UTTAR PRADESH JOURNAL OF ZOOLOGY

43(24): 101-109, 2022
ISSN: 0256-971X (P)

PHYTO-CONSTITUENTS OF CROTON BONPLANDIANUS AS ECO-FRIENDLY BIO-WEAPON AGAINST HUMAN VECTOR MOSQUITOES

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2022/v43i243300

Editor(s):
(1) Dr. Telat Yanik, Atatürk University, Turkey.
Reviewers:
(1) Raja Chakraverty, India.
(2) Erisnaldo Francisco Reis, Brazil.
(3) Iyevhobu Kenneth Oshiokhayamhe, National Open University of Nigeria, Nigeria.

Received: 15 October 2022
Accepted: 24 December 2022
Published: 24 December 2022

Original Research Article

ABSTRACT

The *Croton bonplandianus* various leaf extracts were assessed the presence of phytochemical in which predominant numbers occupied by high polarity solvent (methanol extract). '+' denoted as presence of phytochemical group and '-' denoted as absence of phytochemical group. The major phyto-compounds of Dihydro-pseudosolasodine and Methylsulfonic acid, 2,2,2-trichloroethyl ester were identified from *C. bonplandianus* leaf methanolic extract by using GC-MS analysis. The major phyto-constituents were tested by standard protocol with various concentrations (4-250µg/mL) against 3rd instars larvae of different vector mosquitoes *Ae. aegypti* and *Cx. quinquefasciatus* By GC-MS analysis, confirmed the presence of 15 phyto-compounds in which, Dihydro-pseudosolasodine (15.03%) and Methylsulfonic acid, 2,2,2-trichloroethyl ester (17.72%) were noticed as major phyto-constituent. The lethal toxicity (LC_{50}/LC_{90}) of *C. bonplandianus* leaf methanol extract and Dihydro-pseudosolasodine and Methylsulfonic acid, 2,2,2-trichloroethyl ester tested against 3rd instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* values were 78.48/178.68, 80.33/180.32, 11.46/19.90, 11.72/19.66, 10.66/19.06 and 10.71/19.78 µg/mL, respectively. *C. bonplandianus* leaf methanol extract and selected phyto-compounds were exposed with juvenile stage of medical vector which found the hyper toxicity at lower concentration. Our results, the *C. bonplandianus* phyto-pesticides achieved many folds topper toxic effects on medical vectors which provided eco-friendly approaches to environment.

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Keywords: *Croton bonplandianus*; phyto-constituents; GC-MS analysis; blood sucking vector.

1. INTRODUCTION

Globally, the mosquito-borne diseases (MBDs) are greatest nuisance on many bloods yielding fauna including human and cattle as the results countless mortalities are raised in annually [1]. Primarily, diseases are emerged by pathogenic microbes/ parasites which are mostly transmitted to public with adjacent fauna through by infected vector bites [2,3,4]. Worldwide, MBDs estimated around 20% getting more troubles to publics especially living in tropical and sub-tropical terrains [5,6]. Urban/ semi-urban terrains peoples are highly risked by MBDs because these habitats undoubtedly well favorable situations for tremendous proliferations and very easy to detect the host (human and cattle) for infecting enormously. On top of that, the death rates were comparatively topper in more economically poor people [7].

The mosquitoes (Diptera: Culicidae) are very notorious ecto-parasitic dipterans vectors and they predominantly linked with human and other blood yielding higher vertebrates [8]. They play a main task for effectively transmitting plenty of unwanted pathogens to host fauna including human [9]. *Aedes* species successfully breed in many waters hold natural and artificial containers [10]. The female *Aedes* mosquito adopting in both diurnal and nocturnal habit and it more abundance at crepuscular peak which highly surviving with anthropophilic terrains (Alarcón-Elbal et al., 2021). *Culex* species is a prime vector of Japanese encephalitis (JE) in across of Asian terrains [11]. It can survive, breed and proliferate successfully in instant/ semi-permanent pools, rice fields and other small sewage grounds are more supported in primary life aquatic stage of eggs, larvae and puape [12]. It is a more opportunistic feeder and it can choose the feed whether human/ cattle apart from that its biting strategies are different usually biting in early evening and around midnight [13].

