Bottle Gourd (GC-MS)

by Nur Fitriani Usdyana Attahmid

Submission date: 02-Aug-2021 06:25PM (UTC+0700)
Submission ID: 1626942872
File name: Baghdad_Science_Journal_Bottle_Gourd_Similarity.docx (1.11M)
Word count: 4907
Character count: 31387

Effect of extraction time on a bioactive compound of Bottle gourd *(Lagenaria siceraria)* using Gas chromatography-mass spectrometry

Abstract:

Traditional medical systems have always played an important role in meeting global healthcare needs. They are still doing so and will continue to play important roles in the future. Bottle gourd *(Lagenaria siceraria)* is a vegetable that contains health-promoting secondary metabolites. This study to determine bioactive compounds profiling of Bottle gourd *(Lagenaria siceraria)* fruit extracts in methanol and chloroform using gas chromatography-mass spectrometry (GC-MS). Bottle gourd extract with different solvents methanol and chloroform and variations in extraction time 10, 20, and 30 min utilising GC-MS. Total 91 compounds were tentatively identified, with 55 found in methanol extract and 41 found in chloroform extract. Many peaks with high area percentages were discovered in a methanolic extract of Bottle gourd containing key chemical constituents such as stearic acid, oleic acid, palmitic acid, linoleic acid, Cholesta-4,6-dien-3-one, gamma sitosterol, and Phenol, 2,2'-methylene bis. The corresponding constituents from chloroform extract includes Tetracontane, Dotriacontane, Phenol, 2,2'-methylenebis, and most esters and aromatic derivatives. Most of the bioactive compounds were detected between 20-30 min time of extraction. Moreover, fatty acids, methyl and ethyl esters, and sterols represent 40% of the total extracts. They were dominated by oleic acid, palmitic acid, gamma-sitosterol and its ethyl and methyl esters. Finally, bottle gourd was discovered to contain various valuable compounds, indicating its pharmaceutical, biomedical activities and food functionals potential.

Key words: Bottle gourd, Bioactive compounds, Chloroform, GC-MS, Methanol.

Introduction:

Plants high in antioxidants, such as vitamin C, tocopherols, polyphenols compounds and carotenoids, are gaining popularity in the food industry as alternatives to synthetic antioxidants, whose use is limited concerns about safety. Synthetic antioxidants have long been used in foods to prevent lipid oxidative rancidity, nutritional loss, off-flavor, quality loss, and discoloration. In addition to extending the shelf life of food, these compounds can slow the progression of many oxidative stress-related chronic diseases in humans. Because of their role in protecting the body from reactive nitrogen species, reactive oxygen species, and free radicals that come from either normal metabolic processes or external sources, dietary antioxidants play an essential role as nutraceuticals (1,2). Several mechanisms of action are likely to be involved in this protection, including inhibition of free radical generation, increased scavenging capacity against free radicals, reducing capacity, and metal chelating ability. These reactions are commonly used in antioxidant activity tests. A wide range of activities can be determined using antioxidant activity assays and the lipidic system used as a substrate (2,3).

Bottle gourd *(Lagenaria siceraria)* is relatively easy to plant. The planting area is spread in various parts of the world, from tropical to subtropical climates, from the highlands to the lowlands suitable for planting bottle gourd. Bottle gourd is a vegetable rich in nutrients, so people make bottle gourd as daily food. This plant contains calcium, iron, vitamin C, polyphenols and saponins, beneficial for health. The bottle gourd is a common vegetable because of its high choline, phenolics, vitamin B complex, and vitamin C content (4). Bottle gourd juice is now well-known for its cardioprotective, cardiotonic, aphrodisiac properties, and diuretic, use as an antidote to some poisons. Bottle gourd juice is also beneficial for maintaining the body's alkaline reserve due to its less acidic nature (5). The ability of bottle gourd juice (BGJ) to be used as a health drink, on the other hand, is dependent on the extraction and preservation of functional components like phenolics, carotenoids, and ascorbic acid. As a result, the processing method chosen is essential due to the presence of heat-sensitive components like phenolics, carotenoids, ascorbic acid, and perishable nature of the product. Fresh bottle gourd juice extraction is the only way to use it. To date, no attempt has been made to investigate the effects of processing on bottle gourd juice functional components in order to store and improve their efficiency.

Bottle gourd plants have phytochemicals that are beneficial to the body. This plant can produce reactive oxygen species. Inhibition of reactive oxygen species (ROS) production, direct or indirect scavenging of free radicals and alteration of intracellular redox potential are all biochemical activities of natural antioxidants. Antioxidants, such as carotenoids, flavonoids, polyphenolic compounds, vitamin A, vitamin C, and vitamin E are abundant in vegetables and fruits, preventing free radical damage and lowering the risk of chronic diseases. As a result, consuming dietary antioxidants from these sources may help prevent cardiovascular diseases, especially atherosclerosis (6). Elucidate free radical scavenging activity of ethanolic extract *Lagenaria siceraria* fruit using the FRAP method and ethanol solvents found 1.95 mg/ml (2). In other studies, a combination of the blanching process and sonication extraction to improve the quality of gourd juice bottle showed significant improvements to total phenolics (TP), total carotenoids, total soluble solids (TSS) and physical stability (PS). Other parameters such as titratable acidity (TA), pH, ascorbic acid(AA), browning index (BI), total plate count (TPC) and yeast and mould count experienced a significant decrease (7). Formulations blend juice bottle gourd, aonla, lemon and ginger using response surface methodology (RSM) with minimal thermal process showing quality stability against physicochemical, sensory and microbiology parameters (8). bottle gourd optimization using acetone, ethanol and methanol solvents using Liquid Chromatography-Mass Spectrometry (LC-MS) analysis. The existence of tetracyclic triterpene–cucurbitacin, as well as other pharmaceutically essential compounds, was verified (9). Another study, in vitro analysis wild bottle gourd against antioxidant content, antidiabetic, anti-acetylcholine esterase, anticancer activities using Reversed-Phase-High Performance Liquid Chromatography (RP-HPLC). The study concluded that wild bottle gourd is a rich source of bioactive metabolites with antioxidant, antidiabetic, anti-acetylcholine esterase, and anticancer properties (10).

