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Chemical Composition and Insecticidal Properties of *Lagenaria siceraria* (LS) Cultivated in Egyptian Habitats

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Abstract

Bottle gourd; *Lagenaria siceraria* (LS) has been recognized as the most popular and widely used fruits vegetable due to its immense health-promoting properties. As a continuation of our ongoing research on the chemical composition of the fresh and dry fruits, seeds and leaves *of L. siceraria* cultivated in Egypt, namely, the moisture, ash, pH, total solids, antioxidants, proteins, phenolics, flavonoids, tannins, reducing and non-reducing sugars, carbohydrates, B vitamins (B1, B2, B6, B9, and B12), and vitamins (C, A, D, E and K) were determined intensively. Biologically, the insecticidal properties of the different plant's parts were explored against the mosquito, *Culex pipiens* larvae and adults. Tested materials showed larvicidal activity in particular fresh/roasted seeds and leaves extracts with LC_{50} concentrations in the range of 12.015-16.166 ppm. Fecundity and egg-hatchability percentages were significantly reduced when LC_{50} and LC_{90} concentrations applied. Additionally, tested extracts reduced fecundity to > 6 folds when LC_{90} concentration was applied. Repellency test elucidated that fruits and fresh seeds extracts behave like strong repellents either when LC_{50} (>85%) or LC_{90} (>90%) was applied. Overall, the obtained data provide detailed analysis to the chemical composition of *L. siceraria* cultivated in Egypt and highlight the insecticidal potential of different plant-part extracts as an effective and safe agent.

Keywords: *Lagenaria siceraria*; Chemical Composition; Insecticidal properties; *Culex pipiens*

1. Introduction

The inadequate accessibility of animal protein sources and the elevated cost of the few available plant protein sources has prompted intense research into harnessing the nutrient potentials of lesser-known underutilized legumes and oil crops [1]. *Lagenaria siceraria* (LS), belonging to family *Cucurbitaceae*, represents the most popular fruits vegetable throughout the world [2]. It has a major contribution to the economically domesticated species for vegetable production [2]. It is comprising ~ 118 genera and 825 species, distributed widely in the warmer regions of the world [3-6]. Cucurbits seeds have been reported as strong sources of food, particularly protein and oil, where dehulled cucurbit seeds were reported to contain about 50% oil and up to 35% protein [7,8].

Biologically, the plant parts of Bottle gourd, *L. siceraria* have been used to treat various diseases such as asthma, fever, hypertension, jaundice, ulcer, cardiac, bronchial, and skin problems, in addition to its use as a diuretic, emetic, purgative, cooling sedative and cardiotonic agent [9]. It also possesses further several therapeutic properties such as antidote, aphrodisiac, antioxidant, anti-inflammatory, analgesic and anticancer [6,10,11]. Besides, Bottle gourd is also used to treat various neurological disorders like Alzheimer's disease [5]. Nutritional evaluation reveals that it is a rich source of various nutrients such as vitamins B and C, pectin, fibers, β-carotene, amino acids, proteins, and glycosides [6]. It has been stated that Bottle gourd is an excellent natural gift to human being due to its essential constituents required for normal and healthy life [9]. Previous studies revealed the presence of several bioactive metabolites in the different fruits parts having pharmaceutical potential namely polyphenols, flavonoids, triterpenoids, saponins [12], fucosterol and campesterol [13], C-

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flavone glycosides [14], lagenin [15] and cucurbitacin I [16].

In terms of public health importance, mosquitoes are the most important group of insects since they transmit many arboviruses such as Zika virus, West Nile virus, St. Louis, and other viral encephalitis in addition to malaria and filariasis which widely contribute to health problems, particularly in developing countries. *Culex pipiens* (Diptera: Culicidae) is the main vector of many mosquito-borne diseases in particular the causative agent of lymphatic filariasis, *Wuchereria bancrofti* (Onchocercidae). Thus, there is a critical need to develop an effective, appropriate, and environmentally safe control programs. Targeting the larval stages at its breeding sites reduces mosquito populations more effectively [17]. Moreover, repellents are an ideal tool used to control disease vector mosquitoes on a wide range if they were appropriately used.

Synthetic insecticides have been widely used as pest control agents for many years. However, problems associated with the repeated use of synthetic insecticides such as its harmful impact on the environment, developed resistance, its effects on human health, and the undesirable effects on non-target organisms all together pave the way for searching an environmental-safe and effective pesticidal agents of natural origin [18,19]. Plant resources contain various phytochemicals that may be used as larvicidal, ovicidal, and repellent agents against mosquitoes of public health importance such as our model organism, *C. pipiens* [20].

As a continuation of our ongoing research on *L. siceraria* LS [**21**], the present study is focusing on further chemical compositions exemplified in the moisture, ash, pH, total solids, total antioxidants, total proteins, total phenolics, total flavonoids, total tannins, total (reducing and non-reducing) sugars, total carbohydrates, B vitamins (B1, B2, B6, B9, and B12), and vitamins (C, A, D, E and K) contents in the fresh and dry fruits, seeds and leaves. Biologically, the insecticidal properties of the different plant's parts of Bottle gourd cultivated in Egypt were studied intensively against the mosquito, *C. pipiens* larvae and adults.

2. Experimental Section

2.1 *Bottle gourd extracts preparation*

The collection and working up of the Bottle gourd parts: fruits (A, B), seeds (C, D), and leaves (E, F) were recently reported in our published article [21].

