Asian Journal of Chemistry Vol. 22, No. 9 (2010), 7336-7342

Phytochemical Screening of Ethanolic Extract and Antibacterial Activity of *Eclipta prostrata*

SUNITA DALAL* and SUDHIR K. KATARIA†
Department of Biotechnology, Kurukshetra University, Kurukshetra-136 119, India
E-mail: sudhir_mdu@rediffmail.com

Aerial parts of *Eclipta alba* are used traditionally for the treatment of several diseases of liver, skin and stomach. Ethanolic extract and active principle compound of a well known Indian hepatoprotective herb, *Eclipta prostrata* was tested for *in vitro* antimicrobial studies. It was evaluated using zone of inhibition studies and minimum inhibitory concentration. The extract exhibited activity against all 8 strains studied. Phyto-chemical screening of the extract revealed the presence of tannins, flavonoids, coumestans, saponins, alkaloids, *etc*. Ethyl acetate fraction and further pure isolated wedelolactone showed enhanced antimicrobial activity. *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Salmonella typhimurium* were most susceptible. *Shigella flexneri and Bacillus cereus* were the most resistant bacterial strains. These results suggest wedelolactone as a promising antimicrobial agent.

Key Words: *Eclipta alba*, Wedelolactone, Antimicrobial activity.

INTRODUCTION

The modern medicine has evolved from folk medicine and traditional system only after thorough chemical and pharmaceutical screening. The use of synthetic compounds led to a decline in the use of plants in modern medicine. However, synthetic medicine can cause side effects and as a result people are more favourable to use natural compounds obtained from plants. Thus, plants remain a major source of medicinal compounds. About 20,000 plant species are used for medicinal purposes[1]. Seventy four per cent of 119 plant-derived drugs were discovered as a result of chemical studies to isolate the active substances responsible for their traditional use[2]. So plants, especially the higher plants contain a variety of substances, which are useful as food additives, perfumes and in treatment of various diseases as medicine due to their versatile therapeutic potential[3]. The active secondary metabolites possess various medicinal applications as drugs or as model compounds for drug synthesis. Phytochemical analysis of plants, used in folklore has yielded a number of compounds with various pharmacological activities. In view of the increasing development of resistant microorganisms, treatment of various diseases caused by microorganisms has become a major challenge in the human medical field.

†Department of Zoology (Bioscience), Maharshi Dayanand University, Rohtak-124 001, India.

This may be due on the one hand, to the synthetic nature of these substances, but also to their known side effects and in some cases to their unpleasant smell, taste or the burning sensation felt on the skin. Medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new antiinfectious agents. About 3,000 materials from 2,764 plant species have been screened for their pharmacological and chemotherapeutic properties[4]. India, in particular has yielded an incredible array of plant products that have drawn the attention of ethno pharmacologists from around the world. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties[5-7].

Various biological activities are possessed by *Eclipta* species, such as memory disorders treatment, general tonic, edema, fevers and rheumatic joint pains treatment, digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders[8-10]. Wedelolactone is active principle compound of this liver disorder treating drug[11]. It also exhibits trypsin inhibitory effect[12,13], suppresses LPS-induced caspase-11 expression in cultured cells by directly inhibiting the IKK complex[14], treatment of cirrhosis of the liver and infectious hepatitis[15]. The shoot extract of the plant showed antimicrobial[16-18], antifungal activity[19] and weak cytotoxicity against the M-109 cell lines by alkaloids verazine[20], antiviral activity against Ranikhet disease virus[21], effective against internal and external parasites[22], *G. intestinalis*[23] and a potent antibacterial[24]. Since It is a weed/herb growing in dump, moist puddles distributed in the tropical and subtropical regions of the world. So besides ethnobotanical evidence, it can be hypothesized that plants which survive in media rich in microbes most likely be possessing antimicrobial principles.

However, up to date, research has been done to investigate various pharmacological activities and antimicrobial activity of only crude extracts of this traditionally used herb. We report here the findings on antibacterial effects of wedelolactone (Fig. 1), the principle active compound, extracted from *E. prostrata*.

