Int J Pharm Bio Sci 2015 July; 6(3): (B) 624 - 636

Research Article **Allied science**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EVALUATION OF MOSQUITOCIDAL POTENTIAL OF *DRYPETES ROXBURGHII* (WALL.)GREEN MATURE FRUITS AGAINST IMMATURES OF *CULEX QUINQUEFASCIATUS* SAY AND *ANOPHELES STEPHENSI* LISTON

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ABSTRACT

Mosquito larvicidal potential of mature fruits of *Drypetes roxburghii* was investigated against mosquito *Culex quinquefasciatus* Say and *Anopheles stephensi* Liston. In the larvicidal bioassay with crude extract against all four instars 3rd instar larvae of both species was noticed to be more susceptible to death in terms of LC_{50} and LC_{90} values. Among the solvent extracts of fruits prepared using six solvents [viz. petroleum ether, benzene, ethyl acetate, chloroform: methanol (1:1 v/v), acetone and absolute alcohol] ethyl acetate extract was most potent solvent extract against 3rd instar of both mosquito species with LC_{50} and LC_{90} values 77.03ppm, 205.64 ppm for *Cx. quinquefasciatus* and 49.535ppm, 108.88ppm for *An. stephensi* respectively. Qualitative analysis traced the presence of some phytochemicals. Upon test on non-target organisms like *Diplonychus annulatum*, *Poecilia reticulata*, larvae of *Toxorhynchites splendens* both crude and ethyl acetate extract proved relatively nontoxic.

KEYWORDS: ***Culex quinquefasciatus*, *Anopheles stephensi*, Larvicidal, Solvent extracts, Phytochemicals, Non- target.**

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1. INTRODUCTION

Different strategies as prevention measures of mosquitoes have been devised globally to reduce the prevalence of mosquito borne diseases. An effective vector control program on adult stage faces several challenges that include development of resistance against insecticides, finding of mosquitoes resting indoors before or after feeding etc[1]. Control measures are those directed against the larval stages of mosquito vectors are substantially useful for breeding sites of mosquitoes are generally accessible and relatively limited in number and size, so immature stages of mosquitoes, including the larval stages remain confined within relatively small aquatic habitats and cannot readily escape control measures [2]. As larval stages are relatively delicate than the matures, control of these stages require less concentrations of insecticides in comparison to adult stage, so it charges a little hazard to the ecosystem. Chemical pesticides such as temephos, fenthion, malathion, methoprene etc are used against the mosquitoes but reports show that different levels of physiological resistance against the aforementioned synthetic larvicides have been developed[3]. Therefore, there is a constant need to identify target specific, inexpensive, user-friendly as well as environment- friendly alternative mosquito larvicidal substances from plant origins[4, 5]. The entire floral community of this green planet preserves rich resources in form of a variety of phytochemicals. The effects of plant derived chemicals against bacteria[6] and pests including mosquitoes vary significantly depending on the plant species, plant parts used and developmental stages of plant parts[7]. Depending upon the extraction procedure and the solvents used effectiveness of the phytochemicals produced even from same plant may vary significantly. The polarity of the solvent that is employed to extract the active ingredients of the plant is vital as polar solvent will extract polar molecules and non-polar solvents extract non-polar molecules[8]. During control of mosquito population at their reproduction place i.e. in confined water-bodies, many other creatures living simultaneously at that particular environment come instantaneous exposure of the mosquito-larvicidal agents. Wise choice of appropriate controlling agent of mosquito is primary necessity not to harm those non-target organisms and to maintain a suitable ecological balance. So the controlling agents must be target specific and less hazardous to non-target organisms. For this purpose plant based mosquito control formulations might be the safer choice for their biodegradability and environment-friendly issue in comparison to the laboratory synthesized chemical insecticides. *Drypetes roxburghii* (Wall.) is an evergreen tree. Generally this plant ranges between 12 to 20 m in height and found all over India. The bark is grey with pendent branches. Leaves shiny and dark green in colour. Fruits are ellipsoid in shape and drupe type; number of seed in fruits is one normally. The leaves and the fruits of the plant are considered as procreant and refrigerant. Those parts have use in treatment of sterility and fever [9, 10]. Our objective of the present study was to assess the potentiality of crude extract and six solvent extracts prepared from mature fruits of *D. roxburghii* as a mosquito larvicide. A comparative documentation of changes of mosquito – larvicidal potentiality of extracts of fruits due to change of solvents used during extraction procedure was made. Concurrently we carried out some tests following appropriate protocols to illuminate the presence of some secondary phytochemicals which might be responsible for larvicidal property of fruits of this particular plant. Experiment was executed to evaluate the effect of extracts on some non-target organisms also.