2.2.1 Estimation of moisture and ash contents and total solids of Bottle gourd parts

Moisture, ash and total solids (T.S) were determined according to the methods of A.O.A.C (2012) [22].

2.2.2. *Determination of pH value*

pH value was measured by using Beckman pH meter with glass electrode at 25 °C (A.O.A.C, 2012) [22].

2.2.3. Estimation of minerals, amino acids and fatty acids

Estimation of minerals, Amino acids and Fatty acids has been reported in our recently published article [21].

2.2.4. Estimation of total protein content

The protein analysis was performed according to Kjeldahl method [23]: after digestion in concentrated sulfuric acid, the total organic nitrogen was converted to ammonium sulfate. Ammonia was formed and distilled into boric acid solution under alkaline conditions. The borate anions formed were titrated with standardized hydrochloric acid, by which the content of nitrogen representing the amount of crude protein in the sample was calculated. Most proteins contain 16% of nitrogen, thus the conversion factor was 6.25. However, the nitrogen from nonprotein additives or contaminants in the food, such as melamine in milk, was also measured.

2.2.5. 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) [24].

2.2.6. Determination of total flavonoids content

Total flavonoid content was detected by $AlCl_3$ colorimetric assay by using the method of Tacouri et al [25].

2.2.7. Identification of sugars content

Sugars were determined by High Performance Liquid Chromatography coupled to a Refraction Index detector (HPLC-RI), after an extraction procedure previously according to the method of Barreira et al. (2010) [26] at Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

2.2.8. Determination of total reducing and non-reducing sugars

Total sugars of the Bottle gourd parts were extracted and determined according to the method of Somogy (1952) [27].

2.2.9. Determination of total hydrolysable carbohydrate:

The method of working up and determination of total hydrolysable carbohydrate was done as described by Dubois et al. (1956) [28].

2.2.10. Determination of hydrolysable tannins

Tannins were determined according to the method of Rooney et al., (1981) [29] at Food Technology Research Institute, Agric. Res. Center, Giza, Egypt.

2.2.11. Determination of condensed tannins

Condensed tannins were determined according to the procedure of Bate-Smith, (1973) [30] at Food Technology Research Institute, Agric. Res. Center, Giza, Egypt.

2.2.12. Determination of phenolic, flavonoid and isoflavonoid compounds by HPLC

Flavonoid and isoflavonoid compounds were identified by HPLC as described in our recently published article [21].

2.2.13. Extraction and determination of B complex vitamins (B12, B9, B6, B2, B1)

B-Complex vitamins were determined according to the method described by Batifoulier et al. (2005) [31] with slight modification using variable wavelength detector (VWD) instead of florescence detector, VWD set at 280 nm.

2.2.14. Extraction and determination of vitamins (A, D, E and K) by HPLC

A 10 gm of sample was mixed with 0.5gm of ascorbic acid, 40 ml of methanol and 10 ml of 1:1 potassium hydroxide in water and heated at reflux with stirring for 30 min. the mixture was cooled in an ice bath and quantitatively transferred to a separating funnel with 50 ml water, 10 ml methanol and 50 ml hexane containing 1.5 mg/100 ml butylated hydroxytoluene (BHT). The separating funnel was shaken vigorously for 2 min and the phases allowed separating. The aqueous phase was removed and extracted twice more with 20 ml portions of hexane containing 1.5 mg/100 ml BHT. The hexane extracts were combined and washed three times with 100 ml of water. 10 ml of the hexane solution was then transferred to a glass tube and the solvent removed under a flow of nitrogen at room temperature. The residue was reconstituted with 1.0 ml of methanol and filtered through a 13 mm 0.45 µm Teflon filter disc into vial for analysis by HPLC.

The analysis was performed by HPLC system (Agilent technologies, Germany) 1200 series equipped with a variable wavelength detector (330 nm for vit. A, 295 for vit E, 266 nm for vit. D and 280 nm for vit. K) with a waters series 2695 quaternary solvent delivery system with a cooled autosampler at 4 ºC and heated column compartment at 30 ºC. The compounds were separated on a 10 µm Bondclone 3.9 x 300 mm C18 column (phenomenex, Sydney, Australia) fitted with a C18 guard column. The mobile phase consisted of water: methanol (5: 95), at a flow rate of 1 ml/min [32].

2.2.15. Fractionation and determination of ascorbic acid "vitamin C" by HPLC

Ascorbic acid content was determined according to the titration method using 2,6-dichlorophenol-indophenol as reported by (A.O.A.C., 2012) [22]. For vitamin C determination, samples were prepared according to the method described by Meléndez *et al.* (2004) [33]. The chromatographic procedure used was carried out using an isocratic method [34].

2.2.16. Estimation of total antioxidant using DPPH

The radical scavenging ability of diets was tested on the basis of the radical scavenging effect on the DPPH free radical. Antioxidant assays are based on measurement of the loss of DPPH color at 515 nm after reaction with test compounds [35]. In clean and labeled test tubes, 2 ml of DPPH solution (0.002% in methanol) was mixed with 2 ml of different concentrations of diets extract separately. The tubes were incubated at room temperature in dark for 30 minutes and measured at 515 nm (UV-Vis Jenway 6705 Series Spectrophotometer). The absorbance of the control DPPH was also noted. The scavenging activity of the diets was calculated using the formula:

DPPH scavenging activity % $=A_B–A_S/A_B\times100$, where A_B = absorbance of Blank (all reagents except sample), A_S = the absorbance of extracts.

