



PHYTOCHEMICAL STUDIES OF CRUDE LEAF EXTRACT OF TULSI (OCIMUM TENUIFLORUM)

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ABSTRACT

Ocimum tenuiflorum, also known as *Ocimum sanctum*, holy basil, or tulsi (also spelled thulasi), is an aromatic plant of the family Lamiaceae, which is native to the Indian subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics. It is an erect, many-branched subshrub, 30–60 cm (12–24 in) tall, with hairy stems and simple phyllotaxic green or purple leaves that are strongly scented. Phytochemical screening of the ethyl acetate, ethanol, methanol and aqueous extracts of the leaf, obtained by the cold maceration method, indicated the presence of alkaloids, flavonoids, saponins, phenols, steroids, tannins, triterpenoids, glycosides, carbohydrates, phlobatannins, thiols, anthroquinone, protein and amino acids, resins, fixed oils & fats, and phytosterols. Qualitative estimation of phytochemicals was performed in different solvent extracts, namely ethyl acetate, ethanol, methanol and aqueous extracts. The results were represented as '+' for the presence and '–' for

the absence of phytochemicals. N.P. analyses were done by total Kjeldahl digests by UV-VIS spectrophotometry, and K analysis was done in Kjeldal digests by Flame photometry method.

Key words: *Ocimum tenuiflorum*, Phytochemical, ethyl acetate, ethanol, methanol

INTRODUCTION

Tulsi is cultivated for religious and medicinal purposes, and for its essential oil. It is widely known across the Indian subcontinent as a medicinal plant and a herbal tea, commonly used in Ayurveda, and has an important role within the Vaishnava tradition of Hinduism, in which devotees perform worship involving holy basil plants or leaves. This plant is revered as an elixir of life. The healing powers of the plant have been known since ancient times. In the Ayurvedic system of medicine, traditional healers and folklores contain volumes of materials on the healing potential of *Tulsi* (*Ocimum sanctum*). The plant is a herbaceous perennial; it belongs to the family Lamiaceae and grows to a height ranging from 60 -90 cm. It has large, oblong leaves. Flowers are white in colour, are sterile and do not produce viable seeds. The fresh fruits and leaves have been used in the Indian system of medicine to cure various ailments. It is one of the holiest and most sacred herbs grown in India, and Hindus worship this plant. This plant is known to possess antiseptic, analgesic, anti-inflammatory, antimicrobial, anti-stress, immunomodulatory, hypoglycemic, hypotensive and antioxidant properties [1-2]. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. These metabolites are known by their active substances. For example, the phenolic compounds which are part of the essential oils [3], as well as in tannin [4].

The *Tulsi* has also been recognized by the rishis for thousands of years as a prime herb in Ayurveda treatment. In Ayurveda, *Tulsi* (*Ocimum sanctum* L) has been well documented for its therapeutic potentials and described by Dashemani Shawasharni (anti-asthmatic) properties and antikaphic drugs (Kaphaghna) [5]. In the last few decades several studies have been carried out by Indian scientists and researchers to suggest the role of essential oils and eugenol in the therapeutic potential of *Ocimum sanctum* L [6, 7]. The main chemical ingredients in this plant are eugenol, carvacrol, methyl eugenol and caryophyllene. One of the qualities that make the *Tulsi* plant such a potent medicinal herb is its ability to reduce stress. The different parts of *Tulsi*

plant has been used for curing a variety of illnesses [8]. This plant acts as a biomedicine and as bio-pharmacological resources for treatment of many diseases [9]. The reducing microbial growth was studied at different concentrations for Tulsi leaf extract and showed maximum activity at 600mg^{-1} [10]. Weak appreciation of failures in the control of multidrug resistance strains would be inhuman, which generates the impetus on a systematic global search for new drugs from natural resources such as plants, worldwide[11]. Tulsi is abundant in essential oils and antioxidants, which are tremendously effective in reducing the effects of stress on the body [12]. In view of this background, we performed the extraction and preliminary phytochemical analysis of Tulsi in different solvent extracts, namely ethyl acetate, ethanol, methanol and aqueous extracts of *O.tenuiflorum*.

