Chemicals Composition of Clove and its Anticancer Activity

المكونات الكيمائية لنبات القرنفل ونشاطه ضد الأورام السرطانية

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Abstract

In this paper, the parts of plant *Syzygium aromaticium (*buds) *were*extracted with equal volumes of Pet. Ether/$CHCl_3$ /MeOH .Evaporation of solvents, and the provided oily residue of crude extract was chromatographed to get. (A,B,C)fractions, the fraction eluted with Pet.Ether-CH_2Cl_2 (7:3) afforded compound**1** (Methyl Euginol) and the fraction eluted with Pet. Ether-CH_2Cl_2(5.5:4.5) afforded compound **2**(9E,14E)-2,10,15,23-tetramethyl-6,19-dimethylene tetracosa-9,14-diene-2,3-diol), while the fraction eluted withPet.Ether-CH_2Cl_2 /MeOH (5.5/1.2) gave compounds **3** (Acetoxy Eugenol**).** All fractions were purified by preparative thin layer chromatography technique (PTLC), and Pre-coated TLC glass silica gel with appropriate solvent systems to give compounds (**1**, **2**, **3)**, The column was monitored by TLC, detected by the spray reagent was Vanillin stain. The spots were identified by R_f .the structures of compounds (**1**, **2**, **3)** were elucidated by spectroscopic techniques mainly ^{1}H NMR, ^{13}C NMR, IR and GC/MS analysis enabled the identification of 76 compounds from *Syzygium aromaticium plant,* Fraction of *Syzygium aromaticum* (42, 101, and 663)were further testing its anticancer activity as an inhibitor of cell growth of MCF-7 breast cancer and HEPG-2 liver cancer.

Introduction

Clove are the aromatic flower buds a tree in the family Mytaceae, Syzygium aromaticium, they are native to the Maluku Island (or Moluccas) in Indonesia, and the name comes from the Latin word *clavus*, which means nail since the shape of dried clove resemble that of a nail, Cloves are popular spice that people use in soups, stews, meats, sauces, and rich dishes. Cloves are available throughout the year owing to different harvest seasons in different counties. The clove tree is an evergreen that grows up to 8-12 meters tall, with large leaves and crimson flowers grouped in terminal clusters. Cloves rich in volatile compounds and antioxidants such as eugenol. Clove essential oil has received considerable interest due to its wide application in the perfume, cosmetic health, medical, flavoring, and food industries. Clove essential oil has biological activity relevant to human health, including antimicrobial, antioxidant, and insecticidal activity. The impacts of the extraction methods (hydrodistillation, steam distillation, microwave- assisted extraction, cold pressing, and supercritical fluid extraction).The main component of clove taste is imparted by the chemical, eugenol, has been used as an anti-cancer and as a starting material for synthesis analog L-œ- metil DOPA.

Experiment

The parts of plant *Syzygium aromaticium* (200.0 g) were dried in the shade at room temperature 24℃ and grinded well, then extracted with equal volumes of Pet.Ether/$CHCl_3$/MeOHat room temperature. Evaporation of the solvents under reduced pressure by rotary evaporator provided

92.0 g oily residue, and Only 15.0 g of crude extract was chromatographed on a normal phase silica gel for column chromatography and eluted using Petroleum ether with increasing proportions of CH_2Cl_2 and EtOAc to yield fractions (A, B, C),The fraction eluted with Pet.Ether-CH_2Cl_2 (7:3) afforded compound **1** (Methyl Eugenol), and the fraction eluted with Pet.Ether -CH_2Cl_2 (5.5:4.5) afforded compound **2**، while the fraction eluted withPet.Ether-CH_2Cl_2/MeOH (5.5/1.2) gave compounds **3** (Acetoxy Eugenol**).** All fractions were purified by preparative thin layer chromatography technique (PTLC) by using TLC aluminum sheets and Pre-coated TLC glass plates, silica gel with appropriate solvent systems to give compounds(**1**, **2**, **3)**, The column was monitored by TLC, detected by the spray reagent was Vanillin stain.The spots were identified by R_f

Isolation and identification of the chemical constituents

The fractions **A-C** were detected by TLC. The metabolites were isolated by preparative TLC, employing appreciate solvent systems. The structures of pure isolated compounds were elucidated based on ^1H, ^{13}C NMR, IR and GC/MS spectral data.

The fraction A, R_f = 0.50 (144 mg) was purified by preparative TLC using the solvent system Pet. Ether : $CHCl_3$ (8:2). The spot with R_f = (0.50) compound (**1**) (give Brown colour after sprayed with Vanillin stain) was obtained as yellow oil (57 mg).**IR** υ_{max}(film) cm^{-1}: 2931, 1610, 1510, 1030.

GCMS (70 eV) (**1**): m/z (relative intensity): 178 (97) [M, $C_{11}H_{14}O_2]^+$, 147 (14) [M**–**$OCH_3]^+$, 135 (63) [M**–**$C_2H_3O]^+$, 107 (58) [M**–**$C_4H_7O]^+$, 91 (27) [M**–**$C_4H_7O]^+$, 77 (55) [M–$C_5H_9O_2]^+$, 51 (35) [M–$C_7H_{11}O_2]^+$, 29 (7), 15 (3).

^1H (400 MHz) ^{13}C (100 MHz) NMR in ($CDCl_3$) δ ppm

The fraction B, (42.0 mg) was purified by preparative TLC using the solvent system Pet.Ether: $CHCl_3$ (2:8). The spot with R_f = (0.38) compound (**2**) (give Dark Brown colour after sprayed with Vanillin stain) was obtained as brownish oil (11.0 mg).

GCMS (70 eV) (**2**): m/z (relative intensity): 428 (3) [M-18, $C_{30}H_{54}O_2$ –H_2O], 354 (4) [M–$C_5H_{16}O]^+$, 281 (8) [M –$C_{10}H_{29}O]^+$, 219 (40) [M –$C_{15}H_{31}O]^+$, 191 (17) [M –$C_{17}H_{35}O]^+$, 159 (22) [M –$C_{19}H_{43}O]^+$, 123 (37) [M –$C_{20}H_{51}O_2]^+$, 55 (97).

The fraction C, (91.0 mg) was purified by preparative TLC using the solvent system Ethyl Acetate: MeOH(8:2). The spot with R_f = (0.73) compound (**3**) (give Brown colour after sprayed with Vanillin stain) was

obtained as brownish oil (42.0 mg).**IR** υ_{max} (film) cm^{-1} : 2961, 2930, 1761, 1604, 1600, 1507.

