



RESEARCH ARTICLE

Isolation, identification and Toxicological profiling of bioactive compounds from *Xanthium strumarium* and *Acmella calva* depict the excess reactive oxygen species generation in the *Culex quinquefasciatus* mosquito vector; an insight behind the probable mode of action of bioactive compounds

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ARTICLE HISTORY

Received: 22 August 2021

Accepted: 26 December 2021

Available online

Version 1.0 (Early Access): 19 March 2022



Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, etc. See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

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Vinu R P K, Puthur R K. Isolation, identification and Toxicological profiling of bioactive compounds from *Xanthium strumarium* and *Acmella calva* depict the excess reactive oxygen species generation in the *Culex quinquefasciatus* mosquito vector; an insight behind the probable mode of action of bioactive compounds. Plant Science Today (Early Access). <https://doi.org/10.14719/pst.1443>

Abstract

The diverse field of chemistry like structural and Analytical chemistry has offered the tools that are essential for purifying the plethora of phytochemical constituents. Such an untapped pool of phytochemicals from the plant world can be used as an alternative to synthetic insecticides in mosquito vector control programme. This investigation has used the Bioassay-guided Chromatography, Fourier-transform infrared spectroscopy (FTIR), Nuclear magnetic resonance (NMR) and GC-MS (Gas chromatography-mass spectrometry) to isolate and identify the most prominent toxic phytocompounds from the medicinal plants *Xanthium strumarium* and *Acmella calva*. The Map of the study site has been prepared using the Q-GIS. SPSS was used to perform the probit regression analysis and plot preparation. The isolated compounds such as Undecane (CH₃(CH₂)₉CH₃; 156.31 g/mol) (LC₅₀: 2.599 mg/L (2.251 - 2.867); LC₉₀ : 4.563 mg/L (3.960 - 6.006) and Phthalic acid, butyl undecyl ester (C₂₃H₃₆O₄; 376.5 g/mol) (LC₅₀: 4.072 mg/L (3.680 - 4.462); LC₉₀: 6.894 mg/L (5.821-10.303) those are isolated from the *Xanthium strumarium* and *Acmella calva* could be recognized as an innovative direction for the conception of natural insecticide against the *Culex quinquefasciatus* mosquito vectors since they produced a maximum range of toxicity. Moreover, the production of excessive free radicals in the phytocompounds exposed mosquito strain illustrated the probable role of oxidative stress in larval death. This investigation recommends that the isolated compounds can be used as an eco-friendly approach for mosquito control in the future.

Keywords

Xanthium strumarium, *Acmella calva*, *Culex*, GC-MS, free radicals, compounds

Introduction

Modern chemistry has piloted a new way of an era for the investigation of herbal products of botanical origin to combat several mosquito species that are responsible for transmitting vector-borne diseases such as Chagas disease, leishmaniasis, human African trypanosomiasis (HAT), chikungunya, onchocerciasis, dengue, West Nile fever and Japanese encephalitis (1-5). Control of mosquito vectors is mainly accomplished by removing suitable artificial habitats that provide a favourable place for oviposition and which permit the further development of the various stages of the mosquito species (6). The entry of mosquito vectors into the artificial habitats can easily be prevented by frequently cleaning and emptying the containers, by killing

the adult mosquitoes using insecticides, by removing the developing stages using biological agents or insecticides, or by using the combination of aforesaid strategies (7-9). However, the use of synthetic insecticides has been limited in recent years in many provinces of the world. One of the major problems behind this is the non-biodegradable nature, concern for environmental sustainability, higher rate of biological magnification through the ecosystem, high cost of synthetic insecticides, augmenting insecticide resistance on a global scale and drastic effect on human health and non-target organisms (10-13).

The complications did not end here, the frequent use of other classes of insecticides including organophosphates, pyrethroids, permethrin, deltamethrin, lambda-cyhalothrin, bifenthrin and cypermethrin have also instigated complications allied with resistance in mosquito populations (14). Moreover, an investigation by (15) reported the adverse health effects instigated by the following synthetic elements; Fenevlerate, Decamethrin, Bonthrin, Cyclethrin, Alphamethrin, Dimethrin, Diptrex, caumphos, Phosphomidon, Dimethoate, Trichlorofan, Ptrethrin, Demetox, Oxydemeton-methyl, Malathion, Methyl Parathion, Cypermethrin, Furethrin, Allethrin, Ronnel, enitrothion, Bidrin, Tetramethrin, Phorate, Fenthion, Abate, Dichlorovas, Dimefox, Mipafox, Phosphomidon and Demetox. Therefore the scientific community has shifted the focus towards the development of alternatives in natural compound contexts. One of the most efficient alternative natural strategies is to explore the extensive floral diversity and progressively enter into the discipline where the safer insecticides are considered as the core for a sustainable method of mosquito vector control (16). Medicinal plants such as *Xanthium strumarium* and *Acmella calva* have previously been reported with insecticidal and biological activities (17). However, none have piloted a study concerning the isolation and identification of bioactive compounds with potent toxicity against the *Culex quinquefasciatus* mosquito larvae. Hence, this investigation has merged the structural chemistry towards the vector control research to reveal the phytochemical constituents of the medicinal plants such as *Xanthium strumarium* and *Acmella calva* along with their probable mode of action in the *Culex quinquefasciatus* mosquito larvae.

