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In Vitro Antimicrobial and Haemolytic Studies of *Bambusa arundinaceae* leaves

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ABSTRACT

Bambusa arundinacea locally known as *Bans* or bamboo, a perennial fastest growing plant. This plant is medicinally much famous. The biological activities of this plant were carried out by using standard procedures. The aim of this work was to investigate the *in vitro* antimicrobial and haemolytic activities of *Bambusa arundinaceae leaves*. The GC-MS analysis revealed that the major components determined in *n*-hexane extract were as *n*-Nonane (17.14%), 2,2,3-Trimethylbutane (5.85%), Tridecane (5.42%), and 3,4,5,6-Tetramethyloctane (5.32%). The disc diffusion and modified resazurin microtitre-plate assays were used to evaluate the inhibition zones (IZ) and minimum inhibitory concentration (MIC) of *B. arundinaceae* leaves extracts. The plant extracts showed considerable antimicrobial activity. The heamolytic activity against human blood erythrocytes (RBCs) was studied and the % lysis of RBCs was found to be in the range of 1.03 to 4.81. The results of the present study confirmed the considerable antimicrobial and haemolytic activities of the plant *Bambusa arundinaceae.*

INTRODUCTION

Bambusa arundinacea locally known as *Bans* or bamboo, a perennial fastest growing plant on the earth is presumed to have origin in Asia. It belongs to the family Poaceae. Bamboo leaves are emmenagogue (Kirtikar and Basu, 1990) and have been used clinically in the treatment of hypertension, arteriosclerosis, febrifuge, cardiovascular disease, and cancer, bechic, used in haemoptysis (Slinkard and Singleton, 1977; Khare, 2007).

The leaves, roots, grains and gum of this plant are used reported to be medicinally important. Roots of this plant are used for preparation of an ointment which is said to be a folk remedy for cirrhosis and hard tumors, especially tumors of the abdomen, liver, spleen and stomach (Sala, 1995).

Seeds are acrid laxative, used in strangury and urinary discharges (Chopra *et al*., 1958). Bark is beneficial for treatment of skin troubles (Slinkard and Singleton, 1977). Chemical contents of *Bambusa arundinacea* young shoots have been reported as cholin, betain, urease, cyanogenetic, glucosides, oxalic acid, and benzoic acid (The Wealth of India, 1956).

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The leaves of *Bambusa arundinacea* have been reported to possess anti-inflammatory, antiulcer, antioxidant and antifertility activity (Muniappan and Sundararaj, 2003; Trueba, 2003; Vanithakumari *et al*., 1989). The plant Bamboo is considered as a rich source of flavones glycosides having ability to interact with lipid bilayers by influencing their incorporation rate into cells. Different parts of bamboo plant possess various biological functions due to the presence of different compounds.

The stilbene gulcosides from the root of bamboo has been reported to possess various medicinal properties (Estuko *et al*., 1998). Jeong *et al.* 2007 reported the presence of poly phenols viz., *p*-(hydroxyphenyl) propionic acid, ferulic acid, caffeic acid, and chlorogenic acid in bamboo shoots.

The phenolic compounds derived from the whole plant's extracts showed inhibition of Pglycoprotein in adriamycin resistant human breast cancer cells (Shibata *et al*., 1975). As part of our efforts to explore the flora of Pakistan (Rizwan *et al*., 2012; Mehmood *et al*., 2012: Zubair *et al*., 2011; Bari *et al.*, 2012; Rasool *et al*., 2011 a, b), Bamboo leaves were investigated as a potential source of natural antimicrobial agents and its haemolytic effects against human erythrocytes.

MATERIALS AND METHODS

Collection of Plant Material

The fresh leaves of the plant *Bambusa arundinacea* were collected in April 2011 from the local areas of Faisalabad, Pakistan and further identified by a taxonomist, Dr. Mansoor Hameed from Department of Botany, University of Agriculture Faisalabad, Pakistan.