2.3. Biological activities

2.3.1. Insecticidal and repellent activities

2.3.1.1 Mosquito colony

The mosquito *Culex pipiens* used in this study was obtained from the established colony at the Animal House, Faculty of Science, Al-Azhar University, Cairo, Egypt. Larvae were reared for several generations in 40 cm white enamel bowls containing 1000 ml dechlorinated water held at 27 ± 2 ºC, 75 ± 5% relative humidity (RH), with 14-10 h day/night photoperiod. Larvae were provided *ad libitum* with fish food as a diet. Produced pupae were transferred into cups filled with dechlorinated water and kept in (30×30×30 cm) wooden cadges. For adults, a piece of sponge soaked in sugar solution (10%) was provided in each cage. Three to four days old females were allowed to feed on a pigeon's blood and lay egg rafts for reproduction and development purposes.

2.3.1.2 Larvicidal activity

The larvicidal assay was carried out according to the World Health Organization protocol (WHO 2005) [36] with some modifications. Briefly, twenty-five newly emerged larvae (24 h old) were exposed to serial concentrations of each extract of *Lagenaria siceraria*. Larvae were treated with different concentrations from each extract/fraction according to its tolerance to tested materials. Each replicate was maintained in polyethylene plastic cup containing 25 larvae/250 mL. Each concentration was replicated five times. Larvae in the control groups were reared in dechlorinated water. The same amount of larval diet was supplied to all treatments. Larvae were observed and dead organisms were removed. Larval mortality was recorded to evaluate lethal concentrations.

2.3.1.3 Fecundity and fertility

In a separate experiment and as the same larvicidal activity evaluation protocol, larvae from the 3rd instar were treated with the LC_{50} and LC_{90} concentrations from each tested extract/fraction of *L. siceraria.* An equal number of males and females that survived and emerged from previous treatments were allowed to mate normally in standard cages (alongside the control) to determine the impact of tested materials on the females' fecundity and fertility. Fecundity (No. of eggs laid/female) was recorded and calculated as described by Rak and Ishii (1989) [37]. Fecundity was calculated by counting the total number of laid eggs divided by the number of females mated and survived till the end of the experiment. The fertility (egg-hatchability) percentages were calculated according to the following equation: Egg-hatchability % = (A / B) ×100, where (A) no. of hatched eggs, and (B) no. of laid eggs.

2.3.1.4 Repellent activity

Repellency was carried out according to the protocol of (WHO 2009) [38]. The LC_{50} and LC_{90} concentrations from each tested material of *L. siceraria* were separately applied to the ventral surface of pigeon after abdominal feathers removal and left for 10 min. Pigeons were placed for 2 h in cages containing blood-starved (for 72 h) females during the night from 8.00 to 11.00 pm. The commercial repellent, DEET 15% (Johnson Wax, Egypt) was applied as control. Treatments were replicated five times in separate cages. Later, number of fed and unfed females were counted and calculated. The percentage of repellency was calculated by the following formula: Repellency % = $[(A_c - A_t) / A_c] \times 100$. Where ($A_c$) the number of mosquitoes in the control group, and (A_t) the number of mosquitoes in the treated group.

2.3.1.5 Statistical analysis

Descriptive analysis including mean and standard error (SE) was calculated for each treatment. The larval mortality data were subjected to probit analysis to calculate LC_{50}, LC_{90} at 95% confidence limits. One-Way analysis of variance, lower and upper confidence limits and Chi-square values were calculated using SPSS (ver. 25). Holm Sidak post hoc test was used for pairwise comparisons. Data are presented as Mean ± SE. *P* value was considered significant at < 0.05.

3. Results and Discussion

3.1 Chemical composition of fruits, seeds and leave extracts of Bottle gourd

The Bottle gourd's parts (fruits, seeds and leaves) comprise various antioxidants, protiens, phenolics, flavonoids, tannins, sugars, carbohydrates and vitamins along with omega fatty acids, flavonoids, isoflavonoids, amino acids [39], and minerals [40] have been reported recently [21].

3.1.1 Moisture, Ash, pH and total solids of Bottle gourd fruits, seeds and leaves

The moisture content in Bottle gourd parts namely, fresh fruits, seeds and leaves were estimated to be of 94.27, 8.68 and 89.63 %, respectively, meanwhile the dry ones contain a moisture % of 7.36, 2.68 and 4.09, respectively. Alternatively, the ash content of the fresh plant's parts contain 0.27, 3.99, 2.59 %, respectively**,** and their corresponding dry parts contain 4.36, 4.26 and 23.95 %, respectively. According to our pH visualization, the fresh fruits, seeds, and leaves were estimated as 5.57, 6.58 and 7.59, respectively, while those of the dry parts were of pH 5.44, 5.25 and 7.17, respectively. Moreover, the total solids of Bottle gourd parts, fresh fruits, seeds and leaves were estimated to be 5.73, 91.32, and 10.37%. In contrast, their dry parts displayed very high ratio of total solids, particularly the fruits, leaves and roasted seeds with recorded values of 92.64, 95.91 and 97.66 %, respectively (Table 1). The results of our study reported herein showed a high consistance with literature [41,42].

