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Phytochemical and insecticidal evaluation of agro-waste of *Lagenaria siceraria* (Cucurbitaceae) plant against the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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Abstract:

Lagenaria siceraria (Cucurbitaceae) commonly known as bottle gourd cultivated in Africa, Asia and now cultivated in Egypt. After harvesting fruits of *L. siceraria*, the leaves of the plants are remained as agro-waste in the field. Preliminary phytochemical screening of *L. siceraria* leaves revealed the presence of steroids, alkaloids, flavonoids, terpenoids, tannins and saponnins. The leaves extracted by ethanol and fractionated *via* solvents of different polarities; petroleum ether, ethyl acetate and n-butanol. The toxicity of extracts was tested against the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) under laboratory conditions. The petroleum ether extract exhibited more activity followed by ethanol, ethyl acetate and n-butanol. The LC_{50} were 0.201, 0.352, 0.539 and 0.735%, respectively. The petroleum ether extract was subjected to saponification reaction and its chemical constituents were investigated by Gas Chromatography-Mass Spectrometry. This indicated that the agro-waste of *L. siceraria* plant can be applied as green insecticide to control the cotton leafworm, *S. littoralis.*

Introduction

The Plant kingdom produce a great variety of phytochemicals (secondary metabolites) including flavonoids, saponnins, tannins, steroids, terpenoids and alkaloids are often important for mediating interactions between plants and their biotic environment. They can be models of active defense against phytophagous insects and pathogens. *Cucurbitaceae* family is commonly known as gourd, melon and pumpkin family. This family is composed of 118 genera and 825 species, which are widely distributed in the warmer region of world (Rahman, 2003).

Among all the plants of Cucurbitaceae family *Lagenaria* species is the most popular. *Lagenaria siceraria* (Molina) member of genus *Lagenaria* that is derived from the word lagena, meaning the bottle. *L. siceraria* known as bottle gourd is a common fruit vegetable used throughout the India. This species follows the plant Kingdom : Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Cucurbitales, Family: Cucurbitaceae, Genus: Lagenaria and Species

: *L. siceraria* *L. siceraria* large pubescent, annual, prostrate or climbing herb cultivated in Africa, Asia and now cultivated in Egypt, its fruit is a source of vitamin-B complex and choline along with fair source of vitamin-C and β-carotene, amongst any other vegetable known to man till date, it is also good source of minerals and amino acids (Kirtikar and Basu, 2001). The fruits have antimicrobial, cytotoxicity, anticancer and antihepatotoxicity properties (Answar *et al.*, 1984; Desta, 1993; Furukawa *et al.*, 1995 and Shiwaikar and Sreenivasan, 1996). Lagenin, a ribosome inactivating protein (RIP) isolated from the seeds of *L. siceraria* possesses immune protective, antitumor, anti HIV and anti proliferative properties (Wang and Ng, 2000). After harvesting fruits of *L. siceraria*, the leaves of the plants are remained as agro-waste in the field. Rahman (2003) described the morphology of this plant species as follows: Stem: The stem is prostrate or climbing in nature with angular, ribbed thick, brittle, softly hairy. Leaves: Leaves are simple with long petiole from 25-30 mm long thick, hallow, densely hairy with two small lateral glands located at the leaf base. Leaf lamina: The lamina of leaf is usually five lobed, broad cordate, pubescent with soft hairs and the tendrils are branched. The flowers are solitary axillary, pedicellate, unisexual and monoecious. Petals: Petals are mainly five, white or cream colored which opens in the evening. Fruits: Fruits are green in color which turns to yellow on maturity. Fruits are large densely hairy often cylindrical or flask shaped or globose. They are constricted at the middle. Pulp: Pulp is pale brown in color and the dried fruit has thick hard hollow structure. Seeds: Seeds are embedded in the spongy pulp and compressed with two flat facial ridges (Figure, 1) .

Figure (1): *Lagenaria siceraria* plant

This study was planned to evaluate the insecticidal activity of ethanolic extract of *L. siceraria* agro-waste (leaves) and its fractions in solvents of different polarities against the cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) under laboratory conditions. . Depending on the insecticidal evaluation, identify the chemical constituents in the bioactive fraction using chromatographic techniques.

