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Phytochemical composition of Neem (*Azadirachta indica* A. Juss), and Argel (*Solenostemma argel* L.) and their biocidal activity against mosquitoes larvae

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Abstract

Mosquitoes are worldwide insect-borne disease caused agents. Malaria is wide spread in tropical and subtropical regions. This work aim to determine the phytochemical composition of Neem (*Azadirachta indica*), and Argel (*Solenostemma argel*) and to test their biocidal activity against mosquitoes larvae. Plant materials were brought from within Wad Medani City, Gezira State, Sudan, while *Anopheles* and *Culex* larvae were brought from the insectary of Blue Nile Institute for Communicable Diseases, University of Gezira. In this study the standard methods for phytochemical screening were followed to determine the presence of the main classes, also the WHO (2012) procedure to test the toxic product against mosquitoes larvae was also followed. The results showed that, neem leaves and Argel shared the presence of flavonoids, glycosides, tannins, steroids and alkaloids. The hydro-ethanol extract of Neem leaves reflected LC50 =278, 63 mg/L against *Anopheles larvae*, while that of the *Culex* was 356.22 mg/L after 24 hours. The hydro-ethanol extract of Argel leaves reflected LC50 = 265.49 mg/L against Anopheles larvae, while that of the *Culex* was 349.58 mg/L after 24 hours. Also Anopheles larvae were more susceptible than *Culex* larvae. The effort of testing the available plant product to combat insect pests and vectors should not ignore.

Keywords: Argel; Neem; Phytochemical screening; Mosquito larvae; Biocides

1 Introduction

Herbals are a seed, fruit, root, bark, bud or other substance primarily used for flavoring, coloring or preserving food, and they are a long times used in insect control, medicine, religious rituals, cosmetics or perfume production, or as spices. From these Neem leaves (*Azadirachta indica*) and Argel (*Solenostemma argel*) are available in Sudan.

Neem (*A. indica* A. Juss) family Meliaceae is a tropical evergreen related to mahogany. Native to east India. It contains azadirachtin as the major active ingredient, which is the main constituent for insecticidal activity [1]. The leaves of the plants are repellent against stored grain insect's pests [2]. All parts of the plant are intrinsically reported an internal chemical defense repelling insect pests, and used in effective pest control [3].

Argel (*S. argel* L.) family Apocynaceae is desert plant of traditional medical used in folk medicine in different place in the world especially in Africa country. Argel is considered to be medicinally important in Sudan, Libya and Chad.Argel leaves and its aqueous extracts are used in herbal medicine for the treatment of a large number of diseases. The plant also showed an insecticidal efficiency and hence was used to control insects [4].

Malaria is a mosquito-borne infectious disease caused by *Plasmodium*. It transmitted with a bite from an infected female mosquito, which introduces the parasite via its saliva into the circulatory system, and ultimately to the liver where they

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mature and reproduce. Malaria is wide spread in tropical and subtropical regions in abroad band around the equator, including Africa, Asia and Americas [5].

Natural drugs have been a part of the evolution of human, healthcare for thousands of years. More than 85% of the global populations turn to plant derived medicines as their first line of defenses for maintaining health and compacting diseases. 119 secondary plant metabolites derived from plants are used globally as drugs, 15% of all angiosperms have been investigated chemically and of that 74% of pharmaco-logically active plant derived component were discovered [6].

This work aims to determine the phytochemical composition of Neem (*A. indica*), and Argel (*S. argel*) and their biocidal activity against mosquito larvae.

2 Material and methods

2.1 Materials

The samples of Argel leaves were brought from the local market, whereas the leaves of Neem were collected from the local trees, both from Wad Medani City, Gezira State, Sudan. These samples were shade dried and crushed, then were extracted with 99% ethanol and were used to test the susceptibility of Anopheles arabiensis and Culex quinquefasciatus larvae brought from the insectary of Blue Nile Institute for Communicable Diseases, University of Gezira.

2.2 Phytochemical screening tests

The plant samples were analyzed using the procedures of Vinoth *et al*. [7] to test the presence (+) or absence (-) of the main classes of phytochemical individually.

