Essential Oil of Ocimum Species (OS & OB) and Its Antimicrobial Activity

[*1]SARVESH KUMAR V. ATAL, Dr. J Anuradha[1 @], Prof. Dr. Sandeep Tripathi1[1 &], Prof. Dr. Yatindra Kumar Mathur[1 %]

***Corresponding author - SARVESH KUMAR V. ATAL (PhD Research Scholar of Biochemistry),**

[1]Department of Advanced Science & Technology, NIMS Institute of Engineering & Technology NIMS University Rajasthan Jaipur.

Emails: *sarvesh_ku85@yahoo.co.in, *geensk85@gmail.com, [@]anujram13@gmail.com, [&]sandeeptripathipd@gmail.com, [%]drvymathur@gmail.com

ABSTRACT - The essential oils of *Ocimum sanctum* and *O. basilicum* were obtained from a Clevenger apparatus distilled for 3 to 4hr. Both essential oil of *O. sanctum* and *O. basilicum* were analyzed by GC-MS. The yield of the essential oil obtained from *Ocimum sanctum* (1.45% w/w) and *O. bascillum* (0.98% w/w). Eugenol and Methyl eugenol were found to be major constituents of both fresh leaves of *O. sanctum* and *O. basilicum*. The extracts of *Ocimum sanctum* and *Ocimum basilicum* were subjected to antimicrobial screening against the pathogens such as *S. aureus, S. Typhimurium* and *E. coli.* Both the species were found to possess equipotent zone of inhibition against the selected pathogens

Keywords: Antimicrobial, Essential oils, Eugenol, Lamiaceae (Syn. Labiatae), Methyl eugenol, *Ocimum* species.

I. INTRODUCTION

Lamiaceae (syn. Labiatae) herb family consists of more than 252 genus and 7000 species (Hedge, 1992). Lamiaceae family is known for the wealth of species with medicinal properties, which have been used since early times and many of these species are common in Mediterranean region (Ali *et al*., 2000). The Lamiaceae plants are 24 generally aromatic in all parts including a number of widely used culinary herbs, such as sage, thyme, rosemary, oregano, basil, mint lavender, marjoram, savory, and perilla (Wink, 2003; Celiktas *et al*., 2007; Hussain *et al*., 2008).

The genus *Ocimum*, a wonder member of the Lamiaceae (Syn. Labiatae) family, contains 200 species of herbs and shrubs (Simon et al., 1999). They were naturally occurs in tropical and subtropical regions and is considered an important culinary herb and source of aromatic essential oils (Paton and Putievsk, 1996). Sweet basil, *Ocimum basilicum* L., is the major culinary and essential oil source of this genus (Lawrence, 1992). The essential oils from Ocimum contain many terpenes (linalool, citral, 1, 8-cineole) andphenylpropanoids (e.g. methyl chavicol, eugenol) produced in specialized glandular trichomes (Charles and Simon,1990; Gang *et al*.,2001). It is also a source of aroma compounds and essential oils containing biologically active constituents that possess insecticidal and nematicidal properties (Deshpande and Tipnis, 1997; Chaterje *et al*., 1982). To the best of our knowledge, an investigation of the essential oil of *O. sanctum* and *O. basilicum* leaves in northern India. In the present study, the essential oils were isolated from fresh leaves and the volatiles oil was analyzed by GC/MS method.

II. GEOGRAPHICAL SOURCE:-

It is an herbaceous, much branched annual plant found throughout India, it is considered as sacred by Hindus. The plant is commonly cultivated in garden and also grown near temples. It is propagated by seeds. Tulasi, nowadays, is cultivated commercially for its volatile oil.

Characteristics

It is much branched small herb and 30 to 75 cm in height. All parts of tulasi are used in medicine, especially fresh and dried leaves. Leaves are oblong, acute with entire or serrate margin, pubescent on both sides and minutely gland dotted, The leaves are green in colour with aromatic flavor and slightly pungent taste. Flowers are purplish in colour in the form of racemes. Nut lets are subglobose, slightly compressed, pale brown or red in colour. Seeds are reddish-black and subglobose.

