

Bioefficacy of Solvent Fractions of *Oreosyce africana* and *Piper capense* against the Malaria Vector, *Anopheles arabiensis* with High Performance Liquid Chromatographic and Ultraviolet-Visible Spectroscopic Analysis

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Abstract

The efficacy of synthetic inorganic insecticides to control malaria vector mosquitoes is compromised by increased mosquito resistance to insecticides. Furthermore, use of inorganic insecticides raises serious environmental toxicity concerns. The test plants, *Oreosyce africana* and *Piper capense* were identified in Ethiopia through ethnobotanical leads obtained on the basis formal and informal field interviews and discussions coupled with literature search for sister species tested elsewhere. The plant powder from these species was extracted using 80% methanol and the methanol crude extracts of *Oreosyce africana* and *Piper capense* were sequentially fractionated with solvents (dichloromethane, ethyl acetate and deionized water). Each fraction was dissolved in dimethyl sulfoxide and deionized water; test concentration prepared and tested for their bioactivity against *Anopheles arabiensis* adults. The dichloromethane fraction of *Oreosyce africana* and ethyl acetate fraction of *Piper capense* had higher adulticidal activities with LC₅₀ and LC₉₀ values of 4.27 and 14.12 ppm and 10.72 and 30.59 ppm, respectively. Comparison of dichloromethane fraction of *Oreosyce africana* with ethyl acetate and water fractions showed significant differences at p<0.05. And comparison of ethyl acetate fraction of *Piper capense* with dichloromethane and water fractions showed significant differences at p<0.05. Thus, the bioassays with dichloromethane fraction of *Oreosyce africana* and ethyl acetate fraction of *Piper capense* exhibited higher adulticidal effect against *Anopheles arabiensis* than other solvent fractions. Dichloromethane fraction of *Oreosyce africana* and ethyl acetate fraction of *Piper capense* were examined under HPLC and UV-Vis for the proximate analysis. These plant products would be ideal alternatives for the control of malaria vector mosquitoes upon fractionation and preparation of suitable delivery packages.

Keywords: *Oreosyce africana*; *Piper capense*; *Anopheles arabiensis*; Fractions; Solvent fractions; Adulticidal activity; UV-Vis spectrum; HPLC chromatogram; Ethiopia

Abbreviations: DDT: Dichloro-Diphenyl-Trichloroethane; MeOH: Methanol; DCM: Dichloromethane; EtOAc: Ethyl acetate; DOF: Dichloromethane *Oreosyce* Fraction; EOF: Ethyl Acetate *Oreosyce* Fraction; WOF: Water *Oreosyce* Fraction; DPF: Dichloromethane *Piper* Fraction; EPF: Ethyl Acetate *Piper* Fraction; WPF: Water *Piper* Fraction; DMSO: Dimethyl Sulfoxide; EPHI: Ethiopian Public Health Institute; ppm: parts per million; WHO: World Health Organization; LC₅₀: 50% Lethal Concentration; LC₉₀: 90% Lethal Concentration; HPLC: High Performance Liquid Chromatography; UV-Vis: Ultraviolet-Visible

Introduction

Malaria still remains one of the most devastating diseases occurring in the world today. The five African countries that include Nigeria, Democratic Republic of Congo, Uganda, Ethiopia, and Tanzania accounted for 50% of malaria deaths and 47% of cases [1]. *Anopheles arabiensis* Patton is widely distributed in Ethiopia and is the major mosquito vector responsible for the transmission of most malaria cases including the occasional seasonal outbreaks and the major periodic cyclical epidemics in the country [2,3].

The ecology of malaria vectors can be influenced by human economic activities. Among these factors are constructions of water reservoirs, canals, irrigations systems, agricultural land reclamations and construction of industrial objects. All these factors leads to considerable change in the earlier existing natural conditions and thus could worsen previous malaria situation in an area. A mosquito's life is critically dependent on water, where the female lays her eggs.

Therefore, agricultural water management needs to a specific attention in Africa. For example, the different aspects of irrigation in agricultural water management such as irrigation efficiency [4-7] and importance of the different studies in various aspects of water sciences [8,9] have been investigated in the previous works, which leads to increasing malaria. To protect the environmental change that affects the malaria situation and ecology of the local vector drainage and environmental engineering technical methods of controlling malaria must be implemented. The purpose of implementing of thus malaria control measure is to prevent the existence of new water-loggings suitable for malaria mosquito breeding in process of planning; construction and exploitation of industrial and agricultural objects, to prevent the construction of borrow areas and reserves close to human settlements.

Historically, the use of synthetic insecticides has been very effective in reducing malaria transmission. However, over time, success has been hampered by mosquito resistance to synthetic insecticides and

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this challenged practical mosquito control around the world [10]. According to studies conducted in Ethiopia [11-13], *An. arabiensis* was resistant to an array of insecticides, including Dichloro-Diphenyl-Trichloroethane (DDT), permethrin, deltamethrin and malathion.

