Doi: HTTPS://DOI.ORG/10.23910/2/2023.0515b

Phytochemical Screening of Aqueous, Acetone, Ethanol and Methanol Leaf Extracts of *Ocimum teniflorum*, *Ocimum gratissimum* and *Ocimum sanctum*

D. Kavyamala, P. Jayamma*, N. Usha Rani and M. Mahesh Babu

Dept. of Food Safety and Quality Assurance, College of Food Science and Technology, ANGRAU, Pulivendula, Kadapa, Andhra Pradesh (516 390), India

Corresponding Author

P. Jayamma
e-mail: palurijaya@gmail.com

Article History

Article ID: IJEP0515b
Received on 28th January, 2023
Received in revised form on 24th April, 2023
Accepted in final form on 17th May, 2023

Abstract

The experiment was carried out during November, 2019 to July, 2020 at Department of Food Safety and Quality Assurance, College of Food Science and Technology, Pulivendula, ANGRAU, Andhra Pradesh, India. A large number of plants show enormous versatility in synthesizing complex materials which have no immediate obvious effect on growth or metabolic functions. Phytochemicals are naturally occurring and biologically active components that have potential disease inhibiting capabilities. Phytochemicals are effective in combating or preventing disease due to their antioxidant effect. The most important of these phytochemicals are alkaloids, tannins, flavonoids, saponins and phenolic compounds. *Ocimum* is a well-known medicinal plant that consists of various biochemically active components which have many functional effects. The leaf extracts were prepared by using aqueous, acetone, ethanol and methanol solvents and phytochemical analysis was conducted for tannins, saponins, phlabotannins, flavonoids, terpenoids, glycosides and steroids. The results revealed that tannins were found in all the extracts of three *Ocimum* spp., Saponins were present in all extracts except methanol extract of *Ocimum* spp., the presence of phlabotannins were observed only in aqueous extract *Ocimum* spp., Flavonoids were present in aqueous and methanol extracts of *Ocimum* spp., Glycosides were found in ethanol and methanol extracts of *Ocimum* spp., The presence of steroids was observed in all extracts except aqueous of *Ocimum* spp., Terpinoids were present in all extracts except aqueous extract of *O. teniflorum* and *O. gratissimum*.

Keywords: Acetone, aqueous, aqueous, ethanol, methanol extract, *Ocimum* spp.

1. Introduction

The plant kingdom is known to comprise approximately 500,000 plant species that are found worldwide, of which only 1.0% has been phytochemically investigated with an illimitable potential for discovering novel bioactive compounds mainly in medicinal plants (Rodrigues et al., 2007). The medicinal plants are rich in secondary metabolites [which are potential sources of drugs] and essential oils of therapeutic importance. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (Janssen et al., 1987), as well as in tannin (Saxena et al., 1994). The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability (Siddiqui, 1993). The plants should be investigated to better understand their properties, safety and efficiency (Eloff, 1998). Plants show enormous versatility in synthesizing complex materials which have no immediate obvious growth or metabolic functions. Phytochemicals are effective in combating or preventing disease due to their antioxidant effect. The medicinal and antimicrobial properties of plants lie in their component phytochemicals (Akinmoladun et al., 2007).

The World Health Organization country relies on traditional medicines, mostly plant drugs, for their primary health care needs. Further, a large number of phyto-drugs are popular and or rather harmless effects (Shahavi and Desai, 2008). Ethnobotanical literature of India, several hundreds of plants are known to have the potential to treat many diseases and one of those popular ones is Tulasi traditionally used for the treatment of diseases (Das et al., 2009). Leaves possess antimicrobial activity. Infections with both Gram-positive and Gram-negative bacteria have clinically become intractable, slowly, due to the emergence of multidrug resistant (Naik et al., 2015).

