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Chemical Compositions of Different Extracts of *Ocimum basilicum* Leaves

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Abstract

The chemical compositions of essential oil of *Oscimum basilicum* extracted by hydrodistillation and different solvent extractions were analyzed by GC-MS. Qualitative analysis of the essential oil as well as other extracts showed that majority of these are mono and sesquiterpenes. Most of the identified compounds are biologically important. Further the *Oscimum basilicum* leaf possesses certain characteristics that can be ascribed to cultivation on a domestic plantation.

*Keywords: Ocimum basilicum; E*ssential oil; Hydrodistillation; Different extracts; GC-MS.

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1. Introduction

Among the plants known for medicinal value, the plants of genus Ocimum belonging to family Labiatae are very important for their therapeutic potentials [1]. *Ocimum sanctum* L. (Tulsi), *Ocimum gratissium* (Ram Tulsi), *Ocimum canum* (Dulal Tulsi), *Ocimum basilicum* (Ban Tulsi), *Ocimum kilimandscharicum*, *Ocimum ammericanum*, *Ocimum camphora*, *Ocimum minimum* L., *Ocimum tenuiflorum* L. and *Ocimum micranthum* are examples of known important species of genus Ocimum which grow in different parts of the world and are known to have medicinal properties. *Ocimum sanctum* L., known as 'Tulsi' in Hindi and 'Holy Basil' in English, is an erect softy hairy aromatic herb or under shrub found throughout India. Tulsi plant is a shrub reaching a height of 0.5 to 1.5 m. The leaves are 2-4 cm in length [2]. *Ocimum basilicum* is an important symbol in the Hindu religious tradition and is worshipped in the morning and evening by Hindus at large [3-9]. In traditional systems of medicine the Indian medicinal plants have been used in successful management of various disease conditions like bronchial asthma, chromic fever, cold cough, malaria, dysentery, convulsions, diarrhea, arthritis, emetic syndrome,

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skin diseases, insect bite etc and in the treatment of gastric, hepatic, cardiovascular and immunological disorders [10-15].

The holy basil is an herbal remedy for a lot of common ailments such as healing power, fever, common cold coughs, sore throat, respiratory disorder, kidney stone, heart and vascular protection, children's ailments, stress, mouth infections, insect bites, skin disorders, teeth disorder, headaches, eye disorders, liver support, lung and bronchial support, radiation protection, immunity tune-up, anti-inflammatory Action, antibiotic protection, nutrition, high safety margin, food additive and perfume etc.

But there is no published report in the literature about the chemical compositions of *Ocimum basilicum* essential oil from Bangladesh. So an attempt has been made to investigate the components of essential oil and some other solvents extract.

2. Material and Methods

2.1. *Plant material*

The plant leaves were collected from Dhaka university area and washed with water to remove mud and dust particles. The leaves were first dried at sunlight and then in an oven at 40 ^{0} C. The dried leaves were grinded to powder by a cyclotec grinder (200 meshes) and the powder was stored in an air tight bottle and this was used throughout the investigations.

2.2. *Extraction of Ocimum basilicum*

250 g of the air-dried leaves of *Ocimum basilicum* were subjected to hydrodistillation for 3 h using a Clevenger type apparatus. Sodium chloride (1 g) and 20 mL of diethylether was added with the distillate in a sepratory funnel, shaking were continued for 40 min and allowed to stand for 15 min. The organic layer was separated and concentrated to 5 ml under reduced pressure. The oils were dried over anhydrous sodium sulphate and preserved in a sealed vial at 4 ^{o}C until further analysis.

The dried powder of *Ocimum basilicum* (50 g for each) was subjected to extract separately and exhaustively in Soxhlet apparatus with n-hexane, ethyl acetate, chloroform and butanol. All extracts were filtered followed by evaporation to desire volume by a rota evaporator. The fractions were labeled as n-hexane (H-1), chloroform (C-1), and ethyl acetate (EA-1).

150 g of *Ocimum basilicum* was extracted with methanol by a Soxhlet apparatus at 50 ^{0}C temperature for 36 hours. It was repeated two times. Then the methanol extract was filtered off and evaporated to dryness by rotatory evaporator at 40 ^{0}C. The dried extract was dissolved in methanol/ water 7:3 mixture to remove chlorophyll. It was then partitioned with hexane, chloroform and ethyl acetate followed by evaporation to desired volume by a rotary evaporator. N-hexane(H-1), chloroform (C-1), ethyl acetate (EA-1) extracts from conventional method and chloroform (C-2), and ethyl acetate (EA-2)

extracts partitioned from highly polar solvent methanol extract were subjected to GC-MS analysis.

