RESEARCH ARTICLE

Comparison of Bioactive Components of Clove Buds as Extracted by Two Different Methods and Analyzed by Gas Chromatography Triple Quad Time-Flight Technology.

Kareem A.A. Mohammed[1*], Ashraf Abdel-Hamid F. Wasfy[1], Mohamed S. Bazalou[2]

[1]*Department of Chemistry, Faculty of Science, Benha University, Benha – Egypt.*
[2]*Animal Health Research Institute, Doky,Giza_Egypt.*

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ABSTRACT

This study was carried out to compare the bioactive components of cloves (*Syzygium aromaticum bud*.) ethanolic extract using both maceration and ultrasound-assisted (sonication) techniques. A total of 101 compounds were identified by gas chromatography triple quad time-flight (GC-Q-TOF) analysis including 48 ,43 for Maceration and Sonication respectively. Regardless of the extraction method utilized Epizonarene, Cyperene, 1R, 4R, 7R, 11R-1, 3, 4, 7-Tetramethyltricyclo [5.3.1.0(4,11)]undec-2-ene, δ-Cadinene, 1S, 4R, 7R, 11R-8-Hydroxy-1, 3, 4, 7-tetramethyltricyclo [5.3.1.0(4,11)]undec-2-ene, 2',3',4' Trimethoxyacetophenone , Tributyl aconitate, Desomorphine, trimethylsilyl ether, 4,5,6,7-Tetrahydroxy-1,8,8,9-tetramethyl-8,9-dihydrophenaleno[1,2-b]furan-3-one, Nonacosane Tritriacontane, γ-Sitosterol and Acetic acid, cedrol ester were the main components of cloves. a comparison was made between the two methods , the maceration strategy gave a whole of a chemical component in cloves by long-time extraction; Whereas sonication gave palatable extraction effectiveness and gave comparably comes about to those of maceration with short extraction time, so sonication technique with GC-Q-TOF may well be valuable and suitable for the quick extraction.

Keywords: Analysis, Ethanolic extract, Bioactive, Compounds, Gas chromatography triple quad time-flight (GC-Q-TOF), Cloves (Syzygium aromaticum bud.), maceration method and sonication method.

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Conflict of interest: None

INTRODUCTION

Cloves are belonging to the genus *Syzygium* family *Myrtaceae*, known by the scientific name *Syzygium aromaticum*.[1] Cloves are a spice that has been traditionally used as a natural medicine for the treatment for various ailments, including dental diseases. Clove is utilized to treat different health conditions, counting intestinal parasites, headache , cerebral pains, colds, weakness, and gastrointestinal issues such as sickness, spewing, loose bowels and gas.[2] The extraction of components from cloves depended to a great extent on maceration and sonication procedures. In any case, there have been uncommon studies on the comparison of components from cloves by distinctive extracting methods. The Sonication method was more rapid, sensitive, and solvent-free compared to maceration traditional methods. Maceration is a traditional technique that is conducted at room temperature. It consists of immersing a plant in a liquid (water, oil, alcohol, etc.) inside an airtight container, for a variable time based on the plant material and liquid used,[3] before being processed, the plant must be properly washed and separated from foreign material such as topsoil, pebbles or rocks, weeds, and materials non-suitable for extraction. The plant material can be used fresh or dry based on the desired product. To increase contact between the plant material being extracted and the liquid (solvent), the plant needs to be cut into small pieces while ultrasound-assisted extraction (UAE) (Sonication), phytochemicals are liberated from the plant tissues by high-frequency sound ranging from 20 to 2000 kHz, which damage the cell wall. Ultrasound-assisted extraction can be used with mixtures of immiscible solvents such as hexane with methanol/water.[4] The process creates heat so that heat-labile compounds may decompose. In such cases the extraction container is placed in ice bath to reduce the temperature. The GC-Q-TOF enables compound identification by comparing the analytes' obtained mass spectra with those of authentic standards from the National Institute of Standards and

**Author for Correspondence: Kareemhagag1010@gmail.com*

Technology (NIST). Our study was conducted to compare the components of cloves as extracted by maceration and sonication techniques. GC-Q-TOF was utilized to distinguish compounds within the obtained extracts by the two methods. This study will assist to select more appropriate extraction methods.

MATERIALS AND METHODS

Reagents and Chemical

All chemicals used in this research were of the highest purity. Ethanol absolute was purchased from (ROMIL-SpS™ Super Purity Solvents), and N-Hexane was from (AVONCHEM).

Plant Material

The bud of Cloves (*Syzygium aromaticum bud.*) was purchased from the local vendor and dried at 37°C for 24 hours where exposure to sunlight was avoided to prevent the loss of active components, the plant material was ground in a grinding machine (IKA, Staufen, laboratory mill, Type A 10, screen size 0.5 mm speed max. [rpm] 2000, Germany) where the obtained powder was subjected to extraction using both maceration and sonication techniques.

