June 2009 Volume 3 Issue 3

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 3(3), 2009 [161-164]

Investigation on phytochemical screening and antibacterial potential of methanol extract of *Baccaurea courtallensis* Muell.-Arg and *Prosopis juliflora* DC

M.L.Sujatha[1], Namitha C.Yadav[1], S.H.Jain[2], S.Mohan[2], T.R.Prashith Kekuda[3]*

[1]P.G Dept. of Studies and Research in Biochemistry, School of Chemical Sciences, Jnana Sahyadri, Shankaraghatta-577451, Karnataka (INDIA)

[2]Institute Wood Science and Technology (IWST), 18th Cross, Malleswaram Bangalore-560006, Karnataka, (INDIA)

[3]Dept. of Microbiology, S.R.N.M.N College of Applied Sciences, Shivamogga-577201, Karnataka (INDIA)

E-mail : prashith_kekuda@rediffmail.com

Received: 10th April, 2009 ; Accepted: 15th April, 2009

ABSTRACT

The present investigation highlights phytochemical screening and antimicrobial activity of methanolic extract of bark of *Baccaurea courtallensis* Muell.-Arg and *Prosopis juliflora* DC. The dried and powdered materials were subjected to soxhlet extraction using methanol solvent and subjected to preliminary phytochemical analysis. The extracts were dissolved in DMF and the antimicrobial efficacy of extracts was tested using well diffusion method against bacteria and fungi. Results of phytochemical analysis showed the presence of Tannin, Terpenoids, Saponins and flavonoids in the methanol extracts of both the plants. A marked antibacterial and antifungal activity was observed as revealed by inhibition zone around the well. The antimicrobial property could be attributed to the presence of phytoconstituents present in the extracts. The plant extract could be used against bacteria causing food poisoning, enteric infections, and nosocomial infections. The extract could be employed in the prevention and treatment of opportunistic infections caused by *Aspergillus* species. Further investigations have to be carried to isolate active constituents and validate the antimicrobial potential of crude extracts and individual constituents *in vivo* in animal models.

KEYWORDS

Prosopis juliflora;
Baccaurea courtallensis;
Phytochemical profile;
Antimicrobial activity;
Well diffusion method.

INTRODUCTION

Phytoconstituents present in plants are producing exciting opportunity for the expansion of modern chemotherapies against wide range of microorganisms[1]. The major chemical substances of interest in these surveys have been the alkaloids and steroidal sapogenins (saponins), however, other diverse groups of naturally occurring phytochemicals such as flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils, etc. have also been reported[2]. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics[3]. Scientific analysis of medical plants has led to the discoveries of many important drugs. *Baccaurea courtallensis* Muell.-Arg is a lesser known wild edible tree distrib-

uted in Western Ghats of Karnataka, Kerala and Tamil Nadu and belongs to the family Euphorbiaceae. The plant is commonly called as Mootapalam, Muttithuri, Kalikuki, Muttathuri. The fruits of the plant are edible[4,5,6]. *Prosopis juliflora* belonging to Mimosae and is an evergreen tree native to South America, Central America and the Caribbean. It is fast growing, nitrogen-fixing, and tolerant to arid conditions and saline soils. In some circumstances, *P.juliflora* can provide a variety of valuable goods and services: fuelwood, charcoal, animal feed, construction materials, soil conservation and rehabilitation of degraded and saline soils[7,8]. In the drylands of India, *P.juliflora* is considered one of the most valuable tree species[7]. Literature survey revealed lack of information on *B. courtallensis* while considerable data was available on *P.juliflora*. The present investigation highlights phytochemical screening and antimicrobial activity of methanolic extract of bark of *Baccaurea courtallensis* and *Prosopis juliflora*.

MATERIALS AND METHODS

Collection of plant material

The bark materials of *Prosopis juliflora* and *Baccaurea courtallensis* were collected, authenticated to their identity in Dept. of Botany and voucher specimen was deposited for future reference. Plants were cleaned off adhering soil/dust by shaking properly and using soft brush. Plants were placed in paper bags and brought to the laboratory. Remaining dust particles were removed by quick rinsing with distilled water.

Extraction of plant material using methanol solvent

The plant materials were shade dried and powdered mechanically. About 150g of powdered material was subjected to soxhlet extraction and exhaustively extracted with methanol as solvent for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the dessicator[9]. Yield of 14% was obtained in case of *P.juliflora* while only 1.8% of yield was obtained in *B.courtallensis*. Both the extracts were subjected to preliminary phytochemical tests.

Phytochemical profile of methanolic extracts

Qualitative phytochemical analysis of the methanolic extracts of selected plants 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. 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[10].

