Asian Journal of Chemistry Vol. 22, No. 7 (2010), 5331-5334

Phytochemical and Insecticidal Screening of The Leaf Extract of *Heliotropium indicum* L.

A.K. Dolui, M. Debnath* and B. De†
Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786 004, India
E-mail: manab_d@rediffmail.com

The leaves of *Heliotropium indicum* was extracted with petroleum ether, ethyl acetate and methanol. Different concentrations were made from each crude extract and applied for insecticidal activity (antifeedant activity) against *Helopeltis theivora*. Out of all the extracts methanolic extract has shown highest activity. Fractionation of effective crude extracts (methanolic extract) by column chromatography revealed that the fraction No. **V** is most toxic against the pest. Qualitative phytochemical analysis of most effective crude extract has also been performed.

Key Words: *Heliotropium indicum*, Methanolic extract, Antifeedant activity/insecticidal activity, *Helopeltis theivora*, Phytochemistry.

INTRODUCTION

Insect pests have mainly been controlled with synthetic insecticides in the last 50 years. But due to increased public awareness about immediate and long term hazardous effect of synthetic pesticides, botanical insecticides are presently getting increased attention because they are environmentally safe, bio-degradable and leave no toxic residues in soil, food and fodder[1].

The pool of plants possessing insecticidal substance is enormous[2]. Today over 2000 species of plants are known that possess some insecticidal properties[3-5]. The deleterious effects of plant extracts on insects can be manifested in several manners including toxicity, mortality, antifeedant growth behaviour and reduction of fecundity and fertility[6].

Keeping this in view, the present study was aimed to assess the chemical which is responsible for showing insecticidal activity of leaves of *Heliotropium indicum* against the tea mosquito bug, *Helopeltis theivora* waterhouse which is an important yield reducer of tea that may cause upto 25 % crop loss[7].

EXPERIMENTAL

Preparation of extracts: Leaves of *Heliotropium indicum* (F. Boraginaceae) were collected from the adjoining areas of Dibrugarh University and authenticated by Botanical survey of India, Shibpur, Howrah. The collected leaves were dried at

†Regional Institute of Pharmaceutical Science And Technology, Abhoynagar, Agartala-799 005, India.

room temperature and were then ground to powdered form. The powdered materials were extracted with petroleum ether, ethyl acetate and methanol respectively in Soxhlet apparatus[8]. The solvent from each extract was removed under vacuum. The dried extracts were then dissolved in acetone to prepare the desired concentrations. The insects, *Helopeltis theivora* were collected from the unsprayed tea garden and reared in the laboratory on freshly collected twigs of tea leaves.

Antifeedant test: Different solvent extracts of the leaf of *Heliotropium indicum* were assessed for antifeedant activity against *Helopeltis theivora*. Fresh tea shoots were collected from the unsprayed field and were sprayed with different concentrations (*viz.*, 0.5, 1.0, 2.0 and 4.0 %) of each solvent extracts and air dried for 10 min[9]. Three numbers of treated shoots were then kept in a conical flask of 50 mL having water inside it by wrapping with absorbent cotton[10].

Two numbers of 6 h starved adult *H. theivora* were released on the treated shoots and covered with glass chimney and one end of which was covered with nylon net. There were three replications for each treatment and control. The insects were then allowed to feed for 24 h and the numbers of feeding spots were recorded. The shoots in control were sprayed the acetone only. The average data from triplicate observation were statistically analyzed by ANOVA test.

Fraction of the crude extract: The most effective solvent extracts were chromatographed on a silica gel column with the solvent ethyl acetate. The solvents of all the fractions (I-VI) were allowed to evaporate and the residues were taken as stock. The stocks were dissolved in acetone to form a desired concentration (0.5 %) for further studies. The qualitative preliminary phytochemical studies[11] were performed for the most effective extract. All the reagents used were of analytical grade.

RESULTS AND DISCUSSION

Methanolic extract of *Heliotropium indicum* leaf showed more activity than petroleum ether and ethyl acetate extract against *Helopeltis theivora* (Table-1). The number of spots produced due to methanolic extract at 4 % was 17.67, indicates more effectiveness than other concentration as compared with control. Interestingly found that in increase of concentration, the extract was showing more activity. The number of spots produced by other two solvent extracts *i.e.*, petroleum ether and ethyl acetate at 4.0, 2.0, 1.0 and 0.5 % concentrations were 22.00, 22.67, 25.67, 33.33 and 19.67, 22.00, 25.67, 32.33, respectively.

The most effective methanolic extract was then column chromatographed, where several fractions were collected. Of those, fraction **V** was most effective and then fraction **VI** as compared to others (Table-2). The fractions **V** and **VI** were then subjected for TLC study using benzene as mobile phase and silica gel-G as stationary phase. Two pure spots were observed. One spot of grey colour in a iodine chamber was observed in fraction **V** having R_f value 0.84. Two spots of pale grey and grey colour were observed in fraction **VI** having R_f value 0.72 and 0.84, respectively. Further the fraction **VI** was allowed to run a column having mobile phase carbon

tetra chloride: distilled water (8:1) and two bands distinctly appeared, which were then collected and their R_f value measured again. The R_f value recorded was 0.72 and 0.84 separately as single spot.