GCMS (70 eV) (**2**): m/z (relative intensity): 206 (25) [M, $C_{12}H_{14}O_3$], 191 (97) [M –$CH_3]^+$, 163 (8) [M –$C_3H_7]^+$, 149 (47) [M –$C_4H_9]^+$, 131 (17) [M –$C_4H_{11}O]^+$, 91 (10) [M –$C_5H_7O_3]^+$, 77 (7) [M –$C_6H_9O_3]^+$, 43 (43) [M –$C_9H_7O_3]^+$, 29 (5) [M –$C_{10}H_9O_3]^+$.

Anticancer Activity

Syzygies aromaticum three fractions (fraction 42, fraction 101, and fraction 663) were taken to dryness under reduced pressure at 40°C. One gram from each fractions were dissolved in 10ml dimethyl-sulfoxide (DMSO), To make the final concentration 100 mg/ml. Then several doses (20 µl, 40 µl, 60 µl, 80 µl, and 100µl) were injected and screened for the presence of antitumor

activities by performing MTT assay. **Mosmann T. (1983) and J. Immunol. Meth. 1983**.The cell lines MCF-7 (breast cancer cells) and HepG2 (liver cancer cells) involved was obtained from Molecular Probes. Screening experiments were preliminary carried out to find the effective fractions (42, 101, and 663) and the effective dose.

- **Reagent Preparation:**

A concentration of 12 mL MTT stock solution was prepared by adding one mL of sterile PBS (phosphate-buffered saline) to one 5 mg vial of MTT (Component A). Mix by vortex or sonication until dissolved. Occasionally there may be some particulate material that will not dissolve; this can be removed by filtration or centrifugation. Each 5 mg vial of MTT provided sufficient reagent for 100 tests that using 10 µL of the stock solution per well. Once prepared, the MTT solution can be stored for four weeks at 4°C protected from light. Ten mL of 0.01 M HCl was added to one tube containing 1 gm. of SDS (Component B). Mix the solution lightly by inversion or sonication until the SDS dissolves. Once prepared, the solution should be used promptly. Each tube makes sufficient solution for 100 tests, using 100 µL per well.

- **In vitro cytotoxic assay**

In vitro cytotoxic assay was determined according to **Bhalodia, N. R. and Shukla, V. J. (2011)**

Confluent cell cultures were harvested with trypsin-EDTA solution and plated into 96-well plate at cell density of approximately 1 X 10^4 cells/well. Serial dilutions of sample were carried out on the plate with the highest concentration of extract being 100µl/ml. Each test well was supplied with 100 µl of the diluted extract. Later 100 µl of cells to be tested were added to the wells making up the volume to a total of 200 µl of solution. The plates were then incubated at 37C in the CO_2 incubator. The assay was carried out with exposure times which were 48 hours. At the end of the incubation period 20 µl of MTT solution were added to each test well. The plate was later incubated for 3 to 4 hours to allow the reaction to take place. Following incubation, most of the solution in each well was discarded leaving the purple formazan precipitate at the bottom of the well. Then 100 µl of DMSO was added to each well and the solution was pipetted thoroughly to dissolve the purple formazan crystals. The amount of formazan produced after treatment was read using a DR-200B microplate ELISA reader at the wavelength of 570 nm. The absorbance was recorded. The IC50 values (concentration of tested compound required to inhibit cell proliferation by 50%) were determined from the dose-response inhibition curve.The inhibition rate was calculated as follows: Inhibition rate (%) =1- (absorbance of treatment group/absorbance of the control group) × 100% . The 50% inhibitory concentrations (IC_{50}) of the 48 hours are calculated with Bliss assay.

- **Statistical analysis** Results are presented as the mean ± standard deviation (SD) of three replicates. The statistical analyses were carried out using SPSS (version 22). Data obtained were analyzed statistically to determine the degree of significance using a two-way analysis of variance (ANOVA) at probability level $p \leq 0.05$.

Results and Discussion

Characterization of *Syzygium aromaticium* SA-42as Methyl eugenol.

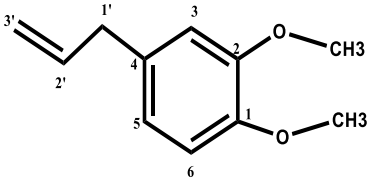
Compound **1** (1,2-Dimethoxy-4-(prop-2-en-1- yl benzene)was isolated asyellow oil with a molecular formula$C_{11}H_{14}O_2$by GC-MS highest fragment peak at *m/z* 178.099 and from the ^{13}C NMR spectrum, thus have five degrees of unsaturation. The IR spectra data clearly indicated the appearance absorption C-O-C linkage of ether around 1030 cm-1, strong absorptions at 1610 and 1510 cm^{-1} were also found from eugenol due to terminal double bond and aromatic moiety.

The structure of Methyl eugenol was elucidated by spectroscopic techniques mainly ^1H NMR, ^{13}C NMR and IR. The 400 MHz (^1H NMR) and 100 MHz (^{13}C NMR) in $CDCl_3$ spectra of Methyl Eugenol showed the presence of 14 protons in the molecule. The presence of an aromatic protons at δ_H 6.98 ppm (d, 1H, *J*= 8.4 Hz), δ_H 6.79-6.78 (m, 2H) could be assigned to be 1,2,4-trisubstituted benzene,together with the singlet peak of methoxy protons at δ_H 3.91 (s, 6H). Moreover, the doublet pattern at δ_H 3.43 (d, 2H-1‘, *J*= 6.7 Hz) proved the presence of CH_2 protons and deshielded by aromatic ring and double bond. The signal at δ_H 5.17-5.22 (m, 2H-3') suggested that was methylene protons of terminal double bond and another proton of this double bond at δ_H 6.06 (m, 1H-3'), indicating the location of terminal double bond.

Analysis of its ^{13}C-NMR and DEPT spectra showed the presence of 11 carbon atoms. It indicated three quaternary, four tertiary, two secondary and two oxygenated methyl carbons. In addition, the ^{13}C-NMR showed the presence of an aromatic moiety (δ_C146.69, 144.02, 137.99, 121.27, 115.57 and 111.32ppm) and a terminal double bond (δ_C 131.99, 114.51 ppm) within the structure; thus, the molecule is a monocyclic.