2. MATERIALS AND METHODS

2.1. Collection of plant material of D. roxburghii

The mature fruits of *D. roxburghii* were gathered from trees grown within the University campus during November to January, 20013-2014. It was authenticated and voucher specimens were deposited in the Mosquito, Microbiology and Nanotechnology Research Units, Department of Zoology, The University of Burdwan.

2.2. Collection and rearing of test mosquitoes and non target organisms

Adult *Culex quinquefasciatus* and *Anopheles stephensi* mosquitoes were collected from the mosquito colonies maintained in pathogen free and hygienic condition in the laboratory of Mosquito, Microbiology and Nanotechnology

Research Units, Department of Zoology, The University of Burdwan. Adult mosquitoes were reared in humidified cages and fed with 10% aqueous glucose solution. Female mosquitoes were periodically blood-fed on restrained albino rat for egg production. Adults of *Diplonychus annulatum*, *Poecilia reticulata* and *Toxorhynchites splendens* were collected from the surrounding ponds and drainage canals. All of them were acclimatized in laboratory conditions for at least 72 hours before subjected to experiments.

2.3. Preparation of crude extract

Green mature fruits were washed thoroughly with distilled water. Crude extract was produced by grinding the plant material in a mixer grinder. Then the ground material was passed through cheesecloth. Required concentrations of crude extract were prepared by mixing the liquid extract with suitable amount of sterilized distilled water.

2.4. Preparation of solvent extracts

Collected fresh mature fruits were dried in shade at room temperature and milled into fine powder with mixer grinder. For solvent extraction procedure 200 gm of finely ground powdered fruit were extracted subsequently with solvents of increasing polarity (non-polar to polar) viz. petroleum ether, benzene, ethyl acetate, chloroform: methanol (1:1), acetone and absolute alcohol, each for 72 hours, in a grease free soxhlet apparatus . The respective solvent extracts were collected separately and the same were filtered through Whattman 41 filter paper. Each of the extracts was subjected to evaporation in a vacuum rotary evaporator below 40°C, and the resulting dry powder of the extracts was stored in a refrigerator within air tight glass container until they were used for bio assay.

2.5. Mosquito larvicidal bio assay

The mosquito larvicidal bioassay was done by following the World Health Organization standard protocols[11] with slight modifications. Batches of 25 first, second, third and fourth instar larvae of *An. stephensi* and *Cx. quinquefasciatus* were transferred from rearing tray to disposable test plastic bowls by droppers, each containing 200 ml of water with required concentration of crude extract (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%) dissolved in it. For larvicidal bio assay with solvent extracts from the stock solutions, some experimental solutions of desired concentrations (50 ppm, 100 ppm, 150 ppm, 200 ppm) were prepared by dilution with distilled water and were applied on mosquito larvae in similar way. Four replicates were carried out for each concentration and an equal number of replicates were performed simultaneously as controls using distilled water in case of each experiments. Mortality percentage was noticed at 24 hours interval up to 72 hours post exposure.

2.6. Phytochemical analysis of the plant extracts

Phytochemical analysis of crude extract of the fruits of *D. roxburghii* was performed according to the methodologies of Trease and Evans[12], Sofowara[13] and Harborne[14]. In our study we searched for the presence or absence of some secondary biochemicals like tannins, saponins etc.

2.7. Effect on non-target organisms

Following the procedure reported by Suwannee *et al*., (2006)[15], the non target creatures were exposed to the sub lethal dose, LC_{50} (24 hours for 3rd instar larvae of *Culex quinquefasciatus*) of the crude (0.13%), ethyl acetate extract (77.03 ppm) of the fruit of *D. roxburghii*. Batches of 25 *D. annulatum*, *T. splendens* were kept into separate 2 L glass aquariums containing 1.5 L tap water and batches of 25 *P. reticulata* were put into 20 L aquarium containing 12 L tap water along with aforementioned doses of extracts. 4 replicates of experiments were performed for each type of organism in parallel with same number of replicates of untreated controls. Effects were screened up to 72 hours of post exposure for any type of physiological or behavioral abnormalities or for mortality.

2.8. Statistical calculations

3. The result of the mosquito larvicidal bio assay was calculated thoroughly by statistical calculations. All the percent of mortality were recorded after 24 hrs, 48 hrs and 72 hrs of exposures and those were corrected with Abbot's formula[16]. LC_{50} and LC_{90} were calculated. Comparisons of effects were analyzed by multivariate ANOVA using SPSS statistical package. Regression equation, regression coefficient (R^2) was also calculated.