This investigation will also evaluate the effects of the solvent extraction on bottle gourd better to understand the physiological, pharmacological, and flavour. The effect of extraction time with sonication and solvents variations has not been reported yet. In general, analysis of bioactive compounds is usually conducted using gas chromatography-mass spectrometry (GC-MS). Bottle gourd is medicinal plants, and both have been attributed to beneficial health effects, but there are only a few studies related to this topic. This work has aimed to investigate the effect of this solvent type, extraction time using sonication and profile of bioactive compounds.

Material and Methods:

Materials

Bottle gourd was purchased from the local market and stored at room temperature until use (Fig. 1). The chemicals used in the study of analytical grade hexane, methanol and chloroform supplied by Merck Millipore (Burlington, Massachusetts, United States). Instrument Shimadzu 2010 GC-MS, Elma Ultrasonic Cleaner S60H, and Buchi Rotary Rotavapor R-300.

Preparation of the extract

Fruits were picked and washed with flowing tap water after separating the fruit into epicarp, mesocarp, and seeds. Fresh fruit homogenized such as mesocarp were ground separately in an electricmixer grinder. To extract the sample (1:2), 20 ml bottle gourd juice mixed with 40 ml hexane (v/v) transferred to conical flask. Conical flasks were immersed in an ultrasonic bath (Elma Ultrasonic) for temperature 40^{o}C at 20 min. Finally, the filtrate will be used for sonication extraction using methanol and chloroform solvents.

Ultra-sonication assisted extraction (UAE)

20 ml filtrate was transferred to a conical flask containing 40 ml solvent methanol or chloroform (1:2 v/v). Further, all the conical flasks were immersed in an ultrasonic bath (Elma Ultrasonic) with temperature 40^{o}C for 10, 20 and 30 min.

Figure 1. Bottle gourd.

Table 1. Extraction time of bottle gourd using *Ultra-Sonication Assisted Extraction (UAE)*.

Solvent	Extraction time (min)
Chloroform	10
	20
	30
Methanol	10
	20
	30

GC-MS determination

Shimadzu 2010 GC-MS, RTX-5 capillary column (30 mm x 0.25 mm x 0.25 μm). Split ratio 40:1 with a temperature of column was 70°C, heating rate 10°C min^{-1}, up to 300°C, maintained for 5 min with a total analysis time of 25 min. Helium was used as a carrier gas flowing at a constant 1.0 ml/min; the temperature of the inlet was 280°C; pre-column pressure was 80 kPa, Ionization voltages 70 eV.

Results and Discussion:

Identification of bioactive compound by GC–MS

Phytochemical constituents of bottle gourd were extracted sequentially using two different organic solvents varying in polarity from 4.1 (chloroform) to 5.1 (methanol) and at a different time extraction (Table 1). The chemical constituents of two different crude extracts of bottle gourd were analyzed using gas chromatography–mass spectrometry. Fig. 2 illustrates the chromatograms of two crude extracts; 100 different compounds were identified in the two crude extracts (Tables 2-3). The identified compounds were classified into ten chemical groups based on their common name, retention time (Rt), and percent peak area. Chemical groups identified include esters derived from fatty acids, fatty alcohols, fatty acids (FA), amines, aromatic, phenolics, hydrocarbons, terpenes, sterols, and among others. The identified compounds were compared in the NIST 2.7 and Willey 8 libraries using GC-MS. The chloroform extract identified the fewest compounds (10 compounds) after 10 min extraction time. In comparison, the highest number of compounds (25 compounds) was identified in the methanol extract after 30 min of extraction (Table 2).

Table 2. GC–MS detection of bioactive compounds from bottle gourd using methanol solvent in 10, 20 and 30 min time of extraction.

