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IMPACT OF MEDICINAL PLANT COMPOUNDS ON *Callosobruchus chinensis* (COLEOPTERA: CHRYSOMELIDAE: BRUCHINAE)

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AUTHORS' CONTRIBUTIONS

The present work was done in collaboration with all authors. Author VRB designed the study, did the experiment, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SG and SD managed the analysis of the study. Author SD managed the literature searches and corrected the grammatical errors. All authors read and approved the final manuscript.

ARTICLE INFORMATION

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Original Research Article

ABSTRACT

A medicinal plant *Boerhavia diffusa* was tested against the pulse beetle *Callosobruchus chinensis* and was found to be effective in controlling the pest. Both HPLC and GC MS analysis of the ethanol extract of the plant showed the presence of certain insecticidal compounds, thus proved to be effective in controlling the pest.

Keywords: *Boerhavia diffusa; Callosobruchus chinensis;* HPLC; GCMS; ethanol extract; pest.

1. INTRODUCTION

A great number of studies during the last decade have linked the consumption of medicinal plants with numerous beneficial health properties, leading to increased popularity and renewed interest in exploring the medicinal properties of traditional medicinal plants. The use of traditional medicine has been encouraged by the World Health Organization (WHO) and United Nation Children`s Fund (UNICEF) for its cultural role, greater availability and acceptability than the modern pharmaceutical agents.

Tropical plants are rich in bio active compounds and these compounds are present as the secondary metabolites in the plant body. Their concentration may vary in the different parts of the plants. These are otherwise known as phytochemicals. Phytochemicals are a fascinating mysterious group of thousands of chemicals found in plants [1]. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds [2].

Plant extracts are commonly referred to as plant botanicals and are the secondary plant metabolites synthesized by the plant for protective purposes. Some of these compounds are toxic to insects. These plant compounds are called botanical pesticides, plant

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pesticides or simply botanicals. They may be contact, respiratory or stomach poisons. Botanicals are not very selective because they target a broad range of insect pests. Higher plants are a rich source of novel insecticides [3]. Plant materials with insecticidal properties have been used traditionally for generations throughout the world [4].

Constituents of many aromatic plants used for flavouring or medicinal purposes have been found to possess insecticidal properties. Neem, *Azadirachta indica* from Meliaceae family, is the most important botanical insecticide presently in use through the world. However, many other plant species, especially from tropical regions, have the potential to be used as botanical insecticide or as font of bioactive compounds [5,6]. The most economically important of the natural plant compounds used in commercial insect control are the pyrethrins from the flower heads of pyrethrum *Chrysanthemum cinerariaefolium* [7]. Nicotine isolated from number of species of *Nicotiana* is also insecticidal. Botanical products like tobacco extract, neem oil and extract, which can be easily and cheaply collected in rural farmers, have been found promising and useful for common bean pest control [8].

The selected medicinal plant, *Boerhavia diffusa* showed a significant effect on the insect and little information so far is available on the components of the leaf of the plant *Boerhavia diffusa*. The plant is easily available also. So this plant was selected for possible purification and identification of compounds present in the leaf.

2. MATERIALS AND METHODS

2.1 Test Insects

Experiments were conducted in the Entomology Research Laboratory, Department of Zoology, University College Thiruvananthapuram. The pulse beetle, *Callosobruchus chinensis* L. adults were obtained from naturally infested green gram seeds from local markets. The adult male and female beetles were reared on clean and un-infested green gram (*Vigna radiata* L). The seeds were made pesticide free by washing with clean water. Newly emerged adults were used for the study.

2.2 Preparation of Ethanol Extracts

Ethanol extract was prepared using the soxhlet apparatus.20 gm of powdered leaves were weighed and tied in a thin cloth and placed in extraction tube.200 ml ethanol was taken in the glass flask. Ethanol was boiled at 55°C continuously. Boiling was continued for six to eight hours till the extract became pale green. On completing the boiling, the extract was allowed to cool and stored in air tight containers for further use under refrigerated condition. The ethanol extract obtained was treated as 100% concentration. These were diluted to different concentrations like 0.5%, 1.5%, 2.5% and 3.5%.

2.3 Treatment with Ethanol Leaf Extracts (Residual Film Method)

The effect of ethanol leaf extract was analysed by using residual film method. No.1 Whatman filter paper was cut in round shape and placed in the plastic containers. Extract was applied in different doses (i.e 0.5%, 1.5%, 2.5% and 3.5%) to these filter papers in different concentrations using a micropipette and allowed to dry so that the solvent may evaporate completely. Then the feed, green gram seeds (50 gm) was weighed out and placed in the containers. Adult insects (25 each) were then transfered to the containers. For each treatment control were also set up without applying plant extract and solvent alone was applied in the filter paper. Five replicates were kept for each treatment and its control. Observations were recorded on the fifth day of treatment.