4. RESULTS

In larvicidal bioassay against *Cx. quinquefasciatus* larvae with crude fruit extract of *D. roxburghii* 0.4% concentration exhibited 100% mortality at 72 hours of exposure in case of 1st and 2nd instars. After 48 hours of exposure to 0.4% concentration of the crude extract 100% mortality was noticed in 3rd instar larvae. At 0.5% concentration highest mortality was achieved against 4th instar that increased with time of exposure (72h > 48h > 24h). In case of *An. stephensi* the 3rd instar larvae showed highest mortality at lowest dose (100% mortality at 48 hour of exposure against 0.4% concentration) (Table 1). Table 2 presents the result of log-probit analysis (at 95% confidence level) and regression analysis of larvicidal activity of *D. roxburghii* mature fruit extract against four larval stages of *Cx. quinquefasciatus* and *An. stephensi*. LC_{50} values were below 0.2% in every case except 24 hours of post exposure in 1st (0.23%) and 4th (0.28%) instars of *Cx. quinquefasciatus* larvae and4th (0.25%) instars of *An. stephensi* . LC_{90} value was highest in 4th instar at 24 hours and lowest for 2nd instar at 72h (0.22% and 0.17% in *Cx. quinquefasciatus* and *An. stephensi* respectively) of post exposure. From regression equation it is evident that for all four larval stages of both type of mosquito larvae Y (mortality rate, dependent variable) was positively related to its corresponding X (dose, independent variable) and the value of R^2 in all cases were nearer to 1 which indicates that the rate of mortality linearly increased with the increasing dose. The results of the three-way factorial ANOVA (Table 3) carried out on mortality of (a) *Cx. quinquefasciatus* and (b) *An. stephensi* larvae by crude extracts using different concentrations, on different instars in different time period revealed significant differences in larval mortality ($P < 0.05$). The efficiency of different solvent extracts of *D. roxburghii* fruits as mortality causing agent on *Cx. quinquefasciatus* have been depicted in Table 4. From LC_{50} and LC_{90} values of different solvent extracts ethyl-acetate extract showed the lowest values against 3rd instar larvae of *Cx. quinquefasciatus* which indicates that it was the most efficient solvent extracts among the tested samples. The pattern of regression analysis followed the equation for straight line and in all cases R^2 values were nearer to 1, which indicates that the results were justified (Table 5). Table 6 represents completely randomized three-way factorial ANOVA of larval forms (instars) of *Cx. quinquefasciatus* using different hours, different solvent extracts and different concentrations of solvent extracts as three variables which indicates that the difference in larval mortality was significant in all cases except in the interaction between hour and concentration ($p > 0.05$). Table 7 represents the mortality percent of *An. stephensi* 3rd instar larvae on exposure to different concentrations of polar to non polar solvent extracts. 100% mortality was noticed after 72 h post exposure at 150 ppm concentration of ethyl-acetate extract whereas 200 ppm concentration of the same extract was responsible for 100% death of the larvae only after 24h. Table 8 embodies the results of Log probit analysis and regression analysis of larvicidal activity of solvent extracts of fruits on third instar of *An. stephensi*. Here also ethyl-acetate extract exhibited highest mortality percentage of larvae at minimal dose. Completely randomized three-way factorial ANOVA using different hours, different solvent extracts and different concentrations of solvent extracts as three variables is represented in table 9. As in all cases p values were less than 0.05 the results can be considered as significant. Table 10 represents the results of qualitative analysis of metabolites in mature fruits of *D. roxburghii*. In the crude fruit extract tannins, flavonoids, terpenoids, saponins, alkaloids, sterols, cardiac glycosides were present but carotinoids were absent. During the test on non-target organisms, in case of *Toxorhynchites splendens* larvae 0.50%, 1.12% and1.56% mortality was observed after 24h, 48h, 72h of exposure in crude extract respectively while corresponding mortality with ethyl acetate extract was 0.56%, 1.88%, 2.14%. In case of *Diplonychus annulatum* mortality percent was nil after 48 h in crude extract exposure and it was1% after 72h of exposure. Mortality percent like 0.00%, 0.67%, 1.87% was noticed after 24h, 48h, 72h exposure to ethyl acetate extract respectively. Absolutely no mortality was observed in case of *Poecilia reticulata* up to 72 h of exposure to crude extract and up to 48 h of exposure in ethyl acetate extract. After 72h of exposure in ethyl acetate extract 1.27% mortality was noted.

