

BIOLOGICAL STUDIES OF *CONYZA* AND *EUPHORBIA* SPECIES

Bashir Ahmad Chaudhry, Khalid Hussain Janbaz, Muhammad Uzair and
Abdul Subhan Ejaz

Department of Pharmacy, Bahauddin Zakariya University, Multan.

Abstract: As a part of our research programme into the exploitation of natural plant sources for their biological activities, we have studied three species namely *Conyza bonariensis* (Compositae), *Euphorbia prostrata* and *Euphorbia helioscopia* (Euphorbiaceae). The arial parts of each species were extracted successively with petroleum ether, dichloromethane and methanol. In the biological screening, brine shrimp toxicity was observed in petroleum ether extracts of all the three species. Dichloromethane extract of *E. prostrata* and methanol extract of *C. bonariensis* displayed significant antifungal activity against *Cladosporium cucumerinum*. Dichloromethane and methanol extracts of *C. bonariensis* and methanol extract of *E. helioscopia* showed antibacterial activity against *Sarcina leutea*. Cholinergic activity was observed in the methanol extract of *E. prostrata* as well as in the dichloromethane and methanol extracts of *C. bonariensis*.

Keywords: Screening, biological activities, *Conyza* and *Euphorbia* species.

INTRODUCTION

Three species namely *Conyza bonariensis* (Compositae), *Euphorbia prostrata* and *Euphorbia helioscopia* (Euphorbiaceae) has been selected for the present study. The genus *Conyza* composed of 50 species which are found on the tropical Himalaya from Nepal to Sikkim, extending to Assam, Khasia hills, Chittagong and Burma. The species *C. bonariensis* is distributed in many parts of the Punjab province along the edges of roads, gardens and maize lands. The genus *Conyza* is found to be very rich in terpenoids like celarodanes [Zdero *et al.* 1990], sesquiterpenes [Bohlmann and Wagner 1982] and diterpenes [Ahmad *et al.* 1992, Mata *et al.* 1997]. Spasmolytic and antiinflammatory activities are shown by some of the species containing these secondary metabolites [De-las *et al.* 1998].

The genus *Euphorbia* considered to be one of the largest genera of the family Euphorbiaceae and it includes 2000 species. Phytochemical reports on the species belonging to this Genus showed the presence of secondary metabolites such as flavonoids [Yoshida *et al.* 1994] and tannins [Amakura *et al.* 1997] but only a few have been studied for their biological activities. Moreover, the biological effects of *Euphorbia prostrata* and *E. helioscopia* have not been studied so far.

MATERIALS AND METHODS

PLANT MATERIAL

The aerial parts of *Conyza bonariensis*, *Euphorbia prostrata* and *Euphorbia helioscopia*, were collected from the surroundings of the Bahauddin Zakariya University, Multan. The plant materials were

identified by Altaf Ahmad Dasti, Associate professor, Department of Botany, B. Z. University, Multan.

EXTRACTION

The air-dried plant materials were ground and extracted successively with petroleum ether, dichloromethane and methanol (thrice with each solvent). The extraction was carried out at room temperature and with occasional shaking for 24 hrs.

BIOLOGICAL METHODS

Antifungal Assay

The antifungal test against *Cladosporium cucumerinum* was carried out on a TLC plate. After developing with suitable solvent system, the TLC plates were well dried with an air dryer in order to remove the solvent completely. The developed and dried TLC plates were sprayed with a conidial suspension of *C. cucumerinum* in a nutrition medium and incubated in moist atmosphere for 2-3 days. Inhibition of the fungal growth was observed as clear zones on the chromatogram that indicates the presence of antifungal compounds.

Brine Shrimp Toxicity Test

Eggs of brine shrimp were incubated in a petri dish containing artificial seawater for 24 hrs. Hatched larvae were transferred into a second petri dish and incubated for another 24 hrs to allow for molting to 2nd instar. This process allows to obtain a homogenous population of 2nd instar larvae. Extracts were solubilized in DMSO with a maximum concentration of 50 µg/ml. The larvae were counted into groups of 10 and placed in 1 ml of artificial seawater to which had been added the sample to be tested in DMSO. Survival was evaluated after incubation at 25°C for 24 hrs by observing under dissection microscope.

Cholinergic Activity

The cholinergic activity of the plant extracts was studied by using isolated guinea-pig ileum and rabbit jejunum preparations. Guinea-pig (500-600 g) of a local breed and of either sex were used for this study. Animals had free access to water but food was withdrawn 24 hrs prior to experiment. Guinea-pig were killed by cervical dislocation and gut segments of 2 cm long were suspended in Tyrode's solution bubbled with a mixture of 95% oxygen and 5% carbon dioxide and maintained at 37°C. Intestinal responses were recorded isotonicly using Bioscience transducers and an oscillograph. Under these experimental conditions, guinea-pig's ileum behaved as a quiescent preparation and is considered more useful for detecting cholinergic activity. Each tissue was allowed to equilibrate for at least 30 minutes before the addition of any drug. Acetylcholine was used as the positive control as spasmogenic agent [Gilani *et al.* 2000].

