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*In vitro* antibacterial activity of *Desmodium gangeticum* (L.) DG

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## ABSTRACT

**Objective:** To identify the antibacterial activity of *Desmodium gangeticum* (*D. gangeticum*). **Methods:** Antibacterial activity of *D. gangeticum* was tested with various solvents viz., methanol, ethanol, chloroform and aqueous extract against various bacterial pathogens such as *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi*, *Streptococcus mutants* and *Pseudomonas aeruginosa*. Antibiotic sensitivity assay was performed with amoxicillin, kanamycin, tetracyclin, ciprofloxacin and penicillin. **Result:** Of the selected several extract, the methanolic extract showed maximum zone of inhibition (24±2.3mm) against *S. mutants* and minimum zone of inhibition was observed with aqueous extract against *P. aeruginosa* (7±0.08). In addition the antibiotic sensitivity was observed with kanamycin, tetracyclin, ciprofloxacin against all bacterias. **Conclusions:** The methanolic extract of *D. gangeticum* be able to use as potential antibacterial source for various infective pathogens.

## 1. Introduction

The discovery, development and clinical use of antibiotics during the nineteenth century have substantially decreased public health hazards resulting from bacterial infections. However, there has been a parallel and alarming increase in bacterial resistance to existing chemotherapeutic agents as a result of their injudicious use. In addition, antibiotics are occasionally associated with adverse effects to the host, including hypersensitivity, immune-suppression and allergic reactions [1]. These developments demand that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. One possible strategy is the rational localization of bioactive products from folk medicines, with the hope that systematic screening of these will result in the discovery of novel effective compounds with potent and useful activities against microbes. There is an ever-increasing demand for plant-based therapeutics in both developing and developed

countries due to a growing recognition that they are natural products, non-narcotic and in most cases, easily available at affordable prices; they also have no side effects.

*Desmodium gangeticum* (L.) DG. (family Leguminaceae) is a small shrub of tropical region which has been used in India system of medicine as a bitter tonic, febrifuge, digestive, anticatarrhal, antiemetic, in inflammatory condition of chest and various other inflammatory condition due to 'vata' disorder [2]. The aqueous of these species has been reported to show severe antiwrithing activity, moderate central nervous system (CNS) depressant activity [3] and antileishmanial activity [4]. Gangetin, a pterocarpnoid from DG, has been shown to possess anti-inflammatory and analgesic activities [5]. Total of this species showed anticholinesterase, smooth muscle stimulant, CNS stimulant and depressant response [6]. It is also know to possess antioxidant activity [7]. Chemical studies on the DG revealed the presence of alkaloids, pterocarpnoid, flavnoid and isoflavanoid glycoside [8, 9].

The sterols, N, N-dimethyltryptamine, 5-methoxy-N, N-dimethyl tryptamine, their oxides and other derivatives have been isolated from aerial parts, three pterocarpenoids, gangetin and desmodin, are the major chemical constituents of the roots [6]. Alkaloid isolated from aerial part comprises indole-3-alkyl-amines and B-Carbolines and has

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anticholinesterase, smooth muscle stimulant, CNS stimulant response [10]. It is reported to possess antiulcer [7], antioxidant [11], cardiotoxic [12], anti-inflammatory, anti-nociceptive activities and useful in neurological disorder [13]. We report here a systematic *in vitro* screening of a wide range of antibiotics with the aim of investigating their potential against five reference bacteria, *E. coli*, *K. pneumoniae*, *S. typhi*, *S. mutans* and *P. aeruginosa*. The study was also designed to determine the potential antibacterial activities of extracts (methanol, ethanol, chloroform and aqueous) of the medicinal plant *D. gangeticum* and compare these against the reference bacteria.

## 2. Materials and methods

### 2.1. Plant materials

*D. gangeticum* (L.) DG plants were obtained from the Herbal Research garden of A.V.V.M. Sri Puhspam College, Poondi (lat: 13° 63'N and lang: 79° 84'E), Thanjavur district, Tamilnadu, India. The freshly collected plant materials were dried in shade at 30 °C for 10 days, ground to a fine powder and stored in air tight bottles at 4 °C.

