Journal of Medicinal Plants Research Vol. 6(11), pp. 2105-2110, 23 March, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1006
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Antimicrobial evaluation, determination of total phenolic and flavoniod contents in *Zanthoxylum armatum* DC

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Accepted 24 August, 2011

The ethanolic and *n*-hexane extracts of leaves, fruits and bark of *Zanthoxylum armatum* were evaluated for their antimicrobial potential against bacteria (*Micrococcus leutus*, *Escherichia coli*, *Staphylococcus aureus*, *Pasturella multcidia*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Streptococcus viridians*) and fungi (*Trichophyton longifusis*, *Candida albicans*, *Fusarium solani*, *Microsporum canis*, *Aspergillus flavus* and *Candida glabrata*). The total flavonoids and phenolic contents were also quantified in ethanolic extract of all parts. The ZLE extract showed highest inhibition against *M. leutus* (18.00 ± 0.71 mm), *P. multocida* (18.00 ± 0.71 mm), *E. coli* (17.00 ± 0.71) and *B. subtilis* (15.33 ± 0.81 mm). The ZFE showed topmost action against *M. leutus* (21.33 ± 0.41 mm) and *P. multocida* (18.33 ± 0.41 mm) while the ZFH showed inhibitory action against *M. leutus* (19.67 ± 0.41 mm) as compared to other tests species. The ZBH extract was found active against *M. leutus* (20.33 ± 0.41 mm). The minimum inhibitory concentration (MIC) values for most of the bacterial species were found to be 0.65 µg/ml. The crude ethanolic and *n*-hexane of all parts were proved a rich source of fungicidal effect. Highest flavonoids was found in ethanolic extract of *Z. armatum* fruit (ZFE) (22.8 ± 1.33 mg/g) followed by ethanolic extract of *Z. armatum* bark (ZBE) (18.33 ± 1.22 mg/g) while highest phenolic contents were found in ZFE (21.68 ± 0.44 mg/g) followed by ZBE (16.48 ± 1.33 mg/g).

Key words: *Zanthoxylum armatum*, antimicrobial, MIC, phenolic, flavoniods.

INTRODUCTION

The indiscriminate use of profitable antimicrobial medications are commonly employed in the treatment of contagious diseases coupled with undesirable side effects and emergence of serious medical problem (Marchese and Shito, 2001; Portillo et al., 2001).This has led the medical chemist and pharmacist to discover new antimicrobial substances from natural sources. The screening of plant extracts for antimicrobial activity has shown that plants are a potential source of novel antimicrobial compounds (Afolayan, 2003; Aliero and Afolayan, 2006). In modern era, herbal medical has drawn attention to identify and isolate easy accessible anti-microbial agents from natural sources to cure various infectious diseases (Sashoo et al., 2008).

Flavonoids are plant secondary metabolites widely distributed in the plant kingdom. More than 6000 flavonoids have been identified in plants (Harborne and Williams, 2000). A number of flavonoids and phenolic compounds from natural botanic extracts and flavors are responsible for antimicrobial, antioxidant and anti-cancer activities (Kale et al., 2010; Greenberg et al., 2008). *Zanthoxylum armatum* DC is a large spiny shrub, which

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Abbreviations: ZLE, Ethanolic extract of *Zanthoxylum armatum* leaves; **ZLH,** n- hexane extract of *Zanthoxylum armatum* leaves; **ZBE,** ethanolic extract of *Zanthoxylum armatum* bark; **ZBH,** n- hexane extract of *Zanthoxylum armatum* bark; **ZFE,** ethanolic extract of *Zanthoxylum armatum* fruit**, ZFH,** n- hexane extract of *Zanthoxylum armatum* fruit.

