

Wedelolactone as an Antibacterial Agent extracted from *Eclipta alba*

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Abstract

Wedelolactone is a naturally occurring coumestan isolated from aerial parts of *Eclipta alba*. *E. alba* is used as a traditional medicine for the treatment of several diseases. The antimicrobial activity of wedelolactone was evaluated using minimum inhibitory concentration and agar well diffusion method. The compound exhibited good activity against *Staphylococcus epidermidis* and *Salmonella typhimurium*. The MIC test showed the growth inhibition of *S. epidermidis* at a concentration of 15.0 µg/ml, ZOI 10.24 mm and of *S. typhimurium* at a concentration of 25.0 µg/ml, ZOI 9.16 mm. *Escherichia coli* was the most resistant bacterial strain. These results suggest the wedelolactone as a promising antimicrobial agent.

INTRODUCTION

Plants are invaluable sources of pharmaceutical products. India, in particular has yielded an incredible array of plant products that have drawn the attention of ethno pharmacologists from around the world. 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 anti-infectious agents. In order to find out new sources of drugs, a number of plants have been screened for wide range of biological activities. About 3,000 materials from 2,764 plant species have been screened for their pharmacological and chemotherapeutic properties (Anon, 1988). Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Iyengar, 1976; Harborne, 1989; Chopra et al., 1992). Plants used in ethno medicine for the production of bioactive compounds are used and rationalize the use of these medicinal plants in health care (Morales et al., 2008). Most of their properties are due to secondary metabolites produced by plants.

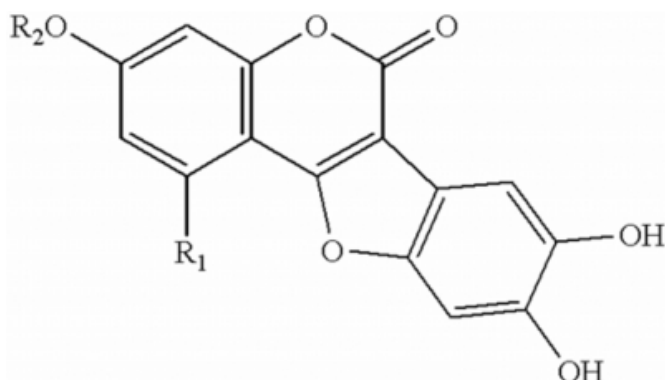
E. alba is reported in literature for its various biological activities such as: calm the mind, removes memory disorders, relieve swollen glands, strengthens spleen, works as a general tonic, useful for treatment of edema, fevers and rheumatic joint pains, stimulate digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders (Chopra et al., 1956; Karnick and Kulkarni, 1990; Karthikumar et al., 2007). Wedelolactone exhibited Trypsin inhibitory effect

(Samiulla et al., 2003; Syed et al., 2003), suppresses LPS-induced caspase-11 expression in cultured cells by directly inhibiting the IKK complex (Kobori et al., 2004), treatment of cirrhosis of the liver and infectious hepatitis (Murphy et al., 1979), possessing potent anti-hepatotoxic activity (Wagner et al., 1986). The shoot extract of *E. alba* showed antimicrobial (Anonymous 1952, Kosuge et al., 1985; Wiart et al., 2004), antifungal activity (Venkatesan and Ravi 2004) and weak cytotoxicity against the M-109 cell lines by alkaloids Verazine (Abdal Kadar et al., 1998), antiviral activity against Ranikhet disease virus (Khin et al., 1978), effective against internal and external parasites (Lans et al., 2001) *G. intestinalis* (Sawangjaroen et al., 2005), antibacterial (Kumar et al., 2007).

The microbes used for the detection of antimicrobial activity were chosen for certain reasons. *E. coli* is best-known member of normal micro biota of the human intestine and a versatile gastrointestinal pathogen. *S. typhimurium* can be found in a broad range of hosts as well as in the environment. Its infection is a serious health problem in developing countries and represents constant concern for the food industry. *S. epidermidis* was used due to its clinical relevance as the major cause of infection in the immunosuppressed prosthetic heart valve in joint implants. *B. subtilis* common saprophytic water and soil bacteria, causes laboratory contamination and conjunctivitis in humans (Ross, 2001).

Figure 1

Figure 1: Chemical structure of Wedelolactone (R-OH, R-CH)



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 our findings on antibacterial effects of wedelolactone (Fig. 1), the principle active compound, extracted from *E. alba*.

MATERIALS AND METHODS

Collection of plant material - Plants of *E. alba* 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 of Department of Biosciences, Maharshi Dayanand University, Rohtak (Haryana) India.