The past few decades' vector mosquitoes and its diseases eradication/ control are very big dispute because of they develop resistance against almost all kinds of synthetic chemical mosquitocides as the results many scientific communities, doctors and researchers are continuously searching the newer path for ending these problems [14,15,16]. Moreover, the usage of unauthorized SCMs initiates many troubles to publics, cattle, and non-target organisms. By the continuous applications of synthetic chemical provides unpredictable multidirectional defects on ecosystem: Food chain/ food web carrying greater levels of poisons which successfully settle in to all living things and its organs; many helpful/ beneficial microbes, decomposer, pollinators, natural predators etc., are considerably extinctions; water, soil, and environment are loss their viability and greater contamination [17,18,19]. But it can be totally rectified by using of phyto-product (phyto-pesticides) which are purely originated from floral parts and they are well efficient tool for dimming the vector population in various lifespan [20]. *Croton bonplandianus* is a green herb, it can grow up to 30cm, branches are procumbent and it has whitish small flower on tip of every branches. It could be highly abundance in many parts of tropical and subtropical terrains of globe. The whole floral parts (leaves, stem, aerial part, flower and root) have been used as a medicinal prepossess for curing different pathogenic diseases [21]. Since, it is an impregnation of foremost information towards the vectors eradication/ control tactics at juvenile aquatic stages and it is an eco-friendly bio-weapon by using *C. bonplandianus* leaf methanol extract and major phyto-compounds on selected target pests.

2. MATERIALS AND METHODS

2.1 *Croton bonplandianus* Floral Collection and Processing

The floral green leaves were collected from Alaveli Village, Mayiladuthurai District, Tamilnadu, India. Diseases free, well matured and cleaned leaves only selected for collection after that the collected leaves were primarily dried under shade in air circulated room which allowed more than 10-15days and maintained 28–34°C daytime. A compete dried leaves were used for powdering with the help of electric blender. In total we gained 500 grams powder which extracted through Soxhlet apparatus using various extracts (Hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts) and run 5-6 hours heating range between 40–55°C. Finally, collected extract was condensed through rotary vacuum evaporator and obtained 10grams (Fig. 1) were stored in deep refrigerator below 1- 4°C [22].

2.2 Vector Mosquitoes Culture Establishment

Ae. aegypti and *Cx. quinquefasciatus* vector mosquitoes premature aquatic life stages (eggs, larvae and puape) were collected from agricultural and stagnant water areas of Mayiladuthurai District, Tamilnadu, India and vector species identified by ICMR-Centre for Research in Medical Entomology, Madurai, Tamil Nadu, India. The colony were carefully reared in laboratory and each species was reared separately which protected with muslin cloth. The eggs, larvae and pupae were kept in well visible glass container as well as allowed required feed (balanced diet) were allowed to larvae and adults as per the method of Krishnappa et al., [6]. The pre mature experimentally selected hale and healthy larvae were kept in glass container and maintained at 28±4°C, relative humidity 70–80% and photophase 12:12 light and dark. Well ground pet biscuit, yeast powder, *Apis florea* real honey and multivitamin (3:1:1:1 ratio) was used as larval diet.

2.3 Larval Vector Toxicity

The larval toxicity test was evaluated by standard protocol [23], the required concentration of *C. bonplandianus* leaf methanol extract and major phyto-compounds of Dihydro-pseudosolasodine and Methylsulfonic acid, 2,2,2-trichloroethyl ester were used to test the larval (3^{rd} Instar larvae, 0-10 hours age-old and hale and healthy) toxicity against selected mosquitoes. Few negligible amendments have done in previous works, twenty-five (25 Nos.) 3^{rd} Instar larvae were transferred in to a small 500 ml capacity glass and crystal-clear beaker composites (450 ml of chlorine free tap water added with1 ml of DMSO and required dosage of proposed floral compositions. The control is strictly followed by without phyto-products and toxicity evaluation was started from lowest concentration to maximum range (4µg/mL to 250µg/mL). The bioassay was maintained in appropriate and optimal physio-chemical parameter as well as the larval mortalities were proved by visualization. Larval deaths were regularly monitored every slot (6 hours interval) and after end of the day (sharply 24 hours of exposure periods) the percentage of death rate were calculated [24] by replication of five times and lethal concentration LC_{50} /LC_{90} calculated by using probit analysis method [25].