Peak	R Time	Area (%)	Bioactive compound
(a) Extraction time 10 min			
Terpenes			
15	33.992	1.55	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-, (all-E)
Esters			
6	16.716	1.63	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester
8	20.918	2.86	Hexadecanoic acid, methyl ester
10	21.843	1.26	Hexadecanoic acid, ethyl ester
11	23.308	0.77	9,12-octadecadienoic acid (z,z)-, methyl ester
12	23.393	0.41	8,11,14-docosatrienoic acid, methyl ester
13	24.291	0.46	Linoleic acid ethyl ester
Fatty alcohols			
7	18.058	0.59	1-hentetracontanol
Fatty acids			
9	21.524	3.18	n-Hexadecanoic acid
Aromatic			
2	5.261	51.86	Ethylbenzene
3	5.433	28.62	P-Xylene
4	5.852	4.18	Benzene, 1,2-dimethyl
Others			
1	5.054	0.73	Cyclotrisiloxane, hexamethyl
5	9.226	0.84	Cyclotrisiloxane, hexamethyl
14	29.924	1.06	Bis(2-ethylhexyl) phthalate
(b) Extraction time 20 min			
Aromatic			
1	5.262	37.59	Ethylbenzene
2	5.333	7.23	Benzene, ethyl-
3	5.433	22.15	P-xylene
4	5.853	1.99	Benzene, 1,2-dimethyl-
Esters			
5	16.712	0.91	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester
7	20.901	2.80	Hexadecanoic acid, methyl ester
8	21.826	1.02	Hexadecanoic acid, ethyl ester
9	23.290	0.85	9,12-octadecadienoic acid (z,z)-, methyl ester
10	24.272	0.63	Linoleic acid ethyl ester
Fatty alcohols			
6	18.018	1.40	1-tetradecanol, acrylate
Phenolics			
11	28.119	2.99	Phenol, 2,2'-methylenebis
Fatty acids			
12	29.913	0.79	1,2-benzenedicarboxylic acid
Sterols			
13	33.314	0.72	Cholesta-4,6-dien-3-one
15	38.965	0.59	Stigmast-5-en-3-ol, oleat
Others			
14	34.021	18.35	Tetrakis (2,3-ditert-butylphenyl)-4,4'-biphenylene diphosphonat
(c) Extraction time 30 min			
Aromatic			
1	5.229	20.11	Ethylbenzene
2	5.310	7.24	Benzene, ethyl-
3	5.400	19.44	P-xylene
4	5.823	1.79	Benzene, 1,2-dimethyl-
5	5.942	0.76	Octane, 2,4,6-trimethyl

6	7.794	0.36	Octane, 3,5-dimethyl
Hydrocarbons			
7	9.563	0.37	Undecane
Fatty acids			
8	16.703	0.25	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-pro
11	21.437	4.56	n-hexadecanoic acid
17	24.025	2.13	6-octadecenoic acid, (z)
19	24.353	1.35	9,12-octadecadienoic acid (z,z)
23	29.910	0.42	1,2-benzenedicarboxylic acid
Esters			
9	18.339	0.33	Tetradecanoic acid, methyl ester
10	20.883	4.32	Hexadecanoic acid, methyl ester
12	21.811	1.60	Hexadecanoic acid, ethyl ester
13	23.273	1.71	9,12-octadecadienoic acid (z,z)-, methyl ester
14	23.367	1.46	8,11,14-docosatrienoic acid, methyl ester
15	23.446	0.37	9-octadecenoic acid, methyl ester
16	23.742	0.51	Octadecanoic acid, methyl ester
20	24.450	0.37	9-octadecenoic acid (z)-, ethyl ester
21	24.742	0.25	Octadecanoic acid, ethyl ester
18	24.255	1.56	Ethyl (9z,12z)-9,12-Octadecadienoate
Sterols			
22	28.460	26.82	Stigmast-5-En-3-Ol, (3.Beta.,24s)
25	38.976	1.45	Stigmast-5-en-3-ol, oleat
Terpenes			
24	33.986	0.47	2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-e)

Table 3. GC-MS detection of bioactive compounds from bottle gourd using chloroform solvent in 10, 20 and 30 min time of extraction.

Peak	R Time	Area (%)	Bioactive compound
(a) Extraction time 10 min			
Esters			
2	20.997	37.36	Hexadecanoic acid, methyl ester
3	21.200	2.16	Beta.-n-acetylneuraminic acid, methyl ester-2-methyl-7,9-methyl-boronate-3,8-di(trimet)
4	21.919	1.57	Hexadecanoic acid, ethyl ester
5	23.466	28.37	9-Octadecenoic acid (Z)-, methyl ester
6	23.831	6.06	Octadecanoic acid, methyl ester
7	29.939	1.51	1,2-benzenedicarboxylic acid, diisooctyl ester
Terpenes			
8	33.995	16.27	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- alkohol
Aromatic amines			
10	35.283	2.18	1-isopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1h-py
Others			
1	6.470	2.53	Ethane, 1,1,2,2-tetrachloro-
9	35.050	1.99	3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol
(b) Extraction time 20 min			
Aromatic			
1	5.435	5.58	Ethylbenzene
Fatty acids			
3	16.782	2.28	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-pro
7	31.842	1.84	22.alpha.-hydroxy-3,4-secostict-4(23)-en-3-oic acid
Phenolics			
5	28.149	2.78	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-

