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Phytochemical and Nutritional Studies of Bottle Gourd (*Lagenaria Siceraria* Ls) Cultivated In Egyptian Habitat

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Abstract

In this study, the phytochemical and nutritional valueofthe different parts of Bottle gourd; *Lagenaria siceraria* LS, cultivated in Egypt has been evaluated. Particularly, extracts of fresh/dried fruits, fresh/roasted seeds, and fresh/dried leaves of Bottle gourd were studied. Analysis of minerals, amino acids, fatty acids, phenolic compounds, flavonoids, and isoflavonoids was investigated together with the GC-MS tentative visualization of the unpolar compounds in fruits, seeds, and leaves, establishing their unique constituents. According to this study, the bottle gourd parts were confirmed to contain eleven minerals, seventeen amino acids, twenty-one phenolic analogs, ten flavonoids and four isoflavonoids. Moreover, the fresh/roasted seeds are rich with seventeen fatty acids where linoleic acid (C18:2 ω6) was the most abundant among them. Based on GC-MS analysis, it has been recognized that dry fruits extract revealed the presence of thirty compounds, meanwhile the fresh seeds indicated the presence of eighteen compounds, while roasted seeds hexane soluble fraction exhibited the existence of sixteen compounds and the dichloromethane soluble fraction of roasted seeds revealed the presence of ten compounds, and finally, the air-dried leaves revealed the presence of fourteen compounds.

Keywords: *Lagenaria siceraria* LS; Phytochemical Profiling; Nutritional Potency; GC-MS analysis

1. Introduction

The deficient availability of sources for animal protein and the high expense of the little available protein plant sources has triggered intense research into utilizing the nutrient capacities of lesser-known underutilized leguminous plants and oil crops [1].Bottle gourd;*Lagenaria siceraria* LS, represents the most widespread fruit vegetable in the world[2]. It is a member of the family Cucurbitaceae [2]. It is planted worldwide for its vast nutrition and medicinal properties [2]. It also has a main contribution to the economically cultivated species to producevegetables. The plant includes about 118 genera and 825 species, which are extensivelyspread in the hotter regions of the World [3-5]. Ethnobotanical,epidemiological,and traditional information related to the medicinal and nutritional capacity of bottle gourd has been extensively reviewed [6-12]. Cucurbits (family *Cucurbitaceae*) seeds have been reported as strong sources of food, particularly protein and oil, where dehulled seeds of cucurbit were reported to have about 50% oil and up to 35 % protein [13,14].

Biologically, Bottle gourd'sdifferent parts have been utilized to cure various diseases such as hypertension,asthma, cardiac, bronchial,fever, ulcer,jaundice, and skin problems [15]. Also, it is used as aemetic,diuretic, cooling sedative,purgative, and cardiotonic. It also possesses various therapeutic properties such as antidiabetic, antidote, antioxidant,aphrodisiac, anti-analgesic, inflammatory, and cardioprotective [16,17]. Numerous studies have also showed that Bottle gourd acted as an anticancer and antidiabetic agent [6]. Besides, Bottle gourd is as wellemployed to treat several neurological disorders as Alzheimer's disease owing to the existence of precursor-molecule 'choline' which is necessaryfor the production of neurotransmitter 'acetylcholine' [5]. Nutritional assessment reveals that bottle gourd is a rich resource of many nutrients as vitamin B, C, fibers, pectin, amino acids, proteins, beta-carotene, and glycosides [6]. It has been confirmed that bottle gourd is an exceptional gift to humansbecause of its important constituents essential for healthy normal

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and life [15]. Previous studies uncovered the presence of flavonoids, triterpenoids, polyphenols, saponins [18], campesterol,and fucosterol[19], C-flavone glycosides [20], lagenin[21] and cucurbitacin I [22]. In addition, various bioactive metabolites possessing pharmaceutical potential have been found in the fruit'sdifferent parts [22].

Because of the occurrence of a large array of bioactive constituents particularly phenolic compounds, biological activities, and applications in medicine, bottle gourd fruit is of important scientific interest and a potential food to be explored for its phenolic substances. Therefore, the present studies were conducted in this context. Based on earlier reports, bottle gourd is having noteworthy medicinal properties, so the present study was concentrated on a detailed assessment of phytochemicals of bottle gourd parts (fruit, seeds, and leaves), cultivated in Egypt.

2. Experimental Section

2.1. Bottle gourd collection and sampling

Bottle gourd parts (fruits [Fresh (A), air dried (B)], seeds [fresh (C), roasted (D)] and leaves [fresh (E), air dried (F)]) were obtained in June 2018 and June 2019 from the greenhouses of Vegetable Research Department Humidity Pollination, Horticultural Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

~Ca 40 kg of the whole fresh fruit of Bottle gourd(A) were harvested, washed, and cut to slices, followed by air-drying at ambient temperature. After complete drying, the obtained pieces of the dried fruits were grinded by mill, affording 2 kg of dried powder [B]. Likely, 50 kg of fresh green leaves (E) of the plant have been applied to air drying and grinding resulting in 2 kg of powder [F]. On the other hand, three kg of untreated dry seeds of bottle gourd were grinded by mill to obtain 1.3 kg approximately after removing the combined peels [C]. Further three kg of the dried seeds of bottle gourd were roasted using a roasting machine and then grinded by mill, giving 1.25 kg net weight after removing of the combined peels [D].