Table 1

Mean larval mortality (%) with standard error of Cx. quinquefasciatus and An. stephensi exposed to different concentrations of crude extracts of D. roxburghii (mean of 4 experiments per trial)

Mosquito	Larval instars	Concentrations (%)	(%) Mortality rate (Mean + Standard error)		
			24h	48h	72h
Cx. quinquefasciatus	First	0.1	12.00 ± 1.60	29.00 ± 1.88	47.00 ± 3.76
		0.2	29.00 ± 1.88	68.00 ± 3.64	80.00 ± 3.24
		0.3	63.00 ± 3.40	82.00 ± 4.16	92.00 ± 4.32
		0.4	86.00 ± 2.56	97.00 ± 1.88	100.00 ± 0.00
		0.5	98.00 ± 1.12	99.00 ± 1.00	100.00 ± 0.00
	Second	0.1	21.00 ± 1.88	42.00 ± 3.80	68.00 ± 3.64
		0.2	45.00 ± 3.40	57.00 ± 2.48	77.00 ± 4.40
		0.3	73.00 ± 3.40	89.00 ± 4.40	96.00 ±2.80
		0.4	94.00 ± 2.56	99.00 ± 1.00	100.00 ± 0.00
		0.5	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	Third	0.1	41.00 ± 4.40	49.00 ± 4.40	59.00 ± 4.40
		0.2	58.00 ± 4.16	65.00 ± 4.12	72.00 ± 3.24
		0.3	77.00 ± 4.40	83.00 ± 3.40	92.00 ± 3.24
		0.4	96.00 ± 2.80	100.00 ± 0.00	100.00 ± 0.00
		0.5	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	Fourth	0.1	25.00 ± 1.88	36.00 ± 1.600	48.00 ± 2.80
		0.2	38.00 ± 2.56	48.00 ± 1.600	55.00 ± 1.00
		0.3	42.00 ± 2.56	53.00 ± 3.40	67.00 ± 3.00
		0.4	59.00 ± 3.00	69.00 ± 1.00	77.00 ± 1.88
		0.5	76.00 ± 1.60	78.00 ± 1.12	81.00 ± 1.00
An. stephensi	First	0.1	21.00 ± 1.88	36.00 ± 1.60	59.00 ± 3.00
		0.2	36.00 ± 1.60	77.00 ± 1.88	83.00 ± 1.88
		0.3	64.00 ± 1.60	86.00 ± 2.56	96.00 ± 1.60
		0.4	88.00 ± 1.60	98.00 ± 2.00	100.00 ± 0.00
		0.5	99.00 ± 1.00	100.00 ± 0.00	100.00 ± 0.00
	Second	0.1	36.00 ± 2.28	52.00 ± 1.60	80.00 ± 1.60
		0.2	53.00 ± 1.88	71.00 ± 2.48	85.00 ± 3.00
		0.3	78.00 ±2.00	89.00 ± 1.88	97.00 ± 1.88
		0.4	87.00 ± 3.00	95.00 ± 1.88	100.00 ± 0.00
		0.5	99.00 ± 1.00	100.00 ± 0.00	100.00 ± 0.00
	Third	0.1	56.00 ± 1.60	68.00 ± 3.64	74.00 ± 3.44
		0.2	74.00 ± 2.56	79.00 ± 1.88	84.00 ± 1.60
		0.3	83.00 ± 1.88	87.00 ± 1.88	97.00 ± 1.00
		0.4	97.00 ± 1.88	100.00 ± 0.00	100.00 ± 0.00
		0.5	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	Fourth	0.1	27.00 ± 3.00	39.00 ± 1.00	55.00 ± 2.48
		0.2	42.00 ± 2.00	53.00 ± 1.00	59.00 ± 3.00
		0.3	49.00 ± 3.40	56.00 ± 2.80	74.00 ± 2.00
		0.4	60.00 ± 3.64	77.00 ± 1.88	76.00 ± 2.28
		0.5	76.00 ± 2.28	79.00 ± 2.48	83.00 ± 1.88

Table 2

Log-probit analysis and regression analysis of larvicidal activity of D. roxburghii mature fruit crude extract against different larval instars of Cx. quinquefasciatus and An. stephensi (mean of 4 experiments)

Mosquito	Larval instars	Period of exposure (hours)	LC_{50} value (% conc.)	LC_{90} value (% conc.)	Regression equation	R^2 value
Cx. quinquefasciatus	First	24	0.23	0.46	Y= -11.10X + 229	0.98
		48	0.14	0.32	Y= 24.30X +169	0.93
		72	0.10	0.24	Y= 46.00X + 126	0.89
	Second	24	0.18	0.38	Y= 4.50X + 207	0.98