Sample preparation

The plant leaves were washed with distilled water and then shade dried. The grinded fine powder of leaves (1000g) was extracted with *n*-hexane (2×1.5L) at room temperature. After 3 days the extract was filtered and concentrated through rotary vacuum evaporator (Eyela, Tokyo Rikakikai Co., Ltd., Japan). This process was repeated thrice to obtain a sufficient quantity of *n*-hexane extract (5.6 g).

The remaining plant residue was further extracted with other different polarity based solvents and obtained successively chloroform (9.8 g), ethylacetate (9.2 g), acetone (7.3 g), *n*-butanol (8.9 g), absolute methanol (11.3 g) , (0.5:9.5) water: methanol (13 g), (1:9) water: methanol (12 g) extracts. All obtained extracts after drying were stored at -4°C till further analysis.

Gas chromatography/mass spectrometry analysis

The GC–MS analysis of the *n*-hexane extract was performed using GC 6850 Network gas chromatographic system equipped with 7683 B series auto injector and 5973 inert mass selective detector (Agilent Technologies USA). Compounds were separated on an HP-5 MS capillary column having 5% phenyl polysiloxane as stationary phase, column length 30.0 m, internal diameter 0.25 mm and film thickness 0.25 µm. The temperature of injector was 300 °C and 1.0 µL of sample was injected in the split mode with split ratio 30:1. Helium was used as carrier gas, with a flow rate of 1.5 ml/min. The temperature program was: initial temperature 150 °C and held for 1 min, then ramping at rate of 10° C/min upto 290 °C and finally hold at this temperature for 5 min. The temperature of MSD transfer line was 300 °C. For mass spectra determination MSD was operated in electron ionization (EI) mode, with the ionization energy of 70 eV, while the mass range scanned was 3-500 m/z.

The temperature of ion source was 230 °C and that of MS quadropole 150 °C. The identification of components was based on comparison of their mass spectra with those of NIST mass spectral library (Massada, 1976; Mass Spectral Library, 2002).

Antimicrobial assay

Test microorganisms

Alternaria alternata ATCC 20084, *Ganoderma lucidum* locally isolated, were used as the fungal tested organisms and *Pasturella multocida* locally isolated, *Escherichia coli* ATCC 25922, *Bacillus subtilis* JS 2004, *Staphylococcus aureus* API Staph tac 6736153 were used as the bacterial tested organisms. The pure bacterial and fungal strains were obtained from the Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan. The bacterial strains were cultured overnight at 37 °C in nutrient agar (Oxoid, Hampshire, UK) while fungal strains were cultured overnight at 28 °C using potato dextrose agar (Oxoid).

Disc diffusion method

Antimicrobial activity of the plant extracts was determined by using the disc diffusion method (NCCLS, 1997). All samples (dry residue) were dissolved in 10% sterile dimethyl sulfoxide. The discs (6 mm diameter) were impregnated with 10 mg/mL extracts (100 µL/disc) placed aseptically on the inoculated agar. Discs injected with 100 µL of respective solvents served as a negative controls, rifampcin (100 µL/disc) (Oxoid) and fluconazole (100 µL/disc) (Oxoid) were used as positive reference for bacteria and fungi, respective ly.

The petri dishes were incubated at 37±0.1 °C for 20-24 h and 28±0.3 °C for 40-48 h for bacteria and fungi, respectively. At the end of period, the inhibition zones formed on the media were measured. The positive antimicrobial activity was read based on growth inhibition zone.

Resazurin microtitre-plate assay

The minimum inhibitory concentration (MIC) of the plant extracts was evaluated by following a resazurin microtitre-plate assay as reported by Sarker *et al*. (2007) with little modifications.

Haemolytic activity

Haemolytic activity of the plant was checked by the reported method of Powell *et al*. (2000). 3ml of freshly obtained heparinized human blood was gently mixed, poured into a sterile 15 ml polystyrene screw-cap tube and centrifuged for 5 min, at 850 *x*g. The supernatant was poured off and the viscous pellet washed three additional times with 5 ml of chilled (4 °C) sterile isotonic phosphate-buffered saline (PBS) solution (pH~ 7.4). The washed cells were suspended in a final volume of 20 ml chilled, sterile PBS and the cells counted on a haemacytometer. The blood cell suspension was maintained on wet ice and diluted with sterile PBS to 7.068×10^{8} cells $m1^{-1}$ for each assay.

