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Antimicrobial activity and cytotoxicity of *Eclipta prostrata*

Mohammad S Rahman[1] and Mohammad A Rashid[1,2,*]

[1]Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh; [2]Centre for Biomedical Research, University of Dhaka, Dhaka-1000, Bangladesh

SUMMARY

The plant *Eclipta prostrata*, a member of the Compositae family, has folkloric reputation of being used as a medicinal agent in Bangladesh. In the present investigation, attempt was taken to explore the antimicrobial potency and cytotoxicity of its extractives and purified compounds. The methanolic extract of the whole plant, its *n*-hexane, carbon tetrachloride, chloroform, aqueous soluble fractions and two purified compounds, eclalbasaponin I (1) and II (2), obtained from *Eclipta prostrata* were subjected to screening for inhibition of microbial growth by the disc diffusion method at 300 and 100 μg/disc for extracts and pure compounds, respectively. In this case, the carbon tetrachloride and chloroform soluble fractions of the methanolic extract appeared very potent in terms of both zone of inhibition and spectrum of activity. However, all the extractives were also subjected to brine shrimp lethality bioassay for preliminary cytotoxicity evaluation. Here, the carbon tetrachloride soluble fraction of methanolic extract revealed the strongest cytotoxicity having LC_{50} of 1.318 μg/ml.

Key words: *Eclipta prostrate*; Compositae; Antimicrobial; Cytotoxicity

INTRODUCTION

With centuries of tireless efforts people have found in plant kingdom the remedy to diverse diseases (Farnsworth, 1990). Bangladesh is a good repository of medicinal plants.

Herbal medicine represents one of the most important fields of traditional medicine in Bangladesh. Thus, phytotherapy is practiced by a large proportion of people of Bangladesh for the treatment of several physical, physiological and mental problems (Rashid *et al*., 1997; Rahman *et al*., 2001). To promote the proper uses of herbal medicine and determine their potential as sources for new drugs it is essential to study medicinal plants, which have folkloric reputation.

Within the recent years, infections have increased to a great extent and antibiotics resistance becomes an ever-increasing therapeutic problem (Austin *et al*., 1999). Natural products from higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action (Hamil *et al*., 2003; Motsei *et al*., 2003). For the initial *in vitro* antimicrobial susceptibility test, the disc diffusion method has been proved to be very efficient and popular (Cowan, 1999). On the other hand, due to the shortcomings of the available modern drugs to treat cancer, research is going on globally to develop more effective, safer and cheaper drugs from plant sources (Cox, 1994). The presence of

*Correspondence: Mohammad A Rashid, Phytochemical Research Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh. Tel: +88028612069, 966190073, Ext. 4363, 4364, 8137; Fax: +88028612069; E-mail: rashidma@aitlbd.net

antitumor and antiproliferative principles in a plant extract can be evaluated by cytotoxicity screening, since it is a standard marker for anticancer activity (Persoone, 1980). Meyer *et al*. (1982) established a positive correlation exists between brine shrimp lethality and 9KB (human nasopharyngeal carcinoma) cytotoxcity. Brine shrimp lethality bioassay is therefore used in many prescreens for potential anti-tumor activity. In addition, this bioassay effectively predict pesticidal activities and respond to a broad range of chemically and pharmocologically diverse compounds (McLaughlin, 1991). In this line, we have been studying indigenous plants of Bangladesh to explore their antimicrobial potency and cytotoxicity in order to discover novel drug candidates.

Eclipta prostrata (Bengali name-Kalokeshi; Family-Compositae) is a herbaceous multibranched annual weed of moist places with long lanceolate leaves, hirsute stem and white flowers in axillary heads, grows all over Bangladesh (Ghani, 2003). The whole plant is astringent, depurative, emetic, febrifuge, ophthalmic, purgative, styptic and tonic. It is used internally in the treatment of dropsy and liver complaints, anemia, diphtheria, tinnitus, tooth loss and premature graying of the hair (Wagner *et al.,* 1986; Saraf *et al*., 1991; Chopra, 1992; Singh *et al*., 1993). Externally, it is used as an oil to treat hair loss and is also applied to athlete's foot, eczema, dermatitis, wounds etc. (Chevallier, 1996). The plant juice, mixed with an essential oil, is used in the treatment of catarrhal problems and jaundice. The roots are emetic and purgative as well as antiseptic. They are applied externally as an antiseptic to ulcers and wounds, especially in cattle (Chopra, 1992). Besides, the plant was reported to act as nervine tonic (Satyavati *et al*., 1976; Vaidya, 1997; Uniyal *et al*., 1998). Previous phytochemical investigations of *E. prostrata* revealed the occurrences of thiophene derivatives (Dictionary of natural products, 2002), glycosides (Yahara *et al*., 1994; Rahman *et al*., 2006), alkaloids (Abdel-kader *et al*., 1998) and triterpenes (Upadhyay *et al.,* 2001). In this paper, we report the preliminary antimicrobial activity and cytotoxicity of *E. prostrata* extractives and two purified compounds eclalbasaponin I (1) and II (2).