Plant extraction and fractionation - The three months old 950 gm lyophilized leaves were Soxhlet extracted with methanol for 36 h. The solvent was removed and the residues were suspended in water separately and heated on steam bath below 80 °C for 30 min. After filtration, the aqueous phase was partitioned with ethyl acetate. The organic phase was dried, filtered and the solvents were evaporated to yield 6.8 gm light brown powder. 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 to 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 demethylwedelolactone 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 x 4.6 mm, Lot No. 59353.

Preparation of samples for testing - The study was conducted with purified wedelolactone diluted with 10% dimethylsulfoxide (DMSO). The serial dilutions were performed in a concentration range from 0.005 mg/ml to 50 mg/ml.

Micro organisms - Standardized strains from the American type culture collection (ATCC) were used in bioassays. The Gram-positive bacteria were *Bacillus subtilis* (ATCC 6633) and *Staphylococcus epidermidis* (ATCC 155). The Gram-negative bacteria were *Escherichia coli* (ATCC 10536) and *Salmonella typhimurium* (ATCC 23564). Organisms 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 - MIC of wedelolactone was determined by microdilution technique as described by the National Committee for Clinical Laboratories standards (2000). 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.

Agar well diffusion method was carried out by allowing perforation of compound dissolved in DMSO at a concentration of 10 mg/ml. Petriplate containing 30 ml nutrient agar medium were kept for the solidification before inoculating the microorganism, desired numbers of holes of uniform diameter of 8mm were made after solidification, using sterile aluminium 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 (10.0 µg/ml) was used as positive control. The negative control was 10% DMSO.

RESULTS

Wedelolactone exhibited significant antibacterial activity against the four tested strains (Table 1). *S. typhimurium* and *S. epidermidis* were found to be highly sensitive. The MIC of *S. epidermidis*, *S. typhimurium*, *B. subtilis* and *E. coli* was 15 µg/ml, 25 µg/ml, 500 µg/ml and 1000 µg/ml respectively. The compound showed the highest antibacterial activity in *S. epidermidis* (10.24 mm), followed by *S. typhimurium* (9.16 mm), *B. subtilis* (9.12 mm) and *E. coli* (8.60 mm) as zone of inhibition in radius. The ZOI of the antibiotic at concentration of 10.0 µg/ml was maximum for *B. subtilis* (9.64 mm) followed by *S. epidermidis* (9.26 mm), *S. typhimurium* (9.22 mm), and *E. coli* (9.06 mm).

Figure 2

Table 1: Antibacterial activity of wedelolactone and gentamycin against bacterial strains

Microorganisms tested	ZOI (mm)		MIC (µg/ml)	
	A	B	A	B
<i>S. epidermidis</i>	10.24	9.26	15.0	5.0
<i>S. typhimurium</i>	9.16	9.22	25.0	5.0
<i>B. subtilis</i>	9.12	9.64	500.0	2.5
<i>E. coli</i>	8.60	9.06	1000.0	5.0

MIC: minimum inhibitory concentration; ZOI: zone of inhibition (Diameter); A: wedelolactone (10.0 mg/ml); B: gentamycin (10.0 µg/ml); Values are mean of three replicates.

DISCUSSION

In Ayurveda, the traditional Indian system of medicine, *E. alba* is used for the treatment of Kapha and Vata imbalances and is considered as a Rasayana for rejuvenation and longevity. In Gujarat and Punjab, it is used externally for ulcers and as an antiseptic for wounds in cattle and is reported to treat many microbial infections in rural areas (Warrier, 1994). 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 anti viral activity (Kosuge et al., 1985; Wiart et al., 2004; Venkatesan and Ravi, 2004; Khin et al. 1978; Karthikumar et al., 2007). Wedelolactone exhibited effective antibacterial activity against all the four strains studied. It proved highly effective

against *S. epidermidis* and *S. typhimurium*, demonstrating the specificity of wedelolactone activity.

MIC and zone of inhibition were performed to evaluate its antimicrobial potential (Table I). Kumar et al., (2007) reported antimicrobial effect of ethanolic extract of *E. alba* against *S. epidermidis*. The MIC was 0.312 mg/ml and ZOI was 10 mm for 100 mg/ml extract and while the present studies exhibited MIC 15.0 µg/ml and ZOI 10.24 mm for 10 mg/ml. In another study on ethanolic extract of *E. prostrata*, Karthikumar et al., (2007), indicated good activity against *S. typhi* upto 20.8 mm at 50 µg concentration and Wiart et al., (2004), reported 10 mm at 1.0 mg against *B. subtilis*. Our results exhibited comparatively less activity against *E. coli* that corroborates with reports of alcoholic extracts of *E. alba* and *E. prostrata* by Wiart et al., (2004) and Karthikumar et al., (2007). Another interesting report on *E. alba* is that it is one of the herb used for treatment of conjunctivitis (Poonam and Singh, 2002). The results from the current studies revealed that the wedelolactone could be the main constituent responsible for this treatment as it exhibited activity against *B. subtilis*.

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.

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