International Scholars Journals

African Journal of Malaria and Tropical Diseases ISSN 2736-173X Vol. 13 (6), pp. 001-006, June, 2025. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Larvicidal Effects and Phytochemical Profile of Acacia nilotica Against *Anopheles arabiensis*

Amal Elsayed Edriss*, Abdalla Abdelrahim Satti and Zuhair Alfadil Alabjar

Sudan Academy of Science (SAS), P. O. Box 2404, Khartoum, Sudan.

Accepted 17 December, 2024

Acacia nilotica **is a natural forest tree with many traditional uses in Sudan. Preliminary laboratory studies were conducted to analyze the phytochemical constituents of three extracts (water, ethanol and petroleum ether) prepared from *A. nilotica* (leaves and fruits), and to evaluate their larvicidal effects against the malaria vector mosquito, *Anopheles arabiensis*. The knockdown and residual effects of the extracts were tested in separate bioassay experiments, compared with two standard insecticides. The results showed wide range of secondary metabolites in water and ethanol extracts, mainly alkaloids, saponins, flavones and tannins, while the petroleum ether extracts were dominated by triterpenes and sterols. The fruits extracts seemed to be richer in active compounds than the leaves extracts. Therefore, the petroleum ether extracts exerted better mortality effects than other extracts, with fruits treatments being superior to leaves in all cases. Moreover, the fruits petroleum ether extract (0.5%) showed similar mortality percent (90.0%) as those of the two insecticides at 24 h post exposure. This treatment also manifested significantly the best residual actions up to three weeks, but lied next in order to both insecticides. Hence, the extract was recommended for further studies to indicate its toxic compound(s) and their possible usage for *A. arabiensis* control.**

Key words: *Acacia nilotica*, *Anopheles arabiensis,* extracts, phytochemical analysis, larvicidal effect.

INTRODUCTION

*Acacia nilotic*a (Fabaceae) is one of about 135 thorny Africa *Acacia* species. It is naturally wide spread in drier area of Africa (Nongonierma, 1976; El Gazali et al., 1998). In Sudan, the species is familiar by local names, “Sunt” for the tree and “Garad” for the fruit pods. Different products of this tree especially the “Garad”, as well as many other plant products, are known to have many traditional uses in the country since earlier times. The tree is naturally grown in form of dense forests mainly along the rivers, streams and rain catchments. The bark, gum, leaves and pods of the tree also have many medicinal uses in Africa for treating cancer, colds, cough, fever, tumors, hemorrhage, typhoid, convalescence, nerve stimulant, intestinal pain and diarrhea (Duke, 1983). Seeds and leaves of *A. nilotica* were reported to contain various active ingredients of multiuse (Pande et al., 1981; Chaubal et al., 2005). Therefore, leaves and fruits extracts of *A. nilotica* have proved to be effective as fungicides, bactericides, molluscicides and insecticides (Umalkar et al., 1976; Fagg and Greaves, 1990; Chaubal et al., 2005).

However, the discovery and use of synthetic chemicals not only overshadowed the use of plant products, but also become the major tactic adopted worldwide for pests and diseases control. Among human diseases, malaria is an endemic pathogen in Sudan. The malaria parasite is transmitted chiefly by the mosquito, *Anopheles arabiensis* Patton (Diptera: Culicidae), which occurs in almost all parts of the country. Chemical insecticides directed against larval or adult stages of mosquitoes are the main control measures applied (Haridi et al., 1975; Abdel Gadir, 1993; Azami et al., 1996). But nowadays, the control of the malaria vector is becoming increasingly difficult due to several reasons, particularly the buildup of insecticide resistance mosquito strains, the high cost of chemicals and the problems of toxicity to non target

*Corresponding author. E-mail: apbc.92@gmail.com.

Table 1. Phytochemical constituents of three extracts prepared from leaves and fruits of *Acacia nilotica*.

Extracts	Chemical groups detected in different extracts							
	Am	Sa	Al	Fl	Fn	Tn	St	Tr
Leaves water extract	-	+	+	-	+	-	-	-
Fruits water extract	+	+	+	-	+	+	-	-
Leaves ethanol extract	-	-	+	-	-	-	-	-
Fruits ethanol extract	+	-	-	+	+	+	+	-
Leaves petroleum ether extract	-	-	-	-	-	-	-	+
Fruits petroleum ether extract	+	-	-	-	-	-	+	+

Am= amino acids; Sa= saponins; Al= alkaloids; Fl= flavonoids; Fn= flavones; Tn= tannins; St= sterols; Tr= triterpenes; (-)= non present; (+)= present.

organisms (WHO, 1992; Ranson et al., 2001, 2009; Matambo et al., 2007). Therefore, these factors, besides the other numerous drawbacks of synthetic insecticides, have necessitated the search for effective and safe biodegradable control agents. According to Potter and Beavers (2005), plant products of potential insecticidal or repellent effects can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at community level. Hence, several plant species were already exploited traditionally long ago as natural insecticides before the discovery of synthetic chemicals (Ahmed et al., 1989; Campbell et al., 1993).