2.2. Bottle Gourd extracts Preparation for biological activity and GC-MS investigation

For performing the biological studies and GC-MS analysis of the Bottle gourd, 20 g powders of the fruits (B), fresh seeds (C), roasted seeds (D) and leaves (F) were individually soaked (3 times) in 1L conical flask each containing ~0.8 L methanol and kept for 48 hrs at ambient temperature. The obtained methanol extracts were filtered, and then concentrated *in vacuo* affording the desired extracts. For the biological activity studies, a fixed weight of each sample of the original extract was obtained and submitted to the desired activity testing. On the other hand, a small part of the original extracts was mixed with n-hexane or dichloromethane, and the soluble hexane/DCM fractions were filtered, and applied to drying at ambient temperature, containing the lipidic/unpolar components for the GC-MS analysis.

2.2.1. Estimation of Minerals

The minerals content was determined according to (AOAC, 2012)[23] using the flame photometer (Gallen, Kamp, FGA 330, England). Meanwhile, magnesium was determined using Perkin-Elmar (Model 80, England) Atomic Absorption Spectrophotometer.

2.2.2. Determination of Amino acids

Bottle gourd different parts, fruits, seeds, and leaves were tested for their amino acids content by employing High Performance Amino Acid Analyzer (Bekman 7300) according to the method of AOAC (2005)[24].Indispensable amino acid (IAA) score was estimatedby comparison with the reference protein pattern of FAO/WHO (1973)[25]bythe equation: <u>Amino acid score = mg amino acid in 1 gm protein / mg amino acid,</u> suggested by FAO/WHO.

2.3. Estimation of fatty acids in Bottle gourd seeds

Bottle gourd seed oil was estimated for the fatty acids as methyl ester by using gas liquid chromatography. BF_3 in methanol (14%) as a methylating agent was used to prepare the fatty acids methyl esters in the seed oil according to AOAC (2005) [24]. The fatty acid methyl esters were analyzed with a GCV Hewlett Packard gas chromatography model 5890 equipped with a dual-flame ionization detector and dual-channel recorder.

2.4. Determination of total phenolic content

Different plant parts of bottle gourd were analyzed for their total phenolic content using a FolinCiocalteu assay according to the method of Singleton and Rossi (1965) [26].

2.5. Determination of phenolic compounds by HPLC

Phenolic and aromatic compounds were evaluated by HPLC according to the method of Goupy *et al*. (1999)[27].

2.6. Determination of total flavonoids and isoflavonoids content

Total flavonoid content was detected by $AlCl_3$ colorimetric assay by using the method of Tacouri et al. (2013)[28].

2.7. Determination of flavonoid and isoflavonoid compounds by HPLC.

Flavonoid fractions were also chracterized by HPLC according to the method of Mattila *et al*. (2000) [29].

2.8. GC-MS Analysis

The chemical constituents of the samples ofbottle gourd were analysed using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness).The components were detected by comparing their mass spectra with those of WILEY 09 and NIST 14 mass spectral database [30].

3. Results and Discussion

3.1 Chemical Composition of fruit, seeds and leave extracts of Bottle gourd

3.1.1 Minerals Evaluation in Fruit, Seeds, and Leaves

The mineral contents in bottle gourd parts: dry fruits, seeds (fresh and roasted) and dry leaves, were estimated, revealing the presence of eleven elements with diverse quantities: Calcium, Phosphorus, Potassium, Magnesium, Sodium, Zinc, Selenium, Chromium,Iron, Manganese, and Copper (Table 1). These elements are occurred in the range between high, moderate, and low contents according to the plant part and element type specifically. Particularly, leaves are the most abundant with Calcium (4000 mg/100 g), Sodium (90 mg/100 g), Iron (110 mg/100 g), Manganese (8.49 mg/100 g), and Selenium (0.038 mg/100 g). On the other hand, seeds contain the highest content of Phosphorus (1400 mg/100 g in roasted, 1200 mg/100g in fresh) and Zinc (10.99 mg/100 g in fresh seeds). Fruits among the different plant parts were shown specifically to be rich in Copper (17.3 mg/100g) and Chromium (0.132 mg/100 g) along with an abundance of Potassium and Iron as well.In contrast with those reported in the literature, selenium is existing herein instead of Cobalt reported by Hassan et al [31]. The difference in such constitution of minerals could be most likely attributed to differences in environments and cultivating habitats. Generally, the mineral composition of the seeds and leaves of bottle gourd was found to be relatively high, indicating them to be good sources of dietary elements [31].

3.1.2 Amino acids study in Fruit, Seeds, and Leaves

The whole contents of amino acids (%) located in fruit, seeds, and leaves were visualized to be 7.15, 30.82/31.34 (fresh/roasted seeds), and 19.24, respectively, signifying the presence of 17 amino acids (isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, valine, alanine, arginine, aspartic acid, glutamicacid,glycine, histidine, proline, and serine) (AA's, Table 2). According to this study, seeds had the highest amount of crude protein. Glutamic acid, a non-essential amino acid [32], was the most abundant amino acid in the whole parts of the plant at where seeds recorded the highest abundance (5.16/5.44 %), meanwhile, the fruits revealed the lowest percentage of it (1.28 %). After glutamic acid abundance, leucine and aspartic acid are being the predominant amino acids. Moreover, arginine was recorded, after glutamic acid, as the second abundant amino acid in seeds (4.66/4.72 %). In contrast, cysteine displayed the lowest abundance among the profile of whole recorded amino acids. It is worthy however, to mention that tryptophane was not recorded in this evaluation as matched with literature [31].