TABLE-1
ANTIFEEDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF LEAVES OF *H. indicum* AGAINST TEA MOSQUITO BUG

Concentrations (%)	No. of spots produced after 24 h (mean ± SE)		
	Petroleum ether	Ethyl acetate	Methanol
4.0	22.00 ± 0.58	19.67 ± 0.33	17.67 ± 0.33
2.0	22.67 ± 0.67	22.00 ± 1.00	20.33 ± 0.33
1.0	25.67 ± 0.33	25.67 ± 0.33	23.33 ± 0.33
0.5	33.33 ± 0.33	32.33 ± 0.88	30.33 ± 0.33
0.0	99.33 ± 0.33	01.67 ± 0.88	00.00 ± 0.58
CD (p = 0.01)	3.20	4.12	3.06

Each figure is the mean ± SE for 3 replicates. Significant at 1 % level since ($p < 0.01$).

TABLE-2
ANTIFEEDANT ACTIVITY OF DIFFERENT FRACTIONS OF METHANOLIC EXTRACTS OF LEAVES OF *H. indicum* AGAINST TEA MOSQUITO BUG

Fractions	No. of spots produced after 24 h (mean ± SE)
I	99.00 ± 1.15
II	82.33 ± 1.45
III	97.66 ± 1.20
IV	86.33 ± 0.66
V	16.67 ± 0.33
VI	26.67 ± 0.88
Control	97.66 ± 0.67

Antifeedant and repellent properties of *P. glabra*, *L. camara* and of some other indigenous plant extracts against *H. theivora* were reported[12,13]. Antifeedant activity reduces pest damages to products even without killing the pest. So, the antifeedant activity can therefore be incorporated in to other insect control techniques in the strategy of integrated pest management control (IPM).

The results of preliminary phytochemical analysis of methanolic leaf extract of *H. indicum* showed the presence of alkaloid, tannin and fixed oil. Tannin combine with protein inhibit the enzyme activity and reduce the availability of protein in haemolymph insect[14]. Tannins in the plant extract also have mosquito larvicidal activity[15]. The alkaloid present in *P. harmala* is responsible for toxic effect against *T. castaneum*[6].

Ultimately two fractions of methanolic extract of leaves of *H. indicum* were showing activity against *H. theivora*. Among these two fraction one *i.e.*, fraction **V** was pure, as observed in TLC and another one *i.e.*, fraction **VI** was the mixture of two component, where one spot was showing R_f value as fraction **V**. Therefore the activity of fraction **VI** might be due to the presence of fraction **V** and it was showing

less activity than fraction **V** because of the presence of another component showing R_f value 0.72.

Now, it should be the further approach to evaluate chemical properties and structure of fraction **V** of the methanolic extract of the leaves of *H. indicum*.

REFERENCES

1. G.S. Dhaliwal and R. Arora, Principles of insect pest management, Kalyani Publishers, Ludhiana, p. 107 (1998).
2. M. Jacobson, Agricultural Hand Book, US Department of Agriculture, Washington D.C., p. 138 (1975).
3. M.F. Balandrin, *Science*, **228**, 1154 (1985).
4. R.L. Rawls, *Chem. Eng. News*, **22**, 2124 (1986).
5. K. Sukamar, M.J. Perich and L.R. Boobar, *J. Am. Mosq. Control Ass*., **7**, 210 (1991).
6. R. Jbilou, A. Ennabili and F. Sayah, *Afr. J. Biotechnol*., **5**, 936 (2006).
7. S. Prasad, Fundamentals of Biostatistics (Biometry), Emkay Publications, p. 70 (1992).
8. N. Roychoudhury, *J. Appl. Zool. Res*., **5**, 13 (1994).
9. N.D. Pandey, L. Singh, Y.P. Singh and E.A. Triparthi, *Indian J. Ent*., **49**, 238 (1987).
10. R.M. Moraes-Cerdeira, J.V. Krans, J.D. McChesney, A.M.S. Pereira and S.C. Franca, *HortSci.*, **30**, 1082 (1995).
11. S.B. Gokhale, C.K. Kokate and A.P. Purohit, Pharmacognosy, Nirali Prakashan, Pune, p. 65 (1997).
12. M.K. Deka, M.K. Karan Singh and R. Handique, *Indian J. Agric. Sci*., **68**, 274 (1998).
13. I. Gogoi, I. Rahaman and A.K. Dolui, *J. Ent. Res*., **27**, 321 (2003).
14. B.G. Chan, A.C. Waiss, R.G. Binder and C.A. Elliger, *Entomol. Exp. Appl*., **24**, 94 (1982).
15. V. Gopieshkhanna and K. Kannabiran, *African J. Biotech*., **6**, 631 (2007).

(*Received*: 22 September 2009; *Accepted*: 22 March 2010) AJC-8558