Preparation of Plant Extract

Maceration Extraction Methods

Five grams of powdered cloves were placed in a glass container, mixed with 50 mL of ethanol as solvent,[5] labeled. A long shaking in the dark for 24 hours. at room temperature was performed using a universal shaker (Analytica Ltd, Ireland). The obtained extract was filtered through a Whatman No. 4 filter paper, centrifuged at 5000 rpm for 10 minutes. Finally, the supernatant was collected and filtered through 0.2 µm filters (Sarstedt, Germany). The filtrates were stored at -30°C until use.[5]

Sonication Extraction Methods

Five grams of powdered Cloves placed in a glass container, mixed with 50 mL of ethanol as solvent[5], labeled and extracted by ultrasound-assisted technique (Sonication Method) at 60°C for 30 minutes[6] using a 20 kHz ultrasonic generator (Misonix S3000; Misonix Inc., New York, NY, USA). The mixtures were filtered through a Whatman No. 4 filter paper, the obtained extract was centrifuged at 5000 rpm for 10 minutes, the supernatant was collected and filtered through 0.2 µm filters (Sarstedt, Germany). The filtered extract was subsequently stored at -30 °C until use.[7]

GC-Q-TOF Analysis

Two milliliters of the previously prepared filtrates for each ethanol plant extracts were evaporated to dryness under a gentle stream of nitrogen at 40°C (Alliance Nitrogen evaporator), reconstituted by 2 mL of hexane, vortexed by vortex mixer (vortex mixer, KMC -1300V) to dissolve the dried parts of plant extract, placed in vials and 1 µL was injected for analysis by GC -Q -TOF.

The GC-Q-TOF analysis using HP-5MS ultra inert column with 30 m × 250 µm × 0.25 µm film thickness, Helium was used as a carrier gas. Injector and detector temperatures were 325 °C. An Agilent Technology gas chromatograph type 7250 accurate-mass quadrupole time-of-flight GC/MS System. The 7250 Q-TOF GC/MS is equipped with three ion vacuum gauges: Vacuum manifold chamber, TOF vacuum manifold chamber, and Turbomolecular vacuum pump exhaust. To Condition a Capillary Column: Materials needed: carrier gas, (99.9995% pure or better), Wrench, open-end, 1/4-inch, and 5/16-inch (8710-0510). The 7250 Accurate-mass Q-TOF GC/MS can operate the LE-EI ion source in standard or low energy modes. The column oven temperature was programmed as follows: start temperature at 60ºC hold for one minute; increased to 120ºC with a ramp of 40ºC/min with a run time 2.5 minutes, increased to 310ºC with a ramp of 5ºC/min with a run time 40.5 minutes. After 15 s the split valves were opened for 3 minutes to purge the injector.

Identification of Components

Components were identified via MassHunter Workstation Software, Qualitative Analysis Navigator, version B.08.00 service pack 1build 8.0.8208.38. Interpretation of mass spectrum of GC-Q-TOF was done using a database of NIST. The mass spectrum of unknown components was compared with the spectrum of the known component stored in the NIST library.

RESULTS AND DISCUSSION

A total of 91 compounds were extracted by maceration and sonication methods as analyzed and identified by the GC-Q-TOF technique. The compounds were shown by Figures 1 and 2 and listed in Tables 1 and 2 where forty-eight compounds and forty-three compounds were identified in *Syzygium aromaticum bud* ethanolic extract by maceration and sonication methods, respectively.

The extracted compounds were identified after analysis by the computerized library (NIST) using the GC-Q-TOF spectra at different retention times where the name, probability, molecular formula, molecular weight, CAS,

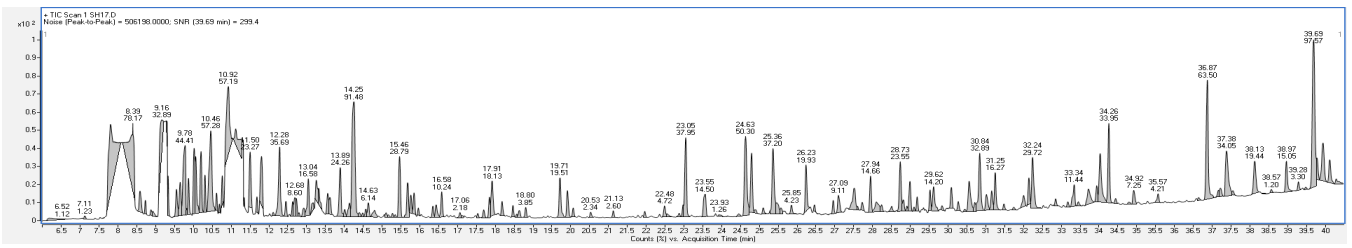


Figure 1: GC-Q-TOF Analysis of Ethanolic Extract of Cloves by Maceration Method

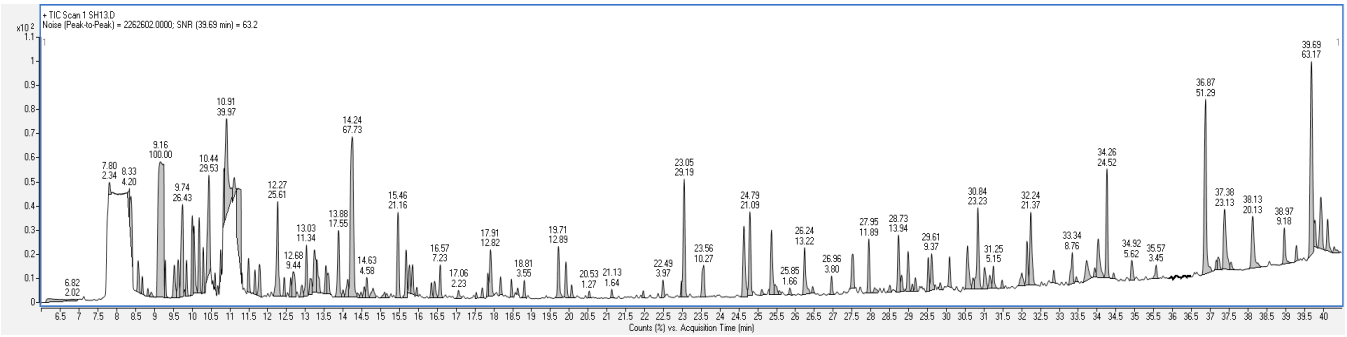


Figure 2: GC-Q-TOF Analysis of Ethanolic Extract of Cloves by Sonication Method

Table 1: Identified Compounds of Ethanoic Extract of Cloves by GC-Q-TOF Analysis (Maceration Method).