Table 1. Estimation of moisture, ash, pH and total solids (T.S) of Bottle gourd fruits, seeds and leaves (gm/100gm [%])

Sample	Moisture (%)	Ash (%)	pH	T.S (%)
Fresh fruits (A)	94.27	0.27	5.57	5.73
Dry fruits (B)	7.36	4.36	5.44	92.64
Fresh seeds (C)	8.68	3.99	6.58	91.32
Roasted seeds (D)	2.34	4.26	5.25	97.66
Fresh leaves (E)	89.63	2.59	7.59	10.37
Dry leaves (F)	4.09	23.95	7.17	95.91

3.1.2 Total antioxidants, and proteins of Bottle gourd fruits, seeds and leaves

Bottle gourd is well known for its rich of antioxidant constituents [43]. In this study, the total antioxidants of fresh fruits, seeds and leaves were found to be 13.15, 5.65 and 11.53 %, meanwhile the dry parts showed high contents of total antioxidants, particularly, 31.51, 13.51 and 71.37%, respectively. Likely, the total protein content of the fresh parts were estimated to be 1.80, 40.11 and 5.51%, while the dry parts showed a great content of the total proteins, uniquely, 29.10, 39.08, and 50.86 %, respectively, as matched with those reported by Hassan et al [44] (Table 2). Antioxidants are only compounds that inhibit or delay the oxidation process by blocking the initiation of oxidizing chain reactions and inhibit the formation of reactive oxygen species (ROS) [45].

Table 2: Estimation of total antioxidants and crude proteins of fruits, seeds and leaves (gm/100gm [%])

Sample	Total Antioxidants (%)	Crude Protein (%)
Fresh fruits (A)	13.51	1.80
Dry fruits (B)	31.35	29.10
Fresh seeds (C)	5.65	40.11
Roasted seeds (D)	13.51	39.08
Fresh leaves (E)	11.53	5.51
Dry leaves (F)	71.37	50.86

Table 3: Estimation of total phenolic compounds, total flavonoids and total tannins Bottle gourd fruits, seeds and leaves (gm/100gm [%])

Sample	Total Phenolic compounds	Total Flavonoids	Total Tannins
Fresh fruits (A)	0.043	0.026	0.021
Dry fruits (B)	0.694	0.412	0.446
Fresh seeds (C)	0.508	0.391	0.110
Roasted seeds (D)	0.541	0.401	0.418
Fresh leaves (E)	0.649	0.057	0.024
Dry leaves (F)	0.756	0.545	0.580

3.1.3 Total Phenolic compounds, flavonoids and Tannins of Bottle gourd fruits, seeds and leaves

Phenolics are considered as widest secondary metabolites in the plant kingdom [46]. Due to redox properties such as reducing agents, hydrogen donators, and singlet oxygen quenchers, phenolics serve as better antioxidants. In addition, they have a metal chelation potential. The amount of total phenolics varied with the plant part and solvent used[47]. By the same manner, the total % of phenolic compounds, flavonoids and tannins of fruits, seeds and leaves of Bottle gourd were studied (Table 3). In particular, the fresh fruits, seeds and leaves revealed the presence of total phenolic compounds of 0.043, 0.508 and 0.649 %, while their corresponding dry parts displayed little bed higher (0.694, 0.541, and 0.756 %), respectively.

Flavonoids are a class of antioxidant agents which act as a free radical scavengers. They have a preventative role in the development of cancer and heart diseases [48]. Visualization of the total flavonoids displayed the presence of 0.026, 0.391, 0.057 % in fresh fruits, seeds and leaves, while those of dry ones showed higher percentages (0.412, 0.401, and 0.545), respectively.

Finally, tannins are the second most abundant polyphenols after lignins found in plants [49]. They are known for the treatment of ulcerated tissues and effective as antimicrobial and anticarcinogenic agents [50]. They can be served as defensives against pathogens and herbivory [51]. Our results showed that the plant parts were established to bear very low contents of tannins, at where the fresh and dry parts showed similarly a big difference in their contents of tannins, particularly, fresh fruits, seeds and leaves which have % of 0.021, 0.110, and 0.024, meanwhile the dry parts showed greater ratio of 0.446, 0.418 and 0.580 %, respectively.

3.1.4. Total sugars, carbohydrate, Vitamins (B, C, A, D, E, and K) of Bottle gourd fruits, seeds and leaves

The total sugars, implying reducing, non-reducing sugars, have been determined as well in the three parts of Bottle gourd. In case of fresh fruits, seeds and leaves, the total sugars were recognized to be 0.44, 4.59 and 0.12 %, meanwhile those of total sugars in dry fruits, seeds and leaves were estimated to be 9.25, 3.14 and 2.90 %, respectively recognizing that the dry fruits is highly abundant with total sugars. According to estimation result of reducing sugars and non-reducing sugars, it has been remarked that the latter are shown to be mostly likely equally distributed in fruits, while they were remarked to be 2:1 in case of seeds, and highly different in leaves with a greater abundance of reducing-sugars than those of non-reducing sugars (Table 4). Likely, the total carbohydrates determined in fruits, seeds and leaves were shown to be similarly great in case of dry fruits (21.03%), followed by seeds (16.21/13.49%), meanwhile they are very low in case of leaves (0.48/11.72%) (Table 4).