Materials and Methods

Study area and Collection of plant materials

The fresh and healthy leaves of medicinal plants were collected from Pulpally (11.79° N, 76.16° E) and Mananthavady (11.80° N, 76.00° E) in Wayanad (Fig. 1), a part of Western Ghats, Kerala India. The identification of the collected plants up to species level was accomplished by experts and a herbarium number was provided for each of the collected specimens. In some instances like when the study obtained a very small plant for consideration, the investigations have chosen the whole plant for extraction and further studies. The voucher specimens that are provided with the herbarium number were stored in the Communicable Disease Research Laboratory (CDRL), Depart-

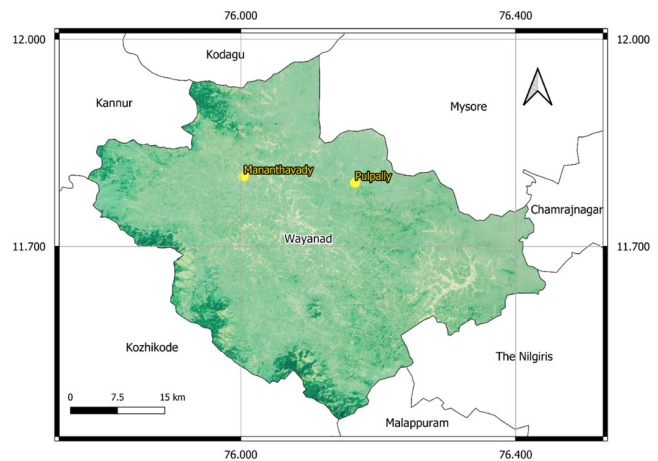


Fig. 1. Map of Wayanad

ment of Zoology, St. Joseph's College, Irinjalakuda, Thrissur, Kerala, India. The photographs of the collected plant specimens were captured using the Canon Digital Camera.

Larvae rearing

Freshly laid egg rafts of *Culex quinquefasciatus* were collected from Communicable Disease Research Laboratory, Department of Zoology, St. Joseph's College, Irinjalakuda, India. The collected egg rafts were then kept in transparent plastic trays (28 L × 39 W × 14 D cm) for 24 hrs at room temperature for incubation. The hatched larvae for provided with fish feed (Coppens®) in such a way that the feed has been sprinkled daily above the water surface at the rate of 0.32 mg/100. The following experimental conditions were maintained during the rearing: 86.00% (relative humidity), and 12:12 (light: dark hours), and 28.00 ± 2.00 °C (temperature).

Preparation of the plant materials

The medicinal plants were shade dried at the environmental temperatures ranging from 27-35 °C day time. Contamination and fungal attack towards the collected specimens were monitored and preserved during this period and if any contamination has been observed, the specific contaminated source will be eliminated. With the help of a commercial electrical stainless steel blender, the shade-dried plant specimens were ground. For the preliminary screening using the Crude extraction approach, the collected plant specimens were thoroughly washed and crushed and grounded with mortar and pestle. The crude extract of the selected plant specimens was kept for 24 hrs. The desired volume of the crude extract of all the collected plant specimens was taken for larval bioassay protocol prescribed by World Health Organization (WHO). The crude extracts that can produce an extensive range of mortality have been chosen for further analysis. Four hundred grams (400 g) of powdered plant specimens were extracted successively using the Soxhlet apparatus with petroleum ether, methanol, acetone and water as the solvents. Four hundred grams of dried powdered plant specimen which contains the phytochemical constituents to be extracted were placed in a thimble. The thimble is made up of filter paper that allows liquids and prominent phytochemical constituents to pass through it. The boiling tem-

perature for the respective solvents was manually adjusted between the ranges of 60–80 °C for eight hrs. The HPLC grade solvents were bought from Merck Co. (Germany). Once the level of the respective solvents reaches the siphon, it discharges back into the round-bottomed flask. The procedure was repeated uninterruptedly until all the phytochemical constituents from the selected plant specimen were extracted into the respective solvent. The Whatman number 1 filter paper was used to filter the output from the Soxhlet extraction.

Larval bioassay

The larval bioassays were performed using the various extracts prepared from the medicinal plants at 1000 mg/l using the method proposed by WHO (18). The experiments were conducted using the early fourth instar larvae of *Culex quinquefasciatus* mosquito vector. For each experiment, twenty-five *Culex quinquefasciatus* larvae were transferred to beakers containing 99 ml of distilled water with 1 ml of the plant extract at the desired concentration. Control groups were maintained for each of the experiments.

After continuous exposure of 24 hrs to the tested extracts, the larval mortality was recorded. The following environmental condition was maintained for all the experiments; temperature: 27 °C and 12-hr light: 12-hr dark photoperiod. The larvae were recognized dead and selected for counting if they were not reactive to gentle prodding with a needle. The larval mortality percentage was calculated using the average of replicates. The toxicity for respective isolated compounds was represented as LC50 and LC90.

Bioassay-guided Chromatographic separation

Repeated chromatographic separation was used for the isolation of bioactive compounds from *Xanthium strumarium* and *Acmella calva*. The HPLC grade acetone, hexane and methanol were used for Column (borosilicate glass) and Thin-layer chromatography (TLC) (Silica Gel60 F254 TLC, Merck, Germany). For Column chromatography, the mobile phase for respective extracts was prepared and loaded onto the column having a length of 30-cm. The Silica used for the Column chromatography was bought from Merck, Germany with a mesh size of 60-120. The speed of the solvent drips has been adjusted using the stopcock situated at the bottom of the column. Glass test tubes were kept below the column to collect the solvent containing the phytochemical constituents of the selected medicinal plants. These steps were repeated many times till enough quantity of the isolated phytochemical constituents were obtained.

FTIR, NMR and GC-MS

The pure bioactive elements have proceeded to FTIR and NMR (Bruker 200 MHz DPX). The FTIR spectrum of the isolated phytochemical constituents such as XAD4 and ACE5 isolated from *Xanthium strumarium* and *Acmella calva* was recorded by PerkinElmer Spectrum Version 10.03.09 (NIOS2 Main 00.02.0009). One ml (1 ml) of the phytochemical constituents such as XAD4, and ACE5 isolated from *Xanthium strumarium* and *Acmella calva* was injected for GC-MS analysis with the following conditions; film thickness 0.25 µm DB-5MS column, 30 m × 0.25 mm, initial temperature 50 °C

(2 min) together with the rate of 20 °C/min to 130 °C; 12 °C/min to a 180 °C; and a final temperature to 280 °C at 3 °C/min. The isolated phytochemical compounds from *Xanthium strumarium* and *Acmella calva* were compared with the retention time (RT) values of reference compounds from REPLIB and MAINLIB library for characterization.

