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Research Article

IN VITRO ANTIBACTERIAL EFFICACY OF A SESQUITERPENE LACTONE, PARTHENIN FROM *PARTHENIUM HYSTEROPHORUS* L (COMPOSITAE) AGAINST ENTERIC BACTERIAL PATHOGENS

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ABSTRACT

A sesquiterpene lactone, Parthenin was isolated from *Parthenium hysterophorus* L (Compositae). The chemical structure was established based on the data obtained from nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) and Mass spectroscopic analysis. Invitro antibacterial activity of parthenin against enteric bacterial pathogens was established. Out of the tested enteric bacterial pathogens, *S. typhi*, *E. coli* and *P. aeruginosa* was more sensitive to the compound (MIC = 50 µg/ml). Parthenin is moderately active against *S. typhimurium, K. pneumoniae, S. epidermidis* and *S. aureus* with a minimum inhibitory concentration of 100 µg/ml.

Key words: Antibacterial; Diarrhoea; Enteric bacteria; Parthenin;

INTRODUCTION

Parthenium hysterophorus Linn grows abundantly as a weed in India and was collected from Machillipatnam region, Andhar Pradesh, India and identified. Phytochemical investigations reported the isolation of parthenin as the major constituent of the plant along with some related pseudoguaianolides[1-5]. Parthenin is known to possess significant allelopathic and cytotoxic properties[6]. C. Ramesh *et al.*,[7] studied the Antibacterial activity of parthenin and its analogues. We targeted the isolated sesquiterpene lactone, parthenin, towords diarrhoeal pathogens.

Infectious diarrhoeal diseases are responsible for considerable morbidity and mortality, especially in developing countries[8]. According to World Health Organization (WHO) bulletin, diarrhoeal infections are major public health problems in developing countries and contribute to the death of millions of children annually[9]. Among the bacterial enteric-pathogens, *Vibrio cholerae*, *Salmonella spp.* and *Shigella spp.* are of special concern because of the severity of the illness they cause and their association with various outbreaks. The problem of antimicrobial resistance in bacterial pathogens causing diarrhoeal diseases continues to be alarming[10-12]. Even though a number of reports were reported on various biological activities of parthenin, the antibacterial activity of the compound against enteric bacterial pathogens was not yet established. In this regard, in order to discover alternative natural products against infective agents, the present study is focused on the *in vitro* antienterobacterial activity of parthenin isolated from an orbonox weed of India, *P. hysterophorus*.

MATERIALS AND METHODS

Extraction and Isolation

Air-dried plant material of *P. hysterophorus* was powdered and macerated with petroleum ether for 72 h at room temperature. After evaporation of solvent to dryness, residue obtained was 9 g. The crude extract (8.5 g) was then fractionated over an open 100-200 mesh silica gel column using EtOAc/petroleum ether as eluents with increasing polarities. Six fractions were collected and the major fraction was subjected to spectroscopy (^{1}H NMR, ^{13}C NMR and Mass) and the chemical structure (Figure 1) of the major compound was determined by comparing spectroscopic data with that of the literature[13,14].

Spectroscopy

^{1}H NMR (300 MHz) and ^{13}C NMR (150 MHz) spectra were measured with Bruker UXNMR/XWIN-NMR (300 MHz) instrument. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI$^+$ software with capillary voltage 3.98 KV and ESI mode positive ion trap detector.

Microorganisms

The test bacterial cultures were procured from microbial type culture collection (MTCC), IMTECH, Chandigarh, India. The bacterial cultures were maintained on Mueller-Hinton Agar and stored in a refrigerator at 4°C.

In vitro Antibacterial activity

Agar cup bioassay was employed for testing preliminary antibacterial activity of the compound following the standard procedure[15]. The Minimum Inhibitory Concentrations were determined using broth dilution method according to the protocols of National Committee for Clinical Laboratory Standards (NCCLS)[16] against various enteric bacterial pathogens.