Although, promising mosquitocidal results were obtained from some indigenous plants in Sudan (Kehail and Bashir, 2004; Satti et al., 2010), screening of botanical extracts is still going on in search for more potent species. In this respect, the current preliminary research was directed to ascertain the major phytochemical constituents and mosquito larvicidal properties of some extracts of *A. nilotic*a against the malaria vector, *Anopheles arabiensis*, under laboratory conditions.

MATERIALS AND METHODS

Rearing of mosquito larvae

Samples of mosquito eggs were collected from stagnant water in Khartoum and brought to the laboratory where *Anopheles arabiensis* were segregated from *Culex* species (Gillett, 1971; Potter and Beavers, 2005). The eggs of the former species and subsequent stages were reared in special glass containers and cadge, fed on natural and artificial diets, according to WHO (1975, 1992). Hence, the second generation 4^{th} instar larvae were secured for the bioassays.

Preparation of plant extracts

Leaves and mature fruit pods of *A. nilotic*a were collected from Khartoum State, cleaned with water, dried under shade and ground into fine powders. For water extraction, the required weights of leaf and fruit powders were mixed in one liter water, stirred vigorously for five minutes with a magnetic stirrer, and left to stand overnight before filtration. Three concentrations (10, 5 and 2.5% w/v), were prepared for the bioassays. However, regarding organic solvents (namely, ethanol and petroleum ether), the extracts were achieved through a soxhlet apparatus (Harbone, 1983) and dried using a rotary evaporator. Also, three concentrations (0.5, 0.25 and 0.125% v/v) were prepared from both extracts.

Phytochemical analysis

The phytochemical analysis of the above water and organic (ethanol and petroleum ether) extracts of *A. nilotica* (leaves and fruits) were done through chemical approach as described by Harbone (1973, 1983). Accordingly, all the necessary reagents were prepared and used for testing the various classes of secondary metabolites. These included; Mayer, Ninhydrin, Potassium hydroxide, Ferric chloride and Vanillin reagents specific for testing alkaloids, amino acids, flavonoids, tannins and sterols/triterpenoids, respectively. Following the chemical testing procedure, such reagents were helped to detect almost all chemical groups based either on a precipitate formation or a colour change, except the saponins which was detected simply through foam characteristics that appeared after shaking of extract samples in water. Hence, the above tests were enabled to tentatively identify the different chemical groups in the studied extracts.

Bioassay experiments

Two bioassay experiments were conducted to test the knockdown (at 24 and 48 h post treatments) and residual effects (at 3, 14 and 21days) of the different extracts concentrations against the 4th instar larvae of *An. Arabiensis*. Two insecticides, Malathoin 50%EC (malathion) (10 ml/L) and Abate 50%EC (temephos) (1 ml/L), were included for comparisons. Metal plates (250 ml) were used to hold the treatments where twenty five larvae were placed in each plate. In case of residual effects, the larvae were introduced in treatments at three consecutive intervals (that is, 3, 14 and 21 days) post application, as mentioned above, following the procedure of WHO (1975). The treatments were replicated four times and assigned in a Completely Randomized design (Gomez and Gomez, 1984). The data recorded on larval mortality were analyzed statistically for both experiments, and means separation was performed according to Duncan's Multiples Range test.

RESULTS

Phytochemical constituents of extracts

Table 1 shows the results of chemical analysis for the

Table 2. Mortality of *Anopheles arabiensis* 4th instar larvae, at two intervals (24 and 48 h) post treatments, with *Acacia nilotica* extracts.