Ocimum spp. is one such medicinal plant that is grassy and annual whose leaves are oval with a sharp tip. It is a native of Iran, Afghanistan and India (Mann et al., 2000). This species belongs to the mint family, *Lamiaceae* spp., commonly known as "holy basil" in English and "Tulasi" in Hindi and Sanskrit. Morphologically, there are many types of tulasi, some of them are a purplecolored leaf or dark variety, commonly known as "krishna tulasi" (*Ocimum teniflorum*), a green colored leaf or light variety known as "rama tulasi" (*ocimum sanctum*) and

other type is "vana tulasi" (*Ocimum gratissimum*) (Kothari et al., 2005). Its oil possesses a pleasant odor characteristic of the plant, with an appreciable note of clove. The chemical composition of the oil of *O. tenuifl orum* has been the subject of previous studies (Lawrence et al., 1972, Lawrence et al., 1980, Dey and Choudhuri, 1983, Malik et al., 1986, Laakso et al., 1990, Pino et al., 1998., Machado et al., 1999, Maheshwari et al., 1987). In last few decades several studies have been carried out by Indian scientists and researchers to suggest the role of essential oils &eugenol in therapeutic potentials of *Ocimum sanctum* L. (Sen, 1993). Eugenol is a phenolic compound and major constituent of essential oils extracted from different parts of Tulsi plant (Gupta et al., 2002, Khanna and Bhatia., 2003).

The blessed Basil or Tulsi is significant in the traditional Ayurvedic and Unani systems (Pattanayak et al., 2010). In India, *Ocimum sanctum* is believed that it can be given the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, increase in body temperature and also insect bite (Xia et al., 2018). Different parts of Tulsi plant e.g. leaves, flowers, stem, root, seeds etc. are known to possess therapeutic potentials and have been used, by traditional medical practitioners, as expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidmic and antistress agents (Prakash and Gupta, 2005). Keeping in views, the study was aimed to find phytochemical compounds responsible for the antimicrobial activities.

2. Materials and Methods

2.1. Collection and preparation of plant material

Tulasi spp., such as *Ocimum santum, Osmium teniflorum, Ocimum gratissimum* were collected from Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telengana, India from November, 2019 to July, 2020. The fresh leaves collected were washed in clean water followed by distilled water. The cleaned leaves were shade dried at room temperature and powdered. The powdered materials were stored in air-tight jars at room temperature.

Soak 20 g of Tulasi powder in 200 ml of solvent for 48 h. Decant the solvent again soak the residue with the same solvent for 24 h. Combine the total extract. Filter the solution by Whatmann's filer paper No.1. Evaporate the solution in a rotary evaporator at 40°C. Add respective solvent to make up for the required volume.

2.2. Phytochemical Screening

Chemical tests were carried out by using the aqueous and organic solvent extracts of Tulasi to identify the presence of phytochemical constituents by using the standard procedure described by Harborne (1998).

2.3. Test for Tannins

Take 2.0 ml of aqueous extract. Stir after adding 2.0 ml of distilled water. Add a few drops of $FeCl_3$ solution. The formation of green color precipitate indicates the presence of tannins

2.4. Test for Saponins

Take 5.0 ml of aqueous extract. Shake it vigorously with 5.0 ml of distilled water and warm. The formation of stable foam indicates the presence of saponins.

2.5. Test for phlobatannins

Take 2.0 ml of 1% HCl and add 2.0 ml of aqueous extract. Boil the mixture. Deposition of a red precipitate indicates the presence of phlobatannins.

2.6. Test for Flavonoids

Take 1.0 ml of aqueous extract and add 1.0 ml of 1.0% lead acetate solution. The formation of yellow color indicates the presence of flavonoids.

2.7. Test for Terpenoids

Take 2.0 ml of organic extract and dissolve it in 2.0 ml of chloroform. Evaporate the mixture to dryness and add 2.0 ml of H_2SO_4 and heat for about 2.0 m. The development of greyish color indicates the presence of terpenoids.

2.8. Test for Glycosides

Take 2.0 ml of organic extract and dissolve it in 2.0 ml of chloroform. Add 2.0 ml of acetic acid to the mixture. Cool this solution with ice and add H_2SO_4 carefully. Color change from violet to blue-green indicates the presence of glycosides.

2.9. Test for Steroids

Take 2.0 ml of organic extract and dissolve it in 2.0 ml of chloroform. Add 2.0 ml concentrate H_2SO_4 to the solution. The formation of red color in the lower chloroform layer indicates the presence of steroids (or) take 2.0 ml of organic extract. Dissolve it in 2.0 ml of chloroform. Treat the solution with H_2SO_4 and acetic acid. The development of greenish color indicates the presence of steroids.

