ORIGINAL ARTICLE

Evaluation of antibacterial activity of different solvent extracts of medicinal plant: *Cleome viscosa* Linn.

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Abstract

Aim: The aim of the study deals with evaluation of antibacterial activity of the medicinal plant *Cleome viscosa* Linn. traditionally used in the treatment of communicable diseases. **Materials and Methods:** The plant was taxonomically identified and dried whole plant of *C. viscosa* Linn. was defatted with n-hexane (60–80°C) in a Soxhlet apparatus. The defatted powder material thus obtained was further extracted successively with chloroform, ethyl acetate, ethanol, and water to afford corresponding fractions. Standard strains of *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis,* and *Staphylococcus aureus* purchased from the Institute of Microbial Technology, Chandigarh, India. Antibacterial activities of the extracts were determined by the microbroth dilution assay. **Results and Discussion:** Plant extracts showed good bactericidal activity. Among the plant extracts, water and ethanol extract showed potent antibacterial activity against all the five bacteria, especially against Gram-negative bacteria *E. coli, S. typhi,* and *P. aeruginosa* followed by ethanol extract, whereas chloroform extract showed minimal bactericidal activity. **Conclusion:** The findings provide support for the use of these plants in traditional medicine for the treatment of infectious disease and are thus potential sources of drugs that would need to be subjected to further studies.

Key words: Antibacterial, medicinal plants, minimum inhibitory concentration

INTRODUCTION

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents.[1] Pathogenic bacteria have always been considered as a major cause of morbidity and mortality in humans. Even though pharmaceutical companies have produced a number of new antibacterials in the past years, resistance to these drugs has increased and has now become a global concern.[2] The global emergence of multidrug-resistant (MDR) bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure.[3] Bacterial resistance to chemically unrelated antimicrobial agents is public health concern[4] and may be caused by overexpression of MDR efflux pumps.[5] In Gram-negative bacteria, the effect of the efflux pumps in combination with the reduced drug uptake (due to the presence of a double membrane barrier) is responsible for the high inherent and acquired antibiotic resistance often associated with this group of organisms.[6]

Antibiotic resistance has increased substantially in the recent years and is posing an ever-increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is using antibiotic resistance inhibitors from plants.[7,8] Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant pathogens.[9] Medicinal plants have been used as traditional treatments for numerous human

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diseases for thousands of years and in many parts of the world. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs.[10,11] Today, there is much interest in studying natural products and their derivatives in search of options for disease treatment and other medical applications. Natural products are especially notorious as anticancer and anti-infective agents.

Cleome viscosa belongs to the family Cleomaceae. It is a small family of flowering plants in the order *Brassicales* having approximately 300 species belong to nine genera. The genus cleome is well recognized and shows subcosmopolitan distribution throughout the tropical and warm temperate regions of the world. It contains about 180–200 species of ethnomedicinal, ethnobotanical, and ecological importance. It contains 170 families among which herbaceous annual or perennial plants and shrubs are represented in different regions. *C. viscosa* Linn. is a widely distributed terrestrial, annual, and sticky herb that attains a height, up to 120 cm. It is a fodder plant commonly known as Hur Hur in Hindi. Plant is characterized by its yellow flowers and long slender pods containing seeds. Seeds are similar to mustard with strong penetrating odor.[12] *C. viscosa* L. is small size herb with yellow flowers and long slender pods containing seeds. Leaf and stem surfaces of *C. viscosa* L. show the presence of secretary glandular trichomes with club cylinder and cylinder morphologies.[13] *C. viscosa* plant is erect, grooved, and aromatic glandular and contains sticky shoots. Stem is rounded, solid, (glandular) and hairy while root is taproot branched white or brown. Plant bears, elliptic-oblong, and obovate leaflets of variable in size (1.5–2.5 cm) broad and petiole up to 5 cm long. Leaves are sessile, compound, trifoliolate, alternate, spiral, stalked, and foliate in shape and contain glandular hairs on both sides, leaflets are elliptic, (glandular) hairy on both sides, margin entire, apex acute, base acute, and pinnately veined. Flowers are pedicellate, bisexual, single, axillary, stalked, yellow, petals 4, and free. These are white or yellow in color. Flowering occurs from May to September and fruiting in August to November. Inflorescence is racemose or corymbose. Stipules are absent. Pedicels are 6–20 mm long, bracts foliaceous, petals 8–15 mm long, 2–4 mm broad, and oblong-obovate. Stamens are 10–12 and rarely occur up to 20 and not exceeding the petals; gynophores absent. Sepals are oblong-lanceolate, 3–4 mm long, 1–2 mm wide, and glandular-pubescent. Fruits are dark brown 30–75 mm long and 3–5 mm broad in size, a capsule, either a slender, linear-oblong capsule, erect, obliquely striated, and tapering at both ends. Its two valves contain hundreds of seeds that are oil producing.[14] The whole plant is sticky in nature and has a strong odor resembling asafetida.