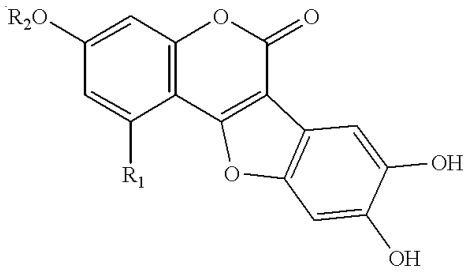

Fig. 1. Chemical structure of wedelolactone (R_1-OH, R_2-CH_3)

EXPERIMENTAL

Collection of plant material: Plants of *E. prostrata* were collected locally from botanical garden and surroundings of Maharshi Dayanand University, Rohtak. The

plant was duly authenticated and voucher specimens were deposited in the herbarium section, Prof. Jaya Parkash Yadav, of Department of Genetics, Maharshi Dayanand University, Rohtak (Haryana) India.

Qualitative estimation of primary and secondary plant metabolites: All estimations were done by reported methods[25,26]. Different aerial parts of the plant were dried at room temperature, powered and extracted with ethanol (70 % v/v) in Soxhlet apparatus for 6 h. The extract was filtered and was tested with different reagents.

Ethanol extract: The three months old 1000 g lyophilized leaves were Soxhlet extracted with ethanol for 36 h.

Ethyl acetate fraction: The ethanol was removed from extract and the residues were suspended in water separately and heated on steam bath below 80 °C for 0.5 h. After filtration, the aqueous phase was partitioned with ethyl acetate. The organic phase was dried, filtered and the solvents were evaporated to yield 7.2 g light brown powder.

Isolation of wedelolactone: The powder was subjected to fractionation by column chromatography on silica gel, eluted with the solvent of increased polarity *i.e.*, non-polar-polar-highly polar. The coumestans are polar compounds so the solvent combination found suitable for their elution was chloroform + methanol (70 + 30). They were eluted simultaneously in 37-48 fractions. The pooled sample was then subjected to TLC, the solvent system (toluene:acetone:formic acid::11:6:1 v/v) showed two spots with R_f values 0.39 and 0.28 which matched with the R_f values of reference wedelolactone and demethyl wedelolactone, respectively (courtesy M/s Natural Remedies, Bangalore, India). The purified sample of wedelolactone was put to HPLC for further qualitative analysis using instrument-Thermo Finnigan from Thermo Electron Corp. USA, with quaternary pump and online degasser system with auto sampler equipped with photo diode array (PDA) detector, ChromQuest Version 5.0 for data interpretation and Supleco C8 Discovery column, 15 cm × 4.6 mm, Lot No. 59353 (Fig. 2).

Preparation of samples for testing: The zone of inhibition studies were conducted with various extracts diluted with 10 % dimethyl sulfoxide.

Micro organisms: Standardized strains from the American/Microbial type culture collection were used in bioassays. *Staphylococcus aureus* (ATCC 9144), *Salmonella typhimurium* (ATCC 23564), *Escherichia coli* (ATCC 10536), *Staphylococcus epidermidis* (ATCC 155), *Shigella flexneri* (ATCC 29508), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 11778) and *Pseudomonas aeruginosa* (ATCC 25668) were cultured at 37 °C on nutrient medium in aerobic conditions for 24 h. These bacteria were obtained from the Institute of Microbial Technology, Chandigarh.

Antimicrobial susceptibility testing: Minimum inhibitory concentration (MIC) of wedelolactone was determined by microdilution technique as described by the National Committee for Clinical Laboratories standards[27]. The MIC was defined as the lowest concentration of the compound to inhibit the growth of microorganism.

The bacteria inoculums were prepared in 5 mL nutrient broth and incubated at 37 °C. The final inoculums were of approximately 5×10^6 CFU/mL. Controls with 0.5 mL of culture medium with out the samples and other without microorganisms were used in the tests. Tubes were incubated at 37 °C for 24 h. The activity was measured as a function of turbidity at 660 nm. Lack of turbidity was further confirmed by pouring suspension aliquot of 0.1 mL into pre-sterilized Petri dishes with nutrient agar medium. The tests were conducted in triplicate.

