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Phytochemical screening of *Calotropis procera* ait flower parts and their larvicidal potentialities against *Anopheles* and *Culex* Larvae, Gezira State, Sudan

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Abstract
Mosquitoes (*Anopheles* and *Culex*), the vector of many diseases, are highly resistant to insecticides in many developing countries. It would be of great relevance to search for alternatives to control mosquito-borne diseases. This study aimed to run phytochemical screening for *Calotropis procera* Ait flower parts, thin layer chromatography (TLC) test, in addition to test their potentialities against *Anopheles* and *Culex* larvae. Fresh flowers of *C. procera* were collected from the gardens of the main campus, University of Gezira and were separated into four main parts: petals, corona, gynostigium and both styles and ovary. The dried plant materials were used to run the phytochemical, TLC and the toxicity tests, in the Faculty of Engineering and Technology, University of Gezira. Samples of *Anopheles* and *Culex* larvae were collected from the breeding sites of some villages near to Wad Medani City. The results showed that, petal and corona parts contain tannins, flavonioids, glycosides and steroids, while the other parts lack tannins and glycosides. Similar number of spots were detected using TLC test among flower parts, but with different *Rf* values. At 1.2 g/L, corona and gynostigium parts resulted in 90%-100% mortalities on *Anopheles* and on *Culex* larvae, while Ovary and style part showed low toxicity (10% - 30%). It was clear that, *Culex* larvae were relatively susceptible to *C. procera* flower parts more than *Anopheles* larvae. Similar studies should be run for all Sudanese aromatic and medicinal plants in order to establish local database.

Keywords: *Calotropis procera, anopheles, culex*

Introduction
Mosquitoes (Diptera, Culicidae), the vector of many diseases are disseminated everywhere within the world. In addition, *Anopheles* and *Culex* population is highly resistant to insecticides (WHO, 2005) [14]. It would be of great relevance to search for alternatives in combating mosquito-borne diseases. One of the methods to control is to control the vectors for eradication of disease transmission.

Use of insecticides to control the insect pests has resulted in development of resistance in some vectors of malaria, filariasis and dengue fever (El Safi, 1994; WHO, 2005) [7, 14]. In last few decades, the findings of various natural plant products against mosquito vectors have proved to be an alternative to the synthetic chemicals (Abdaldafae, 2009; Masaad, 2010; Kehail, 2004; Yousif, 2013; Zarrough *et al.*, 1990) [1, 10, 9, 15, 16].

Natural products especially within the field of organic chemistry are often defined as primary and secondary metabolites. A more restrictive definition limiting natural products to secondary metabolites (Cox and Nelson, 2013) [5]. Plant produces wide range of bioactive molecules via secondary metabolic pathways. Most of these molecules have been developed on the basis of traditional knowledge in health care and in many cases, there is a correlation between the indications of pure substances and those of respective crude extracts used in traditional medicine (Hanson, 2003) [8].

C. procera is the most common species and it grows to about 3 to 6 ft (0.91 to 1.83 m). The leaves are sessile and sub-sessile, opposite, ovate, cordate at the base. The flowers are about 1.5 to 2 in (3.8 to 5.1 cm) in size, with umbellate lateral cymes and are colored white to pink and are fragrant but in rare cases are also light green-yellow or white. The seeds are compressed, broadly ovoid, with a tufted coma of long silky hair. The large, waxy, white flowers have deep purple spots or blotches at the base of each of the five petals, and are grouped in clusters, known as umbels (Brandao, 1995) [4].

Phytochemical screening and biological activity of *C. procera* was investigated by Doshi *et al*., (2011) [6]. The quantitative estimation and identification of active principles of the crude extracts of *C. procera* leaves which performed by TLC method using different solvents as mobile phase was done by Vishwa (2014) [12].

Many natural compounds have been suggested as alternatives against conventional chemical control. The genus *Calotropis* has attained a high repute for its various medicinal properties. The plant *C. procera* belonging to the family Asclepiadaceae was selected for the present work. Many of which possess biologically active compounds. *Calotropis* is a small genus having 6 species of shrubs or small trees, distributed in tropical and subtropical Africa, Asia and America. Two species namely *C. procera* and *C. gigantae* are found in India which closely resembled to each other in structure and in functional uses (Bhat *et al.*, 2005) [3].