The natives and traditional healers of India[15,16] use *C. viscosa* Linn. for various therapeutic purposes. In traditional system of medicine, this plant is used to treat various disorders such as diarrhea, fever, inflammation, liver diseases, bronchitis, skin diseases, and malarial fever. The juice is useful in piles, lumbago, and earache.[15] The leaves are diaphoretic, rubefacient, and vesicant and are used as an external application to wounds and ulcers. The juice of the leaves has been used to relieve earache.

MATERIALS AND METHODS

Identification and Collection of Plant

The plant was taxonomically identified by Dr. V. Chelladurai, M.Sc., Ph.D., Research officer - Botany (Scientist-C) Central Council for Research in Ayurveda and Siddha, Government of India (Rtd), Tirunelveli, Tamil Nadu. The voucher has been deposited in the Department of Pharmacognosy, Swamy Vivekanandha College of Pharmacy, Tiruchengode.

Preparation of Plant Extracts[17]

Dried whole plant of *C. viscosa* Linn. was defatted with n-hexane (60–80°C) in a Soxhlet apparatus. The defatted powder material thus obtained was further extracted successively with chloroform, ethyl acetate, ethanol, and water to afford corresponding fractions. Solvents were evaporated under reduced pressure and stored at 4°C for further use.

Microorganisms

Standard strains of *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Bacillus Subtilis,* and *Staphylococcus aureus* purchased from the Institute of Microbial Technology, Chandigarh, India.

Antibacterial Screening[18]

Antibacterial activities of the extracts were determined by the microbroth dilution assay as described by Buwa and Staden. The water and ethanol plant extracts were dissolved in corresponding extracting solvents at a concentration of 2400 µg/mL, while the other extracts were dissolved in dimethyl sulfoxide. Proper controls were kept for each experiment. The bacterial strains used as inoculums were grown at 37°C to get OD 0.6 at 600 nm and used for susceptibility testing. Lowest concentration, which inhibited any visual growth, was considered to be minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

Whole plants were evaluated for their antibacterial potential against five microorganisms in this study using microbroth dilution assay [Table 1]. Table 2 summarizes