The bases for the insecticidal effects of the plants are the secondary plant products that are known to be less harmful to non-target organisms. As a result, the use of indigenous plants has increasingly become a major subject of research as they contain an array of bioactive chemical compounds, which would be used to kill or repel mosquitoes at various life stages [14,15]. The secondary metabolites which include – anabasine, azadirachtin, d-limonene, nicotine, pyrethrins, quassia and rotenone were among important insecticides widely used [16]. Although several compounds of plant origin have been reported as insecticides, there is still a wide scope for the discovery of more effective plant products [17] particularly in the indigenous flora of lesser studied countries like Ethiopia.

The application of easily degradable botanicals for the control of mosquitoes is recommended [18] because they minimize the accumulation of harmful residues in the environment. Therefore, the alternative control measure is screening of locally available indigenous ethno-medicinal plants as mosquito larvicidal and adulticidal agents that would eventually lead to their usage in plant-based mosquito abatement practices [19]. Although the potency of extracts is less than chemical insecticides, they are safer than the latter [20].

A recent study by Bekele et al. [21] showed that people in Akaki District (east-central Ethiopia) used *O. africana*, *B. nigra* and *Aloe* spp traditionally for mosquito control and for the control of cattle ticks and other arthropod pests. *Oreosyce africana* Hook. f. (Cucurbitaceae) is a slender climbing herb or trailer growing to 3 m and its habitat is in wet or moist *Pouteria* (= *Aningeria*) *adolji-friederici-Syzygium guineense* forest margins, grassland and in plantations at altitude between 1650-2000 m [22]. *Piper capense* L.f. (Piperaceae) is a small shrub 1-2 m high, possibly sometimes sub-scandent, base semi-woody, much branched above and stems are glabrous. It grows in the understorey of moist montane forest; 1600-2400 m [23].

Bowers et al. [24] reported that the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate efforts to enhance public health. The task of developing mosquito control agents from Ethiopian traditional herbal plants with ethnobotanical leads has remained an unaccomplished challenge, though ethnobotanical surveys have catalogued a considerable number of species [25]. There is emergence of resistance to synthetic chemical insecticides which has led to an increase in mosquito population and hence increases in the spread of mosquito-borne diseases like malaria. In addition, there are no reports of potential products derived from plants targeting the adult stages of mosquitoes in an effective way. The results of the present study would be useful in closing on the gap of developing new botanicals from indigenous Ethiopian plant source for possible insecticides. The adulticidal activities of the selected plant species given here against *An. arabiensis* have not been reported so far. The aim of this study was to investigate the mosquitocidal effects of *O. africana* leaf and *P. capense* fruit for malaria vector control in Ethiopia.

Materials and Methods

Collection and identification of plant materials

Leaves of *O. africana* Hook. f (Family: Cucurbitaceae) were collected from Yerer Lencho locality of Akaki district, eastern Ethiopia

(Latitude 08° 50.682' N, longitude 038° 56.630' E, altitude 1936 m asl) and fruits of *P. capense* L.f. (Piperaceae) were collected from Bada Buna locality of Qarsa district, western Ethiopia (Latitude 07°39.730' N, longitude 036°53.213' E, altitude 1779 m asl). These candidate plant species were collected both through initial literature search followed by field searching in a series of ethnobotanical survey. Voucher specimens and plant materials for extraction and testing were collected during field trips at the sites. Voucher specimens of both species were authenticated by a plant taxonomist in the Department of Plant Biology and Biodiversity Management, Addis Ababa University and deposited at the National Herbarium of Ethiopia found at College of Natural Sciences in Addis Ababa University.

Preparation of plant extracts

The crude extraction of plant materials and fractionation were done in the Department of Traditional and Modern Medicine of the Ethiopian Public Health Institute (EPHI). Ground leaves of *O. africana* and fruits of *P. capense* were homogenized with 80% aqueous methanol (3 liters) three times using Erlenmeyer flasks on orbital shaker (VWR, USA) at room temperature for 72 hrs following standard procedures [26,27]. The suspension was then filtered using Whatman no. 1 filter paper (Chatman Int. Ltd, Kent, UK) and the combined filtrate was concentrated using a rotary vacuum evaporator (Staurt®RE300, UK), 22-26 mm Hg below 45°C. The extract thus obtained was concentrated further over water bath (Kottermann, Germany) by evaporating the solvent. The dried extracts were stored in a vial at -20°C in freezing medium until used for fractionation.