The objective of this study

This work aimed to run phytochemical screening for *Calotropis procera* Ait flower parts (petals, corona, gynostigium and the styles and ovary), through the detection of the presence of the main phytochemicals, TLC test (for detection of the active ingredients as spots), in addition to test the potentiality of each flower part against *Anopheles* and *Culex* larvae.

Materials and Methods

Materials

Fresh flowers of *C. procera* were collected from the gardens of the University of Gezira, main campus. The collected flowers were immediately cleaned, and separated into four main parts: petal, corona, gynostigium and both styles and ovary (Plate, 1) in a special clean containers.

The separated parts of the *C. procera* flowers were dried at room temperature under shade away from the direct sunlight. The dried parts were crushed to fine size particles by using mortar. The plant materials were then used for the study experiments (the phytochemical screening, TLC test, and toxicity bioassay) in the Laboratory of Food Analysis, Faculty of Engineering and Technology, University of Gezira.

Samples of *Anopheles* and *Culex* larvae of the third and/or the early fourth instars were brought from the breeding sites of Tayba and Karkoug villages (5 km North Wad Medani City). The larvae were used in the potentiality test, and were not reared.

Phytochimal screening tests

The phytochemical screening tests were run according to Mohamed Nour (2009) [11].

For saponin

A weight of 5 g of the dried powder of each flower part of the *C. procera* plant was extracted with 20 ml ethanol (98%) and filtered. Aliquots of the alcoholic extracts (10 ml) were evaporated to dryness. The residue was dissolved in distilled water (4 ml), shaked and filtered. The filtrate was vigorously shaken, if a voluminous forth was developed and persisted for almost one hour, this indicates the presence of saponins.

For tannins

The dried powder of each part of *C. procera* flower (5 g) was extracted with ethanol (98%) and filtered. 1 ml from ferric chloride gent (5% w/v) in methanol, was added to 5 ml of filtrate, the appearance of green color which changes to a bluish black color or precipitate, indicate the presence of tannin.

For flavonoids

About 2.0 g of the dried powder of each part of *C. procera* flower was macerated in 1% of hydrochloric acid (50 ml) over night, filtered and the filtrate was subjected to the following tests:

a. 10 ml from each filtrate was render alkaline with sodium hydroxide (10% w/v), if a yellow color was formed, that might indicate the presence of flavonoids.
b. Shinoda test: A known volume (5 ml) of each filtrate was mixed with concentrated hydrochloric acid (1 ml) and magnesium was added. The formation of red color indicates the presence of flavonoids, flavonones and/or flavonols.

For alkaloids

The dried powder of each part of *C. procera* flower (5 g) was extracted with ethanol and filtered. Aliquots from the ethanolic extracted (10 ml each) were mixed with HCl (20 ml, 10% v/v) and filtered. The filtrate was rendered alkaline with ammonium hydroxide and extracted with chloroform. The combined chlorformic extract was evaporated to dryness in water bath; the residue was dissolved in HCl (2 ml, 10% v/v) and tested with Mayer's reagent and Dragendorff''s reagent, respectively. If a precipitate was formed, it indicates the presence of alkaloids.

For sterols

The dried powder of each part of *C. procera* flower (1 g) was extracted with petroleum ether (10 m each) and filtered. The filtrate was evaporated to dryness and the residue was dissolved in chloroform (10 ml). Aliquots of chloroformic extract (3 ml each) were mixed with concentrated acetic acid anhydride. 3 ml and few drops of sulphuric acid were added the formation of a reddish violet ring at the junction of the two layers, indicates the presence of unsaturated sterols.