 DOI : 10.35291/2454-9150.2021.0065

III. MICROSCOPY

Tulasi leaf is dorsiventral. Stomata are of diacytic type, particularly abundant on lower surface. Epidermal cells are wavy walled with thin cuticle. A single layer of elongated palisade cells is present below upper epidermis. Mesophyll consists of four to six layers of spongy parenchymatous cells with intercellular spaces and oil glands. Leaf bears both covering and glandular trichomes; covering trichomes, uniseriate, multicellular and often very long (100–400 µ). Glandular trichomes are sessile with radiate head composed of eight cells with common cuticle forming a bladder, typical labiate type trichomes. A few glandular trichomes with unicellular stalk and a spherical unicellular head also occur. The midrib region shows collenchymatous cells below both upper and lower epidermis. Xylem bundles are arranged in an arc. The phloem is arranged on the dorsal side of xylem. (Fig-1)

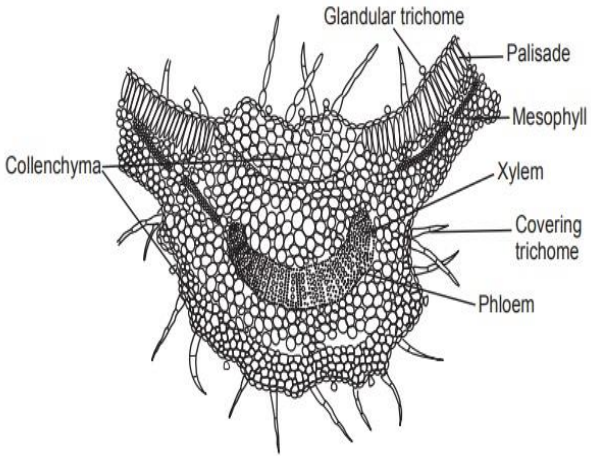


Fig.1:- Transverse section of Tulasi leaf

IV. MATERIALSAND METHODS:-

Collection of Plant Materials -

The fresh leaves of *Ocimum sanctum* and *Ocimum basilicum* were collected from Medicinal/Herbal Garden at Pharmacy college of NIMS University Rajasthan District, Jaipur, India. Voucher specimens of *Ocimum sanctum* and *Ocimum basilicum* were deposited at the Department of Advanced Science & Technology NIMS Institute of Engineering &Technology NIMS University Rajasthan Jaipur.

Isolation of the Essential Oils -

The fresh leaves of *O. sanctum* and *O. basilicum* were chopped and hydrodistilled for 3 to 4h using a Clevenger-type apparatus (Fig. 2) as recommended by British harmacopoeia giving yellowish oils The essential oils were collected separately and stored in well capped bottles prior to analysis(Fig.3). The essential oil was dried over anhydrous sodium sulphate (Merck) until the last traces of water were removed and then stored in a dark glass bottle at 4 ºC prior to GC-MS analysis(Adams, 1991).

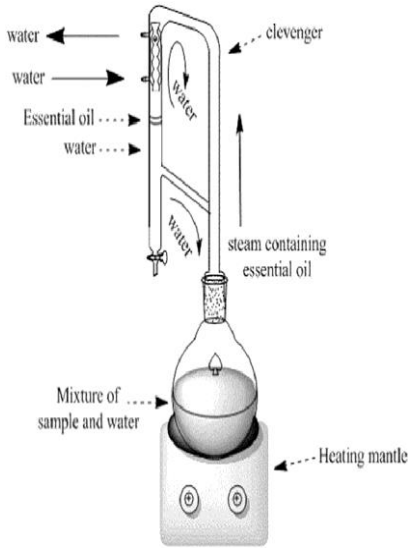


Fig. 2:- A Hydodistilation Clevenger type apparatus used to extract the Essential oils of *O. sanctum and O. basilicum*

