

Phytochemical Screening of Methanolic Extract and Antibacterial Activity of Active Principles of Hepatoprotective Herb, *Eclipta alba*

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Abstract

Aerial parts of *Eclipta alba* are used traditionally for the treatment of several diseases of liver, skin and stomach. Methanolic extract and active principle compound of a well known Indian hepatoprotective herb, *Eclipta alba* 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 six strains studied. Phytochemical screening of the extract revealed the presence of tannins, flavonoids, coumestans, saponins and alkaloids etc. Ethylacetate fraction and further pure isolated wedelolactone showed enhanced antimicrobial activity. *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Salmonella typhimurium* were most susceptible. *Shigella flexneri* was the most resistant bacterial strain. These results suggest coumestans/wedelolactone as a promising antimicrobial agent.

Key words: *Eclipta alba*, wedelolactone, antimicrobial activity.

Introduction

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 favorable 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 (Penos, 1983). Seventy four percent of 119 plant-derived drugs were discovered as a result of chemical studies to isolate the active substances responsible for their traditional use (Farnsworth and Soejarto, 1991). 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 (Mukherjee and Wahile, 2006). 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. 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 anti-infectious agents. About 3,000 materials from 2,764 plant species have been screened for their pharmacological and chemotherapeutic properties (Anon, 1988). 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 (Iyengar, 1976; Harborne, 1989; Chopra *et al.*, 1992).

Various biological activities are possessed by *E. alba*, such as memory disorders treatment, general tonic, edema, fevers and rheumatic joint pains treatment, digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders (Chopra *et al.*, 1956; Karnick and Kulkarni, 1990; Karthikumar *et al.*, 2007). Wedelolactone is active principle compound of this liver disorder treating drug (Wagner *et al.*, 1986). It also exhibits 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). 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). Since *E. alba* 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 our findings on antibacterial effects of wedelolactone (Fig. 1), the principle active compound, extracted from *E. alba*.

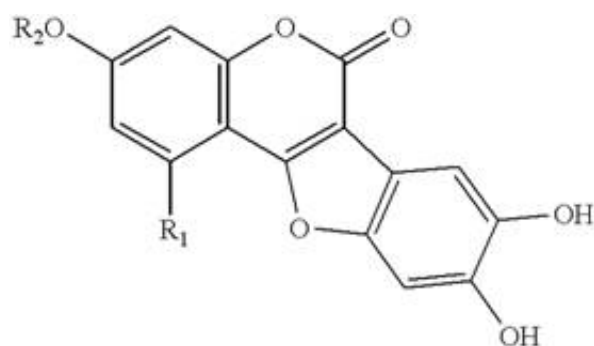


Figure 1. Chemical structure of Wedelolactone (R_1 -OH, R_2 -CH₃).

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 (EA-06/) were deposited in the herbarium section, Dr Jaya Parkash Yadav, of Department of Biosciences, Maharshi Dayanand University, Rohtak (Haryana) India.

Qualitative estimation of primary and secondary plant metabolites

All estimations were done following Hunda *et al.* (1985); Brindha *et al.* (1981). Different aerial parts of the plant were dried at room temperature, powdered, and extracted with methanol (70% v/v) in Soxhlet apparatus for six hours. The extract was filtered and was tested with different alkaloid reagents.

Methanol extract

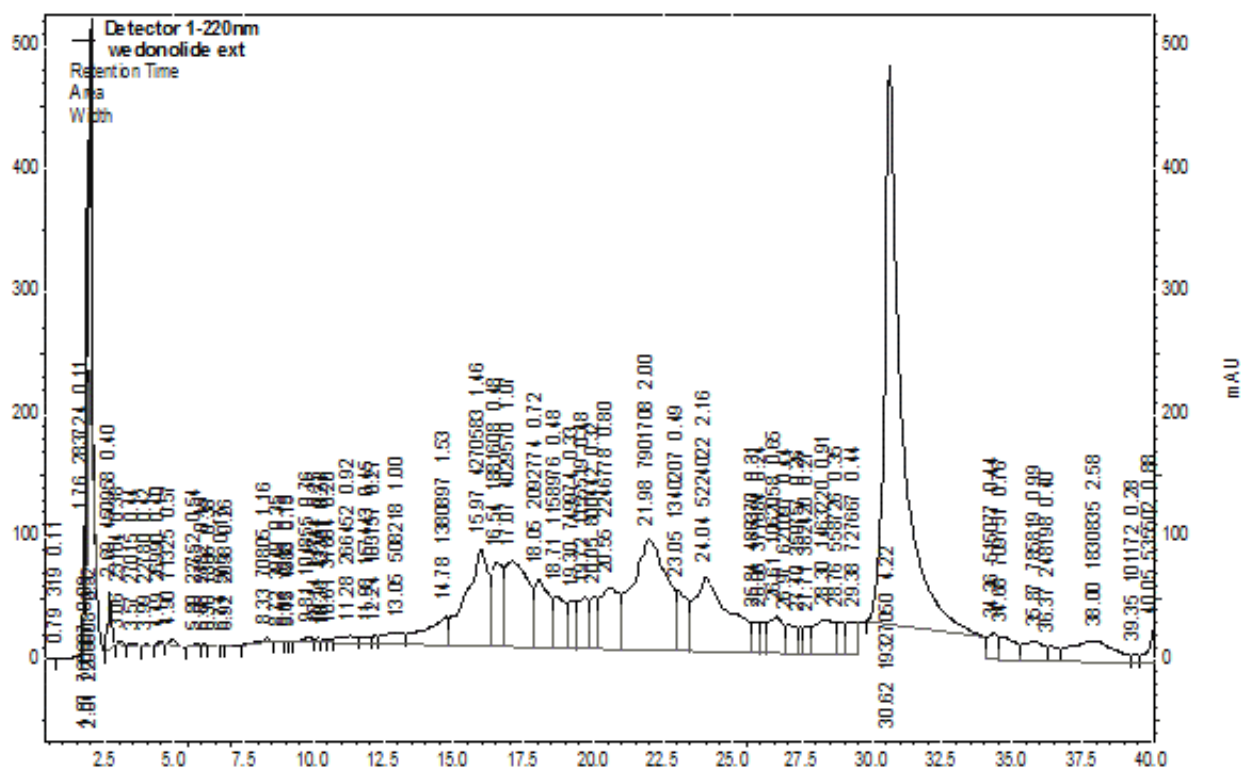
The three months old 950 gm lyophilized leaves were Soxhlet extracted with methanol for 36 h.

