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Authors: Muhammad Yusuf, Sri Indriati, Nur Fitriani Usdyana Attahmid, Rahmawati Saleh, Akhmad Rifai

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EFFECT OF EXTRACTION TIME ON THE BIOACTIVE COMPOUNDS OF BOTTLE GOURD *(LAGENARIA SICERARIA)* USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Abstract

Traditional medical systems have always been played an important role in meeting global healthcare needs. Meanwhile, bottle gourd *(Lagenaria siceraria)* is a vegetable that contains health-promoting secondary metabolites. Therefore, this study aims to determine the bioactive compounds profiling of Bottle gourd *(Lagenaria siceraria)* fruit extracts in methanol and chloroform using gas chromatography-mass spectrometry (GC-MS) with variations in extraction time of 10, 20, and 30 min using GC-MS RTX-5 capillary column. A total of 91 compounds were tentatively identified, with 55 found in methanol and 41 in chloroform extract. The 1:2 (v/v) ratio using methanol solvent at 30 min was suggested as the most suitable time for maximum extraction. Several peaks with high area percentages were discovered in the methanolic extract containing key chemical constituents such as stearic, oleic, palmitic, and linoleic acid, as well as Cholesta-4,6-dien-3-one, gamma sitosterol, and Phenol, 2,2'-methylene bis. Meanwhile, the corresponding constituents from chloroform extract include Tetracontane, Dotriacontane, Phenol, 2,2'-methylenebis, 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, as well as sterols represent 40% of the total extracts and were dominated by oleic, and palmitic acid, gamma-sitosterol along with its ethyl and methyl esters. Based on the results, bottle gourd contains various valuable compounds, indicating its pharmaceutical, biomedical, and food functional potential.

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

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INTRODUCTION

Plants with high antioxidant levels, such as vitamin C, tocopherols, polyphenols and carotenoids, are gaining popularity in the food industry as alternatives to synthetic antioxidants which have limited use due to safety concerns [1]. Meanwhile, synthetic antioxidants have long been used for foods to prevent lipid oxidative rancidity, nutritional loss, off-flavor, quality loss, and discoloration. Aside from extending the shelf life of foods, these compounds also slow the progression of various oxidative stress-related chronic diseases in humans. Furthermore, due to the role in protecting the body from reactive nitrogen species, crystallization, reactive oxygen species, and free radicals from either normal metabolic processes or external sources, dietary antioxidants play an essential role as nutraceuticals [2–4]. Several mechanisms are presumably involved in this protection, including inhibition of free radical generation, increased scavenging capacity against free radicals, reduced capacity, and metal chelating ability. These reactions are commonly used in antioxidant activity tests. A wide range of activities is determined using antioxidant activity assays with the lipidic system as a substrate [3,5].

Bottle gourd *(Lagenaria siceraria)* is relatively easy to plant and the planting area is spread in various parts of the world, ranging from tropical to subtropical climates, as well as highlands to the lowlands. This plant is rich in nutrients containing calcium, iron, vitamin C, polyphenols and saponins which are beneficial for health, therefore, it is taken as daily food. Furthermore, bottle gourd is a common vegetable due to its high choline, phenolics, vitamin B complex, and vitamin C content [6], while the juice is well-known for its cardioprotective, cardiotonic, aphrodisiac, and diuretic properties, as well 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 [7]. Meanwhile, the ability of bottle gourd juice (BGJ) to be used as a health drink, is dependent on the extraction and preservation of functional components such as phenolics, carotenoids, and ascorbic acid. Therefore, the processing method selected is essential due to the presence of heat-sensitive components like phenolics, carotenoids, ascorbic acid, and the perishable nature of the product. The best method is the fresh bottle gourd juice extraction. To date, no

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attempt has been made to investigate the effects of processing on bottle gourd juice functional components to store and improve efficiency.