3.1.3 Fatty Acid Composition of L. Siceraria Roasted and Fresh Seed Oils

Plant oils are one of the key constituents that can be cheaply obtained from biomass and processed readily to afford the appropriate raw material for chemical industries [33]. Plants oils have both edible and non-edible applications [34,35]. Four main categories of fatty acids were identified in the oil of fresh and roasted seeds of Bottle gourd: palmitic acid [C16:1] (15.0 %, 14.23 %), stearic acid [C18:1] (6.62 %, 6.29 %), oleic acid [C18:1] (16.07 %, 15.71 %) and linoleic acid [C18:2] (66.10, 60.34 %)(Table 3, Figure S1). Linoleic acid was the most abundant fatty acid in the oil. Altogether, sixteen different fatty acids were displayed herein along with non-identified ones, which have been started with caprylic acid [C8:0] and ended by erucicacid [C22:1 ω9]. Evaluation of the roasted seed oils revealed the presence of fifteen identified fatty acids. In contrast, only eleven identified fatty acids were shown the fresh seeds oil comparatively, in roasted seed oils, five different fatty acids were shown but did not occur in fresh seeds oil: Caprylic acid [C8:0] (0.29 %), capric acid [C10:0] (0.13%), lauric acid [C12:0] (1.74 %), pentadecanoic acid [C15:0] (0.11%) and heptadecanoic acid [C17:0] (0.12%). On the other hand, only one different fatty acid, erucicacid [C22:1 ω9], was found only in the oils of the fresh seeds. Based on this study, the fatty acid content of the seed's oils recognized that *L. Siceraria* seeds could be arich source of oil for domestic and industrial purposes [36].Palmitic acid and stearic acid are examples of saturated fatty acids, and oleic acid is a monounsaturated fatty acid while linoleic acid is a polyunsaturated fatty acid. Some studies have stated many impacts of SFAs on human health. It has been concluded that lauric acid (C12:0) as well as myristic acid (C14:0), raise plasma total cholesterol concentrations, the first due to an increase in LDL cholesterol while the latter due to a rise of both LDL and HDL cholesterol concentrations [37,38]. However, according to Mensink [39] and Lawrence [40], the ratio of total cholesterol to HDL cholesterol is a more specific marker of coronary artery diseases than the value of LDL cholesterol. Oils rich in lauric acid (C12:0) decreased the ratio of total to HDL cholesterol. On the other hand, myristic (C14:0) and palmitic acids (C16:0) affected this ratio only little, and stearic acid (C18:0) slightly reduced this ratio.

Table 1: Mineral’s visualization in dry fruit (B), fresh (C) and roasted seeds (D), and dry leaves of *Bottle gourd*

Element	**Fresh fruit(A)**		**Dry fruit (B)**		**Fresh seeds (C)**		**Roasted seeds (D**		**Fresh leaves (E)**		**Dry leaves (F)**	
	%-ppm	**mg/100g**	**%-ppm**	**mg/100g**	**%-ppm**	**mg/100g**	**%-ppm**	**mg/100g**	**%-ppm**	**mg/100g**	**%-ppm**	**mg/100g**
Calcium	0.026 **%**	26.470	0.56 %	560	0.22	220	0.23 %	230	0.164 **%**	163.808	4 %	**4000**
Phosphorus	0.029 **%**	28.833	0.61 %	610	1.2	**1200**	1.4 %	**1400**	0.018 **%**	17.609	0.43 %	430
Potassium	0.158 **%**	158.344	3.35 %	**3350**	0.86	860	1.01 %	1010	0.188 **%**	188.379	4.6 %	**4600**
Magnesium	0.016 **%**	16.071	0.34 %	340	0.72	**720**	0.7 %	**700**	0.030 **%**	29.895	0.73 %	**730**
Sodium	0.0014 %	1.418	0.03 %	30	0.02	20	0.02 %	20	0.004 **%**	3.686	0.09 %	**90**
Iron	0.0047 **%**	4.727	0.1 %	**100**	0.044	44	0.045 %	45	0.005 **%**	4.505	0.11 %	**110**
Manganese	0.766 ppm	0.077	16.2 ppm	1.62	35.9 ppm	3.59	34.9 ppm	3.49	3.477 ppm	0.348	84.9 ppm	**8.49**
Zinc	2.198 ppm	0.220	46.5 ppm	4.65	109.9 ppm	**10.99**	67 ppm	6.70	3.448 ppm	0.345	84.2 ppm	8.42
Selinum	0.003 ppm	0.0003	0.06 ppm	0.006	0.23 ppm	0.023	0.14 ppm	0.014	0.016 ppm	0.0016	0.38 ppm	**0.038**
Chromium	0.062 ppm	0.0062	1.32 ppm	**0.132**	0.6 ppm	0.06	0.35 ppm	0.35	0.00041 ppm	0.00004	0.01 ppm	0.001
Copper	8.177 ppm	0.818	**173** ppm	17.3	88.8 ppm	8.88	45.8 ppm	4.58	2.158 ppm	0.216	52.7 ppm	5.27

Table 2: Amino acids estimation in fresh fruit (**A**), dry fruit (**B**), fresh (**C**) and roasted seeds (**D**), fresh leaves (**E**) and dry leaves (**F**) of *Bottle gourd*