2.3. *GC-MS analysis*

The GC–MS analysis of the samples of *Ocimum basilicum* was performed using a Varian GC–MS (Model Varian CP 3800, USA) equipped with a VF-5 fused silica capillary column (30 m × 0.25 i. d. mm .film thickness 0.25 µm, Varian, USA). For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 ml min^{-1}. Injector and mass transfer line temperature were set at 250 °C and 300 °C, respectively. The oven temperature was programmed from 50 °C to 200 °C at 8 °C min^{-1} and then held isothermal for 20 min and finally raised to 300 °C at 10 °C min^{-1}. Diluted samples (prepared in Methanol) of 0.2 µl were manually injected in the split less mode. Identification of compounds of the samples was based on GC retention time on VF-5 capillary column, computer matching of mass spectra with standards (Mainlab, Replib and Tutorial data of GC–MS systems).

3. Results and Discussion

3.1. *Physical properties*

Colour, smell, density and refractive index of essential oil are light yellow, intense scent, 0.928 g/cm^3 at 25 ^{0}C and 1.515, respectively. The color of the H-1 extract was brown in color. The color of the EA-1 extract was orange in color. The color of the EA-1 extract was orange. The color of the C-1 extract was gray in color. The color of the C-2 extract was gray.

3.2. *Chemical composition of essential oils*

The major chemical compounds that were found in essential oil **(**shown in Fig. 1 and Table 1) are eugenol (61.76%), isopropyl palpitate (11.36 %), α-cubene (3.85%), 2, 3-dihydroxy propyl elaidate (5.10%), 1-methyl-3-(1-methyl) benzene (1.73 %), 2-methoxy-4-(1-propyl) phenol (2.65%), vanillin (1.27%), 1, 4-diethyl benzene (1.03%), hexa-decanoic acid methyl ester (2.51%) and [2-methyl-4-(1-propyl) phenoxy] silane (2.01%).

3.3. *Chemical composition of different extracts*

The major chemical compounds that were found in hexane extract (H-1) **(**shown in Fig. 2 and Table 1) are 1,2-dimethoxy-4-(2-propenyl)benzene(35.82%), 2- pentanone(27.06%), caryophyllene oxide(4.64%), acetic anhydride(4.32%), tricyclo[5.2.2.0(1,6)]undecan-3-01,2-me(1.86%), aromadendrene oxide-(2)(1.16%) and 1,3-benzodioxide(0.59%).

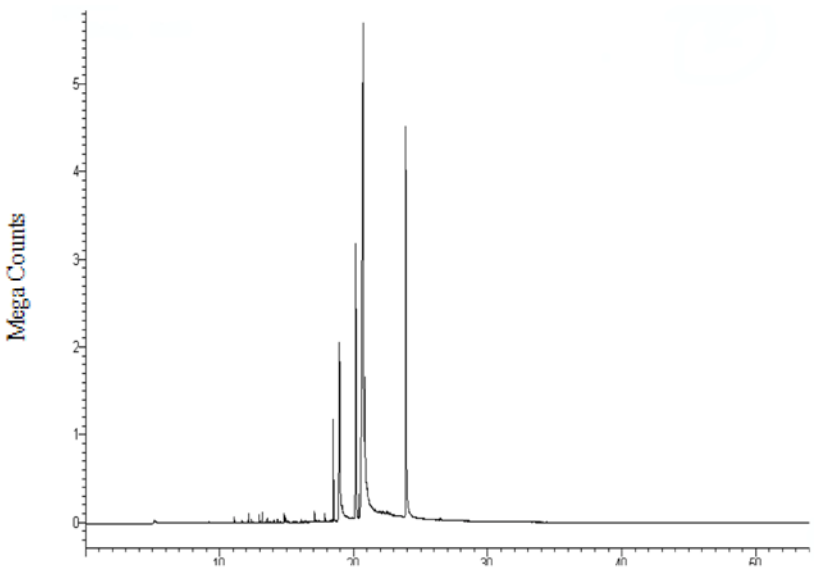

Fig. 1. Gas chromatogram of essential oil.

