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Phytochemical Screening, Isolation, Characterization of Bioactive and Biological Activity of Bungkang, (Syzygium polyanthum) Root-bark Essential Oil

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Abstract

Bungkang (*Syzygium polyanthum*) is a medium to tall plant which produces medicinal root-bark, the plant is normally found along inland river bank and produces small white flowers and fruits. Essential oils are among the most interesting components of the plant extracts consisting mostly of monoterpenoid or sesquiterpenoids. They are used as therapeutic agents in ethno, conventional, and complementary alternative medicines. Investigation and evaluation of the essential oil of *Syzygium polyanthum* as well as the antibacterial, antioxidant and antifungal activity was ascertained. The experiment was performed. 100 chemical constituents were obtained and two pure compound was isolated as Eugenol **(1)** and Farnesol (**2**). Significant growth inhibition of *Staphylococcus aureus*, (ATCC©25923) *Klebsiellia pneumonia* (ATCC©19155), *Salmonella typhi* (ATCC©14028) and *Escherichia coli* (ATCC©.25922) and the fungal strains *Aspergillus flavin*, *Aspergillus niger*, *Candida, tropicalis,* and *Fusarium oxysporium* was observed from the essential oil at concentration of 500 µg/mL. Antioxidant potential was observed to be strong of 18.42 µg/mL when compared to the control of 15.23 µg/mL. The result indicated that the oil obtained from root-bark of *Syzygium polyanthum* can be considered as an agent for antioxidant, antibacterial and antifungal in pharmaceutical food and cosmetic industries trails.

Keywords: Phytochemical, Biological, Activity, Bungkang, Roots-bark, Essential Oil

Major classifications: Health science.

1. Introduction

Plant product was used by man for health care delivery for centuries. The use of plant extract as medicine is wide spread throughout the world. While, plant disease remedies are as old as human history, it was estimated that about 80% of useful bioactive plant derived pharmaceuticals are used around the world as was discovered by University academicians, researcher from the field of traditional herbal medicine (Diba et al., 2013).

Bungkang (Syzygium polyanthum) is a medium to tall plant which produces edible and aromatic leaves, the plant is normally found along inland river bank and produces small white flowers and fruits. It is well known for its small young leaves which are slightly fragrant and are used for flavouring food. The leaves, though commonly used by some communities in various countries to flavour many local dishes, with various medicinal properties. It was reported that Bungkang, (Syzygium polyanthum) Leaf increases the haemoglobin level in rat model with iron-deficiency anaemia (Adyani et al., 2018)

It was also reported in the study of inventory and biodiversity of medicinal Plants from tropical rain forest based on traditional knowledge by ethnic Dayaknese communities in West Kalimantan Indonesia of the potency of stem-bark of Syzygium polyanthum traditionally used for stomach ache (Diba et al., 2013).

Based on the report of Ismail &Ahmad. (2019), the roots are consumed to reverse the hangover effect from alcohol. However, as far as our literature survey could ascertain, no studies have been carried out with the S. polyanthum root-bark extract. The biological activities of these plants part (Root-bark) have not been studied extensively although, Asian people have been consuming it as medicine for a long time (Ismail & Ahmad, 2019).

Essential oils are among the most interesting components of the plant extracts consisting mostly of monoterpenoid or sesquiterpenoids. They are used as therapeutic agents in ethno, conventional, and complementary alternative medicines particularly as analgesic, anti-inflammatory, antispasmodic, local anaesthetic, anthelmintic, antipruritic, and antiseptic as well as many other therapeutic uses and disease control (Umaru et al., 2018).

Several line of studies have also reported that essential oils are used broadly in medicine and cosmeceutical and pharmaceutical industries and as flavouring agents and preservatives in food industry and design (Umaru et al., 2019)

Hence, this study was conducted to extract, Isolate, characterize and verify the potentials of essential oil against pathogen, antioxidant and cytotoxicity of locally grown Bungkang, (Syzygium polyanthum) in Sarawak, Malaysia.

Figure 1: Bungkang, (Syzygium polyanthum) natural habitat

2. Material and Methods

2.1. Reagents and Chemicals

The standard antibacterial agents, tetracycline (30µg), and antifungal standard Fluconazole (30µg), susceptibility discs and Nutrient agar (CM0003) were obtained from Oxoid Ltd, Wade Road, Basingstoke, Hants, RG2 8PW, UK.

2.2. Sample collection

Fresh root-bark of Syzygium polyanthum was collected from the Kampong Singai Sarawak by the river side in Bau. Identification of the species was made by a botanist. The sample was then deposited in the Herbarium Faculty of Resource Science and Technology, Universiti Malaysia Sarawak.

2.3. Isolation and Purification

Isolation and Purification of secondary Metabolites of the essential oil of Bungkang, (Syzygium polyanthum) root-

bark was carried out using chromatographic procedure namely column chromatography (CC), thin layer chromatography (TLC) plate as a medium for visual identification. Procedure adopted was reported by Umaru et al. (2019).

2.3.1. Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) was carried out using aluminium plate of 20 x 20 cm coated with silica gel 60 F254 (Merck 1.05554.0001) and R_f value of each spot was determined (Umaru et al., 2019).

2.3.2 Column Chromatography (CC)

The essential oil extract was examined on TLC plate in different solvent ratio; hexane, hexane-dichloromethane, dichloromethane, dichloromethane-chloroform, chloroform, chloroform - ethyl acetate, ethyl acetate, ethyl acetate–methanol and methanol.

The column was eluted using suitable solvent systems with increasing polarity (Fasihuddin et al., 2010). The column's valve was then opened and about 10-20 mL fraction of the eluate was collected in test tubes. The procedure was repeated using different solvent systems. Samples collected from the column were examined by using Thin Layer Chromatography (TLC) plates. Fractions with similar R_f values were combined. Fraction with single component spot (one spot) that appeared on TLC plate was treated as possible pure secondary metabolite (Umaru et al., 2019).

2.4. Chemical Structure Elucidation

The identification of the isolated secondary metabolite was made by spectroscopy method namely Gas Chromatography-Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance (NMR) and Fourier Transform Infra-Red spectrometry (FTIR) as described by Fasihuddin et al. (2010). The elucidation of chemical structure for the extracted secondary metabolite was made based on the data obtained from various spectroscopy methods and also comparison with published information.

2.4.1. Gas Chromatography-Mass Spectrometry (GC-MS)

The single spot obtained in TLC was further analysed by GC-MS (model Clarus 680) to obtain molecular mass of pure compounds according to mass per charge (m/z) ratio as described by Umaru et al. (2019).

Isolated compound was matched with the retention times with those of authentic compounds information, and identification with obtained mass spectral from library data of the corresponding compounds (Kalaiselvan et al., 2012).

2.4.2. Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectrometry was performed by using JEOL JNM-ECA 500 Spectrometer, based on the method as described by Umaru et al., (2019), Efdi et al., (2010) and Danelutte et al., (2003). Identification of the type of each ^{1}H-NMR and ^{13}C-NMR detected was based on the Table of characteristic NMR absorptions published in Organic Chemistry (Silverstein, 2005) and with the guide of the possible proposed structure given by NIST library.

2.5 Melting Point

The melting point of the compounds isolated was determined as reported by Umaru et al. (2019) using a melting point apparatus (Stuat model SMP3). The heating process was monitored and the temperature at which the sample begins to melt and completely melted was recorded.