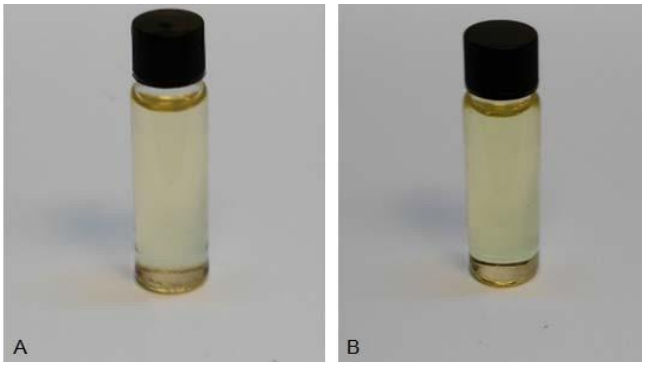


Fig. 3:- (A) *Ocimum sanctum and* (B) O. basilicum essentialoils in 3ml air tight glass vials

Analysis of Essential Oils by (GC/MS) Gas Chromatography /Mass Spectrometry

 DOI : 10.35291/2454-9150.2021.0065

GC-MS was carried out with a Hewlett- Packard 6890/ Hewlett-Packard 5973 instrument. GC conditions were equipped on fused-silica capillary column 20m×0.25 mm i.d., 0.25µm film thickness). Helium (at 0.5 ml/min) was used as a carrier gas. Samples were injected in the split mode at a ratio of 1:10 - 1:100. The injector was kept at 240 °C and the transfer line at 280 °C. The column was maintained at 50 °C for 2 min and then programmed to 260 °C at 5 °C / min and held for 10 min at 260 °C. The MS was operated in the EI mode at 70 eV, in m/z range 42–350. The identification of the compounds was performed by comparing their retention indices and mass spectra with those reported (Adams, 1995) and supplemented by Wiley and Quadlib 1607 GC-MS libraries.

Chemical constituents-

Tulasi leaves contain bright, yellow colored and pleasant volatile oil (0.1 to 0.9%). The oil content of the drug varies depending upon the type, the place of cultivation and season of its collection. The oil is collected by steam distillation method from the leaves and flowering tops. It contains approximately 70% eugenol, carvacrol (3%), and eugenol-methyl-ether (20%). It also contains caryophyllin. Seeds contain fixed oil with good drying properties. The plant is also reported to contain alkaloids, glycosides, saponin, tannins, an appreciable amount of vitamin C and traces of maleic, citric, and tartaric acid.

Antimicrobial activity-

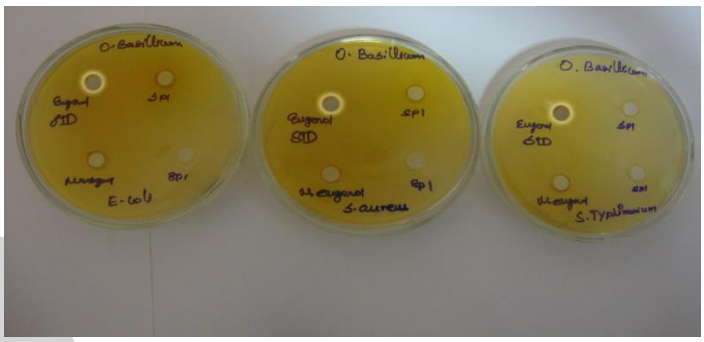
The extracts of *Ocimum sanctum* and *Ocimum basilicum* were subjected to antimicrobial screening against the pathogens such as *S. aureus, S. Typhimurium* and *E. coli*. Both the species were found to possess equipotent zone of inhibition against the selected pathogens (Table-1, Fig.4 and 5). The result of this work however agrees with the findings of Orafidiya *et al*., 2000 which showed that the oil extract of *O. gratissimum* was active against enter aggregative *E. coli*. It is therefore conceivable that this extract can be used to treat different disease caused by these organisms in infected individuals &responsible for various medicinal activities.

Table 1- Antimicrobial Activity of *Ocimum sanctum* and *Ocimum basilicum*

Microorganisms	Zone of Inhibition	
	Ocimum sanctum **(mm)**	***Ocimum basilicum*** **(mm)**
S. aureus	35	32
S. typhimurium	32	35
E. coli	30	30