		48	0.13	0.30	Y= 30.00X + 158	0.94
		72	0.07	0.22	Y= 62.10X + 87	0.93
	Third	24	0.13	0.36	Y= 27.60X + 156	0.98
		48	0.11	0.31	Y= 38.30X + 137	0.97
		72	0.09	0.25	Y= 51.60X + 110	0.94
	Fourth	24	0.28	1.46	Y= 11.10 X + 123	0.98
		48	0.19	1.37	Y= 25.30X + 105	0.99
		72	0.12	1.12	Y= 39.00X + 88	0.98
An. stephensi	First	24	0.20	0.46	Y= - 0.80X+208	0.99
		48	0.12	0.28	Y= 34.70X + 149.00	0.90
		72	0.09	0.21	Y= 57.90X + 99.00	0.89
	Second	24	0.15	0.42	Y= 22.60X + 160.00	0.98
		48	0.11	0.30	Y= 45.40X + 120.00	0.96
		72	0.05	0.17	Y= 75.90X + 55.00	0.93
	Third	24	0.09	0.30	Y= 48.70X + 111.00	0.97
		48	0.07	0.25	Y= 61.30X + 85.00	0.97
		72	0.06	0.19	Y= 70.60X + 68.00	0.92
	Fourth	24	0.25	1.43	Y= 16.00X + 116.00	0.99
		48	0.16	1.12	Y= 29.60X + 104.00	0.96
		72	0.09	1.15	Y= 47.50X + 73.00	0.97

Table 3

Completely randomized three-way factorial ANOVA of larval forms of (a) Cx. quinquefasciatus and (b) An. stephensi using different hours, different instars and concentrations of crude extracts as three variables

(a)

Source of variations	Sum of squares	df	Mean squares	F value	P value
Hour	1030.633	2	515.317	195.175	0.001
Instar	985.046	3	328.349	124.361	0.001
Conc	5784.958	4	1446.240	547.760	0.005
Hour *Instar	113.367	6	18.894	7.156	0.002
Hour * Conc	433.367	8	54.171	20.517	0.001
Instar * Conc	536.975	12	44.748	16.948	0.004
Hour *Instar * Conc	165.300	24	6.887	2.609	0.001
Residual	475.250	180	2.640		
Total	9524.896	239			

(b)

Source of variations	Sum of squares	df	Mean squares	F value	P value
Hour	895.075	2	447.538	439.600	0.020
Instar	1453.646	3	484.549	475.955	0.001
Conc	3928.933	4	982.233	964.813	0.003
Hour *Instar	110.992	6	18.499	18.171	0.002
Hour * Conc	318.467	8	39.808	39.808	0.000
Instar * Conc	251.167	12	20.931	20.931	0.005
Hour *Instar * Conc	137.133	24	5.714	5.613	0.001
Residual	94261.000	180	1.018		
Total	7278.663	239			

All the combinations with P < 0.05 are significant, df - Degrees of Freedom

Table 4

Efficacy of different concentrations of polar and non-polar solvent extracts of D. roxburghii fruit on third instar larvae of Cx. Quinquefasciatus

Solvent extracts	Conc. (ppm)	Mortality rate (%) [Mean ± SE]		
		24h	48h	72h
Petroleum ether	50	4.00±1.63	9.00 ± 1.88	15.00±1.88
	100	15.00±1.91	18.00±2.56	26.00±2.00
	150	25.00±2.51	30.00±2.65	38.00±2.00
	200	34.00±3.82	46.00±3.44	56.00±3.64
Benzene	50	5.00±1.91	8.00±0.00	12.00±2.28
	100	15.00±1.91	21.00±1.00	28.00±3.24
	150	25.00±2.51	32.00±2.8	41.00±3.00
	200	50.00±2.58	56.00±4.32	60.00±4.88
Ethyl acetate	50	34.00±1.12	43.00±3.00	57.00±1.88
	100	54.00±2.00	68.00±2.28	85.00±2.52
	150	77.00±1.88	87.00±1.88	95.00±1.88
	200	96.00±2.28	100.00±0.00	100.00±0.00
Chloroform: Methanol (1:1 v/v)	50	15.00±3.00	26.00±1.15	35.00±4.43
	100	40.00±5.65	52.00±3.65	55.00±3.41
	150	60.00±2.82	70.00±1.15	79.00±1.00
	200	75.00±1.00	78.00±4.16	85.00±3.41
Acetone	50	0.00±0.00	2.00±1.12	3.00±1.00
	100	4.00±0.00	7.00±1.88	9.00±1.88
	150	20.00±0.00	18.00±3.44	22.00±1.12
	200	26.00±0.00	42.00±1.12	51.00±3.4
Absolute alcohol	50	0.00±0.00	0.00±0.00	2.00±1.00
	100	0.00±0.00	2.00±1.12	4.00±0.00
	150	7.00±1.00	9.00±2.48	12.00±1.6
	200	9.00±1.00	19.00±3.4	40.00±4.32