Bottle gourd is rich as well with different types of vitamins, particularly those belonging to vitamin B, such as vitamin B12 (cobalamin), B9 [folic acid], B6 [pyrodoxin], B2 [riboflavin] and B1 [thiamine]) (Table 5), along with vitamins C, A, D, E, and K (Table 6). Therefore, it was important to estimate

their abundance in the different parts of Bottle gourd: fruits, seeds and leaves. In accordance, vitamin B12 (Cobalamin) was diversely distributed showing 1.307, 15.745, and 26.166 mg/100g in fresh fruits, seeds and leaves of Bottle gourd, respectively; meanwhile it displayed 22.814, 8.904 and 252.324 mg/100g in the plant's dry parts, respectively. Vitamin B9 (folic acid) was shown with high abundance in case of roasted seeds (4.722) and leaves (15.646). Vitamin B6 (Pyridoxine) showed high abundance (mg/100g) in dry fruits (6.907) and leaves (4.300), while vitamin B2 (riboflavin) showed high abundance in dry fruits (6.480), fresh seeds (8.782) and dry leaves (41.429). Finally, dry fruits, fresh, roasted seeds and dry leaves showed high abundance of thiamin (4.075, 45.862, 30.437 and 17.78, respectively) (Table 5). Pyridoxin represents a form of vitamin B6 found commonly in food and used as dietary supplement, preventing pyridoxine deficiency, sideroblastic anemia, pyridoxine-dependent epilepsy and certain metabolic disorders [52].

Vitamins C, A, D, E and K were visualized in Bottle gourd parts. Bottle gourd is a rich source of vitamins C and K. Vitamin C showed high abundance in dry fruits (7.97), seeds (4.52) and leaves (14.93). Moreover, vitamin K was found to be the most abundant one in fresh and roasted (25.29, 36.28), respectively, meanwhile vitamins A, D and E were shown in very less quantities ranged between 0.001 and 0.4 (mg/100g) (Table 6).

Biologically, Vitamin B12 (cobalamin) is one of 8 B vitamins (B complex vitamins), which help the body convert food (carbohydrates) into fuel (glucose), producing the required energy of the body. These B vitamins also help the body use fats and protein, and are needed for healthy skin, hair, eyes, liver and the nervous system function properly. Vitamin B12 is an especially important vitamin for maintaining healthy nerve cells, and it helps in the production of DNA and RNA, the body's genetic material. Vitamin B12 works closely with vitamin B9 (folic acid) to help make red blood cells and to help iron work better in the body [53]. Folate and B12 work together to produce S-adenosylmethionine (SAMe), which is involved in immune function and mood. Vitamins B12, B6 (pyridoxin) and B9 work together to control blood levels of the amino acid homocysteine. Vitamin B6 shows the most promise for the treatment of pregnancy-induced nausea, and may be associated with lower risk of cancers, compared to low blood levels. Vitamin B6 in coenzyme forms performs a wide variety of functions in the body and is extremely versatile, with involvement in more than 100 enzyme reactions, mostly concerned with protein metabolism [54,55]. Vitamin B1 (Thiamin) plays alternatively a vital role in the growth and function of various cells [56].

Vitamin C is essential for normal growth, repairing of tissues and development, forming an important protein used to make skin, tendons, ligaments, and blood vessels; heal wounds and form scar tissue; repair and maintain cartilage, bones, and teeth; aid in the absorption of iron. Vitamin C is one of many antioxidants. Antioxidants are nutrients that block some of the damage caused by free radicals. Vitamins A, D, E, and K are called the fat-soluble vitamins. Small amounts of these vitamins are required in the diet to promote growth, reproduction, and health. Particularly, vitamin A plays a vital role in maintaining vision, and is essential for body growth, immune function, hair growth and reproductive health as well. Vitamin D on the other hand, is best known for its beneficial effects on bone health. Vitamin E is a group of related compounds divided into tocopherols and tocotrienols, serving strongly as antioxidant, protecting cells against free radicals and oxidative damage. Finally, Vitamin K is a family of compounds, playing a key role in blood clotting and supports bone health, without which there's a higher risk of excessive bleeding, which could lead to death [57].

It is worthy to refer herein that, the total antioxidants (phenolics and flavonoids, tannins), total protein, carbohydrates (including reducing and non-reducing sugars) and vitamins are higher concentrated dry parts of Bottle gourd than those of fresh parts of the plant, and this is attributed mainly to higher content of moisture in the fresh one than those of the dry, which reach 94 % in fresh fruits for example while it does not exceed 7% in the dry fruit.

3.2. Insecticidal and repellent activities

3.2.1 Larvicidal activity

The *Lagenaria siceraria* extracts namely: fruits (B), fresh seeds (C), roasted seeds (D) leaves (F) along with the subsequently chromatographic fractionated methanol fruits extract (methanol 1) starting with hexane, chloroform, ethyl acetate and then methanol (methanol 2) were screened for their potential larvicidal activity against the mosquito vector, *Culex pipiens*. Data of larvicidal activity given in (Table 7) show that extracts and fractions possess promising larvicidal activity. The larval mortality percentages were directly proportional to the tested concentration. Among tested materials, the highest larvicidal activity was observed in tested extracts (B, C, D, and F) of *L. siceraria* more than the fruits fractions. The LC_{50} concentrations for C, D, and F extracts recorded (12.015, 14.017 and 16.166 ppm, respectively) after 24 h from 3rd larval instar exposure, while the LC_{50} concentrations for the fruits fractions ranged between