Thin layer chromatography test

Identification of the individual components (as separated spots and their retention factor "R_f") from the crude polar (ethanol) or apolar (hexane) extracts by thin layer chromatography was done according to Mohamed Nour (2009). The solution was applied as a band, using a micro-syringe on a TLC plate coated with silica gel (0.5 mm thickness), the plate was developed in a tank containing the solvent mixture (chloroform : methanol; 2:1) for about 45 minutes. After solvent drying at room temperature, the provision was made by using iodine sprays (as detecting reagent) and visualized under UV light. The R_f of the separated spots were measured.

R_f value = the distance driven by component (cm)
the distance driven by solvent

Potentiality test

The potentialities of different parts of *C. procera* flower were evaluated as larvicidal activity against the larvae of *Anopheles* and *Culex* mosquitoes according to WHO (1981) [13]. 20 larvae of *Anopheles* and *Culex* were put separately in a batch of 250 ml beakers 3/4 filled with ordinary tap water. 0.3 g of the powder of each part of usher flower was thrown separately on each beaker. The volume of the water was then completed to 250 ml. The dried powder of each of the freshly collected flowers was tested at the concentration of 0.3 g/250 ml water (1.2 g/L, which equivalent to 1200 ppm). Each flower part was tested for its larvicidal potentiality in triplicate. The beakers were covered with fine mesh and kept at room temperature for 24 hours. A control batch was also design for each *Anopheles* and *Culex* larvae.

Statistical analysis

Microsoft office, Excel program, 2007, was used to present and analyze the obtained data. Simple descriptive statistics and ANOVA two factors were also used to clear the differences observed in the results of the flower parts of *C. procera* plant.

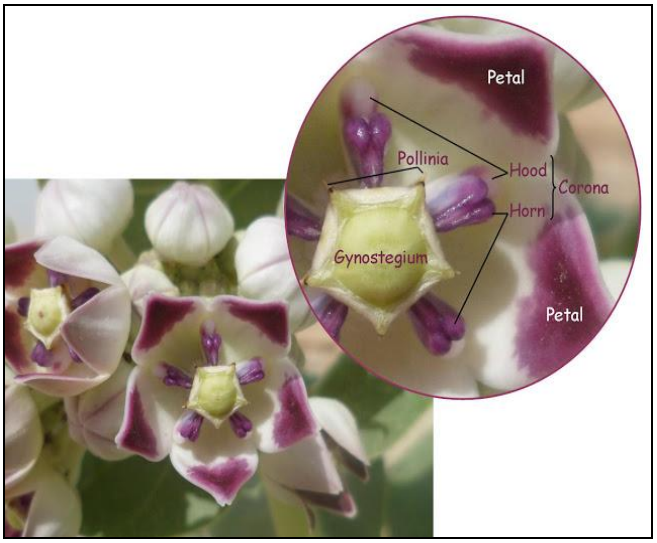

Fig: The parts of usher flower

Results and Discussion

The phytochemical analysis of *C. procera* flower parts

The results of phytochemical analysis (for the main classes) of *C. procera* flower parts, were presented in Table (1). The s.

phytochemical screening showed the presence of tannins and glycosides (in petal and corona), flavonioids (in all parts but in more concentration in petals and corona) and s zteroids (in all flower parts but in more concentration in gynostigium). Saponins and alkaloids were not detected in all parts.

Table 1: The phytochemical screening of *C. procera* flower parts

Class	Petal	Corona	Gynostigium	Ovary and style
Tannins	+	+	-	-
Saponins	-	-	-	-
Alkaloids	-	-	-	-
Flavonoids	++	++	+	+
Glycosides	+	+	-	-
Steroids	+	+	++	+

+ indicated the presence of the class,
++ indicated the presence of the class in relatively high concentration
– indicated the absence of the class.

