



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2019; 7(3): 1256-1258

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Received: 07-03-2019

Accepted: 09-04-2019

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Phytochemical analysis of aqueous extract of *Ocimum sanctum*

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Abstract

Extracts of fresh leaves of Tulsi (*Ocimum sanctum*) were subjected to chemical analysis for the presence of various phytoconstituents viz phenols, flavonoids, flavonolglucosides, saponins, carbohydrate, sugar, terpenoids, amino acids, proteins and quinones tannins and the results showed positive for phenols, flavonoids, flavonolglucosides, saponins, quinines and tannins and negative for terpenoids and amino acids.

Keywords: Tulsi, *Ocimum sanctum*, leaves, aqueous extract, phytoconstituents

Introduction

Ocimum sanctum commonly known as Tulsi belonging to the family Lamiaceae, is grown throughout India. The two most common varieties of Tulsi include Black and Green variety. Different parts of the plants are used traditionally in many domains of medicines like Ayurveda, Siddha, Greek, Roman and Unani medicine system (Pattanayak *et al.*, 2010; Siddiqui, 1993) [1, 2].

Tulsi has been utilized therapeutically since 400-500 BC. Earliest references of tulsi were found in Rigveda (3500-1600 BC). It has been reported to possess many medicinal properties namely anticarcinogenic, antioxidant, antidiabetic etc. Extract of Tulsi is used in ayurvedic treatments for common cold, heart diseases, and stomach disorders, poisoning cases, convulsions, epilepsy, malaria, fever, bronchitis and certain inflammatory problems (Bano *et al.*, 2017) [3]. *Ocimum sanctum* has specific aromatic odour because of the presence of essential or volatile oil, mainly concentrated in the leaf. This aromatic volatile oil mainly contains phenols, terpenes and aldehydes. The oil extracted from seeds is called fixed oil and mainly composed of fatty acids. Besides oil, the plant also contains alkaloids, glycosides, saponines and tannins. The leaves contain ascorbic acid and carotene as well (Anonymous, 1991) [4]. The present work was carried out to know the presence of various phytoconstituents in black variety tulsi grown in Madhavaram, Chennai, Tamil Nadu.

Materials and Methods

Aqueous extract of *Ocimum sanctum*

Fresh and healthy leaves were collected locally and rinsed thoroughly with tap water followed by distilled water to remove all the dust and unwanted visible particles. 30g of leaves were weighed and transferred into 250 mL beakers containing 200 mL distilled water and boiled for about 20 min. The extracts were then filtered thrice through Whatman No. 1 filter paper to remove particulate matter and to get clear solutions which were then refrigerated (4°C) in 250 mL Erlenmeyer flasks for further experiments (Balamurugan *et al.*, 2014) [5].

Tests for identification for active compounds

The tests were carried out to find the presence of the active chemical constituents such as phenol, flavonoids, flavonoglycosides, saponins, carbohydrates, terpenoids, alkaloids, proteins, amino acids, phytosteroids, quinones and tannin by the following procedure.

Test for Flavonoids

Ferric Chloride test

To 5 ml of extract, neutral 5 per cent ferric chloride solution was added drop by drop. The presence of flavonoid was indicated by the appearance of dark green colour.

Alkaline reagent test

To 2 ml of extract, 3 ml of 10 per cent lead acetate solution was added. The presence of flavonoids was indicated by yellow fluorescence.

Magnesium and Hydrochloric acid reduction

The extract solution was treated with 5 ml of alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid (drop wise) were added. Appearance of pink to crimson colour indicated the presence of flavonolglucosides.

Test for Saponins (Foam test)

Twenty ml of extract solution was shaken in a graduated cylinder for 15 minutes. Appearance of a 2 cm layer of foam indicated the presence of saponins.

Tests for Carbohydrate**Molish' s test**

To 2 ml of plant sample extract, two drops of alcoholic solution of α -naphthol were added. The mixture was shaken well and few drops of concentrated sulphuric acid was added slowly along the sides of test tube. Appearance of violet ring indicates the presence of carbohydrates.

Benedict's test

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. Appearance of coloured precipitate indicated the presence of sugar.

Test for Terpenoids

To 2 ml of extract was treated with 1ml of 2, 4- dinitrophenyl hydrazine in 2M HCl. Non appearance of yellow colour indicated absence of terpenoids.

Tests for protein**Millon's test**

To 2 ml of filtrate a few drops of Millon's reagent were added. Failure to develop white precipitate indicated the absence of proteins.

Biuret test

To 2 ml of filtrate, 1 drop of 2 per cent copper sulphate solution was added. To this, 1 ml of ethanol (95 per cent) was added, followed by excess of potassium hydroxide pellets. Non appearance of pink layer indicates the absence of proteins.

Test for Amino acids (Ninhydrin test)

Two drops of Ninhydrin solution (10 mg of Ninhydrin in 200 ml of acetone) were added to 2 ml of aqueous filtrate. The absence of amino acids was indicated by absence of development of pink colour.

Test for Quinones

To 1ml of the leaf extract, 1 ml of concentrated sulphuric acid was added. Appearance of red colour indicated the presence of quinones.

Test for Tannin

1 ml of the extract was added to a test tube containing 2 ml of distilled water. Then 2 to 3 drops of dilute ferric chloride solution was added. Appearance of green to blue-green or a blue-black color indicated the presence of tannin.

Results and Discussion

Phytochemical analysis of tulsi showed positive for phenols, flavonoids, flavonolglucosides, saponins, quinines and tannins and negative for terpenoids, and amino acids (Table-1)

Table 1: Tulsi-Qualitative analysis of various Phytoconstituents

| Sl. No | Tests | Results |
|--------|--|---|
| 1 | Test for Flavonoids: • Ferric Chloride test • Alkaline reagent test • Magnesium and Hydrochloric acid reduction | Presence of phenol, flavonoids and flavonolglucosides |
| 2 | Test for Saponins (Foam test) | Presence of saponins |
| 3 | Tests for Carbohydrate: • Molish' s test • Benedict's test | Presence of carbohydrate and sugar |
| 4 | Test for Terpenoids | Absence of terpenoids |
| 5 | Tests for protein: • Millon's test • Biuret test | Absence of protein |
| 6 | Test for Amino acids (Ninhydrin test) | Absence of amino acids |
| 7 | Test for Quinones | Presence of quinones |
| 8 | Test for Tannins | Presence of tanins |

Rajesh *et al.*, (2013) ^[6] reported the presence of flavonoids, terpenes, saponins and tannins and absence of phenols in the leaves of *Ocimum sanctum*. Joshi *et al.*, (2011) ^[7] reported that *Ocimum sanctum* contain tannins and flavonoids and sugar were absent. The present day information about the chemical properties is based on the various studies that have been done in different parts of the world (Vina and Murillo, 2003) ^[8] and it is likely that chemical constituents might be varying due to edaphic and geographic factors (Bakkali *et al.*, 2008) ^[9].

Summary

Qualitative analysis of phytoconstituents in the leaf extracts of Tulsi (*Ocimum sanctum*) revealed the presence of phenols, flavonoids, flavonolglucosides, saponins, quinines and tannins and absence of terpenoids, and amino acids.

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