MATERIALS AND METHODS

Experimental

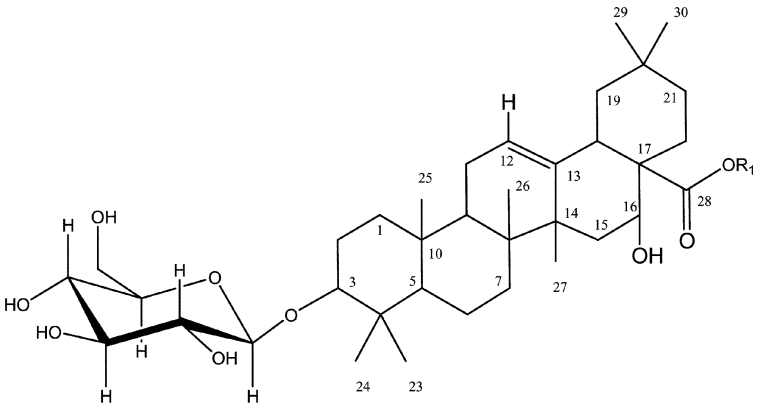
The ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument. The ^{13}C NMR spectra were acquired on the same instrument at 100 MHz while the 2D-NMR spectra were recorded on a Bruker 400 MHz instruments using the standard microprograms. For NMR studies deuterated chloroform and methanol were used and the δ values for ^{1}H and ^{13}C spectra were recorded in respect to the residual non-deuterated solvent signals. Fast atom bombardment mass spectra (FAB-MS) were obtained as a JEOL SX102 spectrometer.

Plant materials

The whole plant of *E. prostrata* was collected from Savar in the month of August 2004. A voucher specimen has been maintained for this collection in Bangladesh National Herbarium (ACC-31253), Dhaka, Bangladesh.

Extraction

The powdered plant (1.00 kg) of *E. prostrata* was soaked in 1.5 l of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator and a portion (5 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol (Vanwagenen *et al*., 1993) into *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions. Subsequent evaporation of solvents afforded *n*-hexane (0.550 g), carbon tetrachloride (1.50 g), chloroform (1.50 g) and aqueous soluble materials. The *n*-hexane soluble fraction was then chromatographed by column chromatography (CC) over silica gel (60 - 120 mesh) using *n*-hexane, ethyl acetate and methanol mixtures of increasing



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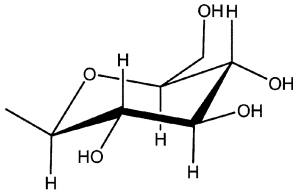

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Table 1. Antimicrobial activity of of *E. prostrata* extractives (300 μg/disc) and Kanamycin (30 μg/disc)