may reach the height of small tree with compound leaves, flowering in March and April, mostly grow in semi shady place. Locally, it is known as Dambara (Pashtu), Dambrary, Tamur (Urdu) and wing leaf prickly ash (English). It is used as antiseptic, disinfectant, deodorant (Shinwariet et al., 2006) antipyretic and anti diarrheal (Ahmad et al., 2006). It is also consider as a tonic, carminative, condiment, stomachic, anthelmintic (Verma and Khosa, 2010), Insecticidal (Tiwary et al., 2007), tootheache reliving, abortifacient, antifertility agent (Shah and Khan, 2006). It also improves speaking power and increase saliva secretion (Ahmad et al., 2006). Fruits and seeds of this plant are used in fever, dyspepsia and skin diseases (Khare, 2007). Locally, it is used for complications caused by germs; therefore it is assumed that this plant may have constituents of antimicrobial potential. This study is carried out to investigate antimicrobial potential of the leaves, bark and fruits of *Z. armatum* as well as their flavonoids and phenolic contents.

MATERIALS AND METHODS

Plant materials

Z. armatum was collected from Charkotli hills Batkhela. Fruits, leaves and bark were separately rinsed, dried and pulverized into powder. The plants parts were then separately macerated with ethanol and *n*- hexane solvent in a tank for fourteen days with constant shaking. The extract was filtered and the filtrate was concentrated using rotary evaporator at low temperature and high pressure. These crude extracts were evaluated for antimicrobial activities, total flavonoids and total phenolic contents.

Microorganism

Antimicrobial activity of *Z. armatum* was carried out using various Gram positive (*Bacillus subtilis, Streptococcus viridines, Micrococcus leutus*), Gram negative (*Escherichia coli, Pasturella multcidia, Pseudomonas aeruginosa*) bacterial strains and some fungal species (*Trichophyton longifusis, Candida albicans, Fusarium solani, Microsporum canis, Aspergillus flavus* and, *Candida glabrata*) fungal strain).

Anti-bacterial activities

Ethanolic and n- Hexane extracts of leaves, bark and fruits of *Z. armatum* were applied to screen out following agar well diffusion method. The experimental bacterial cultures were first grown on nutrient broth and incubated for 24 h prior to experiments. The nutrient agar was melted, then cooled to 40°C, poured to sterilized Petri dishes and allowed to solidify. Wells were then bored in agar using 6 mm diameter with the help of sterile metal cork borer keeping a distance of 24 mm between two consecutive well. 4 to 8 h old bacterial culture was spread on the surface of nutrient agar in petri dishes with the help of sterilized cotton swab. These processes were repeated thrice turning the plate 60°C between each streaking. About l00 µl of 3 mg/ ml of respective extract dissolved in dimethyl sulfoxide (DMSO) were then added to the wells. Other wells were supplemented with DMSO and 10 µg ciprofloxacin. The zones of inhibition were then measured after 24 h incubation period. All the experiments were conducted in triplicate.

Minimum inhibitory concentration (MIC)

The MIC values of the respective extracts were determined following recommended procedure (Sahm and Washington, 1999; Akinpelu and Kolawole, 2004). 1 ml of the respective extract solution at concentration of 20 mg/ml was added to 1 ml of pre sterilized nutrient broth. Subsequently, 1 ml from the first test tube was transferred to the second test tube containing 1 ml of nutrient broth and then these processes were continued up to the seventh test tube. Thereafter, 1 ml of each test bacterium (1.0×10^6cells/ml) was inoculated into each test tube and mixed thoroughly. The test tubes were then incubated at 37°C for 24 h. The MIC was taken as the lowest concentration that prevented the growth of bacterial culture.

