EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 7
243-246
Year: 2017

EUGENOL: EXTRACTION, CHARACTERIZATION AND ANTIBACTERIAL PROPERTY

Jaya Maitra* and Mukesh Kumar

School of Vocational Studies and Applied Sciences, Department of Applied Chemistry, Gautam Buddha University, Greater Noida, U.P 201310.

***Corresponding Author: Dr. Jaya Maitra**
School of Vocational Studies and Applied Sciences, Department of Applied Chemistry, Gautam Buddha University, Greater Noida, U.P 201310.

Article Received on 15/05/2017 **Article Revised on 05/06/2017** **Article Accepted on 26/06/2017**

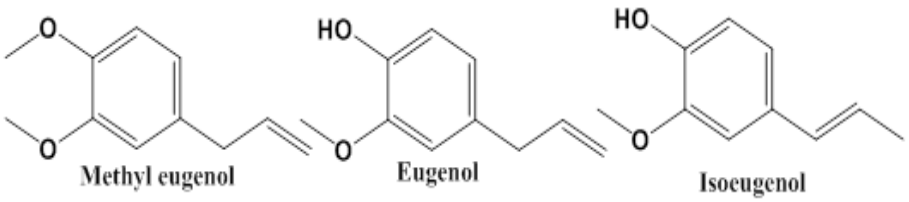
ABSTRACT
Eugenol is a phenyl propene an allyl chain substituted pale yellowish colour liquid, which is used as a flavor in the food industry and as well as in the other industries. Eugenol is generally found in the clove buds (80-90%) and have antibacterial properties. The present work is to extract eugenol form the clove buds by distillation method and to find its antibacterial activity. By the distillation methods extracted eugenol has shown good antibacterial activity against the ***Bacillus subtilis*** **and** ***Escherichia coli.***

KEYWORDS: Eugenol, extraction, *Bacillus subtilis* and *Escherichia coli*, antibacterial property.

1. INTRODUCTION

Eugenol (4-allyl-2-methoxyphenol) is the naturally extracted main component of clove's essential oil. It is inexpensive and easily available phenylpropene that has been known by humankind since antiquity, and used as a medicinal agent, widely used for the industrial application like food flavoring, preservation, irritant, sensitizer and can produce local anesthesia. The natural product is the sources of the major aromatic constituent (up to approximately 80% by weight) of the essential oil of clove [*Eugenia caryophyllata* L. Merr. & Perry (Myrtaceae) = *Syzygium aromaticum*], that can be synthesized by distillation process like hydro-distillation, steam distillation, or Soxhlet (ethanol) extraction from the leaves of corresponding trees, buds, and stems of clove trees.[1] Eugenol is a pale yellowish liquid having solubility in organic solvents like methanol, chloroform etc. Eugenol can be isolated from *Myristica fragrans, Cinnamomum tamala, Zygium aromaticum, Ocimum basilicum, Ocimum grattisimum, Ocimum tenuiflorum, Pimenta racemosa* etc. Eugenol can be extracted from many plants like clove[2] by the different synthetic routes.

On the other hand eugenol derivatives also available which can be synthesised or extracted from the natural products or can be synthesized in laboratory by synthetic routes. Methyl eugenol is also a natural product which is the derivative of the methyl ether and can be extracted from the natural resources.[3],[4] Here are some structures of eugenol and its derivatives, which can be extracted naturally or synthetically synthesized and have importance in various fields.



Methyl eugenol Eugenol Isoeugenol

Eugenol can be extracted from colve buds and leaves of *Eugenia caryophyllata*(clove) for the first time and it is named as eugenol.[5] In the modern trend eugenol can also be synthesized in laboratory scale and industrial scale by the allylation of guaiacol with allyl chloride having the functional properties which is similar to eugenol**.** Eugenol has got much attention by the researchers and opened up a wide area of research in applying it as a medicine to cure various diseases**.** Eugenol have antioxidant properties and properties to diminish formation of free radical, when the concentration of the eugenol increases from a minimum level then it possess the activity of pro-oxidants by forming free radicals.[6],[7] Besides antioxidant properties eugenol exhibits versatile therapeutic properties like antibacterial activity, antiviral activity, anti-parasitic

activity, anti-cancer activity and anti-inflammatory properties. Eugenol can be transformed into the various products. Lambert and co-workers were able to use Eugnol as a feedstock for the production of various valuable compounds.[8] Due to the wide application of Eugnol in the various fields, researchers have good interest and have published a high number of articles in recent years.[9],[10]

In the recent work eugnol is extracted from the clove buds by the distillation process in good yield and its antibacterial property is investigated against *Bacillus subtilis* and *Escherichia coli.*

2. MATERIAL AND METHODS

Clove buds were purchased from the local areas of Greater noida (U.P.) Extraction solvent DMSO was purchased from the Sigma Aldrich. Methanol, Sodium anhydride were purchased from from CDH. TLC was prepared in laboratory.

