WORLD JOURNAL OF PHARMACEUTICAL RESEARCH

SJIF Impact Factor 5.045

Volume 3, Issue 10, 1705-1720. Research Article ISSN 2277– 7105

PRELIMINARY PHYTOCHEMICAL SCREENING AND ACTIVITIES OF *CITRULLUS COLOCYNTHIS* (L.) SCHRAD. AS MOSQUITO LARVICIDES

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Article Received on 26 October 2014,

Revised on 21 Nov 2014,
Accepted on 16 Dec 2014

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ABSTRACT

Citrullus colocynthis is a widespread wild cucurbit herb of multi traditional uses in Sudan. The current laboratory work was aimed to scrutinize the main phytochemical constituents of three extracts (water, ethanol and petroleum ether) prepared from leaves and fruits of *C. colocynthis* and to evaluate their biological activities against the 4th instar larvae of *Anopheles arabiensis* Patton, the main malaria vector in the country. The knockdown and residual effects of botanical treatments were evaluated in comparison with two standard insecticides. The phytochemical analysis revealed various compounds, dominated by; alkaloids, saponins and flavones in water extracts, alkaloids and triterpenes in ethanol extracts and triterpenes and sterols in petroleum ether extracts. Generally, the highest chemical groups were triterpenes, flavones and alkaloids; then followed by sterols and amino acids. Fruits seemed to be richer in active compounds than the leaves. Consequently, petroleum ether extracts showed better mortality results than water and ethanol extracts, but all showed significant effects. The fruits were more potent than the leaves, and in most cases the activities increased in relation to concentration and exposure time. In conclusion, fruits petroleum ether extract (0.5%) was the best botanical treatment which showed comparable significant effect (84.2 ± 6.7% mortality) with those of the two insecticides (90.0 ± 0.0%) at 24 h post exposure. This treatment also manifested the best significant residual actions up to three weeks, but ranked next in order to synthetic insecticides. Such promising product needs to be investigated in advanced research.

KEYWORDS: Citrullus colocynthis; Anopheles arabiensis; phytochemical; mortality; residual.

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are serious medical insects annoying and frustrating human beings worldwide, particularly in tropical and subtropical countries. Several species of the genera *Anopheles*, *Culex* and *Aedes* are vectors of various human diseases including malaria, filariasis, dengue hemorrhagic fever, yellow fever, Rift Valley fever, West Nile virus, Venezuelan equine encephalitis and Japanese encephalitis. [1, 2] Moreover, the mosquitoes' biting habit often makes it very nuisance to enjoy staying in houses, parks and other recreational areas. Such bites effect can deprive many areas of productive people; hence contribute in poverty and immigration. Mosquitoes also attack poultry and farm animals and cause loss of weight and decrease of production. [3]

Anopheline mosquitoes such as *Anopheles gambiae* complex are the most important malaria vectors spreading in Africa. Of these, *Anopheles arabiensis* Patton is the dominant species in central and northern parts of Sudan. [1, 4, 5, 6] The distribution of malaria varies greatly from one area to another and from country to country. However, malaria is endemic in 45 African countries including the Sudan. [7] This disease is still the most important cause of fever, morbidity and mortality in its endemic areas. [8] About 300 – 500 million clinical cases and 1.2 – 2.6 million deaths of malaria were estimated to be recorded each year, the majority of them are children and pregnant women. [7, 9] Thus, one of the approaches to minimize mosquito problems is the interruption of disease transmission either through proper vector control or otherwise by avoiding mosquito bites.

Application of chemical insecticides, though undesirable, is still the major tool adopted globally for mosquito control. Sudan is one of the first African countries used chemical mosquitocides at large scales. Since the mid of the 20th century a number of insecticides from different chemical groups (i.e., organochlorines, organophosphates and pyrethroids) were recommended and applied for outdoor and indoor control of both adult and larval stages of mosquitoes. [10, 11, 12, 13] This long journey of extensive chemicals has been reflected in various drawbacks exemplified largely by; decline of insecticides activities due to emergence of resistant mosquito strains, contamination of environment and mortality of non-target organisms, bio-magnifications in ecosystem, and the increasing prices of chemicals coupled with high cost of control. [14, 15, 16, 17, 18, 19] Thus, ecologically safe and potentially effective control alternatives are being sought carefully in everywhere. Despite the fact that such alternatives are slowly growing, appreciable efforts were devoted to bio-control and natural biocides including botanical products. [20, 21, 22, 23, 24]