Esters			
6	29.935	2.48	1,2-benzenedicarboxylic acid, diisooctyl ester
8	32.025	3.61	Decanoic acid, 8-chloro-, chloromethyl ester
14	34.400	2.86	2,5,9-Trimethyl-12-oxododeca-4,8-dienoic acid, methyl ester
Sterols			
9	32.208	11.56	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24s)-
10	32.342	3.67	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24s)-
Terpenes			
12	33.993	48.88	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)
Hydrocarbons			
13	34.234	4.06	Hexacontane
15	37.556	2.58	Hexacontane
Others			
2	6.472	3.29	Ethane, 1,1,2,2-tetrachloro-
4	18.085	2.49	Spiro(tetrahydrofuryl)2.1'(decalin), 5',5',8'a-trimethyl-
11	33.817	2.04	1-Propanol, 2,3-bis[(3,7,11,15-tetramethylhexadecyl)oxy]-
(c) Extraction time 30 min			
Hydrocarbons			
1	15.419	1.74	Hexadecane, 2,6,10,14-tetramethyl-
17	34.232	6.24	Tetracontane
18	35.765	6.56	Dotriacontane
19	37.570	4.83	Tetracontane
20	39.727	3.54	Dotriacontane
Fatty alcohols			
3	18.103	6.06	1-tetradecanol, acrylate
4	18.404	3.59	1-tridecanol
6	21.583	2.41	1-octadecanol
10	24.777	1.74	1-octadecanethiol
Esters			
2	16.732	8.93	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester
5	20.957	10.31	Hexadecanoic acid, methyl ester
7	21.867	4.60	Hexadecanoic acid, ethyl ester
8	23.413	9.86	9-octadecenoic acid, methyl ester
9	23.782	5.50	Octadecanoic acid, methyl ester
11	25.000	2.00	Acetic acid, octadecyl ester
Aromatic			
12	26.813	1.42	1h-purin-6-amine, [(2-fluorophenyl)methyl]-
Phenolics			
13	28.124	11.46	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-
Terpenes			
16	33.986	5.47	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)
Others			
14	30.813	2.13	Tetracosamethyl-cyclododecasiloxane
15	32.700	1.61	Tetracosamethyl-cyclododecasiloxane

Resource properties of bottle gourd

Terpenes

Terpene compounds were detected in bottle gourd extracts using methanol solvents in extraction times of 10 and 30 min, shown in Table 2. As shown in Table 3, terpene compounds were detected in all bottle gourd extracts using chloroform solvents. The total terpene compounds using methanol and chloroform solvent in 10, 20 and 30 min time of extraction represented by 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-e), were the dominant terpenes and represented 1.55 and 0.47% of total peak area for methanol extracts and 16.27, 4.88, and 5.47% for chloroform extracts. 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-e) were detected in several plants.

Bioactive compounds are identified as strong drug and biomedical activities to strengthen the body's resistance, resist fatigue, improve human immunity and protect the liver, was considered substances with great potential in the nutraceutical and pharmaceutical industries in functional and therapeutic applications (11).

Esters

Twenty-two different ester compounds were identified in the different extraction time of bottle gourd using methanol solvents (Table 2). In contrast, fourteen different ester compounds were identified using chloroform solvents with different extraction time variations (Table 3). Hexadecanoic acid, methyl ester and Hexadecanoic acid, ethyl ester is the dominant derived ester in bottle gourd extract using methanol solvents. Esters derived from Hexadecanoic acid, methyl ester represented about 2.86%, 2.80%, and 4.32%, while ester derived from Hexadecanoic acid, ethyl ester represented about 1.26%, 1.02%, and 1.60% of the total ester peak area. On extract bottle gourd using solvent chloroform, identified dominant derived ester is Hexadecanoic acid, methyl ester (37.36% and 10.31%), 9-Octadecenoic acid (Z)-, methyl ester (28.37% and 9.86%), Propanoic acid, 2-methyl-, 1-(1,1-dimethyl ethyl)-2-methyl-1,3-propanediyl ester (8.93%), Hexadecanoic acid, ethyl ester (1.57% and 4.60%), and Octadecanoic acid, methyl ester (5.50%). Some other esters in extract of bottle gourd contain useful applications, such as Octadecanoic acid, methyl ester, and 9-octadecenoic acid, methyl ester. Octadecanoic acid, methyl ester or stearic acid, methyl ester is indicated that they were suitable for biodiesel production (12). Meanwhile, 9-octadecenoic acid, methyl ester (oleic acid, methyl ester) reported having an essential role in health care, especially the treatment of cancer and other diseases (13). In this case, 9-hexadecenoic acid was used to represent various bottle gourd extracts containing polyunsaturated fatty acids (PUFA/n-7). PUFAs have been shown to play critical roles in tissue metabolism and cellular, including thermal adaptation, electron and oxygen transport, regulating membrane fluidity and may help reduce the risk of coronary heart disease (14).

Fatty acids (FA)

Fatty acids are active substrates and allopathic agents; their antibacterial effect is also well known (15). Saturated fatty acids are synthesized from acetyl coenzyme A by plants and animals as long-term energy storage forms. Saturated fatty acids have affected hypercholesterolemia and inducing cyclooxygenase-2 expression (16). As shown in Table 3, the fatty acid composition of bottle gourd extracted using methanol solvents was represented by five saturated fatty acids; n-Hexadecanoic Acid (3.18% dan 4.56%), 1,2-benzene dicarboxylic acid (0.79% and 0.42%), Propanoic acid, 2-methyl-, 1-(1,1-dimethyl ethyl)-2-methyl-1,3-pro (0.25%), 6-octadecenoic acid, (z) (2.13%), and 9,12-octadecadienoic acid (z,z) (1.35%) (Table 2). Fatty acid composition was identified using methanol solvents represented by two saturated fatty acids; Propanoic acid, 2-methyl-, 1-(1,1-dimethyl ethyl) 2-methyl-1,3-pro (2.28%) and 22.alpha.-hydroxy-3,4-secostict-4(23)-en-3-oic acid (1.84%) (Table 3). n-Hexadecanoic Acid (Palmitic acid) is a saturated long-chain fatty acid with a 16-carbon. Palmitic acid and linoleic acid were the dominant fatty acids bottle gourd extracts. Meat, kernel oil, palm oil, cheese, butter, and milk all contain palmitic acid. This type of fatty acid is reportedly used in pharmaceuticals as an antioxidant, treatment for cancer, and hypercholesterolemic prevention (17). Phthalic acid or 1,2-benzenedicarboxylic acid used in China to clean pollutants and contaminated soils (18). Linoleic acid is a source of PUFA, useful in pharmaceutical and medicine for antihistaminic, anticoronary, antieczemic, antiacne, anticancerous, analgesic and ulcerogenic (19,20).

