Asian Journal of Chemistry; Vol. 23, No. 4 (2011), 1661-1663

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Antimicrobial Potentiality of *Polyalthia cerasoides* Leaf Extracts and Separation of Compounds

CH. SUREKHA[1,*], K.V.V.V. SATYANARAYANA[2] and T. DASARADHA RAM[1]

[1]Department of Biochemistry, GITAM Institute of Science, GITAM University, Visakhapatnam-530 045, India
[2]Department of Chemistry, GITAM Institute of Science, GITAM University, Visakhapatnam-530 045, India

*Corresponding author: Fax: +91 891 2790032; Tel: +91 891 2840464; E-mail: challa_surekha@gitam.edu

(*Received*: 29 June 2010; *Accepted*: 9 December 2010) AJC-9382

The antimicrobial activity of 90 % ethanol, ethyl acetate and hexane leaf extracts of *Polyalthia cerasoides* was investigated on both bacterial and fungal strains using agar gel diffusion method. Commercial antibiotics were used as positive standard references to determine the sensitivity of the strains. Ethyl acetate and ethanol extracts showed significant inhibitory activity against bacteria (*Proteus vulgaris, Klebseilla pneumonia, Bacillus subtilis*) and fungi (*Candida albicans, Aspergillus niger*). The range of zone of inhibition was found to be (14-34 mm) and minimum inhibitory activity was as low as 1 µL/well. The zone of inhibition was higher in ethyl acetate extract than ethanol and no activity was observed with hexane extract. Ethyl acetate extract was analyzed with silica gel column chromatography. The UV spectrum readings at higher absorptions are the sources of bioactive compounds having antimicrobial activity.

Key Words: *Polyalthia cerasoides*, Antimicrobial activity.

INTRODUCTION

The genus *Polyalthia* belongs to Annonaceae family, which include more than 150 species out of which 12 species occur in India. A small to moderate size tree ranging from 5-15 m in height. It is commonly called Gutti in Telugu, Kudumi in Hindi. It is commonly seen in evergreen and deciduous forests in Bihar, Orissa, Assam. It has simple leaves, greenish white fragrant flowers and fruits are dark red in colour, sweet in taste with ovoid fruitlets. *P. cerasoides* reproduces by root suckers with high regeneration.

Gonzalez *et al.*[1] conducted phytochemical studies on *Polyalthia* species lead to the isolation of different types of secondary metabolites *e.g.*, alkaloids, terpenes, flavonoids and diterpenes[2] with clerodane 5 skeleton and some of these secondary metabolites exhibited cytotoxic properties and have two sesquiterpenes benzopyran metabolites namely α-spinosterol, β-sistosterol in leaves and stem. *P. longifolia* leaf aqueous extracts was reported to lower blood pressure, rate of respiration in laboratory animals by Saleem *et al.*[3].

Zafra-polo *et al.*[4] reported Polyalthidin, a new benzopyran derivative from stem bark of *P. cerasoides* which showed potent biological activity as an inhibitor of the mammalian mitochondrial respiratory chain. Gonzalez *et al.*[5] isolated two new 7,8-dihydro-d-oxoproto berbeine alkaloid, namely 'cerasodine and cerasoinine' from stem bark of *P. cerasoides*. The present study investigates the antimicrobial activity of *P. cerasoides* against bacterial and fungi *in vitro* and these extracts were screened for bioactive compounds in them.

EXPERIMENTAL

The leaves of *Polyalthia cerasoides* were collected from medicinal plant nursery from Rajahmundry and are authenticated by taxonomist Prof. M. Venkataratnam Department of Botany, Andhra University, Visakhapatnam.

Preparation of plant extracts: The leaves are collected and air dried for 5 days and then powdered using a mechanical grinding mill. 10 g of ground powder is subjected to Soxhlet extraction with hexane, ethyl acetate and ethanol separately for 36 h each. These extracts were distilled to separate the organic solvents for obtaining concentrated fractions. These fractions were further concentrated by evaporation on water bath and weighed. 1gm extracts were dissolved in dimethyl sulphoxide to give a final concentration of 1 mg/mL which were used for antimicrobial tests.