Materials and methods

1. Chemicals:

The organic solvents of different polarities (petroleum ether, ethyl acetate, ethanol and n-butanol) used in the present work were obtained from Edwic Company.

2. Gas Chromatography-Mass Spectroscopy analysis (GC/MS):

GC-MS analysis was performed on a Varian GC interfaced to Finnegan SSQ 7000 Mass selective Detector (SMD) with ICIS V2.0 data system for MS identification of the GC

components. The column used was DB-5 (J&W Scientific, Folosm, CA) cross-linked fused silica capillary column (30 m. long, 0.25mm. internal diameter) coated with poloy dimethyl-siloxane (0.5µm. film thickness). The oven temperature was programmed from 50 °C for 3 min., at isothermal, then heating by 7 °C /min. to 250 °C and isothermally for 10 min., at 250 °C. Injector temperature was 200 °C and the volume injected was 0.5µl. Transition-line and ion source temperature were 250 °C and 150 °C, respectively. The mass spectrometer had a delay of 3 min. to avoid the solvent plead and then scanned from m/z 50 to m/z 300. Ionization energy was set at 70 eV. (Agricultural Research Center, Dokki, Giza).

3. Experimental insect:

Newly molted 4th instar larvae of the cotton leafworm *S. littoralis* were selected for this study. The culture of laboratory susceptible stain was reared under optimum conditions 25±2°C, 75±5% RH. and (16L:8D) light: dark photoperiod in the plant protection research institute, Dokki, Giza, Egypt. The larvae were reared on castor bean leaves

4. Plant material:

The agro-waste leaves of *L. siceraria* plant were remained after harvesting fruits. *L. siceraria* leaves were collected from field of experimental farm in Zagazig Governorate, Egypt and identified by Dr. Reda Mohamed El-Sayed, Faculty of Agriculture, and Zagazig University. The collected leaves were cleaned, left to dry under shade and homogenized to fine powder using electric mill.

5. Extraction and fractionation

A weighed amount of the powder (250 gm) was soaked in ethanol, the obtained extract from filtration was evaporated under vacuum at 40°C on rotary evaporator to yield (45.32 gm). Part of the crude extract (30 gm) was suspended in distilled water in a separating funnel, petroleum ether was added to the funnel which was shaken gently and allowed to stand for about 2 hrs, the pet. ether layer was separated in a flask, and the process was repeated three times to ensure maximum extraction. In the same manner, ethyl acetate and n-butanol fractions were obtained, leaving behind a residual aqueous fraction. The solvents were evaporated in rotary evaporator and the dried fractions were collected.

6. Preliminary phytochemical screening;

Preliminary phytochemical screening of *L. siceraria* leaves was carried out as the standard prodedures (Sofowara, 1993 and Harborne, 1998).

6.1. Alkaloids: The alcoholic extract (corresponding to 2.5 g leaves powder) was evaporated to dryness and the residue was heated on a boiling water bath with 2N HCl (2 ml). After cooling the mixture was filtered and the filtrate was treated with few drops of Mayer's reagent.

Mayer's reagent: dissolve 136 g $HgCl_2$ in 60 ml water and 5 g KI in 10 ml water. Combine both solutions and make up with water to 100 ml. add a few drops to an acidified solution (HCl or diluted H_2SO4), a yellow to orange precipitate will appear indicated a positive test.

6.2. Terpenoid: Alcoholic extract was evaporated and the residue was treated with anhydrous chloroform and filtered. Conc. sulphuric acid was added to the filtrate and shaken. Yellow color was produced, changed to orange then red.

6.3. Steroids: Alcoholic extract was evaporated and the residue was treated with anhydrous chloroform and filtered. The filtrate was treated with acetic acid and drops of Conc. sulphuric acid were added carefully on the side of test tube. Greenish color appeared (lower layer).

6.4. Flavonoids: A small quantity of the extract was dissolved in diluted sodium hydroxide (5%) and hydrochloric acid was added to the mixture. A yellow solution that turns colorless on addition of hydrochloric acid indicated the presence of flavonoids.