2.4 GC–MS Spectral Analysis

The flora of *C. bonplandianus* leaf methanol extract was analyzed by using Gas Chromatography-Mass Spectroscopy (GC-MS) analysis and it was more helpful for finding the different/ Individual phyto-constituents (PCs) were clearly identified and it was comparatively assessed through NIST library then other values (Peak, Retention Time, Area %, Compound Name etc.,) were authentically proved with isolated reference compounds which compared with already isolated compounds for identification [6].

2.5 Statistical Analysis

The toxicity of vector mosquitoes larvae data were statistically evaluated into Mean, Standard Deviation, LC_{50}/LC_{90}, Regression, Chi-square, etc., The various data were calculated by using probit analysis with the help of IBM-SPSS 26.0 version.

3. RESULTS

3.1 Phytochemical Screening and Spectral Analysis

The *C. bonplandianus* various extracts were started from low polarity to high polarity, they were examined for availability of various phytochemicals and preliminary assessment of phytochemical screening apparently showed in Table 1. The maximum quantities of phytochemicals were shown in higher polarity *C. bonplandianus* leaf methanol extract. '+' represented as occurrence of phytochemical and '-' represented as absence of phytochemical. The medicinal flora of *C. bonplandianus* leaf methanol extract was assessed by GC-MS and availability of phyto-constituents were evidently confirmed by appearing peaks with retention time (RT) then it was comparing with NIST library. By the GC-MS analysis showed 15 phytoconstituents with their other related values were apparently shown in Table 2 and Figs. 2-3. In which, in which, Dihydro-pseudosolasodine (15.03%) and Methylsulfonic acid, 2,2,2-trichloroethyl ester (17.72%) were noticed as major phyto-constituent.

3.2 Vector Larval toxicity

The medical pets: *Ae. aegypti* and *Cx. quinquefasciatus* larval toxic effects and it's the values of phyto-pesticidal agents results were well clearly shown in the Table 3. The phyto-products were compared Dihydro-pseudosolasodine and Methylsulfonic acid, 2,2,2-trichloroethyl ester were provided significant larval toxicity was observed than *C. bonplandianus* leaf methanol extract. The lethal toxicity (LC_{50}/LC_{90}) of *C. bonplandianus* leaf methanol extract and Dihydro-pseudosolasodine and Methylsulfonic acid, 2,2,2-trichloroethyl ester tested against 3^{rd} instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* values were 78.48/178.68, 80.33/180.32, 11.46/19.90, 11.72/19.66, 10.66/19.06 and 10.71/19.78 µg/mL, respectively. The chi-square, regression and other statistical values are denoted in Table 3 whereas all the values were statistically significant.

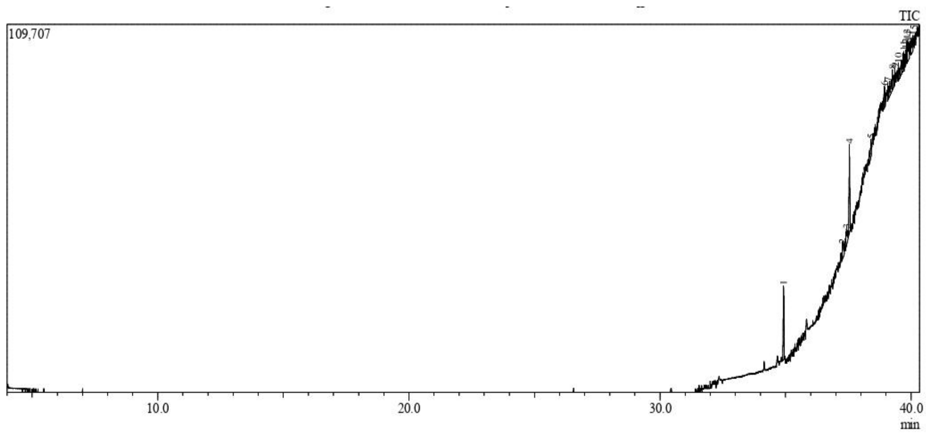

Fig. 1. GC-MS chromatogram of *C. bonplandianus* leaf methanol extract

Table 1. The various qualitative phyto-chemicals detected from leaf extracts of *Croton bonplandianus*

