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A STUDY ON ISOLATION AND PURIFICATION OF EUGENOL FROM CLOVE BY COLUMN CHROMATOGRAPHY

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ABSTRACT

In the present study the phytomedicine Eugenol is extracted from Clove in order to prepare its semisynthetic derivaties bearing 1,2,4-triazole motiff. The clove oil was subjected to isolation of Eugenol by column chromatography. The stationary phase used is silicagel (230-400 mesh size) and the mobile phase was n-hexane:ethylacetate in the ratio of 7:3. About six fractions were collected from the elution. On TLC studies third and fourth fraction collected were found to contain Eugenol on comparision with standard Eugenol. Further these eluents were combined and evaporated to get yellowish oil of pure Eugenol. This isolated oil was characterised by IR, ^{1}H NMR and GC-MS studies. The results of these characterisation studies were in good aggrement with the structural features of Eugenol.

KEYWORDS: Eugenol, Isolation, Column Chromatography, ^{1}H NMR, GC-MS.

INTRODUCTION

Eugenol (fig1) is chemically 4-allyl-1-hydroxy-2-methoxybenzene and is the principle component of clove oil (Family:Myrtaceae Scientific name: *Syzygiumaromaticum*). Clove oil contains 84-95% of phenols ie., eugenol with 3% of acetyl eugenol, sesquiterpenes(α and β caryophyllene) and small quantities of esters, ketones and alcohols.[1] It is found to have a wide range of biological activities such as Anti-inflammatory, antibacterial, aphrodisiac, antifungal, anti-platelet, anti-stress, mosquito repellent, insecticidal, hyperlipidimic, diuretic, antiviral, immunomodulating activity, anti-leishmanial activity.[2,3] This diversified phytomedicine is exploited to synthesise its semisynthetic derivatives in order to counteract inflammation and infections situations.[4] The literature studies in this regard reveals the fact that there is a paucity of information. By keeping this and the importance of Eugenol in therapy, In the present study an attempt has been made to extract and isolate eugenol from clove oil in order to prepare its semi synthetic derivatives bearing 1,2,4-triazole motif for their biological activities as this may results in the library of synergestic compounds.

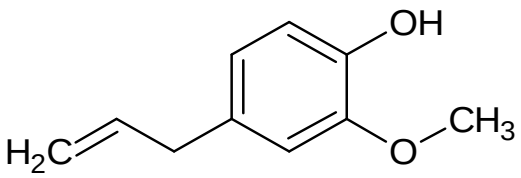

Fig. 1: Eugenol:4-allyl-1-hydroxy-2-methoxybenzene.

MATERIAL AND METHODS

Commercially available clove buds were procured from M/S Amruth Kesari Benagaluru and authenticated by Regional Ayurveda Research Institute for Metabolic Disorders, Bengaluru. The voucher specimen is preserved in our laboratory for further reference. These authenticated clove buds were used to extract clove oil by Hydrodistillation method.[5]

Extraction of clove oil from clove buds

Clove buds (15g) were placed into a 500ml two necked RBF. The apparatus was set up for hydrodistillation using Clavengers apparatus. 300ml distilled water was added to the flask and heated with heating mantle at a temperature of about 50-70◦C. During the distillation, distilled water was added to maintain the original level of the liquid in the flask until the distillate became colorless. The emulsion thus obtained was extracted with 3 volumes of dichloromethane and dried with sodium sulphate to remove the traces of water present. The clove oil was separated from dichloromethane using rotary evaporator.[5] The clove oil was characterised by TLC, IR and GC-MS studies.

TLC: Thin layer chromatography was performed using the mobile phase Dichloromethane:n-hexane in the ratio2:1/ Ethyl acetae :n-hexane in the ratio 3:7. The Rf values were 0.82 / 0.75. The Eugenol spots were located by dipping the eulted plate in saturated KMnO4 solution and the appearance of the brown color indicates the presence of Eugenol.[6,7] Standard Eugenol obtained as a gift sample from M/S NATURAL REMEDIES

bengaluru karnataka was used for the purpose of comparision.