Solvent fractionation procedure of *Oreosyce africana* and *Piper capense*

The dried 80% aqueous methanol crude extracts of *O. africana* and *P. capense* were suspended in deionized water and then partitioned with solvents dichloromethane, ethyl acetate and deionized water using solvent-solvent extraction at room temperature following the methods of Alkofahi et al. [18]. Portions of 60 g and 65 g of the 80% methanol crude extracts of *O. africana* leaf and *P. capense* fruit were suspended in 600 ml deionized water in separatory funnel and, which was extracted three times with 1200 ml dichloromethane (Carlo Erba, France). Extracts of each solvent were filtered using Whatman no.1 filter paper. The mixture was allowed to settle for one day, after which the solvent extract lower layer was slowly drawn off until only the upper layer remained, and partitions were combined and evaporated at 45°C to give dichloromethane *Oreosyce* fraction (DOF) and dichloromethane *Piper* fraction (DPF). Portions of 61 g and 60 g of the crude extracts of *O. africana* and *P. capense* were suspended in 600 ml deionized water in a separatory funnel and each of them was extracted with 1200 ml ethyl acetate (Carlo Erba, France). The ethyl acetate upper layer filtrates were combined and evaporated to give ethyl acetate *Oreosyce* fraction (EOF) and ethyl acetate *Piper* fraction (EPF). Finally, each of the water residual layer and the solution were evaporated and lyophilized to dryness to give water *Oreosyce* fraction (WOF) and water *Piper* fraction (WPF).

Preparation of yield of fraction and test concentration

The percentage yield of fractions of *O. africana* and *P. capense* were determined using the formula $W_2 - W_1 / W_0 \times 100$, which is described by Anokwuru et al. [28], where W_2 is the weight of the extract and the vial, W_1 the weight of the vial alone and W_0 the weight of the initial dried sample. Standard stock solutions were prepared at 1% by dissolving the extracts using the universal solvent dimethyl sulfoxide (DMSO),

Carlo Erba, France) at concentration of 0.05% and diluted in deionized water (deionizer, EasypureII, USA). From this stock solution different concentrations were prepared.

Rearing *Anopheles arabiensis* Patton from the egg stage

Anopheles arabiensis, the major malaria vector in Ethiopia, was selected for the testing of adulticidal bioactivities of the test plant extracts. Eggs of *An. arabiensis* for starting a colony were obtained from the EPHI and reared according to the World Health Organization [29] protocol. The colonies were reared and maintained by using heater and humidifier, which were kept the optimum temperature at about 25°C to 27°C and relative humidity about 70% to 80% and 12 hr light and 12 hr dark photoperiod cycle at the insectary of College of Natural Sciences, Addis Ababa University. Glass petridishes (10.5 cm internal diameter) lined with wet filter paper were kept inside the cages for oviposition, then eggs laid on the filter paper were transferred to plastic and enamel trays containing three liters distilled water and allowed to hatch to first instar larvae and kept until they reach the pupae. The larvae were fed on ground Tetramin® fish food pellets (Tetra holding Inc., Blacksburg, VA, USA); the feed was applied on alternate days for normal development. Water of the larval culture was changed every third day to avoid decay. The trays containing the larvae were kept in the sun during the early morning hours for keeping the water aerated. After attaining pupae, they were transferred to beakers by disposable pipettes and kept inside the mosquito cages for adult emergence. Food for sustaining *Anopheles arabiensis* adults in cages (30 × 30 × 30 cm³) were continuously provided with 10% sucrose solution with cotton wicks by placing on the top of each cage. The cotton-wool was moistened daily and changed twice weekly. Adult female *An. arabiensis* were periodically blood-fed on restrained rabbits shaved on the side of their belly for fertile egg production and the feeding was stimulated by darkening the cage. The *An. arabiensis* reared in the laboratory thus served as the source of adults for the bioassay tests.

Test mosquitoes and experimental design

Stock solutions were prepared at 1.0% by dissolving the dried fractions of *O. africana* and *P. capense* in 0.05% DMSO. The stock solutions were then diluted with deionized water to obtain the different test concentrations – 4, 8, 16 and 32 ppm for fractions coded DOF, EOF and WOF and 6, 12, 24 and 48 ppm for fractions coded DPF, EPF, WPF and the positive control (0.05% lambda-cyhalothrin). The 0.05% DMSO in deionized water was used as a negative control. Adult *An. arabiensis* were exposed to each test concentration together with the positive and negative controls. The bioassay was conducted using WHO test kit consisting of holding and exposure cylindrical plastic tubes both measuring 125 × 44 mm following the procedure of WHO [30]. The tests were conducted on uniformly spread impregnated papers (12 × 15 cm²) with respective concentrations of *O. africana* and *P. capense* by dipping the papers. The papers were left to dry at room temperature overnight and then inserted into the WHO tubes for test.

The test mosquito adults were caught from the rearing net cages with the help of a mouth aspirator (12 mm internal diameter), together with 60 cm of tubing and mouthpiece and released into a plastic holding tube. The WHO bioassay tubes served to expose the mosquito adults to the papers impregnated with fractions and for holding the mosquito adults before and after the exposure period. The bioassays were performed with non-blood-fed mosquitoes of known age (2 to 5 days old post-emergence of *An. arabiensis* adults) in batches of 20 in each concentration. The mosquitoes were allowed to acclimatize in the holding tube for 1 hr and then exposed to the fractions on the

impregnated paper and control for 1 hr. At the end of exposure period, the mosquitoes were transferred back to the holding tube and kept for 24 hrs recovery period. A pad of cotton soaked with 10% sucrose solution was placed on the mesh screen during the holding period of 24 hrs. Mortality rates of the mosquitoes were recorded at the end of 24 hrs post exposure period. Three replicates were maintained at a time. This adulticidal activity was evaluated at 25°C to 27°C and 70% to 80% relative humidity. The fraction which exhibited a pronounced adulticidal activity was chosen for further test. There was no need to correct the data with the Abbott's formula [31].