Considering indigenous works, a good number of important plants manifested variable activities against different mosquito species in Sudan has been listed by Satti *et al*. (2010) which represents rich source for biocidal studies. [22] Among such plants the bitter apple or bitter cucumber [*Citrullus colocynthis* (L.) Schrad.], a member of the family Cucurbitaceae, locally known as “hanzal” or “handal” (Arabic), is a widespread wild herb of multi-traditional uses in the country. It is largely exploited in folk medicine and traditional pests control since immemorial time as a cheap and effective natural product. Therefore, laboratory works were conducted to test the occurrence of main secondary metabolites in some extracts of this plant (*C. colocynthis*) and to assess their larvicidal activities against *Anopheles arabiensis*.

MATERIALS AND METHODS

The current study was conducted as a prelusive research in the project of botanical pesticides adopted at the Environment, Natural Resources and Desertification Research Institute, Khartoum, Sudan. It was aimed to scrutinize the main phytochemical constituents of some extracts (water, ethanol and petroleum ether) prepared from leaves and fruits of *C. colocynthis*, and to evaluate their biological activities against the 4th instar larvae of *Anopheles arabiensis* Patton, the main malaria vector in the country.

Rearing of the test insect, Anopheles arabiensis

Based on morphological characters of mosquito immature stages [3, 25], samples of *A. arabiensis* eggs were taken from stagnant water of Elozozab area, Khartoum, and brought to the laboratory for rearing. A glass container with clean water was used to accommodate the eggs until hatching; whereas the emerged larvae were transferred to another wider glass cage (40 x 40 x 40cm) partially filled with tap water. Larvae were fed on Brewer’s yeast and wheat flour. The obtained pupae were transferred to open glass Petri dishes containing tap water and enclosed in a new cage (40 x 40 x 40cm) covered with muslin cloth to prevent the escape of emerging adults. The first generation adults of both sexes were fed on a honey diet (10%), but blood meals were also secured for egg laying females by introducing a pair of albino rats (*Rattus norvegicus*) in the cage. Glass Petri dishes including 70 ml water and a wetted filter paper were placed inside the latter cage for oviposition. Eggs deposited were transferred to the first rearing cage and followed in the same previous way until the second generation larvae were obtained. [26, 27] Such larvae were used for the different bioassay experiments.

Preparation of botanical extracts

Extracts of two botanical parts (leaves and fruits) from *Citrullus colocynthis* were investigated in this study for their phytochemical constituents and larvicidal effects against *An. arabiensis*. These plant materials were collected during the rainy season 2008 from Khartoum State, cleaned with water and dried under room temperature. Dry samples were firstly crushed manually, and then ground into fine powders using an electric blender (Laboratory Blender: MAH/11/050/0117). Three extracts (water, ethanol and petroleum ether) were prepared from such powders. To obtain water extracts, 200g of powder from each sample was mixed with 1litre distilled water in a conical flask, stirred thoroughly for one hour using a magnetic stirrer, and left over night. In the second day the solution was filtered through a fine muslin cloth to obtain the stock solution. The latter step was prepared in the same day coinciding with each bioassay experiment. Regarding organic constituents, the classical chemical extraction procedure through the soxhlet apparatus was applied. Petroleum ether and ethanol solvents were used to separate apolar and polar compounds, respectively. [28] Accordingly, 200g from each sample was separately extracted for eight hours in the soxhlet. After drying in a rotary evaporator the two extracts were kept in black bottles and stored in the refrigerator (at 5^{o}C) until being used.

Phytochemical analysis

Standard chemical procedures of phytochemical analysis [29, 30, 31] were adopted to test the main secondary metabolites groups in all samples. Accordingly, the necessary reagents were prepared and used for testing seven chemical groups included; alkaloids, amino acids, flavones/flavonoids, tannins and sterols/triterpenoids. The characteristic evidence indicated for each group of chemicals was followed. Moreover, only foam characteristics of shaken samples were considered in testing saponins contents of all extracts.

Bioassay experiments

The above prepared extracts of *C. colocynthis* were bioassayed in two separate laboratory experiments to evaluate their acute toxicity (knockdown) and residual effects, respectively, against the 4^{th} instar larvae of *An. arabiensis*. The treatments included three concentrations of water (2.5%, 5% and 10% w/v) and organic solvents (0.125%, 0.25% and 0.5% v/v) extracts

[32], plus two standard insecticides [Malathion 50%EC (6ml/l) and Abate 50%EC (1ml/l)] and a water control for comparison.