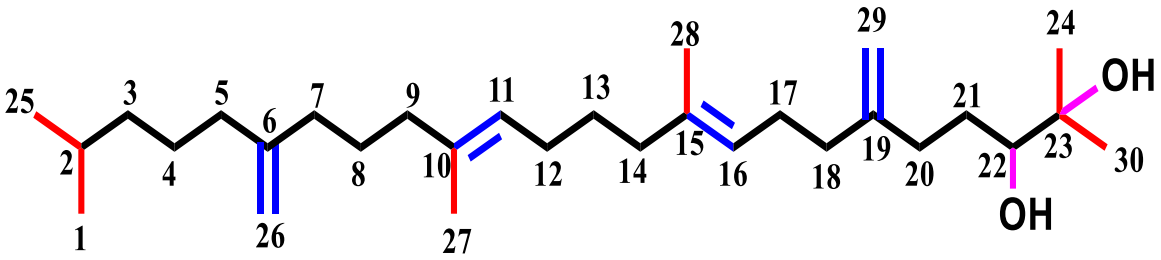
Notably, the chemical shift at δ_C 55.89 ppm was assigned to be two identical methoxy carbons and attached to benzene ring. The downfield quaternary carbon at δ_C 146.69 and 144.02 ppm corresponded to aromatic carbon (C-1, C-2) bearing two methoxy groups, while the upfield quaternary carbon (C-4) at δ_C 137.99 ppm was allylic group confirmed. It showed the interactions between proton of methoxy groups at δ_H 3.911 ppm to C-2, H-3 (δ_H 6.98 ppm) to C-1, C-2, C-4, C-5, C-6, H-1' (δ_H 3.43 ppm) to C-2', C-3', C-4, C-5, C-6 . Consequently, alkoxy group of δ_C 55.89 ppm and allylic group substantiated at C-2 (δ_C 146.6 ppm) and C-4 (δ_C 137.99 ppm). The structure of compound SA-7 was finally confirmed by directed comparison of ^1H, ^{13}C NMR and MS data.

Characterization of SA-101



Methyl Eugenol

Compound (2) (9E,14E)-2,10,15,23-tetramethyl-6,19-dimethylenetetracosa-9,14-diene-2,3-diol) was isolated as a yellow oil with a molecular formula $C_{30}H_{54}O_2$ by GC-MS highest fragment peak at m/z 446 and from the ^{13}C NMR spectrum, thus have four degrees of unsaturation. The structure of compound **2** was elucidated by spectroscopic techniques mainly ^{1}H NMR, ^{13}C NMR and DEPT. The 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) in $CDCl_3$ spectra of compound **2** showed the presence of 54 protons in the molecule as well as 30 carbon atoms. Analysis of its ^{13}C-NMR and DEPT spectra showed the presence of two terminal double bonds (δ_C 154.69, 152.47, 109.70, 109.17 ppm) and two other double bonds (δ_C 151.31, 137.64, 125.64, 113.51 ppm) within the structure; thus, the molecule is open-chain structure.Further analysis of the ^{13}C-NMR data revealed the presence of two oxygenated carbons atoms (δ_C 75.2, 69.6 ppm), six-methyl carbon (CH_3) at 15.6, 21.99, 22.71, 28.53, 29.95, 30.05 ppm and 13 (CH_2) carbon at 54.24, 50.24, 43.86, 42.51, 39.63, 37.02, 34.19, 33.47, 32.86, 32.61, 32.50, 32.45, 30.69 ppm, Analysis of the ^{1}H and ^{13}C NMR spectral data showed that the presence of 4H of two terminal double bonds δ_H 4.70 ppm (2H, m) and δ_H 4.66 ppm (2H, m). In addition, the CH
protons of the two remaining double bonds at δ_H 4.97 ppm (1H, m) and δ_H 4.88 ppm (1H, m). The chemical shift at δ_H 4.01 ppm (1H, m) in ^{1}H NMR proved the presence of CH proton deshielded by an OH group.Further analysis of the ^{1}H-NMR data revealed the presence six methyl protons;



(9*E*,14*E*)-2,10,15,23-tetramethyl-6,19-dimethylenetetracosa-9,14-diene-2,3-diol

two singlet methyl protons at δ_H 1.18 ppm (6H, s), two doublet methyl protons at δ_H 0.94 ppm (6H, d, *J*= 6.8 Hz) and two methyl carbons attached to double bonds at δ_H 1.59 (6H, m). Therefore, the structure of compound **2** confirmed by ^{1}H, ^{13}C NMR and MS spectral data

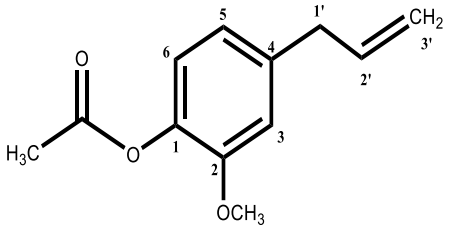
Characterization of SA-663 as Eugenol Acetate

Compound **3** (Eugenol Acetate)was isolated as a brownish oil with a molecular formula $C_{12}H_{14}O_3$ by GC-MS highest fragment peak at *m/z* 206.241 and from the ^{13}C NMR spectrum, thus have six degrees of unsaturation. The IR spectral data indicated the presence of the carbonyl band of the ester bound with the aromatic ring at 1.761 cm^{-1}, and the stretch of the double aliphatic/aromatic carbon bond is around 1,604 and 1,600 to 1,507 cm^{-1}, respectively.
The structure of Eugenol Acetate was elucidated by spectroscopic techniques mainly ^{1}H NMR, ^{13}C NMR and IR. The 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) in $CDCl_3$ spectra of Eugenol Acetate showed the presence of 14 protons in the molecule as well as 12 carbon atoms. Analysis of its ^{13}C-NMR and DEPT spectra showed the presence of an acetate (δ_C 168.88 ppm) and a terminal double bond (δ_C137.8, 115.57 ppm) within the structure; thus, the molecule is a monocyclic. Further analysis of the ^{13}C-NMR data revealed the presence of an aromatic moiety

(δ_C 151.04, 138.93, 138.23, 122.47, 120.40, 112.73 ppm), DE shielded methylene carbon (CH_2) at δ_C 39.92 ppm, a methoxy carbon (OCH_3) at δ_C 55.89 ppm and only one methyl carbon (CH_3) at δ_C 20.76 ppm. Analysis of the ^1H and ^{13}C NMR spectral data showed that the presence of acetoxy (CH_3COO) protons at δ_H 2.29 ppm (3H, s), methoxy protons at δ_H 3.76 ppm, and thearomatic protons at δ_H 6.913 ppm (d, 1H, J= 8.4 Hz), 6.75-6.77 ppm (m, 2H) could be assigned to be 1,2,4-trisubstituted benzene. Moreover, the doublet peak at δ_H3.25 ppm (d, 2H-1', J= 6.7 Hz) suggested that the presence of methylene protons (CH_2) and DE shielded by aromatic ring and double bond.The CH proton at δ_H 5.924 ppm (1H, m) proved that was a proton of a double bond and the high value of the chemical shift because of the deshielding effect of the aromatic system, and the signal at δ_H 5.17-5.22 ppm (2H-3', m) suggested that was methylene protons (═CH2) of this terminal double bond. The downfield quaternary carbon at δ_C 151.04 ppm corresponded to aromatic carbon (C-2) bearing a methoxy group, while the upfield quaternary carbons at δ_C 138.23, 138.93 ppm were aromatic carbons (C-1, C-4) attached to acetoxy and allylic groups respectively. Therefore, the structure of compound SA-663 was finally confirmed by directed comparison of ^1H, ^{13}C NMR and MS spectra data with the value reported

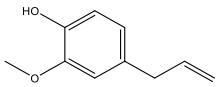
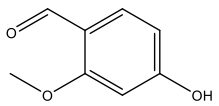
Identification of Pet.Ether/$CHCl_3$/MeOH extract constituents of *Syzygium aromaticium*by using GC/MS technique.