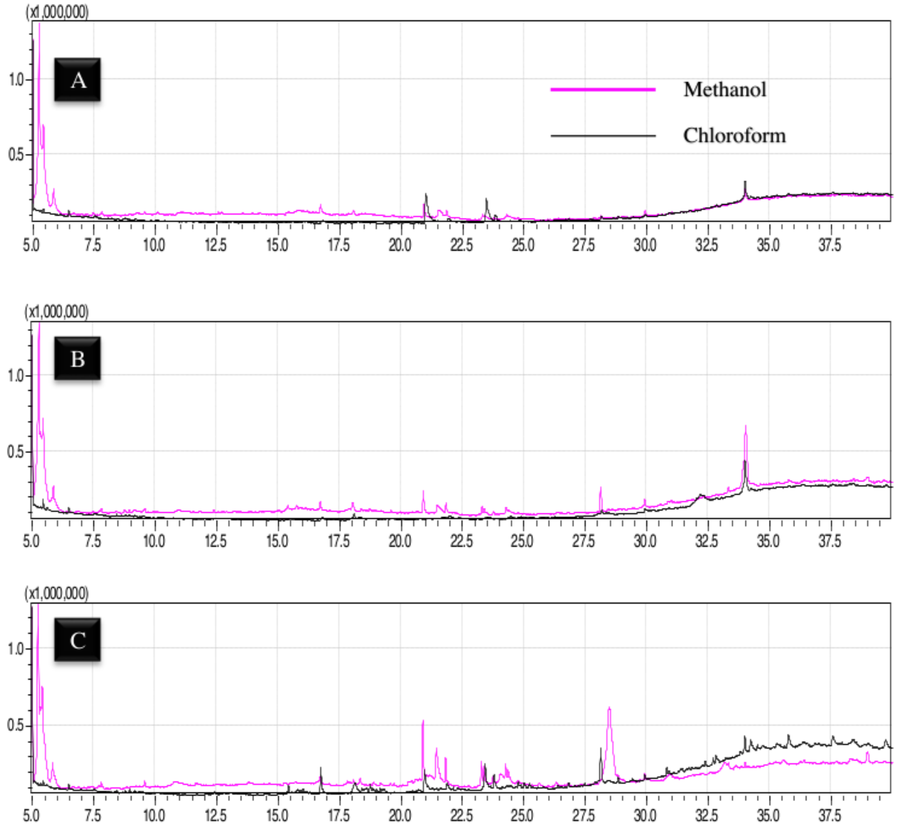

Figure 2. Chromatograms of different crude extract. (A) methanol and chloroform in 10 min time extraction; (B) methanol and chloroform in 20 min time extraction; (C) methanol and chloroform in 30 min time extraction.

Fatty alcohols

Natural fatty alcohols derived from plant or animal lipids are used in detergen, plastics, and pharmaceutical (14,21). Fatty alcohols in bottle gourd were represented by one saturated fatty alcohols; 1-hentetracontanol (0.59%) is extracted from methanol solvents, while four saturated fatty alcohols; 1-tetradecanol, acrylate (6.06%), 1-tridecanol (3.59%), 1-octadecanol (2.41%), and 1-octadecanethiol (1.74%) (Table 2-3). Saturated fat alcohols are chemical intermediates for surfactants and are widely used in pharmaceutical formulations, industrial, agrochemicals, and personal and home care products (14,21).

Table 4. Biological activity identified in bottle gourd.

Compound	Bioactivity	References
Palmitic acid/ Hexadecanoic acid	Hemolytic, 5-α reductase inhibitor, antiandrogenic, antioxidant, hypocholesterolemic, nematicide, flavor	(19,20)
Oleic acid/9-octadecenoic acid	Antiandrogenic cancer-preventive, dermatitigenic hypocholesterolemic, antiinflammatory, 5-α reductase inhibitor, anemiagenic, and flavour	(19,20)

Stearic acid/Octadecanoic acid	Cosmetic, flavour, 5-α reductase inhibitor, hypocholesterolemic	(19,20)
Linoleic acid/9,12-octadecadienoic acid	Nematicide antiarthritic, hypocholesterolemic, hepatoprotective antiandrogenic, antihistaminic, antieczemic, antiacne, anticancerous, diuretic, analgesic, 5-α reductase inhibitor, anticoronary, and ulcerogenic	(19,20)
Gamma sitosterol/Stigmast-5-En-3-Ol	Antihepatotoxic, Anticancer, antiviral, antioxidant, *Antihyperglycemic,* hypocholesterolemic	(19,22)
Myristic acid/ Tetradecanoic acid, methyl ester	Antimicrobial activity	(23)
Phenol, 2,2'-methylenebis	Antioxidant	(14)
Petroselinic acid	Antiaging and antiinflammatory	(24)
Tetracontane	Antibacterial activity	(25)
Dotriacontane	Antioxidant, Antimicrobial activity, antispasmodic	(26,27)