3. Test Organisms

Bacterial strains Staphylococcus aureus, (ATCC©25923) Klebsiellia pneumonia (ATCC©19155), Salmonella typhi (ATCC©14028) and Escherichia coli (ATCC©.25922) and the fungal strains Aspergillus flavin, Aspergillus niger, Candida, tropicalis, and Fusarium oxysporium were selected for the study. The bacterial and Fungal strains were obtained from the Microbiology Laboratory, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, and were used for the antibacterial and antifungal activities. The stock cultures were incubated at 37 ^{o}C for 24 hrs on potato dextrose agar (PDA). (Microcare Laboratory, Surat, India), and was stored at 4 ^{o}C. Plates containing Mueller-Hinton Agar (MHA) and Nutrient Agar were used to grow the bacterial and the fungal strains at 37 ^{o}C. The stock cultures were then kept at 4 ^{o}C until use.

4. Essential Oil

Fresh root-bark of Syzygium polyanthum was collected washed with distilled water, cut into smaller pieces and subjected to hydro distillation for 8 hours using Clevenger apparatus to obtain the essential oil quantitatively. According to the method reported by Umaru et al. (2019). 100 g of the Syzygium polyanthum root-bark was weighed and transferred into 2 litre round flask with 1.35 litre of distilled water. The flask was assembled to the Clevenger trap, connected to the condenser and heated for 8 hours using hydro distillation process. After 8 hours, the collected oil was allowed to cool at (28°c) room temperature. The water of the oil was first drained to separate from the oil. The oily sample was treated with anhydrous sodium sulphate (Na_2SO_4) to remove the remaining trace water. The experiment was performed in triplicates for each sample and the yield was averaged. The essential oil was kept in a vial and then stored in refrigerator at 4°C prior to further analysis. The percentage yield of the oil was calculated based on the formula as reported by Costa et al. (2014).

$$\text{Percentage (\%) yield} = \frac{\text{Weight of the extracted (g)}}{\text{Dried weight of the sample (g)}}$$

4.1 GC-MS Analysis of Essential Oil

The essential oil was characterized by chromatography method. The oil was first analysed on a gas chromatograph. The prepared oil sample exactly 1µL was injected into the GC column. The chemical constituents of the oil sample were identified based on the library obtained from GC-MS.

5. Cytotoxicity Assay of essential oil

5.1 Hatching of Brine Shrimp

1.5 g of Artemia salina cysts was placed in one side in 1 L capacity glass container (hatching chamber) containing seawater (collected from Damai beach in Kuching-Sarawak) and aerated, air pump was fitted to the water in the container to ensure complete aeration of the cysts and fluorescence lamp was placed at the other side of the chamber. After 48 hours of incubation at room temperature (29°C), the newly hatched free swimming nauplii were harvested from the glass container and used for the bioassay as reported by Umaru et al., (2018).

5.2 Preparation of Test Samples

Preparation of the test sample as reported by McLaughlin et al. (1998), alternative dilution procedure was taken in the preparations of the essential oil concentrations. 20 mg of the essential oil was dissolved in 2 ml methanol and from this solution, 5, 25, 50, 250 and 500 µl were transferred into pre-marked vials to give a lower series of chosen concentration of 1, 5, 10, 50 and 100 ppm, respectively. The preparation was left overnight for the solvent to evaporate. 0.2 ml DMSO and about 4 ml of seawater were added to each pre-marked vial in triplicates where10 brine shrimps were carefully added with micropipette and introduced into each vial. The mixture was adjusted to a final volume of 5 ml with seawater. Thymol was used as the control where seawater was added in a different set of 3 pre-marked vials and 10 brine shrimps were carefully introduced into each microplate, If the brine shrimp in these microplates show a rapid mortality rate, then the test is considered invalid as the nauplii might have died due to some reasons other than the cytotoxicity of the essential oil. The numbers of surviving nauplii were then counted with an aid of a hand lens and recorded to ascertain the toxicity of the essential oil (Umaru et al., 2018).

6. Antioxidant Assay of Essential oil (DPPH (2,2-diphenyl-1-picryl-hydrazyl)

The free radical scavenging assay of compound 2,2-diphenyl-1-pycryl-hydrazyl (DPPH) was used to evaluate the antioxidant properties of the essential oil. The measurement was based on the method described by Wang et al. (2008). The sample was prepared by diluting 5 mg of the essential oil into 5 mL of methanol, producing a concentration of 1000 µg/mL. The stock solution was sonicated to ensure the homogeneity of the sample. Three other concentrations were prepared at 10, 50 and 100 µg/mL, diluted from the 1000 µg/mL stock solution. Sample of 5000 µg/mL was prepared separately by diluting 25 mg of the essential oil into 5 mL of methanol.

Approximately 3 mL of 0.1 mM solution of 2,2-diphenyl-1-pycrylhydrazyl (DPPH) in methanol was each added into five series of prepared concentrations (10, 50, 100, 1000 and 5000 µg/mL) of sample solutions (1 mL). Analysis was done in triplicate. The solution was mixed vigorously and left to stand at room temperature for 30 minutes in the dark after which its absorbance was measured spectrophotometrically at 517 nm using Jasco ultra violet spectrophotometer model V-630. Methanol was used as blank (only methanol) and negative control (1 mL methanol mixed with 3 mL DPPH), while ascorbic acid (vitamin C) as the standard. The concentration of the sample required to inhibit 50% of the DPPH free radical was calculated as IC_{50} and the value was determined using Log dose inhibition curve which performed by using PRISM version 3.02 software (Tailor & Goyal, 2014).

7. Antibacterial Assay of Essential oil.

Antibacterial activity of root-bark essential oil Syzygium polyanthum was determined against four pathogenic bacterial strains Staphylococcus aureus, (ATCC©25923) Klebsiellia pneumonia (ATCC©19155), Salmonella typhi (ATCC©14028) and Escherichia coli (ATCC©.25922) using disk diffusion method as reported by various authors (Boyan et al., 2005; Prashanth et al., 2006). The oil was dissolved using methanol and sterilized by filtration and stored at 4 ºC until use. Standard antibiotics (tetracycline) was used for comparison of the zone of inhibition of the pure strains of the bacteria. The oil was then screened for their antibacterial activity against the bacterial strains. Set of five dilutions for antibacterial activity (25, 50, 100, 250, 500 µg/mL) of the root-bark of Syzygium polyanthum and sterile plates containing Mueller-Hinton agar were seeded with indicator bacterial strains and control experiment using tetracycline as standard drug kept for 3 hrs. at 37 ºC. They were then incubated for 18 to 24 hrs. at 37 ºC, and the zones of growth inhibition around the disks were measured in mm. The antibacterial activity of the test organisms on the plant extracts were determined by measuring the diameter of the inhibitory zones on the surface of the agar around the disk, and the values < 9 mm were considered as not active against the microorganism for antibacterial activity (Prashanth et al., 2006). The experiment was carried out in triplicate and the mean values of the diameter of zones of inhibition was calculated using statistical software SPSS 22.

8. Antifungal Assay of essential oil

The antifungal activities of the root-bark essential oil Syzygium polyanthum at varying concentration (25, 50, 100, 250 and 500 µg/mL) prepared in methanol was performed against a standard drug fluconazole (positive control) using standard paper dilution method as described by Aboh et al. (2014). The strains were maintained in culture medium of potato dextrose agar (PDA). Fresh cultures of the Aspergillus flavin, Aspergillus niger, Candida, tropicalis, and Fusarium oxysporium were grown in sterilized potato dextrose broth (PDB) and incubated at 25 ºC for 24 hrs. The cultures were then diluted with PDB until use. The antifungal activity of the test organisms on the oil were determined by measuring the diameter of the inhibitory zones on the surface of the agar around the disk, and the values < 16 mm were considered as active against the microorganism for antifungal activity (Aboh et al., 2014). The experiment was carried out in triplicate and the mean values of the diameter of zones of inhibition was calculated using statistical software SPSS 22.

9. Result and Discussion

9.1. Essential oil constituent

The essential oil constituents of the root-bark of Syzygium polyanthum is shown in Table 1 and Figure 1, the essential oils consist monoterpene, oxygenated monoterpene, sesquiterpene, oxygenated sesquiterpene and the total number of constituent obtained in root-bark are 96 phytochemicals as reported in the gas chromatography chromatogram of which two compound were isolated and characterised.