109.715 ppm and 379.957 ppm. Additionally, the LC_{90} concentrations of tested extracts (B-D, F) showed restless acting with abnormal movement ending with quick larval mortality. For all tested materials, the obtained Chi-square values which consider significant at $P<0.05$ indicates the heterogeneity of the test population (see supplementary file, Tables S1-S9). The comparative lethal concentration values of aqueous extract of *L. siceraria* were (LC_{50} = 185.78; LC_{90} = 389.05 ppm) against the fourth-instar larvae of *Anopheles Stephensi* [58] and (LC_{50} = 261.67; LC_{90} = 606.49 ppm) against the 3rd instar larvae of the same vector [59]. Overall, it was revealed that tested fractions caused a decreased or increased larvicidal activity if compared with previously reported studies, while tested extracts (fruits, seeds (fresh/roasted) and leaves) induced much more pronounced larvicidal activity as compared to tested fractions.

Table 4: Estimation of total (reducing and non-reducing) sugars and total carbohydrates of Bottle gourd fruits, seeds and leaves (gm/100gm [%])

Sample	Total Sugars (%)	Reducing Sugars (%)	Non-Reducing Sugars (%)	Total Carbohydrate (%)
Fresh fruits (A)	0.44	0.28	0.16	0.99
Dry fruits (B)	9.25	5.84	3.41	21.03
Fresh seeds (C)	4.59	2.80	1.79	16.21
Roasted seeds (D)	3.14	2.36	0.78	13.49
Fresh leaves (E)	0.12	0.08	0.04	0.48
Dry leaves (F)	2.90	1.92	0.98	11.72

Table 5: Estimation of Vitamins B of Bottle gourd fruits, seeds and leaves [mg/100gm]

Sample	Vitamin B12 (Cobalamin)	Vitamin B9 (Folic Acid)	Vitamin B6 (Pyrodoxine)	Vitamin B2 (Riboflavin)	Vitamin B1 (Thiamine)
Fresh fruits (A)	1.307	0.168	0.396	0.371	0.234
Dry fruits (B)	22.814	2.925	6.907	6.480	4.075
Fresh seeds (C)	15.745	2.966	0.488	8.782	45.862
Roasted seeds (D)	8.904	4.722	2.655	2.966	30.437
Fresh leaves (E)	26.166	1.623	0.446	4.296	1.844
Dry leaves (F)	252.324	15.646	4.300	41.429	17.780

Table 6: Estimation of Vitamins C, A, D, E and K of Bottle gourd fruits, seeds and leaves (mg/100gm)

Sample	Vitamin C	Vitamin A	Vitamin D	Vitamin E	Vitamin K
Fresh fruits (A)	0.457	0.011	0.004	0.001	0.269
Dry fruits (B)	7.973	0.195	0.067	0.017	4.694
Fresh seeds (C)	6.319	0.398	0.045	0.300	25.287
Roasted seeds (D)	4.516	0.306	0.038	0.123	36.275
Fresh leaves (E)	1.549	0.005	0.0007	0.002	0.179
Dry leaves (F)	14.936	0.053	0.0065	0.015	1.727

Table 7: Larvicidal activity of the 3rd larval instar of the mosquito, *Culex pipiens* treated with different extracts and fruits fractions of *Lagenaria siceraria* after 24 h of exposure.

Extracts/Fractions (ppm)	**LC_{50} (LCL–UCL) (ppm)**	**LC_{90} (LCL–UCL) (ppm)**	***d.f.***	**χ^2**
Fruits (B)	37.099 (32.493 – 43.023)	154.547 (119.139 – 217.510)	5	7.179[a]
Fresh seeds (C)	12.015 (10.629 – 13.705)	46.625 (37.153 – 62.445)	5	9.818[a]
Roasted seeds (D)	14.017 (12.288 – 16.218)	59.131 (45.685 – 82.943)	5	4.862[a]
Leaves (F)	16.166 (14.078 – 18.928)	69.930 (52.901 – 101.380)	5	9.781[a]
Methanol-1	342.873 (300.009 – 397.348)	1502.998 (1154.947 – 2124.993)	5	16.852[a]
Hexane	286.815 (255.152 – 324.524)	1035.248 (841.179 – 1347.881)	5	6.034[a]
Chloroform	133.392 (116.964 – 154.107)	571.557 (442.332 – 799.386)	5	8.567[a]
Ethyl acetate	109.715 (97.147 – 124.758)	428.023 (343.215 – 567.896)	5	11.722[a]
Methanol-2	379.957 (337.114 – 430.876)	1449.145 (1169.294 – 1905.399)	5	33.311[a]

(LC_{50}) concentration that kills 50% of population, (LC_{90}) concentration that kills 90% of population, (LCL) lower confidence limit, (UCL) upper confidence limit, (Df) degree of freedom (χ^2) Chi-square, [a] Significant at $P<0.05$. Five replicates were used in each treatment, n= 625. No mortality was recorded in the control. Methanol-1 = The original fruits (B) extract before chromatographic fractionation; Methanol-2 = the residual fraction obtained from the column after final chromatographic fractionation of the fruits extract (B).

Table 8: Fecundity and egg-hatchability (%) of the mosquito, *Culex pipiens* females after treating the 3rd larval instar with the LC_{50} and LC_{90} values of different extracts and fruits fractions of *Lagenaria siceraria*.