Aliquots of 20 µl of plant extracts were aseptically placed into 2.0 ml microfuge tubes. For each assay, 0.1% Triton X-100 was used as the positive, 100% lytic control and PBS as the negative, 0% lytic control. Aliquots of 180 µl diluted blood cell suspension were aseptically placed into each 2-ml tube and gently mixed three times with a wide mouth pipette tip. Tubes were incubated for 35 min at 37 °C. Immediately following incubation, the tubes were placed on ice for 5 min, and then centrifuged for 5 min at 1310 *x*g. Aliquots of 100 µl of supernatant were carefully collected, placed into a sterile 1.5 ml microfuge tube, and diluted with 900 µl chilled, sterile PBS. All tubes were maintained on wet ice after dilution. Then 200 µl were placed into 96 well plates, and three replicates was taken in well plate which contain one positive

and one negative. Absorbance at 576 nm was then measured on a microquant. The experiment was done in triplicate. Percent hemolysis was calculated by following formula:

% Haemolysis = As (sample absorbance)/Ac (control absorbance) ×100

Statistical Analysis

All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data was performed by analysis of variance (ANOVA), using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) software. A probability value of difference $p \leq 0.05$ was considered to denote a statistically significance. All data were presented as mean values ± standard deviation (SD).

RESULTS AND DISCUSSION

GC/MS Analysis of n-hexane Extract

The GC/MS analysis of *n*-hexane extract enabled the identification of 40 components. This volatile extract consisted of a mixture of different classes of compounds. The major constituents were found to be *n*-nonane (17.14%), 2,2,3-Trimethylbutane (5.85%), Tridecane (5.42%), and 3,4,5,6-Tetramethyloctane (5.32%) (Table 1). The *n*-hexane extract may have some fatty acids/methyl esters which may be implicated in some antimicrobial and haemolytic activities. Various reports (Rizwan *et al*., 2012; Massry *et al*., 2009; Singh *et al*., 2004) about chemical composition of many plants analyzed by GC/MS analysis and their different biological activities are available in literature. During literature review it was found that the some phyto-components such as flavonoids, palmitic acid (hexadecanoic acid, ethyl ester and *n*-hexadecaonoic acid), unsaturated fatty acid, docosatetraenoic acid and octadecatrienoic acid have antimicrobial, anti-inflammatory, and antioxidant activities (Kumar *et al*., 2010). Therefore the chemical constituents found in *B. arundinaceae* leaves may play major roles in the biological activities and pharmacological properties.

***In vitro* Antimicrobial activity of *B. arundinaceae* leaves**

The antimicrobial activity of the various organic extracts of *B. arundinaceae* leaves against a panel of food-borne and pathogenic microorganisms were assessed. The results are presented in Table 2. The plant exhibited considerable antimicrobial activity against most of the bacterial and fungal strains. The results from the disc diffusion method measured in inhibition zone (IZ) followed by measurement of minimum inhibitory concentration (MIC), indicated that *n*-hexane extract showed good inhibitory activity against *E. coli* (IZ= 22.2 mm; MIC = 3.81 mg/mL), *P. multocida, (*IZ = 19.0 mm; MIC = 5.28 mg/mL) and *B. subtilis* (IZ=17.0 mm; MIC = 5.62 mg/mL) respectively. The *n*-hexane extract was inactive against both tested fungal strains (*A. alternata, G. lucidum*). Chloroform extract showed good inhibitory effects against *S. aureus* (IZ=19.0 mm; MIC = 15.5 mg/mL) and *B. subtilis* (IZ=17.0 mm; MIC = 10.7 mg/mL), *E. coli* was resistant against chloroform extract.

Table. 1: The Chemical Composition of *n*-hexane extract of *B.arundinaceae* analyzed by GC-MS.