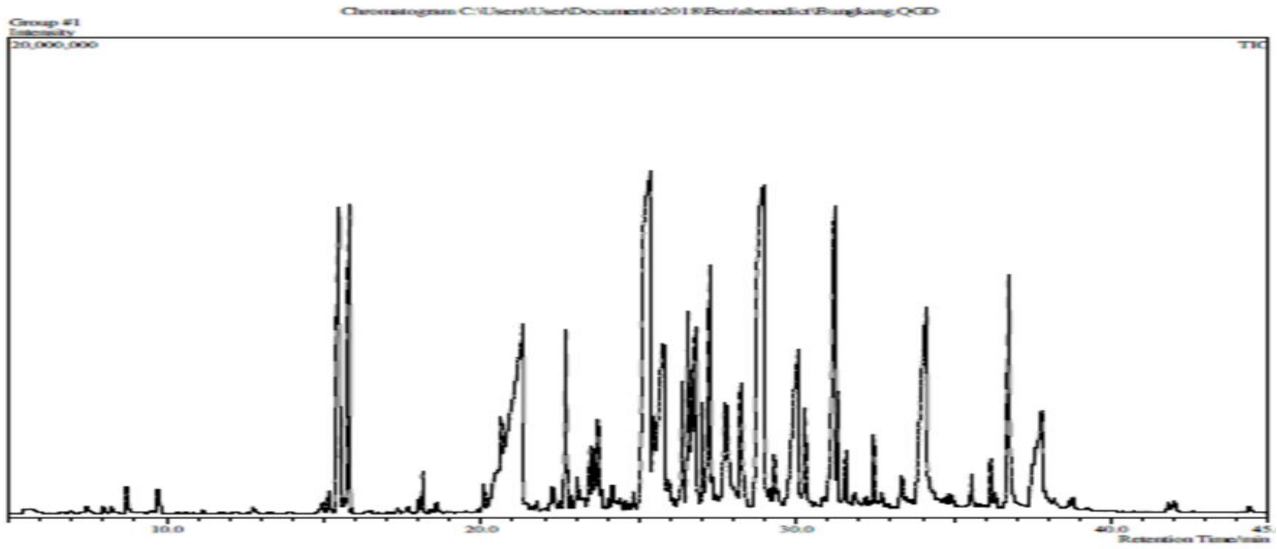


Figure 2: Chromatogram of Bungkang, (Syzygium polyanthum) essential oil

Table 1: chemical constituents of Bungkang, (Syzygium polyanthum) essential oil.

Peak Report TIC

Peak#	R.Time	Area	Height	Name
1	5.510	1587736	160768	Cyclopentanol, 3-methyl-
2	5.669	848668	168320	(R)-(+)-3-Methylcyclopentanone
3	5.760	1048077	136044	Hexane, 2,3,4-trimethyl-
4	6.969	708535	108719	2-Furanmethanol, tetrahydro-5-methyl-
5	7.470	1595017	244883	2-Ethoxyethyl 3-methylbutanoate
6	7.981	1716833	307253	Pentanoic acid, 2-propenyl ester
7	8.252	1423587	285234	1,2,6-Hexanetriol
8	8.736	5675858	1082006	3,3-Diethoxy-1-propyne
9	9.480	458685	76214	Cyclopropane, 1,1,2,2-tetramethyl-
10	9.545	406861	93770	3,3-Diethoxy-1-propyne
11	9.718	6405906	1010420	Octanal
12	10.588	200387	55470	Eucalyptol
13	10.675	148619	35028	1-Hexanol, 4-methyl-, (S)-
14	10.840	62369	24064	1-Hexanol, 4-methyl-, (S)-
15	11.147	624355	146313	2-Octene, 2-methyl-6-methylene-
16	11.705	193652	36925	Sulfurous acid, isobutyl 2-pentyl ester
17	11.823	321246	66047	2-Furancarboxylic acid, tetrahydro-3-methyl-
18	12.020	77719	26744	Bicyclo[3.1.1]heptan-endo-6-ol, syn-7-brom
19	12.268	308918	40631	3,6-Dimethyl-1-heptyn-3-ol
20	12.570	85835	21360	Linalool
21	12.742	1387653	268491	Nonanal
22	12.870	171053	52827	(E)-4,8-Dimethylnona-1,3,7-triene
23	13.242	193682	58499	Decane, 5,6-dimethyl-
24	13.348	174094	54486	Decane, 5,6-dimethyl-
25	14.975	2650723	437902	Octanoic acid
26	15.152	4328512	891361	Terpinen-4-ol

27	15.483	89485574	12878076	4-Decenal, (E)-
28	15.825	85231672	12999834	Decanal
29	16.322	436371	85698	3-Cyclohexene-1-carboxaldehyde, 1,3,4-trim
30	16.419	449905	110174	1-Butanol, 2,3-dimethyl-
31	16.527	440839	117936	trans-2-Pinanol
32	16.744	156775	41921	2,3-Octanedione
33	16.830	53667	19059	Acrylic acid, (5-cyclopropylidenepentyl) este
34	17.011	313807	73356	3-Isopropyl-5-methylhexan-2-one
35	17.137	300815	69185	3-Isopropyl-5-methylhexan-2-one
36	17.335	1189912	210597	Oxirane, decyl-
37	17.600	671115	104227	1-Heptanol, 6-methyl-
38	17.688	1348261	299084	Oxirane, (7-octenyl)-
39	18.006	3069575	578155	trans-Ascaridol glycol
40	18.140	5677676	1751505	2-Undecanone
41	18.397	815045	160793	Oxirane, decyl-
42	18.589	3057272	443741	Cyclohexanol, 2-(1-methylpropyl)-
43	18.750	180702	41208	Fumaric acid, cis-hex-3-enyl pentyl ester
44	18.914	427591	79053	Methyl 6-methyl heptanoate
45	19.029	399189	65128	2,4-Decadienal
46	19.260	574191	53568	1,3-Dioxolane, 2,2-dimethyl-4,5-di-1-propen
47	19.509	551900	98577	Cyclopentane, 1,2-dipropyl-
48	19.620	159803	41073	Benzaldehyde, 3-ethyl-
49	19.870	771472	166283	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)
50	20.088	5377975	1212803	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimeth
51	21.314	250206578	7926874	n-Decanoic acid
52	21.776	1813861	390192	(3S,6R)-3-Hydroperoxy-3-methyl-6-(prop-1-
53	22.275	12620147	1044353	Nerolidol
54	22.695	41041408	7372254	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl
55	23.058	17024474	1391937	1-Heptatriacotanol
56	23.472	15948913	2670443	2-Tridecanone
57	23.720	32530295	3698733	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,
58	24.171	17461398	1128139	(1S,2S,4S)-Trihydroxy-p-menthane
59	24.600	3202339	486095	Cyclohexene, 4-[(1E)-1,5-dimethyl-1,4-hexa
60	24.832	4495179	817759	9,12-Tetradecadien-1-ol, (Z,E)-
61	25.363	240776540	14176855	Nerolidyl acetate
62	25.774	135754157	7058889	Dodecanoic acid
63	26.397	27559711	5361128	Cyclohexene, 4-[(1E)-1,5-dimethyl-1,4-hexa
64	26.576	40983653	8199388	(3E,7E)-1,5,5,8-Tetramethylcycloundeca-3,7
65	26.822	84099050	7735965	Apiol
66	27.031	17320123	4109728	(1aR,4S,4aR,7R,7aS,7bS)-1,1,4,7-Tetrameth
67	27.267	69861202	10075703	Caryophyllene oxide
68	27.738	65615526	4199273	2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H
69	28.255	42407646	5315524	.alpha.-Bisabolol
70	28.963	227807472	13721905	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-
71	29.308	16848895	2090763	2,6,10-Dodecatrienal, 3,7,11-trimethyl-, (E,E