Treatments		Fecundity (Mean ± SE)	Hatchability % (±SE)
Control		160.0 ± 1.70^{a}	91.02 ± 1.01^{a}
Fruits (B)	LC_{50}	106.4 ± 2.25^{b}	73.90 ± 2.26^{b}
	LC_{90}	38.4 ± 1.44^{c}	36.69 ± 3.14^{cdf}
Fresh seeds (C)	LC_{50}	76.4 ± 2.01^{d}	32.99 ± 2.25^{d}
	LC_{90}	18.0 ± 1.41^{e}	15.55 ± 4.87^{e}
Roasted seeds (D)	LC_{50}	84.2 ± 2.06^{f}	41.57 ± 0.63^{f}
	LC_{90}	26.6 ± 1.66^{gi}	31.38 ± 2.91^{gd}
Leaves (F)	LC_{50}	86.4 ± 1.96^{hf}	43.99 ± 1.04^{hfi}
	LC_{90}	25.2 ± 1.46^{ig}	38.03 ± 3.85^{idf}
Methanol-1	LC_{50}	141.0 ± 2.43^{lj}	87.93 ± 2.32^{aj}
	LC_{90}	122.2 ± 2.73^{mks}	86.29 ± 2.62^{al}
Hexane	LC_{50}	138.0 ± 1.92^{j}	88.16 ± 1.35^{aj}
	LC_{90}	118.2 ± 1.56^{km}	84.19 ± 2.13^{al}
Chloroform	LC_{50}	111.0 ± 1.87^{pbn}	79.42 ± 1.52^{lb}
	LC_{90}	75.0 ± 1.58^{qd}	68.09 ± 1.83^{mb}
Ethyl acetate	LC_{50}	106.6 ± 1.89^{nbp}	82.67 ± 1.03^{jlm}
	LC_{90}	$69.8 \pm 1.28o^{q}$	75.06 ± 1.80^{kb}
Methanol-2	LC_{50}	138.6 ± 2.46^{rjl}	89.48 ± 2.53^{aj}
	LC_{90}	125.2 ± 1.32^{sl}	88.41 ± 2.06^{aj}

Within each column, means with different letters are significantly different (P<0.05), (SE) standard error. Five replicates were used in each treatment.

3.2.2 Fecundity and fertility

The LC_{50} and LC_{90} concentrations of different extracts and fruits fractions of *L. siceraria* were tested to evaluate their impact on the total number of eggs laid per female (fecundity) and to assess their effect on the hatchability percentages of *C. pipiens* females treated as larvae. Fecundity and fertility were found to be inversely proportional to the applied concentrations (Table 8). Fecundity was significantly reduced to more than 6 folds when the LC_{90} concentrations of (C, D, and F) extracts were applied and to more than 4 folds when LC_{90} concentration of the fruits extract (B) was applied compared with the untreated control females. Fruits fractions also exhibited promising activity, where fecundity reduced to 69.8 ± 1.28 and 75.0 ± 1.58 eggs/♀ when the LC_{90} concentration of ethyl acetate and chloroform fraction was applied respectively, compared to 160.0 ± 1.70 eggs/♀ for the control group. Egg-hatchability percentages decreased in a concentration-dependent manner and this effect was more pronounced in tested extracts than in fruits fractions. The lowest egg-hatchability percent was recorded among females treated with the LC_{90} concentration of the fresh seeds extract (C) extract, where it reduced significantly (P<0.05) from 91.02 ± 1.01 for the control to 15.55 ± 4.87. Meanwhile, almost all recorded egg-hatchability % among females treated with different fruits fractions were found to be non-significantly decreased except for those treated with the LC_{50} and LC_{90} concentrations of ethyl acetate and chloroform fractions. However, plant extracts and its phytocompounds may acts as potent ovicidal agent particularly when higher concentrations applied revealing maximum egg mortality [60]. In the present study, LC_{90} concentrations significantly reduced both fecundity and egg hatchability (%) more than LC_{50} concentration of all tested extracts/fractions and this pattern may be due to the shell that cover eggs of mosquitoes that may needs higher concentrations to be penetrated.

3.2.3 Repellency test

To investigate the Repellency potential of tested extracts and fruits fractions of *L. siceraria, C. pipiens* starved females were allowed to feed on a pigeon after topical application of the LC_{50} and LC_{90} concentrations from each tested material separately. Results illustrated in (Figures 1 and 2) showed that the B and C extracts behave like strong repellents either for LC_{50} (repellency percent >85%) or LC_{90}

treatments (repellency percent >90%), while the D and F extracts showed relatively low repellent activity for both tested concentrations (repellency percent <70%) in comparison with the commercial standard repellent (DEET) in the four tested times. On the other hand, the fruits fractions showed promising repellent activity, particularly those of ethyl acetate, hexane, and chloroform, where the LC_{50} and LC_{90} concentrations of them showed much higher escape responses (repellency percent >70%) than those of methanol-1 and methanol-2 even when the LC_{90} concentration was applied (repellency percent <45%) in comparison with the commercial DEET (Figures 1 and 2). At all tested concentrations, no mortality was recorded.