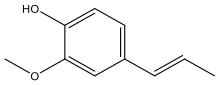
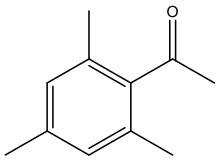
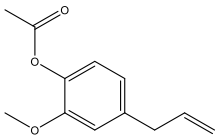
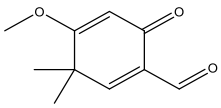
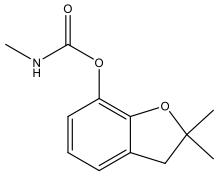
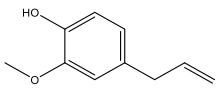
Pet.Ether/$CHCl_3$/MeOH extract was identified by GC/MS technique, three compounds SA-42, SA-101 and SA-663 as well as **76** compounds were identified by comparing their mass spectra with those of their analogous reported by NIST library (Table 1

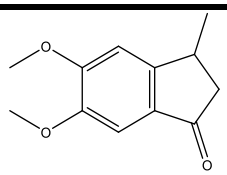
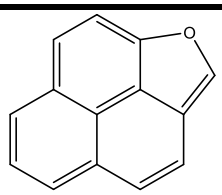
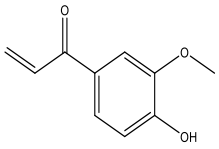
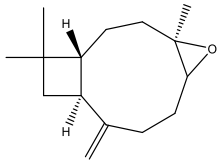
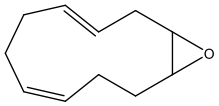
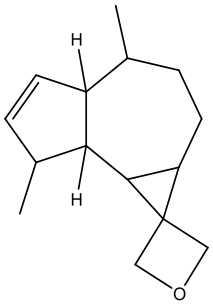
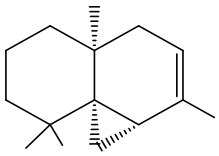


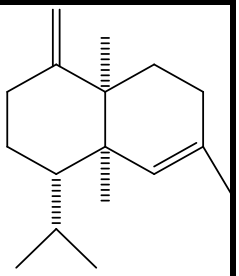
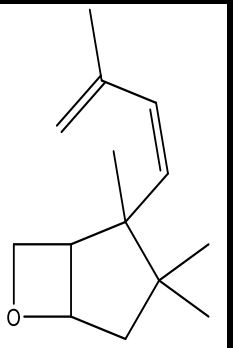
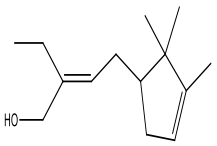
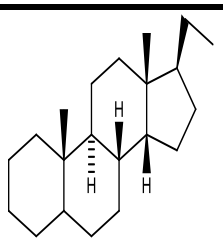
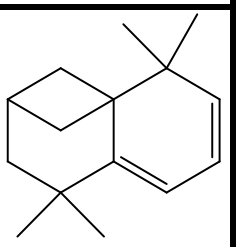
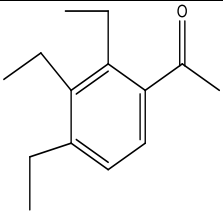
Acetoxy Eugenol

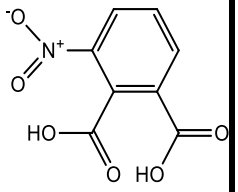
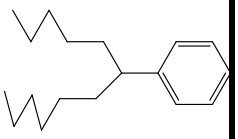
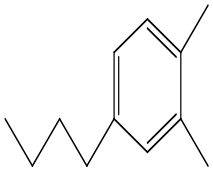
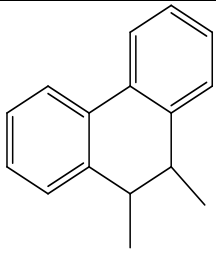
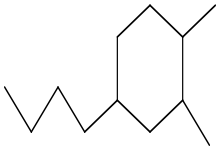
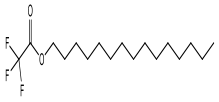
Table (1) Chemical constituents identified by GC/MS technique from Pet.Ether/CHCl3/MeOH extract of *Syzygium aromaticium* whole plant material.

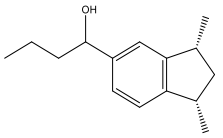
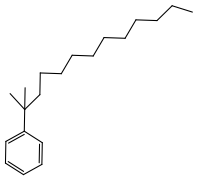
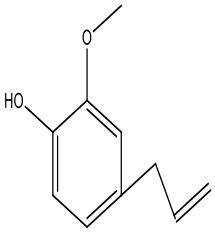
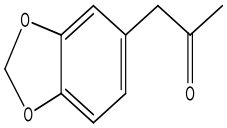
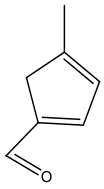
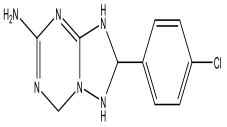
No	Compound Name	Rt	Mol. Wt	Area %	MS-Data	CSBC
1	Phenol, 2-methoxy-4-(2-propenyl)-(CAS) $$ Eugenol	5.842	164.084	2.07	164(µ)(100%),149(40%)131(35%),115(3%),103(43%),91(39%),77(50%).	
2	4-Hydroxy-2-methoxybenaldehyde $$	5.911	152.047	0.86	151(µ)(100%),137(11%),123(43%),109(28%),81(32%)	

