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JIGNA PAREKH

SUMITRA CHANDA

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Jigna PAREKH, Sumitra V. CHANDA

Phytochemical, Pharmacological and Microbiological Laboratory,

Department of Biosciences, Saurashtra University, Rajkot 360 005 - INDIA

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Abstract: The antibacterial effect of some selected Indian medicinal plants was evaluated on bacterial strains like *Bacillus cereus* ATCC11778, *Staphylococcus aureus* ATCC25923, *Enterobacter aerogenes* ATCC13048, *Escherichia coli* ATCC25922 and *Klebsiella pneumoniae* NCIM2719. The solvents used for the extraction of plants were water and methanol. The *in vitro* antibacterial activity was performed by agar disc diffusion and agar well diffusion method. The most susceptible Gram-positive bacteria was *B. cereus*, while the most susceptible Gram-negative bacteria was *K. pneumoniae*. The extracts of *Abrus precatorius*, *Cardiospermum halicacabum* and *Gmelina asiatica* could not inhibit any of the bacterial strains investigated. The most active antibacterial plant was *Caesalpinia pulcherrima*. The significant antibacterial activity of active extracts was compared with the standard antimicrobics, piperacillin (100 µg/disc) and gentamicin (10 µg/disc). The results obtained in the present study suggest that *Caesalpinia pulcherrima* can be used in treating diseases caused by the test organisms.

Key Words: Medicinal plants, antibacterial activity, aqueous extract, methanol extract

Introduction

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern (1). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (2). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (3). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (4). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (5,6).

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties (7).

In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (8). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (9). Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments (10).

The aim of this study was to evaluate the activity of extracts from 12 plants against several Gram-positive and Gram-negative bacterial strains in vitro.

Materials and Methods

Collection and Identification of Plant Material

Fresh plant/plant parts were collected randomly from the semi-arid region of Rajkot, Gujarat, India. The plants and the parts screened, together with their families and vernacular names, are given in Table 1. The taxonomic identities of these plants were confirmed by Dr. P. S. Nagar, Department of Biosciences, Saurashtra University, Rajkot, and the voucher specimen numbers of the plants were preserved. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Preliminary Phytochemical Analysis

Qualitative phytochemical analysis of the crude powder of the 12 plants collected was determined as follows: Tannins (200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate + 2 ml $FeCl_3$, blue-black precipitate indicated the presence of Tannins. Alkaloids (200 mg plant material in 10 ml methanol, filtered); a 2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayor's reagents/Wagner's reagent/Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. Saponins (frothing test: 0.5 ml filtrate + 5 ml distilled water); frothing persistence indicated presence of saponins. Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate + 1 ml glacial acetic acid + $FeCl_3$ + conc. H_2SO_4); green-blue color indicated the presence of cardiac glycosides. Steroids (Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered); a 2 ml filtrate + 2 ml acetic anhydride + conc. H_2SO_4. blue-green ring indicated the presence of terpenoids. Flavonoids (200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate + conc. HCl + magnesium ribbon pink-tomato red color indicated the presence of flavonoids (11).

Extraction of Plant Material

Aqueous extraction

10 g of air-dried powder was added to distilled water and boiled on slow heat for 2 h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 min. The supernatant was collected. This procedure was repeated twice. After 6 hours, the supernatant collected at an interval of every 2 hours was pooled together and concentrated to make the final volume one-fourth of the original volume (12). It was then autoclaved at 121 °C and at 15 lbs pressure and stored at 4 °C.

Table 1. Ethnobotanical information of some traditionally used Indian medicinal plant species selected for antibacterial activity.