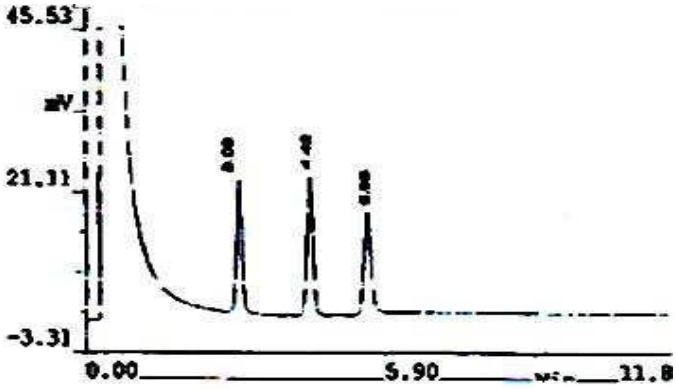
Fig.4- Antimicrobial Activity of *Ocimum sanctum*

Fig.5 - Antimicrobial Activity of *Ocimum basilicum*



V. RESULTS AND DISCUSSION

The percentage of essential oil obtained from *Ocimum sanctum* (1.45%w/w) and *O. basilicum* (0.98%w/w). The gas chromatograms of these three essential oils are given in Figure -1and Figure -2.The essential oils of both plants of *O.sanctum* and *O. basilicum* obtained from the Clevenger apparatus were found to be rich methyl eugenol and eugenol. As shown in Table -2 and 3, essential oils constituents in the both plant sample from Medicinal/herbal Garden of NIMS University Rajasthan, Jaipur India were identified by GC-MS method. The essential oil of *Ocimum sanctum* (L) which was identify as three major constituents such as methyl eugenol (37.5%),eugenol (31.3%) and unknown(31.1%). The *O. basilicum* was dominated by methyl eugenol, which accounted for 99.99%. The major constituents of methyl eugenol (66.66%), eugenol (25.53%) were identified.



Retention Time (Min)

Fig.6:- The GC-MS chromatogram of essential oil from the fresh leaf of *Ocimum sanctum*.

 DOI : 10.35291/2454-9150.2021.0065

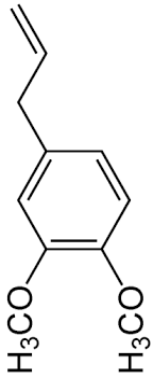
Table- 2: Chemical composition of essential oil from the fresh leaf of *Ocimum sanctum*

SL. No	RT	% Area	Constituents	Methods
1.	3.03	31.1	Unknown	GC-MS
2.	4.42	37.5	Methyl Eugenol	GC-MS
3.	5.55	31.3	Eugenol	GC-MS
Total		99.9		

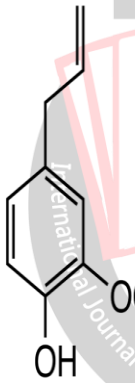
Table- 3: Chemical composition of essential oil from the fresh leaf of *Ocimum bascilicum*

SL. No	RT	% Area	Constituents	Methods
1.	2.22	6.8	Unknown	-
2.	2.78	0.6	Unknown	-
3.	3.01	66.3	Unknown	-
4.	4.07	0.087	Methyl Eugenol	GC-MS
5.	4.43	25.53	Eugenol	GC-MS
6.	5.60	0.35	Unknown	GC-MS
Total		99.9		

Methyl eugenol



Eugenol



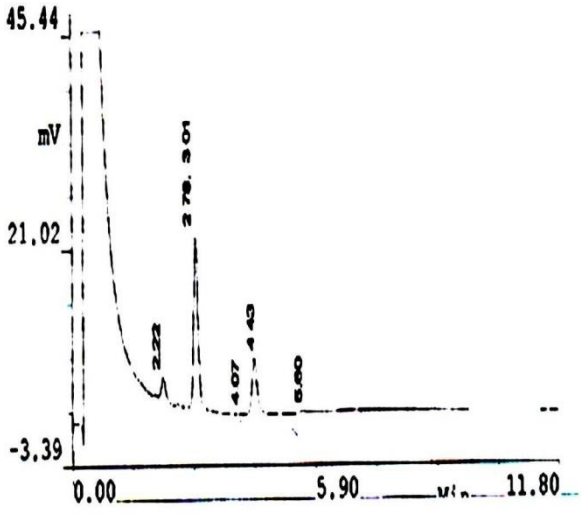


Fig.7:- The GC-MS chromatogram of essential oil from the fresh leaf of *Ocimum basilicum*

Methyl eugenol has been previously reported as the main constituent of the essential oils from *Ocimum selloi* and *Ocimum basilicum* (Ozcani and Chalchat, 2007; de Paula *et al*., 2007). In contrast, trace amount of methyl eugenol have been reported from the essential oil of *Juniperus angosturana* (Adams *et al*., 2009), and a low content in the essential oil from *Pimenta dioica* berries (Park *et al*., 2007). There is currently concern as to the carcinogenic potential of methyl eugenol.

