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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL PROPERTIES OF SELECTED MEDICINAL PLANTS AGAINST BACTERIA ASSOCIATED WITH DIABETIC PATIENTS

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ABSTRACT

The antibacterial activity of ten medicinal plants was screened for different bacterial strains using methanol and water as solvents. The leaves of seven plants (*Emblica officinalis, Achyranthes aspera, Coleus aromaticus, Acacia nilotica, Solanum nigrum, Azadirachta indica, Prosopis spicigera)* and the bark of three plants (*Syzygium cumini, Mangifera indica, Lawsonia inermis)* were dried and powdered before being subjected to soxhlation. All extracts were concentrated by using rotary flash evaporator. The phytochemical screening was carried out to know the compounds responsible for these activities. Methanol and water extracts were tested against *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes* and *Pseudomonas aeruginosa.* The antibacterial assays in this study were performed by the agar-well diffusion methods so that they could be quantified by measuring the zones of growth inhibition diameters, MIC values. The susceptibility of the bacteria to the crude extracts on the basis of zones of growth inhibition varied according to microorganism and extracting solvent. In most of the above mentioned plants, the methanol extract produced the highest activity when compared to aqueous extract. The organisms used for the purpose of this investigation were associated with opportunistic infections in diabetic patients. On the basis of the results obtained, it could be concluded that methanol could be used for extracting antimicrobial compounds from both leaves and bark. The present study showed that the plant extracts possessed the antimicrobial activity against some organisms associated with diabetes.

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KEYWORDS

Antibacterial, Medicinal plants, Diabetes

INTRODUCTION

The medicinal use of plants is probably as old as mankind. Plants have continued to be a valuable source of natural products for maintaining human health, as studies on natural therapies have intensified. More than 150,000 plant species have been studied and several of them contain therapeutic substances and the use of plant compounds for pharmaceutical purposes has gradually increased. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources[1-2.] According to the World Health Organization, medicinal plants are probably the best source of a variety of drugs. About 80 % of individuals in developed countries use traditional medicine containing compounds derived from medicinal plants[3-5.]

India is represented by rich culture, tradition and natural biodiversity and it offers a unique opportunity for drug discovery research[6.] A number of traditional natural products have been increased and much work has been done on selected ethno medicinal plants for antibacterial activity against pathogenic strains of Gram negative and Gram positive bacteria[7.]

Many works have been done which aim at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections (both topical and systemic applications) as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant. Further, natural products as an alternative to conventional treatment in healing and treatment of various diseases have been on the rise in the last few decades[8]. During the last 10 years, the pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance (especially multiple) has increased astronomically[9.]

The bioactive constituents from plant origin show antimicrobial activity against some microorganisms like bacteria, fungi and protozoa. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbiostatic). Thus, the phytomedicines derived from plants have shown great promise in the treatment of various diseases including viral infections. One of the most abundant groups of polyphenol compounds in plants is the flavonoids. Over 4000 different flavonoids occurring in plants have been described, and these compounds have various pharmacological properties such as antioxidative, antiinflammatory, diuretic and antimicrobial activity[10.]

The development of wounds is a serious complication for patients with diabetes. Numerous factors related to diabetes can impair wound healing, including wound hypoxia (inadequate oxygen delivered to the wound) infection, nutrition deficiencies and the disease itself[11.] The aim of the present study is to determine the role of bioactive principle for the control of bacterial infections associated with diabetic patients by testing the antibiotic susceptibility of antibiotics against the predominant bacterial types in comparison to selected plant extracts.

MATERIALS AND METHODS

(i) Collection of plant material

Fresh and healthy leaves and bark of different medicinal plants were collected from in and around Mysore (Karnataka state, India). The plants were identified based on the taxonomical characteristics by Prof. G.R. Shivamurthy, Department of Studies in Botany, Mansagangotri, University of Mysore, Mysore, Karnataka, India. Both leaves and bark were shade dried, powdered and used for extraction.

(ii) Test Microorganisms

The test organisms were clinical isolates obtained from patients previously diagnosed with diabetes from the surgical ward, K.R. Hospital, Mysore, with the aid of the hospital staff following standard procedures described by the hospital administration. Wound exudates were collected using sterile cotton tipped swab before antiseptic dressing was applied to the wound. Normal saline was used to moisten the swab stick before collecting the specimen. Patients were also assessed, using a confidential semi- structured questionaires for medical conditions that might predispose them to developing wound infection. Discrete colonies were isolated and cultured on Mueller Hinton Agar slants and were immediately transported to the Microbiology Laboratory of Manasagangotri, University of Mysore, for identification purposes. All the isolates were sub cultured thrice to obtain pure culture. The cultures were maintained on nutrient agar medium[12].

