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Insecticidal efficacy of aqueous and methanol extract of *Catheranthus roseus* (L) G. Don against an insect model *Drosophila melanogaster* Meigen

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Abstract: **Biopesticides are the integral components of integrated pest management which attain high momentum in recent years in organic farming activities. Insecticidal activity of aqueous and methanol extract of *Catheranthus roseus* were evaluated against an insect model *Drosophila melanogaster* along with determine extraction yeied. The insecticidal activity assayed at various concentrations of both extract under the laboratory condition. The concentrations of aqueous and methanol extract causes 50% and 90% eclosion inhibition in test organism were determined by probit analysis. Both extract showed insecticidal activity and the highest eclosion inhibition were found in the methanol extract (EC_{50}=126.86 mg/ml) than aqueous extract (EC_{50}=364.29 mg/ml). No mortality observed in the control. The methanol and aqueous extract of *Catheranthus roseus* have potential to be used as biopesticide for the control of insect pest in the agricultural field with minimum retention in the environment.**

Keywords: Catheranthus roseus, **eclosion inhibition, insecticidal activity and yield of extract.**

I. INTRODUCTION

Insect pest are the major problem of the world food production during crop growth, harvest and storage. To reduce these problems, different management approaches like chemical, biological and physical methods has been conducted. The use of synthetic organic pesticides particularly chlorinated hydrocarbons has increase the agricultural productivity, but it leads to serious environmental pollution, affecting human health and causing death of non-targeted organisms [1]. Hence it is essential to find out better alternatives to chemical insecticides that should be eco-friendly as well as cost effective in to the community.

In the early 1940, prior to discovery of chemical pesticides the plant based insecticides has been used for insect pest management [2]. Plant extract can be promising alternative source of pesticides due to their eco-friendly and more compatible properties. These are being used to manage the pest and minimise the yield loss. The various types of plant extracts are used as bio pesticides such as neems, garlic, syringe, ginger etc. to control and manage wide range of pests of different plants. Approximately 1,200 plant species have been reported to have insecticidal value [3].

Catharanthus roseus is a well-known medicinal plant, an ornamental shrub belonging to the family Apocynacae and commonly known as Madagascar periwinkle. Fresh biomass of *C. roseus* produce monoterpenoidindole alkaloids as secondary meatabolites that contain the two major vital cytotoxic dimeric alkaloids (vincristine and vinblastine) used for cancer chemotherapy; also many alkaloids have strong medicinal property. *C. roseus* contains a significant number of volatiles and phenolic compounds which can be used both biopesticide and theraputical capability [4]. In plants several

groups of phytochemicals like alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported for their insecticidal activities [5].

Several oral toxicity studies have been reported on the different parts in experimental animals. The uses of aqueous leaf extract as infusion for disease remedy result to hepatocellular damage when consumed in large dose [6]. Ethyl acetate fractions of *C. roseus* was used as larvicidal or anti-feedant agent [7]. This study was undertaken to evaluate the pesticidal efficacy of aqueous and methanol extract of *catheranthusroseus* against an insect model *drosophila melanogaster*.

II. MATERIALS AND METHODS

A. Plant material

The plant *Catheranthus roseus* (L) G.Don were collected from Botanical garden of Calicut University, Malappuram, Kerala, India and identified same by the taxonomist of Department of Botany, Calicut University, Malappuram, Kerala, India. The root, stem and leaves of fresh *C. roseus* were collected, washed in running water and air dried in the shade and powdered using an electric grinder. The dried powder was extracted using Soxhlet extractor with methanol and water.

B. Extraction yield determination

The extraction yield is a measure of the solvent efficiency to extract specific components from the original material. The 50 gram of shade dried sample of *Catheranthus roseus* extracted using 750 ml of 70% methanol and 750 ml of water. The final volume will be 450 ml. Evaporate the extract to dryness by the rotary evaporator (Cyber lab, RE10 CSE84) under vacuum at 40°C. Weigh the obtained dried extract. Calculate the extraction yield as the percentage of the weight of the crude extract to the raw material (50g). A portion of each extract was evaporated and dried in the Laboratory oven, Labline attaining constant weight. The final dry weight was used to calculate extraction yield.

$$\text{Extraction yield (\%)} = \frac{\text{(Weight of the dried extract x 100)}}{\text{(Weight of the original sample)}}$$

C. Experimental organism

Test organisms selected for the present work was *Drosophila melanogaster*. The healthy culture was procured from *Drosophila* stock centre of Mysore University and maintained in the laboratory for the entire work and cultured with culture media prepared according to the standard protocol formulated by [8].