72	30.060	94249581	6589747	Tetradecanoic acid
73	30.280	29471611	4240306	Benzyl Benzoate
74	30.860	9351249	612620	13-Methyltetradecanal
75	31.238	126755988	12804858	Farnesol, acetate
76	31.590	18168557	2327193	6-Octadecenoic acid
77	31.875	8061562	772502	Dibutyl phthalate
78	32.187	6142402	542993	Pentadecanal-
79	32.466	16486037	2930536	Benzoic acid, 2-hydroxy-, phenylmethyl este
80	32.640	9748000	439284	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10
81	33.361	16411692	1340682	Isophytol
82	33.650	4352410	497788	trans-Z-.alpha.-Bisabolene epoxide
83	34.138	149180604	8419178	n-Hexadecanoic acid
84	34.968	3063292	400117	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10
85	35.574	11636086	1493300	geranyl-.alpha.-terpinene
86	35.810	5548680	313464	n-Hexadecanoic acid
87	36.185	15698122	2010702	Bergamotol, Z-.alpha.-trans-
88	36.753	70311456	9617444	Phytol
89	37.794	84109602	4128124	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
90	38.725	7747998	291976	1-Hexacosene
91	39.265	1623425	155022	Phytol, acetate
92	41.832	2804550	368244	2-Dodecen-1-yl(-)succinic anhydride
93	42.010	2968170	425723	Eicosane
94	42.596	381191	42774	Z-28-Heptatriaconten-2-one
95	44.151	411647	43623	Ferruginol
96	44.396	2181233	2517764-olide	4,8,12,16-Tetramethylheptadecan-
		2296189396	207622689	

Table 2: Average death of brine shrimp (Artimia salina) at different concentration of root-bark essential oil of Syzygium polyanthum

Solvent system	Average death of Artemia salina Concentration (µg/mL)						LC_{50} (µg/mL)
	1	10	25	50	100	500	
Control	0	0	0	0	0	0	-
Thymol	5±0.57	7±0.58	10±0.00	10±0.00	10±0.00	10±0.00	1.16
Essential oil	4.33±1.15	4.77±0.58	8.70±2.31	9.33±1.15	10.0±0.00	10.00±0.00	18.53

The result is Mean±SD. N = 30

Table 3: IC_{50} value of fresh leaves essential oil of Syzygium polyanthum

Plant parts	Syzygium polyanthum IC_{50} (µg/mL)
Fresh root-bark essential oil	18.42
Ascorbic acid standard	15.23

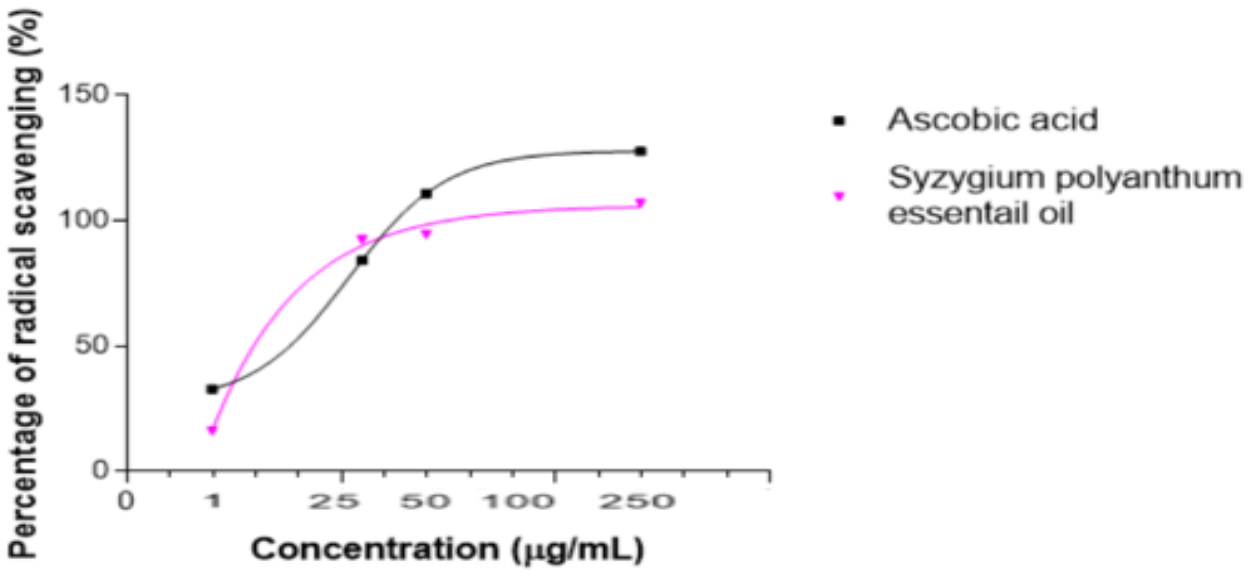


Figure 3: Radical scavenging activities of root-bark Syzygium polyanthum at absorbance of 517 nm.

Table 4: Antibacterial of root-barkeEssential oil of Syzygium polyanthum

Organism	25	50	100	250	500
Tetracycline (control)	19.77± 0.38	19.77±0.38	19.77± 0.38	19.77± 0.38	19.77± 0.38
Salmonella typhi	8.63 ± 0.08	9.70± 0.02	11.73 ± 0.06	14.84 ± 0.02	14.34 ± 0.22
Escherichia coli	8.43 ± 0.13	18.60±0.06	19.62 ± 0.06	23.72±0.07*	15.82 ± 0.02
Staphylococcus aureus	11.73±0.06a	13.93±0.14	16.56 ± 0.06	16.02 ± 0.04	18.13±0.11a
Klebsiella Pneumonia	11.60 ± 0.12	11.73±0.16	13.80±0.10a	12.85 ± 0.06	14.95±0.08a

Table 5: Antifungal activity of root-bark essential oil of Syzygium polyanthum

Organism	25	50	100	250	500
Fluconazole (control)	24.67±0.11	24.67±0.11	24.67±0.11	24.67±0.11	24.67±0.11
Aspergillus flavin,	9.48±0.17ad	10.25±0.27a	10.97±0.03a	14.07±0.08a	19.37±0.05
Aspergillus niger	9.67±0.03	12.04±0.12	15.11±0.04	15.21±0.03	19.78±0.04
Candida, tropicalis	14.11±0.06	14.66±0.03	16.43±0.10	18.24±0.01	20.55±0.08
Fusarium oxysporium	10.13±0.02	13.23±0.11	15.11±0.11	16.78±0.13	18.36±0.11

9.2. Purification and Elucidation of Compound 1

The GC-MS analysis of the combined fraction from the column chromatography an TLC was subjected to GC analysis and the result from the GC showed a single peak at the retention time of 36.11 min. this confirmed a pure compound and named as Compound 1.