2.4 Statistical Analysis of Data

The data obtained were recorded as mean± standard deviation. For testing the significance of the data obtained, statistical analysis was carried out using ANOVA ($p \leq 0.05$) using SPSS software [9].

2.5 HPLC

HPLC of ethanol extract was carried out in a Waters Inc HPLC system consisting of 2695 alliance separation and model 2487 tunable UV detector. 20 µl of the samples were injected to the C18 symmetry column 4.6X250 mm. Detection wave lengths was set as 295 nm.

2.6 GC-MS

The GC-MS of the ethanol extract of the *Boerhavia diffusa* was carried out in GC-MS Varian Saturn 2200 with factor four V.F ms column and GC-MS solution software 5.2. Programme temperature was initially set as 100°C for 1.5 minutes and gradually raised to 270°C for 60 minutes. 1 µl sample was injected for analysis. Helium gas of 99.99% purity was used as carrier gas. The flow rate of gas was set as 1 ml/min. The sample injector temperature was maintained at 250°C and the ionization mass was done with 70eV. Mass spectra were recorded for the mass range 40-600 m/z for about 60 min. Identification of compounds was based on comparison of the mass spectra with library as the compound separated, on dilution through the column, were detected in electronic signals.

3. RESULTS

3.1 Effects of Ethanol Leaf Extract on the Adult Insects

All the experimental cultures had shown a significant mortality. But no mortality was observed in the control set up (Table 1). Percentage of mortality at different doses of 0.5, 1.5, 2.5 and 3.5 was 20, 36, 54 and 66 respectively.

Table 1. Effect of plant leaf ethanol extract on adult insects of *Callosobruchus chinensis*

Plant	Age of insects	Dose (%)	Mortality (%)
Boerrhavia diffusa	Adults	0.5	20±0.00
		1.5	36±0.01
		2.5	54±0.02
		3.5	66±0.01

All values are mean ±S.E of six replicates and significant at p≤0.05 level of significance

3.2 HPLC

HPLC of ethanol extract of the plant analysed at 295 nm showed multiple peaks.Separation of 9 distinct peaks with varying Retention Time was observed.It consists of 3 major peaks and 6 minor peaks. The peak having the retention time 2.476 was analysed as Boerhavinone A as shown in Table 2 and Fig. 1.

Table 2. HPLC analysis

	RT	Area	%Area	Height
1	2.476	19306031	72.76	719202
2	3.101	5787625	21.81	272381
3	3.602	1212002	4.57	41181
4	5.824	36503	0.14	2524
5	6.237	20641	0.08	1463
6	7.944	69067	0.26	2612
7	8.659	23920	0.09	1234
8	9.861	1514	0.01	346
9	10.920	77629	0.29	2270

3.3 GC-MS

The GC-MS analysis of ethanol extract revealed presence of a spectrum of volatile compounds. Approximately 38 compounds with different molecular mass were separated in which 27 compounds are identified Fig. 2.

Serial no.	Name of compound
1.	silicic acid diethyl bis(trimethylsilyl) ester
2.	oxime- methoxy-phenyl
3.	spiro 2.4 hepta-4 6-diene
4.	N methyl 2-(4-chloro phenol)
5.	benzofuran-2-yl ethanol
6.	quinoline
7.	2-butanedoic acid
8.	Digitoxigenin
9.	2h-1 4-benzodiazepin-2-one
10.	butanedioic acid hydroxy- diethyl ester
11.	1-mono linoleoyl glycerol
12.	2-Methoxy-4-vinylphenol
13.	3,5-Dibutoxy-1,1,1,7,7,7
14.	alpha cubebene
15.	5-methoxy uridine
16.	Octanoic acid, tert-butyl ester
17.	Caryophyllene
18.	6,8-Dichloro-1,2,3,4-tetrahydroisoquinoline
19.	1,4,7,-Cycloundecatriene
20.	phenol 2 4-bis(1 1-dimethylethyl)
21.	2,5-Bis(1-naphthamido)
22.	Methyl6-O-[1-methylprop-***1***-en-***1***-yl)cyclopropanecarboxylic acid
23.	9,12-octadecadienoic acid methyl ester
24.	Phytol
25.	Cyclopropaneoctanoic acid
26.	9 ,12, 15-octadecatrienoic acid
27.	*3',8,8'-Trimethoxy-3-pip* eridyl-2,2'-binaphthalene-1,1',4,4'-tetrone.