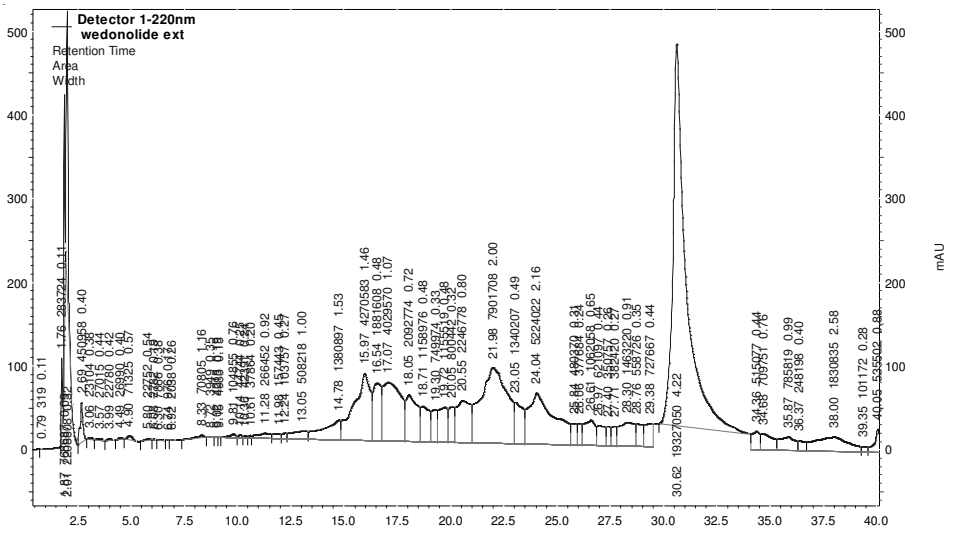

Fig. 2. Chromatogram of extract showing the peaks

Agar well diffusion method was carried out by allowing perforation of extract and wedelolactone dissolved in DMSO at a concentration of 3.5 mg/well and 10 mg/mL, respectively. Petriplate containing 30 mL nutrient agar medium were kept for the solidification before inoculating the microorganism, desired numbers of holes of uniform diameter of 8 mm were made after solidification, using sterile aluminum borer. 0.2 mL of compound, positive (gentamycin) and negative (solvent blank) controls were poured into wells. After incubation for 24 h at 37 °C the plates were observed and the compound activity was evaluated by measuring zone of inhibition (diameter mm). The tests were conducted in triplicate. Gentamycin (10 µg/mL) was used as positive control. The negative control was 10 % DMSO.

RESULTS AND DISCUSSION

The results of the presence of various primary and secondary metabolites in methanol extract (Table-1) reported negative for antraquinones. Ethanol extract and ethyl acetate fraction showed positive signs of antimicrobial activity against all strains (Table-2). Wedelolactone exhibited significant anti-bacterial activity against the tested strains (Table-3). *S. epidermidis* and *S. aureus* were found to be highly sensitive.

TABLE-1
QUANTITATIVE ESTIMATION OF THE VARIOUS PRIMARY AND SECONDARY METABOLITES

Chemical test	Natural plant extract		
	Leaf	Stem	Root
Alkaloids	+	+	–
Coumestans	+	+	+
Anthraquinones	–	–	–
Phenolics	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Proteins	+	+	+
Amino-acids	+	+	+
Reducing sugar	+	+	–
Flavonoids	+	+	+

TABLE-2
ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT AND ETHYL ACETATE FRACTION AGAINST BACTERIAL STRAINS

Extracts tested	Conc. mg/well	*Escherichia coli*	*Salmonella typhimurium*	*Staphylococcus epidermidis*	*Staphylococcus aureus*	*Shigella flexneri*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Bacillus cereus*
Ethanol extract of leaves	3.5	++	+++	++++	+++	+	++	++	+
Ethyl acetate fraction	3.5	++	+++	++++	+++	+	++	++	+