MATERIALS AND METHODS

Fresh leaves of *O.tenuiflorum* were collected from Tain and John's areas, Region 6, Guyana. The leaves of *O.tenuiflorum* were air-dried and pulverized, using Thomas-Wiley Laboratory Mill Model 4 at Central Chemistry Laboratory, Department of Chemistry, Faculty of Natural Science, University of Guyana, Turkeyen Campus. The powdered samples (100g) were extracted with different solvents (300 cm^3 each of ethyl acetate, ethanol, methanol and aqueous extracts) using cold maceration method. The crude extracts were filtered with Whatman No.1 filter paper and concentrated using a rotary evaporator. The extracts were stored in the refrigerator throughout the experiments.

The preliminary phytochemical analysis of leaf extracts of *O.tenuiflorum* was performed by Harbone (1998) and Dey et al. (1987), and these leaf extracts were evaluated for the presence of phytochemicals such as alkaloids, flavonoids, saponins, phenols, steroids, tannins, triterpenoids, glycosides, carbohydrates, phlobatannins, thiols, anthroquinone, protein and amino acids, resins, fixed oils & fats, and phytosterols. The qualitative determination of these secondary metabolites was carried out by standard methods [13]. Chromatographic study of the different extracts was also done using standard methods[14]. The phytochemical screening of alcoholic and aqueous extracts showed alkaloids, steroids, tannins respectively, which are the active antimicrobial components in plant extracts [15].

Nitrogen (N), Phosphorous (P) quantitative test was done by total Kjeldal digests by UV-VIS spectrophotometry and Potassium (K) in Kjeldhal digest by Flame photometry method.

RESULTS AND DISCUSSION

Qualitative estimation of phytochemicals was performed in all the four (ethyl acetate, ethanol, methanol and aqueous extracts) extracts of leaves of *O.tenuiflorum*. The phytochemicals were extracted from leaves using threedifferent solvents, namelyethyl acetate, ethanol, and methanol. The results were represented as ‘+’ for presence and ‘-‘for absence of the phytochemicals.

Table 1. Physical characteristic of the different extracts.

Name of plant	Used part	Name of extract	Consistency	Color	Odour
Tulsi (<i>Ocimum tenuiflorum</i>)	Leaves	Ethanolic	Liquid	Dark green	No
		Methanolic	Liquid	Greenish	No
		Ethyl acetate	Liquid	Light green	No
		Aqueous	Liquid	Dark green	Characteristic

Table 2. Phytochemical analysis in the ethanol, methanol, ethyl acetate and aqueous extracts.

Name of plant	Used part	Phytoconstituents	Ethanol	Methanol	Ethyl acetate	Aqueous
Tulsi (<i>Ocimum tenuiflorum</i>)	Leaves	Alkaloids	+	+	+	-
		Flavonoids	+	+	+	-
		Tannins	-	+	-	+
		Thiols	+	+	+	+
		Amino acids	+	-	+	+
		Carbohydrates	+	+	+	+
		Phenols	+	+	+	+
		Glycosides	+	-	+	-
		Triterpenoids	+	+	-	+
		Fixed oils, fats	+	-	+	-
		Proteins	+	+	+	+
		Saponins	-	+	-	-
		Steroids	-	-	+	-
		Phlobatannins	+	-	-	-
		Anthroquinone	-	+	-	+
		Resins	-	-	-	-
		Phytosterols	+	-	-	+

+ = Present, - = Absent

Table 3. N.P. K. analysis in Tulsi (*Ocimum tenuiflorum*) leaves in percentage (%).