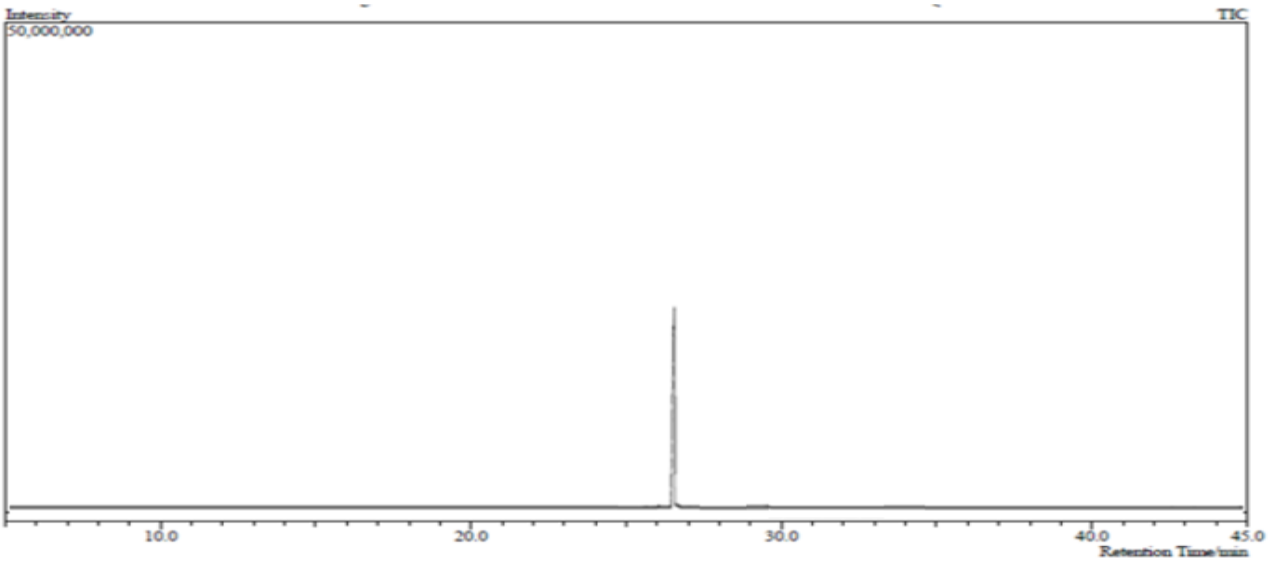


Figure 4: Gas chromatogram of Compound 1

9.2.1. Structural Elucidation

The compound 1 was isolated from essential oil of Syzygium polyanthum root-bark, yellow in colour and a melting point of 10.11°C with molecular formula $C_{10}H_{12}O_2$. The NMR analysis was performed to elucidate the chemical structure of the Compound 1 through the chemical shift of every proton the Compound 1 as reported in Table 6 (^{1}H-NMR) and Table 7 (^{13}C-NMR) as while on Figure 5 and Figure 6. Based on the table in Organic Chemistry by Silverstein et al., (2005), the proton signals were all integrated and were assigned to every proton NMR of Compound 1 as the proposed chemical structure.

^{1}H-NMR of Compound 1 exhibited 9 proton resonates, a signal was observed at δ 3.83 (3H, m, J=2.99) as methyl (CH3) group attached oxygen group of Compound 1 and was assigned to H-4. An ethylene (CH_2) group was observed at δ 5.08 (2H, t, J=2.02) and was assigned to H-9. A singlet was observed at δ 6.92, δ 6.20, δ 6.85 and δ 6.70, they were identified as the methine group of the compound ring and were assigned to H-5 (1H, s, J=1.00), H-8 (1H, m, J=0.93), H-10 (1H, d, J=1.02) and H-11(1H, d, J=1.02). A signal was observed alternate to the oxygen group as shown in Figure 5, attached to carbon one of the compound identified as the only hydroxyl group of the compound and was assigned to H-12 (1H, s, J=1.00)

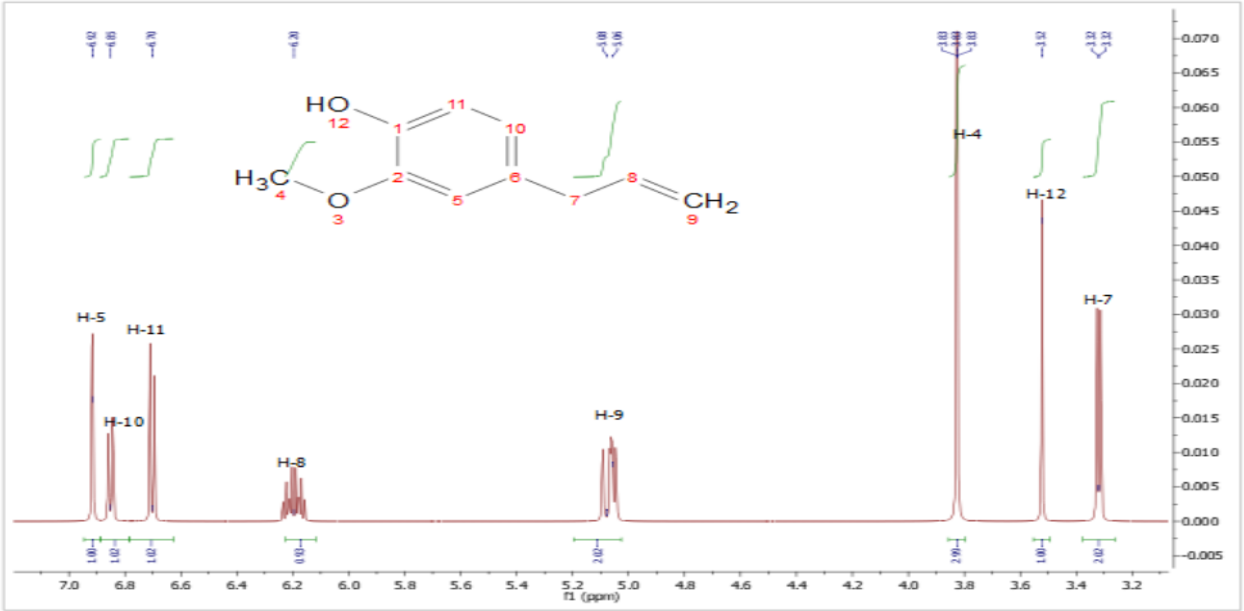


Figure 5: ^{1}H-NMR spectrum of Compound 1 from δ 3.2 to δ 7.0 (500 MHz, $CDCl_3$)

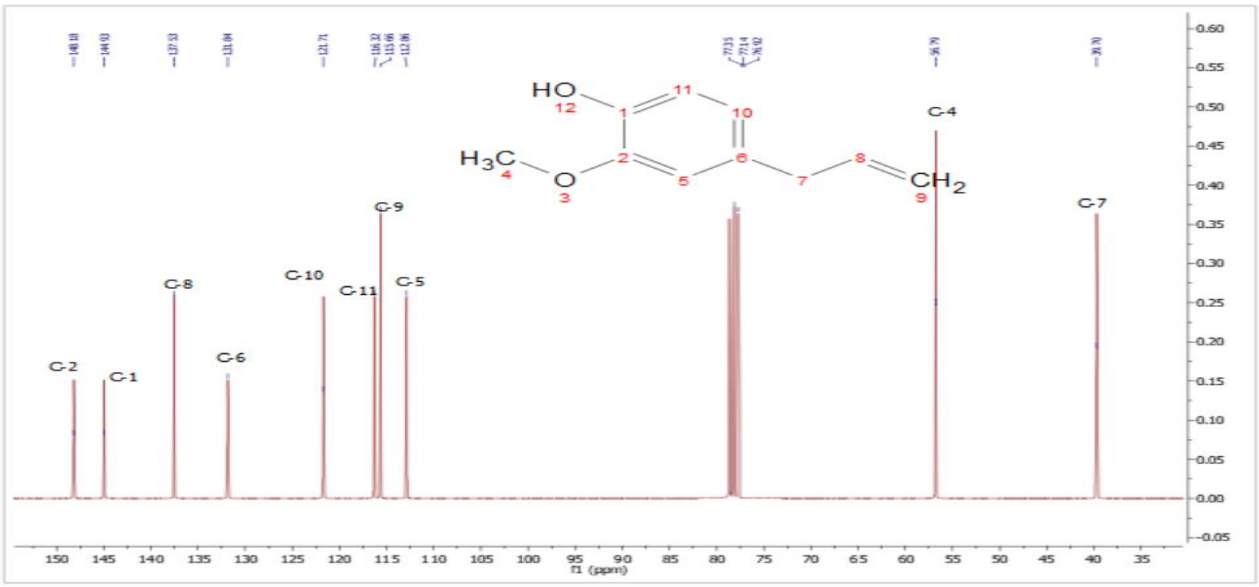


Figure 6: ^{13}C-NMR spectrum of Compound 1 from δ 25 to δ 150 (125 MHz, $CDCl_3$)

^{13}C-NMR spectrum showed a total of 10 signals. Eight signal was observed at down field with a chemical shift at δ 144.93, δ 148.18, δ 112.86, δ 131.84, δ 137.53, δ 115.66, δ 121.71 and δ 116.32, they were assigned to C-1, C-2, C-5, C-6, C-8, C-9, C-10 and C-11. At the up field two signals was observed at δ 56.74 and δ 39.70, they were all assigned to C-5 and C-7.