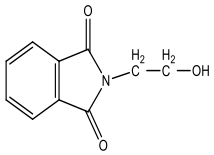
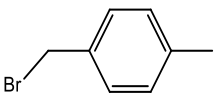
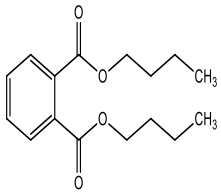
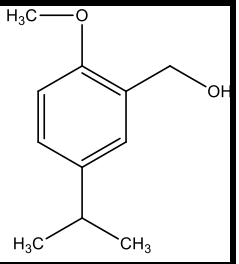
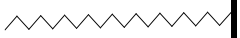
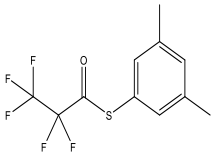
	Benzaldehyde, 4-hydroxy-2-methoxy-					
3	Phenol, 2-methoxy-4-(1-propenyl)- (CAS) $$ Isoeugenol	6.14	164.084	1.20	164(40%),147(µ+)(1 6%),131(17%),119(1 %),91(11%),77(14%)	
4	Ethanone, 1-(2,4,6-trimethylphenyl)- (CAS) $$ 2,4,6-Trimethylacetophenone	6.47	162.104	0.13	162(40%),147(µ+)(100%),119(48%),103(6%), 91(14%),77(8%).	
5	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	6.672	206.094	8.27	206(8%), 164(µ+)(100%),149(21%),131(13%), 107(9%), 91(11%).	
6	4-Methoxy-3,3-dimethyl-6-oxocyclohexa-1,4-diene-1-carboxaldehyde	7.301	180.079	0.49	180(µ+)(100%),165(10%) .	
7	Carbofurane Furadane	7.399	221.105	0.21	221(4%),164((µ+)(100%),149(55%) 131(18%),117(13%),103(8%),77(7%).	
8	Phenol, 2-methoxy-4-(2-propenyl)- (CAS) Eugenol	7.462	164.084	0.07	164(µ+)(100%),149(34%),131(24%),121(14%),103(24%),91(18%),77(24%).	

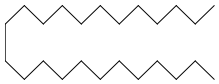
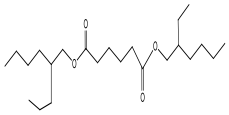
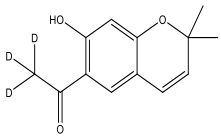
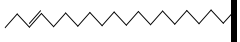
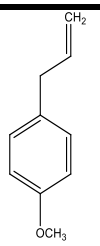
9	1H-Inden-1-one, 2,3-dihydro-5,6-dimethoxy-3-methyl-	7.496	206.094	0.06	192(µ+)(100%),131(50%),77(34%).	
10	phenalenol[1,9bc]furan Phenaleno[1,9-bc]furan (CAS)	7.56	192.058	0.44	192(µ+)(100%),164(90%).	
11	4 - (oxo - allyl) - guaiacol guaiacyl vinyl ketone	7.66	178.063	1.39	178(53%),151(µ+)(100%),137(18%),123(13%),108(9%).	
12	Caryophyllene oxide	7.828	220.183	6.78	220(6%),205(2%),177(6%),161(7%),138(9%),121(25%),95(65%),79(87%),58(38%),43(µ+)(100%).	
13	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)	8.12	220.183	1.09	152(1%),138(32%),123(13%),109(43%),96(28%),81(18%),67(30%),55(24%),43(µ+)(100%).	
14	ALLOAROMADENDRENOXID-(1) $$ AROMADENDRENEPOXIDE-(I) $$ AROMADENDRENEPOXIDE-(II)	8.228	220.183	0.73	220(7%),205(6%),177(30%),149(17%),133(22%),107(35%),91(45%),67(37%),41(µ+)(100%).	
15	Thujopsene	8.268	204.188	0.49	2049(%32),189(10%),175(4%),161(11%),147(10%),133(33%),119(µ+)(	

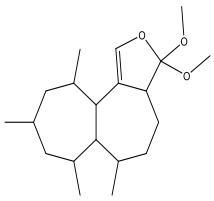
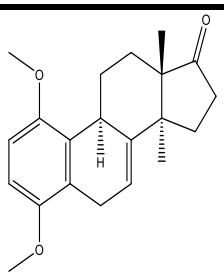
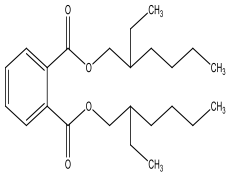
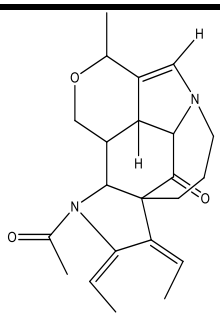
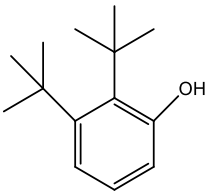
					100%),105(52%), 92(45%),92(48%).	
16	gamma. 1-cadinene	8.383	204.188	0.10	204(16%),189(2%),175(1%),161(41%),133(14%),119(45%),105(55%),93(μ+)(100),77(36%).	
17	2-((Z)-3'-Methyl-1',3'-butadien-1'-yl)-2,3,3-trimethyl-6-oxabicyclo[3.2.0]heptan	8.543	220.146	0.11	220(10%),176(11%),161(33%),135(48%),121(34%), 107(μ+)(100%),93(73%),79(34%).	
18	Tetradeca-2,12-diyne	8.669	190.172	0.46	190(1%),175(33%),161(56%),147(57%),133(66%),119(70%),107(57%), 95(91%),81(88%), 67(μ+)(100%).	
19	Xanthoxylin $$ Ethanone, 1-(2-hydroxy-4,6-dimethoxyphenyl)-	8.772	196.074	0.13	196(40%),181(μ+)(100%),166(6%), 151(2%),138(4%), 121(3%),95(5%).	
20	Isolongifolene, 9,10-dehydro-	8.904	202.172	0.12	202(30%),187(19%),173(6%),145(40%),131(μ+)(100%),105(17%),91(19%),77(14%).	
21	2,3',5-Trimethyldiphenylmethane $$ 2,3',5 -	9.115	210.141	4.69	210(38%),195(μ+)(100%),177(5%), 152(16%),1378(%),109(4%),94(3%),77(6%).	

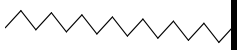
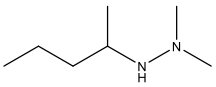
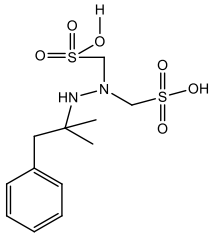
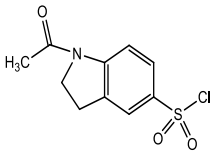
	trimethyl(diphenylmethane)					
22	2-isopropyl-1-cyano-naphthalene	9.356	195.105	0.50	195(40%),180(µ+)(100%),153(15%).	
23	Benzene, (1-pentylheptyl)- $$ Dodecane, 6-phenyl- $$ 6-Phenyldodecane	9.579	246.235	0.07	246(5%),189(31%),161(13%),145(4%),119(6%),91(µ+)(100%).	
24	2-Propenal, 3-(4-hydroxy-3-methoxyphenyl)- $$ 3-Methoxy-4-hydroxycinnamaldehyde	9.985	178.063	1.82	178(µ+)(100%),161(24%),148(52%),135(64%),121(27%),107(57%),91(29%).	
25	5,6-dihydro-5,6-dimethylbenzo[c]cinnoline	10.466	210.116	0.55	210(40%),195(µ+)(100%),180(37%).	
26	2-Propenal, 3-(4-hydroxy-3-methoxyphenyl)- $$ 3-Methoxy-4-hydroxycinnamaldehyde	10.586	178.063	0.13	178(µ+)(100%),161(25%),148(47%),135(50%),12125%),107(52%),91(27%),77(53%).	
27	Trifluoroacetic acid, n-heptadecyl ester $$ Pentadecyl trifluoroacetate	10.889	324.228	0.09	255(2%),210(3%),182(4%),140(5%),111(42%),83(78%),57(µ+)(100%).	
28	Octadecane (CAS) $$ n-Octadecane $$	11.044	254.297	0.05	254(2%),196(2%),141(4%),	