Test microorganisms	Diameter of zone of inhibition (mm)				
	MEWP	CTSF	CFSF	AQF	KAN
Gram positive bacteria					
Bacillus cereus	-	11.23 ± 0.11	14.17 ± 0.15	13.23 ± 0.23	25.30 ± 0.20
Bacillus megaterium	10.0 ± 0.10	13.26 ± 0.05	9.20 ± 0.26	11.40 ± 0.20	24.17 ± 0.15
Bacillus subtilis	9.00 ± 1.00	14.33 ± 0.30	13.47 ± 0.21	15.23 ± 0.25	26.30 ± 0.20
Staphylococcus aureus	8.10 ± 0.17	11.16 ± 0.15	15.37 ± 0.15	12.37 ± 0.15	25.30 ± 0.26
Sarcina lutea	9.13 ± 0.05	12.40 ± 0.26	14.27 ± 0.42	11.43 ± 0.06	23.27 ± 0.23
Gram negative bacteria					
Escherichia coli	-	10.23 ± 0.40	11.30 ± 0.17	8.97 ± 0.15	25.17 ± 0.21
Pseudomonas aeruginosa	9.16 ± 0.05	9.00 ± 0.11	10.00 ± 0.20	16.20 ± 0.63	25.33 ± 0.25
Salmonella paratyphi	8.13 ± .015	15.30 ± 0.17	13.30 ± 0.17	12.10 ± 0.26	23.10 + 0.10
Salmonella typhi	-	11.40 ± 0.15	14.30 ± 0.17	10.13 ± 0.31	24.33 ± 0.15
Shigella boydii	8.23 ± 0.30	12.26 ± 0.25	16.30 ± 0.26	15.33 ± 0.12	20.17 ± 0.25
Shigella dysenteriae	-	17.26 ± 0.20	18.27 ± 0.12	13.13 ± 0.21	24.07 ± 0.38
Vibrio mimicus	8.16 ± 0.20	16.33 ± 0.15	15.43 ± 0.21	12.17 ± 0.31	25.17 ± 0.31
Vibrio parahemolyticus	-	14.13 ± 0.20	17.07 ± 0.25	11.43 ± 0.21	24.27 ± 0.06
Fungi					
Candida albicans	9.16 ± 0.15	16.36 ± 0.20	12.07 ± 0.25	15.20 ± 0.20	24.43 ± 0.38
Aspergillus niger	11.10 ± 0.17	14.36 ± 0.11	17.23 ± 0.29	15.23 ± 0.47	22.27 ± 0.15
Sacharomyces cerevaceae	10.23 ± 0.05	13.43 ± 0.11	13.10 ± 0.30	14.20 ± 0.40	24.27 ± 0.29

The diameter of zone of inhibition are expressed as mean ± S.D. (n = 3); a diameter less than 8 mm was considered inactive; MEWP: methanolic extract of the whole plant; CTSF: carbon tetrachloride soluble fraction of the methanolic extract; CFSF: chloroform soluble fraction of the methanolic extract; AQF: Aqueous fraction of the methanolic extract; KAN: standard kanamycin.

polarities to give a total of 61 fractions, each 25 ml. Preparative thin layer chromatography (stationary phase- silica gel F_{254}, mobile phase- 8% methanol in chloroform, thickness of plates - 0.5 mm) of fraction 53 afforded needle shaped crystals of eclalbasaponin I (1). Similar purification of fraction 55 using 10% methanol in chloroform provided eclalbasaponin II (2).

Bioassays

The antimicrobial activity of the extractives was determined by the disc diffusion method (Bauer *et al*., 1966). The samples were dissolved separately in specific volume of solvent (chloroform or methanol) and applied to sterile discs at a concentration of 300 and 100 µg/disc for crude extracts and pure compounds, respectively and carefully dried to evaporate the residual solvent. The investigation was carried out in triplicate.

For cytotoxicity screening, DMSO solutions of the plant extracts were applied against *Artemia salina* in 1 day *in vivo* assay, the experimental details of which could be found elsewhere (Meyer *et al*., 1982). For the experiment, 4 mg of each of the Kupchan fractions was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/ml were obtained by serial dilution technique. The median lethal concentration LC_{50} of the test samples after 24 h was obtained from a plot of percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration). The bioassay was conducted in triplicate.

Statistical analysis

For each of the extracts, three samples were prepared for each of the bioassays. The zone of inhibition and LC_{50} were calculated as mean ± S.D. (n=3) for the antimicrobial screening and brine shrimp lethality bioassay, respectively.

RESULTS

The extractives of *E. prostrata* demonstrated varying degrees of inhibition to growth of microorganisms and strong cytotoxic activity against *A. salina*. The methanolic extract of the whole plant, its *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions and two purified compounds, eclalbasaponin I (1) and II (2), obtained from *E. prostrata* were subjected to screening for inhibition of microbial growth against 13 bacteria and 3 fungal strains. Table 1 represents the summary of the antimicrobial activities of the samples with respect to each of the test organisms. The average zones of inhibition produced by the crude methanol extract of the whole plant and its carbon tetrachloride, chloroform and aqueous soluble fractions were 8 - 11, 9 - 17, 9 - 18 and 9 - 16 mm, respectively. However, the *n*-hexane soluble partitionate and eclalbasaponin I and eclalbasaponin II showed no significant antimicrobial activity. On the other hand, the cytotoxcity of the samples was evaluated against *A. salina* (Table 2) where significant effects were found by the carbon tetrachloride, chloroform and aqueous soluble fractions of the crude methanol extract with LC_{50} values of 1.318, 2.344 and 3.311 µg/ml, respectively.