### 2.2. Preparation and preservation of plant extract

Aqueous, ethanol, chloroform and methanolic extracts of *D. gangeticum* were obtained from air-dried plant materials according to the standard methodologies [14]. The extracts (2000 mg/ml) were stored as a stock solution in a refrigerator at 4 °C for further use [15]. All extracts were exposed to UV-radiation (200–400nm) for 24 h and checked frequently for sterility by streaking on nutrient agar plates [16].

### 2.3. Test microorganisms

Five reference bacteria *E. coli*, *K. pneumoniae*, *S. typhi*, *S. mutans* and *P. aeruginosa*, were used during the study. The tested strains were procured from the Microbial Type Culture Collection, Chandigarh, India. They were cultured in nutrient broth (Hi-media, M002) at 30±2 °C and stored in nutrient agar slants at 4 °C.

### 2.4. Antibiotics

The following antibiotic sensitivity test discs (Span Diagnostics Limited, Surat, India), with their concentrations shown in parentheses, were used to determine the antibiotic sensitivity profile against the reference bacteria: amoxicillin (30mg), kanamycin (30mg), tetracycline (30mg), ciprofloxacin (5mg), penicillin (10mg).

### 2.5. Sensitivity test

Antibiograms were carried out by the disc diffusion method [17] using commonly used antibiotics. Antibiotic sensitivity was tested in Mueller–Hinton agar plates. The surfaces of the media were inoculated with bacteria from a broth culture, antibiotic-impregnated discs were placed on the solid medium and the plates were then incubated at 37 °C for other bacterial strains for 24h. The clear zones of inhibition formed around the discs were measured and interpreted in accordance with the manufacturer's instruction as indicating the sensitivity, intermediate-sensitivity or resistance of that bacterium to the antibiotic being tested.

### 2.6. Antibacterial assay

This assay was carried out using the agar well diffusion method [18]. Bacterial strains grown on nutrient agar at 37 °C for 18h were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 MacFarland standards (108 CFU/ml). The suspension was used to inoculate 90 mm diameter petri plates. Wells (diameter 6 mm) were punched in the agar and filled with 30 ml of 2000 mg/ml extracts. The dissolution of the organic extracts (methanol, ethanol, and chloroform) was facilitated with the addition of 1% (v/v) DMSO and that of the aqueous extracts with the addition of sterile distilled water, neither of which affected the growth of microorganisms (as shown by our control experiments). The plates were incubated in air at 37 °C for 24h. Antibacterial activities were evaluated by measuring the inhibition zone diameters. The experiments were conducted in triplicate. DMSO was used as a control for the methanol, ethanol, chloroform extracts, and sterile distilled water used as a control for the aqueous extracts.

## 3. Results

### 3.1. Sensitivity test

The antibiotic sensitivity profile of the bacterial strains tested is listed in Table 1. All of the bacteria tested are resistant to Penicillin G, indicating the appearance of multiple drug resistance phenotypes of the bacteria. Consequently, we could not use these antibiotics as therapeutic agents for treating diseases related with reference bacteria. A comparison of data obtained on the inhibition zones of the pathogenic bacteria showed that, kanamycin, amoxicillin, tetracycline and ciprofloxacin and was effective against all five bacterial species tested. The most effective antibiotic against the bacteria was kanamycin and ciprofloxacin against *K. pneumoniae*, *E. coli* and *S. mutans*, tetracycline against *K. pneumoniae* and *E. coli*, amoxicillin against *S. mutans* and *P. aeruginosa* (Table 1).

**Table 1**

Antibiotic sensitivity profile of bacteria against commercially available antibiotics.