Role of free radical generation (ROS) as probable mode of action in the *Culex quinquefasciatus* mosquito larvae

Oxidative stress has been illustrated as the disruption in the balance between the antioxidant defences and the generation of reactive oxygen species. The ability of isolated phytochemical constituents such as XAD4 and ACE5 isolated from *Xanthium strumarium* and *Acmella calva* to generate augmented production of ROS as a probable mode of action of phytochemicals was investigated using the method suggested by (19). Twenty, fourth instar larvae of *Culex quinquefasciatus* treated with different concentrations of *Xanthium strumarium* and *Acmella calva* extracts and isolated compounds were homogenized as the sample. The control group was maintained with distilled water containing the homogenized *Culex quinquefasciatus* larvae that were not treated, lacking phytochemicals. The augmented production of ROS within the *Culex quinquefasciatus* larvae was determined using Ultraviolet-visible spectroscopy.

Statistical Analysis

All data were entered in Microsoft Excel 2010 and 19 spreadsheets. The data were then exported into SPSS version 24.0.0 to find out the MEAN ± SD. The graphical representation of the data has been done by using the SPSS software and Graph pad prism. The molecule editor ChemDraw developed by David A. Evans and Stewart Rubenstein was used to draw the chemical structure of the isolated compounds. Q-GIS was used to illustrate the GIS data for the sampling site.

Results

Isolation of phytochemical constituents from the *Xanthium strumarium* and *Acmella calva*

The methanol extracts of *Xanthium strumarium* have been chosen for the Chromatographic separation and yielded the following subfractions: XA, XB, XC, XD, XE, XF, XG and XH (Acetone: Methanol in the ratio of 3:5 ml). Among them, the 'XA' has been chosen for further repeated Bioassay-guided Chromatographic separation since it shows maximum range of toxicity against the *Culex quinquefasciatus* mosquito larvae and it yielded the following subfraction fractions; XAA, XAB, XAC, XAD, XAE, XAF, XAG, XAH, XAI, XAJ and XAK (Acetone, hexane, methanol in the ratio of 3:2:6). Since the XAD has to produce a maximum range of toxicity against the *Culex quinquefasciatus* mosquito larvae, it was selected for further chromatographic analysis and produced XAD1, XAD2, XAD 3, XAD4 and XAD5 subfractions. Among them, the purified fraction 'XAD4' has been reported with high toxicity against the *Culex quinquefasciatus* mosquito larvae. The same pattern has been followed for *Acmella calva* methanol extract. The Chromatographic separation of *Acmella calva* methanol extract has yielded the following subfrac-

tions: AA, AB, AC, AD, AE, AF, AG, AH, AI, AJ, AK and AL (Acetone, Toluene, methanol, in the ratio of 2:2:4 ml). The AC sub-fraction has then proceeded for further Bioassay-guided Chromatographic separation since it produced a maximum range of toxicity against the *Culex quinquefasciatus* mosquito vector and it yielded the following subfractions; ACA, ACB, ACC, ACD, ACE, ACF, ACG and ACH (Acetone, Toluene, Methanol in the ratio of 7:4:6). Among them, the subfraction ACE has been shown to exhibit a maximum range of toxicity against the mosquito vector. Due to this reason, the subfraction ACE has been subjected for Bioassay-guided Chromatographic fractionation and it yielded the following subfraction fractions; ACE1, ACE2, ACE3, ACE4, ACE5 and ACE6 (Acetone, hexane, methanol in the ratio of 4:8:3). Among them, the ACE5 sub-fraction has been known to produce a maximum range of toxicity against the tested mosquito population. Since the purified bioactive fractions such as 'XAD4' and 'ACE5' produced maximum toxicity against the mosquito population they have proceeded for further structural characterization including the FTIR, NMR and GC-MS. The larvicidal potential of various bioactive fractions and the phytochemical constituents in the two medicinal plants such as *Xanthium strumarium* and *Acmella calva*, has been evaluated in this study against the *Culex quinquefasciatus* larvae. The ability of each of the bioactive fractions and isolated bioactive elements to induce the maximum range of toxicity towards the *Culex quinquefasciatus* larvae has been verified after 24 hrs of continuous exposure (Figs. 2-7).

The bioactive fractions that are failed to produce enough levels of toxicity have been immediately discarded

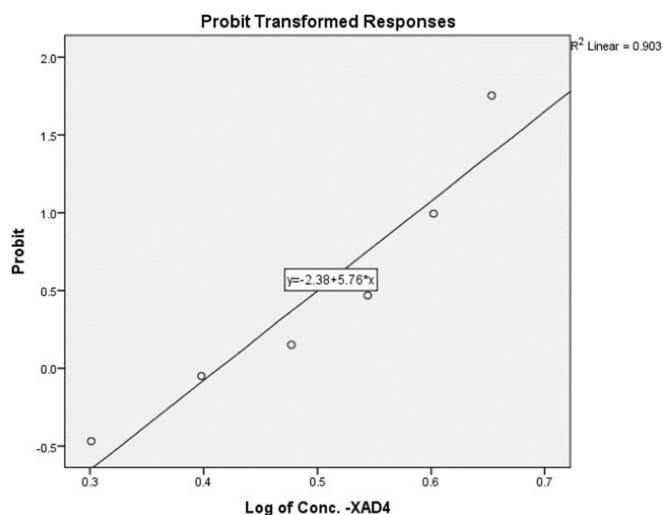


Fig. 2. Probit regression analysis of Isolated Bioactive compound from *Xanthium strumarium*.

during the investigation. The bioactive fractions such as XA, XAD and XAD4 were isolated from *Xanthium strumarium*. Likewise, the bioassay-guided chromatographic fractionation of methanol extract prepared from *Acmella calva* yielded bioactive fractions such as AC, ACE and ACE5.