IR Spectroscopy: The clove oil extracted was subjected to IR spectral studies Fig:3 using IRAffinity-1 Shimadzu and the values are tabulated.(Table:1).

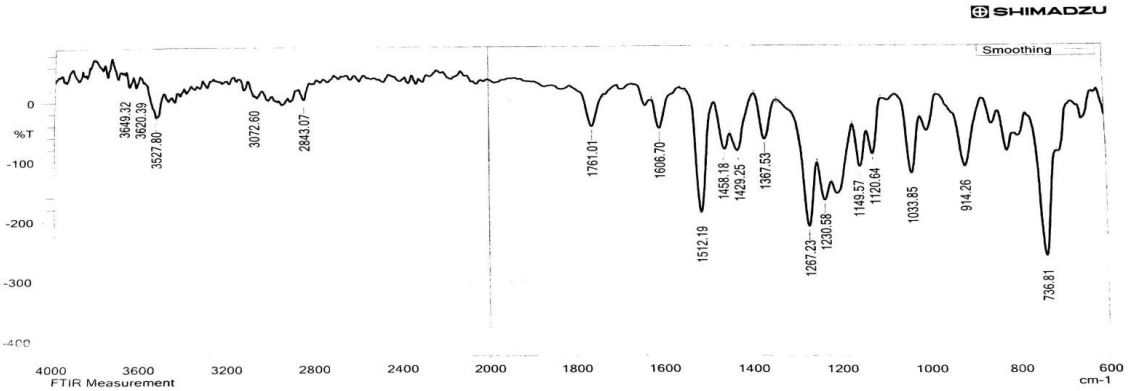

Fig. 2: IR spectroscopy of Clove oil.

Table 1: The IR spectral values of Clove oil (cm-1).

Functional group	Expected values cm-1	Obtained values cm-1
OH	3650-3600	3620
C=C (Aromatic)	1475	1458.18
C-H (Ar stretching)	3150-3050	3072.60
C=C (Alkene)	1680-1600	1606.70
C-H (Alkane stretching)	3000-2850	2843.07
C-O	1300-1000	1512.19

GC-MS: The extracted Clove oil from Clove buds was subjected to GC-MS studies to characterise the presence of Eugenol in it. The chromatogram obtained is depicted in Fig:2.

Column RTX-5 MS(30m*0.25mm)
film thickness 0.25µm
Temperature programme: Rate, Temperature and Hold time.

Conditions
GC-MS model Shimadzu GCMS-QP5050
Auto injector: Shimadzu AOC 5000

Sl no.	Rate	Temperature	Hold time
1	0	60	1
2	10	100	1
3	20	290	3

Injection volume:1µl; Inlet pressure:68.6kPa; Linear velocity:39.2cm/sec; Injection mode:split MS interface temperature:290◦C; MS mode:TIC; Detector voltage:Absolute 1.4kV; Mass range:100-920m/z; Scan speed:1000; Interval time :0.5sec; Library used: NIST MS 2.0.

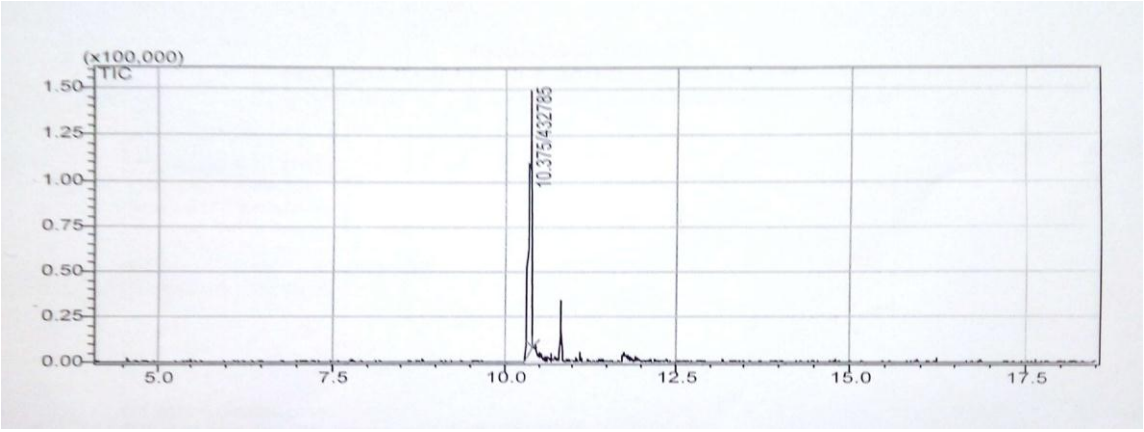

Fig. 3: GC-MS of clove oil with retention time of 10.375.