Sl. No.	Phytochemical screening	*C. bonplandianus* different leaf extracts				
		Hex-e	Dee-e	Dcm-e	Eta-e	Met-e
1.	Alkaloids	+	+	–	+	+
2.	Anthraquinones	–	+	+	+	+
3.	Carbohydrates	–	+	+	+	–
4.	Coumarins	+	–	–	–	+
5.	Flavonoids	–	–	+	–	+
6.	Glycosides	–	–	–	+	+
7.	Protein	+	+	+	–	–
8.	Phenolics	–	–	–	+	+
9.	Resins	+	+	–	+	–
10.	Saponins	–	–	+	–	+
11.	Steroids	–	–	–	+	+
12.	Tannins	+	+	+	+	+
13.	Triterpenes	–	–	–	+	+

Hex-e: Hexane extract; Dee-e: Diethyl ether extract; Dcm-e: Dichloromethane extract; Eta-e: Ethyl acetate extract; Met-e: Methanol extract
+: phytochemical groups zero
–: phytochemical groups abundance

Table 2. GC-MS analysis of *C. bonplandianus* leaf methanol extract

PE	RT	ST	ET	AR	AR %	HE	HE %	CN
1	34.919	34.88	34.96	6261	1.99	5418	4.55	1,2-BENZENEDICARBOXYLIC ACID, DIOCTYL ESTER
2	37.26	37.245	37.335	19321	6.14	4650	3.9	MORPHINAN-17-CARBOXYLIC ACID, 6,7,8,14-TETRADEHYDRO-4,5-EPOXY-3,6-DIMETHOXY-, METHYL ESTER, (5.ALPHA.)-(.+-.)-
3	37.425	37.375	37.5	21621	6.87	4907	4.12	3,4-Dihydroxymandelic acid, 4TMS derivative
4	37.541	37.5	37.58	5469	1.74	4802	4.03	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
5	38.397	38.325	38.415	16811	5.34	5398	4.53	1,1-DIMETHOXY OCTADECANE

PE	RT	ST	ET	AR	AR %	HE	HE %	CN
6	38.934	38.91	38.97	18109	5.75	6453	5.42	Heptasiloxane, hexadecamethyl-
7	39.065	39.05	39.09	47304	15.03	22042	18.5	Dihydro-pseudosolasodine
8	39.253	39.09	39.26	29233	9.29	6172	5.18	2,5-FURANDIONE, DIHYDRO-
9	39.344	39.26	39.355	15500	4.93	5063	4.25	Adipic acid, cycloheptyl tetradecyl ester
10	39.486	39.475	39.505	5939	1.89	5121	4.3	Adipic acid, 2,4-dimethylpent-3-yl tetradecyl ester
11	39.7	39.505	39.715	31358	9.96	5464	4.59	SILIKONFETT
12	39.78	39.755	39.79	55768	17.72	26516	22.25	Methylsulfonic acid, 2,2,2-trichloroethyl ester
13	39.831	39.825	39.835	3462	1.1	6928	5.81	4-NONYN-1-OL
14	39.893	39.835	39.93	22414	7.12	5384	4.52	Methanesulfonylacetonitrile
15	40.053	39.985	40.07	16148	5.13	4850	4.07	2-PROPANOL, 1,1,1-TRIBROMO-3-CHLORO-

PE: Peak, RT: Retention time, ST: Start time, ET: End Time, AR: Area, AR%: Area %, HE: Height, HE%: Height % and CN: Compound Name