Antibacterial Activity

Antibacterial activity was determined by an "Agar Well Diffusion method" on the already prepared plates of the inoculated media. Three wells of 8mm diameter on one plate were cut with a borer and sealed with a drop of inoculated sterile media. All the solutions (0.1 ml each) i.e. the extract, solvent and reference standard (ampicillin) were poured into their respective well by sterilized pipette. The petri dishes were incubated at 37°C for 24-48 hrs. Zones of inhibition were measured with vernier calipers.

RESULTS AND DISCUSSION

BIOLOGICAL SCREENING

Each of the extracts was submitted to a battery of biological tests including cholinergic activity, brine shrimps toxicity, antibacterial and antifungal activity. Antifungal activity against *Claudosporium cucumerinum* was determined by a bioautographic assay. Antibacterial activities against *Sarcina leutea* and *Escherchia coli* were determined by Well diffusion method. The protocol of each test is given in the experimental part. Brine shrimp toxicity was observed in all the petroleum ether extracts. All the petroleum ether extracts were devoid of antifungal and antibacterial activities. Methanol extracts of all the species exhibited no toxicity to brine shrimps. Dichloromethane extract of *E. prostrata* and methanol extract of *C. bonariensis* showed antifungal activity against *C. cucumerinum*. Dichloromethane extract of *E. prostrata* also exhibited cholinergic activity. Antibacterial and cholinergic activities were observed in the dichloromethane and methanol extracts of *C. bonariensis*. Antibacterial activity was also found in the methanol extract of *E. helioscopia*. The details of the results of biological screening of petroleum ether, dichloromethane and methanol extracts of the three plants are summarized in Tables 1, 2 and 3.

Table 1: Results of the biological studies of petroleum ether extracts of the arial parts of *Conyza bonariensis*, *Euphorbia prostrata* and *E. helioscopia*.

Name of plant	Cholinergic activity	Antifungal activity (100 µg)**	Antibacterial activity* (10 mg/ml)	Brine shrimp toxicity
<i>Conyza bonariensis</i>	-ive	-ive	-ive	+ive
<i>Euphorbia prostrata</i>	-ive	-ive	-ive	+ive
<i>Euphorbia helioscopia</i>	-ive	-ive	-ive	+ive

* against *Sarcina leutea*.

** amount applied on TLC plate

Table 2: Results of the biological studies of dichloromethane extracts of the arial parts of *Conyza bonariensis*, *Euphorbia prostrata* and *E. helioscopia*.

Name of plant	Cholinergic activity	Antifungal activity (100 µg)	Antibacterial activity (10 mg/ml)	Brine shrimp toxicity
<i>Conyza bonariensis</i>	+ive	-ive	+ive	-ive
<i>Euphorbia prostrata</i>	-ive	+ive	-ive	-ive
<i>Euphorbia helioscopia</i>	-ive	-ive	-ive	+ive

Table 3: Results of the biological activities of methanol extracts of the arial parts of *Conyza bonariensis*, *Euphorbia prostrata* and *E. helioscopia*.

Name of plant	Cholinergic activity	Antifungal activity (100 µg)	Antibacterial activity (10 mg/ml)	Brine shrimp toxicity
<i>Conyza bonariensis</i>	+ive	+ive	+ive	-ive
<i>Euphorbia prostrata</i>	+ive	-ive	-ive	-ive
<i>Euphorbia helioscopia</i>	-ive	-ive	+ive	-ive

Acknowledgements

We are highly grateful to Professor Dr. Anwar-ul-Hassan Gilani, Department of pharmacology, Aga Khan Medical University, Karachi for the evaluation of cholinergic activity. The antifungal activity and brine shrimp toxicity were determined in collaboration with Professor Dr. Kurt Hostettmann, School of Pharmacy, University of Lausanne, Switzerland. Financial support was provided by the B. Z. University, Multan.

References

- Ahmad, V.U., Khatoon, R.T., Farooqi, A., Ismail, N. and Fizza, K. (1992) *Nat. Prod. Lett.*, 1, 225-232.
- Amakura, Y., Kawata, K., Hatano, T., Agata, I., Nishibe, S., Okuda, T. and Yoshida, T. (1997) *Can. J. Chem.*, 75, 727-733.
- Bohlmann, F. and Wagner, P. (1982) *Phytochemistry*, 21, 1693- 1695.
- De-las, H., Slowing, B.K., Benedi, J., Carretero, E., Toledo, C. and Villar, A. (1998) *J. Ethnopharmacol.*, 61, 161-166.
- Gilani, A.H., Aziz, N., Khurram, I.M., Rao, Z.A. and Ali, N.K. (2000) *Phytother. Res.*, 14, 1-7.
- Mata, R., Rojas, A. and Linares, E. (1997) *Planta Med.*, 63, 31-35.
- Yoshida, T., Amakura, Y., Liu, Z. and Okuda, T. (1994) *Chem. Pharm. Bull.*, 42, 1399-1409.
- Zdero, C., Ahmad, A.A., Bohlmann, F. and Mungai, G.M. (1990) *Phytochemistry*, 29, 3167-3172.