(iii) Preparation of aqueous extract

The finely powdered plant materials (100 grams) were boiled in 500 ml distilled water till one-fourth of the extract initially taken was left behind after evaporation. The solution was first filtered through double layered muslin cloth and centrifuged at 5000 g for 30 min and the supernatant was filtered through whatman No.1 filter paper under strict aseptic conditions and then the filtrate was collected in fresh sterilized bottles and stored at 4^{o}C until further use.

(iv) Preparation of solvent extract

100 grams each of the powdered material was extracted with 500ml of methanol separately for 24hrs. The extract was filtered with sterile whatman filter paper No.1 into a clean conical flask. The solvent along with the sample was transferred into the sample holder of the rotary flash evaporator for the evaporation of the solvent. The evaporated solvent so obtained was weighed and preserved at 4^{o}C in airtight bottles until further use.

(v) Phytochemical screening

Phytochemical screening was carried out to determine the presence of saponins, tannins, flavonoids, glycosides, triterpenoids, phytosterols and cardiac glycosides[13-14]. The solvents used were methanol and distilled water.

1. *Test for Saponins (Foam test*)

About 200 mg of powdered sample was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent broth. Formation of foam indicated the presence of saponins.

2. *Test for Tannins (Ferric chloride test)*

About 200 mg of plant extract was treated with few drops of 0.1% ferric chloride and observed for blue or black colouration. Formation of blue black colour confirmed the presence of tannins.

3. *Test for Alkaloids (Wagner's test)*

About 0.5ml of extract solution was treated with 2-3 drops of Wagner's reagent (solution of Iodine in potassium iodide) and the formation of reddish brown precipitate indicated the presence of alkaloids.

4. *Test for Flavonoids (Alkaline reagent test)*

To the extract solution, few drops of sodium hydroxide was added, formation of an intense yellow colour, which turns to colourless on addition of few drops of dilute acetic acid indicated the presence of flavanoids.

5. Test for Sterols and Triterpenoids (Salkolwski's test)

The extract was treated with chloroform, few drops of concentrated H_2SO_4 was added, the test tube was shaken well and allowed to stand for some time. The appearance of red colour in upper layer indicated the presence of sterol and formation of yellow colour at the

lower layer indicated the presence of triterpenoids.

6. Test for Cardiac Glycosides (Keller Killani test)

The extract was treated with chloroform and allowed to dryness. Then, 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride solution was added. The mixture was transferred to small test tube. 0.5 ml of concentrated H_2SO_4 was added along the sides of the test tube, the appearance of blue colour in acetic acid layer indicated the presence of cardiac glycosides.

(vi) Agar-well diffusion assay:

Suspension of 24 h cultures of *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes and Pseudomonas aeruginosa* was made in sterile normal saline. Each labeled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. A sterile cork borer of 5mm diameter was used to make wells on the medium. 100µL of the various extract concentration were dropped into each, appropriate well[15-16]. Methanol solvent used for extraction apart from water was tested neat for each organism. The inoculated plates were kept in refrigerator for 2 hours to allow the extracts to diffuse into the agar[15]. The agar plates were incubated at 37°C for 24 h. Antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation. 30µg of standard antibiotic streptomycin was used as positive control and respective solvents as negative controls.

(vii) Minimum Inhibitory Concentration (MIC) assay:

Various concentration of the plant extracts ranging between 10 and 100 mg/mL were introduced into different test tubes, each tube was inoculated with an overweight culture of *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes and Pseudomonas aeruginosa* diluted to give a final concentration of 106 cells per ml. The tubes were incubated at 37°C for 24 h. The least concentration of the plant extract that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the MIC in each case[17].

Table 1
List of medicinal plants and their part used for antibacterial studies.

Medicinal Plant	Family	Part used
Emblica officinalis	Amaranthaceae	Leaves
Achyranthes aspera	Euphorbiaceae	Leaves
Coleus aromaticus	Lamiaceae	Leaves
Acacia nilotica	Mimosae	Leaves
Mangifera indica	Anacardiaceae	Bark
Lawsonia inermis	Lythraceae	Bark
Solanum nigrum	Solanaceae	Leaves
Syzygium cumini	Myrtaceae	Bark
Azadirachta indica	Meliaceae	Leaves
Prosopis spicigera	Mimosae	Leaves

RESULTS AND DISCUSSION

Phytochemical screening on the crude methanol and aqueous extracts of 10 medicinal plants were done using test tube. The results (Table 2) revealed the presence of secondary metabolites such as saponins, tannins, alkaloids, phytosterol, triterpenoids and cardiac glycosides. Except *Azadirachta indica*, none of the other plants showed the presence of cardiac glycosides. Alkaloids and flavonoids were present in all extracts except *Lawsonia inermis* and *Prosopis spicigera* respectively.