Anti-fungal activity

Anti-fungal screening of ethanolic and *n*-Hexane extract of *Z. armatum* (Leaves, bark and fruits) were conducted using agar dilution method following Umadevi et al. (2003). Stock solution of each extract was prepared by dissolving at a concentration of 24 mg/ml in DMSO and stored in a refrigerator till further used. Sabouraud dextrose agar (SDA) media for fungal growth was prepared by mixing Sabouraud 40% glucose agar and agar in distilled water with acidic pH (pH 5.5 to 5.6) and then autoclaved at 121°C for 15 min (Atta-ur Rahman et al., 2001). Media was then cooled to 45 to 50°C and 20 ml of molten SDA medium was aseptically transferred into each sterilized Petri dish (4 cm diameter). All dishes were then inoculated with 4 mm diameter piece of inoculums detached from a seven days old culture of fungus (Parekh and Chanda, 2008). Once the agar was hardened, 8 mm wells were bored using a sterile cork borer, then from each stock solution, extracts were transferred to a separate well having a final concentration of 100, 125, and 250 mg/ml and the plates were incubated for 24 h at 29°C. Two wells in each petri dish were supplemented with DMSO and reference antifungal drug Miconazole (0.2 mg/ml) dissolved in DMSO (sigma) serve as negative and positive control respectively. All the process was carried out in triplicate.

Incubation

All these tubes were incubated at 28 ± 1°C for 7 days. Cultures were observed twice weekly during incubation. Growth in the media was estimated by measuring the clear zone (mm) in the media loaded with sample, DMSO and miconozole, and then the percentage inhibition of fungal growth was calculated as follows:

% Mycelia inhibition = Gn-Gt / Gn × 100

where, Gn = mycelial growth in normal, and Gt = Mycelial growth in test (Usmanghani and Shameel, 1986; Ali-Shtayeh and Ghdeib, 1999).

Total flavoniod determination

Aluminum chloride colorimetric method was used for flavonoids determination according to Chang et al.'s (2002) study. Each extract (0.5 ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% Aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min.

Table 1. Antibacterial effect of the ethanolic and *n*-hexane extracts of leaves, fruit and bark of *Z. armatum* DC.

Parameter	*M. leutus*	*E. coli*	*S. aureus*	*P. multocida*	*P. aeruginosa*	*B. subtilis*	*S. viridans*
	Zone of inhibition (mm)						
DMSO(Neg. cont)	-	-	-	-	-	-	-
Cifrofloxacin(P.C)	28 ± 0.11	30 ± 0.05	24± 0.06	32± 0.10	44± 0.14	28± 0.05	22± 0.09
ZLE	18.00 ± 0.71	17.00 ± 0.71	-	18.00 ± 0.71	-	15.33 ± o.81	-
ZLH	11.67 ± 0.41	11.67 ± 0.41		8.67 ± 0.82	-	9.33 ± 1.08	-
ZFE	21.33 ± 0.41	-	17.33 ± 0.41	18.33 ± 0.41	14.67 ± 0.51	11.67 ± 0.41	-
ZFH	19.67 ± 0.41	17.00 ± o.71	-	17.67 ± 0.41	10.33 ± 0.33	11.67 ± 0.41	7.67 ± 0.41
ZBE	13.33 ± 0.41	14.33 ± 0.41	-	9.67 ± 0.41	9.33 ± 0.33	10.33 ± 0.41	7.67 ± 0.42
ZBH	20.33 ± 0.41	11.33 ± 0.41	12.67 ± 0.51	15.33 ± 0.41	-	11.33 ± 0.41	10.00 ± 0.71

values are mean ± SEM of three determination.

Table 2. MIC values for the ethanolic and *n*-hexane extracts of leaves, fruit and extract bark of *Z. armatum* DC.