3. Extraction of Eugenol

15 gm of the clove was weighed on the weighing balance. Cloves were Grind into coarse powder using mortar and pestle. Powder was reweighed and weight was recorded. Powder form of cloves was transferred to the 100 mL round bottom flask. By adding 50 ml water and few boiling chips the mixture was heated to the desired temperature within the distillation setup. When the mixture started boiling, adjusted the heat to maintain a distillation rate of approximately one drop per second. Distillation process was stopped when the 30-40 ml distillate was collected. After the collection of distillate, distillate was cooled down to room temperature. Distillate was carefully poured into a separatory funnel. Added 10mL of saturated NaCl solution. Added 10 mL amount of di-chloromethane (DCM). Separatory funnel was capped gently and contents were swirled for several seconds. Released the separatory funnel frequently. After the pressure has been released, the contents were shaken vigorously to mix the two layers thoroughly. Layers were allowed to separate. CH_2Cl_2 layer was poured into flask or beaker. Repeated the extraction of the aqueous layer three times, each time with 10 mL portion of CH_2Cl_2. Eugnol was collected in the Organic layer of CH_2Cl_2 in the same flask. The collected all combine organic layer is shown in figure-2.

Fig.2. ***The collected all combine CH_2Cl_2 organic layer***

Dried the combined CH_2Cl_2 solution using anhydrous Na_2SO_4. Decanted the CH_2Cl_2 solution into a pre-weighed ceramic evaporating dish, making certain that no Na_2SO_4 is transferred with the solution. Place the evaporating dish on a hot water bath to remove CH_2Cl_2. When all of the CH_2Cl_2 has been evaporated, evaporating dish was allowed to cool to room temperature. Yellowish coloured eugenol was extracted, weighed and characterized by spectroscopic technique.

4. Evalution and characterization of extracted Eugenol

Physical and chemical properties of eugenol

Chemical Formula	***$C_{10}H_{12}O_2$***
Molecular Weight	*164.20*
Colour	*Pale Yellow*
Physical State	*Liquid*
Solubility	*Miscible in organic solvent like Chloroform, Alcohol etc.*

Characteriazation of Eugenol

Characterization of the extracted eugnol was done by the help of spectroscopic technique: ^{1}H NMR, TLC and FTIR.

1-Thin Layer Chromatography (TLC) Analysis

Sample Details: Eugenol
Adsorbent: Precoated Silica gel
Physical properties: Liquid sample
Solvent System: Ethyl Acetate (30): n-Hexane (70)

Procedure

The Eugenol was subjected on to the percolated and activated silica gel TLC plates. The mobile phase is pet-Ethylacetate: n-Hexane in 3:7 ratio. The TLC plates were runs with in two spot (one spot for extracted eugenol and another spot for industrially available eugenol). After the TLC runs, TLC plate put inside the iodine chamber yellowish spot of eugenol were identified. Both spot indicate successful extraction of eugenol. R_f value was calculate.

R_f value = 0.65

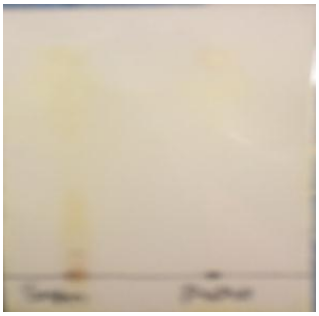


Fig.3. ***TLC of Eugnol***

2. ***Characterization of eugenol by Fourier transform infrared spectroscopy (FTIR)***

For the conformation of eugenol purity, structural analysis of the extract was performed. FTIR spectra for the extracted eugenol were indicating that the extracted product is eugenol which characteristic peak matched with the reported literatures data.