Treatments	Mortality percent means (±S.E.) at two intervals	
	24 h	48 h
Leaves water extract, 2.5%	01.8 ± 0.0^{k}	01.8 ± 0.0^{i}
Fruits water extract, 2.5%	34.1 ± 0.1^{hi}	33.8 ± 0.6^{fg}
Leaves water extract, 5.0%	20.4 ± 0.0^{j}	20.1 ± 0.7^{h}
Fruits water extract, 5.0%	35.7 ± 0.2^{gh}	35.6 ± 0.8^{fg}
Leaves water extract, 10.0%	41.1 ± 0.2^{f}	41.5 ± 0.5^{e}
Fruits water extract, 10.0%	53.9 ± 0.2^{d}	56.2 ± 0.9^{c}
Leaves ethanol extract, 0.125%	01.8 ± 0.0^{k}	01.8 ± 0.0^{i}
Fruits ethanol extract, 0.125%	31.9 ± 0.9^{i}	31.9 ± 0.9^{g}
Leaves ethanol extract, 0.25%	01.8 ± 0.0^{k}	01.8 ± 0.0^{i}
Fruits ethanol extract, 0.25%	41.5 ± 0.5^{f}	41.5 ± 0.5^{e}
Leaves ethanol extract, 0.5%	46.7 ± 0.7^{e}	46.7 ± 0.7^{d}
Fruits ethanol extract, 0.5%	63.6 ± 1.1^{b}	63.6 ± 1.1^{b}
Leaves petroleum ether extract, 0.125%	38.2 ± 0.6^{fg}	37.4 ± 0.6^{ef}
Fruits petroleum ether extract, 0.125%	32.5 ± 0.8^{hi}	32.5 ± 0.8^{g}
Leaves petroleum ether extract, 0.25%	41.5 ± 0.8^{f}	41.5 ± 0.8^{e}
Fruits petroleum ether extract, 0.25%	60.0 ± 0.3^{c}	61.5 ± 0.9^{b}
Leaves petroleum ether extract, 0.5%	65.8 ± 0.7^{b}	65.8 ± 0.8^{b}
Fruits petroleum ether extract, 0.5%	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}
Malathion 50%EC, 10ml/L	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}
Abate 50%EC, 1ml/L	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}
Water control	00.0 ± 0.0^{k}	00.0 ± 0.0^{i}
C.V. %	4.7	5.9

studied extracts. Generally, all the eight tested chemical groups were detected in the tree samples, but at variable levels based on plant part and the extract type. Therefore, five chemical groups were obtained in water extracts, six in ethanol and only three in petroleum ether extracts. This revealed the presence of wide range of polar secondary metabolites in the plant which extracted through water and ethanol solvent, including mainly alkaloids, saponins, flavones and tannins. On the other hand, the apolar materials extracted with petroleum ether were mostly triterpenes and sterols. Nevertheless, the extract yield of each group and their constituents of active com-pounds are yet to be determined. In all extracts, the fruit samples appeared to be richer in active chemicals than the leaf samples. The variability in active compounds and their concentrations in each extract are expected to affect their biological activities against different pests.

Larvicidal effects of treatments

The results of knockdown effects of treatments on the 4th instar larvae of *An. Arabiensis* are shown in Table 2. Significant differences were achieved among the different treatments. In general, it is clear that the petroleum ether extracts gave higher mortality effects than water and ethanol extracts. Also, the fruit treatments showed better effects than leaf treatments. In most cases, the mortality means were increased progressively in relation to doses and exposure time. Therefore, the highest concentration of fruits petroleum ether extract (0.5%) manifested the best significant mortality percent (90.0 ± 0.0%) of all botanicals, a result which was similar to those obtained by the two insecticides.

On the other hand, the results of the second bioassay experiment on residual actions of treatments are given in Table 3. Again, the various fruits extracts performed better residual effects than leaves extracts, and mortality percents increased in relation to concentrations in most cases. The highest doses of all extracts generally exerted the highest significant effects, as compared to the untreated control. At the third day, both fruits petroleum ether (0.5%) and fruits water (10%) extracts showed similar significant effects (58.7 ± 0.6% and 56.9 ± 1.1%, respectively), and followed by fruits ethanol extract (50.8 ± 0.5%). At 14 days interval, the activities of the three extracts dropped nearly to the half, giving 30.0 ± 0.6%, 28.0 ± 0.4% and 22.8 ± 0.4% mortalities, in the same sequence. However, these treatments, except water extract, kept the same effects after 21 days, post treatments. Although, the two insecticides reflected the best residual performances, compared to the tested extracts, they also experienced continuous drop in activities from 90.0 ± 0.0% down to 69.9 ± 0.7% in the

Table 3. The residual effects of different extracts of *Acacia nilotica* on mortality of *Anopheles arabiensis* 4th instar larvae, at different intervals from treatments.