Table 2. LC_{50} data of test samples of *E. prostrata*

Samples	LC_{50} (µg/ml)
VS (positive control)	0.212 ± 0.10
MEWP	16.595 ± 0.25
HSF	10.471 ± 0.40
CTSF	1.318 ± 0.30
CFSF	2.344 ± 0.20
AQF	3.311 ± 0.90
Eclalbasaponin I	16.21 ± 0.35
Eclalbasaponin II	17.78 ± 0.21

The values of LC_{50} are expressed as mean ± S.D. (n = 3). VS: vincristine sulphate (Std.); HSF: *n*-hexane soluble fraction of the methanolic extract; CTSF: carbon tetrachloride soluble fraction of the methanolic extract; CFSF: chloroform soluble fraction of the methanolic extract; AQF: aqueous fraction of the methanolic extract.

DISCUSSION

The carbon tetrachloride, chloroform and aqueous

soluble fractions of the methanolic extract *E. prostrata* exhibited mild to moderate inhibitory activity against the tested microorganisms, while the methanolic crude extract of the whole plant demonstrated mild activity against most of the microbial strains (Table 1). However, the *n*-hexane soluble partitionate and the two purified compounds (eclalbasaponin I and eclalbasaponin II) isolated from *n*-hexane soluble fractions showed no significant antimicrobial activity (data not shown on table). The carbon tetrachloride soluble partitionate of the methanolic extract strongly inhibited the growth of *S. dysenteriae* (17.26 mm), whereas *V. mimicus* (16.33 mm), *S. paratyphi* (15.30 mm), *V. parahemolyticus* (14.13 mm), *B. subtilis* (14.33 mm), *B. megaterium* (13.26 mm), *S. lutea* (12.40 mm) and *S. boydii* (12.26 mm) was moderately inhibited. Again, the chloroform soluble partitionate strongly inhibited the growth of *S. dysenteriae* (18.27 mm) and *V. parahemolyticus* (17.07 mm). It also showed moderate activity against *S. boydii* (16.30 mm), *V. mimicus* (15.43 mm) *S. aureus* (15.37 mm) and *B. subtilis* (13.47 mm). The aqueous fraction showed strong inhibitory activity against the growth of *P. aeruginosa* (16.20 mm), *B. boydii* (15.33 mm) and *S. subtilis* (15.23 mm), whereas moderate activity was found against *B. cereus* (13.23 mm), *S. dysenteriae* (13.13 mm), *S. aureus* (12.37 mm), *V. mimicus* (12.17 mm) and *S. paratyphi* (12.10 mm).

In case of fungi, the carbon tetrachloride soluble fraction showed moderate to strong inhibitory activity having the average zone of inhibition 13 - 16 mm. On the other hand, chloroform soluble fraction strongly inhibited the growth of *A. niger* (17.23 mm). The aqueous fraction revealed moderate to strong inhibitory activity against the fungal growth (14 - 15 mm).

Following the procedure of Meyer *et al*. (1982), the lethality of the methanolic extract of the whole plant (MEWP), *n*-hexane soluble fraction (HSF), CCl_4 soluble fraction (CTSF), $CHCl_3$ soluble fraction (CFSF), aqueous fraction (AQF) of the methanolic extract and pure eclalbasaponin I and II to brine shrimp was determined and the results are summarized in the Table 2. The LC_{50} obtained from the best-fit line slope were 16.595, 10.47, 3.311, 2.344 and 1.318, 16.21 and 17.78 μg/ml for MEWP, HSF, AQF, CLSF, CTSF, eclalbasaponin I and II, respectively. In comparison with the positive control (vincristine sulphate), the cytotoxicity exhibited by the HSF, CTSF, CLSF and AQF was significant. This clearly indicated the presence of potent bioactive priciples in these extractives which might be very useful as antiproliferative, antitumor, pesticidal and other bioactive agents (Meyer *et al*., 1982; McLaughlin, 1991).

As the *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions of the methanolic extract showed potent cytotoxicity and the carbon tetrachloride, chloroform and aqueous soluble fractions of the methanolic extract exhibited significant antimicrobial activity, these bioactivities substantiate the folk use of *E. prostrata* in various diseases.

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