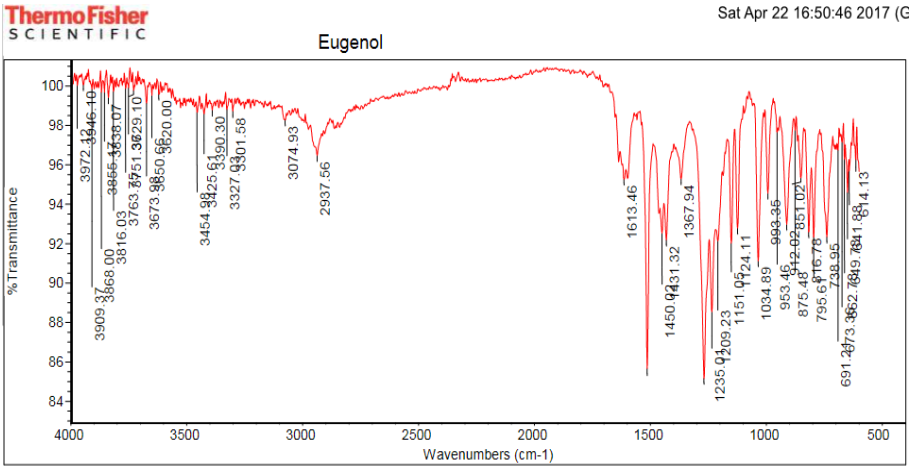


Fig.4. ***FTIR of Eugnol***

IR (KBr pellet) cm^{-1}: 3327(O-H), 3074 (C=CH-Ar), 2937 ($CH=CH_2$), 1613 (C=C), 1209 (C-O).

3. ***Characterization of eugenol by ^1H NMR (Nuclear Magnetic Resonance Spectroscopy)***

For the conformation of eugenol purity, structural analysis of the extract was performed. ^1H NMR spectra of the extracted eugenol was taken using $CDCl_3$ as a solvent. It was indicated that the extracted product is eugenol which is matched with the reported literature data.

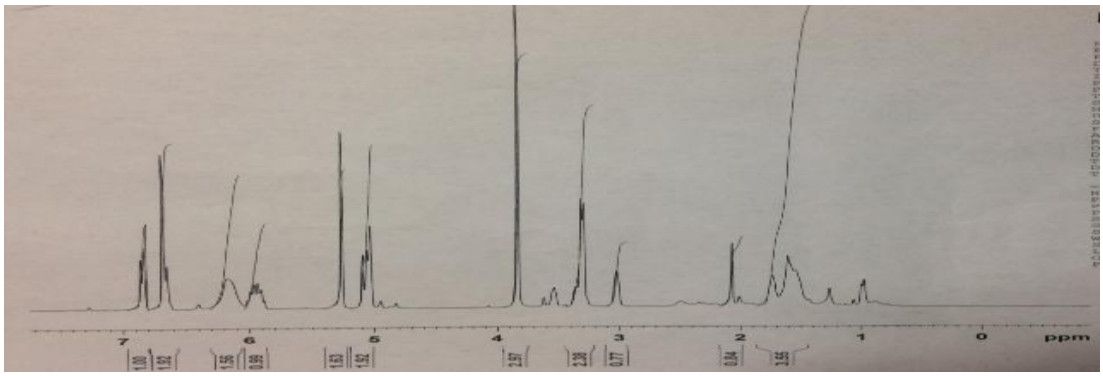


Fig.5. ***NMRof Eugnol***

^1H NMR (400.1 MHz, $CDCl_3$)

d 3.35 (2H); 3.93 (3H, s, OCH_3); 5.13 (2H, m); 5.91 (1H, m); 6.96 (1H, s); 7.50 (1H, d); 10.67 (1H, s, OH).

$^{[13]}C$ NMR (100.6 MHz, $CDCl_3$): d 39.4; 56.7 (OCH_3); 115.1; 117.1; 118.6; 131.2; 133.6; 135.9; 144.9; 149.8.

Antibacterial Activity of Eugenol

A preliminary study on clove oil (Eugenol) shows that it had potent antibacterial activities against the test *Bacillus subtilis and Escherichia coli.* The Disc diffusion method was performed to find the antibacterial activity against gram-positive organism which is *Bacillus subtilis* (ATCC 6633), and a gram negative organism *Escherichia coli* (ATCC 10536).

Procedure

The fresh species were dried, and make in the powdered form, 2gm was extracted with ethanol. The resultant was placed on the rotary shaker for 10 h and the process was repeated for some times until the extract was colour and odour free. The resultant was then centrifuged to a fixed

volume and stored in vials at 10°C. The pH of the extract was adjusted near to 6.5-6.8 in order to eliminate the possible interfaces of growth of the test organism due to pH shifts.

