

Antibacterial Activity of Seed and Flower Parts of *Crotalaria juncea* Linn

Hemendra S. Chouhan and Sushil K. Singh

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

Abstract: *Crotalaria juncea* Linn. (leguminoceae), is used as medicine in the traditional system of Indian medicine for the treatment of anaemia, impetigo, menorrhagia, psoriasis etc. The objective of this study was to evaluate antibacterial activity of the seed and flower parts of *C. juncea*. The ethanol extract of flowers part (CJFEE) and seeds part (CJSEE) were evaluated for the antibacterial activity by the agar disc diffusion method. The presence of steroids, triterpenes, flavonoids, phenolics and glycosides were reported in the extracts by the preliminary chemical tests. Results revealed that CJSEE possess significant antibacterial activity against the *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus* and *V. cholerae*. Hence, study concluded that seed part possess significant antibacterial activity which may be linked to its phenolic content.

Key words: Antibacterial activity • Agar disc diffusion method • *Crotalaria juncea*

INTRODUCTION

Medicinal plants have played a pivotal role in the primary healthcare and formed the basis of traditional systems of medicines. Plants have been bestowed us with food, spices, flavours, fragrances, medicines, etc. Plant are being used to treat many diseases or ailments viz. infectious diseases, inflammatory disorders, skin diseases, etc. since ancient time [1-3]. Infectious diseases are the major cause of death across the world. Infections due to pathogenic microorganisms cause a severe concern to human health and hence constitute an important area of drug discovery. Increasing cases of drug resistance, unwanted side effects of existing antibiotics and the reappearance of earlier known infections have demanded need of new, safe and effective antimicrobial agents [4-7]. Plant products such as phenolics are being considered to be safe and produce fewer chances for the resistance development in the microorganism [8-9]. Therefore, current scientific investigation dwells in identifying new leads from the plant sources possessing significant antimicrobial activity.

Crotalaria juncea Linn. (leguminoceae), used as medicine, edible, culinary purpose by many tribal communities. It is commonly known as sunn hemp and is widely distributed in the tropical and subtropical regions of India, Nepal, Sri Lanka and Southern Africa. In the folk and Ayurvedic medicines, *C. juncea* is used as blood purifier, abortifacient, astringent, demulcent, emetic, purgative and in the treatment of anaemia, impetigo, menorrhagia and psoriasis [10-15]. Studies on the seeds of *C. juncea* revealed that it possess significant antispermatogenic activity, anti-ovulatory and contraceptive activity [16-20]. Prakash *et al.* reported that the daily administration of ethanol extract of *C. juncea* to experimental rats causes severe hepatotoxicity after thirty days [21]. Moderate antifungal activity has been reported in the methylene chloride and methanol extract of aerial parts of this plant of Indonesian origin [22]. Current knowledge on chemistry of *C. juncea* is limited to the isolation of riddelline, seneciphylline, senecionine, trichodesmine, chodesmine alkaloids, galactose-specific lectin and cardiogenin 3-*O*-[β]-*D*-xylopyranoside [23-24]. However, there is no report available on the antimicrobial activity of the plant and hence this study aims to evaluate the antibacterial activity, if any, of the seed and flower parts of *C. juncea*.

Corresponding Author: Sushil K. Singh, Department of Pharmaceutics, Pharmaceutical Chemistry Research Lab.,
Institute of Technology, Banaras Hindu University, Varanasi - 221005. India.
Tel: +91-542-6702736, Fax: +91-542-2316428, E-mail: sksingh.phe@itbhu.ac.in.

MATERIALS AND METHODS

Plant Material: *Crotalaria juncea* flowers were collected during September–October 2008 from the fields of Guna district, Madhya Pradesh, India. *C. juncea* Seeds were purchased from the local market. Both flowers and seeds parts were identified morphologically at Department of Botany, Banaras Hindu University, Varanasi and voucher specimen (PCRL-40 & 41 respectively) were 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 flowers and seeds were pulverized separately to course crude powder and stored in air tight containers at room temperature till the extraction.

Preparation of Extract: Course pulverized flowers of *C. juncea* (1.0 kg) were defatted with petroleum ether (fraction collected from 60–80°C) by soxhlation for 24 h followed by ethanol for 36 h. Ethanol extract (CJFEE) was concentrated under vacuum and yielded semisolid mass (10.5% w/w). Similarly, ethanol extract from *C. juncea* seeds (1.0 kg) was obtained (9.0% w/w). Both extracts were separately stored in air tight container in the refrigerator till the use.

Preliminary Phytochemical Screening: Qualitative determinations of CJFEE and CJSEE for the chemical constituents were carried out using standard procedures as described by Trease and Evans [25].

Microorganism Strain: Antibacterial activity of CJFEE and CJSEE were evaluated by using bacterial strains of *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Vibrio cholerae*.

Evaluation of Antibacterial Activity: The antibacterial activity of CJFEE and CJSEE was evaluated by the disc diffusion method and performed in the accordance with the guidelines of National Committee for Clinical Laboratory Standards [26]. 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 [$\sim 10^6$ 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 μ l/disc; 500 μ g extract/disc) were

placed on the inoculated plates and petri dishes were incubated for 24 h at 37°C. A paper disc impregnated with Ciprofloxacin (5 μ g/disc) and dimethylsulfoxide (DMSO) was used as positive and negative control respectively. The inhibition zone diameters were measured in millimeters.

RESULT AND DISCUSSION

Plants of genus *crotalaria* have been found to possess significant antimicrobial activity which is linked to their constituents' viz. phenolic, protein [27-30]. In this study, preliminary phytochemical analysis of CJFEE and CJSEE revealed the presence of steroids, triterpenes, flavonoids, phenolics and glycosides. Abundance of phenolics was found in the CJSEE. The antibacterial activity of these extracts was evaluated by the paper disc agar diffusion method against both Gram-positive and Gram-negative strains of bacteria [26]. Results of the study are summarized in Table 1.

Results showed that both CJFEE and CJSEE possess antibacterial activity against the *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *V. cholerae* and no activity was observed against the *C. freundii*, *E. faecalis* and *S. dysenteriae*. However, the ethanol extract of seeds part have higher antibacterial than that to ethanol extract of flower parts of *C. juncea*. The difference in antibacterial activity of the extracts may be due to the variation in the composition of extracts, structure of bioactive constituents, their interactions with bacterial cell wall components, etc. [31-32].

In conclusion, study analyse the antibacterial activity of the seed and flower parts of *C. juncea* and reports the significant antibacterial activity of CJSEE against *K. pneumoniae*, *P. aeruginosa*, *S. aureus* strains of bacteria. However, activity may be linked to its higher phenolic content and or constituents of seed part. Further, phytochemical studies on the plant are required to isolate a new lead from the plant.

Table 1: Antibacterial activity of CJFEE and CJSEE (zone of inhibition in mm)

Microorganism	CJFEE	CJSEE	Ciprofloxacin
<i>C. freundii</i>	-	-	24-29
<i>E. coli</i>	13	16	24-29
<i>E. faecalis</i>	-	-	19-21
<i>K. pneumoniae</i>	14	18	23-26
<i>P. aeruginosa</i>	10	15	24-27
<i>S. flexneri</i>	-	-	23-24
<i>S. aureus</i>	13	18	25-28
<i>S. dysenteriae</i>	-	-	27-29
<i>V. cholerae</i>	8	14	17-20

- No zone of inhibition

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