Phytochemical analysis

Dichloromethane fraction of *O. africana* and ethyl acetate fraction of *P. capense* were subjected to chromatography and spectrophotometer analysis by using high performance liquid chromatography (HPLC) and ultraviolet-visible (UV-Vis) at the traditional medicine and drug research Department of Ethiopian public health institute. The chemicals/reagents were all analytical and HPLC grades. Chromatographic fingerprints of dichloromethane (DCM) fraction of *O. africana* leaf and EtOAc fraction of *P. capense* fruit were done by injecting into analytical HPLC (WATERS LC-2000 model equipped with Waters 600 pump controller, Waters in-line mobile phase degasser AF, heated column thermostat, and Waters 2484 dual absorbance UV detector operated by millennium 32 software, WATERS, USA). The isocratic elution was done with the mobile phase containing a mixture of water and acetonitrile in 0.1% trifluoroacetic acid (TFA) (20:80, v/v) at a flow rate of 1.00 ml/min. The detection was done at 254 nm. Separation was carried out by low pressure gradient. The chromatographic tests were performed at 20°C on a Tracer Extrasil ODS (octadecylsilane), TR416059 (5 µm, 0.4 × 25 cm), Teknokroma (Barcelona, Spain), and the injection sample volume was 10 µl. The mobile phase and the samples were screened with 0.45 µm membrane filter. In all data acquisitions, the running time was 30 min.

Isolation and purification of solvent fractions of *O. africana* and *P. capense* Sephadex LH-20 column chromatography

The portion of EtOAc fraction of *P. capense* was dissolved in chloroform-methanol (1:1, v/v) and put into a column of Sephadex LH-20 (5 × 74 cm; Fluka, Switzerland) and eluted with a gradient of increasing polarity of n-hexane-chloroform (100:0, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, and 50:50, v/v). The purified fractions 5 and 6 were combined based on their thin layer chromatography analysis. The combined filtrates were concentrated using a rotary evaporator, and used for UV-Vis measurement.

Preparative thin layer chromatography

The portion of DCM fraction of *O. africana* was dissolved in chloroform and applied to eight sheets of silica gel 60 F₂₅₄ TLC plates (20 × 20 cm, 0.25 mm thickness; Merck, Germany). These were developed in a solvent system of chloroform-methanol (1:15) in a 15 cm height. Each 1.5 cm zone was scraped off from its plate and extracted three times with 100 ml of chloroform-methanol (1:9), and each combined filtrates was concentrated in vacuum to dryness. The purified fraction was subjected to UV-Vis measurement.

Ultraviolet/visible light (UV-Vis) spectroscopy measurement

To detect the UV-Vis absorption spectrum profile of the purified fractions of *O. africana* and *P. capense*, the fractions were measured in the wavelength ranging from 200-800 nm by using a double beam Ultraviolet-Visible spectrophotometry (UV-Vis) (Shimadzu, Japan)

and the characteristic peaks were detected and the peaks values recorded.

Data analysis

To test variations in *An. arabiensis* adult mortalities using crude methanol extracts of *O. africana* and *P. capense*, and fractions including DOF, EOF, WOF, DPF, EPF and WPF, the LC₅₀ and LC₉₀ values were determined using probit regression analysis of the statistical package PoloPlus (version 2.0, LeOra Software, Petaluma, California, USA; 2007). The chi-square (χ^2) probability for goodness of fit test was used to estimate how well the data of each concentration-mortality rate curve fit the assumption of the probit model using statistical package. The 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) for the lethal concentration (LC₅₀ and LC₉₀) in ppm for adulticidal effects was used to measure variations among the *O. africana* and *P. capense* fractions and lethal concentration ratio confidence limits (95%) that did not include 1.0 were considered significant ($p < 0.05$) [32].

Results

Yields of *O. africana* and *P. capense* fractions

The percentage yields of the fractions DOF, EOF and WOF were 24.5%, 12.8%, and 15.7%, respectively and the yields of DPF, EPF and WPF were 16.3%, 26.9% and 15.6% respectively (Table 1).