	M. leutus	*E. coli*	*S. aureus*	*P. multocida*	*P. aeruginosa*	*B. subtilis*	*S. viridans*
	Minimum inhibitory concentration (MIC) (mg/ml)						
ZLE	1.25	1.25	-	0.65	-	2.5	-
ZLH	5	-	-	5	-	-	-
ZFE	0.65l	-	0.65	0.65	1.25	1.25	-
ZFH	0.65	0.65		1.25	2.5	2.5	-
ZBE	5	5	-	-	-	5	-
ZBH	1.25	2.5	2.5	1.25	1.25	1.25	-

The absorbance of the reaction mixture was measured at 415 nm using Spectronic 20D (Milton Roy). The calibration curve was prepared by comparing with standard curve of quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

Total phenolic contents

Total phenol concentration of ethanolic extracts was determined in accordance with Khan et al. (2006) and Okwu (2005). About 2 g of ethanolic extract were treated with about 20 ml of Folin-Denis reagent, 30 ml of 20% Na_2CO_3 and diluted by a factor of 100 with distilled water. The resulting mixture was kept as such at room temperature for 30 min. It was then filtered and the absorbance was measured at 770 nm against the blank using Spectronic 20D (Milton Roy). The total phenol content of each sample was calculated by comparing with a standard curve of tannic acid using as blank.

RESULTS AND DISCUSSION

The ethanolic and *n*-hexane extracts of leaves, fruits and bark were tested against various Gram positive and Gram negative bacteria that is, *M. leutus, E. coli, Staphylococcus aureus, P. multcidia, P. aeruginosa, B. subtilis,* and *S. viridines*as (Table 1). Results obtained during the study, showed a dose and test species dependency. The antibacterial actions of the extracts were compared with ciprofloxacin as positive control. The ZLE extract showed highest inhibition against *M. leutus* (18.00 ± 0.71 mm), *P. multocida* (18.00 ± 0.71 mm), *E. coli* (17.00 ± 0.71) and *B. subtilis* (15.33 ± 0.81 mm). The antibacterial action of ZLH was non significant as compared to ZLE and ciprofloxacin. The ZFE showed the topmost action against *M. leutus* (21.33 ± 0.41 mm) and *P. multocida* (18.33 ± 0.41 mm) while the ZFH showed inhibitory action against *M. leutus* (19.67 ± 0.41 mm) as compared to other tests species. The zone of inhibition for ZBE showed less potential against bacteria, but the ZBH extract was found active against *M. leutus* (20.33 ± 0.41 mm). Form the above results; it is proved that *M. leutus* is most sensitive strain to most of the extracts. Other bacterial strains such as *P. aeruginosa*, *S. aureu s*and *S. viridines* also showed its inhibition to different extract. The MIC for all extract was also determined as shown in Table 2. In most cases the MIC was found 0.65 mg/ml against most bacteria. *B. subtilis* was appeared the most resistant bacterial strain as it has highest MIC values in comparison with other species. The ethanolic and *n*-hexane Extracts of ZL, ZF and ZB were evaluated for antimycotic potential against various fungal strains like *Trichophytonlongifusis, C. albicans, Fusariumsolani, Microsporumcanis, A. flavus* and *C. glabrata* as shown in Table 3. All the extracts were showed anti fungal activities in dose dependent manner, as the extracts were tested in three various doses that is, 125, 250 and 500

Table 3. Antifungal effect of ethanolic and activities of ethanolic and *n*-hexane extracts of leaves, fruit and bark of *Z. armatum* DC.

	Concentration	Percent Inhibition of mycelial growth					
	(µg/ml)	*T. longifusis*	*C. albicans*	*F. solani*	*M. canis*	*A. flavus*	*C. glabrata*
	125	0	34.33	35.33	8.66	32.44	7.33
ZLE	250	0	47.32	45.33	12.65	44.56	7.66
	500	8.82	70.97	59.26	46.15	45.16	20.59
	125	20.93	26.98	12.45	7.88	19.32	19.22
ZLH	250	30	39.33	37.33	8.33	42.34	36.76
	500	32.35	45.16	40.74	26.92	54.84	44.12
	125	34.5	56.33	66.67	54.33	44.29	12.56
ZBE	250	53.33	67.33	70.33	61.22	56.97	34.77
	500	55.88	74.19	74.07	69.23	61.29	67.65
	125	29.33	18.33	23.78	7.33	23.33	19.33
ZBH	250	50.63	44.33	49.33	18	56	32.76
	500	70.59	70.97	59.26	26.92	58.06	44.12
	125	35.5	32.31	21.76	0	44.29	13.46
ZFE	250	54.33	57.43	41.22	10.54	56.97	34.77
	500	55.88	58.06	48.15	11.54	64.52	44.12
	125	29.33	18.33	23.78	7.33	23.33	19.33
ZFH	250	50.63	42.33	31.66	47.33	24.74	32.76
	500	52.94	58.06	37.04	57.69	29.03	52.94