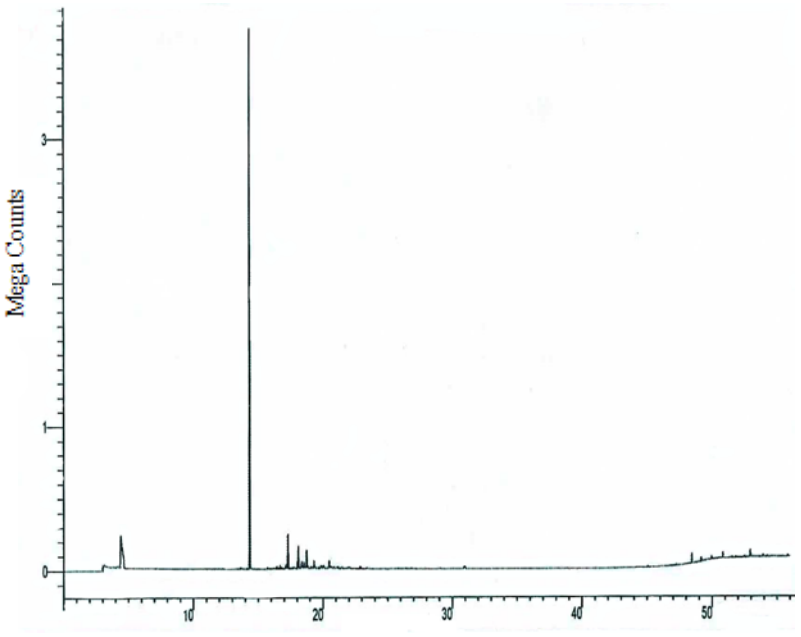

Fig. 2. A typical gas chromatogram of the constituents of extracts H-1.

The major chemical compounds that were found in ethyl acetate extract (EA-1) **(**Fig. 3 and Table 1) are 1,2-dimethoxy-4-(2-propenyl)benzene(53.06%), 2-pentanone(18.06%), 4-methyl-2-pentyl acetate(3.11%), aromadendrene oxide-(2)(1.16%), caryophyllene oxide – 1(1.44%), 1,2-benzenedicarboxylic acid(0.44 %) and 10-heneicosene(0.30%).

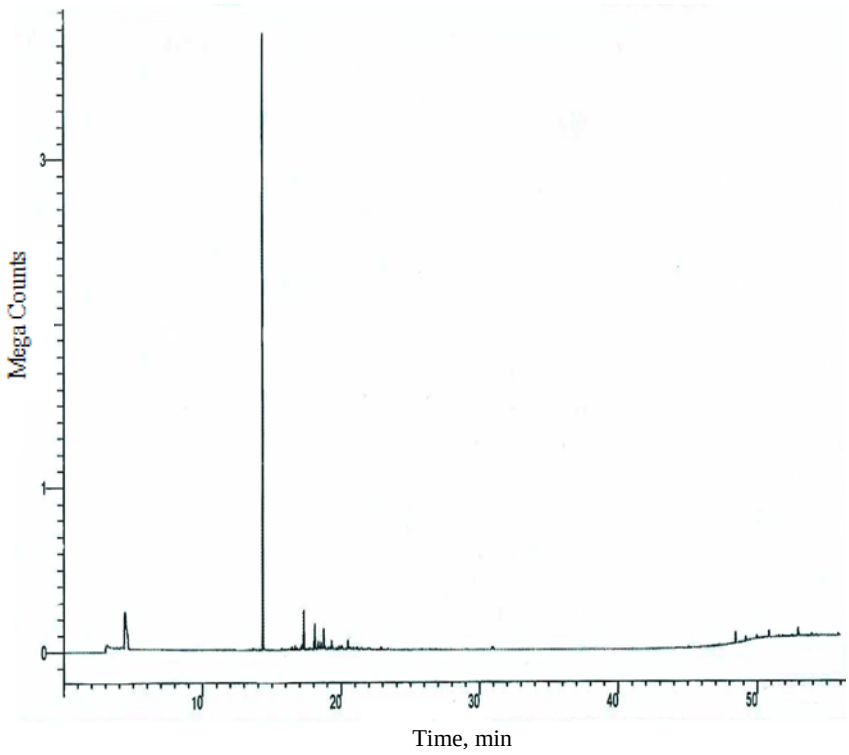

Fig. 3. A typical gas chromatogram of the constituents of extracts EA-1.