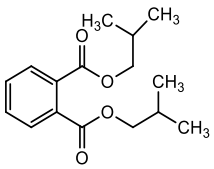
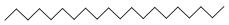
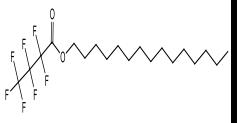
	Octadecan $$ n - octadecane $$ AI3-06523				113(9%),85(45%) 57(µ+)(100%).	
29	1-[(cis)-1',3'-Dimethylindan-5'-yl]butan-1-ol	11.25	218.167	0.10	218(2%),203(1%), 175(µ+)(100%),145(6%),105(82%), 91(48%).	
30	Benzene, (1-pentyloctyl)- $$ Tridecane, 6-phenyl- $$ (1-Pentyloctyl)benzene #	11.639	260.25	03	260(2R%),189(7%),161(12%),119(8%).91(µ+)(100%).	
31	Phenol, 2-methoxy-4-(1-propenyl)- (CAS) $$ Isoeugenol	11.833	164.084	0.08	164(µ+)(100%),149(35%),131(23%),115(2%),103(26%),91(23%),77(31%).	
32	2-Propenal, 3-(4-hydroxy-3-methoxyphenyl)- $$ 3-Methoxy-4-hydroxycinnamaldehyde	12.417	178.063	0.07	178(µ+)(100%),161(26%),135(66%),121(25%),107(57%),91(27%).	
33	1,3-Cyclopentadiene, 5,5-dimethyl-1,2-Dipropyl-	12.503	178.172	0.18	178(26%),163(1%),149(µ+)(100%), 135(6%),121(6%), 107(34%),91(6%), 79(%4).	
34	5-amino-2-(p-chlorophenyl)-2,3-dihydro-[1,2,4]triazolo[1,5-a][1,3,5]triazine	12.892	248.058	0.19	248(11%),137(µ+)(100%).	

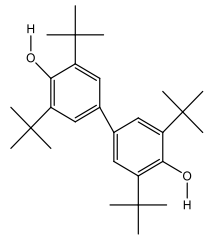
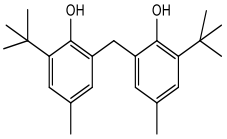
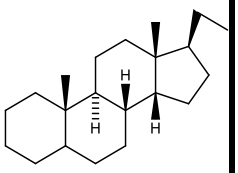
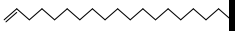
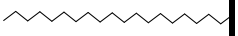
35	1H-Isoindole-1,3(2H)-dione, 2-hydroxy- $$ Phthalimide, N-hydroxy-	13.544	163.027	0.12	163(µ+)(100%),147(24%),133(28%),119(7%),104(48%),89(4%),76(48%).	
36	Benzene, 1-(bromomethyl)-4-methyl- $$ p-Xylene, .alpha.-bromo-	13.836	183.989	0.08	184(7%),105(µ+)(100%),91(1%),77(10%).	
37	Dibutyl phthalate $$ 1,2-Benzenedicarboxylic acid, dibutyl ester	14.963	278.152	0.08	223(4%),205(3%),149(µ+)(100%),121(2%),104(5%),76(4%).	
38	Propan-2-one, 1-(4-isopropoxy-3-methoxyphenyl)-	15.461	222.126	0.49	222(5%),168(4%),180(38%),137(µ+)(100%),122(38%),94(2%).	
39	5-Eicosene, (E)- $$ (5E)-5-Icosene $$ [E]-5-Eicosene	16.342	280.313	0.08	139(3%),111(12%),83(44%),55(µ+)(100%).	
40	Eicosane $$ n-Eicosane $$ Icosane # $$ n-Icosane	16.554	282.329	0.03	282(3%),225(1%),197(2%),169(3%),141(7%),113(13%)85(62%),57(µ+)(100%).	
41	3,5-Dimethylthiophenol, S-pentafluoropropionyl-	20.159	284.029	0.05	284(50%),165(4%),137(µ+)(100%),111(11%),91(14%),	
42	1-Nonadecene $$ Nonadec-1-ene	23.191	266.297	0.08	266(1%),238(1%),154(3%),125(24%),97(µ+)(100%).	

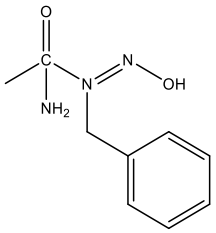
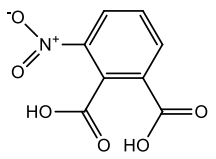
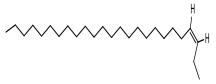
43	Docosane (CAS) $$ n-Docosane $$ C22H46 STANDARD $$ Normal-docosane	23.414	310.36	0.07	310(9%),197(4%),169(6%),141(10%),113(17%),85(72%),57(µ+)(100%).	
44	Tetracosane (CAS) $$ n-Tetracosane $$ n-tetracosane $$ AI3-52698	27.077	338.391	0.09	338(4%),309(0%),281(2%),253(2%),225(2%),197(2%),169(3%),141(5%),113(9%),85(5%),57(µ+)(100%).	
45	Hexanedioic acid, bis(2-ethylhexyl) ester (CAS) $$ Bis(2-ethylhexyl) adipate	30.252	370.308	0.07	241(6%),199(1%)157(22%),129(µ+)(100%),106(11%),83(21%).	
46	1-Docosene $$ Docos-1-ene	30.573	308.344	0.19	308((31%),280(4%),238(2%),209(3%),181(5%),153(10%),125(29%),97(90%),57(µ+)(100%).	
47	6-TRIDEUTEROACETYL-7-HYDROXY-2,2-DIMETHYLBENZOPYRAN	31.888	221.113	0.01	221(22%),206(µ+)(100%),187(13%),175(3%).	
48	3-Eicosene, (E)- $$ (3E)-3-Icosene #	33.519	280.313	0.02	280(2%),167(1%),139(4%),111(31%),83(76%),97(µ+)(100%).	
49	Estragole $$ Tarragon $$ Anisole, p-allyl- $$ Chavicol, O-methyl-	33.903	148.089	0.03	148(µ+)(100%),133(25%),121(4%),105(23%),91(23%),77(30%).	