Uses:

The fresh leaves, its juice and volatile oil are used for various purposes. The oil is insecticidal insect repellent and antibacterial. The leaves are used as stimulant, aromatic, spasmolytic, and diaphoretic. The juice is used as an anti-periodic and as a constituent of several preparations for skin diseases and also to cure earache. Infusion of the leaves is used as a stomachic. The drug is a good immunomodulatory agent.

VI. CONCLUSION

The result of the present investigation reveals that the hydro-distillation of *O. sanctum* & *O. basilicum possessed* significant content of eugenol & methyl eugenol. The extract of *O. sanctum & O. basilicum* and subjected to antimicrobial screening against the pathogens such as *S. aureus, S. Typhimurium* and *E. coli* Both the species were found to possess equipotent zone of inhibition against the selected pathogens . The results from the present investigation are very encouraging and indicate that herb should be studied more extensively to explore its potential in the treatment of infectious diseases as well. In future we will work towards the hydroponic agricultural practices for aromatic, endangered, essential oil bearing medicinal plants from India, so as to maintain the quality and quantity of their essential oil and also to protect threatened aromatic medicinal plant's germplasm as a whole.

ACKNOWLEDGEMENTS

The authors are thankful to my supervisor & Principal Director of Pharmacy College Analytical Lab, & NIET NIMS University for utilizing GC-MS analysis.

REFERENCES

[1] Adams RP. Identification of Essential Oil Components by Gas chromatography/Mass Spectrometry, 2nd edn. Allured: Carol Stream, IL, 2001

[2] Paton, A. and Putievsky, E.1996. Taxonomic problems and cytotaxonomic relationships between and within varieties of *Ocimum basilicum* and related species (Labiateae). *Bull*, 51: 509-524.

[3] Shatar S., Altantsetseg Sh., Sarnai I., Zoljargal D., Thang Tran Dinh, Nguyen Xuan Dung.Chemical composition of the essential oil of *Ocimum basilicum* cultivated in Mongolian Desert-Gobi, *Chemistry of Natural Compounds*, 2007,43, 6, 726-727.

 DOI : 10.35291/2454-9150.2021.0065

[4] Benedec D., Oniga I., Oprean R., Tămaş M. Chemical composition of the essential oils of *Ocimum basilicum* L. cultivated in Romania. *Farmacia* 57(5), 2009, 625-629.

[5] Lawrence, B.M.1992. Chemical components of Labiatae oils and their exploitation. In: Advances in Lamiaceae Science. Edits., R.M. Harley and T. Reynolds, pp 399-436, Royal Botanic Gardens, Kew.

[6] Lawrence B.. A further examination of the variation of *Ocimum basilicum* L. In: B.M. Lawrence, B.D. Mookerjee and B.J. Willis, Editors, Flavors and fragrances: A world perspective, Elsevier Sci. Publ. B.V, Amsterdam, 1988, 161-170

[7] Charles, D.J. and Simon, J.E.1990. Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. *J. Amer. Soc. Hort. Sci*., 115,458-462.

[8] Gang, D.R., Wang, J., Dudareva, N., Nam, K.H., Simon, J.E., Lewinsohn, E. and Pichersky, E.2001. An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. *Plant Physiol*., 125: 539-555.

[9] Gang, D,R., Wang, J., Dudareva, N., Nam, K.H., Simon, J.E., Lewinsohn, E. and Pichersky, E. 2001. An investigation of the storage and biosynthesis of phenylpropenes an sweet basil. *Plant Physiol*., 125: 539–555.

[10] Deshpande, R.S. and Tipnis, H.P.1997. Insecticidal activity of *Ocimum Fbasilicum* L. *Pesticides*,11: 1-12.