Phenolic compounds

GC–MS analysis of bottle gourd extracts revealed the major phenolic compounds in methanol and chloroform extracts. The percentage of total phenolic compounds that varies depending on the extraction solvent, from 2.99% in methanol extract (20 min) to 2.78% and 11.46% in chloroform with extraction time 20 and 30 min (Table 2-3). Phenolics compound were identified Phenol, 2,2'-methylenebis[6-(1,1-dimethyl ethyl)-4-methyl. Solubility of phenolic compounds, type of solvent, and polarity in the extraction had an impact on phenolic compound recovery (28). Furthermore, the polarity of the solvent is important in increasing phenolic solubility (29). Phenolic compounds have been reported for pharmacological that serve as antimicrobial activity, effects against neurodegenerative pathologies and anticarcinogenic). Research (30), antioxidant capacity in different extracts of *Lagenaria siceraria* (bottle gourd) relates directly to their phenolic content. The study identified and isolated six phenolics compounds, one of which is phenolic glycoside (*E*)-4-hydroxymethyl-phenyl-6-*O*-caffeoyl-β-d-glucopyranoside; invitro analysis showed high antioxidant activity of the compound. In addition, the fruit of *Lagenaria siceraria* (Molina) could be a rich source of natural radical scavengers (2). Analysis of free radical scavenging activity in ethanol extract show percentage of inhibition was 89.21%.

Hydrocarbons

The hydrocarbon content in bottle gourd differed between extraction solvents, representing an Undecane (0.37%) for methanol extracts (20 min). Hydrocarbons identified from chloroform solvents are represented by hexacontane (4.06 and 2.58%) with an extraction time of 20 min, while for extraction time 30 minutes more identify hydrocarbons; Hexadecane, 2,6,10,14-tetramethyl (1.74%), Tetracontane (6.24 and 4.83%), and Dotriacontane (6.56 and 3.54%), most of the identified hydrocarbons were Alkanes (5 hydrocarbons). Tetracontane and Dotriacontane were detected in *Caralluma retrospiciens (Ehrenb)* and *Asclepias Curassavica L* as antimicrobial, antifungal, and antibacterial (25,27).

Sterols

C29 sterols, also known as phytosterols, are important precursors to vitamin D. Sterols, some of their derivatives played a major role in reducing low-density lipoprotein cholesterol in vivo (31). Phytosterols in bottle gourd were represented by three different steroid compounds, for an extract time of 20 min identified Cholesta-4,6-dien-3-one (0.72%) and Stigmast-5-en-3-ol, oleat (0.59%), while for the extraction of 30 min identified more components Stigmast-5-en-3-ol, oleat (26.82 and 1.45%). Chloroform sterols identified as Stigmast-7-en-3-ol (11.56 and 3.67%) of total peak area with an extraction time of 20 min (Table 2-3). Stigmast-5-En-3-Ol, (3.Beta.,24s) or Gamma Sitosterol is the sterols with the largest amount identified in bottle gourds using methanol solvents. Cholesta-4,6-dien-

3-one and gamma sitosterol investigated from Alginate *Glycyrrhiza glabra L* and *Bidens pilosa* L was used in pharmaceuticals as a antihepatotoxic, antiviral, antioxidant, cancer preventive and hypocholesterolemic (32,33). Stigmast-7-en-3-ol investigated from Djulis (*Chenopodium formosanum Koidz.)*, can be developed in the industry as enriched functional foods and nutraceuticals. Based on literature studies, phytosterols identified by GC–MS were biologically active compounds; for example, these compounds were scientifically proven to have many health benefits, including antioxidant activities and anti-cancer (34).

Aromatic, amine and Others

Using methanol extract, two compounds from different chemical groups were identified in bottle gourd extracts and are listed in Table 2, namely 1 phthalate derivative (1.06%) and Tetrakis (2,3-ditert-butylphenyl)-4,4'-biphenylene diphosphonat (18.53%), meanwhile four compounds were identified from choloroform extract are shown in Table 3, namely Ethane, 1,1,2,2-tetrachloro (2.53% and 3.29%), Spiro (tetrahydrofuryl)2.1'(decalin), 5',5',8'a-trimethyl (2.49%), and 1-Propanol, 2,3-bis[(3,7,11,15-tetramethylhexadecyl)oxy] (2.04%). Otherwise, also identified six aromatic compounds in methanol extract, namely Ethylbenzene (51.86, 37.59, and 20.11%), P-xylene (28.62, 22.15, and 19.44%), Benzene, 1,2-dimethyl (4.18, 1.99, and 1.79%), Benzene, ethyl (4.18, 7.23, and 7.24%), Octane, 2,4,6-trimethyl (0.76%), and Octane, 3,5-dimethyl (0.36%). Two aromatic in chloroform extract, name Ethylbenzene (5.58%) and 1h-purin-6-amine, [(2-fluorophenyl)methyl] (1.42%). One amines 1-isopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1h-py (2.18%). Ethylbenzene, p-xylene and Benzene, 1,2-dimethyl is an aromatic compound that is often found in plants type cucurbitaceous one of them in *Lagenaria siceraria* fruits. Tetracosamethyl-cyclododecasiloxane that is used in the cosmetics and fragrance industry (35). The 3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol was detected in *Eucalyptus granlla* wood and used as pesticide to protect the environment (36).

