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POTENTIAL LARVICIDES IN NIGERIAN HERBAL RECIPES

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ABSTRACT

Investigations into the larvicidal potential of crude extracts of ten Nigerian plants were carried out against the fourth instar larvae of *Anopheles gambiae* mosquito. The phytochemical screening revealed that both anthraquinones and cyanogenic glycosides were absent in all the plants. However, alkaloids, saponins, tannins, cardiac glycosides, terpenes and flavonoids were either present or absent. The larvicidal activity expressed as % LA was concentration and incubation-time dependent. At 5%w/v (12 and 24h), only *Carica papaya* and *Dacryodes edulis* demonstrated remarkable larvicidal activity of 40% and 55% and 50% and 70% respectively while the rest were largely inactive. However, at 10%w/v (12 and 24h), seven of the ten plants namely; *Antholeisia djalonensis* (60% and 80%), *Calotropis procera* (50% and 70%), *Carica papaya* (70% and 80%), *Cyathula prostrata* (37% and 67%), *Dacryodes edulis* (90% and 100%), *Pycanthus angolensis* (45% and 50%) and *Viscum album* (33% and 73%) gave comparably stronger activities especially after 24h incubation time. This study indicates a potential use of these plants in the control of vector mosquitoes which cause malaria.

INTRODUCTION: Malaria is a very rampant and devastating disease in the tropical regions of the world. Numerous efforts have been made in the past to control its morbidity and mortality [1, 2, 3]. Treatment of the vector at the various developmental stages with insecticides has received wide acceptability, though these synthetic products suffer from major disadvantages of resistance and environmental pollution [4].

The use of plants as alternative sources of potent chemicals for vector control has been extensively studied and documented [3, 5, 6, 7, 8].In view of the continued search for potent mosquito larvicides of natural origin, this present investigation was carried out with some Nigerian plants against the fourth instar larvae of *Anopheles gambiae* mosquito.

MATERIALS AND METHODS: Collection of materials: Ten medicinal plants native to Nigeria were collected in the March, 2011 from various Local Government Areas of Akwa Ibom State, Nigeria. The plants were identified by Dr. (Mrs.) M. Bassey, of the Department of Botany and Ecological Studies, University of Uyo, Nigeria and voucher specimens labelled No H62 to No H71 were deposited in the herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria. *Anopheles gambiae* larvae were bred in plastic buckets and appropriately identified.

Extraction and processing of Plant Materials: The plants were individually oven-dried (40^{o}C) and then separately ground into coarse powders. The resultant ground powders were then extracted with cold 96% aqueous ethanol at room temperature (27$\pm$2^{o}C) for

72h. The filtrates were also separately evaporated to dryness using a rotary evaporator (Buchi CH-920, Laboratorium Technic, Flawk/SG, Switzerland). The obtained residues were stored in amber bottles in a refrigerator (-4^{o}C) prior to the further tests.

Phytochemical screening: The dried crude ethanolic extract of each plant was separately investigated for secondary metabolites (alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, terpenes, flavonoids and cyanogenic glycosides) according to the laid down rules [9, 10, 11, 12, 13, 14, 15, 16, 17].

Bioassay for larvicidal activity:

The breeding of larvae of *Anopheles gambiae:* The larvae were bred by keeping outdoor basins of water under growing shrubs near houses for about two weeks. After this period, at least three groups of mosquito's larvae were identified accurately in a container using classical methods [18].

Anopheles gambiae, *Aedes aegypti* and *Culex piper-fatigans* responsible for the transmission of malaria, yellow fever and filariasis respectively were so identified. The fourth instar larvae of *Anopheles gambiae* were later selected, separated and the species authenticated at the Department of Entomology, Michael Okpara University of Agriculture, Umidike, Abia State, Nigeria before further work. The method employed for the determination of larvicidal activity was adopted from that described by several authors [19] and WHO directives on such assay with modifications [20]. Thirty (30) *Anopheles gambiae* larvae in their fourth stage were put in recovery cups (250ml plastic jars) containing 10ml de-ionized water (pH 7.0) at room temperature (27$\pm$ 2^{o}C). Three (3ml) volume each of the graded concentrations of the extracts (5 and 10 %w/v) were added to 90ml de-ionized water, mixed thoroughly and then poured into exposure cups (250ml plastic jars containing larvae food). Each aqueous solution of the extract was set up in triplicates. Negative control (containing

90ml de-ionized water, larvae food and larvae) and as well as positive control (containing 3ml absolute alcohol, 90ml de-ionized water, larvae food and larvae) were also set up in triplicates. Both the test controls were set up, and maintained at room temperature (27$\pm$2^{o}C). The *Anopheles* larvae in each recovery cup were scooped and transferred by means of small nets into test exposure cups containing the sample solutions and or control, larvae food and de-ionized water [7]. The larvae in the test and controls set-up were incubated for a period of 12 and 24h at room temperature (27$\pm$2^{o}C).

Therefore, the larvae were gently scooped into small nets, washed with de-ionized water, transferred into recovery cups containing 100ml of de-ionized water, maintained at pH 7.0 and allowed to settle. Prior to mortality determinations, the larvae in recovery cups were gently disturbed and made to go below the water surface by agitating the water with a sterile pipette. The dead and dying larvae which started to float on the surface were pushed down the recovery cups. The living larvae which were able to swim to the surface were allowed to do so within 5minutes following agitation. The larvae remaining and or staying at the bottom of the recovery cups unable to swim to the surface were regarded as dead.