6.5. Saponins: one gram of the leaves was boiled with 10 ml water for few minutes and filtered. The filtrate was vigorously shaken. The persistent froth (1 cm height) was observed for 1 hr. indicates the presence of saponins.

6.6. Anthraquinones: The alcoholic extract (corresponding to 5g plant material) was evaporated to dryness and the residue was heated on a boiling water bath with 2N HCl (10 ml). After cooling the mixture was filtered and the filtrate was extracted with benzene. The benzene extract was shaken with NH4OH (5 ml) and a positive reaction was evidenced by the formation of a red color in the alkaline layer.

6.7. Tannins: About one gram of the powdered sample was boiled with 10 mL of distilled water for five minutes, filtered while hot and a few drops of ferric chloride reagent was added to the filtrate. A red color indicates a positive test.

7. Preparation of tested concentrations:

The prepared fractions of *L. siceraria* were diluted in distilled water containing one drop of an emulsifier Tween®20 (Tween®20; Sigma-Aldrich), to ensure complete solubility of constituents. The applied concentrations were selected after preliminary bioassays with a wide range of concentrations to determine the proper range. Two negative control treatments were used in each experiment. One of them was for one drop of emulsifier (Tween®20) in water, where the Tween®20 solutions showed no significant mortality effect against *S. littoralis* larvae (data not shown) compared with water.

8. Toxicological evaluation:

Leaf dipping technique was applied; Castor bean leaves were dipped for 30 seconds in each concentration of tested extracts then left to dry. The treated leaves were offered to newly molted 4th instar larvae of *S. littoralis* for 48 hrs. then replaced by untreated ones. Accumulative mortality percentages were recorded, then corrected according Abbott's formula (1925). From the corrected mortality percentages, the corresponding toxicity lines (LC-P lines) were estimated in addition to determine LC_{25} and LC_{50} values and their confidence limits, slope values of tested extract were also estimated.

9. Chromatography and identification of chemical constituents:

Petroleum ether fraction, which showed the highest toxicity activity against the 4th instar larvae, was selected for phytochemical investigation through GC/MS technique.

Pet. ether extract was subjected to Saponification reaction; hydrolysis with 10% alcoholic NaOH over water bath under reflux for 30 min., cooling, then diluted with water and extracted wit diethyl ether afforded un-saponifiable fraction, while the saponifiable fraction was acidified with diluted HCl then extracted with diethyl ether. The saponifiable fraction was analyzed by GC/MS technique for characterization the petroleum ether constituents.

Results and discussion

L. siceraria plant was cultivated in Africa, Asia and now cultivated in Egypt. After harvesting fruits of the plant, the leaves are remained as agro-waste. Phytochemical screening of *L. siceraria* leaves showed the presence of flavonoids, saponins, tannins, steroids, terpenoids and alkaloids active phytoconstituents as shown in Table (1).

L. siceraria leaves were extracted by ethanol and fractionated via solvents of different polarities; petroleum ether, ethyl acetate and n-butanol, and examined as natural insecticides against newly molted 4th instar larvae of *Spodoptera littoralis* under laboratory conditions. , the tested extracts of *L. siceraria* leaves exhibited a high degree of efficiency as insecticide against *S. littoralis* larvae.

The petroleum ether extract exhibited a high degree of efficiency as insecticide followed by ethanol, ethyl acetate and finally n-butanol extracts. The activity was concentration dependent of the tested

extracts. LC_{25} and LC_{50} values and their confidence limits obtained from probit analysis for mortality values are showed in Table (2).

The obtained results agree with those obtained by Amit and Sangh (2012) who suggested that the crude methanol extract of *L. siceraria* leaves produced anthelmintic activity against Indian earthworm, *Pheretima posthuma* when compared with the conventionally used drug (Albendazole).

Table (1): Preliminary phytochemical screening of *Lagenaria siceraria* leaves.