Treatments	Mortality percent means (±S.E.) at different intervals		
	3 days	14 days	21 days
Leaves water extract, 2.5%	12.8 ± 0.6^{i}	01.8 ± 0.0^{f}	01.8 ± 0.0^{e}
Fruits water extract, 2.5%	33.7 ± 1.4^{f}	01.8 ± 0.0^{f}	01.8 ± 0.0^{e}
Leaves water extract, 5.0%	19.7 ± 1.4^{h}	01.8 ± 0.0^{f}	01.8 ± 0.0^{e}
Fruits water extract, 5.0%	37.5 ± 0.6^{ef}	16.2 ± 0.9^{d}	01.8 ± 0.0^{e}
Leaves water extract, 10.0%	40.2 ± 1.9^{de}	01.8 ± 0.0^{f}	01.8±0.0^{e}
Fruits water extract, 10.0%	56.9 ± 1.1^{b}	28.0 ± 0.4^{b}	01.8 ± 0.0^{e}
Leaves ethanol extract, 0.125%	01.8 ± 0.0^{j}	01.8 ± 0.0^{f}	01.8 ± 0.0^{e}
Fruits ethanol extract, 0.125%	19.2 ± 0.9^{h}	01.8 ± 0.0^{f}	24.2 ± 0.8^{c}
Leaves ethanol extract, 0.25%	01.8 ± 0.0^{j}	01.8 ± 0.0^{f}	01.8 ± 0.0^{e}
Fruits ethanol extract, 0.25%	36.1 ± 1.6^{ef}	01.8 ± 0.0^{f}	01.8 ± 0.0^{e}
Leaves ethanol extract, 0.5%	46.2 ± 0.5^{cd}	01.8 ± 0.0^{f}	01.8±0.0^{e}
Fruits ethanol extract, 0.5%	50.8 ± 0.5^{c}	22.8 ± 0.4^{c}	23.5 ± 0.6^{c}
Leaves pet.eth. extract, 0.125%	32.6 ± 0.3^{f}	01.8 ± 0.0^{f}	01.8 ± 0.0^{e}
Fruits pet.eth. extract, 0.125%	26.5 ± 0.6^{g}	01.8 ± 0.0^{f}	01.8 ± 0.0^{e}
Leaves pet.eth. extract, 0.25%	36.9 ± 0.5^{ef}	01.8 ± 0.0^{f}	01.8 ± 0.0^{e}
Fruits pet.eth. extract, 0.25%	49.6 ± 1.0^{c}	11.6 ± 1.7^{e}	11.6 ± 1.7^{d}
Leaves pet.eth. extract, 0.5%	42.1 ± 0.9^{de}	01.8 ± 0.0^{f}	01.8 ± 0.0^{e}
Fruits pet.eth. extract, 0.5%	58.7 ± 0.6^{b}	30.0 ± 0.6^{b}	30.0 ± 0.6^{b}
Malathion 50%EC, 10ml/l	90.0 ± 0.0^{a}	71.9 ± 1.1^{a}	69.9 ± 0.7^{a}
Abate 50%EC, 1ml/l	90.0 ± 0.0^{a}	73.8 ± 0.9^{a}	70.8 ± 0.9^{a}
Water control	00.0 ± 0.0^{j}	00.0 ± 0.0^{f}	01.8 ± 0.0^{e}
C.V. %	9.3	16.7	16.9

Pt.eth. = Petroleum ether.

third week.

DISCUSSION

Phytochemical constituents of extracts

The use of chemical reagents approach in analyzing the phytochemical constituents of the studied *A. nilotica* extracts seemed to be reasonable at this preliminary stage for screening different extracts. It tentatively revealed the presence of all tested secondary metabolites groups, but advanced techniques may be necessary to characterize the specific active compounds in each group at least for the most promising extracts based on biological assays as mosquito larvicides. Since local studies on *A. nilotica* are rarely found, the results more or less proved the occurrence of different active compounds distributed in different parts of the tree as reported in scattered literature elsewhere. However, as detected in the study, Harbone and Turner (1984) explained that generally the petroleum ether and hexane solvents are involved in the extraction of non polar compounds (for example, triterpenes and sterols), while alcohol (ethanol) for polar components like alkaloids and polar flavonoids. According to Schmutterer (1990), different plant parts contain different compounds and concentrations of active chemicals.