Ethyl acetate fraction

The methanol was removed from extract 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.

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 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 (Fig. 2).



Preparation of samples for testing

Micro organisms

Antimicrobial susceptibility testing

Agar well diffusion method was carried out by allowing perforation of extract and wedelolactone dissolved in

DMSO at a concentration of 3.5mg/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 8mm 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 (10.0 µg/ml) was used as positive control. The negative control was 10% DMSO.

Results

The results of the presence of various primary and secondary metabolites in methanol extract (Table 1) reported negative for antraquinones throughout herb. Methanol extract and Ethyl acetate fraction showed positive signs of antimicrobial activity against all six strains (Table 2). Wedelolactone exhibited significant antibacterial activity against the six tested strains (Table 3). *S. epidermidis* and *S aureus* were found to be highly sensitive. The MIC of *S. epidermidis*, *S. typhimurium*, *S. aureus*, *P. aeruginosa*, *S. flexneri* and *E. coli* was, 15 µg/ml, 25 µg/ml, 20µg/ml, 1250 µg/ml ,1300 µg/ml and 1000 µg/ml respectively. The compound showed the highest antibacterial activity (ZOI) in *S. epidermidis* (10.24 mm), followed by *S. typhimurium* (9.16 mm), *S. aureus*(9.14 mm),*E.coli* (8.60mm) *P. aeruginosa* (8.00 mm) and *S. flexneri* (7.60 mm) as zone of inhibition in radius.

Table 1. Quantitative estimation of the various primary and secondary metabolites.

| S.No. | Chemical Test | Natural plant extract | | |
|-------|----------------|-----------------------|------|------|
| | | Leaf | Stem | Root |
| 1 | Alkaloids | + | + | - |
| 2 | Coumestans | + | + | + |
| 3 | Anthraquinones | - | - | - |
| 4 | Phenolics | + | + | + |
| 5 | Saponins | + | + | + |
| 6 | Steroids | + | + | + |
| 7 | Proteins | + | + | + |
| 8 | Amino-acids | + | + | + |
| 9 | Reducing sugar | + | + | - |
| 10 | Flavonoids | + | + | + |

Table 2. Antibacterial activity of Methanol extract and Ethyl acetate fraction against bacterial strains.

| Extracts tested | Conc. mg/well | <i>E. coli</i> | <i>S. typhimurium</i> | <i>S. epidermidis</i> | <i>S.aureus</i> | <i>Shigella flexneri</i> | <i>Pseudomonas aeruginosa</i> |
|----------------------------|---------------|----------------|-----------------------|-----------------------|-----------------|--------------------------|-------------------------------|
| Methanol extract of leaves | 3.5 | + | +++ | ++++ | +++ | + | ++ |
| Ethylacetate fraction | 3.5 | + | +++ | ++++ | +++ | + | ++ |

Discussion

The primary and secondary metabolites were analyzed in methanolic 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. In an earlier study, the extracts of the leaves of *E. alba* tested positive for steroid, reducing sugars, alkaloids, phenolics, saponins and tannins, but no anthraquinones and flavonoids were detected (Gopalakrishnan and Solomon, 1992).

In Gujrat and Punjab, *E. alba* 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 six strains studied. It proved highly effective against *S. epidermidis* and *S. typhimurium* demonstrating the specificity of wedelolactone activity.

Table 3. 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>S. aureus</i> | 9.14 | 9.24 | 20 | 5.0 |
| <i>P.aeruginosa,</i> | 8.00 | 9.06 | 1250 | 2.5 |
| <i>S.flexneri</i> | 7.60 | 9.00 | 1300 | 5.0 |
| <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.

Karthikumar *et al.*, (2007) reported *in vitro* antimicrobial activities of ethanolic extract of *E. prostrate*. It indicated good activity against *S aureus* 7.2mm (ZOI) and MIC 70µl/ml, and for *P. aeruginosa* 8.8mm (ZOI) and MIC 65µl/ml, at 50 µg concentration. While the present studies exhibited respective ZOI at 9.14 mm, 8.00mm and MIC 20 µg/ml and 1250 µg/ml respectively. Traditional reports on *E. alba* indicate that it is one of the herb used for treatment of stomach and digestion disorders, skin diseases and conjunctivitis (Poonam and Singh, 2002).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.

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