Table 5

Log probit analysis and regression analysis of larvicidal activity of polar and non-polar solvent extracts of D. roxburghii fruit on third instar of Cx. quinquefasciatus

Solvent extract	Period of bioassay (hours)	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	R^2 value
Petroleum ether	24	305.14	1172.67	Y= -5.5X+ 0.2	0.99
	48	246.93	1018.42	Y= -5X+ 0.24	0.98
	72	192.13	884.38	Y= 10X+ 0.27	0.98
Benzene	24	225.96	657.91	Y= -12.5X+ 0.29	0.93
	48	198.11	632.49	Y= -9.5X+ 0.31	0.96
	72	170.95	608.90	Y= -4X+0.31	0.99
Ethyl acetate	24	77.03	205.64	Y= 13X+ 0.41	0.99
	48	61.53	152.07	Y=27 X+ 0.38	0.98
	72	45.64	109.60	Y= 49.5X+ 0.27	0.87
Chloroform: Methanol (1:1 v/v)	24	119.52	339.67	Y= -2.5X+ 0.40	0.98
	48	93.40	321.56	Y= 13X+ 0.34	0.94
	72	76.58	257.91	Y= 20X+ 0.34	0.95
Acetone	24	279.89	617.39	Y= -11 X+ 0.18	0.94
	48	248.74	586.20	Y= -15.5X+ 0.26	0.99
	72	219.60	511.06	Y= -18X+0.31	0.90
Absolute alcohol	24	419.72	905.91	Y= -4.5 X+ 0.06	0.87
	48	330.06	693.18	Y=-8.5X+ 0.12	0.92
	72	263.82	519.76	Y=-16X+ 0.24	0.80

Table 6

Completely randomized three-way factorial ANOVA of 3rd instars larval form of Cx. quinquefasciatus using different hours, different solvent extracts and different concentrations of solvent extracts as three variables

Source of variations	Sum of squares	df	Mean squares	F value	P value
Hour	540.271	2	270.135	174.307	0.000
Extract	9400.823	5	1880.165	1213.191	0.000
Conc	4364.844	3	1454.948	938.816	0.000
Hour * Extract	39.688	10	3.969	2.561	0.006
Hour * Conc	15.812	6	2.635	1.701	0.122
Extract * Conc	474.052	15	31.603	20.392	0.000
Hour * Extract * Conc	183.729	30	6.124	3.952	0.000
Residual	334.750	216	1.550		
Total	15353.969				

All the combinations with P < 0.05 are significant

Table 7

Efficacy of different concentrations of polar and non-polar solvent extracts of D. roxburghii fruit on third instar larvae of An. stephensi

Solvent extracts	Conc. (ppm)	Mortality rate (%) [Mean ± SE]		
		24h	48h	72h
Petroleum ether	50	6± 1.15	11±1.88	20± 1.6
	100	18± 1.12	25±3.4	28±2.8
	150	32± 3.2	36±3.24	42± 2.0
	200	36±1.63	50± 2.56	61± 1.88
Benzene	50	8± 2.82	20± 1.63	28± 1.60
	100	22±1.91	35± 3.41	40± 4.32
	150	32±2.82	40± 3.26	55± 3.4
	200	55±1.00	65± 3.00	72± 4.32
Ethyl acetate	50	53± 1.9	69± 1.91	77± 1.91
	100	83± 1.00	88± 2.82	94± 2.00
	150	97± 1.91	99± 1.00	100± 0.00
	200	100± 0.00	100.00± 0.00	100 ± 0.00
Chloroform: Methanol (1:1 v/v)	50	22± 2.00	30± 2.51	40± 4.61
	100	43± 2.51	56± 3.26	60±1.63
	150	67± 3.00	75± 3.78	82± 3.82
	200	70± 3.46	80± 1.63	89± 3.41
Acetone	50	2± 1.12	5± 1.91	7±1.0
	100	8± 1.60	15± 3.00	18±1.12
	150	20±1.60	27± 1.00	30±1.12
	200	30± 2.56	35± 3.00	47±2.48
Absolute alcohol	50	0± 0.00	0± 0.00	2± 1.12
	100	5± 1.12	9± 1.00	11± 1.00
	150	10± 1.12	22± 2.58	24± 3.64
	200	18± 2.00	25± 1.91	35± 2.48