Peak NO	*RT*	*Compound name*	*Prob*	*Molecular formula*	*CAS no*	*MWT*	*Area%*
1	6.52	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	21.0%	$C_{16}H_{50}O_7Si_8$	19095-24-0	578	0.10%
2	7.11	Cyclohexasiloxane, dodecamethyl-	90.6%	$C_{12}H_{36}O_6Si_6$	540-97-6	444	0.11%
3	8.39	Epizonarene	9.69%	$C_{15}H_{24}$	41702-63-0	204	7.03%
4	9.16	1R,4R,7R,11R-1,3,4,7-Tetramethyltricyc lo[5.3.1.0(4,11)]undec-2-ene	26.0%	$C_{15}H_{24}$	137235-59-7	204	2.96%
5	9.78	Cyperene	16.7%	$C_{15}H_{24}$	2387-78-2	204	4.00%
6	10.46	1R,4R,7R,11R-1,3,4,7-Tetramethyltricyc lo[5.3.1.0(4,11)]undec-2-ene	10.6%	$C_{15}H_{24}$	137235-59-7	204	5.15%
7	10.92	δ-Cadinene	22.4%	$C_{15}H_{24}$	483-76-1	204	5.15%
8	11.50	2-sec-Butylphenol, tert-butyldimethylsilyl ether	24.4%	$C_{16}H_{28}OSi$		264	2.09%
9	12.28	1S,4R,7R,11R-8-Hydroxy-1,3,4,7-tetramethyltricyc lo[5.3.1.0(4,11)]undec-2-ene	57.1%	$C_{15}H_{24}O$		220	3.21%
10	12.68	Isoeugenol, acetate	39.5%	$C_{12}H_{14}O_3$	93-29-8	206	0.77%
11	13.04	Epizonarene	24.2%	$C_{15}H_{24}$	41702-63-0	204	1.49%
12	13.89	1S,4R,7R,11R-8-Hydroxy-1,3,4,7-tetramethyltricyc lo[5.3.1.0(4,11)]undec-2-ene	45.4%	$C_{15}H_{24}O$		220	2.18%
13	14.25	2',3',4' Trimethoxyacetophenone	32.0%	$C_{11}H_{14}O_4$	13909-73-4	210	8.23%
14	14.63	6-Methoxyeugenyl isovalerate	33.6%	$C_{16}H_{22}O_4$	957467-00-4	278	0.55%
15	15.46	Benzo[b]dihydropyran, 6-hydroxy-4,4,5,7,8-pentamethyl-	26.3%	$C_{14}H_{20}O_2$	50442-70-1	220	2.59%
16	16.58	3β,9β-Dihydroxy-3,5α,8-trimethyltricyclo[6.3.1.0(1,5)] dodecane	29.2%	$C_{15}H_{26}O_2$		238	0.92%
17	17.06	Clovene	8.05%	$C_{15}H_{24}$	469-92-1	204	0.20%
18	17.91	Eugenin	68.6%	$C_{11}H_{10}O_4$	480-34-2	206	1.63%
19	18.80	Benzeneacetamide, N-(aminocarbonyl)-4-hydroxy-3-methoxy-	40.0%	$C_{10}H_{12}N_2O_4$	15324-70-6	224	0.35%
20	19.71	10-Methylanthracene-9-carboxaldehyde	70.1%	$C_{16}H_{12}O$	7072-00-6	220	1.76%
21	20.53	(Z)-4-(3-Acetoxyprop-1-en-1-yl)-2-methoxyphenyl isobutyrate	26.7%	$C_{16}H_{20}O_5$	160788-11-4	292	0.21%
22	21.13	Benzene, 1,2,3-trimethoxy-5-(1-acetoxy-2-propenyl)-	41.9%	$C_{14}H_{18}O_5$		266	0.23%
23	22.48	(Z)-4-(3-Acetoxyprop-1-en-1-yl)-2-methoxyphenyl isobutyrate	38.9%	$C_{16}H_{20}O_5$	160788-11-4	292	0.42%
24	23.05	Tributyl aconitate	95.3%	$C_{18}H_{30}O_6$	7568-58-3	342	3.41%
25	23.55	Silane, methylvinyl(adamant-2-yloxy)isopropoxy-	10.2%	$C_{16}H_{28}O_2Si$		280	1.30%
26	23.93	Hydromorphone, TBDMS derivative	8.24%	$C_{23}H_{33}NO_3Si$		399	0.11%
27	24.63	Desomorphine, trimethylsilyl ether	22.6%	$C_{20}H_{29}NO_2Si$		343	4.53%