Table 1: Selected Indian medicinal plants used to treat various diseases

Plant (Voucher no)	Part	Use
Abutilon indicum (245)	Whole plant	The plant is used to treat impotency, rheumatism, menorrhea, polyuria, gout and hemorrhagic diseases
Acacia leucophloea (3218)	Bark	Bark of plant is used as antimicrobial, anthelmintic, expectorant, and blood purifier. It is also used to treat skin diseases (leprosy), ulcer, gum bleeding, mouth ulcer, dry cough, dysentery, diabetes, and fever
Acacia nilotica (591)	Bark	Bark is used to treat cough, acute gonorrhea dysentery, diarrhea, cancers, syphilitic affections, and genitourinary affections
Aegle marmelos (346)	Fruit	Fruits are used in diarrhea and dysentery
Bacopa monnieri (371)	Leaves	Leaves of plant are used to treat epilepsy, insanity, and other nervous disorders
Bombax ceiba (579)	Bark	Bark of plant is demulcent, tonic, and expectorant and used to treat ulcer
Calotropis procera (97)	Whole plant	Plant is used to treat leprosy
Chlorophytum borivilianum (577)	Root	Roots are used to treat diarrhea and dysentery and also used as demulcent and galactagogue
Chlorophytum laxum (574)	Root	Roots are used to treat diarrhea and dysentery and also used as demulcent and galactagogue
Chlorophytum tuberosum (372)	Root	Roots are used to treat diarrhea and dysentery and also used as demulcent and galactagogue
Holoptelea integrifolia (146)	Stem bark	Stem bark is externally used in inflammation and internally used to treat piles, skin disease, anthelmintic, and obesity
Jatropha gossypifolia (3325)	Latex and leaf	Root is used in diarrhea and dysentery. Oil used as purgative and locally applies in skin disease and arthritis. Latex and leaf juice are used to treat ulcer, skin disease (leprosy), and gum infections
Justicia zeylanica (3128)	Leaf	Leaf of the plants is used in microbial infections, bronchitis, asthma, fever, and arthritis
Lantana camara (507)	Leaf flower	Leaf juice is used as antimicrobial in skin diseases
Mangifera indica (122)	Root	Roots are used in menorrhea, leucorrhea, and scabies
Phyllanthus emblica (37)	Fruit and seed	Fruits and seeds are used to treat asthma, bronchitis, and biliousness
Phyllanthus urinaria (342)	Whole plant	Plants are used to treat cough, bronchitis, skin disease, enlarged spleen and liver, jaundice, and fever
Pithecellobium dulce (3306)	Root	Root and bark decoctions are taken orally to treat diarrhea; fruit pulp is taken orally to stop blood flow in case of hemoptysis. The seed juice is inhaled into the nostrils against chest congestion
Saraca asoca (119)	Bark	Bark is used to treat menorrhea, bowel, pimple, weakness, hemorrhage, dropsy, and uterine sedative
Solanum surattense (99)	Whole plant	Plant is used to treat skin disease, cough, cold, bronchitis, and asthma

Table 2: MIC of plant extract against the microorganism by microdilution broth assay

Plant extracts	MIC (µg/ml)				
	E. coli	***S. typhi***	***P. aeruginosa***	***B. subtilis***	***S. aureus***
n-Hexane	ND	ND	ND	ND	ND
Chloroform	150	75	300	600	600
Ethyl acetate	75	75	150	300	300
Ethanol	18.75	18.75	75	37.5	75
Water	18.75	18.75	18.75	37.5	150

E. coli: *Escherichia coli*, *S. typhi*: *Salmonella typhi*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *B. subtilis*: *Bacillus subtilis*, *S. aureus*: *Staphylococcus aureus*, ND: Means not determined, MIC: Minimum inhibitory concentration

the results obtained. Antimicrobial activity of plant extract was considered to be good if its MIC was <100.0 µg/mL, moderate if MIC was from 100.0 to 500.0 µg/mL, and poor over 500.0 µg/mL. The water extracts of *C. viscosa* Linn. found to be the most active against bacteria followed by ethanol extract showed moderate activity, but chloroform extract showed minimal bactericidal activity. Water extract showed potent antibacterial activity against both Gram-positive and Gram-negative bacteria but very potent against Gram-negative bacteria. The screenings of this medicinal plant showed that is a potential source of antibacterial agents. This synergy could lead to new options for the treatment of infectious diseases and emerging drug resistance. There is a need for more studies on the molecular basis of the synergistic interaction to understand the synergistic mechanism that is fundamental for the development of pharmacological agents to treat bacterial infections using medicinal plants. Therefore, research should be focused in that direction to identify medicinal plants that have a synergistic behavior.

CONCLUSION

In this study we evaluated the antibacterial activity of *Cleome viscosa* Linn. Among the plant extracts water and ethanol extract showed potent antibacterial activity against all the five bacteria especially against Gram negative bacteria *E.coli, S.typhi* and *P.aeruginosa*. Further studies are necessary for this potent plant extracts to evaluate the other parameters of antimicrobial efficacy (e.g., *in vivo* efficacy, toxicity and antimycobacterial, antiviral, and antiparasitic activity).

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