Isolation

Clove oil thus obtained was purified by column chromatography using silica gel of mesh size 230-400. A slurry of preactivated silica gel was prepared using n-hexane. This was transferred to a dried and cotton plugged (50cmX3cm) glass column. Once the slurry was packed to the 1/10th of the length of packed column the oil was added and allowed to adsorb on the bed followed by solvent and then packed with wetted cotton dipped in n-hexane, to this mobile phase was added in fraction of 100ml. 5-6 fractions were collected and the fraction which shows the presence of eugenol was confirmed by TLC and recovered by rotary evaporator.[8]

The isolated eugenol was characterised by TLC, IR,'H NMR.

TLC of Isolated Eugenol was performed by adopting the methodology followed in TLC of Clove oil using using the mobile phase Dichloromethane:n-hexane in the ratio2:1/ Ethyl acetae :n-hexane in the ratio 3:7. The Rf values obtained were 0.82 / 0.75.[6,7]

IR Spectroscopy of the isolated Eugenol was similarly subjected to IR studies Fig: 4 using IRAffinity-1 Shimadzu.

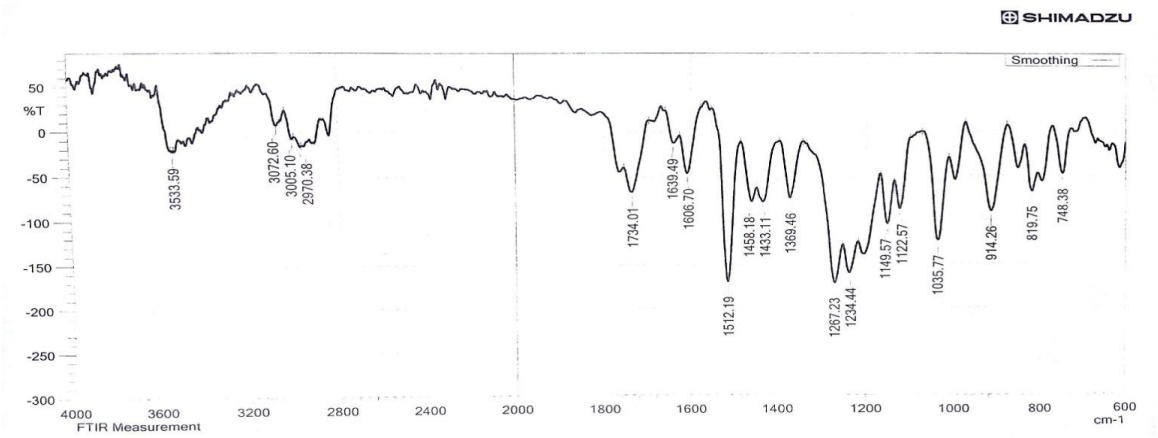

Fig. 4: IR spectroscopy of isolated Eugenol.

Table 2: The IR spectral values of Isolated Eugenol (cm-1).