3. Results and Discussion

3.1. Phytoconstituent screening

Phytochemicals in the plant are essential oil from leaves like αThujene, Octane, Nonane, Benzene, (Z)-3-hexanol, Ethyl 2- methyl butyrate, α-pinene, βpinene, Toluene, citronellal, Camphene, Sabinene, Dimethyl benzene, Myrecene, Ethyl benzene, Limocene, 1,8,-cineole, cis-β-ocimene, p-cymene, Terpiniolene, Allo-oc-imene, Butylbenzene, α-cubebene, Linalool, Eugenol, Methyl eugenol, β-elemene, (E)-cinnamy, Lactate, Isocaryophyllene, β-caryophyllene, Iso-eugenol, α-guaiene, α-amorphene, α-humulene, γ- umulene, 4,11-seinadiene, α-terpeneol, Isoborneol, Carvacrol, Borneol, germacrene-D, α- selinene, β-selinene, Myrtenylformat, α-murolene, cadinene, δ - Cuparene, Calamene, Geraneol, Nerolidol, Caryophyllene oxide, Iedol, Humulene oxide, α-guaiol, τ - cadinol, α- bisbolol, (EZ)- famesol, Cissesquisainene

hydrate, Elemol, Tetradecanal, Selin-11-en-4-α-ol, 14-hydroxy-αhumulene.Alcohalic extract of leaves / aerial parts contain Urosolic acid, Apigenin, Luteolin, Apigenin-7-Oglucuronide, Luteolin-7-O-glucuronide, Isorientin, Orientin, Molludistin, Stigmasterol, Triacontanol ferulate, Vicenin-2,Vitexin, Isovitexin, Aesculetin, Aesculin, Chlorgenic acid, Galuteolin, Circineol, Gallic acid, gallic acid methyl ester, Procatechuic acid, Vallinin acid, 4-hydroxybenzoic acid, Caffiec acid, Chlorogenic acid, Phenylpropane glucosides, β-Stigmasterol, urosolic acid. Fixed Oil from Seeds contain Palmitric acid, Stearic acid, Linolenic acid, Oleic acid, Sitosterol, Dilinoleno-linolins, Linodilinolin, Hexourenicacid. Mineral Content 100 g^{-1} are vitamin C (83 µg), Carotene (2.5 µg), Ca (3.15%), P (0.34%), Cr (2.9 µg), Cu (0.4 µg), Zn (0.15 µg), V (0.54 µg), Fe (2.32 µg), Ni (0.73 µg) (Mondal et al., 2007).

In the present study, the Phytoconstituent screening showed the presence of Tannins, Saponins, Phlabotannins, Flavonoids, Terpinoids, Glycosides and Steriods. From Table 1 results revealed that tannins were found in all the extracts of three *Ocimum spp.,* Saponins were present in all extracts except methanol extract of *Ocimum* spp*.,* the presence of phlabotannins were observed only in aqueous extract *Ocimum* spp., Flavonoids were present in aqueous and methanol extracts of *Ocimum* spp*.*, Glycosides were found in ethanol and methanol extracts of *Ocimum* spp*.,* The presence of steroids was observed in all extracts except aqueous *Ocimum* spp*.,* Terpenoids were present in all extracts except aqueous extract of *O. teniflorum and O. gratissimum*.

Table 1: Phytochemical properties of the leaf extracts of Ocimum spp.

Sl. No	Treatment	*Ocimum* spp.	Tannin	Saponnin	Phlabotannin	Flavonoid	Terpinoid	Glycoside	Steroid
1.	Aqueous	*O. teniflorum*	+	+	+	+	-	-	-
		O.gratissimum	+	+	+	+	-	-	-
		O. sanctum	+	+	+	+	-	-	-
2.	Acetone	*O. teniflorum*	+	+	-	-	+	-	+
		O. gratissimum	+	+	-	-	+	-	+
		O. sanctum	+	+	-	-	-	-	+
3.	Ethanol	*O. teniflorum*	+	+	-	-	+	+	+
		O. gratissimum	+	+	-	-	+	+	+
		O. sanctum	+	+	-	-	-	+	+
4.	Methanol	*O. teniflorum*	+	-	-	+	+	+	+
		O. gratissimum	+	-	-	+	+	+	+
		O. sanctum	+	-	-	+	-	+	+