	Fresh fruit (A)		**Dry fruit (B)**		**Fresh seeds (C)**		**Roasted seeds (D)**		**Fresh leaves (E)**		**Dry leaves (F)**	
	%	**U***	**%**	**U***	**%**	**U***	**%**	**U***	**%**	**U***	**%**	**U***
Asparic Acid	0.036	-	0.77	-	2.61	-	2.62	-	0.078	-	1.90	-
Therionine	0.014	-	0.29	-	1.07	-	1.09	-	0.044	-	1.08	-
Serine	0.017	-	0.36	-	1.32	-	1.42	-	0.034	-	0.84	-
Glutamic acid	**0.061**	**-**	**1.28**	**-**	**5.16**	**-**	**5.44**	-	**0.096**	**-**	**2.35**	-
Proline	0.015	-	0.32	-	1.08	-	1.20	-	0.045	-	1.09	-
Glycine	0.017	-	0.35	-	1.87	-	1.80	-	0.053	-	1.30	-
Alanine	0.026	-	0.54	-	1.83	-	1.89	-	0.049	-	1.20	-
Valine	0.020	-	0.42	-	1.51	-	1.52	-	0.038	-	0.92	-
Methionine	0.005	-	0.11	-	0.91	-	0.87	-	0.016	-	0.39	-
Isoleucine	0.015	-	0.32	-	1.37	-	1.39	-	0.047	-	1.15	-
Leucine	0.024	-	0.50	-	2.11	-	2.14	-	0.066	-	1.61	-
Tyrosine	0.014	-	0.30	-	1.13	-	1.15	-	0.038	-	0.92	-
Phenylalanine	0.016	-	0.34	-	1.74	-	1.97	-	0.050	-	1.22	-
Histidine	0.009	-	0.19	-	0.87	-	0.90	-	0.020	-	0.50	-
Lysine	0.021	-	0.45	-	0.98	-	0.90	-	0.051	-	1.25	-
Argnine	0.025	-	0.52	-	**4.66**	**-**	**4.72**	-	0.049	-	1.19	-
Cysteine	0.004	-	0.09	-	0.60	-	0.32	-	0.014	-	0.33	-
Whole content	**0.338**		**7.15**		**30.82**		**31.34**		**0.788**		**19.24**	

*Estimated Uncertainty

Table 3: Fatty acids composition and abundance in fresh (C) and roasted seed (D) oils of *Bottle gourd*

#	Fatty acid	Name	Relative Distribution (%)	
			Roasted seeds (D)	Fresh seeds (C)
1	C8:0	Caprylic acid (**AC1**)	0.29	
2	C10:0	Capric acid (**AC2**)	0.31	
3	C12:0	Lauric acid (**AC3**)	1.74	
4	C14:0	Myristic acid (**AC4**)	1.35	0.24
5	C15:0	Pentadecanoic acid (**AC5**)	0.11	
6	C16:0	Palmitic acid (**AC6**)	15.05	14.23
7	C16:1 ω7	Palmitoleic acid (**AC7**)	0.26	0.19
8	C17:0	Heptadecanoic acid (**AC8**)	0.12	
9	C18:0	Stearic acid (**AC9**)	6.62	6.29
10	C18:1 ω9	Oleic acid (**AC10**)	16.07	15.71
11	C18:1 ω7	Vaccinic acid (**AC11**)	0.68	0.62
12	C18:2 ω6	Linoleic acid (**AC12**)	56.10	60.34
13	C18:3 ω3	Linolenic acid (**AC13**)	0.53	0.27
14	C20:0	Arachidic acid (**AC14**)	0.30	0.34
15	C20:1 ω9	Gadolic acid (**AC15**)	0.15	0.36
16	C22:1 ω9	Erucic Acid (**AC16**)		1.17
17	NIFA*		0.32	0.24

*Non-Identified Fatty Acids

3.1.4 Phenolic Compounds Estimated in Fruit, Seeds, and Leaves

Phenolic compounds are the most widely spread secondary metabolites and are ubiquitously present in fruits and vegetables and form an essential constituent of human and animal diets. Phenolic compounds can range from simple phenolic acids (e.g., hydroxybenzoic acids, hydroxycinnamic acids, gallates, hydroxybenzoates) to complex natural polymers such as tannins [41]. This diverse group of compounds has received much attention to servingas protective factors against oxidative cellular damage which is associated with the generation of reactive oxygen species (ROS) [42].It has been reported that the ROS scavenging activity of phenols is mainly due to their redox properties, hydrogen donors, and singlet oxygen quenchers [43].The phenolics are claimed to have a series of potential biological activities, including antioxidant activity [44] and anticancer [45,46], antimutagenic [47], anti-inflammatory [48], and anti-HIV [49] properties. Phenolics also have a beneficial role in wound healing and skin disease, reduce the risk of cardiovascular diseases and protect from drug toxicity [50,51].

The levels of total phenolic compounds present in the crude extract of fruits, seeds, and leaves of bottle gourd are shown in Table 4 and Figure S2 (compounds **S1-S21**). Twenty-one phenolic compounds were estimated with diverse quantities according to the source part of the plant.