Peak NO	*RT*	*Compound name*	*Prob*	*Molecular formula*	*CAS no*	*MWT*	*Area%*
28	25.36	4,5,6,7-Tetrahydroxy-1,8,8,9-tetramethyl-8,9-dihydrophenaleno[1,2-b]furan-3-one	48.9%	$C_{19}H_{18}O_6$	2582-86-7	342	3.35%
29	25.85	4,6-Bis(1,1-dimethylethyl)-2,'4'-dimethoxy-1.1'-biphenyl-2-ol	36.1%	$C_{22}H_{30}O_3$		342	0.38%
30	26.23	4,5,6,7-Tetrahydroxy-1,8,8,9-tetramethyl-8,9-dihydrophenaleno[1,2-b]furan-3-one	23.6%	$C_{19}H_{18}O_6$	2582-86-7	342	1.79%
31	27.09	δ9-Tetrahydrocannabivarin	37.4%	$C_{19}H_{26}O_2$	31262-37-0	286	0.82%
32	27.94	N-(2-Methyl-5-trimethylsilylphenyl)acetamide	10.1%	$C_{12}H_{19}NOSi$	30127-86-7	221	1.32%
33	28.73	Dehydrodieugenol	43.0%	$C_{20}H_{22}O_4$	4433-08-3	326	2.12%
34	29.62	Eugenol acetate	18.0%	$C_{12}H_{14}O_3$	93-28-7	206	1.28%
35	30.84	Guaiol acetate	7.26%	$C_{17}H_{28}O_2$	134-28-1	264	2.96%
36	31.25	Glycerol 2-acetate 1,3-dipalmitate	46.4%	$C_{37}H_{70}O_6$	58546-06-8	610	1.46%
37	32.24	Allocryptopine	10.4%	$C_{21}H_{23}NO_5$	485-91-6	396	2.67%
38	33.34	Supraene	16.0%	$C_{30}H_{50}$	7683-64-9	410	1.03%
39	34.26	Nonacosane	20.0%	$C_{29}H_{60}$	630-03-5	408	3.05%
40	34.92	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	26.7%	$C_{16}H_{50}O_7Si_8$	19095-24-0	578	0.65%
41	35.57	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	40.5%	$C_{16}H_{50}O_7Si_8$	19095-24-0	578	0.38%
42	36.87	Tritriacontane	14.9%	$C_{33}H_{68}$	630-05-7	646	5.71%
43	37.38	β-Tocopherol, O-methyl-	22.2%	$C_{29}H_{50}O_2$		430	3.06%
44	38.13	Vitamin E acetate	51.6%	$C_{31}H_{52}O_3$	7695-91-2	472	1.75%
45	38.57	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	60.2%	$C_{16}H_{50}O_7Si_8$	19095-24-0	578	0.11%
46	38.97	Stigmasterol	18.6%	$C_{29}H_{48}O$	83-48-7	412	1.35%
47	39.28	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	55.0%	$C_{16}H_{50}O_7Si_8$	19095-24-0	578	0.30%
48	39.69	γ-Sitosterol	92.6%	$C_{29}H_{50}O$	83-47-6	414	8.78%

Table 2: Identified compounds of Ethanoic Extract Cloves by GC-Q-TOF analysis (Sonication method).

Peak NO	*RT*	*Compound name*	*Prob*	*Molecular formula*	*CAS*	*MWT*	*Area%*
1	6.82	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	12.1%	$C_{16}H_{50}O_7Si_8$	19095-24-0	578	0.26%
2	7.8	Eugenol acetate	30.0%	$C_{12}H_{14}O_3$	93-28-7	206	0.30%
3	8.33	Eugenol acetate	20.2%	$C_{12}H_{14}O_3$	93-28-7	206	0.54%
4	9.16	1R,4R,7R,11R-1,3,4,7-Tetramethyltricyclo[5.3.1.0(4,11)]undec-2-ene	28.4%	$C_{15}H_{24}$	137235-59-7	204	12.81%
5	9.74	Cyperene	16.1%	$C_{15}H_{24}$	2387-78-2	204	3.38%
6	10.44	Acetic acid, cedrol ester	10.9%	$C_{17}H_{28}O_2$	77-54-3	264	3.78%
7	10.91	δ-Cadinene	21.2%	$C_{15}H_{24}$	483-76-1	204	5.12%
8	12.27	1S,4R,7R,11R-8-Hydroxy-1,3,4,7-tetramethyltricyclo[5.3.1.0(4,11)]undec-2-ene	59.1%	$C_{15}H_{24}O$		220	3.28%
9	12.68	Isoeugenol, acetate	33.5%	$C_{12}H_{14}O_3$	93-29-8	206	1.21%
10	13.03	Epizonarene	24.1%	$C_{15}H24$	41702-63-0	204	1.45%
11	13.88	1S,4R,7R,11R-8-Hydroxy-1,3,4,7-tetramethyltricyclo[5.3.1.0(4,11)]undec-2-ene	45.7%	$C_{15}H_{24}O$		220	2.25%
12	14.24	2',3',4' Trimethoxyacetophenone	32.9%	$C_{11}H_{14}O_4$	13909-73-4	210	8.67%
13	14.63	6-Methoxyeugenyl isovalerate	36.5%	$C_{16}H_{22}O_4$	957467-00-4	278	0.59%
14	15.46	Precocene 2	30.4%	$C_{13}H_{16}O_3$	644-06-4	220	2.71%