Bottle gourd contains phytochemicals that are beneficial to the body and also produce reactive oxygen species. Meanwhile, the 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. Furthermore, antioxidants, such as carotenoids, flavonoids, polyphenolics, vitamin A, vitamin C, and vitamin E are abundant in vegetables and fruits, preventing free radical damage and lowering the risk of chronic diseases. Therefore, the consumption of dietary antioxidants from these sources potentially prevents cardiovascular diseases, especially atherosclerosis [8]. Bottle gourd analysis using the ohmic thermal method with variations in temperature and time combined with Gas and Liquid chromatography-mass spectrometry was used to detect volatile and non-volatile phenolics. The ohmically blanched samples exhibited maximum extraction of phenolics and better color of BG juice compared to other samples [9], while the free radical scavenging activity of *Lagenaria siceraria* fruit ethanolic extract using the FRAP method and ethanol solvents was 1.95 mg/ml [3]. In other studies, a combination of the blanching process and sonication extraction to improve the quality of gourd juice bottle showed significant improvements in the total phenolics (TP), 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), as well as yeast and mold count experienced a significant decrease [10]. The formulations of blended bottle gourd juice, aonla, lemon, and ginger using response surface methodology (RSM) with minimal thermal process showed quality stability against physicochemical, sensory, and microbiology parameters [11]. Moreover, bottle gourd optimization using acetone, ethanol, and methanol solvents with Liquid Chromatography-Mass Spectrometry (LC-MS) analysis found the presence of tetracyclic triterpene–cucurbitacin, as well as other pharmaceutically essential compounds [12]. Another study performed an in-vitro analysis of wild bottle gourd against antioxidant content, antidiabetic, anti-acetylcholine esterase, and anticancer activities using Reversed-Phase-High Performance Liquid Chromatography (RP-HPLC) and FTIR spectroscopy. It was concluded that wild bottle gourd is a rich source of bioactive metabolites [13,14].

Therefore, this study aims to investigate and characterize the bioactive compounds in the different crude extracts of Bottle gourd *(Lagenaria siceraria)* to determine the physiological, pharmacological, and flavor. Meanwhile, bottle gourd is a medicinal plant and has been attributed to beneficial health effects, but there are only a few studies related to this topic. However, the effect of extraction time with sonication and solvents variations has not been reported. In general, the analysis of bioactive compounds is usually conducted using gas chromatography-mass spectrometry (GC-MS).

MATERIAL AND METHODS

Materials

Bottle gourd was purchased from the local market and stored at room temperature for 24 h (Fig. 1), while the chemicals used include analytical grade hexane (emsure 99%), methanol (emsure 99.8%), and chloroform (supelco 99%) supplied by Merck Millipore (Burlington, Massachusetts, United States). Moreover, the instruments used include Shimadzu 2010 GC-MS, Elma Ultrasonic Cleaner S60H, and Buchi Rotary Rotavapor R-300.

Preparation of the extract

Bottle gourd was picked and washed with flowing tap water after separating the fruit into epicarp, mesocarp, and seeds. The fresh fruit was then homogenized, for example, the mesocarp was ground separately in an electric mixer grinder. To extract the sample with a ratio of 1:2, 20 ml of bottle gourd juice was mixed with 40 ml hexane (v/v) and transferred to a conical flask which was then immersed in an ultrasonic bath (Elma Ultrasonic) at 40ºC for 20 min. Finally, the filtrate was used for sonication extraction using methanol and chloroform solvents.

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Fig. 1: Bottle gourd.

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). Furthermore, all the conical flasks were immersed in an ultrasonic bath (Elma Ultrasonic) with a temperature of 40ºC, for 10, 20, and 30 min.

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

This was carried out using Shimadzu 2010 GC-MS and RTX-5 capillary column (30 mm x 0.25 mm x 0.25 µm) with a split ratio of 40:1 and a temperature of 70ºC, heating rate of 10ºC min^{-1}, up to 300ºC, maintained for 5 min with a total analysis time of 25 min. Helium was used as a carrier gas flowing constantly at 1.0 ml/min and the temperature of the inlet was 280ºC, pre-column pressure was 80 kPa, and ionization voltage of 70 eV [15].

RESULTS AND DISCUSSION

Identification of bioactive compound by GC–MS

The 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), while the chemical constituents were analyzed using gas chromatography–mass spectrometry. Fig. 2 illustrates the chromatograms of two crude extracts, with 100 different identified compounds (Tables 2-3), which were then classified into ten chemical groups based on the common name, retention time (Rt), and percent peak area. The chemical groups identified include esters derived from fatty acids, fatty alcohols, fatty acids (FA), amines, aromatic, phenolics, hydrocarbons, terpenes, and sterols, among others. Furthermore, the bioactive compounds were identified using NIST 2.7 and Willey 8 libraries in GC-MS. The chloroform extract contained the fewest compounds (10) after 10 min extraction time, while the highest (25) was identified in the methanol extract after 30 min (Table 2).