RESULTS:

TABLE 1: PHYTOCHEMICAL SCREENING OF CRUDE ETHANOLIC EXTRACTS OF PLANTS

	PLANT METABOLITES							
Plant	**ALKA**	**SAPO**	**TANN**	**ANTR**	**CARD**	**TERP**	**FLAV**	**CYAN**
Acalypha wilkesiana	+ +	-	+ + +	-	+	+ +	+ +	-
Antholeisia djalonensis	-	+ +	-	-	+ +	-	+ +	-
Bryphyllum Pinnatum	+	+ +	+ +	-	+ +	+ +	+ +	-
Calotropis procera	+ +	+ +	+	-	+ + +	+ + +	+	-
Carica papaya	+	+ +	+ +	-	+	-	+ +	-
Cyathula prostrata	-	+ + +	+ + +	-	+ + +	+ + +	+ +	-
Dacryodes edulis	-	+ +	-	-	+	-	-	-
Pycanthus angolensis	-	+	-	-	+	+	-	-
Nymphaea odorata	+	+ + +	+ + +	-	+	-	+ + +	-
Viscum album	-	+ +	+	-	+ +	-	-	-

ALKA = Alkaloids; SAPO = Saponins; TANN = Tannins; ANTR = Anthraquinones; CARD = Cardiac glycosides; TERP = Terpenes; FLAV = Flavonoids; CYAN = Cyanogenic glycosides; - = Absent, + = Trace,+ + = Moderately present

TABLE 2: LARVICIDAL ACTIVITY CRUDE ETHANOLIC EXTRACTS OF PLANTS AT 5%W/V AND 10%W/V AT 12 AND 24H INCUBATION PERIODS

	→ (LA% at 5w/v%) ←		→ (LA% at 10%w/v) ←	
Plant/plant part	**12h**	**24h**	**12h**	**24h**
Acalypha wilkesiana (leaves)	10	20	30	40
Antholeisia djalonensisa (leaves)	20	35	60	80
Bryphyllum pinnatum (leaves)	15	20	30	40
Calotropis procera (stem)	20	38	50	70
Carica papaya (roots)	40	55	70	80
Cyathula prostrata (aerial parts)	10	23	37	67
D. edulis (stem)	50	70	90	100
Pycanthus angolensis (leaves)	20	35	45	50
Nymphaea odorata (leaves)	10	20	30	40
Viscum album (leaves)	07	17	33	73
Negative standard (larvae without extract)	0	0	0	0
Positive standard (absolute alcohol)	100	100	100	100

LA= Percentage Larvicidal Activity

DISCUSSION: The plants used in this present study were identified, authenticated and collected observing basic guidelines of plant collection. Also, the rules governing extraction and phytochemical screening of extracts were strictly adhered to, thus preventing any changes to the chemical composition of the crude extract [10, 11].

The phytochemical screening revealed that all the plants tested negative to [10] both anthraquinones and cyanogenic glycosides. However, each plant showed either the presence or absence of alkaloids, saponins, tannins, cardiac glycosides, terpenes and flavonoids (Table 1). Secondary metabolites such as saponins, cardiac glycosides, alkaloids, tannins and flavonoids have demonstrated in several previous studies [21, 22, 23, 24, 25, 26, 27,28, 29] to be responsible for the cure or management of many ailments caused by microbes and different kinds of disease conditions in the ethno-medicine of plants. Larvicidal assay was carried out on the crude ethanolic extracts of plants at 5%w/v and 10%w/v at 12 and 24h incubation periods.

The larvicidal activity (LA %) was calculated in terms of percentage mortality. The lethality furnished was incubation was concentration and time-dependent as displayed in Table 2. At 5%w/v (12 and 24h), the crude extracts of *Carica papaya* and *Dacryodes edulis* demonstrated remarkable larvicidal activities of 40% and 55% and 50% and 70% respectively. However, the remaining eight plants gave comparably weaker activities with *Viscum album* and *Acalypha wilkesiana* furnishing the poorest larvicidal activities of 7% and 17% and 10% and 20% respectively.

Generally, the larvicidal activities furnished by extracts at 10%w/v (12 and 24h) were comparably stronger when compared with the activities given at 5%w/v (12 and 24h) as seen in Table 2. Furthermore, the larvicidal activities given by *Carica papaya* and *Dacryodes edulis* were profound at 70% and 80% and 90% and 100% showing some consistency in their activities. Also, it was observed that at 10%w/v, there was marked improvement in the larvicidal activities demonstrated by the remaining plants compared with those given at 5%w/v.

This observation is reflected in the larvicidal activities given by [11] *Pycanthus angolensis, Calotropis procera, Cyathula prostrata, Viscum album and Antholeisia djalonensis* at 45% and 50%, 50% and 70%, 37% and 67%, 33% and 73% and 60% and 80% respectively. The negative and the positive standards gave larvicidal activities of 0% and 100% both at 5%w/v and 10%w/v at 12 and 24h respectively. The negative standard was not expected to record any deaths because the larvae were incubated without the plant extracts.

However, total lethality was achieved with the positive standard because the larvae were kept in absolute alcohol which is known for its toxicity and antimicrobial activity. The phytochemical screening carried out on the crude extracts revealed the presence of saponins and flavonoids in seven to nine of the plants. Hence, the results from the larvicidal assay are not surprising because these classes of metabolites had been shown in separate studies [5, 6, 7, 8, 18, 19, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40] to be lethal to the fourth in star larvae of *Anopheles gambiae* which prevent the emergence of adult

mosquitoes responsible for the transmission of malaria scourging huge populations of people around the world. In the light of this reality, further work is presently on-going in our laboratories with fractions obtained from the crude ethanolic extracts to determine if further improvements could be obtained in the observed larvicidal activities of the plants.

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