Metabolite analysis: (i) Silica gel in TLC was of "Lichrosolv" grade from Merck, India. (ii) Solvent mixture (Petroleum ether: acetone =7:3). (iii) Spraying reagent: 0.3 % ninhydrin in butanol containing 3 mL of acetic acid.

Antimicrobial studies

Test microorganisms: The following test microorganisms were used in this study are: *Bacillus subtilis* (NCIM2063), *Proteus vulgaris* (NCIM 2027), *Escherichia coli* (NCIM 2066)

Klebseilla pneumonia (NCIM 2957), *Candida albicans* (NCIM 3557), *Asperigillus niger* (NICM1054). The bacterial and fungal strains are obtained from National Chemical Laboratory, PUNE except *Rhizopus*.

Maintenance of microorganisms: The bacterial cultures were maintained on Luria Bertani Agar (LB) and fungal cultures were maintained on Sabouraud Dextrose Agar (SDA) at 4 °C temperature until used for the study.

Pour plate method: Inoculum of test organisms was prepared by growing pure isolate in nutrient broth at 37 °C for overnight. The overnight broth cultures was subcultured in fresh nutrient broth and grown for 3 h to obtain log phase culture. The agar plates were prepared by pour plate method using 20 mL nutrient agar medium for bacteria and SB medium for fungi. The molten sterile medium is cooled to 45 ºC and mixed thoroughly with 10 µL of growth culture of concerned test organism (1×10^8 cells) and then poured into sterile petriplates and allowed to solidify. Fungal cultures are grown on Sabourand Dextrose Agar and suspension in one fourth strength ringers solution was used to prepare the seeded Sabourand Dextrose Agar plates.

Wells of 6 mm size were made with sterile gel puncture and 10 µL of plant extract was added to each well aseptically. Streptomycin (10 µL/mL) and fucanazole (10 µL/mL) were added as standard positive antimicrobial agents for bacteria and fungi, respectively and tested along with plant extract samples. Nutrient agar plates were incubated at 37 ºC for 24 h. For bacterial culture, while Sabourand Dextrose Agar plates at 28 ºC for 48 h for fungal growth. The diameter of zones of inhibition was measured in mm using Himedia zone reader.

Determination of minimum inhibitor concentration (MIC): The MIC was determined by agar diffusion method. The extracts were incorporated into nutrient agar and SB dextrose agar at concentration of 1 to 10 µL. A control without the extract was also set up. The lowest concentration of extract that inhibited the growth of micro organisms was considered as MIC.

Chromatographic studies

Thin layer chromatography: Spot 2.5 µL volume of hexane, ethyl acetate and ethanol fractions on the baseline at one end of the glass plate (20 × 7.5 cm) using a capillary tube. The solvent used for development of chromatogram, is petroleum ether and acetone mixed in ratio 7:3 v/v solvent was allowed to raise up to ¾ of plate and then observed under UV tranilluminator.

Column chromatography: A glass column (30 × 0.5 diameter) was packed with 12 g of silica gel 60-100 mesh from SISCO chemicals, which was previously dried at 120 ºC for 1 h and used to prepare slurry in benzene. 1 mL ethyl acetate fraction was eluted with 20 mL each of 5, 10, 30, 40, 50 % acetone in benzene (v/v) and finally with 20 mL pure acetone. The fractions collected are read at UV-range 200-335 nm and visible range 400-650 nm on a UV-visible spectrophotometer (HITACHI).