Table 3. LC values of *C. bonplandianus* leaf methanol extract and its derived major bio-active compounds against larvae of medical pests

Species tested	LC_{50} (μg/ml)	95% FL (μg/ml)		LC_{90} (μg/ml)	95% FL (μg/ml)		Regression	χ^2 value
		LCL	UCL		LCL	UCL		
***C. bonplandianus* leaf methanol extract**								
Ae. aegypti	78.48	65.76	89.25	178.68	159.44	198.71	y=1.14+0.02x	3.516
Cx. quinquefasciatus	80.33	67.90	91.83	180.32	165.80	202.43	y=1.22+0.02x	3.645
Dihydro-pseudosolasodine								
Ae. aegypti	11.46	9.56	11.82	19.90	17.48	21.60	y=1.32+0.17x	4.721
Cx. quinquefasciatus	11.72	9.73	11.78	19.66	17.25	21.79	y=1.40+0.18x	4.478
Methylsulfonic acid, 2,2,2-trichloroethyl ester								
Ae. aegypti	10.66	9.47	11.60	19.06	18.53	22.49	y=1.43+0.21x	4.722
Cx. quinquefasciatus	10.71	9.92	11.93	19.78	18.72	22.21	y=1.44+0.43x	4.911

LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit

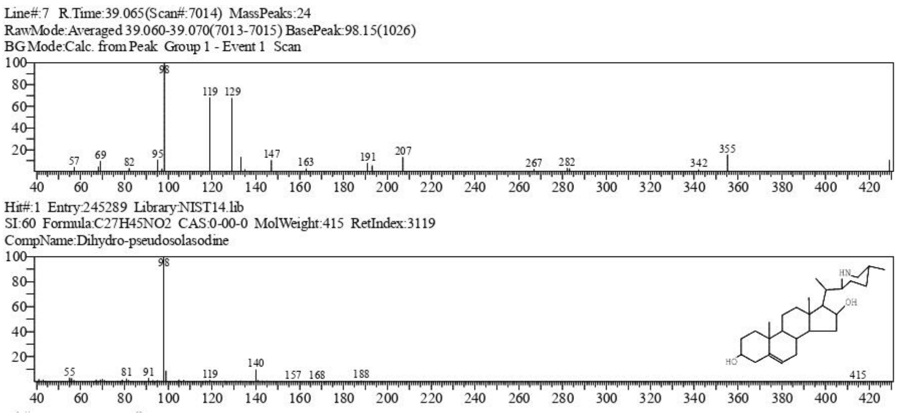

Fig. 2. Mass spectrum and structure of Dihydro-pseudosolasodine isolated from *C. bonplandianus* leaf methanol extract

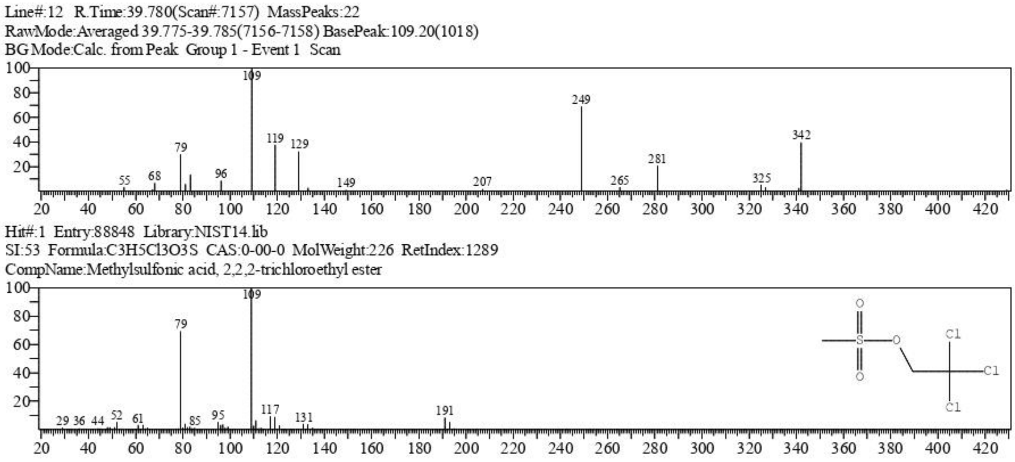

Fig. 3. Mass spectrum and structure of Methylsulfonic acid, 2,2,2-trichloroethyl ester isolated from *C. bonplandianus* leaf methanol extract