Resource properties of bottle gourd

Terpenes

Terpenes were detected using methanol solvents in extraction times of 10 and 30 min as shown in Table 2. Furthermore, Table 3 shows that terpenes were detected in all bottle gourd extracts using chloroform solvents. The total terpenes using both solvents in 10, 20 and 30 min extraction time represented by 2,6,10,14,18,22-tetracosahexaene, and 2,6,10,15,19,23-hexamethyl-, (all-e), were the most dominant and represented 1.55 and 0.47% of total peak area for methanol extracts as well as 16.27, 4.88, and 5.47% for chloroform extracts. Meanwhile, 2,6,10,14,18,22-tetracosahexaene, and 2,6,10,15,19,23-hexamethyl-, (all-e) have been detected in several plants. These bioactive compounds were identified as strong drugs with biomedical activities to strengthen the body's resistance, resist fatigue, improve human immunity, protect the liver, and were considered substances with great potential in the nutraceutical and pharmaceutical industries in functional and therapeutic applications [16].

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

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Peak	R_t	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_t	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

Esters

Twenty-two different esters were identified with varying extraction times using methanol solvents (Table 2) and fourteen using chloroform (Table 3). Hexadecanoic acid, methyl and ethyl esters were the most dominant derivative using methanol solvent amounting to 2.86%, 2.80%, and 4.32%, while ester derived from Hexadecanoic acid, ethyl ester represented approximately 1.26%, 1.02%, and 1.60% of the total ester peak area. Meanwhile, the extract with chloroform solvent, identified 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%) as the most dominant derivatives. Other beneficial esters in the extract of bottle gourd, include Octadecanoic acid, methyl ester, and 9-octadecenoic acid, methyl ester. Octadecanoic acid, methyl ester or stearic acid, is suitable for biodiesel production [17], while 9-octadecenoic acid, methyl ester (oleic acid, methyl ester) reportedly plays an essential role in health care, especially in the treatment of cancer and other diseases [18]. In this case, 9-hexadecenoic acid was used to represent various bottle gourd extracts containing polyunsaturated fatty acids (PUFA/n-7). Besides, PUFAs have been shown to play critical roles in tissue metabolism and cellular, including thermal adaptation, electron and oxygen transport, regulation of membrane fluidity and potentially reduce the risk of coronary heart disease [19].

Fatty acids (FA)

Fatty acids are active substrates and allopathic agents with a widely known antibacterial effect [20]. Saturated fatty acids are synthesized from acetyl coenzyme A by plants and animals as long-term energy storage forms, while saturated fatty acids affect hypercholesterolemia and induce cyclooxygenase-2 expression [21]. As shown in Table 3, the fatty acid composition of bottle gourd, extracted using methanol solvents was represented by five saturated fatty acids namely n-Hexadecanoic Acid (3.18% and 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). Meanwhile, the fatty acid composition identified using chloroform as a solvent showed two saturated fatty acids namely 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. Based on the results, palmitic 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 [22]. Meanwhile, Phthalic acid or 1,2-benzenedicarboxylic acid is used in China to clean pollutants and contaminated soils [23], while Linoleic acid is a source of PUFA, useful in pharmaceutical and medicine for antihistaminic, anticoronary, antieczemic, antiacne, anticancerous, analgesic and ulcerogenic [15,24].

Fatty alcohols

Natural fatty alcohols derived from plant or animal lipids are used as detergents, plastics, and in pharmaceuticals [19,25]. The bottle gourd extracted from methanol solvent contains one saturated fatty alcohols namely 1-hentetracontanol (0.59%), while four saturated fatty alcohols namely 1-tetradecanol, acrylate (6.06%), 1-tridecanol (3.59%), 1-octadecanol (2.41%), and 1-octadecanethiol (1.74%) were identified with chloroform (Table 2-3). Saturated fat alcohols are chemical intermediates for surfactants and are widely used in pharmaceutical formulations, agrochemicals, as well as personal, and home care products [19,25].