2.2.1 Saponnins

In a 10 ml test tube, 5 ml of distillated water was added to 0.5 g of each powdered plant materials, and the tube was shaken vigorously for 1 minute. The presence of saponnins was indicated by the formation of froth that lasted for about 15 minutes.

2.2.2 Tannins

In test tube, 3-4 drops of 10% ferric chloride solution were added to 2 ml of each aqueous diluted extract. A blue or green color indicates the presence of tannins.

2.2.3 Glycosides

In a 40 ml test tube, 10 ml of dilute H2SO4 were added to 1 ml extract and boiled for 15 minutes, and then 10 ml of Fehling's solution were also added. The appearance of brick red precipitate indicated the presence of glycosides.

2.2.4 Alkaloids

In a 10 ml test tube, 3 drops of dragendoff's reagent were added to 2 ml of extract. An orange coloration indicated the presence of alkaloids.

2.2.5 Flavonoid

in a 10 ml test tube, a piece of magnesium ribbon and drop of concentration HCL were added to 4 ml of each extract. A color change from orange to red indicates the presence of flavonones; red to crimson indicated the presence of flavonoids.

2.2.6 Terpenoids

in a 10 ml test tube, 2 m of hexane extract was mixed with 0.1 ml dilute NaOH and 2 drops of concentrated H2SO4 were added. The appearance of red violet color indicated for terpenoids.

2.2.7 Steroids

In a 10 ml test tube, 2 m of hexane extract was left to dry. 4 ml chloroform and 2 drops of concentrated H2SO4 and acetic anhydride were added. The formation of brown ring indicated the presence of steroids.

2.3 The biocidal potentiality

Following the instruction of WHO [8], the susceptibility of *Anopheles* and *Culex* larvae were tested at different concentrations of Neem leaves and Argel leaves hydro-ethanol extracts. The potentiality test period was 24 hours and based on three replicates. Control batch was designed in addition to calculate the % polar contents for each part. The percentages dead larvae were counted and the survived larvae were separated in new rearing cups filled with tap water. The probity analysis was used to detect the toxicity parameters.

3 Results

3.1 The phytochemical screening

3.1.1 Neem contained terpenoids, flavonoids, glycosides and tannins, while Argel contained steroids, flavonoids, saponnins, glycosides and alkaloids (Table 1).

Table 1 Phytochemical analysis of Neem and Argel leaves

The main class	Neem leaves	Argel leaves
Saponnins	-	+
Flavonoids	+	+
Glycosides	+	+
Tannins	+	+
Steroids	+	+ +
Alkaloids	+	+
Terpenoids	+	-

(-)means absent of the main class; (+)means present of the main class; (++)means present of the main class in relatively more concentrated amount

3.2 Biocidal test

The ethanol extract of Neem leaves (polar contents = 27.6%) was tested at concentration between 138 - 828 mg/L on *Culex* and *Anopheles* larvae. The tested mortalities ranged between 0 - 95% for the *Culex* larvae, while they ranged between 5 -100% for *Anopheles* after 24 hrs. The calculated LC_{50}'s were 278.63 mg/L and 365.22 mg/L, respectively, after 24 hrs (Table 2 and 3).

The ethanol extract of Argel leaves (polar content = 19.2%) was tested at concentrations between 96 - 576 mg/L on *Culex* and *Anopheles* larvae. The tested mortalities ranged between 0 - 100% for the *Culex* larvae, and between 15 - 100% for *Anopheles* after 24 hrs. The calculated LC_{50}'s were 265.49 mg/L and 349.58 mg/L, respectively, against *Anopheles* and *Culex* after 24 hrs (Table 4 and 5).

Table 2 Larvicidal activity of Neem leaves hydro-alcohol extract on *A. arabiensis* larvae after 24 hrs

Concentration		% tested Mortality	Probit
mg/L	Log		
138.8	2.14	15	3.96
276.0	2.44	20	4.16
414.0	2.62	30	4.48
552.0	2.74	55	5.13
690.0	2.84	80	5.84
828.0	2.92	100	-

% Polar contents: 27.6%

Control mortality: 0
Regression equation: Y= -1.65 + 2.48X
R2 : 0.797
LC50 : 278.63 mg/L
LC95 : 2201.62 mg/L