µg/ml. ZLE was found effective against *F. solani* (35.33%) followed by *C. albicans* (34.33 %) and *Aspergillusflavus* (32.44 %) at dose of 125 ug/ml while ZLH was active against *C. albicans* (26.98%) followed by *T. longifusis* (20.93%) and showed week effect against remaining fungal strain. *F. solani* (66.67%), *C. albicans* (56.33%) and *Microsporum canis* (54.33%) were sensitive to ZBE at the dose of 125 µg/ml as shown in Table 3 and the ZBH extract showed weak activities against all fungal species however with increasing dose and the activity was found significant. ZFE was active against *A. flavus* (44.12%) and *T. longifusis* (35.50%) while the effect of ZFE was found less against fungal strains as compared to high concentration, which proved a dose dependant inhibition. The total flavoniods and phenolic contest were quantified in ethanolic extract of leaves, bark and fruits (Figures 1 and 2 and Table 4). The result of flavoniod was similar to phenolic contents and the highest quantity was found in ZFE (22.80 mg/g) followed by ZBE (18.33 mg/g). The highest phenolic contents were found in ZFE (21.68 mg/g) followed by ZBE (16.48 mg/g) while remaining samples also showed variable amount of the phenolic contents. Flavonoids and other pheolics exhibit a wide range of fascinating biological activities like antimicrobial, antiviral, antioxidant, anticancer properties (Havsteen, 2002; Harborne and Williams, 2000). These biological and pharmacological activities are usually associated with their free radical scavenging efficacies and their ability of binding proteins with a high degree of specificity (Fotie, 2008). These findings support our results of antimicrobial inhibition as all parts of *Z. armatum* contains flavonoids and phenolic compound, which might be responsible for antimicrobial properties.

The *P. multocida* which is a Gram negative bacterium is responsible for a variety of human infections and in our results; it was found the most sensitive species to all extracts. The tested plant extracts of all parts have enormous potential as antimicrobial, therefore the tested plant extracts can be used in the treatment of infections caused by the tested microbes. The screening of these plant extracts, as a source of new antimicrobial agents confirms the knowledge of traditional herb practitioners. These findings will not only authenticate the traditional knowledge of folk healers but there is also dire need to commercialize this plan in production of cheap plant based products in third world countries. *Z. armatum* in this study confirmed a broad-spectrum of activity against both Gram-positive and Gram-negative bacteria and fungi.

ACKNOWLEDGMENT

We are thankful to Higher Education Commission (HEC) of Pakistan for providing financial support for this part of