Peak No	Chemical compounds	Retention time (Min.)	% composition
1	2,2,3-Trimethylbutane	5.046	5.85
2	Trans-1,4-Dimethylcyclohexane	5.465	2.98
3	2,4-Dimethyhexane	5.530	4.13
4	Trans-1,3-Dimethylcyclohexane	5.725	1.02
5	Isononane	6.400	1.37
6	2,6-Dimethylheptane	6.455	1.54
7	2,4-Dimethy-1-decene	6.589	2.43
8	5,5-Dimethyl-1-hexene	6.644	2.11
9	1,1,4-Trimethylcyclohexane	6.733	1.49
10	1,1,3-Trimethylcyclohexane	6.792	1.05
11	4,5-Diethyloctane	7.338	1.52
12	2,3-Dimethylheptane	7.389	2.59
13	1,4-Dimethylbenzene	7.517	0.86
14	Isononane	7.617	1.54
15	2,3,4-Trimethylhexane	7.683	3.99
16	3,4,5,6-Tetramethyloctane	7.742	5.32
17	1,2-Dimethylbenzene	7.842	2.53
18	2,2,4-Trimethylpentane	7.917	3.57
19	2,5-Dimethyldecane	7.964	4.59
20	Isobutylcyclopentane	8.417	0.69
21	1-Ethyl-3-methylcyclohexane	8.583	2.25
22	O-Methyltoluene	8.651	2.90
23	*n*-nonane	9.035	17.14
24	*n*-Propylcyclohexane	10.108	0.94
25	2,6-Dimethyloctane	10.277	1.32
26	2,5-Dimethyloctane	11.320	1.31
27	1,2,3-Trimethylbenzene	12.467	0.59
28	*n*-undecane	12.772	3.60
29	2,5,5-Trimethylheptane	13.561	0.59
30	2,5-Dimethylnonane	14.788	0.55
31	2-Methyldodecane	15.077	0.85
32	Tridecane	16.317	5.42
33	2-Methylundecane	18.440	0.85
34	3-Methylundecane	18.638	0.57
35	*n*-pentadecane	19.602	4.95
36	3,6-Dimethylundecane	19.991	0.75
37	*n*-tetradecane	22.649	1.48
38	*n*-hexadecanoic acid, methyl ester	35.357	1.03
39	Eicosatrienoic acid, methyl ester	37.675	1.00
40	Gamma-Sitosterol	45.019	0.75
			100%

Acetone extract of the plant did not show any inhibitory activity against *A. alternata, E. coli,* and *S. aureu*s while strong inhibitory action was observed against *G. lucidum* (IZ=2.4.2 mm; MIC = 4.00 mg/mL). Absolute methanol and (1:9) water: methanol extracts were inactive against all tested bacterial strains (*B. subtilis, P. multocida, S. aureus and E. coli*) while both these extracts showed considerable activity against *A. alternata* and *G. lucidum. n*-butanol and (0.5:9.5) water: methanol extracts of the plant showed no activity against *B. subtilis* and *P. multocida.* Jaimik *et al*., 2012 reported the presence of various phytochemicals flavonoids, saponins, carbohydrates, and terpenoids in methanol extract of *B. arundinaceae* leaves. From the literature review it was observed that the phytochemical components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. These compounds have variously been reported to have antimicrobial activity (Scalbert *et al*., 1991; Field and Lettinga, 1992; Siddhuraju and Becker, 2003). The rifampicin and fluconazole were used as positive control for bacterial and fungal strains respectively.

Table. 2: Antimicrobial activity in terms of inhibition zones and minimum inhibitory concentration of *B. arundinaceae* leaves against selected bacterial and fungal strains"