4. DISCUSSION

4.1 Phytochemical Screening and Spectral Analysis

The different *C. bonplandianus* leaf extracts (Hexane, diethyl ether, dichloromethane, ethyl acetate and methanol) were assessed for detecting the abundance of qualitative phyto-chemical and our results were compared with variety of different leaf extracts but the higher numbers of qualitative phyto-chemical were noticed in high polarity solvent extract. Earlier, many research works found on different floral origin and they were potential vector controlling tool on egg, juvenile and adult stages of life [17,18,26,5]. The naturally available phyto-constituents were an excellent eco-friendly mosquitocides by the same way *C. bonplandianus* leaf methanol extract under subjected into GC-MS spectral analysis, a total of 15 phyto-compounds were isolated, among these, Dihydro-pseudosolasodine (15.03%) and Methylsulfonic acid, 2,2,2-trichloroethyl ester (17.72%) were found maximum percentage which tested on 3rd instars juvenile stage of vector mosquitoes. The selected phyto-products showed outstanding output toward the control of vector mosquitoes. Previously, similar type of observations were noticed by using *C. limetta Cl*-LME under visualized into GC-MS analysis as results *C. limetta* major phyto-compound: Corynan-17-0l,18,19-didehydro-10-methoxy-,acelate (ester) identified and it was applied with against 3rd instars larvae *Ae. albopictus, An. maculatus* and *Cx mimulus. C. limetta* major phyto-compound at lower concentration itself showed topper mortality apart from that the same *C. limetta* major phyto-compound showed considerably countable/ lesser mortality were showed on non-target fauna [27]. *J. repens* leaf ethanol extract assessed by GC-MS analysis found *J. repens limetta* major phyto-compound: 4-Piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl]-1H-indol-2-yl]-á-methyl-, methyl ester the both natural compositions were showed predominant aquatic juvenile (larvae) toxicity on medical vectors: *Ae. albopictus, An. stephensi* and *Cx. quinquefasciatus* [6]. GC-MS spectral analysis is a basic/ fundamental assessment for finding the naturally available functional groups from various floral communities [28-31].

4.2 Larval Toxicity on Target Pests

The lethal concentration (LC_{50}/LC_{90}) of *C. bonplandianus* leaf methanol extract and Dihydro-pseudosolasodine and Methylsulfonic acid, 2,2,2-trichloroethyl ester were exposed with juvenile stages of medical vectors which found topper toxicity at lower concentration itself. The current investigation outputs are compared with previously published similar reports, the various medicinal floral extracts and its major phyto-constituents showed a prime toxic effect on aquatic juvenile (various larval stage) of vectors: Corynan-17-0l,18,19-didehydro-10-methoxy-, acelate (ester) (*C. limetta*); 4-Piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl]-1H-indol-2-yl]-á-methyl-, methyl ester (*J. repens*) [27,6]. Previously, several reported outcomes extremely supported with present research, the mosquito larval toxicity potential of various phyto-compositions against different mosquitoes [17,18,15,32-38].

5. CONCLUSION

The *C. bonplandianus* leaf methanol extract and Dihydro-pseudosolasodine and Methylsulfonic acid, 2,2,2-trichloroethyl ester persuaded high level larval toxicities were noted in vector mosquitoes. The selected medical vectors are very serious and urgently eradicated from their living habitats because which are very critically life-threatening vector pathogen to human and other fauna then the naturally obtainable phyto- products are well effective potential tool for thoroughly eradicating vectors mosquitoes.

ACKNOWLEDGEMENT

The authors are grateful to the Professor and Head, Departmental of Zoology, Annamalai University and University Authorities, Annamalai University, Tamilnadu, India, for providing various facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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