Peak NO	*RT*	*Compound name*	*Prob*	*Molecular formula*	*CAS*	*MWT*	*Area%*
15	16.57	3β,9β-Dihydroxy-3,5α,8-trimethyltricyclo[6.3.1.0(1,5)]dodecane	25.8%	$C_{15}H_{26}O_2$		238	0.93%
16	17.06	Clovene	10.3%	$C_{15}H_{24}$	469-92-1	204	0.29%
17	17.91	Eugenin	69.0%	$C_{11}H_{10}O_4$	480-34-2	206	1.64%
18	18.81	Benzeneacetamide, N-(aminocarbonyl)-4-hydroxy-3-methoxy-	45.8%	$C_{10}H_{12}N_2O_4$	15324-70-6	224	0.45%
19	19.71	10-Methylanthracene-9-carboxaldehyde	68.6%	$C_{16}H_{12}O$	7072-00-6	220	1.65%
20	20.53	(Z)-4-(3-Acetoxyprop-1-en-1-yl)-2-methoxyphenyl isobutyrate	26.4%	$C_{16}H_{20}O_5$	160788-11-4	292	0.16%
21	21.13	Benzene, 1,2,3-trimethoxy-5-(1-acetoxy-2-propenyl)-	67.6%	$C_{14}H_{18}O_5$		266	0.21%
22	22.49	(Z)-4-(3-Acetoxyprop-1-en-1-yl)-2-methoxyphenyl isobutyrate	49.6%	$C_{16}H_{20}O_5$	160788-11-4	292	0.51%
23	23.05	Tributyl aconitate	92.9%	$C_{18}H_{30}O_6$	7568-58-3	342	3.74%
24	23.56	Silane, methylvinyl(adamant-2-yloxy)isopropoxy-	11.0%	$C_{16}H_{28}O_2Si$		280	1.32%
25	24.79	Tributyl acetylcitrate	96.5%	$C_{20}H_{34}O_8$	77-90-7	402	2.70%
26	25.85	4,6-Bis(1,1-dimethylethyl)-2,'4'-dimethoxy-1.1'-biphenyl-2-ol	24.7%	$C_{22}H_{30}O_3$		342	0.21%
27	26.24	4,5,6,7-Tetrahydroxy-1,8,8,9-tetramethyl-8,9-dihydrophenaleno[1,2-b]furan-3-one	18.0%	$C_{19}H_{18}O_6$	2582-86-7	342	1.69%
28	26.96	1-(1-Adamantyl)-2-tripropylsilyloxyethane	28.1%	$C_{21}H_{40}OSi$		336	0.49%
29	27.95	Eugenol, TBDMS derivative	21.7%	$C_{16}H_{26}O_2Si$	214330-24-2	278	1.52%
30	28.73	Dehydrodieugenol	38.6%	$C_{20}H_{22}O_4$	4433-08-3	326	1.79%
31	29.61	Eugenol acetate	22.7%	$C_{12}H_{14}O_3$	93-28-7	206	1.20%
32	30.84	Guaiol acetate	7.66%	$C_{17}H_{28}O_2$	134-28-1	264	2.97%
33	31.25	Glycerol 2-acetate 1,3-dipalmitate	34.6%	$C_{37}H_{70}O_6$	58546-06-8	610	0.66%
34	32.24	Triptophenolide	12.0%	$C_{20}H_{24}O_3$	74285-86-2	312	2.74%
35	33.34	Supraene	24.2%	$C_{30}H_{50}$	7683-64-9	410	1.12%
36	34.26	Nonacosane	19.9%	$C_{29}H_{60}$	630-03-5	408	3.14%
37	34.92	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	17.0%	$C_{16}H_{50}O_7Si_8$	19095-24-0	578	0.72%
38	35.57	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	32.5%	$C_{16}H_{50}O_7Si_8$	19095-24-0	578	0.44%
39	36.87	Tritriacontane	16.1%	$C_{33}H_{68}$	630-05-7	464	6.57%
40	37.38	β-Tocopherol, O-methyl-	22.0%	$C_{29}H_{50}O_2$		430	2.96%
41	38.13	Vitamin E acetate	57.0%	$C_{31}H_{52}O_3$	7695-91-2	472	2.58%
42	38.97	Stigmasterol	16.1%	$C_{29}H_{48}O$	83-48-7	412	1.18%
43	39.69	γ-Sitosterol	91.8%	$C_{29}H_{50}O$	83-47-6	414	8.09%

and peak area for each peak were investigated. The mass spectrometer analyzes the compounds eluted at diverse times to distinguish the compounds' nature and structure. The large compound parts into small compounds giving rise to the appearance of peaks at diverse m/z proportions. These mass spectra are unique mark of that compound, which can be recognized from the data library.[8]

A total of 45 compounds were identified in *Syzygium aromaticum bud* ethanolic extract where 31, 8 and 6 compounds were extracted by both maceration and sonication methods, maceration and sonication respectively as listed in Table 3.

Comparison of the two extraction methods in terms of extraction time, solvent volume, and temperature, the outcomes were itemized in Table 4. The table revealed that the sonication method utilizes a shorter time than maceration method (30 minutes in front of 24 hours) but need sonication equipment.

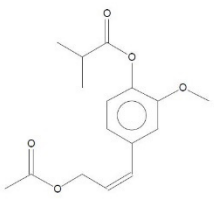
Sonication method presented significant advantages over maceration method in qualitative analysis at analytical level due to clearly fast and efficient (30 minutes) method in comparison with 24 hours extraction time required for maceration in spite of the fact that it was moderately costly to buy the ultrasound-assisted equipment.