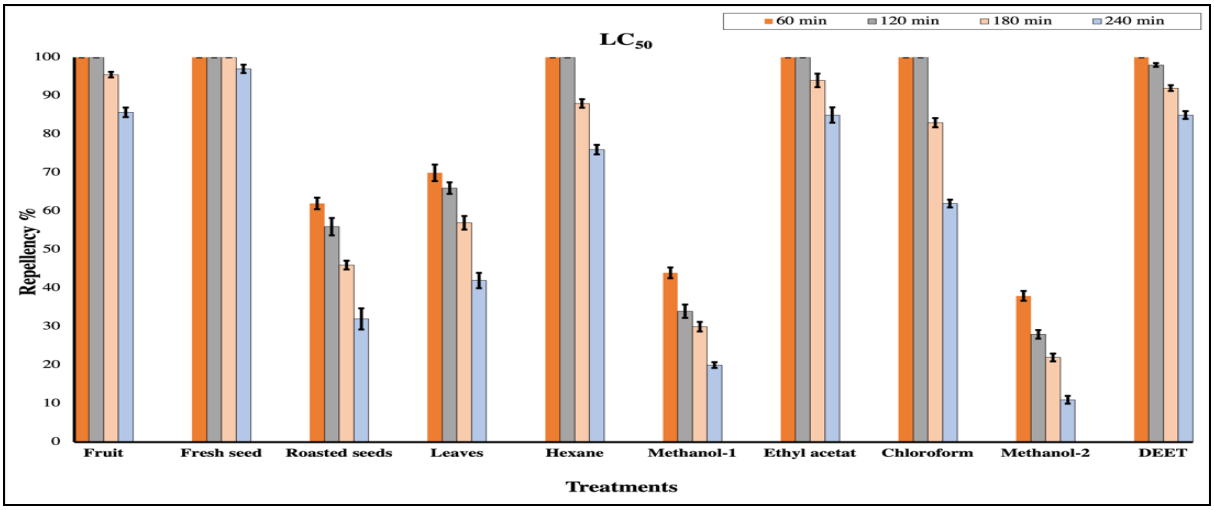

Figure 1. Repellency of the LC_{50} concentrations of different extracts and fruits fractions of *Lagenaria siceraria* against *Culex pipiens* adult females.

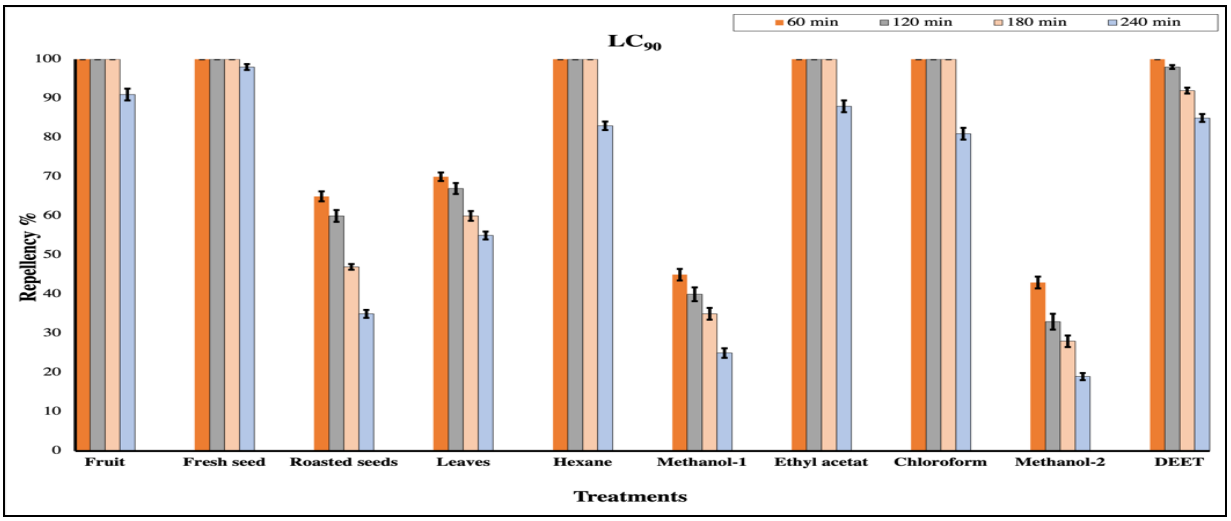

Figure 2. Repellency of the LC_{90} concentrations of different extracts and fruits fractions of *Lagenaria siceraria* against *Culex pipiens* adult females.

4. Conclusions

During our continual research evaluation of the chemical composition of the fresh and dry fruits, seeds and leaves of *L. siceraria* cultivated in Egypt, we have intensively determined the moisture, ash, pH, total solids, antioxidants, proteins, phenolics, flavonoids, tannins, reducing and non-reducing sugars, carbohydrates, B vitamins (B1, B2, B6, B9, and B12), and vitamins (C, A, D, E and K). Moreover, the insecticidal properties of the different plant's parts were investigated against the mosquito, *Culex pipiens* larvae and adults. In accordance, tested materials showed larvicidal activity in particular fresh/roasted seeds and leaves extracts with LC_{50} concentrations ranging from 12.015 to 16.166 ppm. Fecundity and egg-hatchability percentages were significantly reduced when LC_{50} and LC_{90} concentrations applied. Additionally, tested extracts reduced fecundity to > 6 folds when LC_{90}

concentration was applied. Repellency test elucidated that fruits and fresh seeds extracts behave like strong repellents either when LC_{50} (>85%) or LC_{90} (>90%) was applied. On the whole, obtained data provide detailed analysis to the chemical composition of L. siceraria cultivated in Egypt and highlight the insecticidal potential of different plant-part extracts as an effective and safe agent.

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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