Regarding the acute toxicity experiment, metal plates each with 250ml of the extracts solutions were used to run the bioassay test against the mosquito (*An. Arabiensis*) 4th instar larvae. Twenty five larvae were introduced in each plate. All treatments were replicated four times and arranged in a Completely Randomized design (CRD). Records of larval mortality were taken at three consecutive days (24h, 48h and 72h) post treatments. The failure of a larva to swim to the surface or its inability to go to the bottom in response to mechanical probing was taken as indicators of larval mortality. Such data were subjected to analysis of variances, followed by means separations based on Duncan's Multiple Range (DMR) test. [33]

On the other hand, the residual effects of the same previous treatments were assessed in another experiment where a standard procedure was applied. [26] The materials and the experimental design (CRD) used were similar to what have been mentioned for the first experiment, but the larvae were introduced at differed times (3, 7, 14 and 21 days) after treatments. The treatments were replicated four times. Twenty-five larvae (4th instar) of *An. arabiensis* were placed per each test solution according to the mentioned intervals. That is to say the larvae in each treatment solution were left for 24 h, after which they transferred into distilled water for another 24 h, so as to check for any sign of recovery. New 4th instar larvae of *An. arabiensis* were added every time after the removal of the previous population, so as to investigate the residual activities of treatments at the indicated durations. The mortality sign was indicated as stated above. The recorded mortality data were statistically analyzed, followed by means separation using the DMR test.

RESULTS AND DISCUSSION

Phytochemical analysis

The results of phytochemical screening test were depicted in table 1. It is clear that all the eight tested chemical groups were detected at variable levels. Such variability appeared to be occurred as a result of differences in plant parts studied and the solvent used in extraction. Generally, water and ethanol extracts revealed wider range of chemical classes than that of apolar petroleum ether extracts. Namely, all the tested chemicals, except flavonoids, were appeared in the former two extracts, while only five groups were found in petroleum ether extracts (oils) but dominated by triterpenes and sterols. Alkaloids were the main compounds among the polar extracts (water and ethanol) which found in both plant parts (leaves and fruits). Triterpenes were shared between ethanol and petroleum ether extracts of leaves and fruits. The two plant parts (leaf and seed) also showed saponins and flavones in water extracts, and sterols in petroleum ether. However, the rest of recorded compounds in the different extracts were merely restricted to fruit part. This may reflected the richness of fruits in diversified chemical groups as compared with leaves. However, comparing the percentages of chemicals appeared in the whole extracts, alkaloids, flavones and triterpenes were the most frequently detected (66.7%), followed by sterols and amino acids (50.0%), then saponins (33.3%) and lastly flavonoids and tannins (16.7%).

Table 1: Phytochemical constituents of three extracts prepared from leaves and fruits of Citrullus colocynthis.

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	+	-	-	+	+	-	+	+
Chemical groups (%)	50.0	33.3	66.7	16.7	66.7	16.7	50.0	66.7

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

Higher plants are described as untapped sources of various biologically active secondary metabolites, especially in the field of drugs and bio-pesticides. [34] However, in spite of being classical approach, the phytochemical analysis adopted in this research revealed well panorama of secondary compounds in the studied plant, and suggesting their importance in mosquito control which may necessitates application of advanced techniques to be fractionated and characterized based on concurrent bio-assays. Though, meager research is found regarding phytochemical analysis of indigenous flora in Sudan, the present preliminary findings proved previous results in some globally scattered literature. Different alkaloid compounds, flavonoids, saponins, triterpenes and flavones glycosides were recorded in *C. colocynthis.* [35, 36, 37] Gurudeeban *et al*. (2010) reviewed the chemical composition of *C. colocynthis*, and showed that seed kernels of the plant contain good sources of amino acids such as arginine, tryptophan and methionine, and almost all parts (including leaf and fruit) were found to possess flavonoids and flavones, but fruits in particular contain seventeen different compounds under five classes of chemicals. [38] These authors also reviewed the oil yield (ranged from 24.86 – 35.66%) of seeds and its various characteristics and content of fatty acids, sterols and other constituents. [38] The current research also came in consistency with previous literature which showed qualitative diversity in secondary metabolites of botanical extracts based on different morphological parts used [22, 39], besides the richness of fruit parts in secondary compounds as compared to other parts. [40, 41, 42]

According to Sayed *et al*. (1973), the oil of *C. colocynthis* contains oleic and linoleic acids. These substances are known to be more effective against aquatic insects. [43] The uses of different polarity solvents for extracting the fruits of *C. colocynthis* have yielded different amounts of chemical compounds including triterpenes and sterols. [44, 45] Alkaloids as well as saponins were detected in water extracts of this plant. [46, 47, 48, 49] The saponin compounds were preferably used against aquatic insects. [46, 50] Therefore, the detection of alkaloids and saponins in the studied parts, especially in water and ethanol extracts, may justify their larvicidal activities achieved.