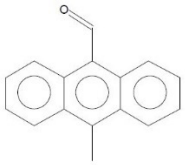
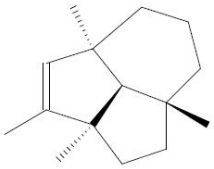
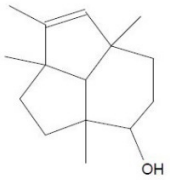
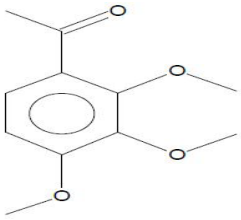
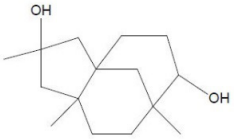
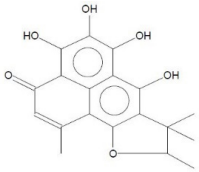
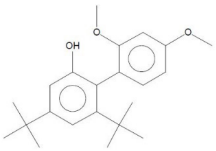
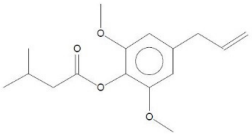
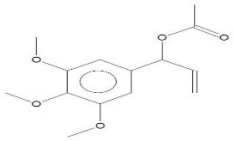
Similar studies used the hydrodistilled extraction method of cloves for 4 hours using a Clevenger-type apparatus then investigated by GC and GC-MS. In total, 22 components were identified by this method. Eugenol was found to be the major component of the essential oil, followed by b-caryophyllene,

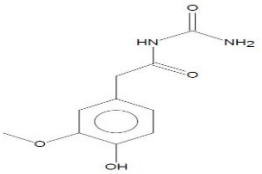
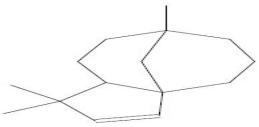
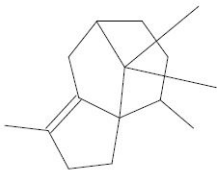
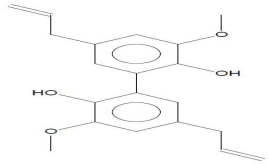
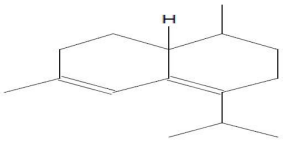
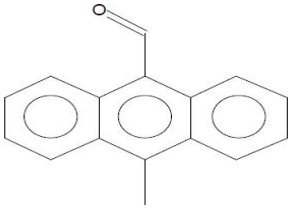
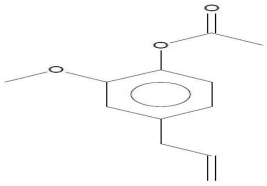
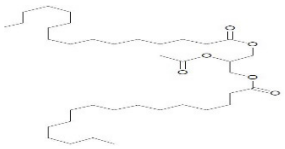
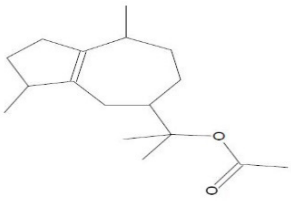
Table 3: Comparison of identified compounds of ethanoic extract cloves by GC-Q-TOF analysis

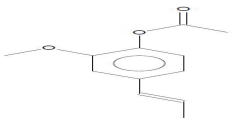
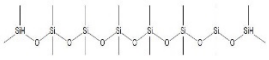
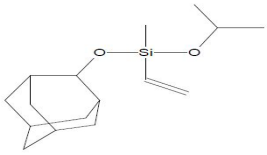
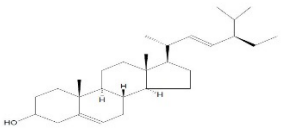
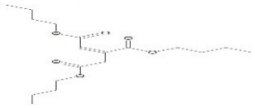
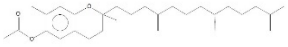
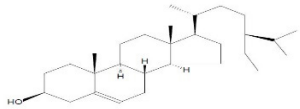
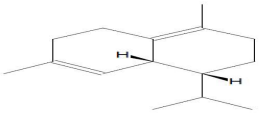
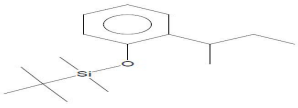
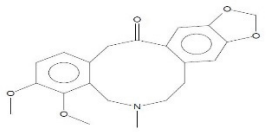
#	*Compounds identified by Maceration and sonication methods*	*Compounds identified by Maceration method*	*Compounds identified by Sonication method*
1	(Z)-4-(3-Acetoxyprop-1-en-1-yl)-2-methoxyphenyl isobutyrate	2-sec-Butylphenol, tert-butyldimethylsilyl ether	1-(1-Adamantyl)-2-tripropylsilyloxyethane
2	10-Methylanthracene-9-carboxaldehyde	Allocryptopine	Acetic acid, cedrol ester
3	1R,4R,7R,11R-1,3,4,7-Tetramethyltricyclo[5.3.1.0(4,11)]undec-2-ene	Benzo[b]dihydropyran, 6-hydroxy-4,4,5,7,8-pentamethyl-	Eugenol, TBDMS derivative
4	1S,4R,7R,11R-8-Hydroxy-1,3,4,7-tetramethyltricyclo[5.3.1.0(4,11)]undec-2-ene	Cyclohexasiloxane, dodecamethyl-	Precocene 2
5	2',3',4' Trimethoxyacetophenone	Desomorphine, trimethylsilyl ether	Tributyl acetylcitrate
6	3β,9β-Dihydroxy-3,5α,8-trimethyltricyclo[6.3.1.0(1,5)]dodecane	Hydromorphone, TBDMS derivative	Triptophenolide
7	4,5,6,7-Tetrahydroxy-1,8,8,9-tetramethyl-8,9-dihydrophenaleno[1,2-b]furan-3-one	N-(2-Methyl-5-trimethylsilylphenyl) acetamide	
8	4,6-Bis(1,1-dimethylethyl)-2,'4'-dimethoxy-1.1'-biphenyl-2-ol	δ9-Tetrahydrocannabivarin	
9	6-Methoxyeugenyl isovalerate		
10	Benzene, 1,2,3-trimethoxy-5-(1-acetoxy-2-propenyl)-		
11	Benzeneacetamide, N-(aminocarbonyl)-4-hydroxy-3-methoxy-		
12	Clovene		
13	Cyperene		
14	Dehydrodieugenol		
15	Epizonarene		
16	Eugenin		
17	Eugenol acetate		
18	Glycerol 2-acetate 1,3-dipalmitate		
19	Guaiol acetate		
20	Isoeugenol, acetate		
21	Nonacosane		
22	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-		
23	Silane, methylvinyl(adamant-2-yloxy)isopropoxy-		
24	Stigmasterol		
25	Supraene		
26	Tributyl aconitate		
27	Tritriacontane		
28	Vitamin E acetate		
29	β-Tocopherol, O-methyl-		
30	γ-Sitosterol		
31	δ-Cadinene		