[11] Chaterje, A, Sukul, N.C., Laska, S., and Ghoshmajumdar, S .1982. Nematicidal principles from two species of Lamiaceae. *J. Nematol*.,14: 118-120.

[12] Adams, R.P.1995. *Identification of essential oil components by gas chromatographylmas spectroscopy*. Carol Stream, USA: Allured Publishing Corp.; 1995.

[13] Ozcani, M. and Chalchat, J.C.2002. Essential oil composition of Ocimum basilicum L. and *Ocimum minimum* L. in Turkey. *Czech J. Food Sci.*, 20(6):223-28.

[14] de Paula, J.P., Farago, P.V., Ribas, J.L.C., Spinardi, G.M.S., Doll, P.M., Artoni, R.F. and Zawadzki, S.F.2007. *In vivo* evaluation of the mutagenic potential of estragole and eugenol chemotypes of Ocimum selloi Benth. essential oil. *Lat. Am. J. Pharm*., 26(6):846-51.

[15] Adams, R.P., Beauchamp, P.S., Dev, V., Dutz, S.M.2007. New natural products isolated from one-seeded *Juniperus* of the south western United States: Isolation and occurrence of 2-ethenyl-3-methyl phenol and its derivatives. *J. Essent. Oil. Res.*,19:146-52.

[16] Park, I.K., Kim, J., Lee, S.G., Shin, S.C.2007.Nematicidal activity of plant essential oils and components from ajowan (*Trachyspermum ammi*), allspice (*Pimenta dioica*) and *Litsea* (*Litsea cubeba*) essential oils against pine wood nematode (*Bursaphelenchus xylophilus*). *J Nematol.*,39:275-79.

[17] Schecter, A., Lucier, G.W., Cunningham, M.L., Abdo, K.M., Blumenthal, G., Silver, A.G, *et al*.,2004. Human consumption of methyleugenol and its elimination from serum.*Environ.HealthPerspect.*,12:678-80.

[18] Hodişan V., Tibori G., Tămaş M. Cercetări asupra uleiului volatil de *Ocimum basilicum*,*Farmacia* 31(1), 1983

[19]Pavel M., Rădulescu V., Ilieş D.C. GC-MS analysis of essential oil obtained from the species *Thymus comosus* Heuff. ex Griseb. (*Lamiaceae*). *Farmacia* 57(4), 2009, 479-484.

[20]Tranchant J. Manuel Pratique de Chromatographie en Phase Gaseuse. Masson: Paris, 1995.

[21] O. Politeo, M. Jukic, M. Milos, Chemical composition and antioxidant capacity of free volatile aglycones from basil (Ocimum basilicum L.) compared with its essentialoil,Food Chem. 31 (2007) 379–385.

[22] N. Savithramma, M.L. Rao, D. Suhrulatha, Screening of medicinal plants for secondary metabolites, Middle East J. Sci. Res. 8 (2011) 579–584.

[23]S.E. Sajjadi, Analysis of the essential oils of two cultivated basil (Ocimum basilicumL.) from Iran, Daru 14 (2006) 128–130.

[24] S. Sugumar, A. Mukherjee, N. Chandrasekaran, Nanoemulsion formation and
characterization by spontaneous emulsification: investigation of its antibacterial
effects on Listeria monocytogenes, Asian J. Pharm. 23 (2013).

[25] Rauber C S, Guterres S S and Schapoval E E S 2005 *J. Pharm. Biomed. Anal.* 37 587

[27] Buiarelli F, Cartoni G P, Coccioli F and Ravazzi E 1991 *Chromatographia* 31 489

[28] Háznagy-Radnal E, Czigle S and Máthé I 2007 *J. Planar. Chromatogr.* 20 189

[29] Cong Z, Meiling Q, Qinglong S, Shan Z and Ruonong F 2007 *J. Pharm. Biomed. Anal.* 44 464

[30] Wang S Y, Wu C L, Chu F H, Chien S C, Kuo Y H, Shyur L F and Chang S T 2005 *Holzforschung* 59 295

[31] Surducan E and Surducan V 2008 *Procedure and device for dynamic processing of materials* Romanian Patent, RO-00112063 B1

 DOI : 10.35291/2454-9150.2021.0065