Screening for antibacterial activity

The pure cultures of Gram positive bacteria namely *Staphylococcus aureus* and *Bacillus cereus* and Gram negative bacteria namely *Escherichia coli* and *Salmonella typhi*, obtained from Dept. of Microbiology, were screened for their sensitivity towards the methanol extracts by Agar well diffusion method[11]. In this method, 24 hours old standardized Muller-Hinton broth cultures of test bacteria were swabbed uniformly on solidified sterile Muller-Hinton agar plates using sterile cotton swab. Then wells of 6mm diameter were bored in the inoculated plates and the extract (50mg/ml of DMF), Standard (Chloramphenicol, 10mg/ml) and Control (DMF) were added into the wells. The plates were incubated at 37^0C for 24 hours in upright position and the zone of inhibition around the well was recorded. The experiment was carried in triplicates to get average reading.

Screening for antifungal activity

The antifungal activity of methanol extracts was tested against two species of the genus *Aspergillus* namely *A.niger* and *A.oryzae*. The suspension of spores of the test fungi was prepared in a test tube containing 0.85% sterile normal saline containing 0.01% Tween 80 detergent[12]. The test fungi were screened for their sensitivity towards the methanol extracts by Agar well

diffusion method[10]. In this method, the spore suspension of tset fungi were swabbed uniformly on solidified sterile Sabouraud's dextrose agar plates using sterile cotton swab. Then wells of 6mm diameter were bored in the inoculated plates and the extract (50mg/ml of DMF), Standard (Chloramphenicol, 10mg/ml) and DMF (control) were added into the wells. The plates were incubated at room temperature for 72 hours in upright position. After incubation, the diameter of zone of inhibition was recorded. The experiment was carried in three trials to get average reading.

RESULTS AND DISCUSSION

In this study, preliminary phytochemical analysis of the bark extracts of the plants showed positive tests to tannin, saponins, terpenoids and flavonoids. Alkaloids and steroids were not detected in the methanolic extracts of *B.courtallensis* while Alkaloid was detected in *P.juliflora* (TABLE 1). TABLE 2 shows result of antibacterial activity of methanol extract of plants selected and the standard drug. Among extracts tested, more inhibition of test bacteria was observed in *B.courtallensis* except in *B.cereus*. A zone of inhibitoin of 2.4cm and more was recorded in case of standard antibiotic. Among bacteria tested, Gram negative bacteria were found to be more affected by the extracts. Both the extracts were found to be effective against species of *Aspergillus*. *A.niger* was more inhibited by *B courtallensis* followed by *P.juliflora* while more inhibitoin of *A.oryzae* was observed by *P.juliflora* (TABLE 3). The antibacterial and antifungal activity of the extracts of selected plants could be attributed to the presence of variety of phytochemicals present in them as detected by screening of extracts.

The antimicrobial activity of plant extracts may be attributed to secondary metabolites present in them like flavonoids, phenolics, polyphenols, tannins, alkaloids, quinones, triterpenoids etc. These phytoconstituents are found to be effective antimicrobial agents against wide range of microbes[8]. Search for literture did not reveal any information about *B.courtallensis* while considerable data is available in *P.juliflora*. The genus *Prosopis* (mesquite) are known to possess medicinal value[13]. Juliflorine and julifloricine, the main alkaloids of Prosopis juliflora, have been isolated for the first time by Ahmad et al.[14] and the antibacterial and antifungal activities were reported by Khan K.A. and Ahmad et al.[15-18]. From P. juliflora, a benzene insoluble alkaloidal fraction (containing 2 major and 3 minor alkaloids) have also been isolated and reported to possess antibacterial and antifungal activities[19,20]. Antibacterial therapeutic efficacy of juliflorine, julifloricine and a benzene insoluble alkaloidal fraction of *Prosopis juliflora* has been studied[21]. *P. juliflora* has gained tremendous popularity among forest officials due to its easy establishment, low mortality rate and fast growth rate on mine spoil, compared to other woody species[22]. People from remote communities are usually treated by traditional healers because of their expertise in making herbal medicines. Traditional healers claim that their medicine is cheaper and more effective than modern medicine. Patients of tribal communities have a reduced risk to get infectious diseases from resistant pathogens than people from urban areas treated with traditional antibiotics. However, if they are treated in a hospital the chance of contracting a nosocomial infection is increased[23]. A knowledge of the chemical constituents of plants is de-

TABLE 1: Phytochemical groups detected in methanol extracts of selected plants

Group	*Prosopis juliflora*	*Baccaurea courtallensis*
Tannin	+	+
Alkaloid	+	N.D
Saponins	+	+
Steroids	N.D	N.D
Terpenoids	+	+
Flavonoids	+	+

'+' present; 'N.D' Not detected

TABLE 2 : Antibacterial activity of methanol extracts of selected plants

Treatment	*Zone of inhibition in cm*			
	S.typhi	*E.coli*	*S.aureus*	*B.cereus*
Control (DMF)	1.0	1.0	1.1	1.1
Chloramphenicol	2.5	2.6	2.4	2.4
P.juliflora	1.7	1.8	1.6	1.6
B.courtallensis	1.8	1.9	1.7	1.5

Chloramphenicol - 10mg/ml; Extract- 50mg/ml

TABLE 3 : Antifungal activity of methanol extracts of selected plants

Treatment	*Zone of inhibition in cm*	
	A.niger	*A oryzae*
Control (DMF)	1.1	1.1
P.juliflora	2.0	2.3
B.courtallensis	2.1	2.1

Extract- 50mg/ml

sirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies.