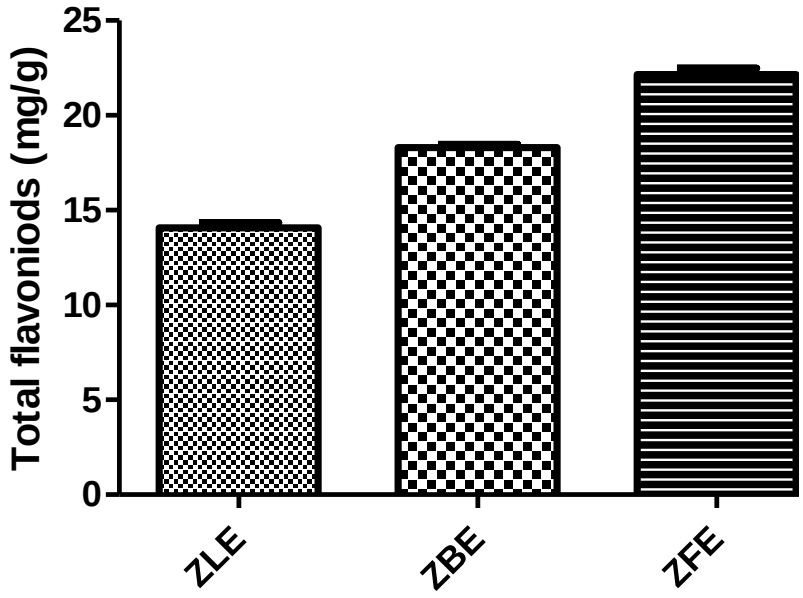

Figure 1. Total flavoniod contents in ethanolic extract of leaves, bark and fruit of *Z. armatum* DC.

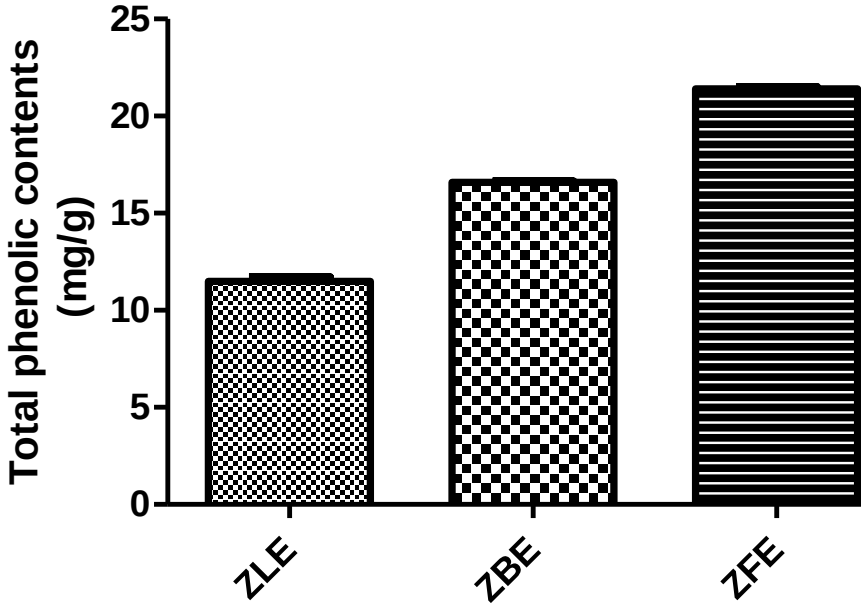

Figure 2. Total phenolic contents in ethanolic extract of leaves, bark and fruit of *Z. armatum* DC.

Table 4. Flavonoids and phenolic contents of leaves, fruit and bark of *Z. armatum*.

S/N	Extract	Flavonoids (mg/g)	Phenol (mg/g)
1	ZLE	13.68 ± 0.66	11.66 ± 0.33
2	ZBE	18.33 ± 1.22	16.48 ± 1.33
3	ZFE	22.8 ± 1.33	21.68 ± 0.44

All values are mean ± SEM of three determinations.

PhD research work. We are also grateful to PCSIR labs, Lahor, for their help in performing antibacterial activities.

REFERENCES

Afolayan AJ (2003). Extracts from the shoots of Arctotisartotoides inhibit the growth of bacteria and fungi. Pharm. Biol., 41: 22-25.

Ahmad S, Ali A, Beg H, Dasti AA, Shinwari ZK (2006). Ethnobotanical studies on some medicinal plants of Booni valley, district Chitral, Pakistan. Pak. J. Weed Sci. Res., 12 (3): 183-190.

Akinpelu DA, Kolawole DO (2004). Phytochemical and antimicrobial activity of leaf extract of *Piliostigma thonningii*(Schum.). Sci. Focus J., 7: 64-70.