Functional group	Expected values cm-1	Obtained values cm-1
OH	3650-3600	3533.59
C=C (Aromatic)	1600 and 1475	1606.70,
C-H (Ar stretching)	3150-3050	3072.60
C=C (Alkene)	1680-1600	1639.49
C-H (Alkane stretching)	3000-2850	2970.38
C-O	1300-1000	1267.23

'H NMR

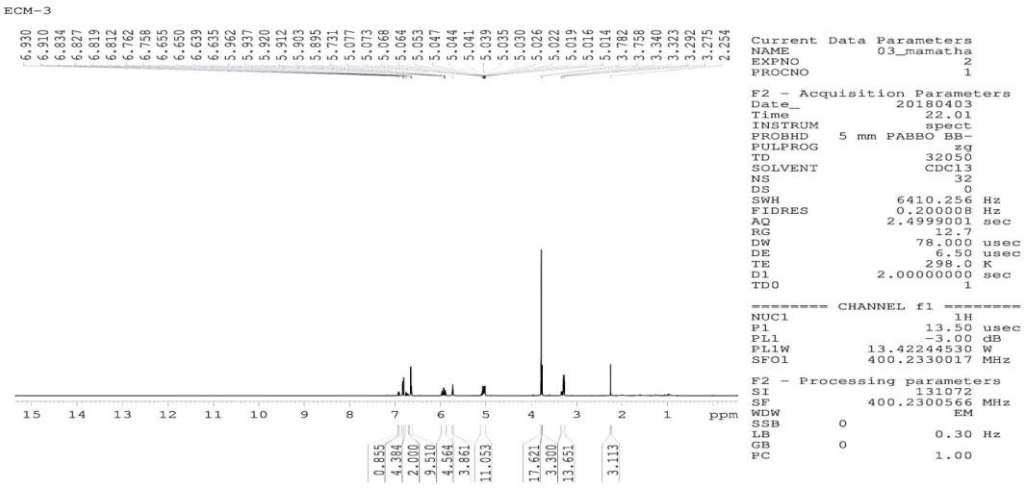

Fig. 5: 'H NMR of Isolated Eugenol.

Table 3: Chemical shift values δ in ppm.

COMPOUND	SIGNAL GROUP	CHEMICAL SHIFT δ (ppm)	NUCLIDES	MULTIPLICITY
EUGENOL	CH2-Ar	3.27 -3.29	2H	doublet
	CH3-O	3.78	3H	singlet
	CH2=CH	5.039-5.108	3H	multiplet
	OH	5.731	1H	singlet
	Aromatic protons	6.930	1H	singlet
		5.878-5.979	2H	doublet of doublet

RESULTS AND CONCLUSION

In the present study an attempt was made to extract clove oil using clove buds from *Syzygium aromatic* belonging to the family Myrtaceae. The crude drug was authenticated from Regional Ayurveda Research Institute for Metabolic Disorders to extract clove oil by Hydrodistillation method. The yellow viscous clove oil percentage yield was found to be 82.3% w/v. The presence of Eugenol in extracted clove oil was confirmed by GC-MS model Shimadzu GCMS-QP5050 (Fig:3). Futher the clove oil was subjected to column chromatography to isolate eugenol using the silica gel of meshsize 230-400. TLC pattern of both extracted clove oil and Eugenol was simillar in nature and Rf values obtained were 0.82 in Dichloromethane:n-hexane(2:1) and 0.75 in Ethyl acetae :n-hexane in the ratio 3:7. The IR spectroscopy of clove oil and Eugenol were recorded using IRAffinity-1 Shimadzu and are in line with each other (Fig:2 and 4). The spectral values are tabulated in table: 1 and 2. The ^{1}H NMR spectrum (Fig:5) of Eugenol showed the presence of 12 protons. The presence of 3 downfield protons at δ 6.930, 5.878, 5.979 ppm indicated the presence of three aromatic protons in the molecule. The presence of a singlet for 3 protons at δ 3.78 suggested the presence of a methoxy group on the aromatic ring. Further, the presence of doublet at δ 3.27-3.29 for 2 protons suggested the presence of a -CH_2 attached to an aromatic ring. The presence of downfield doublet at δ5.039-5.108 further suggested the presence of an exocyclic double bond which was supported by the presence of one proton. The singlet at δ 5.731 for a aromatic OH proton(Table:3). All the above facts are in good agreement with the unique structure of Eugenol(Fig:1). This isolated and characterised Eugenol will be used in our further studies to prepare its semisynthetic derivatives bearing 1,2,4- triazole motiff. These synthesised novel molecules will be exploited pharmacologically for their possible biological activities.

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