50	rac-5,5-Dimethoxy-9,11,13,15-tetramethyl-4-oxatricyclo[8.5.0.0(2,6)]pentadeca-2-ene	34.555	326.152	7.96	326(µ+)(100%),311(13%),295(19%),280(10%),267(45%),251(46%),235(80%),220(13%),207(46%),192(38%),178(38%),165(46%).	
51	1,4-dimethoxy-14-methyl-estra-1,3,5(10),7-tetraen-17-one	35.093	326.188	1.43	326(µ+)(100%),311(31%),293(11%).	
52	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS)	35.774	390.277	15.70	279(26%),149(µ+)(100%),113(19%),83(10%),57(43%).	
53	14-.beta.-Hydroxystrychnobrasiline	35.888	382.189	0.49	382(µ+)(100%),354(9%),323(12%),296(4%),255(5%),210(5%),186(17%),144(85%),108(39%).	
54	Phenol, 2,4-bis(1,1-dimethylethyl)-$$ Phenol, 2,4-di-tert-butyl-	6.134	206.167	5.54	238(3%),210(2%),168(3%),140(6%),111(32%),83(93%),57(µ+)(100%).	

55	Phenol, 2,4-bis(1,1-dimethylethyl)- $$ Phenol, 2,4-di-tert-butyl-	7.141	238.266	3.26	206(16%),191(µ+)(100%),163(7%),147(3%),115(3%),91(5%),74(5%).	
56	Hexadecane (CAS) $$ n-Hexadecane $$ Cetane $$ n-Cetane $$ Isohexadecane	7.244	226.266	0.96	226(1%),169(1%),141(1%),113(7%),85(51%),57(µ+)(100%)	
57	Hydrazine, 1,1-dimethyl-2-(1-methylbutyl)- $$ 1,1-Dimethyl-2-sec-amylhydrazine	7.525	130.147	1.08	130(28%),115(3%),87(22%),71(1%),59(µ+)(100%).	
58	2-Thiopheneacetic acid, 3-tetradecyl ester $$ 1-Ethyldodecyl 2-thienylacetate #	8.892	338.228	2.39	338(6%),196(2%),142(7%),97(97%),57(µ+)(100%).	
59	1-Octadecene (CAS) $$ .alpha.-Octadecene $$ Octadecylene .alpha.-	10.815	252.282	5.48	252(9%),224(3%),196(2%),168(4%),139(9%),111(4%).83(94%),57(µ+)(100%).	
60	1-Octadecanesulphonyl chloride	10.963	352.22	0.82	288(1%),161(3%),133(5%),105(11%),85(4%),57(µ+)(100%).	

61	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	12.434	278.152	4.47	223(7%),205(2%).167(3%),149(µ+)(100%),104(5%),76(3%),57(27%).	
62	5-Eicosene, (E)- $$ (5E)-5-Icosene $$ [E]-5-Eicosene	16.325	280.313	8.36	280(2%),139(3%),111(22%)83(64%),55(µ+)(100%).	
63	EICOSANE $$ ICOSANE $$ ICOSANE # $$ AI3-28404 $$ CCRIS 663 $$ EICOSAN	16.531	282.329	1.65	169(1%),141(3%),113(6%),85(41%),57(µ+)(100%).	
64	Heneicosane $$ n-Heneicosane $$ Henicosane #	19.861	296.344	0.91	169(2%)141(4%),113(3%),85(56%),57(µ+)(100%).	
65	Heptafluorobutyric acid, pentadecyl ester	23.18	424.221	6.22	255(1%),210(6%),169(12%),125(18%),125(40%),95(71%)57(µ+)(100%).	
66	Docosane (CAS) $$ n-Docosane $$ C22H46 STANDARD $$ Normal-docosane	23.403	310.36	2.44	310(6%),281(3%),239(3%),211(3%),183(6%),141(22%),1113(17%),85(64%),57(µ+)(100%).	
67	Tricosane $$ n-Tricosane	26.682	324.376	1.91	324(14%),267(3%),239(3%),197(4%),169(5%),141(8%),1113(13%),85(52%),57(µ+)(100%).	

68	[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)-	28.742	410.318	1.96	410(40%),336(66%),283(53%),241(31%),241(10%),241(40%),207(40%),176(12%),176(14%)148(14%)119(6%),91(7%),57(µ+)(100%).	
69	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	30.561	340.24	6.03	340(82%),284(17%),228(2%),177(µ+)(100%), 149(40%),101(22%),91(9%),57(23%).	
70	Tetracosane $$ n-Tetracosane	30.756	338.391	6.48	338(3%),169(3%), 141(8%),113(8%), 85(55%),57(µ+)(100%).	
71	14-.BETA.-H-PREGNA $$ 14-.BETA.-PREGNA $$ 14B-PREGNANE	32.009	288.282	0.66	288(3%),219(3%), 179(4%),137(11%),97(48%),57(µ+)(100%).	
72	1-Nonadecene $$ Nonadec-1-ene	32.564	266.297	0.37	266(2%),139(6%), 111(33%),83(68%),41(µ+)(100%).	
73	EICOSANE $$ ICOSANE $$ ICOSANE # $$ AI3-28404 $$ CCRIS 663 $$ EICOSAN	33.394	282.329	0.46	141(3%),113(7%), 85(41%),57(µ+)(100%).	

74	N-Benzyl-1-(hydroxyimino)-1-aminoacetamide	33.811	193.085	1.23	193(3%),176(µ+)(100%),159(5%),148(8%),132(9%),120(47%),105(92%).	
75	1,2-Benzenedicarboxylic acid, 3-nitro- (CAS) $$ 3-Nitrophthalic acid	35.207	211.012	11.81	167(28%),149(µ+)(100%),113(7%),99(1%),84(1%),71(20%),57(28%).	
76	n-Pentacos-3-ene	36.066	350.391	4.51	281(1%), 253(1%),225(1%),197(12%),169(3%),141(5%),113(12%),85(46%),57(µ+)(100%).	