Different organic extracts	Tested microorganisms (Diameter of inhibition Zone (IZ), mm)					
	B. subtilis	***P. multocida***	***S. aureus***	***E. coli***	***A. alternata***	***G. lucidum***
n-hexane	17.0±0.81^{b}	19.0±0.70^{b}	15.5±1.11^{d}	22.2±1.47^{b}	n.d	n.d
Chloroform	17.0±0.70^{b}	16.0±1.80^{d}	19.0±0.70^{c}	n.d	8.5±1.23^{c}	9.0±1.11^{c}
Ethyl acetate	22.7±2.27^{b}	18.0±0.70^{bc}	n.d	n.d	n.d	n.d
Acetone	18.5±0.5^{b}	16.2±1.47^{cd}	n.d	n.d	n.d	24.2±5.26^{a}
n-butanol	n.d	n.d	19.5±1.08^{c}	22.0±3.11^{b}	6.5±0.03^{d}	6.5±1.11b
Absolute Methanol	n.d	n.d	n.d	n.d	11±1b	8.5±0.02^{b}
(0.5:9.5) water: methanol	17.7±0.82^{b}	n.d	22.0±1.82^{b}	n.d	7.75±1.47^{cd}	8.5±1.11^{b}
(1:9) water: methanol	n.d	n.d	n.d	n.d	6.0±1.58^{d}	n.d
Control	29.0±1.08^{a}	34±1.08^{a}	28.5±0.82^{a}	28.5±1.08^{a}	26.0±5.71^{a}	26±2.38^{a}
Minimum inhibitory concentration (MIC) mg/mL.						
Extracts	***B. subtilis***	***P. multocida***	***S. aureus***	***E. coli***	***A. alternata***	***G. lucidum***
n-hexane	5.62±0.12	5.28±0.16	22.4±0.32	3.81±0.5	n.d	n.d
Chloroform	10.7±0.07	12.5±0.21	15.5±0.21	n.d	10.6±0.38	10.5±0.40
Ethyl acetate	3.02±0.25	6.48±0.29	n.d	n.d	n.d	n.d
Acetone	12.4±0.11	12.1±0.12	n.d	n.d	n.d	4.00±0.36
n-butanol	n.d	n.d	15.8±0.22	2.81±0.23	18.4	15.0±0.04
Absolute Methanol	n.d	n.d	n.d	n.d	7.4±0.40	11.4±0.04
(0.5:9.5) water: methanol	10.4±0.17	n.d	5.89±0.10	n.d	11.8±0.07	11.4±0.46
(1:9) water: methanol	n.d	n.d	n.d	n.d	18.4±0.36	n.d
Control	2.04±0.32	2.04±0.32	2.14±0.32	2.04±0.32	3.05±0.04	3.05±0.04

Data are expressed as the mean ± standard deviation; values having different letters differ significantly ($p < 0.05$). n.d= not detected. Rifampcin and Fluconazole used as control for bacterial and fungal strains respectively

The standard drugs showed higher activity on the organisms than the tested plant extracts (Table 2). The standard antibiotics were refined industrial products so there activity was more as compared to crude extracts.

As per literature review it was observed that the extracts tested were active against the fungus strains (Verastegui *et al*., 2008) and their results are comparable with our results with some variations. Zhang *et al*. (2010) reported the antibacterial activity of the plant *B. arundinaceae*. So the *B. arundinaceae* plant may be used in future to cure the diseases caused by bacteria and fungi.

In vitro Haemolytic activity of *B. arundinaceae* leaves

Haemolytic activity was performed because the plant possessing potent animicrobial potential may not be useful in pharmacological preparations if they possess haemolytic effect. The total haemolysis (%) was obtained using 20 μL of Triton X-100 (0.1%). The results of haemolytic activity are presented in Table 3.

Chloroform extract showed highest haemolytic effects (4.81%), followed by acetone (4.43%), ethylacetate (3.83%), *n*-hexane (3.52%), (0.5:9.5) water: methanol (3.03%), absolute methanol (2.80%), *n*-butanol (2.41%) and (1:9) water: methanol (1.03%) extracts respectively. These results are in agreement with findings of Rizwan *et al.* (2012) where also chloroform fraction of *A. attenuata* showed highest haemolytic effects against human erythrocytes. The stability of the red blood cells membrane is a good indicator of the effect of various *in vitro* studies by various compounds for the screening of cytotoxity.

The percentage lysis of human erythrocytes was less than 5.0 % for all samples, so it can be predicted that the plant extracts have a minor cytotoxitcity (Sharma and Sharma, 2001).

All these results were in safe range. So pharmacologically this plant may be safe to use for human beings as a source of potential drug.