RESULTS AND DISCUSSION

NMR spectral data of Parthenin

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.46 (1H, d, J = 6.4 Hz), 6.25 (1H, d, J = 2.1 Hz), 6.14 (1H, d, J = 6.4 Hz), 5.54 (1H, d, J = 2.1 Hz), 4.76 (1H, d, J = 9.4 Hz), 3.45 (1H, m), 2.33-2.13 (2H, m), 2.11-2.08 (1H, m), 1.83-1.65 (2H, m), 1.23 (3H, s), 1.13 (3H, d, J = 7.2 Hz)

^{13}C NMR (150 MHz, $CDCl_3$): δ 208.7, 172.4, 162.8, 139.2, 130.1, 120.8, 85.1, 78.2, 60.1, 44.9, 39.6, 30.8, 27.6, 19.6, 16.2; MS (ESI): m/z 263 $[M+H]^+$.

Agar cup bioassay was employed for testing preliminary antibacterial activity of the compounds and tabulated (Table 1). Parthenin (100 µg/ml) was highly active against S. typhi, E. coli and P. aeruginosa with zone of inhibition diameter (IZD) of 20mm. At a concentration of 100 µg/ml of parthenin, moderate antibacterial active was observed against the bacteria, S. typhimurium, K. pneumoniae, S. epidermidis, S. aureus, V. cholerae and S. *flexneri* with IZD value in between 16 - 18mm. E. aerogenes and P. vulgaris were least sensitive to parthinin with a zone of inhibition diameter of 12 mm at the concentration of 100 µg/ml.

From the results of Minimum inhibitory concentration can be interpreted that the parthenin was active against all the tested bacterial strains with degree of variation. Parthenin was highly active against S. typhi, E. coli and P. aeruginosa with MIC value of 50µg/ml. Against *S. typhimurium*, *K. pneumoniae*, *S. epidermidis*, *S. aureus*, *V. cholerae* and *S. flexneri* the MIC value is of 100 µg/ml. *E. aerogenes* and *P. vulgaris* were least sensitive to parthenin with an MIC value of >200 µg/ml. From the biological activity it can be noticed that parthenin is significantly active against the bacterial strains, *S. typhi*, *E. coli* and *P. aeruginosa*. Biological activities including Antidiarrheal activity of many sesquiterpene lactones was clearly stated by Anna K. Picman[17] and Eloy Rodriguez *et al.*[18]. *In silico* analysis of the parthenin biological activity, synthesis of its analogues and identifying mode of action of the compound against the enteric bacterial pathogens is further warranted to get best results in the area of drug discovery from phytochemicals.

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Table 1: Antibacterial activity of parthenin against enteric bacterial pathogens

Microorganism	Zone of inhibition		Minimum Inhibitory Concentration (MIC) µg/ml
	50 µg/ml	100 µg/ml	
Salmonella typhi (MTCC 733)	16	20	50
Salmonella typhimurium (MTCC 98)	14	18	100
Enterobacter aerogenes (MTCC 111)	10	12	>200
Klebsiella pneumoniae (MTCC 109)	14	16	100
Escherichia coli (MTCC 739)	16	20	50
Proteus vulgaris (MTCC 744)	10	12	>200
Pseudomonas aeruginosa (MTCC 424)	16	20	50
Staphylococcus epidermidis (MTCC 435)	14	18	100
Staphylococcus aureus (MTCC 96)	14	18	100
Vibrio cholerae (MTCC 3905)	12	16	200
Shigella flexneri (MTCC 1457)	12	16	200

Zone of inhibition diameters are in mm; DMSO – no activity;
MIC - Minimum Inhibitory Concentration.

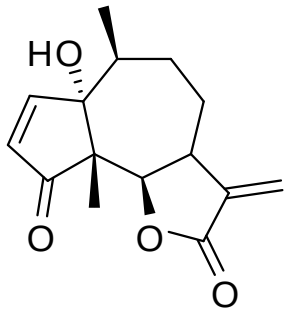

Fig. 1: Chemical structure of Parthenin

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