Among the various tested bioactive fractions including two phytochemical compounds, the XAD4 (Undecane) (0.642g) exhibited a maximum range of larvicidal potential with an LC 50 value of 2.599 mg/L (Fiducial Limit: 2.251 -

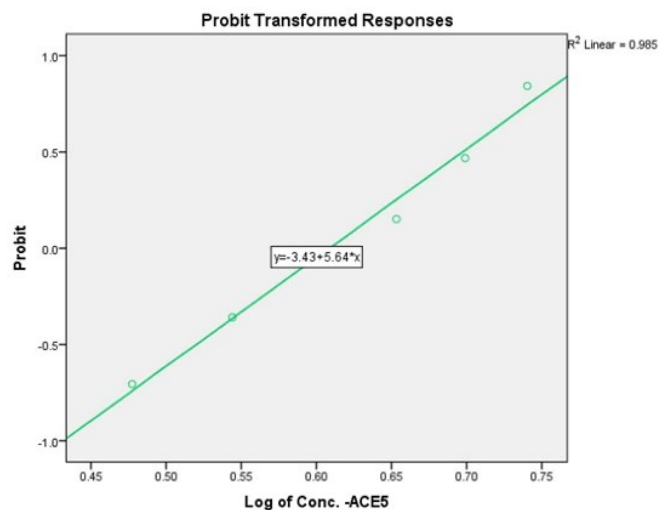


Fig. 3. Probit regression analysis of Isolated Bioactive compound from *Acmella calva*.

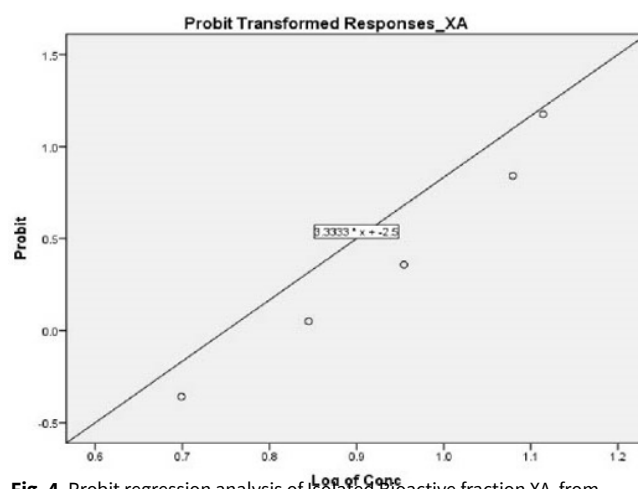


Fig. 4. Probit regression analysis of Isolated Bioactive fraction XA from *Xanthium strumarium*

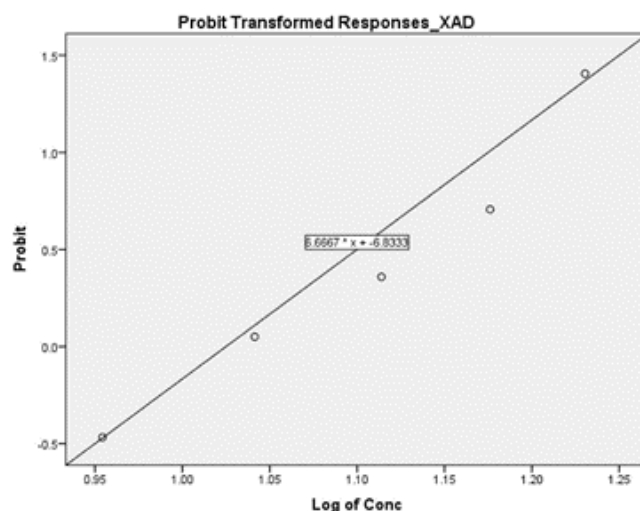


Fig. 5. Probit regression analysis of Isolated Bioactive fraction XAD from *Xanthium strumarium*

2.867) and LC 90 value of 4.563 mg/L (Fiducial Limit: 3.960 - 6.006) (Fig. 8). The active bioactive element ACE5 (Phthalic acid, butyl undecyl ester) (0.826 g), which is isolated from *Acmella calva* has verified its strong larvicidal potential against the *Culex quinquefasciatus* fourth instar larvae with an LC50 value of 4.072 mg/L (Fiducial Limit: 3.680 - 4.462) and an LC90 value of 6.894 mg/l (5.821-10.303).

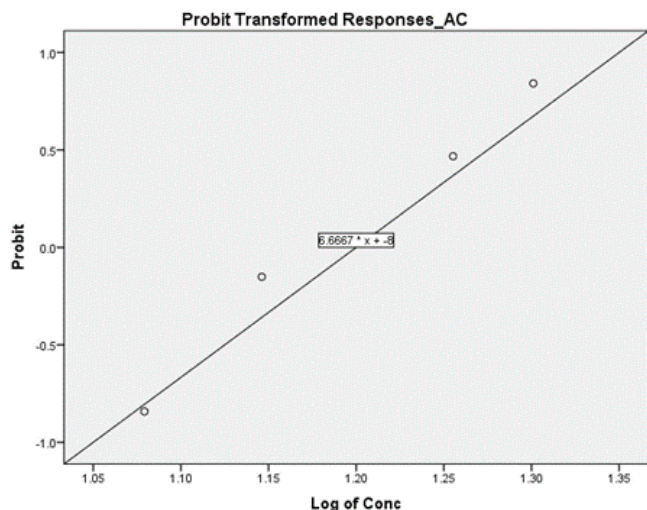


Fig. 6. Probit regression analysis of Isolated Bioactive fraction AC from *Acme-la calva*