The major chemical compounds that were found in ethylacetate extract (EA-2) **(**Fig. 4 and Table 1) are 1, 2-benzene dicarboxylic acid (49.44%), 1, 2, 3, 4-tetramethyl benzene (9.9%), eugenol (7.72%), 1-methyl-3-(1-methylethyl) benzene (5.11%), 1, 2, 4, 5-tetramethyl benzene (3.18%), diethyl phthalate (2.07 %), heneicosene (c, t) (2.16%) and vanillin (2.27%).

The major chemical compounds that were found in chloroform extract (C-1) **(**Fig. 5 and Table 1) are 1,2-dimethoxy-4-(2-propenyl)benzene(29.69%), 4-methyl-2-pentyl acetate(7.48%), ledene oxide(1.12%), longipinocarvone(0.65%), phthalic acid,butyl hexyl ester(0.62%) and 2-pentanone(0.44%).

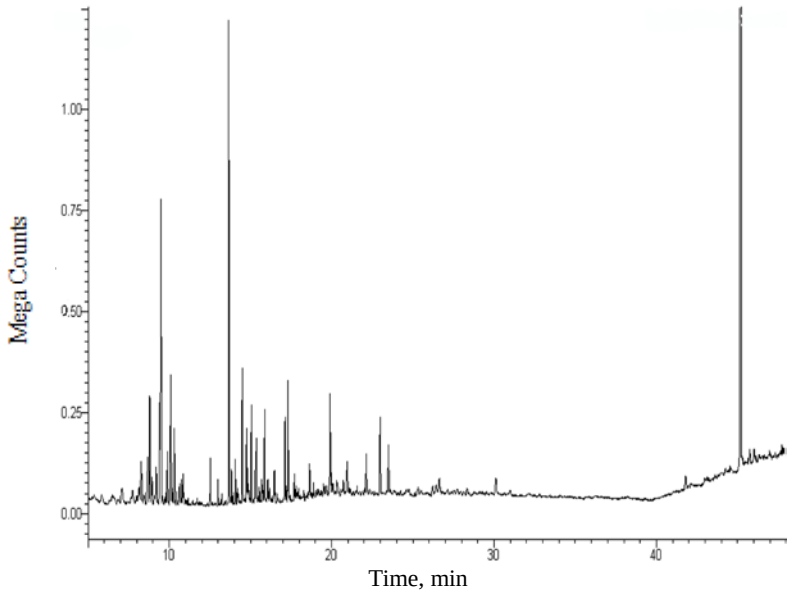

Fig. 4. A typical gas chromatogram of the constituents of extracts EA-2.

The major compounds that were found in chloroform extract (C-2) **(**Fig. 6 and Table 1) are eugenol (88.18%), 1, 2-benzene dicarboxylic acid (3.49%) and 2-methoxy-4-(1propyl)-(E)-phenol (1.46%).

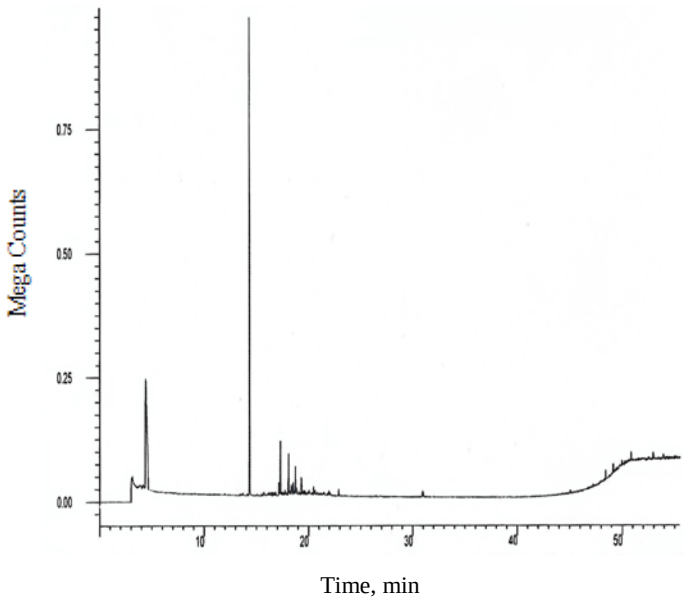

Fig. 5. A typical gas chromatogram of the constituents of extracts C-1.