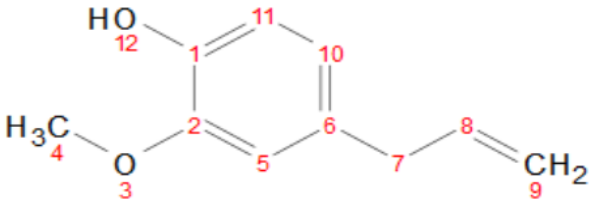
Table 6: Proton NMR signal of compound 1 and that reported by Nova. (2006)

Proton assigned to Compound 1	Proton chemical shift (ppm) of Compound 1	Proton assigned to Eugenol (Nova, 2006)	Proton chemical Eugenol (Nova, 2006)
H-4	3.83 (3H, m, J=2.99)	H-4	3.81
H-5	6.92 (1H, s, J=1.00)	H-5	6.82
H-7	3.32 (2H, t, J=2.02)	H-7	3.29
H-8	6.20 (1H, t, J=0.93)	H-8	5.91
H-9	5.08(2H, t, J=2.02)	H-9	5.06
H-10	6.85 (1H, d, J=1.02)	H-10	6.67
H-11	6.70 (1H, d, J=1.02)	H-11	6.82
H-12	3.52 (1H, s, J=1.00)	H-12	5.53

Table 7: Carbon NMR signal of Compound 1 and that reported by Fujisawa et al. (1988).

Carbon assigned to Compound 1	Carbon chemical shift (ppm) of Compound 1	Carbon assigned to Eugenol (Fujisawa et al., 1988).	Carbon chemical shift (ppm) of Eugenol (Fujisawa et al., 1988).
C-1	144.93	C-1	150.30
C-2	148.18	C-2	145.90
C-4	56.74	C-4	58.90
C-5	112.86	C-5	116.00
C-6	131.84	C-6	136.20
C-7	39.70	C-7	41.80
C-8	137.53	C-8	141.40
C-9	115.66	C-9	118.20
C-10	121.71	C-10	124.10
C-11	116.32	C-11	118.50

The chemical shift of every proton NMR and carbon NMR for Compound 1 correspond to one methyl, two ethylene and four methine as shown in Table 6 and Table 7 and in comparison with the NMR data reported by Fujisawa et al. (1988).



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9.3. Purification and Elucidation of Compound 2

The GC-MS analysis of the combined fraction from the column chromatography and the TLC result of one spot was subjected to GC analysis and the result from the GC showed a single peak at the retention time of 15.5 min. this confirmed a pure compound and its named as Compound 2.

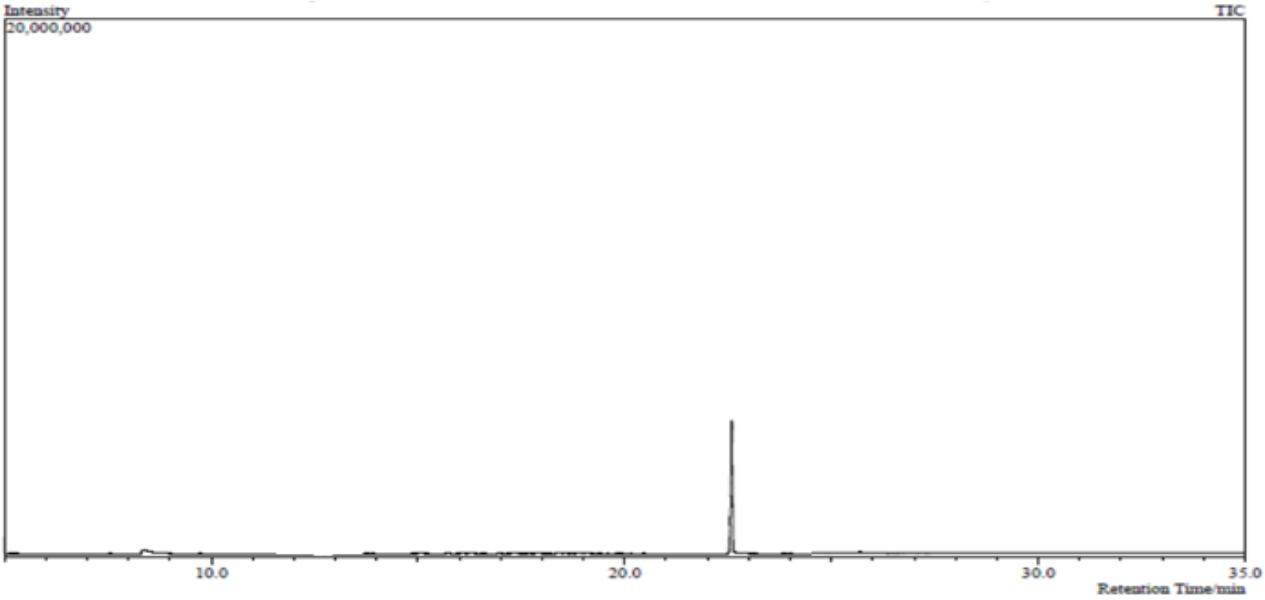


Figure 7: Gas chromatogram of Compound 2

9.3.1. Structural Elucidation

Compound 2 was isolated from essential oil of Syzygium polyanthum root-bark, as a yellow colour compound with a melting point of 25 ^{o}C of molecular formula of $C_{15}H_{26}O$ and molecular weight of 222 mg.

NMR analysis of Compound 2 was performed to elucidate the chemical structure. The result in Figure 8 and Figure 10 represent the ^{1}H-NMR spectrum and ^{13}C-NMR of the proposed structure of Compound 2. The proton NMR of the compound were integrated and assigned to the proposed chemical structure which is based on the table of ^{1}H-NMR characteristics absorption and ^{1}H-NMR peaks splitting pattern reported in Organic Chemistry by Silverstein (2005).

The ^{1}H-NMR spectrum of Compound 2 exhibited 13 proton resonates of characteristic of aliphatic carboxylic alcohol at region between δ 0.0 to δ 2.10. The spectrum indicated the presence of a branch chain of four methyl protons at δ 1.84, δ1.87, 1.81 and 1.81. they were assigned to H-4, H-9, H-14 and H-15. Four proton of long chain of ethylene was observed on the spectrum and as on the chemical structure at δ 4.18, δ 2.03, δ 2.02, and δ 2.02 respectively. they were assigned to H-1, H-5, H-6 and H-11 as shown in Table 7 and Figure 8 and Figure 9. Three proton was observed at δ 5.31, δ 5.29 and δ 5.25, they were identified as methine group of Compound 2. They were assigned to H-2, H-7 and H-12. At a chemical shift of δ 0.54 (1H, s) a singlet proton was observed which was identified as a demonstrative of an OH of the compound 2 and was assigned to H-16.