Mosquito larvicidal effects

The results of acute mortality effects of the different treatments against the 4th instar larvae of *An. arabiensis* were demonstrated in table 2. Significant variations in mortality results were obtained at the three count intervals based on differences in botanical parts, extract types used and the concentration applied. Fruit extracts generally showed better results than leaf extracts, with the highest effects mainly recorded for the petroleum ether extracts (oils). For most extracts, the mortality percent was increased with an increase in concentration. To show the conformity of these results with the literature, Ghosh *et al*. (2012) stated that insecticidal activities of plant extracts vary not only according to plant species, mosquito species, geographical varieties and parts exploited, but also due to extraction methodology adopted and the polarity of solvents used. [51]

Regarding the first count (24 h post treatments), fruit petroleum ether extract at 0.5% and the two insecticides showed significantly the highest comparable mortality levels as compared with the other botanical treatments and the untreated control. The same trend was maintained during the second (48 h) and the third (72 h) intervals. Moreover, most treatments showed progressive increases in mortality levels in relation to exposure time, hence almost all extracts treatments gained non significant differences from that of fruit petroleum ether extract (84.2 ± 6.7% mortality) and the insecticides (90.0 ± 0.0%), at the third count. This may proved the delayed insecticidal effects of botanical extracts which mainly work through stomach action rather than contact effect. [39] According to Mullal *et al*. (2008), certain cucurbit extracts were found to induce 60% and 100% mortalities at 48 h and 72 h post treatments, respectively. [52] Similar results were also shown by other botanical extracts tested against some species of agricultural insect pests and mosquitoes. [53, 54]

Table 2: Acute mortality effects of different Citrullus colocynthis extracts on Anopheles arabiensis 4th instar larvae, at three intervals post treatments (August 2009).

Treatments	Mortality percent means (±S.E) at three intervals		
	24 h	48 h	72 h
Leaves water extract, 2.5%	01.8 ± 0.0k	01.8 ± 0.0m	56.4±83.1abcd
Fruits water extract, 2.5%	32.6 ± 2.5hi	32.6 ± 2.5jk	36.3 ± 2.3abcd
Leaves water extract, 5%	33.2 ± 3.2gh	33.9 ± 4.5jk	33.9 ± 4.5abcd
Fruits water extract, 5%	49.6 ± 4.0de	49.6 ±4.0fg	49.6 ± 4.0abcd
Leaves water extract, 10%	41.0 ± 2.2f	41.0 ± 2.2i	41.0 ± 2.2abcd
Fruits water extract, 10%	50.2 ± 2.9d	50.2 ± 2.9ef	58.1 ± 2.1abcd
Leaves ethanol extract, 0.125%	01.8 ± 0.0k	01.8 ± 0.0m	01.8 ± 0.0d
Fruits ethanol extract, 0.125%	30.0 ± 2.6i	30.0 ± 2.6k	31.2 ± 4.4abcd
Leaves ethanol extract, 0.25%	17.1 ± 4.1j	17.1 ± 4.1L	22.7 ± 3.0bcd
Fruits ethanol extract , 0.25%	41.0 ± 3.5f	41.0 ± 3.5i	44.4 ± 4.8abcd
Leaves ethanol extract, 0.5%	43.8 ± 3.3ef	43.9 ± 3.3gh	45.0 ± 4.8abcd
Fruits ethanol extract , 0.5%	57.5 ± 2.5c	57.5 ± 2.5cd	60.1 ± 3.3abc
Leaves pet.eth. extract, 0.125%	41.7 ± 2.4f	41.7 ±12.4hi	47.9 ± 2.9abcd
Fruits pet.eth. extract, 0.125%	41.7 ± 6.1f	41.9 ± 6.8hi	43.3 ± 5.8abcd
Leaves pet.eth. extract, 0.25%	56.9 ± 3.2c	56.9 ± 3.2de	64.4 ± 4.5ab
Fruits pet.eth. extract, 0.25%	56.2 ± 3.2c	56.2 ± 3.2de	62.1 ± 1.6abc
Leaves pet.eth. extract, 0.5%	65.0 ± 3.1b	65.0 ± 3.1b	72.1 ± 5.2ab
Fruits pet.eth. extract, 0.5%	84.2 ± 6.7a	84.2 ± 6.7a	84.2 ± 6.7a
Malathion 50% EC	90.0 ± 0.0a	90.0 ± 0.0a	90.0 ± 0.0a
Abate 50% EC	90.0 ± 0.0a	90.0 ± 0.0a	90.0 ± 0.0a
Control (water only)	00.0 ± 0.0L	00.0 ± 0.0n	00.0 ± 0.0e
CV%	7.5	09.7	6.5

