Research Article

In Vitro Antibacterial Activity and Phytochemical Studies of Some Medicinal Plants

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ABSTRACT

The extract of many plants used in traditional medicine contain a wide range of curative agents that are used in many modern medicines. In the present study phytochemical analysis and *in vitro* antibacterial activities of the n-butanolic extracts of *Terminalia arjuna, Cleome viscosa* and *Leucas aspera* having ethnomedicinal uses collected from the Kadapa district were tested. The phytochemical studies of the extracts revealed the presence of carbohydrates, tannins, alkaloids, flavonoids, steroids, glycosides in all plants. The extracts were subjected for screening of *in vitro* antibacterial activity against selected major human pathogenic bacterial strains like *Bacillus substilis, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, and Staphylococcus aureus* by agar well diffusion method. The results of antibacterial activity revealed that all the extracts showed excellent inhibitory activity against all the tested pathogens and the *Cleome viscosa* extract showed comparatively better activity than the other extracts.

Keywords: Antibacterial, Phytochemical, *Terminalia arjuna*, *Cleome viscosa*, *Leucas aspera.*

INTRODUCTION

According to World Health Organization, medicinal plants are the best source to obtain a variety of newer herbal medicine. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants[1, 2]. Nature has very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Traditional medicine has been improved in developing countries as an alternative solution to health problems and costs of pharmaceutical products. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated, a search for new antimicrobial substances from other sources, including plants. Secondary metabolites such as flavonoids, alkaloids, tannins and phenolic compounds have been established as the bioactive compounds of plants[3]. The present study is aimed to carry out to screen *in vitro* antimicrobial activity and phytochemical analysis of selected medicinal plants of *Terminalia arjuna, Cleome viscosa and Leucas aspera.*

Terminalia arjuna Wight & Arn is a deciduous and evergreen tree belonging to combretaceae family[4] (Table 1). It is found in abundance throughout Indo-sub-Himalayan tracts of Uttar Pradesh, South Bihar, Madhya Pradesh, Delhi and Deccan region near ponds and rivers. The bark is said to be sweet, acrid, cooling and heating, aphrodisiac, expectorant, tonic, styptic, antidysenteric, purgative and laxative. Its use has been advocated in urinary discharge, strangury, leucoderma, anaemia, hyperhydrosis, asthma and tumours. It provides a significant cardiac protection in myocardial infarction commonly known as, heart attack. It helps in lowering cholesterol levels and maintaining it to normal levels, nourishes heart muscles, which is very helpful in regular expansion and contraction of heart, helps in both vasoconstriction as well as vasodilatation. It works as a wonderful antioxidant therefore helps in stopping early aging signs and helps in maintaining youth.

Leucas aspera is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid acutely quadrangular stem and branches belonging to the family of lamiaceae (Table 1). The plant is used traditionally as an antipyretic and insecticide. Flowers are valued as stimulant, expectorant, aperient, diaphoretic, insecticide and emmenagogue. Larvicidal activity has also been reported for leaf extract of the plant against the mosquito species, *Leucas aspera* is reported to have antifungal, prostaglandin inhibitory, antioxidant, antinociceptive and cytotoxic activities[5]. It is used in the traditional medicine of the Philippines to treat scorpion bites. In some forms of traditional medicine, the plant's flowers, seeds, roots, berries, bark or leaves, can be inhaled to help treat nasal congestion, coughing, cold, headache and fever.

The *Cleome viscosa* is commonly known as Asian spider flower or yellow spider flower. It belongs to capperaceae family (Table 1). Traditionally, this plant is used in various disorders such as diarrhoea, fever, inflammation, liver diseases, bronchitis, skin diseases, and malarial fever[6]. The juice is useful in piles, lumbago and earache. The analgesic, antipyretic and anti-diarrhoeal activities of the extract have been reported by researchers, it was noted that the fresh leaves of *Cleome viscosa* are widely used as medicine for jaundice.

Table 1: Plant species used for screening of antibacterial activity

Name of the plant	Kingdom	Family	Genus	Species	Local name
Terminalia arjuna	Plantae	Combretaceae	*Terminalia*	*arjuna*	Yerramaddi
Cleome viscosa	Plantae	Cleomaceae	*Cleome*	*viscosa*	Kukkuvaminta
Leucas aspera	Plantae	Lamiaceae	*Leucas*	*aspera*	Thummachettu

MATERIALS AND METHODS

Selection and collection of plant material

We have selected the healthy, disease free and mature plants of *Terminalia arjuna, Cleome viscosa* and *Leucas aspera* from Kadapa district.