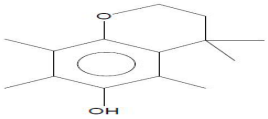
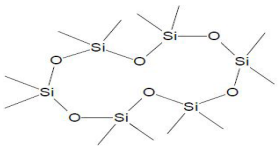
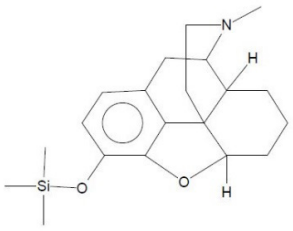
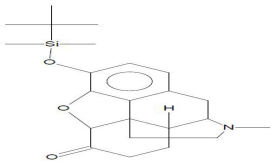
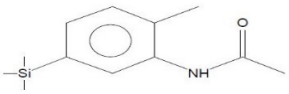
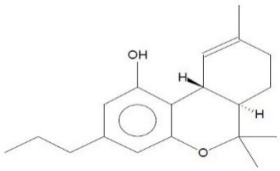
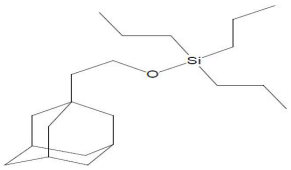
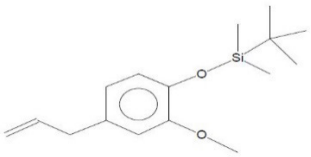
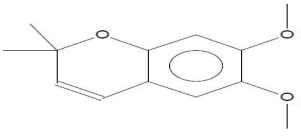
Table 4: Biological activity and structure formulae of identified compounds of ethanoic extract cloves by GC-Q-TOF analysis

#	*Compound*	*Biological activity*	*Structure formula*
1	(Z)-4-(3-Acetoxyprop-1-en-1-yl)-2-methoxyphenyl isobutyrate	Not reported	

2	10-Methylanthracene-9-carboxaldehyde	inhibitors of metallo-aminopeptidases(9)	
3	1R,4R,7R,11R-1,3,4,7-Tetramethyltricyc lo[5.3.1.0(4,11)]undec-2-ene	The anti-microbial activity(10)	
4	1S,4R,7R,11R-8-Hydroxy-1,3,4,7-tetramethyltricyc lo[5.3.1.0(4,11)]undec-2-ene	antibacterial effects against gram negative bacteria compare to gram positive bacteria(11)	
5	2',3',4' Trimethoxyacetophenone	antiinflammatory agents(12)	
6	3β,9β-Dihydroxy-3,5α,8-trimethyltricyclo[6.3.1.0(1,5)] dodecane	Not reported	
7	4,5,6,7-Tetrahydroxy-1,8,8,9-tetramethyl-8,9-dihydrophenaleno[1,2-b]furan-3-one	Not reported	
8	4,6-Bis(1,1-dimethylethyl)-2,'4'-dimethoxy-1.1'-biphenyl-2-ol	Not reported	
9	6-Methoxyeugenyl isovalerate	antioxidant, anti-inflammatory, anti-microbial, herbicidal, and genotoxicity of the essential oil which was compared with pure methyl eugenol compound.13	
10	Benzene, 1,2,3-trimethoxy-5-(1-acetoxy-2-propenyl)-	antiinflammatory(14)	

11	Benzeneacetamide, N-(aminocarbonyl)-4-hydroxy-3-methoxy-	Anti-microbial activity (15)	
12	Clovene	Antimicrobial(16)	
13	Cyperene	Anti-microbial and antioxidant activities(17)	
14	Dehydrodieugenol	Anti-parasitic agents(18)	
15	Epizonarene	Antimicrobial(19)	
16	Eugenin	An immunomodulator(20)	
17	Eugenol acetate	Antibacterial and antioxidant agent(21)	
18	Glycerol 2-acetate 1,3-dipalmitate	Not reported	
19	Guaiol acetate	Anti-microbial activity(22)	