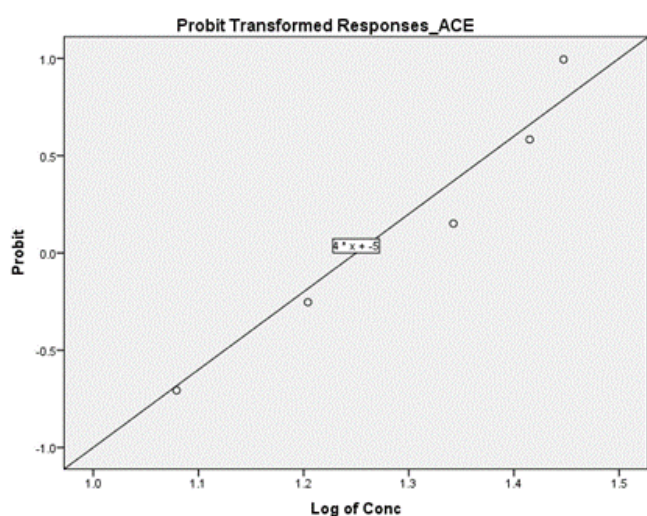


Fig. 7. Probit regression analysis of Isolated Bioactive fraction ACE from *Acme-la calva*.

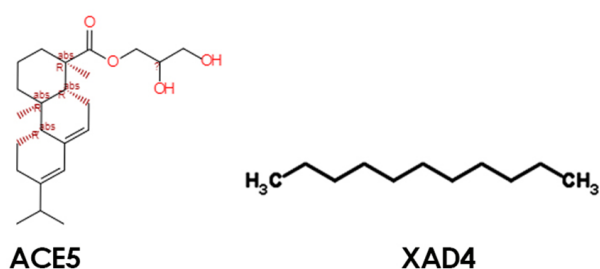


Fig. 8. Chemical structure of isolated compounds.

NMR - Undecane (XAD4)

¹H NMR

δ 0.87 (6H, t, $J = 7.0$ Hz), 1.19-1.32 (14H, 1.23 (quint, $J = 7.0$ Hz), 1.28 (h, $J = 7.0$ Hz), 1.23 (quint, $J = 7.0$ Hz), 1.23 (quint, $J = 7.0$ Hz)), 1.24 (4H, quint, $J = 7.0$ Hz) (Table 1).

NMR - Phthalic acid, butyl undecyl ester (ACE5)

¹H NMR

δ 0.84-0.92 (6H, 0.88 (t, $J = 6.5$ Hz), 0.89 (d, $J = 7.0$ Hz)), 1.17-1.50 (13H, 1.40 (dq, $J = 11.9, 2.8$ Hz), 1.32 (ddt, $J = 12.6,$

Table 1. NMR - Undecane (XAD4)

Atom IDs	delta	nb atoms	multi-plici-ty	cou-plings
11, 12, 13, 32, 33, 34	0.8650000000000001	6	t	7
22, 23	1.229	2	quint	7
20, 21, 24, 25	1.229	4	quint	7
18, 19, 26, 27	1.228	4	quint	7
16, 17, 28, 29	1.238	4	quint	7
14, 15, 30, 31	1.277	4	h	7

10.3, 2.8 Hz), 1.24 (dt, $J = 10.3, 4.8, 2.8$ Hz), 1.29 (dq, $J = 12.6, 2.8$ Hz), 1.40 (dq, $J = 12.9, 2.8$ Hz), 1.41 (dtd, $J = 12.9, 10.3, 2.8$ Hz), 1.30 (h, $J = 6.5$ Hz), 1.30 (h, $J = 6.5$ Hz), 1.25 (td, $J = 6.5, 4.8$ Hz), 1.25 (td, $J = 6.5, 4.8$ Hz), 1.38 (dq, $J = 11.0, 2.8$ Hz), 1.33 (dt, $J = 11.0, 10.3, 2.8$ Hz), 1.29 (dt, $J = 11.9, 10.3, 2.8$ Hz)), 1.57 (1H, qq, $J = 7.0, 2.8$ Hz) (Table 2).

The spectroscopic data acquired using the FTIR and NMR in this study were consistent with those found in the various databases including the NIST library, thereby confirming the identity of the isolated phytochemical constituents such as XAD4 and ACE5. As depicted in Figs. 9-10, GC-MS analysis clearly validated the presence of the phytocom-

Table 2. NMR - Phthalic acid, butyl undecyl ester (ACE5)

Atom IDs	delta	nb atoms	multi-plici-ty	cou-plings
27, 28, 29	0.875	3	t	6.499
16	1.398	1	dquint	11.928, 2.79
15	1.294	1	dt	11.928, 10.26, 2.79
14	1.331	1	dt	10.983, 10.26, 2.79
13	1.376	1	dquint	10.983, 2.79
24	1.252	1	td	6.501, 4.781
23	1.252	1	td	6.501, 4.781
26	1.296	1	h	6.499
25	1.296	1	h	6.499
20, 21, 22	0.8930000000000001	3	d	6.997
18	1.405	1	dtd	12.918, 10.26, 2.79
17	1.397	1	dq	12.918, 2.79
12	1.291	1	dq	12.639, 2.79
11	1.319	1	ddt	12.639, 10.26, 2.79
19	1.235	1	dt	10.26, 4.781, 2.79
10	1.568	1	qq	6.997, 2.79

pounds Phthalic acid, butyl undecyl ester and "Undecane" with the following details : Peak#:1, R.Time : 17.857, Area: 28431, Area%:3.68 and Peak#:1, R.Time : 5.924, Area: 10977, Area%:1.42. Based on the aforementioned results the following two compounds were isolated and identified from *Xanthi-um strumarium* and *Acme-la calva*; 1), Undecane (XAD4; $\text{CH}_3(\text{CH}_2)_9\text{CH}_3$; 156.31 g/mol) 2), and Phthalic acid, butyl undecyl ester (ACE5; $\text{C}_{23}\text{H}_{36}\text{O}_4$; 376.5 g/mol) (Figs. 9-11).