Sampling of plant material

The fresh and healthy leaves of medicinal plants were washed with running tap water, then with distilled water (three times) and air dried under shade, dried mass was grounded to a fine powder. The powder obtained was kept in small plastic bags with proper labeling.

Preparation of plant leaf extract

Dried powdered plant material was extracted in soxhlet's extractor for 12 hrs and all extracts were concentrated using rotary evaporator and dry residue was preserved at 4^0C in air tight bottles until further use.

Bacterial strains

In vitro antimicrobial activity was examined for n-butanol extracts of plants. Bacterial strains used were, *Bacillus subtilis (*G+ve), *Escherichia coli* (G-ve), *Klebsiella pneumonia* (G-ve), *Proteus vulgaris* (G-ve), *and Staphylococcus aureus* (G+ve).

Media preparation and antibacterial activity (Agar well diffusion method)

The antibacterial activities of the leaves were tested against the selected bacterial strains. Sterile agar medium was poured into each sterile Petri plate and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile spreader. The antibacterial assay was performed by Agar well diffusion method for solvent extracts. The well of 0.5 cm was made by using a sterile tip. 100 µl of n-butanol plant extracts were added into two wells and 100 µl of tetracycline (antibiotic) was added into one well for control. After these plates was incubated at 37°C for 24 hours. After incubation period the results were observed and antibacterial activities were measured by measuring the diameter of the zones of inhibition around the each well and were compared with the zone of inhibition of standard drug (Tetracyclin).

Phytochemical screening of the extracts

Plant extracts collected were characterized biochemically by qualitative analysis.

Detection of alkaloids

About 2 ml each of the extracts were stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath for 10 minutes. 1 ml of the extract was treated with a few drops of Mayer's reagent, precipitation with these reagents was seen as evidence for the presence of alkaloids[7].

Detection of carbohydrates

Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates[8].

Detection of glycosides

Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides[8].

Detection of phenols:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols[8].

Detection of tannins:

Two milliliters each of the methanolic extracts were separately boiled for ten minutes in 10 ml of water in a test tube. A few drops of 0.1% ferric chloride were added to each test tube and observed for 10 minutes for a brownish green or a blue black coloration[9].

Detection of Flavonoids

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids[8].

Detection of proteins and amino acids

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid[8].

Detection of diterpenes

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes[8].

Detection of steroids

1 ml each of the extracts was dissolved in 2 ml of chloroform. A few drops of concentrated sulphuric acid were carefully added to form a lower layer. A reddish colour formed at the interphase indicates the presence of a steroid ring[7].

RESULTS AND DISCUSSION

In the present investigation the antibacterial properties of n-butanolic extracts of three medicinal plants such as *Terminalia arjuna, Cleome viscosa, Leucas aspera* were tested against five human pathogenic bacteria.

Antibacterial activity of *Terminalia arjuna:* The n-butanol extracts of *Terminalia arjuna* showed maximum zone of inhibition against *Bacillus subtillis* (18 mm), *Escherichia coli* (20 mm), *Klebsiella pneumonia* (20 mm), *Pseudomonas vulgaris* (20 mm), and *Staphylococcus aureus* (21 mm), (Table 2).

Antibacterial activity of *Cleome viscosa:* The n-butanol extract of *Cleome viscosa* showed maximum zone of inhibition against *B. subtillis* (24 mm), *E. Coli* (23 mm), *K. pneumonia* (23 mm), *P. vulgaris* (22 mm), and *S. aureus* (24 mm) (Table 2).

Antibacterial activity of *Leucas aspera:* The n-butanol extracts of *Leucas aspera* showed maximum zone of inhibition against *B. subtillis* (19 mm), *E. Coli* (20 mm), *K. pneumonia* (18 mm), *P. vulgaris* (21 mm), and *S. aureus* (20mm) (Table 2).