The ^{13}C-NMR spectrum of Compound 2 indicated the presence of 11 carbon resonates in the chemical structure. A recognizable signal was observed at a chemical shift of δ 24.85 which indicated the presence of terminal methyl carbon attached to a methane carbon and is therefore assigned to terminal carbon C-15. Another methyl was observed at the

long chain of the compound of the carbon resonate at δ 17.99, δ 16.53, and δ 17.99 at up field and was assigned to C-4, C-9, and C-14 as shown Table 7 and Figure 10.

A methylene carbon resonate was observed at δ 59.34, δ 30.87, δ 22.28, and δ 26.97, respectively. They were assigned to C-1, C-5, C-6, and C-1 which indicated the presence of alpha and beta carbon. A long chain peak signal observed at δ 125.09, δ 125.33 and δ 124.51 which contain alpha and beta carbon were identified as a long chain of methine carbons was observed at δ 130.94 of the structure, and assigned to C-13 as shown Figure 10 and Table 7.

The chemical shift of every ^{1}H-NMR and ^{13}C-NMR for Compound 2 is shown in Table 6 and Table 7. The comparison was made with ^{1}H-NMR and ^{13}C-NMR data of similar compound reported by Ishaq et al. (1985)

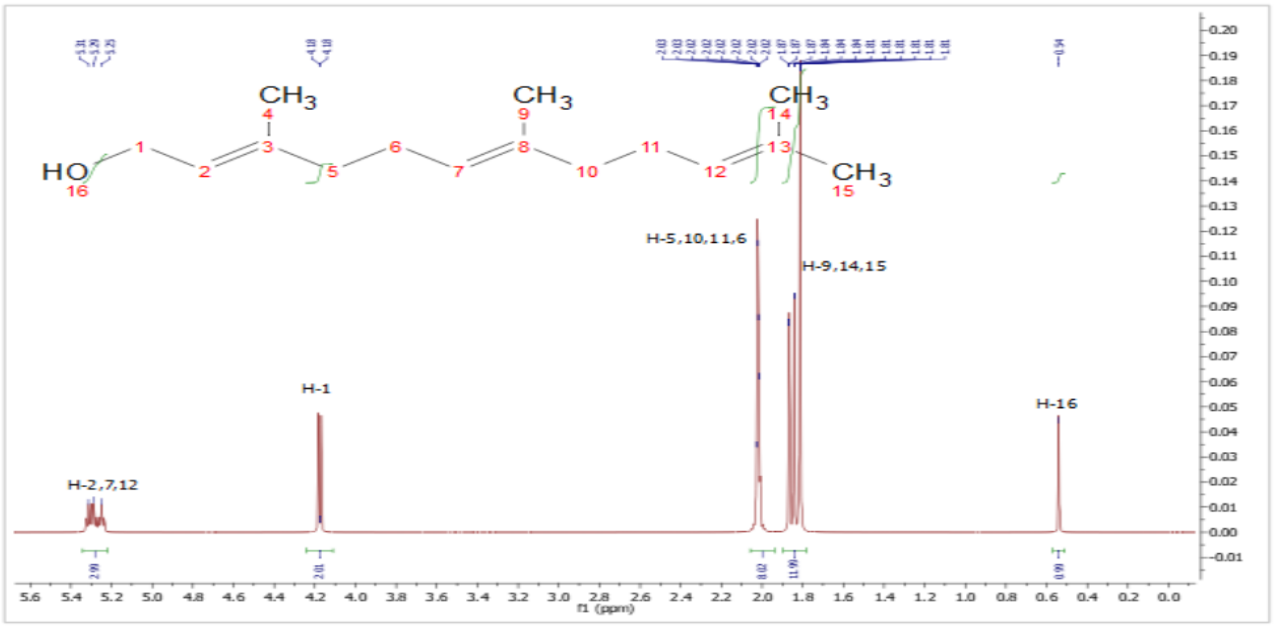


Figure 8: ^{1}H-NMR spectrum of Compound 2 from δ 0.0 to δ 5.6 (500 MHz, $CDCl_3$)

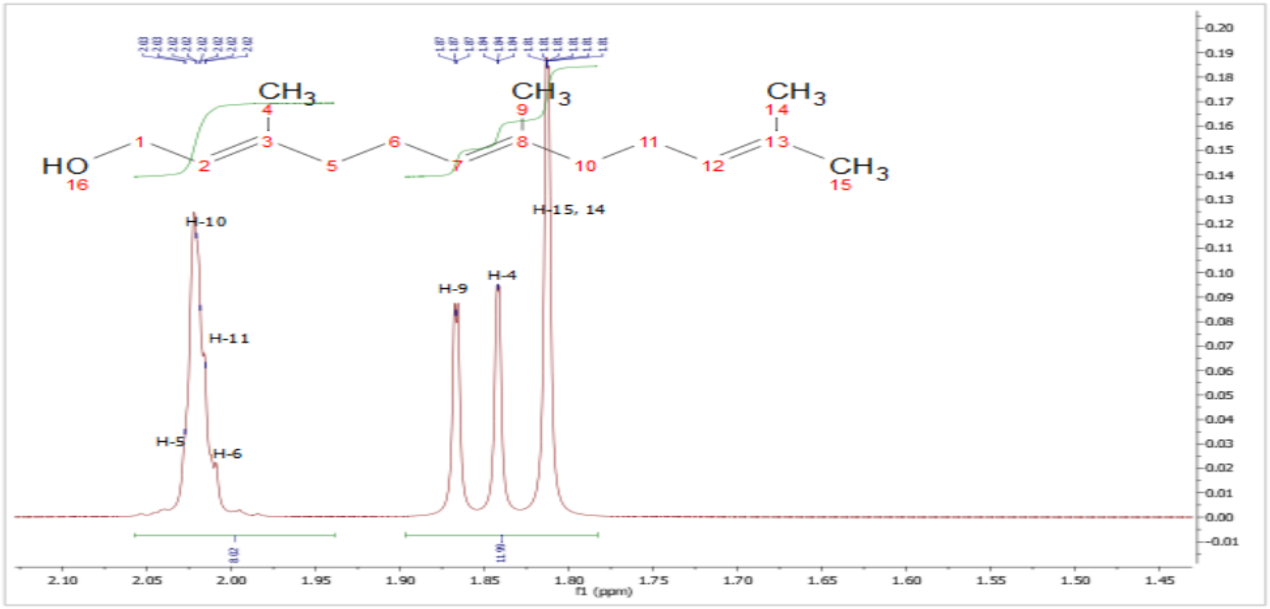


Figure 9: ^{1}H-NMR spectrum of Compound 2 from δ 0.0 to δ 5.6 (500 MHz, $CDCl_3$)

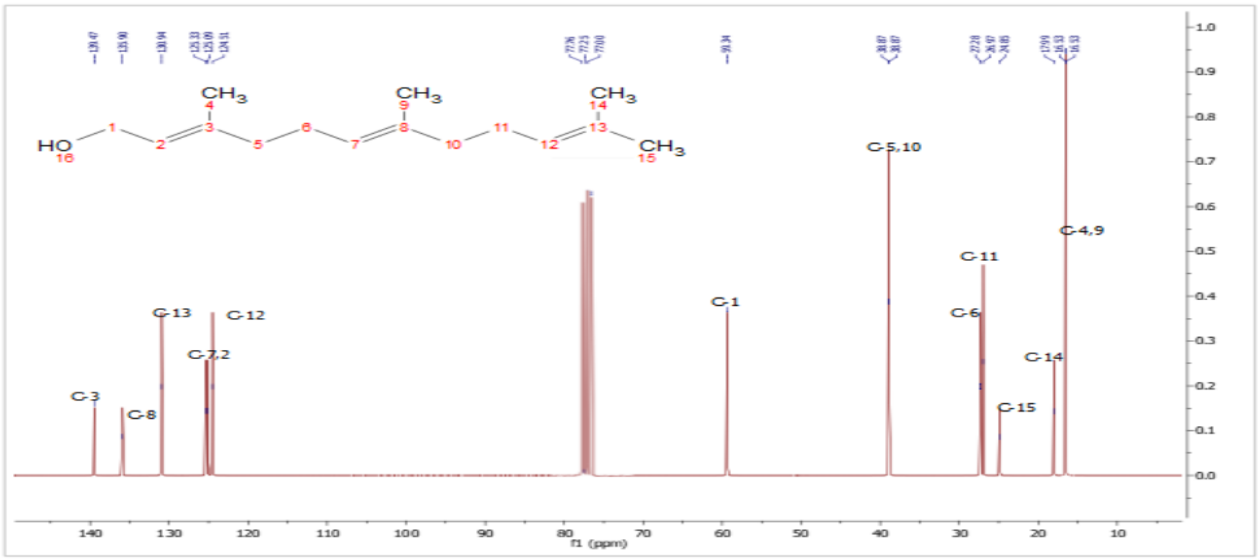


Figure 10: ^{13}C-NMR spectrum of Compound 2 from δ 20 to δ 140 (125 MHz, $CDCl_3$)