Table 8

Log probit analysis and regression analysis of larvicidal activity of polar and non-polar solvent extracts of D. roxburghii fruit on third instar of An. stephensi

Solvent extract	Period of bioassay (hours)	LC_{50} (ppm)	LC_{90} (ppm)	Regression equation	R^2 value
Petroleum ether	24	255.07	976.54	Y = - 5 X + 0.23	0.98
	48	212.00	920.81	Y = -1.5 X + 0.25	0.99
	72	170.68	869.46	Y = 3.5X + 0.27	0.96
Benzene	24	200.21	658.27	Y = -8.5X + 0.30	0.97

	48	156.72	634.78	Y = 5X + 0.28	0.93
	72	115.99	569.78	Y = 12X + 0.29	0.99
Ethyl acetate	24	49.535	108.88	Y = 44.5X + 0.31	0.86
	48	37.08	90.25	Y = 63X + 0.20	0.86
	72	31.80	72.78	Y = 74X +0.15	0.79
Chloroform: Methanol (1:1 v/v)	24	110.31	399.49	Y = 8.5X + 0.33	0.92
	48	83.66	293.21	Y = 18X + 0.33	0.92
	72	67.71	224.72	Y = 25X + 0.22	0.96
Acetone	24	316.06	956.37	Y = -9X + 0.19	0.98
	48	296.61	879.60	Y = -5X + 0.20	0.99
	72	232.64	821.35	Y = -7.5X + 0.26	0.98
Absolute alcohol	24	407.49	1124.94	Y = -6.5X + 0.11	0.98
	48	308.17	846.31	Y = -8X + 0.17	0.95
	72	272.88	786.73	Y = -10X + 0.22	0.99

Table 9

Completely randomized three-way factorial ANOVA of 3rd instars larval form of An. stephensi using different hours, different solvent extracts and different concentrations of solvent extracts as three variables

Source of variations	Sum of squares	df	Mean squares	F value	P value
Hour	538.563	2	269.281	192.121	0.000
Extract	11615.281	5	2323.056	1657.408	0.000
Conc	3519.983	3	1173.328	837.122	0.000
Hour * Extract	48.438	10	4.844	3.456	0.000
Hour * Conc	8.382	6	1.397	.997	0.428
Extract * Conc	306.955	15	20.464	14.600	0.000
Hour * Extract * Conc	95.618	30	3.187	2.274	0.000
Residual	302.750	216	1.38		
Total	16435.969				

All the combinations with P < 0.05 are significant

Table 10

Qualitative analysis of secondary metabolites in mature fruits of D. roxburghii

Plant part	Saponins	Tannins	Flavonoids	Alkaloids	Phytosterols	Terpenoids	Cardiac glycosides	Carotenoids
Mature fruits	+	+	+	+	+	+	+	-

+ = presence of phytochemicals, - = absence of phytochemicals

5. DISCUSSION

Several plant species have been established to have mosquitocidal potentiality[, 17, 18, 19, 20,21]. Plants contain a vast array of secondary metabolites [22, 23] which are somehow responsible for producing toxicity to other creatures. During the present study, the larvicidal potentiality of fruit extract of *D. roxburghii* has been well established in the laboratory condition against larvae of *Cx. quinquefasciatus* and *An. stephensi*. The results of this study showed that the mortality of mosquito larvae exposed to the fruit extract increased with concentration of extract as well as the time of exposure, which is supported by the report of Obomanu *et al.*, (2006)[24]. The increased mortality with time may be due to individual or synergistic effect of