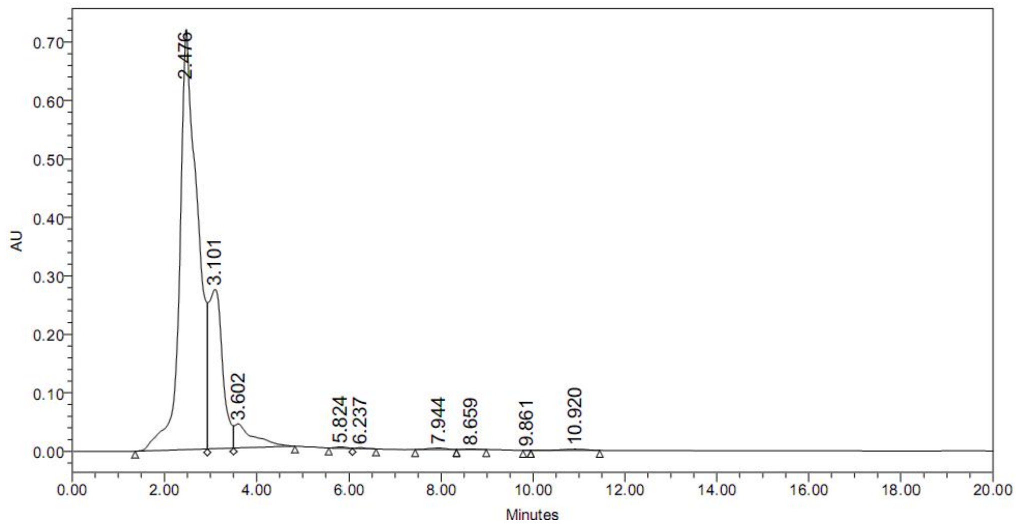

Fig. 1. Chromatogram of HPLC at 295 nm of ethanol extract of the plant *Boerhavia diffusa*

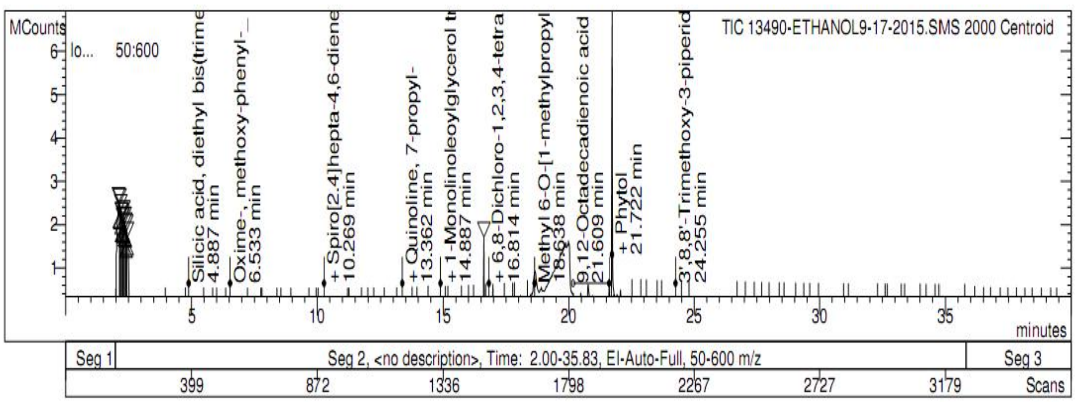

Fig. 2. Graph showing GC MS analysis of ethanol extract of the plant *Boerhavia diffusa*

4. DISCUSSION

In the present study, HPLC and GC MS analysis of the ethanol extract of the plant *Boerhavia diffusa* showed the presence of many compounds. Preliminary phytochemical analysis of the plant revealed that terpenoids, flavanoids, steroids, alkaloids and quinolines are present in the ethanol extract.

Alkaloids are beneficial chemicals to plants with predator and parasite repelling effects [10]. GC-MS analysis of *Boerhaavia diffusa* leaf extract was done by Umamenaka et al. [11]. Juna Beegum et al. [12] has reported that the whole plant contains compounds like alkaloids, flavanoid, terpenoid, steroid, tannin, phlobatannin and phenolic compounds. Isman [13] reported that natural pyrethrins, the neem extract, *Azadirachta indica* and *Khaya senegalensis* were effective in controlling the cotton bollworm. Extracts from marigold was also used against bruchid beetles from cowpeas in storage [14].

Ogunsina et al. [15] has also investigated plant extracts from *Lantana camara*, African nutmeg and Enuopiri against bean weevil *Callosobruchus maculatus* (F.) and maize weevil, *Sitophilus zeamais.* Crude methanolic extracts of *Trichilia americana* and ethanolic extracts of *Annona squamosa* seeds reduced pupal weight and exhibited toxic activity against the Asian armyworm, *Spodoptera litura* (Fabr.) [16]. In addition, ethanolic seed extracts of *A. squamosa* and *A. muricata* reduced larval growth of *S. litura* and the cabbage looper, *Tricho plusiani* (Hbn.) [17] while acetonic seed extracts of *A. squamosa* showed insecticidal activity against the cabbage head caterpillar, *Crocidolomia binotalis* Zeller [18].

Detailed study of the ethanol extract showed that it had many volatile and nonvolatile compounds which have insecticidal activity. Presence of insecticidal compounds was confirmed by HPLC and GC MS.

5. CONCLUSION

HPLC and GC MS analysis of ethanol extract of the plant *Boerhavia diffusa* confirmed the insecticidal activity of the compounds present in the leaf by causing mortality in the insects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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