The thin layer chromatography *C. procera* flower parts

The R_f values of the detected spots of *C. procera* flower parts were presented in Table (2). The obtained results showed that, each of the apolar (hexane extract) and polar (ethanol extract) separated 2 spots (active ingredients) from each flower part. Accordingly, similar number of spots were detected among flower parts, but with different *Rf* values, although there were some similarities among the detected spot

Table 2: The active ingredients screening of *C. procera* flower parts through the TLC

Spot No.	Hexane extract			
	Petal	Corona	Gynostigium	Ovary and style
1	0.45	0.45	0.40	0.48
2	0.50	0.55	0.48	0.55
3	-	-	-	-

Spot No.	Ethanol extract			
	Petal	Corona	Gynostigium	Ovary and style
1	0.58	0.55	0.45	0.45
2	0.63	0.60	0.53	0.50
3	-	-	-	-

Toxicity test

Concerning toxicity (Table, 3), and at the concentration of 1.2 g/L, corona part resulted in 100% mortality on *Anopheles* and on *Culex* larvae. Gynostigium part resulted in 100% mortality on *Culex* larvae, while it resulted in a mean of 90% mortality in *Anopheles* larvae. Petal part produced a mean of 90% on *Culex* larvae and a mean of 85% on *Anopheles* larvae. Ovary and style part showed low toxicity against *Anopheles* larvae (mean mortality was 10%) and *Culex* larvae (mean mortality was 30%).

It was clear that, *Culex* larvae (mean mortality was 80%) were relatively susceptible to *C. procera* flower parts more than *Anopheles* larvae (mean mortality was 71.3%). Also, there was a significant difference (f= 9.28; f-crit= 76.88) of the larvicidal potentiality among *C. procera* flower parts (i.e. some parts are more toxic towards mosquitoes larvae than the others). Accordingly, corona is the more potent part (mean mortality was 100%), followed by gynostigium part (mean of 95% mortality), then petal part (mean of 87.5% mortality), and at the last ovary and style (mean of 20% mortality).

Table 3: Mortality of *Anopheles* and *Culex* larvae at 1.2 g/L of *C. procera* flower parts

Target	Petal	Corona	Gynostigium	Ovary and style
Anopheles	85	100	90	10
	90	100	85	10
	80	100	95	10
Culex	90	100	100	30
	80	100	100	25
	100	100	100	35

Variance	Average	Sum	Count	Summary
1706.25	71.25	285	4	Anopheles
1133.33	80	320	4	Culex
12.5	87.5	175	2	Petal
0	100	200	2	Corona
50	95	190	2	Gynostigium
200	20	40	2	Ovary and style

ANOVA

F crit	P-value	F	MS	df	SS	Source
10.13	0.133	4.2	153.13	1	153.13	Rows
9.28	0.003	76.88	2803.13	3	8409.38	Columns
			36.46	3	109.38	Error
				7	8671.88	Total

Discussion

Plant produces a wide range of bioactive molecules via secondary metabolic pathways. Most of these molecules have been developed on the basis of traditional knowledge in health care and in many cases, as antimicrobial and antifungal agents and in pest control (Hanson, 2003) [8].

The detection of the main phytochemical classes in plant materials usually depended on several chemical factors including the methods and the solvents used. Phytochemical screening and biological activity of *C. procera* was investigated by Doshi *et al*., (2011) [6]. In that study, tannins, carbohydrates, phenols and alkaloids were detected in both Usher stems and leaves, while saponins, flavonoids, steroids, and resins were not detected in both stems and leaves. In this study, the detected phytochemicals were mostly responsible for the observed toxicity of each tested part.

Similar quantitative estimation and identification of active principles of the crude extracts of *C. procera* leaves were performed by TLC method using different solvents as mobile phase. In that study, Vishwa (2014) [12] found that, 9 spots were separated when chloroform, benzene, ethanol were used as a mobile phase, whereas, acetone separated 7 spots, petroleum ether separated 14 spots and water separated only 6 spots. The differences observed in the TLC results of this study from that of Vishwa (2014) [12] can be attributed to the solvent system used, part used, varieties and environmental and seasonality factors.

In Gezira State, Sudan, insecticides were used for many years for controlling mosquitoes and other agricultural pests; insecticides are effective and can be easily applied. The use of insecticides resulted in many ecological and environmental problems (Abdel Karim *et al.*, 1985) [2]. In addition, many strains of mosquitoes developed resistance to these insecticides. Efforts are directed towards finding some natural products alternatives than the use of conventional insecticides. Ushar leaves and stem parts has been used against mosquitoes larvae (Masaad, 2010 and Kehail, 2004) [10, 9].