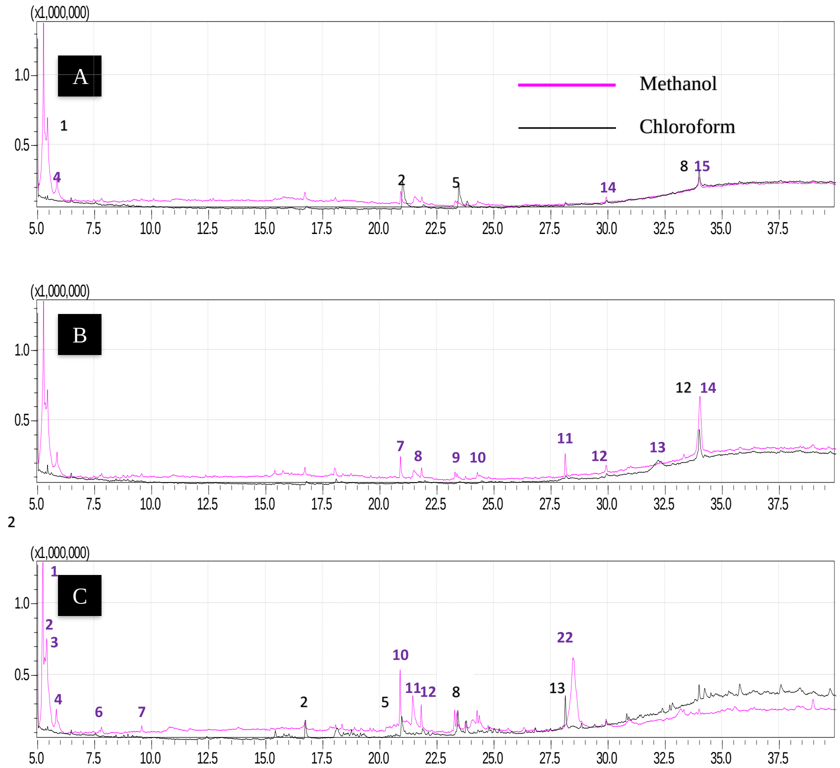

Fig. 2: Chromatograms of different crude extracts. (A) methanol and chloroform in 10 min time extraction; (B) methanol and chloroform in 20 min time extraction; (C) methanol and chloroform in 30 min time extraction.

Phenolic compounds

The GC–MS analysis of bottle gourd extracts showed the major phenolics in methanol and chloroform extracts. The percentage of total phenolics varies depending on the extraction solvent, ranging from 2.99% in methanol extract (20 min) to 2.78% and 11.46% in chloroform with an extraction time of 20 and 30 min (Table 2-3). The identified phenolics include Phenol, and 2,2'-methylenebis-6-(1,1-dimethyl ethyl)-4-methyl. Meanwhile, solubility, type of solvent, and polarity in the extraction influence phenolics recovery [32]. Furthermore, the polarity of the solvent is important in increasing solubility [33]. Phenolic compounds have been used pharmacologically as antimicrobial, against neurodegenerative pathologies, and anticarcinogenic. Another study by [34], reported that the antioxidant capacity of different *Lagenaria siceraria* (bottle gourd) extracts relates directly to the phenolic content. The study identified and isolated six phenolics compounds, including phenolic glycoside (*E*)-4-hydroxymethyl-phenyl-6-*O*-caffeoyl-β-d-glucopyranoside which has high antioxidant activity according to the in-vitro analysis. In addition, the fruit of *Lagenaria siceraria* (Molina) is a potentially rich source of natural radical scavengers [3]. Analysis of free radical scavenging activity in ethanol extract showed that the percentage of inhibition was 89.21%.

Hydrocarbons

The hydrocarbon content in bottle gourd differed between extraction solvents, with Undecane (0.37%) for methanol and hexacontane (4.06 and 2.58%) for chloroform with an extraction time of 20 min. Meanwhile, for the 30 min, more hydrocarbons were identified namely Hexadecane, 2,6,10,14-tetramethyl (1.74%), Tetracontane (6.24 and 4.83%), and Dotriacontane (6.56 and 3.54%), with the majority being Alkanes (5 hydrocarbons). Tetracontane and Dotriacontane have been detected in *Caralluma retrospiciens (Ehrenb)*, while *Asclepias Curassavica L* has antimicrobial, antifungal, and antibacterial effects [29,31].