The percentage yields of ethyl acetate and water fractions were lower in *O. africana*, whereas the percentage yields of water and dichloromethane fractions in *P. capense* were lower as compared with dichloromethane fraction of *O. africana* and ethyl acetate fraction of *P. capense*. The EtOAc fraction yields for the *P. capense* was the highest

Plant species	Plant part (g)	Solvent used	Percent yield of fraction
<i>Oreosyce africana</i>	Leaves (905)	Dichloromethane	24.5% DOF ^a
		Ethyl acetate	12.8% EOF ^b
		Water	15.7% WOF ^c
<i>Piper capense</i>	Fruits (815)	Dichloromethane	16.3% DPF ^d
		Ethyl acetate	26.9% EPF ^e
		Water	15.6% WPF ^f

Note: ^a Dichloromethane *Oreosyce* fraction, ^b Ethyl acetate *Oreosyce* fraction, ^c Water *Oreosyce* fraction, ^d Dichloromethane *Piper* fraction, ^e Ethyl acetate *Piper* fraction, ^f Water *Piper* fraction.

Table 1: Percentage yields of *Oreosyce africana* and *Piper capense* fractions obtained using solvents of different polarities from their 80% methanolic crude extracts.

Extract Tested*	LC ₅₀ ppm (95% CL)	LC ₉₀ ppm (95% CL)	Slope ± SE	$\chi^2(p)$	LC ₅₀ ratio (95% CL)
DOF	4.267 (1.325-6.451)	14.123 (9.268-49.248)	2.466 ± 0.289	3.127 (0.47) ^a	3.051 (2.339-3.980) ^b
EOF	14.562 (13.720-25.292)	82.57 (69.094-98.728)	1.701 ± 0.206	5.086 (0.53) ^a	0.894 (0.692-1.154)
WOF	16.973 (13.456-17.209)	117.877 (88.475-157.372)	1.523 ± 0.204	6.65 (0.48) ^a	0.767 (0.580-1.1014)
Negative control**	0.0	0.0	0.0	0.0	0.0

*The codes used for the fractions was the same as in Table 1, ** DMSO (0.05%) in deionized water, ^a Good fit of the data to the probit model ($p > 0.05$), ^bLC₅₀ ratio significant at $p < 0.05$; 95% confidence interval did not comprise the value 1.0.

Table 2: Evaluation of the effect of solvent fractions of *Oreosyce africana* against *An. arabiensis* adults after 24 hrs post exposure on impregnated papers in WHO test tubes (n = 60 in each test).

Extracts tested*	LC ₅₀ ppm (95% CL)	LC ₉₀ ppm (95% CL)	Slope ± SE	$\chi^2(p)$	LC ₅₀ ratio (95%CL)
DPF	27.661 (22.75-35.504)	173.493 (107.212-380.843)	1.607 ± 0.207	1.815 (0.46) ^a	0.923 (0.695-1.224)
EPF	10.715 (9.349-12.100)	30.591 (25.796-38.413)	2.813 ± 0.261	0.569 (0.56) ^a	2.382 (1.908-2.972) ^b
WPF	28.196 (21.534-36.876)	150.614 (131.959-208.212)	1.761 ± 0.213	0.988 (0.45) ^a	0.905 (0.692-1.185)
Negative control**	0.0	0.0	0.0	0.0	0.0

* The codes used for the fractions were the same as in Table 1, ** DMSO (0.05%) in deionized water, ^a Good fit of the data to the probit model ($p > 0.05$), ^bLC₅₀ ratio significant ($p < 0.05$), 95% confidence interval did not comprise the value 1.0.

Table 3: Evaluation of the effect of solvent fractions of *P. capense* against *An. arabiensis* after 24 hrs post exposure on impregnated papers in WHO test tubes (n = 60 in each test).

followed by DCM fraction yields for *O. africana* (Table 1). These fractions were subjected to adulticidal test against *An. arabiensis* adults.

Adulticidal activity

Quantitative estimation in all the solvent fractions of *O. africana* was carried out to determine the lethal concentrations (LC₅₀ and LC₉₀) of a particular fraction (Table 2). All chi-square values were not significant ($p = 0.05$) in goodness of fit test on the probit model, indicating a good fit of regression line (Table 2). A comparison of the various solvent fractions of *O. africana* in regard to LC₅₀ and LC₉₀ values showed the dichloromethane fraction with 4.267 and 14.123 ppm, respectively to be significantly different at $p < 0.05$ and it was the most potent adulticidal activity against *An. arabiensis* adults than the solvent fractions of ethyl acetate and water (Table 2).

With regard to *P. capense* fractions, all chi-square values were not significant ($p = 0.05$) in goodness of fit test on the probit model, indicating a good fit of regression line (Table 3). The ethyl acetate fraction of *P. capense* with LC₅₀ at 10.715 and LC₉₀ at 30.591 ppm showed potent adulticidal effect against adults of *An. arabiensis* and it differed significantly at $p < 0.05$ than its dichloromethane and water fractions (Table 3).

