as nerving tonic. Tulsi leaves extract reduces pimples, acne and scars effectively (Figure 2).

Organoleptic characterization of aqueous extract- The colour, odour, texture, taste, fracture of *ocimum sanctum* (Tulsi) were characterized [9].

Phytochemical analysis of aqueous extract of *ocimum sanctum*- The aqueous extract of *ocimum sanctum* was subjected to phytochemical analysis find out the presence and



Figure 1: *Ocimum sanctum* leaves.

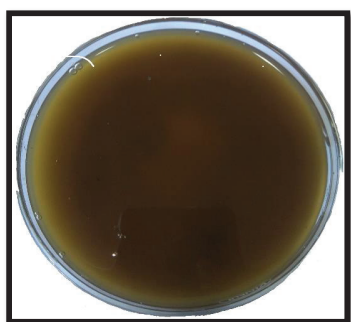


Figure 2: Aqueous extract of *Ocimum sanctum* leaves.

absence of phytochemical constituents. Flavonoid synthesis in plants is induced by light colour spectrums at both high and low energy radiations. Low energy radiations are accepted by phytochrome, while high energy radiations are accepted by carotenoids, flavins, cryptochromes in addition to phytochromes. The phytochemical tests employed for alkaloids, flavonoids, glycosides, proteins, fixed oil, carbohydrate and tannins, Cardiac glycosides, saponins and flavonoids and terpenoids [10-13].

Test for alkaloids

Mayer's test- 5 mg extract of *Ocimum Sanctum* (Tulsi) was transferred in the test tube and then added 1% hydrochloric acid HCl, the obtained solution was gently heated. Red colour indicate the presence of alkaloids because Potassium mercuric iodine are present in Mayer's reagent.

Wagner's test- In this test 5 mg extract of *ocimum sanctum* was taken in a test tube than 0.5 of wagner reagent was added in a solution shaken well. Apperance of reddish brown colour showing the alkaloids are present. Reddish brown colour because of iodine forms a complax is insoluble and has the colour brown redish.

Dragendorff test-5 mg extract of *ocimum sanctum* tulsi was taken in tube. And then one drop of dragendroff reagent was addedin the test tube. orange-red colour, showing the presence of alkaloids. Dragendroff reagent was prepared using Bismuth nitrate, Nitric acid, iodine and water because of these chemicals it gives orange red colur in the preseance of alkaloids.

Test for flavanoids

Shinoda test: Firstly 5mg extract was added in the test tube then small amount of magnesium was mixed in this solution, also added the the few drops of concentrated Hydrochloric acid. It should be indicate the pink colour with the flavonoids. Colours varying from orange to red indicated flavones, red to crimson indicated flavonoids, crimson to magenta indicated flavonones. Catechins when treated with vanillin solution in hydrochloric acid give red pink colour.

Lead ethanoate test for flavonoids- placed 5 mg of aqueous extract of tulsi in test tube then 1ml of lead ethanoate solution was added. It gives the buff coloured solution if the alkaloids are present.

Sodium hydroxide test for flavonoids- 5 mg extract was taken in this 1 ml of the 10% solution of sodium hydroxide was added for appearance of yellow colour solution after addition of 1ml of dilute Hydrochloric acid, in the presence of alkaloids the colour should be changed from yellow to colourless after addition of 2 ml of dilute hydrochloric acid.

Alkaline reagent test for flavonoids- 5 mg extract of *ocimum sanctum* was placed in the test tube mixed than the 2ml of 2% solution of Sodium hydroxide was poured in it, if the formation of yellow which turned into colourless after addition of few drops of diluted acetic. It means that alkaloids are present in the holy basil.

Ferric chloride test- Ferric chloride test was performed for checking the presence of flavonoids in the aqueous extract of *ocimum sanctum*. Firstly 5 mg extract was mixed with 1ml of distilled water than 0.5ml of dilute ammonia solution was added into it. After addition of dilute ammonia few drops of concentrated Sulfuric acid was mixed later. Formation of yellowish with flavonoids.

Test for glycoside

Liebermann's test- Liebermann' test for the analysis of glycoside are present or not in aqueous extract of *ocimum sanctum* in this test 5 mg extract of *ocimum sanctum* was mixed properly with 2ml of chloroform and then 2ml of acetic acid were mixed in the. Solution than it was cooled in ice. After cooling 1 ml of concentrated Sulfuric acid was added. The colour will be change from violet to green with the presence of alkaloids in the extract.

Salkowski's test- for the analysis of glycoside 2ml of chloroform were with 1ml of extract. Then 2ml of concentrated Sulfuric acid were added and shaken gently. A reddish brown colour indicated the presence of glycoside.

Keller-kilani test for cardiac glycosides- for the confirmation of the glycoside in the extract 5 mg extract was taken in the test tubes than the 1 ml of glacial acetic acid was added. Few drop of 2% solution of ferric chloride were mixed into it. Then 1 ml of concentrated Sulfuric acid were into the mixture. A brown colour ring at the edge will be formed in the presence cardiac glycosides

Test for tannins

Ferric chloride test- 5 mg aqueous extract of *ocimum sanctum* was mixed with 0.5 ml of ferric chloride solution. Formation of blackish precipitate in the presence of tannin.

Gelatine test- gelatine test was performed for checking the presence of tannin in the extract. In this test 5 mg extract was mixed with gelatine and 1ml of water was added into the solution. White precipitate should be produced.

Lead acetate- lead acetate test was performed to estimate the presence of tannin in which 5 mg of test samples was taken in test tubes. Few drops of basic lead acetate was added in the sample solution, if brown bulky precipitate will be found it means tannin are present in test sample.