3.1.5 Flavonoids and Isoflavonoids Estimated in Fruit, Seeds, and Leaves

Flavonoids represent one of the largest and most studied classes of phenylpropanoid-derived plant specialized metabolites, with an estimated 10,000 different members. Structurally, they consist of two main groups, the 2-phenylchromans (the flavonoids, including flavanones, flavones, flavonols, flavan-3-ols, and anthocyanidins) and the 3-phenylchromans (the isoflavonoids, including isoflavones, isoflavans, and pterocarpans). Flavonoids act as attractants to pollinators and symbionts, as sunscreens to protect against UV irradiation, as allelochemicals, and as antimicrobial and antiherbivory factors. Their importance in plant biology goes beyond their specific functions within the plant. For example, the early advances in floral genetics were primarily the result of the ease of screening for mutations impacting flavonoid-derived flower colors, and the first demonstration of epigenetic gene silencing in plants was likewise associated with flavonoid biosynthesis [52]. Flavonoids have been ascribed positive effects on human and animal health and are central to the current interest in "botanicals" for disease therapy and chemoprevention.

The phytochemical estimation of the bottle gourd vegetable parts showed the presence of considerable amounts of flavonoids and isoflavonoids (Tables 5 and 6), at where ten flavonoid compounds, namely naringin ((**S22)**, rutin(**S23)**, hesperidin (**S24)**, quercetrin(**S25)**, quercetin (**S26)**, naringenin (**S27)**, hespirtin(**S28)**, kampferol(**S29)**, apigenin (**S30**), andIsorhamentin (**S31**) were estimated. On the other hand, five iso-flavonoid derivatives were characterized in the same six parts of bottle gourd, and defined asDaidasein (**S32)**, genistein (**S33)**, isoformononetin(**S34)** and biochanin (**S35)** (Figures 3-S4).

Table 4: Phenolic compounds estimated in fresh and dried fruits (**A, B**), seeds (**C, D**) and leaves (**E, F**) of Bottle gourd (mg /100g)

Name of Phenolic Compound	Fresh Fruit (A)	Dry Fruit (B)	Fresh seeds (C)	Roasted Seeds (D)	Fresh Leaves (E)	Dry Leaves (F)
Gallic acid (**S1**)	0.047097	1.171002	0.209442	0.381341	0.325425	0.395523
Pyrogallol (**S2**)	1.594584	15.21838	12.34478	14.77553	1.35375	4.974984
3-Hydroxy Tyrosol (**S3**)	0.367739	3.745045	1.028833	3.731178	29.88518	137.6077
Protocatechuic acid (**S4**)	0.384811	4.249789	0.629381	0.606762	1.750678	27.01080
Catechin (**S5**)	1.201924	38.74079	8.087963	5.321826	2.896425	82.92647
Chlorogenic acid (**S6**)	0.111030	7.407983	0.130140	0.672275	2.370382	5.061764
Catechol (**S7**)	0.306872	1.681142	0.823516	0.567774	2.249159	4.223113
P-OH benzoic acid (**S8**)	0.491452	1.494478	0.821393	1.204304	1.204092	8.574009
Caffeic acid (**S9**)	0.025791	0.794651	0.138080	0.355062	0.109141	1.578913
Vanillic acid (**S10**)	0.305519	0.791840	0.145020	0.772955	1.110788	1.250836
P-Coumaric acid (**S11**)	0.056582	0.324667	0.077957	0.147734	0.111596	3.609774
Ferulic acid (**S12**)	0.158650	0.603235	0.011151	0.193464	1.918197	12.04241
Isoferulic acid (**S13**)	0.084443	1.652536	0.495565	0.338559	0.600955	2.982975
Ellagic acid (**S14**)	0.731807	4.060516	0.701309	1.075695	0.960424	14.22925
Oleuropein (**S15**)	1.266424	3.640949	1.438789	1.403370	6.172524	16.20500
Coumaric acid (**S16**)	0.012228	0.047581	0.027634	0.099620	0.026619	0.200779
Benzoic acid (**S17**)	2.292886	2.116965	1.032558	0.624328	4.539336	8.84159
Salicylic acid (**S18**)	2.791670	1.221088	3.409383	3.191733	1.138767	8.741998
3,4,5-trimethoxy Cinnamic acid (**S19**)	0.082383	0.561388	0.272895	0.061221	0.233234	1.232966
Coumarin (**S20**)	0.058110	0.160405	0.169950	0.226582	0.076786	0.793893
Cinnamic acid (**S21**)	0.019562	0.039940	0.078328	0.069477	0.016557	0.077680

Table 5: Flavonoid compounds estimated in fresh and dried fruits (A, B), seeds (C, D) and leaves (E, F) of Bottle gourd (mg /100g)

Name of Flavone	Fresh Fruit (A)	Dry Fruit (B)	Fresh Seeds (C)	Roasted Seeds (D)	Fresh Leaves (E)	Dry Leaves (F)
Naringin (**S22**)	4.966212	15.84058	0.887948	0.718548	5.220319	137.7926
Rutin (**S23**)	1.642006	17.46924	1.212280	0.998750	3.713592	22.98165
Hesperidin (**S24**)	4.899785	73.80399	5.061029	5.386672	19.68636	115.9560
Quercetrin (**S25**)	0.435871	4.269135	1.693261	1.222343	1.731798	8.572887
Quercetin (**S26**)	0.051352	0.131052	1.016233	0.181032	0.086941	0.768288
Naringenin (**S27**)	0.023951	0.029214	0.049242	0.182044	0.079589	0.273550
Hespirtin (**S28**)	0.127605	0.145576	0.123093	0.557698	0.413692	2.829390
Kampferol (**S29**)	0.027152	0.210540	0.461695	0.252590	0.117249	1.152218
Apigenin (**S30**)	0.027152	0.041306	0.127023	0.257830	0.045917	0.373958
Isorhamnetin (**S31**)	0.871404	193.6670	2.546442	2.369051	3.262088	22.13231