RESULTS AND DISCUSSION

The antimicrobial activity of *P. cerasoides* leaf extracts are presented in Table-1. It was found that the hexane extract of leaf of *P. cerasoides* did not exhibit any activity against the microorganisms tested whereas ethyl acetate and ethanol extracts of leaf of *P. cerasoides* studied exhibited different levels of antimicrobial activity against bacteria and fungi. It was found that ethyl acetate extract of leaf of *P. cerasoides* exhibited significant activity on microorganisms like *B. subtilis*, *K. pneumonia, P. vulgaris* with zone of inhibition ranging from 14-18 mm and fungi *C. albicans, A. niger, Rhizopus* with zone of inhibition ranging from 15-34 mm, respectively (Fig. 1). The extracts did not show any inhibition towards *E. coli*. The minimum inhibitory concentration of ethyl acetate extract fraction is shown in Table-2. The minimum inhibitory concentration was 1 µg against *Rhizopus*, 2 µg against *A. niger, P. vulgaris* and *K. pneumoniae* and 3 µg against *C. albicans* and *B. subtilis*.

TABLE-1
ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF *Polyalthia cerasoides* LEAF EXTRACTS ON MICROBIAL STAINS

Micro organism	Zone of growth inhibition (mm)			
	Fucanazole streptomycin +ve control 10 µL/well	Ethanol extract 10 µL/well	Ethyl acetate extract 10 µL/well	Hexane extract 10 µL/well
Fungi				
Rhizopus	25 ± 2	21 ± 1	34 ± 1	ND
P. crysogenum	21 ± 1	11 ± 1	18 ± 1	ND
C. albicans	19 ± 2	8 ± 1	15 ± 1	ND
A. niger	18 ± 2	10 ± 1	20 ± 1	ND
Bacteria				
P. vulgaris	22 ± 2	10 ± 1	18 ± 1	ND
K. pneumonia	33 ± 1	9 ± 1	17 ± 1	ND
B. subtilis	35 ± 1	7 ± 1	14 ± 1	ND
E. coli	21 ± 1	ND	ND	ND

ND = Not detected.

TABLE-2
MINIMUM GROWTH INHIBITORY CONCENTRATION OF ETHYL ACETATE LEAF EXTRACT OF *P. cerasoides*

Organism	MIC (µL/well)
C. albicans	3
Rhizopus	1
A. niger	2
P. vulgaris	2
K. pneumonia	2
A. subtilis	3
E. coli	Not determined

The ethyl acetate extract of plant showed 3 spots on TLC plates when seen under UV light, two are greenish yellow and one was blue suggesting that present compound of interest has fluorescence.

The column chromatographic sample readings have maximum absorbance at 335 nm. The highest absorbance in every gradient fraction was read between 200-335 nm and graph was plot (Table-3). The peak absorbance at 335 nm for all fractions ranging from 2.484-4.364 and they may be source of bioactive compounds having antimicrobial activity.

In the present work *P. cerasoides* showed higher activity against *P. vulgaris* and *Rhizopus*. Thus it may be attributed due to two reasons firstly the nature and potentiality of biological active components (alkaloids, flavanoids, biterpenoids) which could be enhanced in the presence of ethyl acetate and