Test for saponins

Foam test was performed for identification of saponin in the aqueous extract in which 1ml extract was dissolved into the 5ml of distilled water. After addition of distilled water it was shaken for proper mixing till foam was observed. Few foam was added with 2 drops of olive oil and it was shaken vigorously. It should be produced emulsion with the saponins.

Test for oil

Stain test- few quantity of aqueous extract was spread onto the filter paper formation of oil on the filter paper will indicate the presence of oil in aqueous.

Saponification test- Few drops of alcoholic potassium hydroxide and 0.5 ml of extract were taken into test tube and mixed well. 1-4 drops of phenolphthalein were added into the mix solution. It was heated on water bath hours for 1 hour. Formation of partial neutralization of alkali which indicates the presence of oils and fats.

Test for carbohydrates

Benedict's test-Benedict's reagent was taken for the analysis of carbohydrate. the 5 mg extract was mixed with few drops of benedict's reagent, than allowed to boiled, the reddish brown precipitate are found with the presence of the carbohydrates (absent).

Molisch's test- initially 5 mg extract was taken in test tube than the 1 ml of Molisch's reagent was added into it. Mixture was shaken properly. After that, 2ml of concentrated Sulfuric acid was poured carefully along the side of the test tube. Appearance of a violet ring at the interface indicated the presence of carbohydrate.

Test for steroids

5 mg extract of *ocimum sanctum* was mixed with 1 ml of chloroform then few drops of concentrated Sulfuric acid and acetic acid were added into it. The greenish colour was indicate the presence of steroids.

Salkowski's test- 3 drops of concentrated sulphuric acid was added into the 5 mg extract. The formation of red colour indicates the presence of steroids.

Test for proteins

Biuret's test- 5 mg extract was added with the few drops of biuret's reagent. The obtained mixture was shaken well and allowed to warm for 1-5 min. Appearance of red or violet colour indicated presence of proteins

Million's test- 5 mg extract was mixed with 2ml of Mallon's reagent. The solution was heated for 5 min red colour precipitated turns into red colour which confirmed the presence of protein

Ninhydrin test- aqueous extract of tulsi was mixed with 2 ml of 0.2% solution of Ninhydrin and boiled for 2 min on water bath, if violet colour appeared with the presence of amino acids and proteins in the aqueous extract.

Result and Discussion

Phytochemical studies Qualitative phytochemical investigation discovered presence of alkaloids compounds [Appearance of red colour]; flavonoids and tannins [The pink colour shows the presence of flavonoids and blackish precipitate indicated the presence of tannins] and absence of flavonoids [Not observed pink coloration] in all mentioned extracts of plant. Salkowski's test- [formation of brownish-red colour] showed positive result for aqueous extract. It showed negative result in case of protein, saponin, oil, steroids. The presence of these phytochemical components may be responsible for the observed antibacterial activity of the plant leaf extract. Flavonoid has also been reported to have greater

potential benefit to human Health. The medicinal plants *Ocimum sanctum* is being used traditionally for the treatment of inflammation, wound healing, toothache, antiseptics, carminative, cough, expectorant, stomatitis and some fungal infection. The antibacterial activity has been attributed to the presence of some active constituents in the extracts. It showed aromatic- odour, taste – slightly pungent, the texture- smooth of *Ocimum sanctum* were found. The phytochemical screening of aqueous leaf extract of *O. sanctum*, revealed the presence and absence of alkaloids, flavonoids and tannin compounds (Tables 1,2) showed that organoleptic characteristics of *Ocimum sanctum*.

Conclusion

The obtained result from whole study confirm the validity of the use of *Ocimum sanctum* plant as medicine in ancient medicinal traditions and suggest that some of the plant extracts possess compounds with antimicrobial properties. *Cirsilineol*, *circimaritin*, *isothymusin*, *apigenin* and *rosamerinic acid*, are present in isolated aqueous extract of *Ocimum sanctum* which may be useful against fever, syphilitic, ulcer, inflammatory disease wounds, such as antimicrobial infection,

Table 1: Qualitative analysis of phytochemical in the *Ocimum sanctum* extract.

Phytochemical	Test	Inference
Alkaloids	Mayer's test	Present
	Wagner's test	Present
Flavonoids	Sodium hydroxide test	Present
	Shinoda test	Present
	Alkaline reagent test	Present
	Ferric chloride test	Present
Glycosides	Liebermann's test:	Absent
	Salkowski's test:	Absent
	Keller-kilani test	Absent
Tannin	Ferric chloride test	present
	Gelatine test	present
Saponin	Foam test	Present
Oil	Stain test	Absent
Carbohydrates	Saponification test	Absent
	Molisch's test	Absent
Steroids	Benedict's test	Absent
	Chloroform test	Absent
	Salkowski's test	Absent
Test for proteins	Biuret's test	Absent
	Million's test	Absent
	Ninhydrin test	Absent

Table 2: Organoleptic characterization of *Ocimum sanctum*.

S.no	Organoleptic properties	Observation
1	Colour	Green
2	Odour	Aromatic
3	Taste	Slightly pungent
4	Texture	Smooth

analgesic, antifungal, arthritis, anticancer, eye disease, antifertility, hepatoprotective, chronic fever, antispasmodic, antiemetic, cardio protective etc. In protective antioxidant supplement *Ocimum sanctum* leaf extract may be used after the analysis of certain tests. After this study it is assumed that the extract could be used for the new formulations and potent antimicrobial drugs of natural origin.

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