Plant species	Family	Common name	Part used	Therapeutic use
Abrus precatorius L.	Fabaceae	Chanothi	Leaf and stem	Purgative, emetic, aphrodisiac, nervous disorders
Caesalpinia pulcherrima Swartz	Caesalpiniaceae	Galtoro	Aerial parts	Reclamation plant
Cardiospermum halicacabum L.	Sapindaceae	Kagdonio	Aerial parts	Rheumatism, fever, nervous disorders, piles, diaphoretic, diuretic
Casuarina equisetifolia L.	Casuarinacea	Sharu	Leaf, stem, fruit	Diarrhea, dysentery, beriberi, headache, fever, dropsy
Cynodon dactylon (L.)Pers.	Poaceae	Durva	Whole plant	Diuretic, dropsy, syphilis, wound infection, piles
Delonix regia L.	Fabaceae	Gulmohar	Pod	Not reported
Euphorbia hirta L.	Euphorbiaceae	Dudhli	Whole plant	Worms, bowel complaints, cough, colic, dysentery
Euphorbia tirucalli L.	Euphorbiaceae	Thor	Stem	Infections of spleen, blood complaints, whooping cough, asthma
Ficus benghalensis L.	Moraceae	Vad	Branching root	Leukorrhea, diarrhoea, dysentery, diabetes, skin disorders
Gmelina asiatica L.	Verbenaceae	Shivan	Leaf	Diabetes
Santalum album L.	Santalaceae	Shwet chandan	Leaf and stem	Gastric irritability, dysentery, gonorrheal, urethral, bronchial disorders
Tecomella undulata (Sm.) Seem.	Bignoniaceae	Ragat rohido	Leaf and stem	Remedy for syphilis spleen diseases

Methanol extraction

10 g of air-dried powder was taken in 100 ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 hours the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (12) and stored at 4 °C in airtight bottles.

Bacterial Strains

In vitro antimicrobial activity was examined for aqueous and methanol extracts from 12 medicinal plants used by traditional healers. Microorganisms were obtained from the National Chemical Laboratory (NCL), Pune, India. Amongst five microorganisms investigated, two Gram-positive bacteria were *Bacillus cereus* ATCC11778 and *Staphylococcus aureus* ATCC25923, while three Gram-negative bacteria were *Enterobacter aerogenes* ATCC13048, *Escherichia coli* ATCC25922 and *Klebsiella pneumoniae* NCIM2719. All the microorganisms were maintained at 4 °C on nutrient agar slants.

Media Preparation and Antibacterial Activity

The antimicrobial assay was performed by two methods viz. agar disc diffusion method (13) for aqueous extract and agar well diffusion method (14) for solvent extract. The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum (1 x 10^8 cfu/ml) and poured into the Petri plate (Hi-media). For agar disc diffusion method, the disc (0.7 cm) (Hi-Media) was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer (0.85 cm). 100 µl of the test compound was introduced into the well. The plates were incubated overnight at 37 °C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented.

Results and Discussion

The antibacterial activity of 12 plant species extract was assayed in vitro by agar disc diffusion and agar well diffusion method against 6 bacterial species. Table 3 summarizes the microbial growth inhibition of both aqueous and methanol extracts of the screened plant species. The aqueous extract of only three plants showed antibacterial activity (*Caesalpinia pulcherrima*, *Casuarina equisetifolia, Euphorbia hirta*); the other aqueous extracts did not show any antibacterial activity. On the other hand, methanol extracts of almost all the plants exhibited antibacterial activity towards one or another bacterium.

The maximum antibacterial activity was shown by *Caesalpinia pulcherrima*, followed by *Euphorbia hirta* and *Casuarina equisetifolia*, respectively. The methanol extracts of the investigated plants showed maximum antibacterial activity against Gram-negative *K.*

Table 2. Preliminary phytochemical analysis of screened medicinal plant species.

Plant Species	Tannins	Saponins	Flavonoids	Steroids	Cardiac glycosides	Alkaloids		
						Mayor's test	Wagner's test	Dragendorff's test
Abrus precatorius L.	-	+	-	-	-	-	++	-
Caesalpinia pulcherrima Swartz	+	+	-	-	-	-	+	-
Cardiospermum halicacabum L.	-	+	-	-	-	-	+	-
Casuarina equisetifolia L.	+	+	+++	-	-	-	+	-
Cynodon dactylon (L.)Pers.	-	-	-	-	-	-	++	-
Delonix regia L.	+	+	+	+	-	-	+	-
Euphorbia hirta L.	+	+	-	+	+	-	+	+
Euphorbia tirucalli L.	+	-	-	+	-	-	++	-
Ficus benghalensis L.	-	+	+	-	-	+	++	-
Gmelina asiatica L.	-	+	-	+	+	+	-	-
Santalum album L.	+	+	-	+	+	-	-	-
Tecomella undulata (Sm.) Seem.	-	+	-	-	+	-	-	-

Table 3. Antibacterial activity of aqueous and methanol extracts of screened medicinal plants.