Key: + Present, - Absent

Borah and Biswas (2018) results found that various bioactive molecules were in Tulsi leaf extract from the phytochemical screening. The amount of extraction is more in the case of organic solvent than that of water. From the quantitative analysis, it was found that a high amount of phenols is present in Tulsi leaf ranging from 1.6 to 7.6%. Consequently, the amount of alkaloids and flavonoids ranged from 0.91 to 1.28% and 1.56 to 2.24%, respectively. The phytochemical constituents such as alkaloids, steroids, flavanoids, tannins, phenols and several other aromatic compounds of plants serve a defense mechanism against predation by many microorganisms, insects and other herbivore (Bonjar et al., 2004). Singh et al. (2013) on phytochemical investigation discovered the presence of steroidal compounds (appearance of blue or green color or a mixture of the two shades); alkaloids and tannins (the turbidity or yellow precipitation shows the presence of alkaloids and greenish precipitate indicated the presence of tannins) and absence of flavonoids (not observed yellow coloration) in all mentioned extracts of the plant. Fehling test (formation of yellow or brownish-red precipitate) showed a positive result for aqueous extract (Root and Leaf) only. The phytochemical characteristics of the leaf extract of *Ocimum tenuflorum* were investigated. The results reveal the presence of medicinally active constituents like tannins, alkaloids, terpenoids, steroids and Flavnois, Phlobatannins, Glycosides in the leaves of *ocimum tenuflorum.* While saponins were absent in these plants (Naik et al., 2015). The present study provides evidence that leaf extracts of *Ocimum spp.*, contain medicinally important bioactive compounds and their effect on selected bacteria. This justifies the use of plant species as a traditional medicine for various diseases and the need to consider them.

4. Conclusion

The identified phytochemical compounds could be the bioactive constituents responsible for the efficacy of the leaves of the studied plants. Thus, the plant extracts could be a source for industrial production of drugs useful for chemotherapy of some microbial infections.

5. Reference

Akinmoladun, A.C., Ibukun, E.O., Dan-Ologe, I.A., 2007. Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata*. Scientific Research and Essays 2(6), 191–194.

Bonjar, G.H.S., Nik, A.K., Aghighi, S., 2004. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of South East regions of Iran. Journal of Biological Science 4(3), 405–412.

Das, S., Khan, M.L., Rabha, A., Bhattacharjya, D.K., 2009. Ethnomedicinal plants of Manas National Park, Asam, Northeast India. Indian Journal of Traditional Knowledge 8(4), 514–517.

Dey, B.B., Choudhuri, M.A., 1983. Effect of leaf development stage on changes in essential oil of *Ocimum sanctum* L. Biochemie und Physiologie der Pflanzen 178(5), 331–335.

Eloff, J.N., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants. Journal of Ethnopharmacology 60(1), 1–8.

Gupta, S.K., Prakash, J., Srivastava, S., 2002. Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant. Indian Journal of Experimental Biology 40(7), 765–773.

Harborne, A.J., 1998. Phytochemical Methods a Guide to Modern Techniques of Plant Analysis. Springer Science & Business Media, Springer Dordrecht, 2–12.

Janssen, A.M., Scheffer, J.J.C., Svendsen, A.B., 1987. Antimicrobial activity of essential oils: 1976–1986 literature review. Aspects of the test methods. Planta Medica 53(5), 395–398.

Khanna, N., Bhatia, J., 2003. Antinociceptive action of *Ocimum sanctum* (Tulsi) in mice: Possible mechanisms involved. Journal of Ethnopharmacology 88(2–3), 293–296.

Kothari, S.K., Bhattacharya, A.K., Ramesh, S., Garg, S.N., Khanuja, S.P.S., 2005. Volatile constituents in oil from different plant parts of methyl eugenol-rich *Ocimum tenuiflorum* Lf (syn. *O. sanctum* L.) grown in South India. Journal of Essential Oil Research 17(6), 656–658.

Laakso, L., Seppanen-Laakso, T., Hermann-Wolf, B., Knobloch, K., 1990. Constituents of the essential oil from the holy basil or tulsi basil, *Ocimum sanctum*. Planta Medica 56(6), 527–527.