Chromatographic analysis

The qualitative HPLC fingerprint profile of the DCM fraction of *O. africana* and EtOAc fraction of *P. capense* was selected at a wavelength of 254 nm due to the sharpness of the peaks and proper baseline. The HPLC profile of DCM fraction of *O. africana* displayed two prominent peaks with 58.98% and 30.73% area under the peak at retention times of 12.60 and 5.04 min, respectively and some moderate peaks were also observed at retention times of 13.26 and 5.32 min (Figure 1). The HPLC chromatographic profile of EtOAc fraction of *P. capense* is shown in Figure 2. The three major first, third and tenth peaks with area percentage of 15.68, 14.99, and 14.82 appeared at the retention times of 8.37, 3.54, and 3.08 min, respectively.

Spectrophotometric analysis

The UV-Vis measurement of the dichloromethane fraction of *O. africana* and ethyl acetate fraction of *P. capense* was taken at the 200 to 800 nm wavelength due to the sharpness of the peaks and proper baseline. The UV-Vis measurement of dichloromethane fraction of *O. africana* showed the maxima peaks at 663.00, 605.00, 534.00, 410.00 and 368 nm at absorbance of 0.096, 0.025, 0.035, 0.380 and 0.332,

respectively (Figure 3) with low intensity region of 382.00 nm and 358.00 nm at absorbance of 0.317 and 0.330, respectively. The UV-Vis measurement of ethyl acetate fraction of *P. capense* showed the maxima peaks at 268.00 and 212.00 nm with low intensity region of 247.00 nm at absorbance of 1.289, 3.686 and 1.108, respectively (Figure 4).

Discussion

The leads supplied by local traditional medicine practitioners and knowledgeable elders from the geographical areas where the plants were collected were the most critical guides in the study. The approach helped to identify the plants whose extracts possessed anti-*An. arabiensis* potency. The rationale for considering plant materials for anti-mosquito effects was because plants are rich sources of bioactive secondary metabolites and offer an advantage over synthetic insecticides as their extracts are less toxic, less prone to development of resistance, and easily biodegradable thereby reducing the possible accumulation of toxic residues in the environment [33]. The plant fractions that showed activities in the study are expected to offer advantages as insecticides over synthetic products.

Crude methanol extracts of *O. africana* and *P. capense* were further extracted with a less polar organic solvent, ethyl acetate and

dichloromethane and a polar solvent (water) to give six extracts. Among the three fractions isolated from *O. africana*, dichloromethane fraction showed the maximum yield, and of the three fractions isolated from *P. capense*, ethyl acetate fraction showed the maximum yield. The results of the percentage yield suggested that dichloromethane was a better solvent for the fractionation of *O. africana*, while ethyl acetate was a better solvent for the fractionation of *P. capense*.

Therefore, it is to be expected that since different phytoconstituents dissolve in specific solvents [34], the DCM fraction of *O. africana* and ethyl acetate (EtOAc) fraction of *P. capense* would contain constituents with demonstrated adulticidal activity in the present study. Furthermore, since the yields of the active component, in addition to its bioactivity, would determine the suitability of plant extracts for mosquito control.

The rationale for extract fractionation by using solvents with different polarity gradients to water, the most polar (polar index, P=9.0), ethyl acetate (P=4.4), and dichloromethane (P=3.1) was because different organic solvents show difference in dissolving the bioactive components present in the plant materials. In this connection, Shaalan et al. [35] reported that the different solvents can significantly affect the potency of extracted plant compounds. This study is in line with

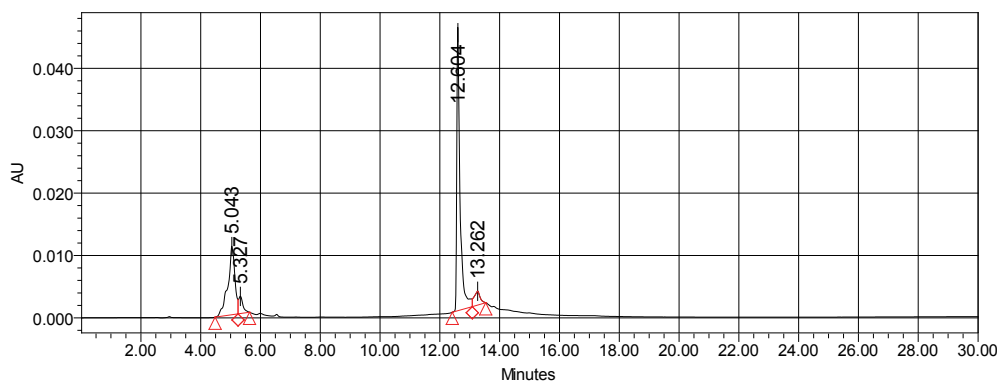


Figure 1: HPLC Chromatogram of DCM fraction of *O. africana* leaf. (Elution was done with water-acetonitrile in 0.1% TFA (20:80) at a flow rate of 1.00 ml/min; detection, absorbance at 254 nm).