Sterols

C29 sterols, also known as phytosterols, are important precursors of vitamin D, while some of the derivatives play a major role in reducing low-density lipoprotein cholesterol in-vivo [35]. Phytosterols in bottle gourd were represented by three different steroids, for an extraction time of 20 min, Cholesta-4,6-dien-3-one (0.72%) and Stigmast-5-en-3-ol, oleat (0.59%) were identified, while more components such as Stigmast-5-en-3-ol, oleat (26.82% and 1.45%) were identified at 30 min. Furthermore, chloroform sterols were identified as Stigmast-7-en-3-ol (11.56 and 3.67%) at total peak area with an extraction time of 20 min (Table 2-3), while stigmast-5-En-3-Ol, (3.Beta.,24s) or Gamma Sitosterol is the most predominant in bottle gourd using methanol solvent. Cholesta-4,6-dien-3-one and gamma sitosterol investigated from Alginate *Glycyrrhiza glabra L* and *Bidens pilosa* L are used in pharmaceuticals as an antihepatotoxic, antiviral, antioxidant, cancer preventive, and hypocholesterolemic [36,37], while Stigmast-7-en-3-ol investigated from Djulis (*Chenopodium formosanum Koidz.)*, has great potential to be developed in the industry as enriched functional foods and nutraceuticals. Based on literature studies, the phytosterols identified by GC–MS are biologically active compounds and have several health benefits, including antioxidant activities and anti-cancer [38].

Aromatic, amine and Others

Using methanol extract, two compounds from different chemical groups were identified and are listed in Table 2, namely 1 phthalate derivative (1.06%) and Tetrakis (2,3-ditert-butylphenyl)-4,4'-biphenylene diphosphonat (18.53%), while four compounds were identified from choloroform extract as 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%). In addition, six aromatic compounds were identified in the 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%) while Ethylbenzene (5.58%) and 1h-purin-6-amine, [(2-fluorophenyl) methyl] (1.42%) were found in the chloroform extract. Also one amines namely 1-isopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1h-py (2.18%) was found. Meanwhile, ethylbenzene, p-xylene and benzene, 1,2-dimethyl are aromatic compounds often found in cucurbitaceous plants such as *Lagenaria siceraria* fruits. Tetracosamethyl-cyclododecasiloxane is commonly used in the cosmetics and fragrance industry [39], while 3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)] undecan-1-ol was detected in *Eucalyptus granlla* wood and is used as pesticide to protect the environment [40].

CONCLUSION

Several valuable compounds were found in the methanol and chloroform extracts of bottle gourd, including fatty acids and alcohols, terpenes, phenolics, hydrocarbons, amines, and esters. The solvent used affects the extracted compounds, particularly fatty acids, esters, amines, phenolics, and sterols, while the presence of antioxidants and sterols (PUFA) in bottle gourd suggests its potential as a source of nutrient in human and animal foods. Furthermore, bottle gourd potential in industrial fragrance and cosmetics was demonstrated by the high concentration of hydrocarbons, esters, and amines. The extraction time of 20-30 min is the optimal range for maximum retention of bioactive compounds. Meanwhile, the identified compounds play an essential role in the development of food functionals and pharmaceutical prospects. However, further studies are needed to identify the various biological activities for the better development of novel pharmaceuticals and food functionals.

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	15,24
Oleic acid/9-octadecenoic acid	Antiandrogenic cancer-preventive, dermatitigenic hypocholesterolemic, antiinflammatory, 5-α reductase inhibitor, anemiagenic, and flavor	15,24
Stearic acid/Octadecanoic acid	Cosmetic, flavor, 5-α reductase inhibitor, hypocholesterolemic	15,24
Linoleic acid/9,12-octadecadienoic acid	Nematicide antiarthritic, hypocholesterolemic, hepatoprotective antiandrogenic, antihistaminic, antieczemic, antiacne, anticancerous, diuretic, analgesic, 5-α reductase inhibitor, anticoronary, and ulcerogenic	15,24
Gamma sitosterol/Stigmast-5-En-3-Ol	Antihepatotoxic, Anticancer, antiviral, antioxidant, *Antihyperglycemic*, hypocholesterolemic	15,26
Myristic acid/ Tetradecanoic acid, methyl ester	Antimicrobial activity	27
Phenol, 2,2'-methylenebis	Antioxidant	19
Petroselinic acid	Antiaging and antiinflammatory	28
Tetracontane	Antibacterial activity	29
Dotriacontane	Antioxidant, Antimicrobial activity, antispasmodic	30,31

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Competing Interests

The authors declare that there are no competing interests.

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Authors' Contributions

The author(s) read and approved the final manuscript.

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Manuscript Title: **EFFECT OF EXTRACTION TIME ON THE BIOACTIVE COMPOUNDS OF BOTTLE GOURD (LAGENARIA SICERARIA) USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY**

Authors: Muhammad Yusuf, Sri Indriati, Nur Fitriani Usdyana Attahmid, Rahmawati Saleh, Akhmad Rifai

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