Ali-Shtayeh MS, Abu GSI (1999). Antifungal activity of plants extracts against dermatophytes. Mycoses*,* 2: 665-672.

Aliero AA, Afolayan AJ (2006). Antimicrobial activity of *Solanum tomentosum.* Afr. J. Biotechnol., 5 (4): 369-372.

Atta-ur R, Choudhary MI, Thomsen WJ (2001). Bioassay Technique for Drug Development. Harwood Academic Publishers.

Chang C, Yang M, Wen H, Chem J (2002). Estimation of total flavonoid content in propolisby two complementary colorimetric methods. J. Food Drug Anal., 10: 178-182.

Fotie J (2008). The antiprotozoan potential of flavonoids: A review. Pharmacogn. Rev., 2(3): 6-19.

Greenberg M, Dodds M, Tian M (2008). Naturally Occurring Phenolic Antibacterial Compounds Show Effectiveness against Oral Bacteria by a Quantitative Structure−Activity Relationship Study. J. Agric. Food Chem., 56(23): 11151–11156.

Harborne JB, Williams CA (2000). Advances in flavonoid research since 1992. Phytochemistry, 55: 481-504.

Havsteen BH (2002). The biochemistry and medical significance of flavonoids. Pharmacol. Ther., 96(2-3): 67-202.

Kale A, Gaikwad S, Mundhe K, Deshpande I, Salvekar J (2010). Quantification of phenolics and flavonoids by spectrophotometer from –*Juglans regia*. Int.. J. Pharm. Biosci., 1(3): 1-4.

Khan T, Ahmad M, Khan R, Khan H, Ejaz A,Choudhary MI (2006). Evaluation of phytomedicinal potentials of selected plants of Pakistan*.* Am. Lab., 38(9): 20–22.

Khare CP (2007). Indian Medicinal Plants.An Illustrated Dictionary. Springer-VerlagBerlin Heidelberg, p. 730.

Marchese A, Shito GC (2001). Resistance patterns of lower respiratory tract pathogens in Europe. Int. J. Antimicrob. Agents, 16: 25–29.

Okwu DE (2005). Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. Int. J. Mol. Med. Adv. Sci., 1(4): 375–381.

Parekh J, Chanda S (2008). *In vitro* antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. Afr. J. Biotechnol., 7(23): 4349-4353.

Portillo A, Vila R, Freixa B, Adzet T, Canigueral S (2001). Antifungal activity of Paraguayan plants used in traditional medicine. J. Ethnopharmacol., 76: 347-354.

Sahm DF, Washington JA (1999). Antimicrobial susceptibility test dilutions methods. In Manuals of Clinical Microbiology.

Shinwari ZK, Watanabe T, Rehman M, Yoshikawa T (2006). A pictorial guide to medicinal plants of Pakistan. KUST, Kohat., p. 400.

Tiwary M, Naik SN, Tewary DK, Mittal PK, Yadav S (2007). Chemical composition and larvicida l activities of the essential oil of Zanthoxylumarmatum DC (Rutaceae) against three mosquito vectors. J. Vector Borne Dis., 44 (3): 198 - 204.

Umadevi S, Mohanta GP, Chelladurai V, Manna PK, Manavalan R (2003). Antibacterial and antifungalactivity of Andrographisechiodes. J. Nat. Remedies., 3: 185-188.

Usmanghani K, Shameel M (1986). Studies on the antimicrobial activity of certain seaweads from Karachicoast.*In*Ahmad R and San Pietro A (eds.): Prospects for Biosaline Research Proc. US-Pak Biosal. Res. Workshop, Karachi, pp. 519-526.

Verma N, Khosa RL (2010). Hepatoprotective activity of leaves of *Zanthoxylum armatum* DC in CCl4 induced hepatotoxicity in rats. Indian J. Biochem. Biophys., 47(2): 124-127.