several factors like time provides the best chance for accumulation of active moiety of the compound in the larval body or with time the active compound turns into more toxic substance for the larvae of mosquitoes[25]. Solvent types used during extraction of plant parts impart great effects on chemo-profile of the plant species. As different biochemical affect the physiology of the target organisms in dissimilar ways, the phytochemicals extracted from the same plant parts with different solvents may have differences in their potentiality against the particular organism. Mathew *et al.,* (2009)[26] screened three potential plant extracts viz., *Saraca indica, Nyctanthes arbor- tristis* and *Clitoria ternatea* for mosquito larval control against three major mosquito vectors *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi.* The petroleum ether extract of the leaves and chloroform extract of the bark of *S. indica* were effective against the larvae of *Cx. quinquefasciatus* with respective LC_{50} values 228.9 and 291.5 ppm. The LC_{50} values of chloroform extract of *N. arbor-tristis* leaves were 303.2, 518.2, and 420.2 ppm against *A. aegypti, An. stephensi* and *Cx. quinquefasciatus* respectively. The methanol and chloroform extracts of flowers of *N. arbor- tristis* showed larvicidal activity against larvae of *An. stephensi* with the respective LC_{50} values of 244.4 and 747.7 ppm. Among the methanol extracts of *C. ternatea* leaves, roots, flowers, and seeds, the seed extract was effective against the larvae of all the three species with LC_{50} values 65.2, 154.5, and 54.4 ppm, respectively, for *A. stephensi, An. aegypti* and *Cx. quinquefasciatus*. Chowdhury *et al*., (2007)[27] studied on efficacy of *Solanum villosum* Mill. as a biocontrol agent against larvae of *Cx. quinquefasciatus* Say and reported that LC_{50} values of the leaves with biologically active different solvent extracts like petroleum ether, absolute alcohol, benzene, acetone, and chloroform: methanol (1:1 v/v) were 645.745 ppm, 321.890 ppm, 204.302 ppm, 107.657 ppm, and 39.192 ppm respectively after 24 h of exposure period. Mortality rate with chloroform: methanol (1:1 v/v) extract was significantly higher ($P < 0.05$) than other extracts. Rawani *et al.,* (2010)[28] worked on mosquito larvicidal activities of *Solanum nigrum* L. leaf extract against *Cx. quinquefasciatus* Say. They extracted mature leaves with six different solvents [viz. petroleum ether, benzene, ethyl acetate, chloroform: methanol (1:1 v/v), acetone and absolute alcohol] to determine the best extractant for subsequent isolation and characterization of active ingredient. The corresponding LC_{50} value of acetone, absolute alcohol, petroleum ether, chloroform: methanol (1:1 v/v), benzene and ethyl acetate extracts were 72.91 ppm, 59.81 ppm, 54.11 ppm, 32.69 ppm, 27.95 ppm and 17.04 ppm, respectively, after 24 h of exposure period. They found that mortality rate of mosquito larvae with ethyl acetate extract was significantly higher ($p<0.05$) than other extracts. In 2014 Rawani *et al.,*[29] reported the presence of a glucosinolate compound, [1-thio-ß-D-glucopyranose-1-[(R)-3-hydroxy-2-ethyl-*N*-hydroxysulfonyloxy propanimidate] having the molecular formula of $C_{11}H_{21}NO_{10}S_2$, which was isolated from ethyl acetate extract of mature leaves of *Solanum nigrum* L. In this experiment the authors found that among the extracts prepared by using different solvents, ethyl acetate extract was most efficient against 3rd instars larvae of both *Cx. quinquefasciatus* and *An. stephensi*. The comparative efficacy of the used solvent extracts were as follow: ethyl acetate > chloroform: methanol (1:1 v/v) > benzene > acetone > petroleum ether > absolute alcohol in respect to LC_{50} value after 24h of exposure in case of *Cx. quinquefasciatus*. In case of *An. stephensi* efficacy of solvents were as follows: ethyl acetate > chloroform: methanol (1:1 v/v) > benzene > petroleum ether > acetone > absolute alcohol. The varying results obtained in lethal concentrations were probably due to presence of toxicity causing ingredients in different levels in different solvent extracts. Secondary metabolites or phytochemicals are used in plants defence mechanisms. Several studies reports that phytochemicals like steroids [30], alkaloids [31], triterpenes[32], saponins[33] etc could be used to control mosquito vectors. In our study we identified presence of some secondary metabolites which indicates that one or more compounds among them may be responsible for death of mosquito larvae.

Several studies report on negative impacts of insecticides on non- target organisms [34,35], though some mosquitocidal agents originated from plant extract proved to be non-toxic on them [36, 37]. In this aspect the fruit extracts of *D. roxburghii* could help us by being less toxic to the tested non-target creatures.

6. CONCLUSION

So without any chemical treatment the use of crude fruit extract from the aboriginal plant *D. roxburghii* can obviously and effectively be used in mosquito population control. Amongst the solvent extracts the ethyl acetate extract is highly efficient agent for the war against mosquitoes. As the fruit extract of *D. roxburghii* is relatively less toxic to tested non-target organisms besides being effective against larvae of mosquitoes, it can be experimented in integrated pest control programmes.

7. CONFLICT OF INTEREST

We declare that we have no conflict of interest.

8. ACKNOWLEDGEMENT

The authors are grateful to UGC for providing financial support to K M Haldar [fellowship No.F.17-8/08 (SA-I)] and also to UGC-DRS. Authors extend their gratitude to Dr. Ambarish Mukherjee, Department of Botany, The University of Burdwan, West Bengal, for the taxonomic identification of the selected plant.

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