Name of plant	Used part	Parameter	(%)
Tulsi (<i>Ocimum tenuiflorum</i>)	Leaves		
		N	3.76
		P	0.99
		K	0.97

DISCUSSION

In *O.tenuiflorum*, alkaloids are present in ethyl acetate, ethanol, and methanol leaf extracts, and could be extracted with the solvents readily, whereas alkaloids are absent in aqueous extracts. Flavonoids are also present in ethyl acetate, ethanol, and methanol leaf extracts, and absent in aqueous extracts. Tannins are present in methanol aqueous leaf extracts but absent in ethanol and

ethyl acetate leaf extracts. Thiols, Carbohydrates, phenol and proteins were found be present in all the four leaf extracts, i.e ethanol, methanol, ethyl acetate and aqueous, of *O.tenuiflorum*, whereas resins were absent in all four of these leaf extracts. Except for ethyl acetate leaf extract, triterpenoids were present in the other three leaf extracts of ethanol, methanol and aqueous; on the other hand phlobatannins were absent in three leaf extracts, viz. methanol, ethyl acetate and aqueous *O.tenuiflorum*, and present in only the ethanol leaf extract. Fixed oils and fats showed present in ethanol and ethyl acetate leaf extracts, and absence in methanol and aqueous leaf extracts *O.tenuiflorum*. While saponins present only in methanol leaf extract, steroids were present in only ethyl acetate leaf extract. Phytosterols exhibited presence in ethanol and aqueous leaf extracts, and were absent in methanol and ethyl acetate leaf extracts *O.tenuiflorum*.

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REFERENCES

1. Vermani, KS, Garg (2002). Herbal medicine for sexually transmitted disease and AIDS. J. Ethnopharmacol. 80:49-66.
2. Nair R, Kalariya T, Chanda S (2005). Antibacterial activity of some selected Indian medicinal flora. Turk. J. Biol., 29: 41-47
3. Parekh. J, Jadeja D, Chanda S (2005). Efficacy of aqueous and methanol extracts of some medicinal plants of potential antibacterial activity. Turk. J. Biol., 29; .203-210.
4. Chopra. RN, Nayar SI, Chopra IC (1956). Glossary of Indian Medicinal Plants: Published by CSIR, New Delhi.
5. *Ocimum sanctum*. The Indian home remedy. In: Current Medical Science. March –April (1992). (Edited and published by S. Rajeshwari. Cipla Ltd. Bombay, Central, Bombay).
6. Gupta. AK, Prakash J, Srivastava S. (2002), Indian Journal of Experimental Biology; 40(7), 756-773.
7. Sen. P (1993) Drugs News and Views, 1(20), 15-21.

8. Dohare, L., Shuaib, M., Ahmad, M. and Naquvi, J. (2012). Chemical composition of volatile oil of *Ocimum sanctum* linn. International Journal of Biomedical and Advance Research. 03(02): 129- 131.
9. Goyal, P. and Kaushik, P. (2013). In Vitro Evaluation of antibacterial activity of various crude leaf extracts of Indian sacred plant, *Ocimum sanctum* linn. Bri. Micro. Res. J. 1(3): pp 70 - 78.
10. Pavithra, S, Saranya D, Sankar, P, Murugesan S. (2012). Effect of Tulsi (*Ocimum sanctum*) as a Disinfectant for Water Treatment. Hitek. J. Bio. Sci. Bioengg., 1 (1):1-7.
11. Ekta, S, Sheel, S, Jaya, D, Swapnil, S. (2012). Diversified potentials of *Ocimum sanctum* Linn (Tulsi). An Exhaustive Survey. J. Nat. Prod. Plant Res., 2 (1):39-48.
12. Dubey D, Rath S, Sahu MC, Debata NK, Padhy RN (2012). Antimicrobials of plant origin against TB and other infections and economics of plant drugs—Introspection. Indian J Trad Know; 11: 225-233.
13. Khanna. N,BhatiaJ, (1999) Journal of Sustainable Agriculture and Environment, 1(2), 198-202.
14. ChiangLC, ChengPW, ChiangW, LinCC (2005) Antiviral activity of extracts and selected pure constituents of *Ocimum basilicum*; Cli. Exp. Pharmacol Physiol. 9320, 811-816.
15. Sadul Rama R, Gidde Milind R, Bipinraj NK (2012). Comparative study of antimicrobial activity and phytochemical screening of aqueous and alcoholic leaf extract of *Ocimum sanctum* on *E.coli* (Faecal Indicator of Water Pollution). J. Environ. Res. Develop. 7 , 1A, July-September, 312-320.