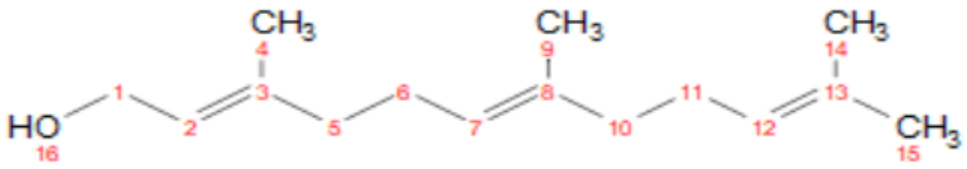
Table 7: Proton NMR signal of compound 2 and that reported by Ishaq et al. (1985)

Proton assigned to Compound 2	Proton chemical shift (ppm) of Compound 2	Proton assigned to Farnesol (Ishaq et al., 1985)	Proton chemical Farnesol Ishaq et al. (1985)
H-1	4.18	H-4	4.18
H-2	5.31	H-2	5.39
H-4	1.84	H-4	1.79
H-5	2.03	H-5	2.00
H-6	2.02	H-6	2.00
H-7	5.29	H-7	5.20
H-9	1.87	H-9	1.79
H-10	2.02	H-10	2.00
H-11	2.02	H-11	2.00
H-12	5.25	H-12	5.20
H-14	1.81	H-14	1.70
H-15	1.81	H-15	1.83
H-16	0.54	H-16	0.62

Table 8: Carbon NMR signal of Compound 1 and that reported by Ishaq et al. (1985)

Carbon assigned to Compound 1	Carbon chemical shift (ppm) of Compound 1	Carbon assigned to Farnesol Ishaq et al. (1985)	Carbon chemical shift (ppm) of Farnesol Ishaq et al. (1985)
C-1	59.34	C-1	65.03
C-2	125.09	C-2	119.80
C-3	139.47	C-3	141.56
C-4	17.99	C-4	17.67
C-5	30.87	C-5	32.04
C-6	22.28	C-6	25.73
C-7	125.33	C-7	124.59
C-8	135.90	C-8	135.41
C-9	16.53	C-9	16.05
C-10	38.87	C-10	39.77
C-11	26.97	C-11	26.77
C-12	124.51	C-12	124.40
C-13	130.94	C-13	131.29
C-14	17.99	C-14	16.43
C-15	24.85	C-15	26.64

Compound 2 is shown to have four methyl, four methylene, one methane and three methine carbon with hydroxyl group. Based on mass spectrum, ^{1}H-NMR and ^{13}C-NMR data and comparison with published information (Ishaq et al., 1985) Compound 2 was identified as Farnesol (**2)** with chemical formula $C_{15}H_{26}O$.



The result in Table 2 shows a remarkable cytotoxicity activity of the essential oil of Syzygium polyanthum root-stem. It was observed to be 18.53 µg/mL significant when compared to the thymol (+ve control).

The IC_{50} value of the essential oil was observed to be 18.42 µg/mL as against the control ascorbic acid of 15.23 µg/mL Table 3 and Figure 2. This agrees with the report of Hidayati et al., (2016) that the plant syzygium polyanthum extract has a potential value of 44.35 µg/mL. Assessed essential oils was observed to have more antioxidant potential when compared to the antioxidant potential of Hidayati extract. Antioxidant activities of the essential oils was as while reported by Amalina et al., (2013) that essential oil exhibited a potential antioxidant activity. Thus, the radical scavenging capacity of Syzygium polyanthum root-bark essential oil from Kampong Bau, in Sarawak Malaysia showed significantly higher antioxidant potential when compared to the value obtained in leaf by Hidayati et al. (2016).

The antibacterial activity of the essential oil was tested using disc diffusion method against four selected bacteria two Gram-positive and two Gram-negative bacterial strains, where their inhibitory growth value was determined. The oil was found to be active against all the selected bacteria, however it was observed to be more active on Gram-positive bacteria (Staphylococcus aureus) with growth inhibition value of 18.13±0.11 mm when compared to the control tetracycline of 19.77± 0.38 mm. However, the oil showed significant activity on both the Gram –ve and Gram +ve bacteria.

The antifungal potential of the essential oil was tested using diffusion method on four fungal strain (Aspergillus flavin, Aspergillus niger, Candida, tropicalis, and Fusarium oxysporium). High inhibition was observed on Candida, tropicalis at 500 µg/ mL of 20.55±0.08 mm as shown in Table 5.

In the study two compounds were isolated from the essential oil; Eugenol (**1**) and Farnesol (**2**) which was reported by various researcher to have significant potentials on diseases and ailment.

Eugenol (**1**) is a phenolic compound from the class of phenylpropanoids and as one of the isolated component of Syzygium polyanthum, its essential oil is used in the food industry as a preservative, mainly due to its antioxidant property (Zhang et al., 2009).

Eugenol (**1**) was reported by Guy et al. (2012), and Barboza et al., 2018, to possess significant antioxidant, anti-inflammatory and cardiovascular properties as while as penetration enhancer.

Farnesol (**2**) was reported by Bandara et al. (2016) that incorporation of natural occurring Farnesol (**2**) significantly increases the efficacy of liposomal ciprofloxacin against pseudomonas aeruginosa. Hence, developing antibiotic resistance to eliminate pseudomonas aeruginosa. It was also reported, the potential of farnesol as a safe insecticide against locusts, since it decreases food consumption, changes the digestive enzymes activities and affects positively protein synthesis in the fat body (Awad et al., 2013). Farnesol (**2**) was reported to have antibacterial potential on Staphylococcus aureus (Jabra-Razk et al., 2006), Candida albicans (Ramage et al., 2002)

In light of the result obtained essential oil from the root-bark of Syzygium polyanthum could be used as an agent for resistance diseases, ailment and for pharmaceutical, cosmetics and agricultural industries.

11. Conclusion

Essential oil of Syzygium polyanthum, obtained through hydrodistillation of mainly root-bark constitute 100 chemical constituents. It is a remarkably versatile oil which could be incorporated as a functional ingredient in numerous products and will find application in the pharmaceutical, agricultural, fragrance, flavour, cosmetic and various other industries. With its vast range of pharmacological activities researched which includes antibacterial, antifungal, antioxidant and cytotoxicity activities, as while as the isolation of Eugenol and farnesol. In addition, with the presence of eugenola dnfarnesol it will be widely used in agricultural applications to protect foods from micro-organisms during storage, which might have an effect on human health. Also as antibacterial, as a pesticide, fumigant

and antioxidant. As a functional ingredient, it is significant to discuss the general toxicity with special reference to its potential as anticancer. This study requires a further research on the potential of the essential oil on cancer cell with emphasis on mechanism of action of the oil.

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