Test	Observation	Result
Test for Flavonoids Filtrate + NaOH (5%)	Yellow color was observed in test tube, then turned to colorless on addition of HCl	Presence of Flavonoids
Test for Tannins 5g of leaf extract + 10mL of distilled water. Filter + drops of ferric chloride (0.1%)	Brownish green to blue black precipitate was observed in test tube.	Presence of Tannins
Test for Saponins (Frothing test) 1g of leaves + distilled water + gentle warming and shaking vigorously	Froth was observed in the test tube, while disappeared after about one hour.	Presence of Saponnins
Test for Alkaloids alcoholic extract + 5mL Aqueous HCl (2N) + drops of Mayer's solution	An yellowish precipitate was observed in test tube	Presence of Alkaloids
Test for Terpenoids alcoholic extract + 2 ml $CHCl_3$ + 3 ml Conc. H_2SO_4	yellow layer then turned to orange was observed in test tube	Presence of Terpenoids
Test of Steroids alcoholic extract + 2 ml $CHCl_3$ + 3 ml Conc. H_2SO_4 + acetic acid	Greenish color layer was observed on test tube	Presence of Steroids
Test for Anthraquinones 5g of leaves + 10mL Benzene. Filtrate +5mL 10% NH_4OH solution	No color change was observed.	Absence of Anthraquinones

Table (2): Insecticidal activity of *Lagenaria siceraria* leaves against the 4th instar larvae of *Spodoptera littoralis.*

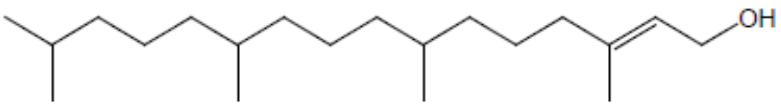
Tested extract	LC_{25} (%) Confidence limits at 95%	LC_{50} (%) Confidence limits at 95%	Slope ± SE	Toxicity index
Petroleum Ether	0.083 (0.057 – 0.108)	0.201 (0.164 – 0.238)	1.754 ± 0.177	100
Ethanol	0.159 (0.125 – 0.191)	0.352 (0.305 – 0.407)	1.947 ± 0.179	56.94
Ethyl acetate	0.213 (0.166 – 0.257)	0.539 (0.457 – 0.654)	1.669 ± 0.179	37.22
n-butanol	0.310 (0.254 – 0.365)	0.735 (0.618 – 0.915)	1.801 ± 0.196	27.34

The obtained insecticidal evaluation data indicated that pet. Ether fraction is the most toxic extract. For this reason, this extract was subjected to saponification reaction, and its chemical constituents were chemically investigated using GC-MS technique (Table ,3).

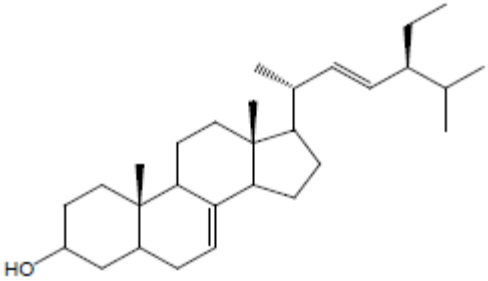
Table (3): Chemical constituents of petroleum ether extract.

Component name	R.T	Area%	Molecular formula
Dodecane	10.42	0.41	$C_{12}H_{26}$
Hexadecene	16.81	0.43	$C_{16}H_{23}$
Octadecane	20.96	0.20	$C_{18}H_{36}$
Neophytadiene	24.21	0.35	$C_{20}H_{38}$
6,10,14-trimethyl-1,2-pentadecanone	25.05	1.16	$C_{18}H_{36}O_2$
3,7,11,15-tetramethyl-1,2-hexadecen-1-ol (Phytol)	28.51	53.01	$C_{20}H_{40}O$
9,12,15-octadecatrienal	32.82	2.05	$C_{18}H_{30}O$
Eicosene	34.06	1.09	$C_{20}H_{40}$
Docosene	36.49	2.38	$C_{22}H_{44}$
Hexacosene	40.11	2.24	$C_{26}H_{52}$
Squalene	42.33	3.02	$C_{30}H_{50}$
Cholesterol	44.38	0.32	$C_{27}H_{46}O$
Campesterol	46.48	1.04	$C_{28}H_{48}O$
Spinasterol	47.11	7.89	$C_{29}H_{48}O$
β-Sitosterol	47.76	1.38	$C_{29}H_{50}O$
Fucosterol	48.01	1.65	$C_{29}H_{48}O$
22-dihydro-spinasterol	48.78	5.78	$C_{29}H_{50}O$