CONCLUSION

The findings of this investigation offer a scientific support to the ethnomedicinal use of the plants. The results of the study indicate the efficacy of methanolic extracts of selected plants against bacteria and fungi. The extracts could be used against bacteria causing food poisoning as test bacteria, known to cause food poisoning, were found to be inhibited. As the study made use of enteric bacteria, the distillate may also be employed to treat infections caused by enteric bacteria like *E.coli* and *S.typhi*. As inhibition of *Aspergillus* species was observed, the extracts could also be used against opportunistic mycotic infections. Since the study made use of bacteria which are known to produce nosocomial infections, the extracts may also be employed against drug resistant bacteria in hospitals. Further investigations have to be carried to validate the antimicrobial potential of crude extracts and individual constituents in vivo in animal models.

ACKNOWLEDGMENTS

The authors express their sincere thanks to the Director, IWST, Bangalore, Head of the Dept. of Microbiology and Principal of S.R.N.M.N College of Applied Sciences and N.E.S, Shivamogga for providing all facilities and moral support to conduct the work.

REFERENCES

[1] S.Dewanjee, M.Kundu, A.Maiti, Majumdar, R.A.Majumdar; Tropical Journal of Pharmaceutical Research, **6(3)**, 773-778 **(2007)**.
[2] F.Mojab, M.Kamalinejad, N.Ghaderi, H.R. Vahidipour; Iranian Journal of Pharmaceutical Research, 77-82 **(2003)**.
[3] P.M.Shah; Clinical Microbiology and Infection, **11**, 36-42 **(2005)**.
[4] R.Vardhana; Floristic Plants of the World., Sarup and Sons, **(2006)**.
[5] http://foodplantsinternational.org/index.php?sec=plants&page=simple_info&plantid=2050&nocache=1
[6] http://wgbis.ces.iisc.ernet.in/biodiversity/database/?q=node/104
[7] N. Pasiecznik; European Tropical Forest Research Network Newsletter, **28**, 12–14 **(1999)**.
[8] N.Pasiecznik, P.Felker, P.J.C.Harris; The Prosopis juliflora-Prosopis pallida Complex: A Monograph. HDRA, Coventy, UK, **(2001)**.
[9] B.K.Manjunatha, H.S.R.Patil, S.M.Vidya, T.R.P. Kekuda, S.Mukunda, R.Divakara; Indian Drugs, **43(2)**, 150-152 **(2006)**.
[10] J.Parekh, S.V.Chanda; Turk J.Biol., **31**, 53-58 **(2007)**.
[11] B.Tepe, E.Donmez, M.Unlu, F.Candan, D. Daferera, G.Vardar-Unlu, M.Polissiou, A.Sokmen; Food Chemistry, **84(4)**, 519-525 **(2004)**.
[12] Z.Rihakova, V.Filip, M.Plockova, J.Smidrkal; Czech.J.Food Sci., **20(2)**, 48-52 **(2002)**.
[13] K.P.Kirtikar, B.D.Basu; 'Indian Medicinal Plants', Leader Press Allahabad, **2**, 910 **(1935)**.
[14] V.U.Ahmad, A.Basha, W.Haque; Z.Naturforsch, **33b**, 347 **(1978)**.
[15] A.Ahmad, K.A.Khan, V.U.Ahmad, S.Qazi; Planta Med., **4**, 285 **(1986)**.
[16] A.Ahmad, S.U.Kazmi, K.A.Khan, V.U.Ahmad, S. Qazi; JPMA., **38**, 20 **(1988)**.
[17] K.A.Khan, A.H.Faruqi, S.A.Rasool, V.U.Ahmad, S.Qazi, T.S.Haroon; Arzeim-Forsch/Drug Res., **36**, 17 **(1986)**.
[18] A.Ahmad, K.A.Khan, V.U.Ahmad, S.Qazi; Arzeim-Forsch/Drug Res., **39**, 625, **(1989)**.
[19] A.Ahmad, K.A.Khan, V.U.Ahmad, S.Qazi; Fitoterapia., LIX, 481 **(1988)**.
[20] A.Ahmad, K.A.Khan, V.U.Ahmad, S.Qazi; Fitoterapia., LX, 86 **(1989)**.
[21] A.Ahmad, V.Ahmad, S.M.Khalid, S.A.Siddiqui, K.A.Khan; Journal of Islamic Academy of Sciences, **8(3)**, 131-136 **(1995)**.
[22] A.Singh; Current Science., **93(9)**, 1204 **(2007)**.
[23] S.Ospina, M.P.Arbeláez, L.A.Paniagua, M.C. Pelaez, J.C.RamIrez, L.C.Sánchez, V.Tuiran, C.E. Villegas; Enfermedades Infecciosasy Microbiologia Infecciones Nosocomiales., **22**, 127 **(2002)**.