TABLE-3
ANTIBACTERIAL ACTIVITY OF WEDELOLACTONE AND GENTAMYCIN AGAINST BACTERIAL STRAINS

Microorganisms tested	ZOI (mm)		MIC (µg/mL)	
	A	B	A	B
S. epidermidis	10.24	9.26	15	5.0
S. typhimurium	9.16	9.22	25	5.0
S. aureus	9.14	9.24	20	5.0
P.aeruginosa,	8.00	9.06	1250	2.5
S.flexneri	7.60	9.00	1300	5.0
E. coli	8.60	9.06	1000	5.0
B. subtilis	9.00	9.64	500	2.5
B. cereus	8.00	9.62	500	5.0

MIC: Minimum inhibitory concentration; ZOI: zone of inhibition (diameter); A: wedelolactone (10 mg/mL); B: gentamycin (10 µg/mL); values are mean of three replicates.

The primary and secondary metabolites were analyzed in ethanolic extracts. Anthraquinones were found to be absent in the natural plant. While the alkaloids and reducing sugars were absent in root extract of the natural plant. The extracts of the leaves tested positive for steroid, reducing sugars, alkaloids, phenolics, saponins and tannins, but no anthraquinones and flavonoids were detected.

In Gujrat and Punjab, the plant is used externally for ulcers and as an antiseptic for wounds in cattle and is reported to treat many microbial infections in rural areas[28]. The results from the current studies revealed that the wedelolactone may be the main constituent responsible for antimicrobial activity. There are various reports that crude extract from *E. alba* and *E. prostrata* showed antibacterial, antifungal and antiviral activity[18,19]. Wedelolactone exhibited effective antibacterial activity against all the strains studied. It proved highly effective against *S. epidermidis* and *S. typhimurium* demonstrating the specificity of wedelolactone activity.

Another report[10] indicated *in vitro* antimicrobial activities of ethanolic extract of *E. prostrate*. It indicated good activity against *S. aureus* 7.2 mm (zone of inhibition) and MIC 70 µL/mL and for *P. aeruginosa* 8.8 mm (zone of inhibition) and MIC 65 µL/mL, at 50 µg concentration. While the present studies exhibited respective zone of inhibition at 9.14 mm, 8.00 mm and MIC 20 and 1250 µg/mL, respectively. Traditional reports indicate that it is one of the herb used for treatment of stomach and digestion disorders, skin diseases and conjunctivitis[29]. Since *S. typhimurium*, *S. flexneri* and *S. aureus* are pathogens responsible for stomach disorders,while *P. aeruginosa* is common in skin flora and *S. aureus* is responsible for most common bacterial conjunctivitis. The results from the current studies revealed that the wedelolactone could be the main constituent responsible for these treatments as it exhibited good activity against them.

Conclusion

On the basis of the antibacterial studies of wedelolactone, it can be suggested that this can be used effectively to treat *S. epidermidis* and *S. typhimurium* infections. However, the compound must be studied in animal models to determine the efficacy *in vivo* against these pathogens and to elucidate their mechanism of action. *In vivo* data may be helpful in determining the real potential usefulness of this plant for the treatment of infectious diseases.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. T. Velpandian, Ocular Pharmacology Laboratory, All India Institute of Medical Sciences, New Delhi, India for HPLC analysis and the members of Department of Biosciences, M.D. University, Rohtak, India.