Mode of action of the isolated bioactive compounds in context with Reactive oxygen species (ROS) generation in the bioactive compounds exposed strains

The present investigation has assessed ROS's augmented

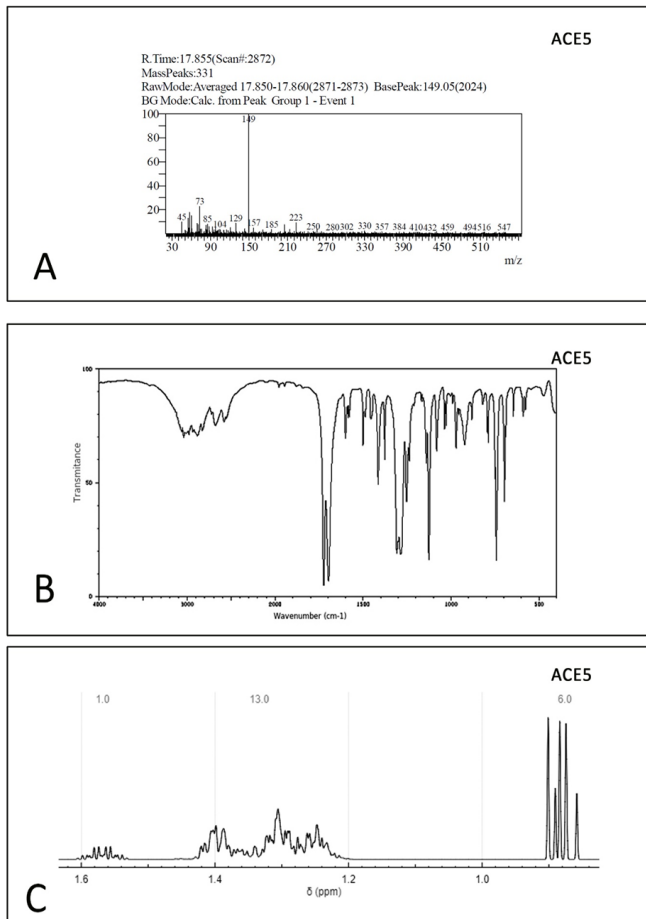


Fig. 9. GC-MS (A), FTIR (B) and NMR spectrum of isolated bioactive element from *Xanthium strumarium*

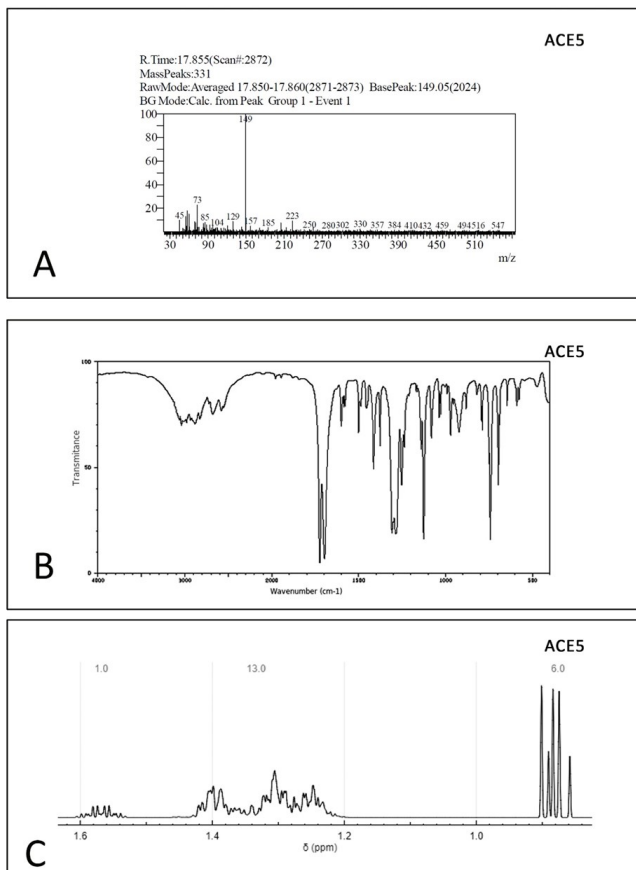
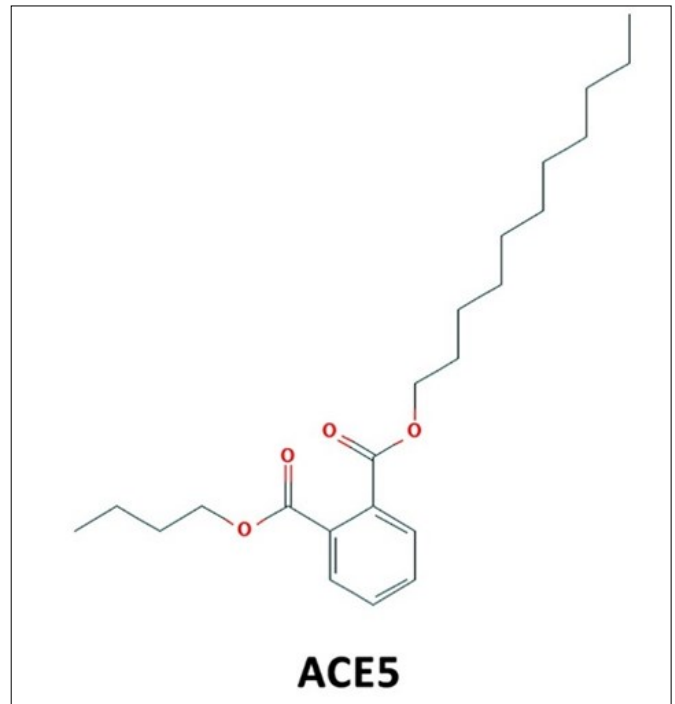
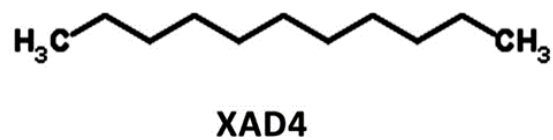