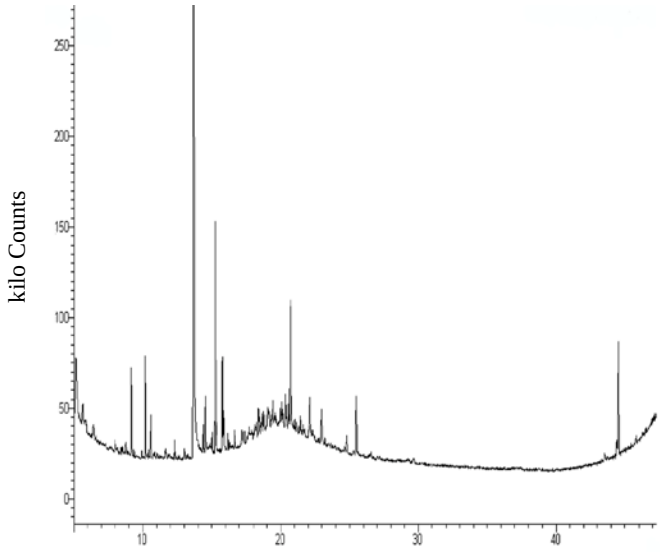

Fig. 6. A typical gas chromatogram of the constituents of extracts C-2.

Table 1. Chemical compositions of essential oil as well as different extracts of *Ocimum basilicum.*

Essential oils compounds	RT, min	Area, %
1, 4-diethyl benzene	8.261	1.03
1-methyl-3-(1-methylethyl) benzene	8.676	1.73
Champhor	10.339	0.56
Broneol	10.566	0.20
Napthalene	10.853	0.53
2-Methylbicycol[4.3.0]non-1(6)-ene	11.101	0.14
Isobornyl acetate	12.521	0.63
α-Cubene	12.789	3.85
Thujopsene	13.200	0.23
γ - Element	13.468	0.62
Eugenol	13.671	61.76
Vanillin	14.499	1.27
2 - Methoxy- 4 -(1-propenoyl) phenol	14.631	2.65
Benzoic acid, 2-hydroxy-, pentyl ester	14.815	0.20
9-Nonadecene	14.874	0.14
Diethyl Phthalate	14.933	0.15
[2-methyl-4-(1-propenyl) phenoxy] Silane	15.355	2.01
Benzyl Benzoate	17.085	0.26
Acetic acid, chloro-, octadecyl ester	17.117	0.12
Galaxolide 1	17.843	0.23
Hexadecanoic acid, methyl ester	18.490	2.51
Isopropyl palmitate	18.947	11.36
1-(+)-Ascorbic acid 2, 6-dihexadecanoate	19.151	0.10
2, 3-Dihydroxypropyl elaidate	20.190	5.10
Octadecanoic acid, methyl ester	20.407	0.57
Oleic Acid	20.828	0.15
Dibutylphthlate	22.983	1.90

Table 1 (contd.)

H-1 **Compounds**	RT, min	Area, %
Acetic anhydride	3.253	4.32
2-Pentanone	4.398	27.06
2-Decyn-1-ol	11.978	0.12
1,2-Dimethoxy-4-(2-propynel)-benzene	14.337	35.82
Cyclohexane methanol	16.690	0.30
Longipinocarvone	17.147	0.28
Caryophyllene oxide	17.287	4.64
12-Oxabicyclo(9.1.0)dodeca-3,7-diene	17.695	0.20
Aromadendrene oxide-(2)	18.520	1.16
Caryophyllene oxide	18.601	0.22
Tricyclo[5.2.2.0(1,6)]undecan-3-01,2-me	18.740	1.86
6-Isopropeny1-4,8a-dimethyl-1,2,3,5,6,7	18.834	0.47
Murolan-3,9(11)-diene-10-peroxy	19.668	0.36
1,3-Benzodioxide,4-methoxy-6-(2-prope)	19.994	0.59
3,7,11,15-Tetramethyl-2-hexadecen-1-0l	20.474	0.46
EA-1 **Compounds**		
4-Methyl-2-pentyl acetate	3.117	3.11
2-Pentanone	4.408	18.72
2-Decenal	11.990	0.05
2-Methoxy -3-(2-propenyl)-phenol	13.637	0.18
1,2-dimethoxy-4-(2-propenyl)benzene	14.346	53.06
Cyclohexane methanol	16.686	0.37
Caryophyllene oxide	16.810	0.19
Tricyclo[5.2.2.0(1,6)]undecan-3-0l	17.195	0.15
12-Oxabicyclo(9.1.0)dodeca-3,7-diene	17.695	0.15
Aromadendrene oxide-(2)	18.520	1.16
Caryophyllene oxide – 1	18.351	1.44
6-Isopropeny1-4,8a-dimethyl-1,2,3,5,6,7	19.053	0.25
Aristolene epoxide	19.661	0.22
10-Heneicosene	19.847	0.30
3,7,11,15-Tetramethyl-2-hexadecen-1-0l	21.167	0.19
1,2-Benzenedicarboxylic acid	22.897	0.41
Tetracontane	47.238	0.15
EA-2 **Compounds**		
1,4- Diethyl benzene	8.621	1.28
1-Methyl-3-(1-metrhylethyl)-benzene	8.737	5.11
2-Ethyl-1, 4-methyl benzene	9.186	0.85
1, 2, 3, 4-Tetramethybenzene	9.447	9.9
4-Ethyl-1, 2-dimethyl benzene	9.889	1.35
1, 2, 4, 5-Teramethyl benzene	10.084	3.18
Acetic acid phenylmethyl ester	10.303	1.70
Naphthalene	10.853	0.89
Isobornyl acetate	12.521	0.82
Eugenol	13.671	7.72
7-Tetadecene	14.067	0.81
Vanillin	14.499	2.27
1H-3a, 7-methanoazulene	14.742	1.14
Thujopsene	15.047	1.49
[2-methoxy-4-(1-propenyl)] phenoxy silane	15.355	1.91
9-Ethyl-3, 6-dimethoxy phenanthrene	15.868	1.34
10-Heneicosene (c, t)	19.899	2.16
Diethyl phthalate	22.983	2.07
1, 2-Benzene dicarboxylic acid	45.205	49.44