Table. 3: Haemolytic activity, as a percentage of haemolysis caused by *B. arundinaceae* leaves extracts.

Organic extracts	% of haemolysis
n-hexane	3.52±0.12
Chloroform	4.81±0.08
Ethyl acetate	3.83±0.16
Acetone	4.43±0.14
n-butanol	2.41±0.14
Absolute Methanol	2.80±1.06
(0.5:9.5) water: methanol	3.03±0.11
(1:9) water: methanol	1.03±0.18
Phosphate Buffer Saline (PBS)	0
Triton X-100	100±0.99

Values are mean ±SD of three separate experiments

CONCLUSION

The collected data suggested that *Bambusa arundinacea* leaves possesses significant antimicrobial activity. It justifies its folklore use in curing microbial infections. Haemolytic activity of plant extracts against human erythrocytes was checked and it was in safe range so the investigated plant may be safe to use for pharmaceutical and natural therapies. Further studies are needed to establish the mechanism of action and isolation of phyto-constituents responsible for the concerned activity.

REFERENCES

Bari, M. N., Zubaır, M., Rızwan, K., Rasool, N., Bukhari, I. H., Akram, S., Bokhari, T. H., Shahid, M., Hameed, M., Ahmad, V. U. Biological activities of *Opuntia Monacantha* cladodes. J. Chem. Soc. Pak. 2012; 34: 990–995.

Chopra RN., Chopra IC., Handa KL., Kapur LD. Indigenous Drugs of India, U.N.Dhur and Sons Pvt. Ltd., Calcutta (1958) 289, 665.

Estuko, K., Nobuyuki, K. and Hironobu, T. A. comparative evaluation of *in vitro* antioxidant properties of bamboo *bambusa arundinacea* leaves extracts. jpn. soc. hortic. sci. 1998; 67: 805-881.

Field, J. A. and Lettinga, G. Toxicity of tannic compounds to microorganisms. Plants Polyphenols: Synthesis, Properties, Significance. Basic Life Sci. 1992; 59: 673-692.

Jaimik, D. R., Nimish, L. P., Ritesh, G. P., Nuruddin, P. J., Laxman, D. P., Vijay, C. Ameliorative Effect Of *Bambusa Arundinacea* Against Adjuvant Arthritis-With Special Reference To Bone Erosion And Tropical Splenomegaly. J. of Drug Deli. & Therap. 2012; 2(3): 141-145.

Jeong, Y. H., Chung, S. Y., Han, A. R., Sung, M. K., Jang, D. S., Lee, J., Kwon, Y., Lee, H. J. and Seo, E. K. P-glycoprotein inhibitory activity of two phenolic compound, (-)- syrigaresinol and tricin from *Sasa borealis*. Chem and Biodiver. 2007; 4: 12-16.

Khare, CP. Indian Medicinal Plants. An Illustrated Dictionary. Springer publication. New Delhi, India (2007) 90.

Kirtikar KR and Basu BD. Indian Medicinal Plants IV, Lalit Mohan Basu

publications, Allahabad (1990) 2724-2727.

Kumar, P, S., Kumaravel. and Lalitha, C. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. *African J. Biochem.Res.* 2010; 4: 191-195.

Massada, Y. *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry*; John Wiley and Sons: New York, NY, USA, (1976).

Mass Spectral Library. Available online: http://www.sisweb.com/software/ms/nist.htm (accessed on 23 May 2002).

Massry, K. F., Ahmed, H., Ghorab, E., Hamdy, A., Shaaban, and Shibamoto, T. Chemical Compositions and Antioxidant/Antimicrobial Activities of Various Samples Prepared from *Schinus terebinthifolius* Leaves Cultivated in Egypt. J. Agric. Food Chem. 2009; 57: 5265–5270.

Mehmood, N., Zubair, M., Rızwan, K., Rasool, N., Jamil, M., Shahid, M. and Ahmad, V. U. Antioxidant, antimicrobial and phytochemical analysis of *Cichorium intybus* seeds extract and various organic fractions. Iran J. of Pharm. Res. 2012; 11: 1145-1151.