Table 2: Antibacterial activity of medicinal plants against bacteria

Name of the organism	Zone of inhibition (mm)			
	Tetracycline	*Terminalia arjuna*	*Cleome viscosa*	*Leucas aspera*
Bacillus subtillis	30±0.35	18±0.4	24±0.15	19±0.71
Escherichia coli	15±0.51	20±0.23	23±0.34	20±0.52
Klebsiella pneumonia	16±0.4	20±0.3	23±0.23	18±0.15
Proteus vulgaris	29±0.3	20±0.15	22±0.31	21±0.6
Staphylococcus aureus	27±0.15	21±0.52	24±0.18	20±0.5

Table 3: Phytochemical analysis of medicinal plants

Name of the compound	Name of the test	Name of the plants		
		Terminalia arjuna	*Cleome viscosa*	*Leucas aspera*
Alkaloids	Mayer's test	++	+	++
Flavonoids	Alkaline Reagent Test	++	++	++
Carbohydrates	Molisch's Test	++	-	+
Phenols	Ferric Chloride Test	++	++	++
Tannins	5% Ferric chloride	++	++	++
Steroids	Chloroform + Acetic acid + H_2SO_4	++	++	++
Glycosides	Legal's Test	++	++	++
Proteins	Ninhydrin Test	++	++	+
Diterpenes	Copper acetate Test	+	++	++

⁻= absent; + = present; ++ = more quality

Plant based antibacterial activity have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antibacterials[10]. The potential of developing antibacterial activity from higher plants appears rewarding as it will lead to the development of phytomedicine to act against microbes. Among the extracts *Cleome viscosa* exhibited maximum antibacterial activity against all the tested strains. It showed highest activity against Bacillus and Staphylococci and the lowest activity against Proteus. *Terminalia arjuna* showed highest activity against Staphylococci and the lowest activity against Bacillus. Highest activity was found against Proteus and lowest activity against Klebsiella by *Leucas aspera*.

Phytochemical constituents such as alkaloids, flavonoids, glycosides and several other aromatic compounds are secondary metabolites in plants that alleviate the pathogenic and environmental stress [11,12]. The results of preliminary qualitative phytochemical study of the three plants showed the presence of alkaloids, tannins, cardiac glycosides, steroids, Flavonoids, proteins and diterpenes (Table 3). Thus, the plants studied here can be seen as a potential source of new useful drugs.

CONCLUSION

The observed antibacterial activity may be due to the presence of potent phytochemical constituents in the extracts. In conclusion all the plant extracts posses 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. The qualitative phytochemical analysis of the three plants showed the presence of alkaloids, tannins, cardiac glycosides, steroids, flavonoids, proteins and diterpenes. The phytochemical characterization of the extracts, the identification of responsible bioactive compounds and quality standards are necessary for future study.

REFERENCES

1. Prusti A, Mishra SR, Sahoo S, and Mishra SK, Antibacterial activity of some Indian medicinal plants. Ethnobotanical Leaflets, 12, 2008, 227-230.
2. Mahalingam R, Bharathidasan R, Ambikapathy V and Panneerselvam A, Studies on antibacterial activity of some medicinal plant against human pathogenic micro organism. Asian journal of plant science and research, 2011, 1, 86-90.
3. Zakir Ullah, Ali Rehman, Najeeb Ullah, Shakeel Ahmad Khan, Shahid Ullah Khan and Ijaz Ahmad, Antibacterial study of *Phyla nodiflora* Linn, Journal of Chemical and Pharmaceutical Research, 5, 2013, 86-90.
4. Nadkarni AK, Nadkarni KM, Indian Materia Medica, 1st ed. Popular Book Depot, Bombay India, 1954, 1198.
5. Prajapati MS, Patel JB, Modi K, Shah MB. *Leucas aspera*, A review, Phcog Rev 2010.
6. Henty EE, Pritchard GH, Weeds of New Guinea and their control. 2nd edition, Department of Forests, Division of Botany, Botany Bull. No. 7. Lae, Papua New Guinea. 1975, 180.
7. Sofowora A, Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd (Pub.), Ibadan, 1993.
8. Pardhasardhi M and Sindhu GS, Obtusifoliol, Syringetin and dihydrosyringetin from *Soymida febrifuga.* Phytochemistry, 11, 1972, 1520-1522.
9. Okwu DE, Morah FNI and Anam EM, Isolation and characterization of phenanthrenic alkaloid uvariopsine from *Dennettia tripetala* fruits. J. Med. Aromatic Plant Sci., 27, 2005, 496 – 498.
10. Lutterodt GD, Ismail A, Basheer RH, Baharudin HM, Antimicrobial effects of *Psidium guajava* extracts as one mechanism of its antidiarrhoeal action. Malaysian J Med Sci, 6, 1999, 17-20.
11. Marjorie MC, Plant products as antimicrobial agent. Clin Microbiol Rev, 12, 1999, 564-582.
12. Edreva A, Velikova V, Tsonev T, Dagnon S, Gürel A , Akta L, Gesheva E ,Stress-protective role of secondary metabolites, diversity of functions and mechanisms. Gen Appl Plant Physiology -Special Issue, 34, 2008, 67-78.

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