Table 6: Isoflavonoid compounds estimated in fresh and dried fruits (A, B), seeds (C, D) and leaves (E, F) of *Bottle gourd* (mg /100g)

Name of Isoflavone	Fresh Fruit (A)	Dry Fruit (B)	Fresh seeds (C)	Roasted Seeds (D)	Fresh Leaves (E)	Dry Leaves (F)
Daidasein (**S32**)	1.782869	5.149291	5.810452	4.230135	6.571985	21.81892
Genistein (**S33**)	0.111465	1.362505	0.692154	0.582569	0.470885	1.997426
isoformononetin (**S34**)	0.357115	1.104637	3.051104	0.353144	0.120756	1.533818
Biochanin (**S35**)	0.017447	0.068076	0.191006	0.099524	0.015700	0.153938

3.1.5 GC-MS Analysis

Gas Chromatography/Mass Spectrometry (GC/MS) analysis is an analytical method that combines the features of gas chromatography and mass spectrometry to identify different substances within a sample component matrix. GC/MS analysis is generally considered one of the most accurate analyses available. It is particularly beneficial for materials where low detection limits are required and can be used to evaluate samples in any size chemical state, and even when the sample quantity is limited.

The volatile constituents of different parts of Bottle gourd were tentatively assessed by comparing their mass spectra with related counterparts reported by NIST, Wiley9, Mainlib, and Replib libraries [30]. Organic extracts of dry fruits (B), fresh/roasted seeds (C and D), and dry leaves (F) were analyzed by GC-MS. Analysis of dry fruitsextract (Figures S5a-b, Table 7) revealed the presence of thirty compounds accounting for97.33 % of the total extract composition. 9,12-octadecadienoic acid methyl ester (25.68 %), (z,z)-9,12-octadecadienyl chloride (18.62 %), n-hexadecanoic acid (15.40 %), 9-hexadecanoic acid methyl ester (12.60 %), 9-octadecenoic acid methyl ester (6.72 %), and Methyl stearate (5.44 %) were the main components in the fruits extract. GC-MS analysis of fresh seeds extract (Figures S6a-b, Table 8) indicated the presence of eighteen compounds with a total area of 98.95 %. The main constituents were (Z)-9-octadecenoic acid methyl ester (31.32 %), (Z,Z)-9,12-octadecadienoic acid (12.63 %), 9,12-octadecadienoic acid methyl ester (11.07 %), hexadecanoic acid methyl ester (9.42 %), Butyl 9,12-octadecadienoate (9.13 %) and methyl stearate (8.50 %). Hexane soluble fraction of roasted seeds (Figures S7a-b, Table 9) exhibited the existence of sixteen compounds including hexadecanoic acid methyl ester (28.13 %), 9, 12-octadecadienoic acid methyl ester (16.42 %), and methyl stearate (13.46 %) as the major constituents, while the dichloromethane soluble fraction of roasted seeds (Figures S8a-b, Table 10) showed only the presence of ten compounds with hexadecanoic acid methyl ester (28.81 %) as the main constituent followed by (Z)-9-octadecenoic acid (22.42 %), cis-vaccenic acid (16.80 %), methyl stearate (11.84 %) and n-hexadecanoic acid (10.60 %). Finally, the extract from air-dried leaves was also analyzed by GC-MS (Figures S9a-b, Table 11) revealing the presence of fourteen compounds with a total area of 99.58 %. Main volatile constituents from the leaves included (Z,Z)-9,12-Octadecadienoic acid (20.19 %), (Z,Z,Z)-9,12,15-Octadecatrienoic acid methyl ester (19.22 %), n-hexadecanoic acid (17.55), hexadecanoic acid methyl ester (9.52 %) and phytol (8.80 %).From the above evidence, it can be elucidated that *Lagenaria siceraria* LS plant consists of the enormous potential of pharmacological constituents, and therapeutic phytocompounds responsible for various pharmacological actions. These major chemical compounds identified from different crude extracts are considered to be a part of plants' defense systems and they may be grouped as protective compounds found in this plant. Thus, the identification ofvarious phytochemical compounds from extracts of bottle gourd different parts displays significant medicinal properties of the plant. Further studies like bioprospecting are essential to support its biological properties and the biological importance of these innovative biomolecules will be interesting to be studied.