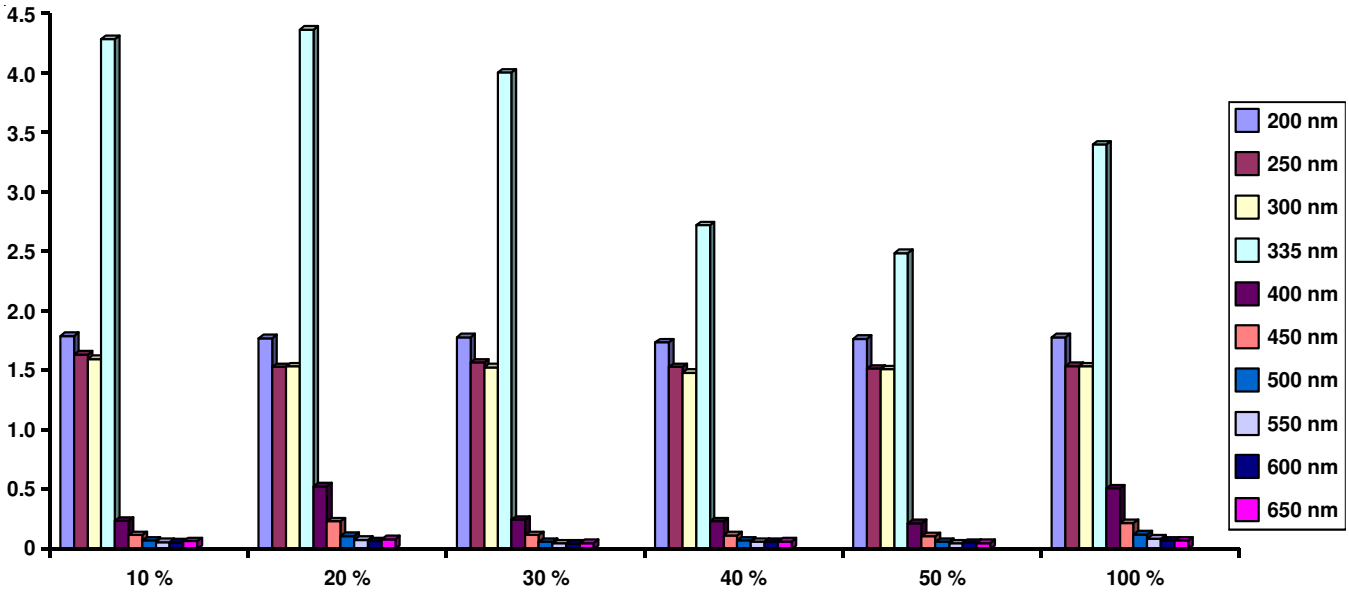

Fig. 1. UV visible spectrophotometer readings in each concentration selected from ethyl acetate leaf extract with concentrations plotted on X-axis and OD on Y-axis

TABLE-3
UV VISIBLE SPECTROPHOTOMETER READINGS IN EACH CONCENTRATION SELECTED FROM ETHYL ALCOHOL LEAF EXTRACT OF *P. cerasoides*

Conc. (%)	nm									
	200	250	300	**335**	400	450	500	550	600	650
10	1.789	1.632	1.595	**4.285**	0.235	0.114	0.067	0.057	0.052	0.065
20	1.771	1.527	1.533	**4.364**	0.524	0.229	0.108	0.076	0.065	0.080
30	1.780	1.564	1.526	**4.003**	0.245	0.114	0.060	0.044	0.042	0.047
40	1.736	1.528	1.479	**2.717**	0.230	0.113	0.069	0.058	0.058	0.061
50	1.767	1.514	1.510	**2.484**	0.214	0.106	0.060	0.044	0.049	0.048
100	1.780	1.536	1.534	**3.398**	0.507	0.216	0.122	0.084	0.069	0.068

secondly the stronger extraction capacity of organic solvent could have produced greater number/amount of active constituents responsible for antimicrobial activity.

Plants have an almost limitless ability to synthesize aromatic substances. Most of them are secondary metabolites of which at least 1200 have been isolated. Identification of plant extracts and phytochemicals with known antimicrobial properties can be great significance in therapeutic treatment, development of phytomedicine against resistant microorganisms.

ACKNOWLEDGEMENTS

The authors acknowledged the support from Department of Biochemistry, GITAM University for providing the necessary research facilities.

REFERENCES

1. M.C. Gonzalez, A. Serrano, M.C. Zafra-Polo, D. Cortes, K.S. Rao, *J. Nat. Prod.*, **58**, 1278 (1995).
2. M.M. Murthy, M. Subramanyam, M.H. Bindu and J. Annapurna, *Fitoterapia*, **76**, 336 (2005).
3. R. Saleem, A. Muhammad, A.S. Iqbal, A. Mohammad, A.R. Khan, N. Rasool, H. Saleem, F. Noor and S. Faizi, *Phytother. Res.*, **19**, 881 (2005).
4. M.C. Zafra-Polo, M.C. Gonzalez, J.R. Tormo, E. Estornell and D. Cortes, *J. Nat. Prod.*, **59**, 913 (1996).
5. M.C. Gonzalez, M.A. Sentandreu, K.S. Rao, M.C. Zafra-Polo and D. Cortes, *Phytochemisty*, **43**, 1361 (1996).