REFERENCES

1. R.P. Samy and P. Gopalakrishnakone, Nature Precedings : hdl:10101/npre.2007.1176.1; 28 Sep (2007).
2. N.R. Farnsworth and D.D. Soejarto, in eds.: O. Akerele, V. Heywood, H. Synge, Global Importance of Medicinal Plants, The Conservation of Medicinal Plants, Cambridge University Press, Cambridge, UK, p. 25 (1991).
3. P.K. Mukherjee and A. Wahile, *J. Ethnopharmacol*., **103**, 25 (2006).
4. P. Anon, Patent-Israel, 78(820), 15 (1988).
5. M.A. Iyengar, Bibliography of Investigated Indian Medicinal plants, edn. 1, Manipal College of Pharmacy, Manipal Medical College, pp. 1950-1975 (1976).
6. J.B. Harborne, *Nat. Prod. Rep*., **8**, 85 (1989).
7. R.N. Chopra, S.L. Nayar and I.C. Chopra, In Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research New Delhi, edn. 3, pp. 7-246 (1992).
8. R.N. Chopra, S.L. Nayar and I.C. Chopra, In Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research, New Delhi, India, p. 104 (1956).
9. C.R. Karnick and M. Kulkarni, *Maharasthra Med. J*., **37**, 131 (1990).
10. S. Karthikumar, K. Vigneswari and K. Jegatheesan, *Sci. Res. Essay*, **2**, 101 (2007).
11. H. Wagner, B. Geyer, K. Yoshinobu and S.R. Govind, *Planta Med*., **5**, 370 (1986).
12. S. Samiulla, D. Mundkinajeddu, Y. Shivanna, C. Arun, M. Keerthi, D. Prashanth, A. Amit and B.V. Venkataraman, *Phytoter Res*., **17**, 420 (2003).
13. S.D. Syed, M. Deepak, S. Yogisha, A.P. Chandrashekar, K.A. Muddarachappa, P. D'Souza, A. Agarwal and B.V. Venkataraman, *Phytother Res*., **17**, 420 (2003).
14. M. Kobori, Z. Yang, D. Gong, V. Heissmeyer, H. Zhu, Y.K. Jung, M.A. Gakidis, A. Rao, K. Sekina, F. Ikegami, C. Yuan and J. Yuan, *Cell. Death Differentiation*, **11**, 123 (2004).
15. R.C. Murphy, S. Hammerarstrom and B. Samuelsson, *Proc. Nat. Acad. Sci. (USA)*, 1976, 4275 (1979).
16. Anonymous, The Wealth of India, Raw Materials, CSIR, New Delhi, 3, p. 127 (1952).
17. T. Kosuge, M. Yokota, K. Sugiyama, T. Yamamoto, M. Ni and S. Yan, *Yakugaku Zasshi*, **105**, 791 (1985).
18. C. Wiart, S. Mogana, S. Khalifah, M. Mahan, S. Ismail, M. Buckle, A.K. Narayana and M. Sulaiman, *Fitoteropia*, **75**, 68 (2004).
19. S. Venkatesan and R. Ravi, *Indian J. Pharm. Sci*., **66**, 97 (2004).
20. K.M.S. Abdal, B.D.B. Malone, S. Werkhoven, M.C. Van, T.F. David, J.H. Wisse, I. Bursuker, K.M. Neddermann, S.W. Mamber and D.G. Kingston, *J. Nat. Prod.*, **61**, 1202 (1998).
21. Ma-Ma. Khin, N. Nayunt and M.T. Khin, *Toxico. Appl. Pharmacol.*, **45**, 723 (1978).
22. C. Lans, T. Harper, K. Georges and E. Bridgewater, *BMC Comp. Altern. Med*., **1**, 10 (2001).
23. N. Sawangjaroen, S. Subhadhirasakul, S. Phongpaichit, C. Siripanth, K. Jamjaroen and K. Sawangjaroen, *Parasitol Res*., **95**, 17 (2005).
24. G.S. Kumar, K.N. Jayaveera, A. Kumar, C.K. Sanjay, B.M.V. Swamy and D.V.K. Kumar, *Trop. J. Pharm. Res.*, **6**, 717 (2007).
25. S.S. Hunda, P. Prakash and B. Roy, *Indian J. Pharm. Sci.*, **13**, 50 (1985).
26. P. Brindha, B. Sasikala and K. Purushothaman, *Biotech. Molecul. Biol. Rev.*, **3**, 84 (1981).
27. National Committee for Clinical Laboratories Standards, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standards (M7-A5), edn. 5, Wayne Pa. (2000).
28. P.K. Warrier, Indian Medicinal Plants, Orient Longman Ltd., Chennai Vol-II, p. 350 (1994).
29. S. Poonam and G. Singh, *Phytother. Res.*, **16**, 1 (2002).

(*Received*: 20 February 2010; *Accepted*: 24 June 2010) AJC-8836