Plant Extracts	Zone of Inhibition (mm)[a]				
	B. cereus	*S. aureus*	*E. aerogenes*	*E. coli*	*K. pneumoniae*
Abrus precatorius L.					
Aq.	-	-	-	-	-
Me.	11	-	10	-	-
Caesalpinia pulcherrima Swartz.					
Aq.	12	9	-	-	13
Me.	20	18	15	14	19
Cardiospermum halicacabum L.					
Aq.	-	-	-	-	-
Me.	-	11	-	-	12
Casuarina equisetifolia L.					
Aq.	13	11	-	-	10
Me.	19	17	12	12	17
Cynodon dactylon (L.)Pers.					
Aq.	-	-	-	-	-
Me.	11	10	11	-	12
Delonix regia L.					
Aq.	10	-	-	-	-
Me.	17	15	-	-	14
Euphorbia hirta L.					
Aq.	13	9	-	-	11
Me.	24	16	-	-	21
Euphorbia tirucalli L.					
Aq.	9	-	-	-	-
Me.	19	11	18	11	21
Ficus benghalensis L.					
Aq.	-	-	-	-	-
Me.	16	12	-	-	15
Gmelina asiatica L.					
Aq.	-	-	-	-	-
Me.	12	-	-	-	14
Santalum album L.					
Aq.	-	-	-	-	-
Me.	11	18	11	-	14
Tecomella undulata (Sm.) Seem.					
Aq.	9	-	-	-	-
Me.	16	11	-	-	15
Piperacillin[b]	18	28	12	13	25
Gentamicin[b]	14	17	10	25	22

[a]: Inhibition zones are the mean including disc (7 mm) and cup-borer (8.5 mm) diameter

[b]: Antimicrobics = Piperacillin (100 µg/disc); Gentamicin (10 µg/disc)

Aq = Aqueous extract; Me = Methanol extract.

pneumoniae. Similar results were also reported by Venkatesan et al. (16), Prescott et al. (17), and Stainer et al. (18), who reported diseases such as pneumonia, urinary and respiratory tract infection, nosocomial pathogens and opportunistic infections caused by *Klebsiella* species. The studied plants were most active against Gram-positive bacteria *B. cereus*. The extracts of *Abrus precatorius*, *Cardiospermum halicacabum* and *Gmelina asiatica* could not inhibit any of the bacterial strains studied. The significant antibacterial activity of the active plant extracts was comparable to the standard antimicrobics, piperacillin (100 µg/disc) and gentamicin (10 µg/disc).

Preliminary phytochemical analysis revealed the presence of alkaloids (+ve test for Wagner's - Table 2) and saponins. The other secondary metabolites like tannins, flavonoids, steroids, cardiac glycosides, etc. were present in trace amounts in some of the plants (Table 2). It is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. It is quite possible that some of the plants that were ineffective in this study do not possess antibiotic properties, or the plant extracts may have contained antibacterial constituents, just not in sufficient concentrations so as to be effective. It is also possible that the active chemical constituents were not soluble in methanol or water (18). The drying process may have caused conformational changes to occur in some of the chemical constituents found in these plants.

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 have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999). Continued further exploration of plant- derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy. However, the present study of in vitro antimicrobial evaluation of some plants forms a primary platform for further phytochemical and pharmacological studies.

In conclusion, *Caesalpinia pulcherrima* extracts possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds.

Corresponding author:

Sumitra CHANDA

Phytochemical, Pharmacological and

Microbiological Laboratory,

Department of Biosciences, Saurashtra University,

Rajkot 360 005 - INDIA

E-mail: sumitrachanda@yahoo.com

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