Muniappan, M. and Sundararaj, T. Antiinflammatory and antiulcer activities of *Bambusa arundinacea* J. Ethnopharmacol. 2003; 88: 161-167.

NCCLS (National Committee for Clinical laboratory Standards). *Performance standards for antimicrobial disc susceptibility test.* 5th (ed), approved Standard. M2-A6, Wayne, PA. (1997).

Powell, W. A., Catranis, C. M. and Maynarrd, C. A. Design of self processing antibacterial peptide of plant protection. *Lett. in appli. Microbio.*, 2000; 31: 163-165.

Rasool, N., Rızwan, K., Zubaır, M., Gulzar, T., Bukhari, I. H., Gilani, M. A., Amjad, A. Antioxidant activity of various extracts and organic fractions of *Ziziphus jujuba*. *Int. J. of Phytomed*. 2011; 3: 346–352.

Rasool, N., Rizwan, K., Zubair, M., Naveed, K. R., imran, I., Ahmed, V. U. Antioxidant potential of different extracts and fractions of *Catharanthus roseus* shoots. Int. J. of Phytomed. 2011; 3: 108–114.

Rizwan, K., Zubair, M., Rasool, N., Riaz, M., Zia-Ul-Haq, M., and Vincenzo, de Feo. Phytochemical and Biological Studies of *Agave attenuata*. Int. j. of Mol. Sci. 2012; 13: 6440-6450.

Sala AV. Indian Medicinal Plants. Orient Langman ltd. Chennai (1995) Vol-III: 244.

Sarker, S. D., Nahar, L., Kumarasamy, Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods. 2007; 42: 321–324.

Scalbert, A. Antimicrobial Properties of Tannins. Phytochem. 1991; 30: 3875-3883.

Shibata, M., Yamatake, Y., Sakamoto, M., Kanamori, M. and Takagi, K. and Zasshi, N. Y. Pharmacologcal studies on bamboo grass, acute toxicities, anti-inflammatory and anti-ulcerogenic activities of water soluble fraction (Folin) extracted from *sasa albomarginata*. Nihon Yakurigaku Zasshi. 1975; 71: 481-490

Siddhuraju, P. and Becker, K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*M. Oleifera Lam.*). J. Agric. Food Chem. 2003; 51: 2144-2155.

Singh, G., Maurya, S., Catalan, C. and De lampasona, M. P. Chemical Constituents, atifungal and antioxidative effects of ajwain essential oil and Its acetone extract. J. Agric. Food Chem. 2004; 52: 3292-3296.

Sharma, P. and Sharma, J. D. *In vitro* hemolysis of human erythrocytes by plant extracts with antiplasmodial activity. *J. of ethnopharmaco*. 2001; 74: 239-243.

Slinkard, K and Singleton, V. L. Total phenol analyses automation and comparison with manual methods. Am. J. Enol. Viticult. (1977); 28: 49-55.

The Wealth of India. Raw Materials, CSIR Delhi (1956) Vol- II: p. 33.

Trueba, P. G. Los flavonoides: antioxidantes o prooxidantes. Rev. Cubana Invest. Biomed. 2003; 22: 48-57.

Vanithakumari, G., Manonayagi, S., Padma, S. and Malini, T. Antifertility effect of *Bambusa arundinacea* shoot extracts in male rats. J Ethnopharmacol. 1989; 25: 173-180.

Verastegui, A., Verde, J., Garcia, S., Heredia, N., Oranday, A. and Rivas, C. Species of Agave with antimicrobial activity against selected pathogenic bacteria and fungi. World J. Microbiol. Biotech. 2008; 24: 1249-1252.

Zhang, J., Gong, J., Ding, Y., Lu, B., Wu, X., Zhang, Y. Antibacterial activity of water-phase extracts from bamboo shavings against food spoilage microorganisms, African J. of Biotech. 2010;. 9(45): 7710-7717.

Zubair, M., Rızwan, K., Rasool, N., Afshan, N., Shahid, M. and Ahmed, V. D. Antimicrobial potential of various extract and fractions of leaves of *Solanum nigrum.* Int. J.of Phytomed. 2011; 3: 63–67.

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