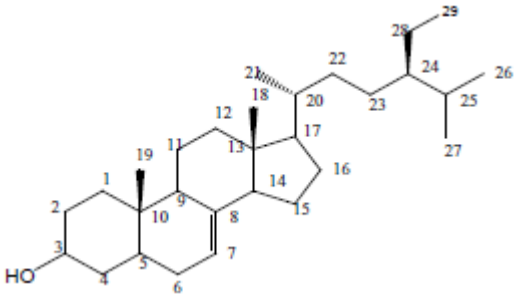
The qualitative and quantitative compositions of petroleum ether extract were analyzed GC/MS where, the most abundant constituents were phytol (53.1%), spinasterol (7.89%) and 22-Dihydrospinasterol (5.78%).



Phytol



Spinasterol



22-Dihydrospinasterol

The insecticidal activity of petroleum ether extract of *L. siceraria* leaves against the 4th instar larvae of *S. littoralis* may due to the presence of natural phytosterols as spinasterol, 22-dihydrospinasterol, Fucosterol, β-Sitosterol and Campesterol., Where spinasterol exhibited different biological activities including anti-ulcerogenic (Klein *et al.,* 2010), anti-inflammatory (Borges *et al.,* 2014), and antiproliferative activities (Ntie-Kang and Yong, 2014). Spinasterol, 22,23-dihydrospinasterol possess pharmacological and cytotoxic exertions likewise, it was isolated from *Bougainvillea spectabillis* and exhibited strong inhibition of xanthine oxidase being IC_{50} of 39.21 µM, (Chang *et al.*, 1994).

Meneses-Sagrero *et al.* (2017), identified spinasterol from the methanol extract of *Stegnosperma halimifolium* and evaluated against cancer cell line, they found that spinasterol exhibited potential activity as antiproliferative against two cell lines of cervical cancer such as HeLa and RAW 264.7. The petroleum ether, chloroform, methanol, absolute alcohol and water of *Lagenaria siceraria* plant showed moderate to potent antimicrobial activity against the bacterial strains: *Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Salmonella typhi, Staphylococcus aureus* and antifungal strains: such as *Aspergillus flavus, Aspergillus oryzae and Trichoderma harzianum* (Nagaraja *et al.*, 2012). Cucurbitaceae family is major source of medicinal agents since ancient time. Various plants parts of this family have been established for their pharmacological potential. The use of *L. siceraria, Cucumis sativus* and *Cucurbita maxima* leaves as useful anti-oxidant and cytotoxic agents (Alamgir *et al.,* 2016). The potency of methanol, acetone, chloroform and petroleum ether extracts of *Lagenaria siceraria* leaves against the 3rd instar larvae of the housefly, *Musca domestica* (Diptera: Muscidae) which consider as a diseases vector was evaluated by Mostafa *et al.,* (2018), where all plant extracts showed a larvicidal activity against the 3rd instar larvae of *M. domestica* larvae; however, the petroleum ether extract was found that to be the more effective than chloroform, acetone and methanol extracts. The LC_{50} values of methanol, acetone, chloroform and petroleum ether extracts

recorded 468.5, 432.1, 433. 8 and 101.4 ppm; respectively. As well, at the LC_{50} values of the tested extracts exhibited repellent activity against *M. domestica* adults. The effective plant extract that exhibited high antifeedant or repellency action was petroleum ether extract as compared to chloroform, acetone and methanol extracts. From all mentioned results, it can be concluded that the petroleum ether extract of *L.siceraria* leaves (Agro-waste) can applied as natural insecticide to control the cotton leafworm, *S. littoralis*.

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