Means followed by the same letter(s) in each column are not significantly different at 5% level according to Duncan's Multiple Range test; pet.eth. = petroleum ether.

The better results obtained by fruit extracts as compared to leaves extracts can be attributed to the fact that fruiting parts, especially the seeds, are the storage bodies where all the heredity characters of the species are embedded. In this respect, the results proved several studies on different plants which showed superior effects of fruiting components over those of other botanical parts. [39, 40, 41, 42, 53, 54, 55] On the other hand, the findings also agreed with different results showing higher activities by apolar extracts of petroleum ether and hexane solvents than by other polar extracts, as previously evaluated against different insects including certain mosquitos' larvae. [42, 53, 54, 56, 57, 58] This solvent seemed to extract the most potent larvicidal ingredients in *C. colocynthis* fruits as part of some chemical groups, probably of triterpenes or sterols. In this occasion, different plant ingredients were reviewed by Kishore *et al*. (2011), who concluded that essential oils, fatty acids, terpenes and sterols, isoflavonoids, alkaloids and many other compounds are potential mosquito larvicides. [59]

In general, hundreds of higher plants were reviewed to have mosquito larvicidal properties worldwide. [22, 51, 59, 60] Among such plants, *C. colocynthis* showed insecticidal effects against some agricultural pests [61, 62] as well as larvicidal activities against some mosquito species [22, 63], besides its antibacterial and antifungal properties which gave the plant its medicinal importance. [64] Thus, it seems that the plant is attracting global attention as a rich source of bioactive materials for different purposes.

The results of residual mortality effects of the studied treatments were demonstrated in table 3. Significant differences were also achieved among the different plant parts, extracts and concentrations. Generally, fruits shoed potent effects compared to leaves, as mentioned for the first experiment. However, apolar petroleum ether extracts (oil) also gave superior results compared to other extracts, and the activities were always dose dependent, but almost all treatments revealed declining effects as the residual period increased.

Table 3: Residual mortality effects of different Citrullus colocynthis extracts on Anopheles arabiensis 4th instar larvae, at different intervals from treatments (August 2009).

Treatments	Mortality percent means (±S.E.) at different intervals			
	3days	7days	14days	21days
Leaves water extract, 2.5%	21.7 ± 4.4o	01.8 ± 0.0n	01.8 ± 0.0f	01.8 ± 0.0e
Fruits water extract, 2.5%	27.3 ± 1.4mn	07.9 ± 7.3m	01.8 ± 0.0f	01.8 ± 0.0e
Leaves water extract, 5%	35.5 ± 6.4 ijk	01.8 ± 0.0n	01.8 ± 0.0f	01.8 ± 0.0e
Fruits water extract, 5%	43.3 ± 2.9g	37.4 ± 4.5de	01.8 ± 0.0f	01.8 ± 0.0e
Leaves water extract, 10%	39.8 ± 2.9hi	32.5 ± 3.2fg	01.8 ± 0.0f	01.8 ± 0.0e
Fruits water extract, 10%	52.0 ± 1.4de	43.9 ± 3.3cd	01.8 ± 0.0f	01.8 ± 0.0e
Leaves ethanol extract, 0.125%	01.8 ± 0.0p	01.8 ± 0.0n	01.8 ± 0.0f	01.8 ± 0.0e
Fruits ethanol extract, 0.125%	28.6 ± 2.6lmn	23.0 ± 4.2hi	01.8 ± 0.0f	01.8 ± 0.0e
Leaves ethanol extract, 0.25%	30.0 ± 2.6lmn	18.4 ± 2.2jk	06.7 ± 5.6e	01.8 ± 0.0e
Fruits ethanol extract , 0.25%	34.4 ± 2.0jk	01.8 ± 0.0n	01.8 ± 0.0f	01.8 ± 0.0e
Leaves ethanol extract, 0.5%	33.2 ± 3.2 jkl	23.5 ± 2.6hi	19.2 ± 3.5c	01.8 ± 0.0e