Table 7: Tentatively identified compounds (hexane soluble) from the air-dried fruits of *Bottle gourd* by GC-MS

No.	Compound Name	MW	MF	R_t(min)	Area(%)
1	1-chlorooctadecane	288	$C_{18}H_{37}Cl$	8.66	0.12
2	9-octadecenoic acid	282	$C_{18}H_{34}O_2$	10.58	0.19
3	1-tetradecanol	214	$C_{14}H_{30}O$	10.72	0.21
4	2-methyl-1-hexadecanol	256	$C_{17}H_{36}O$	14.54	0.16
5	1,3,5-triazine-2,4-diamine-6-chloro-N-ethyl	173	$C_5H_8ClN_5$	14.66	0.11
6	Cis-vacenic acid	282	$C_{18}H_{34}O_2$	18.17	0.16
7	2-methyl-1-hexadecanol	256	$C_{17}H_{36}O$	18.27	0.11
8	Pentadecanoic acid	242	$C_{15}H_{30}O_2$	19.12	0.15
9	9-hexadecenoic acid methyl ester	268	$C_{17}H_{32}O_2$	20.13	0.32
10	9-hexadecanoic acid methyl ester	270	$C_{17}H_{34}O_2$	20.64	12.60
11	Oleic acid; 9-octadecenoic acid	282	$C_{18}H_{34}O_2$	21.49	0.09
12	9-hexadecanoic acid ethyl ester	284	$C_{18}H_{36}O_2$	21.59	0.14
13	n-hexadecanoic acid	256	$C_{16}H_{32}O_2$	22.34	15.40
14	9,12-octadecadienoic acid methyl ester	294	$C_{19}H_{34}O_2$	23.38	25.86
15	9-octadecenoic acid methyl ester	296	$C_{19}H_{36}O_2$	23.46	6.72
16	Methyl stearate	298	$C_{19}H_{38}O_2$	23.75	5.44
17	9,12-octadecadienoic acid (z,z)-,2-hydroxy-(1-hydroxmethylethyl ester	354	$C_{21}H_{38}O_4$	24.14	0.28
18	9,12-octadecadienyl chloride (z,z)	298	$C_{18}H_{31}ClO$	24.74	18.62
19	Octadecanoic acid	284	$C_{18}H_{36}O_2$	24.97	2.17
20	9,12,15-octaecatrienoic acid, 2,3-dihydroxypropyl ester (z,z,z)	352	$C_{21}H_{36}O_4$	25.37	0.11
21	6,9,12-octaecatrienoic acid, methyl ester (z,z,z)	292	$C_{19}H_{32}O_2$	25.94	0.19
22	9,12-octadecadienoic acid	282	$C_{18}H_{34}O_2$	25.85	0.27
23	[1,1'-bicyclopropyl]-2-octanoic acid, 2'-hexyl, methyl ester	322	$C_{21}H_{38}O_2$	26.01	0.42
24	Eicosanoic acid, methyl ester	326	$C_{21}H_{42}O_2$	26.49	0.90
25	Butyl 9,12-octadienoate	336	$C_{22}H_{40}O_2$	28.11	0.97
26	Diisooctyl phthalate	390	$C_{24}H_{38}O_4$	29.39	4.11
27	Dotriacontane	450	$C_{32}H_{66}$	31.12	0.25
28	Pentatriacontane	492	$C_{35}H_{72}$	31.51	0.93
29	1-heptatriacotanol	536	$C_{37}H_{76}O$	32.58	0.17
30	9,10-secocholesta-5,7,10-treiene-3,24,25-triol	416	$C_{27}H_{44}O_3$	35.44	0.16
Total area %					**97.33**

Table 8: Tentatively identified compounds (hexane soluble) from fresh seeds of *Bottle gourd* by GC-MS

No.	Compound Name	MW	MF	R_t(min)	Area(%)
1	Hexanoic acid	116	$C_6H_{12}O_2$	4.94	0.71
2	Methyl tetradecanoate	242	$C_{15}H_{30}O_2$	17.07	0.32
3	Hexadecanoic acid methyl ester	270	$C_{17}H_{34}O_2$	20.83	9.42
4	Hexadecanoic acid ethyl ester	284	$C_{18}H_{36}O_2$	21.61	0.27
5	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	22.73	0.48
6	9,12-octadecadienoic acid methyl ester	294	$C_{19}H_{34}O_2$	23.49	11.07
7	(Z)-9-Octadecenoic acid methyl ester	296	$C_{19}H_{36}O_2$	24.01	31.32
8	Methyl stearate	298	$C_{19}H_{38}O_2$	24.22	8.50
9	Linoleic acid ethyl ester	308	$C_{20}H_{36}O_2$	24.47	1.28
10	(Z,Z)-9,12-Octadecadienoic acid	280	$C_{18}H_{32}O_2$	26.27	12.63
11	Octadecanoic acid	284	$C_{18}H_{36}O_2$	26.38	1.94
12	Eicosanoic acid, methyl ester	326	$C_{21}H_{42}O_2$	26.78	0.80
13	Hexadecanoic acid, hexyl ester	340	$C_{22}H_{44}O_2$	27.48	2.45
14	Pentyl linoleate	350	$C_{23}H_{42}O_2$	28.46	6.49
15	Octadecanoic acid, pentyl ester	354	$C_{23}H_{46}O_2$	28.78	0.51
16	Butyl 9,12-octadecadienoate	336	$C_{22}H_{40}O_2$	29.69	9.13
17	Octadecanoic acid, hexyl ester	368	$C_{24}H_{48}O_2$	29.94	0.78
18	Squalene	410	$C_{30}H_{50}$	32.68	0.75
Total area %					**98.95**

Table 9: Tentatively identified compounds (hexane soluble) from roasted seeds of *Bottle gourd* by GC-MS