Lawrence, B.M., Hogg, J.W., Terhune, S.J., Pichitakul, N., 1972. Essential oils and their Constituents. IX. The oils of *Ocimum sanctum* and *Ocimum basilicum* from Thailand. Flavor Industry 3(1), 47–49.

Lawrence, B.M., Powell, R.H., Peele, D.M., 1980. Variation in the genus Ocimum. Paper no. 34. In: Proceedings of VIIIth International Congress of Essential Oils. Cannes, France, October 12–17.

Machado, M.I.L., de Vasconcelos Silva, M.G., Matos, F.J.A., Craveiro, A.A., Alencar, J.W., 1999. Volatile constituents from leaves and inflorescence oil of *Ocimum tenuiflorum* L. f. (syn. *O. sanctum* L.) grown in Northeastern Brazil. Journal of Essential Oil Research 11(3), 324–326.

Maheshwari, M.L., Singh, B.M., Gupta, R., Chien, M.J., 1987. Essential oil of sacred basil (*Ocimum sanctum*). Indian Perfumery 31(2), 137–145.

Malik, M.S., Rafi, M., Ahmed, R., 1986. Essential oils of the species of Labiatae 2. Studies on the essential oil of *Ocimum sanctum*. Pakistan Journal of Scientific and Industrial Research 29(2), 111–112.

Mann, C.M., Cox, S.D., Markham, J.L., 2000. The outer membrane of *Pseudomonas aeruginosa* NCTC 6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). Letters in Applied Microbiology 30(4), 294–297.

Mondal, S., Mirdha, B.R, Naik, S.N, Mahapatra, S.C., 20017. Antimicrobial activities of essential oils obtained from fresh and dried leaves of *O. sanctum* (L) against enteric bacteria and yeast. Acta Horticulturae 756, 267–269.

Naik, L.S., Shyam, P., Marx, K.P., Baskari, S., Devi, V.R., 2015. Antimicrobial activity and phytochemical analysis of *Ocimum tenuiflorum* leaf extract. International Journal of Pharmtech Research 8(1), 88–95.

Pattanayak, P., Behera, P., Das, D., Panda, S.K., 2010. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Pharmacognosy Reviews 4(7), 95–105.

Pino, J.A., Rosado, A., Rodriguez, M., Garcia, D., 1998. Composition of the essential oil of *Ocimum tenuiflorum* L. grown in Cuba. Journal of Essential Oil Research 10(4), 437–438.

Prakash, P., Gupta, N., 2005. Therapeutic uses of *Ocimum sanctum* Linn. (Tulsi) with a note on eugenol and its pharmacological actions: A short review. Indian Journal of Physiology and Pharmacology 49(2), 125–125.

Rodrigues, F., Lehmann, M., do Amaral, V.S., Reguly, M.L., de Andrade, H.H.R., 2007. Genotoxicity of three mouthwash products, Cepacol, Periogard, and Plax, in the *Drosophila* wing-spot test. Environmental and Molecular Mutagenesis 48(8), 644–649.

Saxena, G., McCutcheon, A.R., Farmer, S., Towers, G.H.N., Hancock, R.E.W., 1994. Antimicrobial constituents of

Rhus glabra. Journal of Ethnopharmacology 42(2), 95–99.

Sen, P., 1993. Therapeutic potentials of Tulsi: from experience to facts. Drugs News and Views 1(2), 15–21.

Shahavi, V.M., Desai, S.K., 2008. Anti-inflammatory activity of *Butea monosperma* flowers. Fitoterapia 79(2), 82–85.

Siddiqui, H.H., 1993. Safety of herbal drugs-an overview. Drugs News and Views 1(2), 7–10.

Singh, A.R., Bajaj, V.K., Sekhawat, P.S., Singh, K., 2013. Phytochemical estimation and antimicrobial activity of aqueous and methanolic extract of *Ocimum sanctum* L. Journal of Natural Products and Resources 3(1), 51–58.

Xia, K.Z., Perveen, N., Khan, N.H., 2018. Phytochemical analysis, antibacterial and antioxidant activity determination of *Ocimum sanctum*. Pharmacy and Pharmacology International Journal 6(6), 490–497.