Fruits ethanol extract , 0.5%	55.6 ± 2.0cd	16.2 ± 3.6jkl	12.7 ± 3.0d	07.9 ± 7.3d
Leaves pet.eth. extract, 0.125%	32.5 ± 3.2klm	01.8 ± 0.0n	01.8 ± 0.0f	01.8 ± 0.0e
Fruits pet.eth. extract, 0.125%	39.8 ± 2.2hi	29.3 ± 3.8g	20.1 ± 2.92c	01.8 ± 2.9e
Leaves pet.eth. extract, 0.25%	36.9 ± 2.0ijk	15.2 ± 2.5kl	01.8 ± 0.00f	01.8 ± 0.0e
Fruits pet.eth. extract, 0.25%	49.6 ± 4.0ef	40.6 ± 15.6de	11.6 ± 6.89d	11.6 ± 6.9c
Leaves pet.eth. extract, 0.5%	42.1 ± 3.5g	21.0 ± 3.4ij	1.8 ± 0.00f	01.8 ± 0.0e
Fruits pet.eth. extract, 0.5%	58.7 ± 2.5bc	42.1 ± 2.2d	30.0 ± 2.6b	30.0 ± 2.6b
Malathion50% EC	90.0 ± 0.0a	90.0 ± 0.0a	71.9 ± 4.4a	69.9 ± 2.9a
Abate50% EC	90.0 ± 0.0a	76.0 ± 2.8b	73.8 ± 3.6a	70.8 ± 3.5a
Control (water only)	00.0 ± 0.0q	01.8 ± 0.0n	01.8 ± 0.0f	01.8 ± 0.0e
CV%	7.6	14.9	14.8	9.9

Means followed by the same letter(s), in each column, are not significantly different at 5% level according to Duncan's Multiple Range test; Pet.eth. = petroleum ether.

In the first count (after 3days) the fruits petroleum ether extract (0.5%) manifested significantly the highest residual mortality percent (58.7±2.5%) compared with the other botanical treatments, but was relatively inferior to the two insecticides (90.0±0.0%). Nearly similar trends were observed during the subsequent residual periods. It is clear that all botanical treatments revealed progressive decreases in activities as the residual durations increased. Such diminishing residual effects were also occurred at relatively slower rates in the two insecticides. Nevertheless, after 21 days the 0.5% petroleum ether extract still showed significant effect (30.0±2.6% mortality) compared with the other botanicals and control, as the second best treatment after the two insecticides. Mullai and Jebanesan (2006) reported that, plants extracts were proved to have lower residual effects as they resulted in negligible mortality percents after 30 days. [65]

However, the superior effects (acute and residual) of fruits petroleum ether extract which was attained in the two experiments can be attributed to different factors as stated before. Moreover, it was believed that triterpenes, sterols and other apolar components in oil extract can remain longer in water than polar chemicals found in ethanol and water extracts, and hence more exposure time is secured by such extract for better performance. Since natural products are always characterized by shorter degradation time [39] than formulated synthetic chemicals, the current residual results achieved from crude petroleum ether extract of *C. colocynthis* fruits were considered promising to proceed forward in advanced research for better exploitation of this plant as potential mosquito larvicide. Again, as water is the most important habitat of life for several living organisms, it should not be treated with harmful materials such as hazardous synthetic chemicals. The use of conventional pesticides in the water sources imposes many risks to people and to the environment. [18, 19] Therefore, the current results were encouraging to go forward in evaluating phytochemical compounds as rich natural sources of potent and safe mosquitocidal alternatives.

CONCLUSION

The overall results of phytochemical screening and bioassays of *Citrullus colocynthis* extracts verified the presence of potentially active substances with mosquito larvicidal properties against the malaria vector *Anopheles arabiensis*. However, the best results of acute toxicity and residual effects shown by the fruit petroleum ether extract suggested the occurrence of certain apolar active ingredients with higher larvicidal properties among the detected chemical groups of the extracted oil portion. Such chemicals should be focused in advanced research to be fractionated and characterized based on concurrent bioassays. This might lead to practical application of some products as alternatives or supplementary measures to those costly and hazardous synthetic chemicals.

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