The efficacy of methanol and aqueous extracts of these medicinal plants against pathogenic bacteria associated with diabetic infections showed varied level of inhibition (Table 3). It was revealed from the result that each medicinal plant showed different degrees of inhibition against different microorganisms.

The maximum zone of inhibition was observed in the case of *Enterobacter aerogenes* and *Pseudomonas aeruginosa* (21mm) due to action of methanol extracts of *Emblica officinalis*, *Syzygium cumini* and *Azadirachta indica* and minimum was against aqueous extract of *Enterobacter aerogenes* (5mm) shown by *Coleus aromaticus*.

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Table 2
Classes of compounds identified in different plant extracts.

Compound	Result									
	E. o	*A. a*	*C .a*	*A. n*	*M. i*	*L. i*	*S. n*	*S. c*	*A. i*	*P. s*
Saponins	+	+	-	+	+	-	+	-	+	+
Tannins	+	+	+	+	-	+	+	+	+	-
Alkaloids	+	+	+	+	+	-	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	-
Phytosterol	+	-	-	-	-	-	-	+	-	-
Triterpenoids	-	-	-	-	-	-	-	-	+	-
Cardiac glycosides	-	-	-	-	-	-	-	-	+	-

E. o = E. officinalis A. a = A. aspera C. a = C. aromaticus A. n = A. nilotica M. i = M. indica L. i = L. inermis S. n = S. nigrum S. c = S. cumini A. i = A. indica P. s = P. spicigera

+ positive - negative.

Table 3
Zone of inhibitory activity (in mm) of methanol and aqueous extracts of medicinal plants against clinically isolated bacteria. Streptomycin, a standard drug, is used for purpose of comparison.

Medicinal plant	Solvents and antibiotic	*E. coli*	*E. aerogenes*	*S. aureus*	*P. aeruginosa*
Emblica officinalis	Methanol (control)	--	--	--	--
	Methanol Extract	20	21	17	19
	Aqueous (control)	--	--	--	--
	Aqueous Extract	16	16	13	18
	Streptomycin	27	25	26	26

	Methanol (control)	--	--	--	--
	Methanol Extract	16	ND	15	13
Achyranthes aspera	Aqueous (control)	--	--	--	--
	Aqueous Extract	14	ND	12	10
	Streptomycin	27	16	26	22
	Methanol (control)	--	--	--	--
	Methanol Extract	16	8	15	9
Coleus aromaticus	Aqueous (control)	--	--	--	--
	Aqueous Extract	9.4	5	8.2	7
	Streptomycin	27	13	26	15
	Methanol (control)	--	--	--	--
	Methanol Extract	15	13	14	10
Acacia nilotica	Aqueous (control)	--	--	--	--
	Aqueous Extract	10.8	9	9.7	8
	Streptomycin	24	17	25	15
	Methanol (control)	--	--	--	--
	Methanol Extract	16	14	14	17
Mangifera indica	Aqueous (control)	--	--	--	--
	Aqueous Extract	12	9	11	8
	Streptomycin	27	12	26	11
	Methanol (control)	--	--	--	--
	Methanol Extract	18	ND	13	16
Lawsonia inermis	Aqueous (control)	--	--	--	--
	Aqueous Extract	15.6	ND	12.5	7
	Streptomycin	22	14	20	21
	Methanol (control)	--	--	--	--
	Methanol Extract	19	ND	18	11
Solanum nigrum	Aqueous (control)	--	--	--	--
	Aqueous Extract	15	ND	14	5.5
	Streptomycin	25	11	24	15
	Methanol (control)	--	--	--	--
	Methanol Extract	16	21	15	11
Syzygium cumini	Aqueous (control)	--	--	--	--
	Aqueous Extract	10.2	15	9	8
	Streptomycin	27	22	26	21
	Methanol (control)	--	--	--	--
	Methanol Extract	20	18	13	21
Azadirachta indica	Aqueous (control)	--	--	--	--
	Aqueous Extract	11	13	6	17
	Streptomycin	18	20	26	18
	Methanol (control)	--	--	--	--
	Methanol Extract	12	ND	11	17
Prosopis spicigera	Aqueous (control)	--	--	--	--
	Aqueous Extract	2	ND	12	15
	Streptomycin	27	17	26	28

Each value is the average diameter of triplicates, --: No inhibition, ND: Not detected

The results revealed variability in the bactericidal concentration of each extract for given bacteria. It was clear from the present result that methanol extract exhibited pronounced activity against all the tested four bacteria. The highest antibacterial activity as seen with methanol extract might be due to the presence of alkaloids and tannins[18]. Broad spectrum activity of methanol extract tended to show that the active ingredients of the leaves were better extracted with methanol. Earlier studies had also shown the greater antibacterial activity of methanol extracts than other solvent extracts[19-20]. With least or no antibacterial activity as seen with other solvent extracts, might be due to loss of some active compounds during extraction process of the sample and lack of solubility of active constituents in the solvent[21].