D. Toxicity bioassay

EC_{50} was determined by administering five different concentrations of each extract to five groups of *Drosophila* (Each group contain 30 eggs of 3 experiment sets) through culture medium. Concentrations were fixed through sequences of range finding experiments and a set of geometric concentration were fixed using the formula a/r^2, a/r, r, ar, ar^2 from 50% (r) and 100% (ar^2) mortality concentration. Cumulative mortality was monitored and 50% eclosion failure (EC_{50}) calculated by probit analysis. Healthy adult *D. Melanogaster* flies were sorted and allowed to lay eggs on normal medium for one hour, followed by transferring the eggs to culture medium with different concentrations of the aqueous (27.5, 137.5, 275, 550, 825 mg/ml) and methanol extract (14.2, 71, 142, 284, 568). Along with the experiment group, a control and appositive control was maintained in the similar condition. All experiment was performed in 15/9 photoperiod.

E. Statistical analysis

The average eclosion inhibition data were subjected to probit analysis were calculating EC_{50}, EC_{90} and other statistics at 95 % confidence limits of upper fiducidal limit and lower fiducidal limit and chi-square values were calculated by using SPSS software (Version 20).

III. RESULTS

The yield of **e**xtractions was expressed as the grams extract per gram of sample and are presented in Fig.1. Results of aqueous and methanol extract showed different amounts of extractable soluble compounds. The highest percentage of extraction yield was exhibited by methanol extract (25.56 mg/g) when n = 6.

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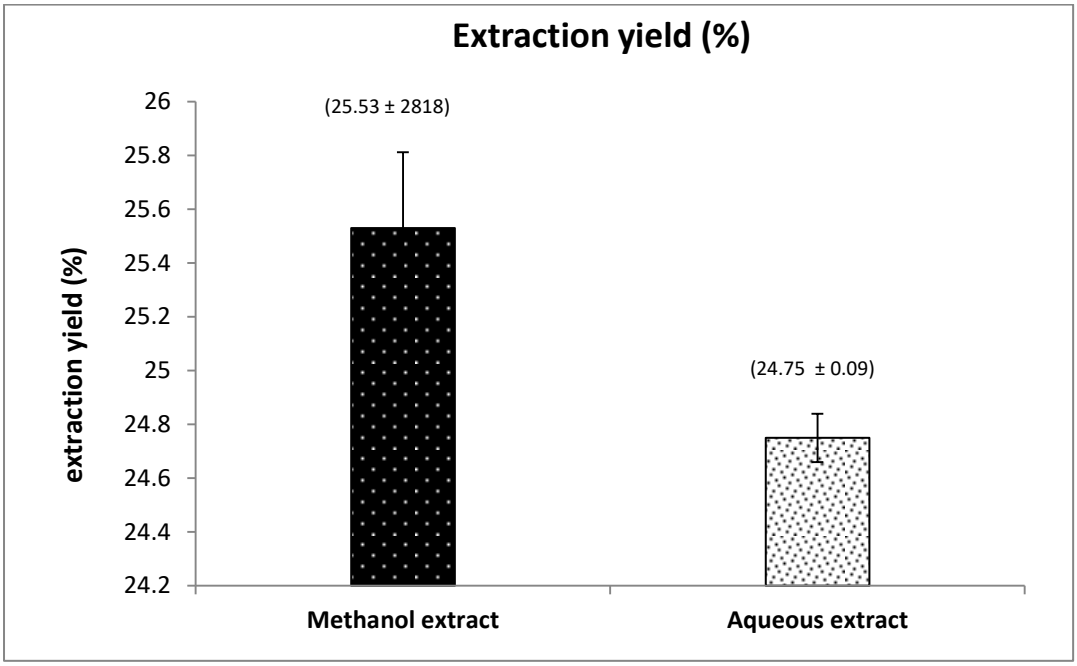

Fig.1. Percentage yield of Methanol and Aqueous extract

Aqueous and methanol extract of *C. roseus* were tested for their insecticidal effect on *D. melanogaster*. Table 1 illustrate that, the dose dependent percentage eclosion inhibition after the treatment with different concentrations of both extract of *C.roseus.*The recorded eclosion inhibition in aqueous extract, were 6.67%, 26.67%, 60%, 70% and 83.33% and methanol extract 14.2%, 71%, 142%, 284% and 568 % according to concentration. The differences between eclosion inhibition readings were statistically significant ($P < 0.001$). The slope and chi^2 data reflected homogeneity between insect individuals. The figure 1 shows that eclosion inhibition increasing with increasing concentrations of both extract and 100 % emergence failure observed in 568 mg/ml of methanol extract whereas 83.33% emergence failure in 825mg/ml of aqueous extract.