Anticancer activity

Fraction of *Syzygium aromaticum* (42, 101, and 663)were further testing its anticancer activity as an inhibitor of cell growth of MCF-7 breast cancer and HEPG-2 liver cancer. *In vitro* cytotoxicity test was conducted as the first step in the screening of potential anticancer compounds. This test using a cell line that provides advantages, such as test material needed is less and it requires short time. Anticancer activity is represented by percentage of inhibition and IC_{50}value. The higher percentage of inhibition is the stronger inhibitory activity. The lower IC_{50} values, the greater anticancer activity. Anticancer activity is represented by an IC_{50} value (µg/mL). IC_{50} value <100 is considered as an active compound with anticancer activity. IC_{50} value ranging from 100 to 300 is considered as weak anticancer activity, whereas the IC_{50} value over than 300 is considered as inactive compounds, the three fractions (42, 101, and 636) had weak cytotoxic activity on the HEPG which has IC_{50} value > 100 mg/ml and it is assigned as a weak active against HEPG cells. While fraction (42) and fraction (101) showed aweak inhibitory activity against MCF-7 cells with IC_{50} values (183.9 mg/ml and 161.7 mg/ml respectively. The strongest inhibitory activity against MCF-7 cells has shown by fraction (663)with IC_{50} value of (122.6 mg/ml)

In this study, it can concluded that the three fractions of cloves have weak lethality, which indicates that it has insufficient ability to inhibit cell proliferation, fraction (42) and fraction (101) of cloves were observed to have intermediate capability. Although a high death rate was observed at concentrations of 40 mg/ml and 60 mg/ml for both fraction (42) and fraction (663) against MCF-7 cells. These suggest that the fractions of cloves inhibit MCF-7 human breast and HEPG liver cancer cell lines in a time and dose-dependent manner. These finding are supported by Prashar,

A.; Locke, I. C. and Evans, C. S. (2006). Cytotoxicity of clove (Syzygium aromaticum) oil and its major components to human skin cells. who reported that clove oil exhibits cytotoxicity towards human fibroblast and endothelial cells.The results of other researches on cloves further support the ability of this spice as an excellent cytotoxic agent. Cloves are being hailed as the future of cancer treatment because of their capability to induce apoptosis and work in various cancer cells ، Cloves are also a source of betulinic acid and other triterpenes, which can act as chemo-preventive agents against breast cancer Various researches have concluded that cloves are ideal for cancer treatment because they enhance apoptosis and inhibit cell proliferation the two key properties that are ideally required for cancer treatment.

Table (2) Anticancer activity of *Syzygium aromaticum* fractions (42, 101, 663) against MCF-7 and HEPG Cell lines.

Sample		**IC_{50} (mg/ml)**	
		MCF-7	HEPG
Syzygium aromaticum	Fraction (42)	126.6	183.6
	Fraction (101)	152.7	184.4
	Fraction (663)	122.6	180.2

CONCLUSION

- ons were purified by preparative thin layer chromatography technique (PTLC) by using TLC aluminum sheets (20 × 20 cm) Silica gel G_{60} F_{254} (Merck, Darmstadt, Germany) and Pre-coated TLC glass plates SIL G-25 UV254 , 0.2 mm silica gel with appropriate solvent systems and detected by *Vanillin* stain to isolate three compounds (**1**, **2**, **3**).
- The structures of the compounds (**1**, **2**, **3**) were determined by interpretation of their spectroscopic data 1D NMR (1H, 13C, DEPT NMR), IR and MS led to elucidation of (SA)-42S as Methyl eugenol (**1**), SA-101 as (9E,14E)-2,10,15,23-tetramethyl-6,19-dimethylenetetracosa-9,14-diene-2,3-diol (**2**) and SA-663 as Euginol Acetate (**3**).
- The Pet.Ether/$CHCl_3$/MeOH extract was identified by GC/MS technique, three compounds (SA)-42S as Methyl eugenol (**1**), SA-101 as (9E,14E)-2,10,15,23-tetramethyl-6,19-dimethylenetetracosa-9,14-diene-2,3-diol (**2**) and SA-663 as Euginol Acetate (**3**) as well as **76** compounds were identified by comparing their mass spectra with those of their analogous reported by NIST library.

تم تجفيف أجزاء نبات L *Syzygium aromaticium*. (200.0 جم) في الظل عند درجة حرارة الغرفة 22 درجة مئوية وطحنها جيدًا ، ثم استخلاصها بكميات متساوية من Pet.Ether / CHCl3 / MeOH (3 × 1 لتر) عند درجة حرارة الغرفة لمدة أسبوعين. وفر تبخير المذيبات تحت ضغط منخفض بواسطة المبخر الدوار 92.0 جم من المستخلص الخام. تم إجراء تحليل كروماتوجرافي لـــ 15.0 جم فقط من المستخلص الخام على السيليكا جل العادي (60-120 شبكة ، Fluka) لعمود كروماتوجرافيا العمود (45 × 2 سم) وتم التصفية التتابعية باستخدام الإيثر البترولي بنسب متزايدة من الكلورفورم و الايثيل اسيتات للوصول إلى ثلاثة كسور (أ ، ب ، ج).

تمت تنقية جميع االتقسيمات باستخدام تقنية كروماتوغرافيا الطبقة الرقيقة التحضيرية (PTLC) باستخدام صفائح الألومنيوم TLC (20 × 20 سم) جل السيليكا G60 F254 (ميرك ، دارمشتات ، ألمانيا) وألواح زجاجية TLC مطلية مسبقًا SIL G-25 UV254، 0.2 ملم السيليكا مع أنظمة المذيبات المناسبة والكشف عن طريق صبغة فانيلين لعزل ثلاثة مركبات (1 ، 2 ، 3).

تم تحديد هياكل المركبات (1 ، 2 ، 3) من خلال تفسير البيانات الطيفية من تحليلات البرتون والكربون وتحليل الاشعه تحت الحمراء وتحليل التكسير الكتلي الطيفي وأدت إلى توضيح 40S- (SA) كمركب ميثيل يوجينول (**1**) ومركب SA 101-وايضا المركب SA-663 كمركب اجينول أسيتات.

تم تحديد والتعرف علي مستخلص بتروليم ايثير /كلورفورم / ميثانول بواسطة تقنية GC / MS ،فوجد ان ثلاثة مركبات 40S- (SA) كمركب ميثيل يوجينول (**1**) ومركب SA 101-وايضا المركب SA-663 كمركب اجينول اسيتات.بالإضافة إلى **76** مركبًا من خلال مقارنة أطيافها الكتلية مع أطيافها المماثلة التي تم الإبلاغ عنها بواسطة مكتبة نيست.

وأخيرا تم تقييم النشاط السام للخلايا السرطانية ل fractions القرنفل مقابل نوعين مختلفين من الخلايا السرطانية التى تصيب الانسان وهما سرطان الكبد، سرطان الثدىباستخدام مقايسة MTT اللونية. في هذه الدراسة ، نستنتج أن fractions الثلاثة (42 ، 101 و 663) من القرنفل كان لها نشاط سام للخلايا ضعيف على HEPG ، مما يشير إلى أن لديها قدرة غير كافية على منع تكاثر الخلايا. على الرغم من ملاحظة معدل وفيات مرتفع بتركيزات 40 مجم / مل و 60 مجم / مل لكل من (42) و (663) ضد خلايا MCF-7.

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