The resultant media was then allowed to solidify in the form of a slant and then seeded with the test organisms. After that nutrient agar media *Bacillus subtilis* and *Escherichia coli* was used.

The testing results of the antibacterial activity of eugenol are shown below and indicate that eugenol has antibacterial activity.

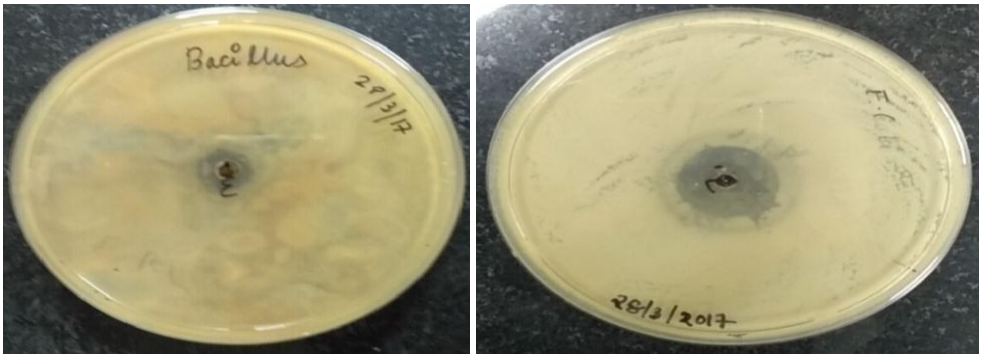


Fig.6. ***Antibacterial activity of Eugnol against i) Bacillus subtilis and ii)E.coli***

DISCUSSION AND CONCLUSION

Eugenol has shown an antibacterial activity against gram negative(*Escherichia coli)* and gram positive *(Bacillus subtilis) so* it can be concluded that clove can reduce the growth of *Bacillus subtilis* and *Escherichia coli* and has potent antibacterial properties and needs to be investigated thoroughly.

REFERENCES

1. W. Guan, S. Li, R. Yan, S. Tang, and C. Quan, "Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods," *Food Chem.*, 2007; 101(4): 1558–1564.
2. M. T. Athar, E. T. Tamboli, S. H. Ansari, and S. Ahmad, "Quantification of eugenol in hydro-distilled clove oil (eugenia caryophyllus) and its marketed products by validated gc-ms method," *J. Herbs, Spices Med. Plants*, 2013; 19(4): 365–376.
3. M. Miele, R. Dondero, G. Ciarallo, and M. Mazzei, "Methyleugenol in Ocimum basilicum L. cv. Genovese Gigante," *J. Agric. Food Chem.*, 2001; 49(1): 517–521.
4. R. I. Vargas, T. E. Shelly, L. Leblanc, and J. C. Piñero, *Recent advances in methyl eugenol and cue-lure technologies for fruit fly detection, monitoring, and control in hawaii*, 2010; 83.
5. M. R. C. Raja, V. Srinivasan, S. Selvaraj, and S. K. Mahapatra, "Versatile and synergistic potential of eugenol: a review," *Pharm. Anal. Acta*, 2015; 6(5): 367.
6. S. K. Mahapatra and S. Roy, "Phytopharmacological approach of free radical scavenging and anti-oxidative potential of eugenol and Ocimum gratissimum Linn.," *Asian Pac. J. Trop. Med.*, 2014; 7(S1): S391–S397.
7. J. B. Chogo and G. Crank, "Chemical Composition and Biological Activity of the Tanzanian Plant Ocimum Suave," *J. Nat. Prod.*, 1981; 44(3): 308–311.
8. K. P. Dhake, D. D. Thakare, and B. M. Bhanage, "Lipase: A potential biocatalyst for the synthesis of valuable flavour and fragrance ester compounds," *Flavour and Fragrance Journal*, 2013; 28(2): 71–83.
9. K. Chaieb *et al.*, "The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae): A short review," *Phytotherapy Research*, 2007; 21(6): 501–506.
10. A. F. de Oliveira Araujo *et al.*, "Larvicidal activity of Syzygium aromaticum (L.) Merr and Citrus sinensis (L.) Osbeck essential oils and their antagonistic effects with temephos in resistant populations of Aedes aegypti," *Mem. Inst. Oswaldo Cruz*, 2016; 111(7): 443–449.