20	Isoeugenol, acetate	Anti-microbial activity(23)	
21	Nonacosane	Anti-microbial activity(24)	
22	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	Anti-microbial activity(25)	
23	Silane, methylvinyl(adamant-2-yloxy)isopropoxy-	Not reported	
24	Stigmasterol	Anti-inflammatory impact and cytotoxic against a few cancer cell line(26)	
25	Supraene	Antioxidant activity(27)	
26	Tributyl aconitate	Not reported	
27	Tritriacontane	Not reported	
28	Vitamin E acetate	Antioxidant (28)	
29	β-Tocopherol, O-methyl-	Antioxidant (28)	
30	γ-Sitosterol	Antidiabetic medicate (29)&(30)	
31	δ-Cadinene	Anti-microbial activity(31)	
32	2-sec-Butylphenol, tert-butyldimethylsilyl ether	Not reported	
33	Allocryptopine	Protection against biotic stressors(32)	

34	Benzo[b]dihydropyran, 6-hydroxy-4,4,5,7,8-pentamethyl-	Not reported	
35	Cyclohexasiloxane, dodecamethyl-	Antifungal activity(33)	
36	Desomorphine, trimethylsilyl ether	Not reported	
37	Hydromorphone, TBDMS derivative	Not reported	
38	N-(2-Methyl-5-trimethylsilylphenyl)acetamide	Not reported	
39	δ9-Tetrahydrocannabivarin	Decreases appetite, increases satiety, and up-regulates energy metabolism(34)	
40	1-(1-Adamantyl)-2-tripropylsilyloxyethane	Not reported	
41	Acetic acid, cedrol ester	Not reported	
42	Eugenol, TBDMS derivative	Used as a medicinal agent, but also for food flavoring and preservation(35)	
43	Precocene 2	Inhibits juvenile hormone biosynthesis(36)	

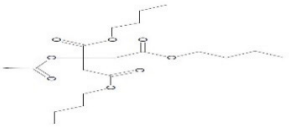
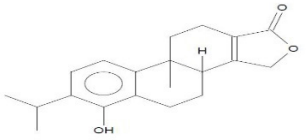
44	Tributyl acetylcitrate	Anticancer activity and anti-microbial activity(37)	
45	Triptophenolide	Antagonist of androgen receptor(38)	

Table 5: Comparison of Extraction Parameters Among the Extraction Methods

Parameter	*Maceration method*	*Sonication method*
Extraction time (min)	24 hrs.	30 Mins
Solvent	Ethanol	Ethanol
Temperature	Room temp (27°C)	60°C
Solvent volume (mL)	50 mL	50 mL
Material amount (g)	5 gm	5 gm
Cost	Not expensive	Expensive

caryophyllene oxide, and eugenyl acetate.[39] Similar studies used sonication extraction method of cloves for 45 minutes then investigated by HS-GC/MS analysis. The best result was obtained, sonication methods application efficiently and easily extracted.[40]

CONCLUSION

Essential oils are generally derived from one or more plant parts, such as stems (e.g., geranium,cinnamon), bark (e.g., cinnamon, cassia), leaves (e.g., mint, lemongrass, jamrosa), roots (e.g., angelica, sassafras, vetiver, saussurea, valerian), flowers (e.g., rose, jasmine, rosemary, lavender, cloves), leaves and wood (e.g., cedar, sandal, pine), seeds (e.g., fennel, coriander, caraway, dill, nutmeg), fruits (bergamot, orange, lemon, juniper), rhizomes (e.g., ginger, curcuma), and gums or oleoresin.[41] In this study, two extraction strategies (maceration and sonication) were compared in terms of time factor and components extracted from cloves. Cloves (*Syzygium aromaticum bud*) was extracted by both methods using ethanol as a solvent individually, then filtrated and identified by 7250 Q-TOF GC/MS equipment. The total ion chromatograms of the extracts obtained by maceration and sonication were shown in Figures 1 and 2 the differences in extract composition among different methods.

It was found that 48 and 43 components were extracted by maceration and sonication method, respectively, and successfully identified. Thirty of these compounds has been reported as biologically active as shown in Table 4. The most important identified compounds due to their biological activity were 1S,4R,7R,11R-8-Hydroxy-1,3,4,7-tetramethyltricyclo[5.3.1.0(4,11)]undec-2-ene , 6-Methoxyeugenyl isovalerate, Cyperene, Dehydrodieugenol, Eugenin, Eugenol acetate, Guaiol acetate, Isoeugenol, acetate, δ9-Tetrahydrocannabivarin, Eugenol, TBDMS derivative. Meanwhile, Epizonarene ,Cyperene,1R,4R,7R,11R-1,3,4,7-Tetramethyltricyclo[5.3.1.0(4,11)]undec-2-ene, δ-Cadinene, 1S,4R,7R,11R-8-Hydroxy-1,3,4,7-tetramethyltricyclo[5.3.1.0(4,11)]undec-2-ene , 2',3',4' Trimethoxyacetophenone , Tributyl aconitate, Desomorphine, trimethylsilyl ether, 4,5,6,7-Tetrahydroxy-1,8,8,9-tetramethyl-8,9-dihydrophenaleno[1,2-b]furan-3-one, Nonacosane. Tritriacontane, γ-Sitosterol and Acetic acid, cedrol ester were the major components in cloves.

The above results showed that the sonication method could be a suitable, fast more efficient extraction method than the maceration method for the extraction of biologically active ingredients in cloves buds. Sonication gave a relatively entire profile of Cloves' chemical constituents by short time extraction that is nearly similar to that obtained by maceration method. The study demonstrated that sonication was appropriate for fast qualitative examination for the components in cloves. This method could be utilized for the standard quality control examination of therapeutic plants at a logical level.

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