The minimum inhibitory concentration (MIC) of these plant extracts was found against *E. coli, E. aerogenes, S. aureus* and *P. aeruginosa* were (10- 80 mg/mL), as could be seen from Table 4. All the plant extracts showed antimicrobial against *E. coli* and *S. aureus*. Whereas, *Emblica officinalis*, *Coleus aromaticus, Acacia nilotica, Lawsonia inermis, Syzygium cumini, Azadirachta indica* and *Prosopis spicigera* did not show inhibition against *E. aerogenes* and so are *C. aromaticus* and *A. nilotica* against *P. aeruginosa*.

Table 4
Minimum inhibitory concentration (MIC) of methanol and aqueous extract of medicinal plants.

Medicinal plant	Solvents	*E. coli*	*E. aerogenes*	*S. aureus*	*P. aeruginosa*
Emblica officinalis	Methanol (control)	--	--	--	--
	Methanol Extract	35	ND	30	10
	Aqueous (control)	--	--	--	--
	Aqueous Extract	75	ND	60	ND
Achyranthes aspera	Methanol (control)	--	--	--	--
	Methanol Extract	40	10	20	10
	Aqueous (control)	--	--	--	--
	Aqueous Extract	80	30	80	40
Coleus aromaticus	Methanol (control)	--	--	--	--
	Methanol Extract	80	ND	60	ND
	Aqueous (control)	--	--	--	--
	Aqueous Extract	20	ND	30	ND
Acacia nilotica	Methanol (control)	--	--	--	--
	Methanol Extract	40	ND	35	ND
	Aqueous (control)	--	--	--	--
	Aqueous Extract	60	ND	50	ND
Mangifera indica	Methanol (control)	--	--	--	--
	Methanol Extract	48	40	60	40
	Aqueous (control)	--	--	--	--
	Aqueous Extract	80	60	32	60
Lawsonia inermis	Methanol (control)	--	--	--	--
	Methanol Extract	50	ND	30	50
	Aqueous (control)	--	--	--	--
	Aqueous Extract	60	ND	45	100
Solanum nigrum	Methanol (control)	--	--	--	--

	Methanol Extract	60	30	60	25
	Aqueous (control)	--	--	--	--
	Aqueous Extract	80	70	40	60
Syzygium cumini	Methanol (control)	--	--	--	--
	Methanol Extract	50	ND	30	40
	Aqueous (control)	--	--	--	--
	Aqueous Extract	60	ND	40	60
Azadirachta indica	Methanol (control)	--	--	--	--
	Methanol Extract	50	ND	60	25
	Aqueous (control)	--	--	--	--
	Aqueous Extract	85	ND	80	40
Prosopis spicigera	Methanol (control)	--	--	--	--
	Methanol Extract	60	ND	60	40
	Aqueous (control)	--	--	--	--
	Aqueous Extract	70	ND	80	75

Each value is the average concentration of triplicates, --: No inhibition, ND: Not detected.

Antibacterial activity due to all these plants was previously reported by investigators. Considering that, in this study, only crude methanol and aqueous extracts were employed. The MIC values for methanol extract indicated that *P. aeruginosa* was more susceptible than any other bacteria followed by *S. aureus*. In spite of this permeability difference between Gram positive and Gram negative bacteria, the methanol extract had a broader spectrum of inhibitory activity. This showed the involvement of more than one active principle of biological significance[22].This study does not only show the scientific basis for some of the therapeutic uses of these plants in traditional medicine, but also confirms the fact that ethnobotanical approach should be considered when investigating antimicrobial properties of plants[23-24].

The implication of the broad spectrum action of some of these extracts may help in selection of plants with antimicrobial activities for further phytochemical work on the isolation and the identification of the active compounds.

CONCLUSION

On the basis of the results obtained, it can be concluded that methanol can be used for extracting antimicrobial compounds from both leaves and bark. The present study shows that plant extracts possessed the antimicrobial activity against some organisms associated with diabetes. Therefore, it suggests that the plant can be a source of oral drugs to be used in the treatment of opportunistic infections and may be a source for future drug formation.

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