Table 1 (contd.)

C-1 Compounds	RT, min	Area, %
4-Methyl-2-pentyl acetate	3.113	7.48
2-Pentanone	4.139	0.44
2-Decen-1-ol	9.110	0.13
1,2-dimethoxy-4-(2-propenyl)benzene	14.334	29.69
Longipinocarvone	17.142	0.65
Tricyclo[5.2.2.0(1,6)]undecan-3-0l	17.195	0.15
Caryophyllene oxide	18.522	1.11
Ledene oxide	18.734	2.14
Phthalic acid,butyl hexyl ester	22.894	0.62
C-2 Compounds		
1,2-Benzenedicarbixylic acid, diisooctyl	5.134	3.49
N-Acetylpyrroliclone	10.197	0.74
Borneol	10.566	0.33
Eugenol	13.696	88.18
2-methoxy-4-(1-propenyl)- Phenol	14.531	0.44
2-methoxy-4-(1-propenyl)-, (E)- Phenol	15.233	1.46
2-methoxy-4-(1-propenyl) phenoxy Silene	15.344	0.50
6-methoxy-3-methylbenzofuran	15.766	0.59
Isoaromadendrene epoxide	22.123	0.37
Biphenyl-4,4'-dicarboxylic acid	44.530	1.78

RT= Retention time.

There are many essential oil constituents such as borneol, camphor, vanillin, napthalene, ledene oxide, caryophyllene oxide and eugenol. But extracted essential oil of this experiment contains only borneol, vanillin, napthalene, and eugenol. H-1 and EA-1 contains only caryophyllene oxide. EA-2 contains vanillin, napthalene, and eugenol. C-1 contains ledene oxide, caryophyllene oxide. C-2 contains borneol and eugenol.

Both H-1 and EA-1 contains 2-pentanone and 1, 2-dimethoxy-4-(2-propynel)-benzene where C-1 contains only 1, 2-dimethoxy-4-(2-propynel)-benzene. But H-1 contains more 2-pentanone (27.06%) and EA-1 contains more 1, 2-dimethoxy-4-(2-propynel)-benzene (53.06%). Essential oil, EA-2 and C-2 contains eugenol. But C-2 contains more (88.18%).

H-1, EA-1 and C-1 contain caryophyllene oxide but H-1 contains more caryophyllene oxide (4.64%) comparative to EA-1 and C-1.

4. Conclusion

The suitable extracts for respective compounds can be chosen on the basis of above GC-MS analysis: essential oil contains isopropyl palmitate (11.36 %) and eugenol (61.76 %); H-1 contains 2-pentanone (27.06 %) and caryophyllene oxide (4.64%); EA-1 contains more 1, 2-dimethoxy-4-(2-propynel)-benzene (53.06 %); EA-2 contains 1, 2-benzene dicarboxylic acid (49.44 %) and C-2 contains eugenol (88.18 %).

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