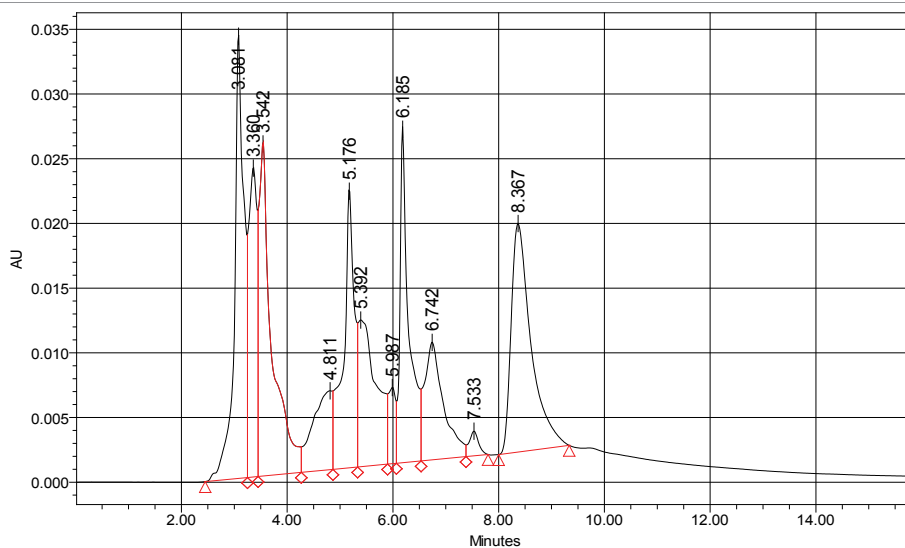


Figure 2: HPLC Chromatogram of ethyl acetate fraction of *P. capense* fruit. (Elution was done with water-acetonitrile in 0.1% TFA (20:80) at a flow rate of 1.00 ml/min; detection, absorbance at 254 nm. HPLC was done as described in materials and methods).

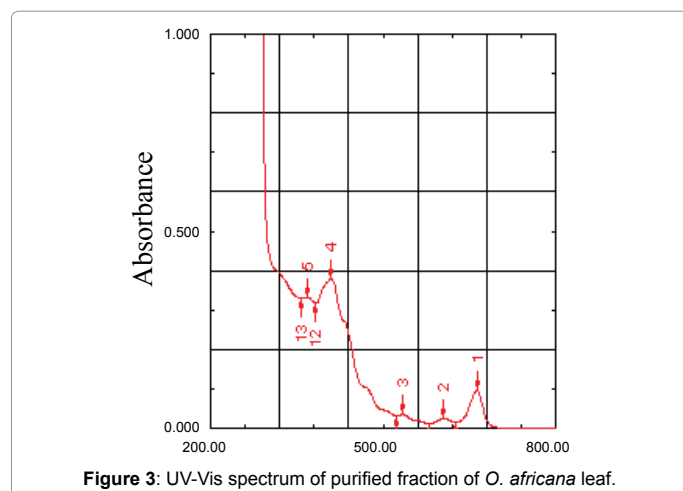


Figure 3: UV-Vis spectrum of purified fraction of *O. africana* leaf.

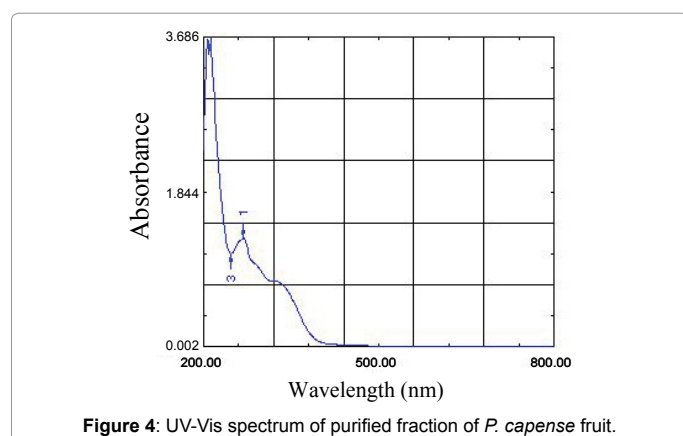


Figure 4: UV-Vis spectrum of purified fraction of *P. capense* fruit.

the observation of Aivazi and Vijayan [36] in oak gall extracts and Mulla and Su [37] in neem plant extracts, who report that a converse relationship between extract effectiveness and solvent polarity where the efficacy increases with decreasing polarity.

Hidayatulfathi et al. [38] also indicates that the bioactive components from *Acorus calamus* (Acoraceae) responsible for the lethal effect on the adults were extracted in greater measures with certain solvents. However, this is not consistent due to differences between the characteristics of active chemicals among plants. From this, it is clear that the bioactive components responsible for the lethal effect on mosquitoes were extracted in greater measures with certain solvents only and not with all.

