ANTIBACTERIAL ACTIVITY OF *PHYLLANTUS SIMPLEX* LINN.

Hemendra S. Chouhan and Sushil K. Singh*

Pharmaceutical Chemistry Research Lab., Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi – 221005. India

Summary

Phyllanthus simplex (*Euphorbiacae*) is used in the traditional medicine for the treatment of diarrhoea, jaundice, liver diseases, hyperglycemia, itching, pruritus etc. In this study, the whole plant of *P. simplex* was extracted successively with petroleum ether and ethanol. The petroleum ether extract (PSPE) and ethanol extract (PSEE) were evaluated for the antibacterial activity by the agar disc diffusion method. PSEE was found to display significant antibacterial activity against *S. flexneri* (gram negative bacteria) responsible for acute dysentery/ shigellosis in human. The zone of inhibition displayed by PSEE was found comparable to that of ciprofloxacin used as standard in the experiment. Preliminary phytochemical test suggested the abundance of phenolics in PSEE. Therefore, it may suggest that antibacterial activity of PSPE is primarily due to its phenolics content.

Keywords: Antibacterial activity; Agar disc diffusion method; *Phyllanthus simplex*

*Corresponding author:
Tel. No.: +91-542-6702736; Fax: +91-542-2316428
E-mail: sksingh.phe@itbhu.ac.in

Introduction

Pathogenic microbes present a substantial hazard to the human health [1]. The development of drug resistance, recurrences of old diseases and side effects of existing antibiotics have been created a daunting situation. Consequently, surge an imperative need of searching new antimicrobial agents and/or their source which are safe and effective [2-4]. Natural compounds such as plant phenolics are always attracting researchers as they offer little risk for resistance development by the pathogenic microorganisms. Many reports have been demonstrated that the crude extracts obtained from various plants possess antimicrobial activity and hence it may be a good source of antimicrobial agent(s) [5-6]. Studies also demonstrated that phenolics found in food, vegetables and plants have immense potential to display overwhelming antimicrobial activity [7-8]. The potential of plant phenolics depend upon their nature, structure and interactions with other molecules of mixture [9].

Plants of family *Euphorbiacae* are rich source of phenolics. Phenolic compounds are found to be exhibit significant antimicrobial activity. *Phyllanthus simplex* (*Euphorbiacae*) is used in traditional medicine for the treatment of diarrhoea, jaundice, gonorrhea, hyperglycemia, liver disease, mammary abscess [10] itching and pruritis [11]. Astringent, diuretic and cathartic activity of *P. simplex* is also reported [12]. In our previous studies, the ethanol extract of the whole plant of *P. simplex* was found to possess good lipid peroxidation inhibitory activity [13] and demonstrated its effect on the antioxidative enzymes viz. superoxide dismutase, catalase, glutathione peroxidase in the liver and kidney of alloxan-induced diabetic mouse [14]. However, there is no report found on the antimicrobial activity of this plant as per our knowledge. Hence, this study was aims to determine the antibacterial activty, if any, in the petroleum ether and ethanol extracts of the whole plant of *P. simplex.*

Materials and Methods

Plant Material

Whole plants of *P. simplex* were collected from the campus of Banaras Hindu University, Varanasi in the month of June-July 2009 and were identified morphologically by Prof. N.K. Dubey, Department of Botany, Banaras Hindu University, Varanasi. A voucher specimen (PCRL-43) was deposited in the Pharmaceutical Chemistry Research Lab, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi for the future reference. Air and shade dried plant material was pulverized to course crude powder and stored in air tight container at room temperature till the extraction.

Preparation of extract

Course pulverized *P. simplex* whole plant (1.0 kg) was extracted (Soxhlation) with petroleum ether (fraction collected from 60-80ºC) for 24 h and followed ethanol for 36 h. The petroleum ether extract (PSPE) and ethanol extract (PSEE) were concentrated separately in vacuo yielding semisolid masse (8.5 and 14 % w/w respectively). Both extracts were stored separately in air tight container and kept in the refrigerator till the use.

Preliminary phytochemical screening

Qualitative determinations of PSPE and PSEE for the chemical constituents were carried out on the PSPE and PSEE using standard procedures as described by Trease & Evans [15].

Microorganism strain

Antibactrial activity of the extracts were evaluated by using bacterial strains of *Escherichia coli, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella flexneri, Shigella dysenteriae, Staphylococcus aureus, Vibrio cholare.*

Evalatuion of antibacterial activity

The antibacterial activity of PSPE and PSEE was evaluated by the disc diffusion method and performed in the accordance with the guidelines of National Committee for Clinical Laboratory Standards [16]. A 24/48 h-old culture of selected bacteria was mixed with sterile physiological saline (0.85%) and the turbidity was adjusted to the standard inoculum of MacFarland scale 0.5 [~106 colony forming units (CFU) per millilitre]. Petri dishes containing 20 mL of Mueller-Hinton agar were used to inoculate bacterial suspension. Filter paper discs (Whatman no. 1, diameter = 6 mm) impregnated with the extract solution prepared in DMSO (10 µl/disc; 500 µg extract/disc) were placed on the inoculated plates and petri dishes were incubated for 24 h at 37ºC. A paper disc impregnated with Ciprofloxacin (5 µg/disc) and dimethylsulfoxide (DMSO) was used as positive and negative control respectively. The inhibition zone diameters were measured in millimeters.

Result and discussion

Preliminary phytochemical analysis of PSPE and PSEE revealed the presence of steroids, triterpenes, lignans, flavonoids, glycosides and phenolics. The antimicrobial effect of PSPE and PSEE was evaluated against two species of gram-positive bacteria and six species of gram-negative bacteria and are presented in table 1. The antibacterial activity of both extract was found in the range of 7-20 mm (zone of inhibition) against the tested species of bacteria were. PSEE was found to be exhibit significant antibacterial activity against *S. flexneri* and moderate antibacterial activity against *E. coli and V. chlorae*, However, modest or no activity were found against the other bacterial species studied. The antibacterial activity of PSEE against *S. flexneri* was found comparable to that of ciprofloxacin used as standard in the experiment.

Table 1: Antimicrobial activity of PSPE and PSEE (zone of inhibition in mm).

Microrganism	PSPE	PSEE	Ciprofloxacin
E. coli	8	16	24-29
E. faecalis	-	-	19-21
K. pneumoniae	-	-	23-26
P. aeruginosa	-	8	24-27
S. flexneri	9	20	23-24
S. aureus	7	12	25-28
S. dysenteriae	-	-	27-29
V. cholare	9	14	17-20

S. flexneri (*Enterobacteriaceae*) is a gram negative bacilli bacteria responsible for the gastrointestinal infections in human, occasionally associated with the extraintestinal manifestation. *S. flexneri* is commonly associated with the acute dysentery/shigellosis and is endemic in many parts of the world. Globally, approx 165 million cases of shigellosis occur annually along with 1.1 million deaths in the developing countries [17-19]. Thus, significant antibacterial showed by the ethanolic extract of *P. simplex* against *S. flexneri* suggested its usefulness in the treatment of shigellosis.

In the conclusion, this study demonstrated that the polar extract (PSEE) possess significant antibacterial activity than that to non polar extract (PSPE). This implies that polar constituents such as phenolics are primarily responsible for the antibacterial activity of the *P. Simplex*. Moreover, this study also vindicated the claims of use of *P. simplex* in treatment diarrhoea. Further, phytochemical exploration of the plant is requisite to isolate a new lead with promising antibacterial activity.

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