Conclusions

1. According to the phytochemical screening, the petal and corona parts contain tannins flavonioids, glycosides and steroids, while the other parts lack tannins and glycosides.
2. According to the TLC test, similar number of spots were detected among flower parts, but with different *Rf* values, although there were some similarities among detected spots.
3. At the concentration of at 1.2 g/L, corona part Corona and gynostigium parts resulted in high mortality on *Culex* and *Anopheles* larvae, while ovary and style showed low toxicity against *Anopheles* and *Culex* larvae.
4. *Culex* larvae were relatively susceptible to *C. procera* flower parts more than *Anopheles* larvae. Also, some flower parts are more toxic against mosquito larvae than the others.

Recommendations

1. The larvicidal potentiality of each detected phytochemical class must be tested.
2. Similar studies should be run for all the Sudanese aromatic and medicinal plants in order to establish local database.

References

1 Abdaldafae AA. The effect of, Neem Azadirachta indica A. Juss, Leaves powder on Anopheles arabiensis Patton larvae. M.Sc. Thesis. University of Gezira. 2009.

2 Abdel Karim AA, Haridi MA, El Fateh A. The environmental impact of four insecticides on non- target organisms in the Gezira irrigation Scheme Canals. J. Trop. Med. Hyg. 1985; 88(2):161-168.

3 Bhat SV, Nagasampagi BA, Sivakumar M. Chemistry of Natural Products (2ed.). Berlin: Springer. ISBN 3-540-40669-7, 2005.

4 Brandao M. Calotropis procera (Ait.) R. Br.: a problem pasture invader in Minas Gerais). Daphne, 1995; 5:68-71.

5 Cox DL, Nelson MM. Lehninger Principles of biochemistry (6th ed.). New York, N.Y.: W.H. Freeman. ISBN 978-1-4641-0962-1, 2013.

6 Doshi HS, Mukund CT, Farzin P, Arif K. Phytochemical Screening and Biological Activity of Calotropis procera (Ait). R. Br. (Asclepiadaceae) Against Selected Bacteria and Anopheles Stephansi Larvae. International J. of Plant Research. 2011; 1(1):29-33.

7 El Safi SH. The use of deltamethrin impregnated mosquito nets for malaria control in Gezira irrigated area. Proc. Water-Borne Diseases, Wad Medani, Sudan 1994.

8 Hanson JR. Natural products: the secondary metabolite. Cambridge: Royal Society of Chemistry. ISBN 0-85404-490-6, 2003.

9 Kehail MA. Larvicidal potentialities of 20 plant species from Wad Medani. Gezira State on Anopheles arabiensis

and Culex quinquefasiatus (Culicidae, Diptera). Ph.D. Thesis, University of Gezira, 2004.
10 Masaad NY. Susceptibility of Culex quinquefasciatus Say (Diptera, Culicidae) larvae to some preparations of Calotropis procera Ait. Latex. M.Sc. thesis, University of Gezira, 2010.
11 Mohamed Nour AAA. Chemical component of the essential oil of basil (Ocimum basilicum L.) active against Salmonella typhi. M.Sc. Thesis, University of Gezira, 2009.
12 Vishwa NV. The Chemical Study of Calotropis. International Letters of Chemistry, Physics and Astronomy. 2014; 1:74-90.
13 WHO Instruction for determining susceptibility or resistance of mosquito larvae to insecticides, 1981. WHO/VBC/81.807
14 WHO Malaria Control in Complex Emergencies, 2005. WHO/ HTM/ MAL/ 1107
15 Yousif H. Susceptibility of Anopheles arabiensis Patton and Culex quinquefasciatus Say Larvae to Ixora (Ixora coccinea L.) Leaves and Flowers Extracts. M.Sc. thesis, University of Gezira, 2013.
16 Zarrough IM, Nugud AD, Bashir AK, Mageed AA. Balanites aegyptiaca as mosquito larvicide. Inter. J. Crude Res. 1990; 28(4):267-271.