The high mosquitocidal activity of DCM fraction of *O. africana* is consistent with the report of Broussalis et al. [39] for the dichloromethane extracts of *Tagetes erecta* L. (Fabaceae/Compositae) which showed a significant pesticidal activity against *Sitophilus oryzae*. In the current study, the higher activity of DCM fraction of *O. africana* and EtOAc fraction of *P. capense* may be due to the presence of bioactive components against adult stage of *An. arabiensis*. In line with a study by Asghari et al. [40], DCM is a semi-polar solvent that had the ability to dissolve polar and non-polar compounds in the extract of *O. africana*. This indicates that the bioactive components in this plant had adulticidal properties against *An. arabiensis* and were better soluble in DCM than in other solvents.

The reason for the higher adulticidal activity of DCM fraction may be due to presence of both polar and non-polar active compounds. The

solvent-specific nature of extraction of bioactive constituents was also shown by the ethyl acetate fraction of *P. capense* that also had a superior adulticidal effect on adult mosquitoes compared to the aqueous fraction. The finding that *O. africana* and *P. capense* aqueous methanol crude extract fractions against *An. arabiensis* adult mosquito effects were solvent-specific and each had different efficacies; and shows the difference in the nature of the insecticidal chemical constituents in the two plants. Furthermore, the adulticide activities of the plant fractions tested against *An. arabiensis* were concentration dependent.

Among the three fractions isolated from *O. africana*, dichloromethane fraction showed the most potent adulticide (LC_{50} at 4.35 ppm), while the least effective one was the water fraction (LC_{50} at 55.14 ppm). This indicates that constituents active against adult mosquitoes were eluted in the dichloromethane fraction. The finding in the present study that dichloromethane partitioned of *O. africana* had superior toxicity against adults *An. arabiensis* is supported by the report of Joseph et al. [41] who show DCM extracts of the plant *Neorautanenia mitis*, to have the highest (LC_{50} 3.05 ppm) anti-adult *An. gambiae* activity. The high potency of the DCM fraction of *O. africana* and EtOAc fraction of *P. capense* against *An. arabiensis* adults at low concentrations could reasonably be attributed to the active constituent of secondary metabolites with mosquitocidal activity.

Fractionation of the extracts allowed to minimize the number of compounds in each solvent extract tested for mosquitocidal effect. As the purpose of fractionating crude extracts for bioactivity is to extract as many potentially active constituents as possible, the observed weak to very strong adulticidal effects of the different solvent fractions was an indication that the plant extracts consisted of different phytochemicals with varying adulticidal potencies. Similarly, a study by Uthayarasa et al. [42] report that sequential extraction directed to minimize compounds in plant extract thereby the antagonistic effect can be reduced and also the isolation and purification can easily be undertaken to find out natural mosquitocides from plant origin.

Furthermore, such variation in the bioactivities of different solvent fractions of crude extracts that screened with ethyl acetate, n-butyl alcohol and water fractions of alcoholic extracts of leaves and stems of *Vanilla fragrans* against *Cx. pipiens* larvae found that both n-butyl alcohol and ethyl acetate fractions were active in bioassays, while the water fraction appeared to contain no substances that inhibited larval growth [43]. This shows that the activity of some crude extracts may be attributed to the complex mixture of bioactive compounds.

On the other hand, the demonstration in the present study that the activity of DCM fraction of *O. africana* was much higher as a mosquito adulticide compared to the ethyl acetate and water fractions, shows that the potency of the active constituents in the crude extracts may also be masked by other, less active or completely inactive, minor constituents. This was further evident from *P. capense* extracts whereby the ethyl acetate fraction was more potent as a mosquito adulticide as compared with its crude methanol extract and the dichloromethane and water fractions. This is consistent with a previous report that showed mortality rates of mosquitoes declined with increasing polarity of the solvent. These authors showed that water extracts of *Zanthoxylum heitzii* (Rutaceae) produced the lowest adult mortalities whereas its ethyl acetate and hexane extracts produced higher mortalities against *Anopheles gambiae* [44].

The peaks obtained in UV-VIS spectrum of *O. africana* fraction has absorption bands at 368.0, 410.0, 534.0, 605.0 and 663.0 nm, and *P. capense* fraction has absorption bands at 212.0 and 268.0 nm. The Flavonoids and Terpenoids spectra typically consist of two absorption

maxima, the first in the ranges 230-290 nm (band I) and the second in the ranges 400-500 nm (band II). The chlorophyll spectra typically consist of two absorption maxima in the ranges 600-700 nm. The precise position and relative intensities of these maxima give valuable information on the nature of the Flavonoids [45,46].

Conclusion

Plant-derived natural insecticides could be useful as an alternative for synthetic insecticides. In the present study, *O. africana* and *P. capense* are easily available, accessible and affordable. Therefore, in addition to encouraging the continued uses of these traditional medicinal plants among the local residents, the use of these plant extracts should be promoted in order to reduce the human-vector contact as well as vector-borne diseases. Further studies on the toxicity test of dichloromethane fraction of *O. africana* and ethyl acetate fraction of *P. capense* against non-target organisms may provide futuristic lead products for field application for routine mosquito control.

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