Table 3 Larvicidal activity of neem leaves hydro-alcohol extract on *C. quinquefasciatus* larvae after 24 hrs

Concentration		% tested Mortality	Probit
mg/L	Log		
138	2.14	5	3.36
276	2.44	30	4.48
414	2.62	65	5.39
552	2.74	75	5.67
690	2.84	85	6.04
828	2.92	95	6.64

% Polar contents: 27.6%
Control mortality: 0
Regression equation: Y= -5.36 + 4.06X
R^2 : 0.991
LC_{50}: 356.22 mg/L
LC_{95} : 902.95 mg/L

Table 4 Larvicidal activity of Argel leaves hydro-alcohol extract on *A. arabiensis* larvae after 24 hrs

Concentration		% tested Mortality	Probit
mg/L	Log		
96	1.98	15	3.96
192	2.28	25	4.33
288	2.46	40	4.75
384	2.58	55	5.13
480	2.68	80	5.84
576	2.76	95	6.64

% Polar contents: 19.2%
Control mortality: 0
Regression equation: Y= -2.66 + 3.16X
R^2 : 0.842
LC_{50} : 265.49 mg/L
LC_{95} : 877.08 mg/L

Table 5 Larvicidal activity of Argel leaves hydro-alcohol extract on *C. quinquefasciatus* larvae after 24 hrs

Concentration		% tested Mortality	Probit
mg/L	Log		
96	1.98	5	3.36
192	2.28	15	3.96
288	2.46	25	4.33
384	2.58	55	5.13
480	2.68	65	5.39
576	2.76	85	6.04

% Polar contents: 19.2%

Control mortality: 0

Regression equation: Y= -3.47 + 3.33

R^2 : 0.930

LC_{50}: 349.58 mg/L

LC_{95}: 1086.52 mg/L

4 Discussion

Neem possesses sterols, flavonoids and sesquiterpenes derivatives [9]. Nimbin, nimbinin, nimbidin, nimbosterol, essential oil, tannins and bitter margosin are also detected in neem [10]. The detected phytochemicals in this study are not differed from that of Saini *et al*., [9] and Singh [10].

Concerning Argel leaves, numerous biochemical ingredients such as pyrgene glycosides, flavonoids, kaempferol, quercetin, rutin, flavonols, flavonones and alkaloids were detected [11]. The detected phytochemicals in the local Argel leaves samples are not differed than the mentioned study.

From the above data, it was observed that, the *Anopheles* mosquitoes have relatively high sensitivity than*Culex* mosquitoes. Also, neem is the first choice as *Culex*biocidal, while Argel and neem are the best choices against*Anopheles*.

Neemcontainsmainly azadirachtin as the major active ingredient, which is the main constituent for insecticidal activity [1].

The main detected compound from Neem leave was Phytol which is an aromatic ingredient used in the medical field because it has an antioxidant activities, as well as antiflammitory and antiallergic effects and often used in pharmaceutical field in synthetic of Vitamin E and K. Also Octadecadienoic acid and glcopyranoside are inhibition agents for the *E. coli* bacteria. Di-n-octyl phthalate has a pesticide efficiency.

The morbidity and the mortality from microbial disease have been reduced. The phenomenon of resistance imposes series constrains on the option for their medical treatment.

In our study has demonstrated that Neem, Argel and Black seed extracts effectively inhibited the growth of *E. coli*, and this can be due to the chemical composition.

In last studies, Neem has proven its efficacy in medicinal field as antibacterial, antiyeast, antiulcer, antifertility, antifilarial, antifungal [12]. While Argel also known as antimicrobial [13], antispasmodic [14], anti-inflammatory [15] and anti-oxidant [16].

5 Conclusion

The phytochemical screening showed that, Neem leaves and Argel shared in flavonoids, glycosides, tannins, steroids, alkaloids. The hydro-ethanol extract Neem reflected LC_{50} =278, 63mg/L against *Anopheles* larvae, while that of the *Culex*

was 356.22mg/L after 24 hours. The hydro-ethanol extract of Argel reflected LC_{50} = 265.49mg/L against *Anopheles* larvae, while that of the *Culex* was 349.58 mg/L after 24 hours.*Anopheles* larvae were more susceptible than *Culex*larvae.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding the publication of this paper.

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