Conclusion:

Many valuable compounds were found in the methanol and chloroform extracts of bottle gourd, including fatty acids, fatty alcohols, terpenes, phenolics, hydrocarbons, amines, and esters. The extraction solvent has an effect on the extracted molecules, particularly fatty acids, esters, amines, phenolics, and sterols. The presence of antioxidants and sterols (PUFA) in bottle gourd suggests potential to be used in human and animal food as a source of nutraceuticals and food functionals. Furthermore, bottle gourd potential in industrial fragrance and cosmetics was revealed high concentration of hydrocarbons, esters, and amines.. Extraction time of 20-30 min is the optimal time for maximum retention of bioactive compounds in bottle gourd. These molecules play an essential role in the development of food functionals and pharmaceutical prospects. More research is being conducted to identify various biological activities to develop better novel pharmaceutical and food functionals.

References:

1. Bera D, Lahiri D, Nag A. Studies on a natural antioxidant for stabilization of edible oil and comparison with synthetic antioxidants. J Food Eng. 2006;74(4):20–6.
2. Mayakrishnan V, Veluswamy S, Sundaram KS, Kannappan P, Abdullah N. Free radical scavenging potential of Lagenaria siceraria (Molina) Standl fruits extract. Asian Pac J Trop Med. 2013;6(1):20–6.
3. Foti MC. Antioxidant properties of phenols. J Pharm Pharmacol. 2007;59(12):1673–85.
4. Bhat S, Saini CS, Kumar M, Sharma HK. Effect of Thermal and Alternate Thermal Processing on Bottle Gourd (Lagenaria siceraria) Juice. J Food Process Preserv. 2017;41(3):1–9.
5. Upaganlawar A, Balaraman R. Cardioprotective effects of Lagenaria siceraria fruit juice on isoproterenol-induced myocardial infarction in wistar rats: A biochemical and histoarchitecture study. J Young Pharm. 2011;3(4):297–303.
6. Uttara B, Singh A, Zamboni P, Mahajan R. Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options. Curr Neuropharmacol. 2009;7(1):65–74.
7. Bhat S, Sharma HK. Combined effect of blanching and sonication on quality parameters of bottle gourd (Lagenaria siceraria) juice. Ultrason Sonochem. 2016;33:182–9.
8. Gajera RR, Joshi DC. Development and quality evaluation of bottle gourd, lagenaria siceraria (Mol.) standl. based blend juice. Indian J Nat Prod Resour. 2015;6(3):194–9.

9. Attar UA, Ghane SG. Optimized extraction of anti-cancer compound – cucurbitacin I and LC–MS identification of major metabolites from wild Bottle gourd (Lagenaria siceraria (Molina) Standl.). South African J Bot. 2018;119:181–187.
10. Attar UA, Ghane SG. In vitro antioxidant, antidiabetic, antiacetylcholine esterase, anticancer activities and RP-HPLC analysis of phenolics from the wild bottle gourd (Lagenaria siceraria (Molina) Standl.). South African J Bot. 2019;125:360–370.
11. Peng W, Li D, Zhang M, Ge S, Mo B, Li S, et al. Characteristics of antibacterial molecular activities in poplar wood extractives. Saudi J Biol Sci. 2017;24(2):399–404.
12. Cubas ALV, Machado MM, Pinto CRSC, Moecke EHS, Dutra ARA. Biodiesel production using fatty acids from food industry waste using corona discharge plasma technology. Waste Manag. 2016 Jan 1;47(Part A):149–54.
13. Ukwubile CA, Ahmed A, Katsayal UA, Ya'u J, Mejida S. GC–MS analysis of bioactive compounds from Melastomastrum capitatum (Vahl)Fern. leaf methanol extract: An anticancer plant. Sci African. 2019 Mar 1;3:1–8.
14. Abdel-Aal EI, Haroon AM, Mofeed J. Successive solvent extraction and GC-MS analysis for the evaluation of the phytochemical constituents of the filamentous green alga Spirogyra longata. Egypt J Aquat Res. 2015;41(3):233–246.
15. McGaw LJ, Jäger AK, Van Staden J. Antibacterial effects of fatty acids and related compounds from plants. Vol. 68, South African Journal of Botany. National Research Foundation; 2002. p. 417–23.
16. Lee JY, Sohn KH, Rhee SH, Hwang D. Saturated Fatty Acids, but Not Unsaturated Fatty Acids, Induce the Expression of Cyclooxygenase-2 Mediated through Toll-like Receptor 4. J Biol Chem. 2001 May 18;276(20):16683–9.
17. Ahmad M, Baba WN, Gani A, Wani TA, Gani A, Masoodi FA. Effect of extraction time on antioxidants and bioactive volatile components of green tea (Camellia sinensis), using GC/MS. Cogent Food Agric. 2015;1(1):1–11.
18. Ma T, Teng Y, Christie P, Luo Y, Chen Y, Ye M, et al. A new procedure combining GC-MS with accelerated solvent extraction for the analysis of phthalic acid esters in contaminated soils. Front Environ Sci Eng China. 2013;7(1):31–42.
19. Yusuf M, Atthamid NFU, Indriati S, Saleh R, Latief M, Rifai A. Optimization ultrasonic assisted extraction (UAE) of bioactive compound and antibacterial potential from sea urchin (diadema setosum). Curr Res Nutr Food Sci. 2020;8(2):556–69.
20. Vadivel E, Gopalakrishnan SB. GC-MS analysis of some bioactive constituents of Mussaenda frondosa Linn. Int J Pharma Bio Sci. 2011;2:313–20.
21. Roose P, Eller K, Henkes E, Rossbacher R, Höke H. Amines, Aliphatic in Ullmann's Encyclopedia of Industrial Chemistry. Ullmann's Encyclopedia of Industrial Chemistry. 2015.
22. Yusuf M, U.A. NF, Mahyati L, Imran M. Phytochemical and antibacterial properties of sea cucumber (Muelleria lecanora) from Barrang Lompo Islands, Makassar South Sulawesi. Food Res. 2020;4(6):1885–95.
23. Shobier AH, Abdel Ghani SA, Barakat KM. GC/MS spectroscopic approach and antifungal potential of bioactive extracts produced by marine macroalgae. Egypt J Aquat Res. 2016;42(3):289–299.
24. Beyzi E, Karaman K, Gunes A, Buyukkilic Beyzi S. Change in some biochemical and bioactive properties and essential oil composition of coriander seed (Coriandrum sativum L.) varieties from Turkey. Ind Crops Prod. 2017;109:74–8.
25. Alqahtani SS, Makeen HA, Menachery SJ, Moni SS. Documentation of bioactive principles of the flower from Caralluma retrospiciens (Ehrenb) and in vitro antibacterial activity – Part B. Arab J Chem. 2020;13(10):7370–7377.
26. Soosairaj S, Dons T. Bio-active compounds analysis and characterization in ethanolic plant extracts of Justicia tranquebariensis L. (Acanthaceae) – Using GC-MS. Int J ChemTech Res. 2016;9(7):260–5.
27. Bihana S, Dhiman A, Singh G, Satija S. Gas chromatography-mass spectroscopy analysis of bioactive compounds in the whole plant parts of ethanolic extract of Asclepias Curassavica L. Int J Green Pharm. 2018;12(2):107–14.
28. Dent M, Dragović-Uzelac V, Penić M, Brñić M, Bosiljkov T, Levaj B. The effect of extraction