Name of the antibiotics	Inhibition Zone Diameter (mm)				
	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>S. mutans</i>	<i>P. aeruginosa</i>
Amoxicillin	17 (IM)	16 (IM)	13 (IM)	24 (S)	20 (S)
Kanamycin	28 (S)	26 (S)	20 (S)	18 (S)	24 (S)
Tetracycline	20(S)	21 (S)	10 (S)	12(R)	16 (IM)
Ciprofloxacin	23 (S)	25 (S)	18(S)	26 (S)	22 (S)
Penicillin	10 (R)	11 (R)	12 (R)	10 (R)	10 (R)

Denotes R= Resistant, S= Sensitive, IM= Intermediate

### 3.2. Antibacterial assay

The antibacterial activity of specific concentrations of aqueous, chloroform alcohol and methanolic extract of the plant *Desmodium gangeticum* is given in (Table 2). The methanolic extract showed the highest antibacterial potentiality, followed by the ethanol, chloroform and aqueous extracts of *D. gangeticum*. We also found the highest inhibition zone in *K. pneumoniae*, *S. mutans*, followed by *S. typhi* and *E. coli*. The aqueous extract did not show high antibacterial activity against all tested bacterias.

## 4. Discussion

The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem [19]. The presence of antifungal and antimicrobial substances in the higher plants is well established as they have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine have been used for the treatment of diseases as in done in cases of Unani and Ayurvedic system of medicines, a natural blueprint for the development of new drugs. Much of the exploration and utilization of natural product as microbial arise from microbial sources. The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Plant based

antimicrobials represents the vast untapped source for medicine. Plant based antimicrobials have enormous therapeutic potential as they can survive the purpose without any side effects that are often associated with synthetic antimicrobials, continued further research and exploration of plant derived antimicrobials is needed today. Many of the studies were useful in identifying the active principle responsible for such potentials and to develop clinically important therapeutic drugs for mankind. Hence an attempt has been to identify the antibacterial activity of different solvents extracts of *D. gangeticum* against five human pathogenic Gram positive and Gram negative bacterias. Previously, many researchers carried out the antimicrobial activity in several medicinal plants [20–28].

The methanolic extract showed the highest antibacterial potentiality, followed by the ethanol, chloroform and aqueous extracts of *D. gangeticum*. Two reasons accounting for the higher antibacterial activity of methanolic extracts may be (i) the nature of biological active components (alkaloids, sterols, flavonoids, essential oil, terpenoids, etc., which may be enhanced in the presence of methanol (ii) the stronger extraction capacity of methanol may have produced a greater number of active constituents responsible for antibacterial activity than the aqueous, ethanol and chloroform. Our results agree with the findings of several of these studies, although, some conflicting observation are worthy of note. In contrast, the aqueous extracts of *P. emblica*, and *L. alba* exhibited, in most cases, a higher antibacterial potentiality than their methanolic counterparts. The aqueous extract did not show high antibacterial activity against all tested bacterias. When the individual sensitivity pattern of all the bacterial strains were compared, the methanolic extract of *D. gangeticum* was equally potent – in terms of antibacterial activity – as the most effective antibiotics, such as amoxicillin, ciprofloxacin, kanamycin, tetracycline and penicillin.

In conclusion, the different solvent plant extracts tested in this study had potential antibacterial activities against with the reference strains. Our results support the use of these plants as traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antimicrobial agents in the search of new drugs.

**Table 2**

Antibacterial activity of a specific concentration (30 ml 2000mg/ ml extract) of aqueous, methanol, ethanol and chloroformic extracts of *Desmodium gangeticum* determined by the well diffusion method.

Name of the organism	Size of Zone of inhibition in diameter (mm±S.E)				
	Methanol extract	Ethanol extract	Chloroform extract	Aqueous extract	Control
<i>K. pneumoniae</i>	23±1.3	20±1.2	15±0.8	12±1.6	0
<i>E. coli</i>	22±0.7	18±0.6	20±1.8	15±1.1	0
<i>S. typhi</i>	20±1.1	16±1.7	14±1.3	10±0.2	0
<i>S. mutans</i>	24± 2.3	21±0.8	18±0.7	14±0.9	0
<i>P. aeruginosa</i>	18±1.7	19±1.3	14±0.3	7±0.08	0

Control set consisting of distilled water and DMSO respectively all values are an average of three determinations.

## Conflict of interest statement

We declare that we have no conflict of interest.

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