The results agreed with some previous studies which showed the richness of fruiting bodies over leaves in different plants, considering their contents of secondary metabolites (El Tayeb et al., 2009; El Kamali, 2001). Pande et al. (1981) recorded that seeds and leaves of *A. nilotica* contain various compounds such as crude proteins, saponins, tannins, galactose, sulphides, pentosan, arabienose, catechol, galacton, silica, phosphorous and calcium. Moreover, the ethanol extract of *A. nilotica* aerial part was isolated and a compound structure determined by spectral methods showed D-pinitol (=3-0 methyl-D-chiro-inositol) with lipophilic nature (Chaubal et al., 2005). The results obtained by Fagg and Greaves (1990) indicated that tannin compounds are powerful biocidal ingredients found in *A. nilotica*. In this respect, the pods of all indigenous *A. nilotica* subspecies were reported to contain > 10% tannins of commercial interest (Ahmed et al., 2005), with the highest amount (54%) was recorded form the premature pods of subspecies *nilotica* at Elfaw area in eastern Sudan (Elkhalifa et al., 2005). This justifies the various traditional uses of such products especially in leather tanning.

Larvicidal effects of treatments

The current findings obtained on mortality effects of *A. nilotica* extracts may prove the previous results of phytochemical analysis which reflected the richness of fruits in active principles as compared to the leaves. Moreover, it may also suggest the possible occurrence of certain potent mosquito larvicidal compounds in the detected chemical classes, triterpenes or sterols. This needs further investigations to ascertain such active compounds and their potential application for larval control of *An. Arabiensis*.

Studies on botanical extracts against mosquitoes in general are very scanty in Sudan, and the present work seems to be one of few attempts evaluated the use of *A. nilotica* against *An. Arabiensis*. However, the study partially confirmed the results of Ali (1987) on the insecticidal effects of *A. nilotica* fruits (Garad) water extract against some *Culex* and *Anopheles* species. Chaubal et al. (2005) recorded chronic larval toxicity from *A. nilotica* acetone extracts against *Aedes aegypti* and *Culex quinquefaciatus*. More recently, comparable results were achieved in Saudi Arabia by Zaitoun et al. (2012), who showed acute (212.1ppm) and chronic (144.2ppm) effects of *A. nilotica* acetone extract against *Culex pipienis*, which induced 93.33% larval mortality plus reduction of egg hatchability and suppression of adult emergence. Moreover, some studies proved the potential activities of *A. nilotica* fruit pods in controlling some aquatic pests such as schistosomiasis transmitting snails, which found to be affected mainly by saponins, tannins and terpenoids (Ayoub and Yankov, 1984; Fagg and Greaves, 1990).

Generally, several authors recorded superior mosquitocidal effects of apolar constituents of some plants extracted with petroleum ether or hexane, attributed mainly to triterpenoids constituents (Mullai and Jebanesan, 2007; Abdul Rahuman et al., 2008; Abdu Zahir et al., 2009). Also, the results regarding the mortality effects of *A. nilotica* treatments in relation to concentration levels and exposure time may suggest that the active compounds in the tested extracts work mostly through stomach actions rather than contact actions. In this context, the more the concentration and exposure time the more chance for the larvae to ingest lethal doses. The results were in consistency with a number of previous works investigating different botanical extracts (Schmutterer, 1990; Tonk et al., 2006; Abdul Rahuman et al., 2008; Edriss et al., 2008; Mullal et al., 2008; Abdu Zahir et al., 2009; El Tayeb et al., 2009).

No similar studies were detected considering the residual insecticidal effects of *A. nilotica* extracts, but the results obtained are promising to proceed forward in studying this plant under laboratory and field levels. The specific larval toxicant compounds in potent extracts are needed to be identified and evaluated. More specifically, it can be stated that the highest concentration (0.5%) of fruits petroleum ether extract was the best botanical treatment which recommended for more advanced studies to indicate its bioactive compounds, LC_{50s} and field stability in order to be utilized in control programs of *An. arabiensis* larvae.

CONCLUSION AND RECOMMENDATIONS

The results of the current initial research on natural products proved variable significant insecticidal properties of *A. nilotica* extracts against the larvae of *An. Arabiensis*, under laboratory conditions. Such activities were attributed to one or more of the various secondary metabolites detected in the plant, which need further investigation. Accordingly, the fruits petroleum ether extract at 0.5% concentration was the best treatment showing similar knockdown effect as those of synthetic insecticides, and with satisfactory residual larvicidal action continued for up to 3 days following application. Since this extract also remained significantly with fair residual activities from control up to three weeks after treatments, it may need some supplementary applications to enhance its residual effects before suitable formulation is prepared. These aspects await future studies applying modern analytical and evaluation techniques for proper exploitation of such abundant safe biocides.

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