TABLE I: DOSE DEPENDENT ECLOSION INHIBITION OF AQUEOUS AND METHANOLIC EXTRACT OF *C.ROSEUS*

	Aqueous extract		**Methanol extract**	
Concentration dependent responses	Concentration (mg/ml)	% eclosion inhibition	Concentration (mg/ml)	% eclosion inhibition
	Control	0	Control	0
	27.5	6.67	14.2	20
	137.5	26.67	71	40
	275	60	142	66.67
	550	70	284	83.33
	825	83.33	568	100
EC_{50}	364.29 mg/ml		128.86 mg/ml	
EC_{90}	847.47 mg/ml		281.651 mg/ml	
P value	0.000003		0.000002	
Chi^2 value	28.169		32.165	

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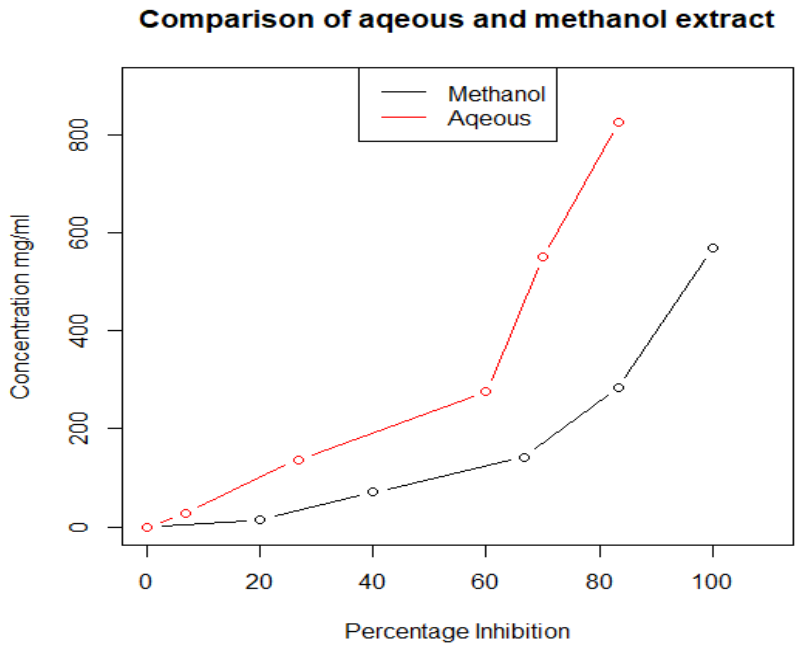

Fig.2. Dose dependent eclosion inhibition of aqueous and methanol extracts of *C.roseus* in *D. melanogaster.*

The Fig.2 shows that eclosion inhibition increasing with increasing concentrations of both extract and 100 % emergence failure observed in 568 mg/ml of methanol extract whereas 83.33% emergence failure in 825mg/ml of aqueous extract.

IV. DISCUSSION

The yield of extraction might be influenced by the polarities of solvents [9]. In this study, the highest extraction yield observed in methanol extract (25.53 %) than aqueous extract (24.75) and the higher concentration (89.33%) of organic molecules are also extracted by methanol when compared to water. Klejdus *et al*., (2004) [10] and [11] Klejdus *et al*., (2005) are reported that methanol is a good solvent when comparing different solvents and technique for extraction of iso-flavo soybean sample. The methanol could be absorb much of the microwave energy and transform it in to heat better than other solvents. That is methanol has high dissipation factor [12]. The result of study of [13] also suggested that absolute methanol was a better solvent for the extraction of *A. wilkesiana* and *S.scabrum*. The magnitude of extraction yield of *Hieracium pilosella* leaf was higher in methanol when compared to water [14].

The study demonstrated that both aqueous and methanolic extract of *C. roseus* have insecticidal activity on *D. melanogaster*, and both extracts are able to suppress the emergence of flies. The reduction in the emergence rate could be due to extract's repellence and toxic effect, since they contain phenolics and alkaloid constituents with pesticidal property [15]. Preliminary phytochemical screening of *C. roseus* revealed the presence of tannin, tri-terpenes, alkaloids, flavonoids and saponin [16]. The petroleum ether extract of the same plant was used to control *An.stephensi* mosquitos [17]. Vincristine and vinblastine important anticancer alkaloids present in the *C. roseus*, which have been to cause neurotoxicity, that influence motor and neuronal function along with bone marrow depression in human [18]

The suppression rate of emergence was higher in methanol extract when compared to aqueous extract. This due to methanol extract has high total phenolic content [19] and the efficiency of the phenolic extraction depends on the type of the plant and kind of solvent used [20]. *Kevin et al.*, (2012) [21] also reported that methanol leaves extract of *C. roseus* caused diarrhoea and mortality in female SD rat. The magnitude of extraction yield of *Hieraciumpilosella* leaf was higher in methanol when compared to water [14]. Hence the efficacy of methanolic extraction increases due to its higher yield.

Methanol was most potent solvent for extraction, recording the lowest EC_{50} value (126.86 mg/ml) when compared to aqueous extract (364.29 mg/ml) (Fig.1).100% eclosion inhibition noted in 568 mg/ml of methanolic extract, whereas 83.3% noted in 825 mg/ml of aqueous extract.

V. CONCLUSION

Aqueous and methanolic extract of *C. roseus* have been supress the adult emergence of *D. melanogaster* and methanolic extract show high yield and high insecticidal activity than aqueous extract. Therefore *C. roseus* extract is one of the best alternatives for chemical insecticides and also cost effective and eco-friendly bio pesticides which create a healthy environment.

Conflict of Interest:

For this research paper there is no conflict of interest as confirmed by the authors.

ACKNOWLEDGMENT

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