Fig. 10. GC-MS (A), FTIR (B) and NMR spectrum of isolated bioactive element from *Acmella calva*



A. Phthalic acid, butyl undecyl ester (**ACE5**) isolated from *Acmella calva*



B. Undecane (**XAD4**) (isolated from *Xanthium strumarium*)

Fig. 11. Isolated Bioactive Elements (A). Phthalic acid, butyl undecyl ester (**ACE5**) isolated from *Acmella calva* (B). Undecane (**XAD4**) (isolated from *Xanthium strumarium*).

production in response to the continuous exposure of phytochemical constituents of 2 medicinal plants such as *Acmella calva* and *Xanthium strumarium* towards *Culex quinquefasciatus* fourth instar larvae. The augmented production of ROS determined in this investigation has been considered as a probable mode of action of the phytochemical constituents (**XAD4**, **ACE5** and **JAB2**) that may be recognized as the major reason for oxidative stress that happened in *Culex quinquefasciatus* fourth instar larvae.

Discussion

The findings of this investigation have clearly illustrated the fact that the two compounds such as Undecane (**XAD4**; $\text{CH}_3(\text{CH}_2)_9\text{CH}_3$; 156.31 g/mol) and Phthalic acid, butyl undecyl ester (**ACE5**; $\text{C}_{23}\text{H}_{36}\text{O}_4$; 376.5 g/mol) those are isolated from the *Xanthium strumarium* and *Acmella calva* could be recognized as an innovative direction for the conception of natural insecticide against the *Culex quinquefasciatus* mosquito vectors (Table 3 and 4). It also directed the way of mosquito vector control research towards the development of alternative strategies against synthetic insecticides as reported in the previous investigations (20). The ability of various extracts prepared from the *Xanthium strumarium* to produce toxicity against the *Aedes aegypti* mosquitoes that has been previously reported. However, none have isolated

Table 3. Larvicidal efficacy of isolated compounds and fractions from *Xanthium strumarium* and *Acmella calva*

Sl. No.	Plant	Isolated compound	LC 50 (Fiducial limit) mg/L	LC 90 (Fiducial limit) mg/L	df	Chi square
1	<i>Xanthium strumarium</i>	Undecane (XAD4)	2.599 (2.251 - 2.867)	4.563 (3.960 - 6.006)	4	2.585
2	<i>Acmella calva</i>	Phthalic acid, butyl undecyl ester (ACE5)	4.072 (3.680 - 4.462)	6.894 (5.821-10.303)	4	0.316

Table 4. Larvicidal efficacy of isolated bioactive fractions from *Xanthium strumarium* and *Acmella calva*

Sl. No.	Plant	Isolated subfractions	LC 50 (Fiducial limit) mg/L	LC 90 (Fiducial limit) mg/L	df	Chi square
1	<i>Xanthium strumarium</i>	XAD	6.626 (5.005-7.784)	15.582 (12.272-27.632)	3	0.512
		XA	10.924 (9.467-11.974)	17.794 (15.549-23.929)	3	0.832
		ACE	15.366 (14.053-16.624)	23.221 (20.311-31.801)	3	0.476
2	<i>Acmella calva</i>	AC	18.362 (15.614-20.928)	37.142 (29.926-60.261)	3	0.765

the above-said compounds from the medicinal plant *Xanthium strumarium* with special inference on the inhibition potential towards the *Culex quinquefasciatus* mosquito larvae along with the probable mode of action of the same compound in the mosquito vector.

To date, vaccinations and specific medications are not existing commercially for treating a number of mosquito-borne diseases and this has indicated the truth that one of the major ways to combat such diseases would be vector control targeting at the larval stage (6, 19). Hence the present investigation has verified the ability of Undecane (XAD4; $\text{CH}_3(\text{CH}_2)_9\text{CH}_3$; 156.31 g/mol) and Phthalic acid, butyl undecyl ester (ACE5; $\text{C}_{23}\text{H}_{36}\text{O}_4$; 376.5 g/mol). The toxic potential of undecane against *Aedes aegypti* (L.) (22) has been previously reported in several studies (7, 21). Likewise, the bioactivity of the compound Phthalic acid, butyl undecyl ester (ACE5; $\text{C}_{23}\text{H}_{36}\text{O}_4$; 376.5 g/mol) has been previously investigated by several authors (22-24). The larvicidal potential of the following compounds were previously investigated by (25); Mahanimbine (LC50: 0.68 $\mu\text{g}/\text{m}$), Girinimbine (LC50: 0.79 $\mu\text{g}/\text{ml}$), Murrayacine (LC50: 1.51 $\mu\text{g}/\text{ml}$), Murrayanine (LC50: 1.14 $\mu\text{g}/\text{ml}$) and Murrayafoline (LC50: 0.43 $\mu\text{g}/\text{ml}$). Another investigation by revealed that the compound pectolinarigenin exhibited potent toxicity against *Aedes aegypti* (LC50: 0.79 ppm and LC90:5.31 ppm) and *Culex quinquefasciatus* (LC50: 0.62 ppm and LC50: 2.87) mosquito vectors. However, none have reported the isolation of Undecane and Phthalic acid, butyl undecyl ester from *Xanthium strumarium* and *Acmella calva* respectively with special inference on the toxic potential towards the *Culex quinquefasciatus* mosquito larvae along with the mode of the aforesaid compounds in the target insect, which justifies the uniqueness of this investigation. Studies based on the natural compounds to combat the mosquito vectors are recognized as important and promising than chemical strategies (27).