No.	Compound Name	MW	MF	R_t(min)	Area(%)
1	Hexanoic acid	116	$C_6H_{12}O_2$	4.94	2.93
2	Tetradecane	198	$C_{14}H_{30}$	10.72	0.48
3	Methyl tetradecanoate	242	$C_{15}H_{30}O_2$	17.09	0.43
4	Hexadecanoic acid methyl ester	270	$C_{17}H_{34}O_2$	20.80	28.13
5	Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$	21.61	0.33
6	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	22.65	8.94
7	9,12-octadecadienoic acid methyl ester	294	$C_{19}H_{34}O_2$	23.47	16.42
8	Methyl stearate	298	$C_{19}H_{38}O_2$	23.87	13.46
9	cis-Vaccenic acid	282	$C_{18}H_{34}O_2$	24.57	8.68
10	Octadecanoic acid	284	$C_{18}H_{36}O_2$	25.13	8.53
11	Hexadecanoic acid, pentyl ester	326	$C_{21}H_{42}O_2$	25.94	4.25
12	Eicosanoic acid, methyl ester	326	$C_{21}H_{42}O_2$	26.52	0.98
13	9-Octadecenoic acid (Z); oleic acid	282	$C_{18}H_{34}O_2$	26.91	0.29
14	trans-9-Octadecenoic acid, pentyl ester	352	$C_{23}H_{44}O_2$	28.20	2.26
15	Octadecanoic acid, pentyl ester	354	$C_{23}H_{46}O_2$	28.55	1.37
16	Diisooctyl phthalate	390	$C_{24}H_{38}O_4$	29.38	2.04
Total area %					**99.52**

Table 10: Tentatively identified compounds (dichloromethane soluble) from fresh seeds of *Bottle gourd* by GC-

No.	Compound Name	MW	MF	R_t(min)	Area(%)
1	2-Aminobiphenyl	169	$C_{12}H_{11}N$	16.43	0.93
2	Diphenylaniline	169	$C_{12}H_{11}N$	16.47	2.54
3	Hexadecanoic acid methyl ester	270	$C_{17}H_{34}O_2$	20.49	28.81
4	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	21.87	10.60
5	(*Z*)-9-Octadecenoic acid	282	$C_{18}H_{34}O_2$	23.18	22.42
6	Methyl stearate	298	$C_{19}H_{38}O_2$	23.60	11.84
7	*cis*-Vaccenic acid	282	$C_{18}H_{34}O_2$	24.66	16.80
8	Isopropyl palmitate	298	$C_{19}H_{38}O_2$	25.81	1.64
9	2,6-dimethyl-N-(2-methyl-α-phenylbenzyl)aniline	301	$C_{22}H_{23}N$	29.35	1.65
10	7,8-Epoxylanostan-11-ol, 3-acetoxy	502	$C_{32}H_{54}O_4$	34.41	2.75
Total area %					**99.98**

MS:

Table 11: Tentatively identified compounds (hexane soluble) fourteen from air-dried leaves of *Bottle gourd* by GC-MS

No.	Compound Name	MW	MF	R_t(min)	Area(%)
1	Caryophyllene	204	$C_{15}H_{24}$	11.91	1.02
2	Neophytadiene	278	$C_{20}H_{38}$	18.94	5.75
3	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	19.36	4.13
4	Hexadecanoic acid, methyl ester	904	$C_{17}H_{34}O_2$	20.48	9.52
5	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	21.81	17.55
6	(*Z,Z*)-9,12-Octadecadienoic acid methyl ester	294	$C_{19}H_{34}O_2$	23.08	3.86
7	(*Z,Z,Z*)-9,12,15-Octadecatrienoic acid methyl ester	292	$C_{19}H_{32}O_2$	23.20	19.22
8	Phytol	296	$C_{20}H_{40}O$	23.41	8.80
9	Octadecanoic acid methyl ester	298	$C_{19}H_{38}O_2$	23.59	2.44
10	(*Z,Z*)-9,12-Octadecadienoic acid	280	$C_{18}H_{32}O_2$	24.38	20.19
11	(*Z,Z,Z*)-8,11,14-Eicosatrienoic acid	306	$C_{20}H_{34}O_2$	24.67	1.44
12	Di-n-octyl phthalate	390	$C_{24}H_{38}O_4$	29.33	2.94
13	Stigmast-5-en-3-ol	414	$C_{29}H_{50}O$	35.76	1.32
14	Ethyl iso-allocholate	436	$C_{26}H_{44}O_5$	36.78	1.84
Total area %					**99.58**

4. Conclusions

The phytochemical and nutritional value for the different parts of Bottle gourd; *Lagenaria siceraria* LS, cultivated in Egypt were studied. Mainly, extracts of fresh/dried fruits, fresh/roasted seeds, and fresh/dried leaves of Bottle gourd were studiedon a broad scale. According to this study, the bottle gourd parts were confirmed to contain eleven minerals, seventeen amino acids, twenty-one phenolic analogues, ten flavonoid and four isoflavonoids. Moreover, the fresh/roasted seeds are rich with seventeen fatty acids detected, where linoleic acid (C18:2 ω6) is the most abundant among them. According to GC-MS analysis, dryfruits extract revealed the presence of thirty compounds, meanwhile, the fresh seeds indicated the presence of eighteen compounds, while roasted seeds hexane soluble fraction exhibited the existence of sixteen compounds, while the dichloromethane soluble fraction of roasted seeds revealed the presence of ten compounds, and finally, the air-dried leaves afforded fourteen diverse compounds.

Conflicts of interest

The authors declared no conflict of interest.

Authors' contributions

All the participant researchers contributed to this work. All authors read and approved the final manuscript.

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