solvents, temperature and time on the composition and mass fraction of polyphenols in dalmatian wild sage (Salvia officinalis L.) extracts. Food Technol Biotechnol. 2013;51(1):1–10.

29. Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. Vol. 41, Journal of Pharmaceutical and Biomedical Analysis. 2006. p. 1523–42.
30. Mohan R, Birari R, Karmase A, Jagtap S, Bhutani KK. Antioxidant activity of a new phenolic glycoside from Lagenaria siceraria Stand. fruits. Food Chem. 2012;132(1):244–51.
31. Francavilla M, Trotta P, Luque R. Phytosterols from Dunaliella tertiolecta and Dunaliella salina: A potentially novel industrial application. Bioresour Technol. 2010 Jun 1;101(11):4144–50.
32. Akhtar R, Shahzad A. Alginate encapsulation in Glycyrrhiza glabra L. with phyto-chemical profiling of root extracts of in vitro converted plants using GC-MS analysis. Asian Pac J Trop Biomed. 2017;7(10):855–61.
33. Shen Y, Sun Z, Shi P, Wang G, Wu Y, Li S, et al. Anticancer effect of petroleum ether extract from Bidens pilosa L and its constituent's analysis by GC-MS. J Ethnopharmacol. 2018 May 10;217:126–33.
34. Huang CY, Chu YL, Sridhar K, Tsai PJ. Analysis and determination of phytosterols and triterpenes in different inbred lines of Djulis (Chenopodium formosanum Koidz.) hull: A potential source of novel bioactive ingredients. Food Chem. 2019 Nov 1;297:124948.
35. Fadle N, Abdalbasit Adam Mariod, Hiba Abdel Rahman Ali, Alfatih Ahmed. TLC and GC-MS analysis of fermented wood "Nikhra" petroleum ether fraction of Combretaceae spp. Combretum hartmannianum and Terminalia laxiflora. Eurasian J For Sci. 2018;6(3):1–7.
36. Ge S, Peng W, Li D, Mo B, Zhang M, Qin D. Study on antibacterial molecular drugs in Eucalyptus granlla wood extractives by GC-MS. Pak J Pharm Sci. 2015;28(4):1445–1448.

Bottle Gourd (GC-MS)

ORIGINALITY REPORT

15%	13%	16%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	s3-eu-west-1.amazonaws.com Internet Source	3%
2	s3.amazonaws.com Internet Source	2%
3	Suheela Bhat, Harish Kumar Sharma. "Combined effect of blanching and sonication on quality parameters of bottle gourd (Lagenaria siceraria) juice", Ultrasonics Sonochemistry, 2016 Publication	2%
4	www.tandfonline.com Internet Source	2%
5	www.cepi.org Internet Source	1%
6	Xiaoyun Tao, Hongnan Sun, Jian Chen, Luning Li, Yin Wang, Aidong Sun. "Analysis of Polyphenols in Apple Pomace using Gas Chromatography-Mass Spectrometry with Derivatization", International Journal of Food Properties, 2014 Publication	1%

7	dlisv03.media.osaka-cu.ac.jp Internet Source	1 %
8	U.A. Attar, S.G. Ghane. "Optimized extraction of anti-cancer compound – cucurbitacin I and LC–MS identification of major metabolites from wild Bottle gourd (Lagenaria siceraria (Molina) Standl.)", South African Journal of Botany, 2018 Publication	1 %
9	bmcresnotes.biomedcentral.com Internet Source	1 %
10	Eman I. Abdel-Aal, Amany M. Haroon, Jelan Mofeed. "Successive solvent extraction and GC–MS analysis for the evaluation of the phytochemical constituents of the filamentous green alga Spirogyra longata", The Egyptian Journal of Aquatic Research, 2015 Publication	1 %
11	patents.google.com Internet Source	1 %
12	www.toxicology.org Internet Source	1 %

Exclude quotes On Exclude matches < 1%