In agreement with our findings, investigations reported the probable mode of action of isolated bioactive compounds against the *Aedes aegypti* with special inference on the excessive production of reactive oxygen species. They also validated the fact that the excessive produc-

tion of reactive oxygen species may results in DNA damage thereby disturbing the normal biological activities leads to oxidative stress. From the results obtained and the previous investigations, it was clearly evident that one of the probable reasons behind the death of the Undecane and Phthalic acid, butyl undecyl ester exposed strains of *Culex quinquefasciatus* is the development of oxidative stress caused by the excessive production of reactive oxygen species in the mosquito larvae (Table 5 and 6).

Table 5. Determination of free radicals from *Xanthium strumarium* exposed *Culex quinquefasciatus*

Sample	Extract Dose	Absorbance
<i>Culex quinquefasciatus</i> Larvae + Distilled water ($\mu\text{g}/\text{ml}$)	50	0.000 \pm 0.000
	100	0.001 \pm 0.002
	150	0.002 \pm 0.002
	200	0.003 \pm 0.002
<i>Culex quinquefasciatus</i> Larvae + <i>Xanthium strumarium</i> Acetone extract ($\mu\text{g}/\text{ml}$)	50	0.014 \pm 0.004
	100	0.021 \pm 0.004
	150	0.034 \pm 0.004
	200	0.046 \pm 0.004
<i>Culex quinquefasciatus</i> Larvae + <i>Xanthium strumarium</i> Water extract ($\mu\text{g}/\text{ml}$)	50	0.009 \pm 0.004
	100	0.016 \pm 0.004
	150	0.022 \pm 0.002
	200	0.029 \pm 0.004
<i>Culex quinquefasciatus</i> Larvae + <i>Xanthium strumarium</i> Methanol extract ($\mu\text{g}/\text{ml}$)	50	0.010 \pm 0.002
	100	0.017 \pm 0.004
	150	0.024 \pm 0.004
	200	0.034 \pm 0.004
<i>Culex quinquefasciatus</i> Larvae + <i>Xanthium strumarium</i> Petroleum ether extract ($\mu\text{g}/\text{ml}$)	50	0.007 \pm 0.004
	100	0.014 \pm 0.002
	150	0.019 \pm 0.002
	200	0.025 \pm 0.002
<i>Culex quinquefasciatus</i> Larvae + Isolated bioactive element Undecane (XAD4) ($\mu\text{g}/\text{ml}$)	50	0.017 \pm 0.006
	100	0.025 \pm 0.002
	150	0.039 \pm 0.004
	200	0.054 \pm 0.004

Conclusion

The findings of this investigation clearly demonstrated that the isolated bioactive compounds such as Undecane (XAD4; $\text{CH}_3(\text{CH}_2)_9\text{CH}_3$; 156.31 g/mol) and Phthalic acid, butyl undecyl ester produced an extensive range of toxicity in the *Culex quinquefasciatus* mosquito larvae. In addition, this

Table 6. Determination of free radicals from *Acmella calva* exposed *Culex*

Sample	Extract Dose (µg/ml)	Absorbance Mean ± SD
<i>Culex quinquefasciatus</i> Larvae + Distilled water (µg/ml)	50	0.000 ± 0.000
	100	0.001 ± 0.002
	150	0.002 ± 0.002
	200	0.003 ± 0.002
<i>Culex quinquefasciatus</i> Larvae + <i>Acmella calva</i> Acetone extract (µg/ml)	50	0.011 ± 0.004
	100	0.017 ± 0.004
	150	0.023 ± 0.004
	200	0.031 ± 0.004
<i>Culex quinquefasciatus</i> Larvae + <i>Acmella calva</i> Water extract (µg/ml)	50	0.012 ± 0.004
	100	0.018 ± 0.004
	150	0.026 ± 0.002
	200	0.032 ± 0.004
<i>Culex quinquefasciatus</i> Larvae + <i>Acmella calva</i> Methanol extract (µg/ml)	50	0.013 ± 0.008
	100	0.020 ± 0.002
	150	0.029 ± 0.006
	200	0.037 ± 0.004
<i>Culex quinquefasciatus</i> Larvae + <i>Acmella calva</i> Petroleum ether extract (µg/ml)	50	0.008 ± 0.004
	100	0.013 ± 0.002
	150	0.019 ± 0.002
	200	0.025 ± 0.002
<i>Culex quinquefasciatus</i> Larvae + Isolated bioactive element Phthalic acid, butyl undecyl ester (ACE5) (µg/ml)	50	0.015 ± 0.006
	100	0.024 ± 0.002
	150	0.037 ± 0.004
	200	0.043 ± 0.004

study has unveiled the probable mode of action of the aforesaid compounds in the target insect with special inference on the excessive production of reactive oxygen species. Finally, this investigation suggests that future studies can be performed on the field applications of isolated compounds to test for its long term potency on other non-target organisms.

Acknowledgements

The authors would like to thank Principal, St. Joseph's College, Irinjalakuda for the laboratory facilities provided.

Authors contributions

The authors confirm contribution to the paper as follows: Conception or design of the work: RKP; Experiment, Data analysis and interpretation. Article writing: VPRK. All authors reviewed the results and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical issues: None.

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