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Antimicrobial and Antioxidant Screening of *Flueggea Virosa*

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The antimicrobial and antioxidant activity of *Flueggea virosa* (Roxb. ex Willd.) Voigt was assessed by preparing extracts in petroleum ether, chloroform, methanol and water. By using agar well diffusion and dilution method the Zone of Inhibition and MIC assay was measured. Maximum activity showed by aqueous extract of leaf of *F. virosa* against *S. aureus,* i.e. 45±1.0 mm. The bark extract had exhibited greater potential as compared to leaf against respective strain. The antifungal assessment had revealed that the leaf and bark extracts had revealed satisfactory potential. Methanolic extract of bark of *F. virosa* showed the highest antifungal activity against *A. oryzae,* i.e 15.6±0.5 mm. The highest total phenolic contents (TPC) content was provided by methanolic extract of leaf of *F. virosa,* i.e. 3029.25 GAE µg/mL and wide range of flavonoid con tents. The significant DPPH free radical scavenging was exhibited by methanolic extract of bark whereas the value of percentage of bound iron ranging from -60.84±0.9 to 56.75±1.0. The ABTS radical cation was range in 5.79±1.0-10.56±0.7 mM of TE and maximum capacity was displayed by the aqueous extract of bark.

Keywords: *Flueggea virosa*, Antimicrobial, Antioxidant, MIC

INTRODUCTION

In this planet about 50,000 people died due to infectious diseases per day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world N'guessan et al. (2007). Natural products from plants could be the best option with increased incidence of resistance to antibiotics (Lu et al. 2007); Mbwambo (et al. 2007). Some plant extracts and phytochemicals are known to have antimicrobial properties and can be of great significance in therapeutic treatments (Ajaib et al., 2016). In the last few years, a number of studies have been carried in different countries to demonstrate such efficacy (Benoit- Vical et al., 2006; Senatore et al. 2007; Singh et al. 2007). On the other hand, free radicals are known to be the major cause of various chronic and degenerative diseases. Oxidative stress is related with pathogenic mechanisms of many diseases including atherosclerosis, neurodegenerative diseases, cancer, diabetes and inflammatory diseases, as well as aging processes (Kamran et al. 2016). It is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants, and it also includes their elimination by protective mechanisms, referred to as antioxidative systems (Mazhar et al. 2015).

***Flueggea virosa* (Roxb. ex Willd.) Voigt**

Medicinal plants are rich sources of antimicrobial and antioxidant agents (Ajaib et al. 2020). Many infectious diseases have been treated with herbal extracts. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Ajaib et al. 2017). Although many plant species have been tested for antimicrobial properties, the majority of them have not been sufficiently evaluated (Balandrin, 1985). As an alternate source to the existing antibiotics, there is an urgent need to discover new antimicrobial compounds from various biological resources (Ajaib et al. 2015).

The Family Euphorbiaceae provides various compounds of medicinal importance. Members of this Family are used in treatment of various ailments such as respiratory infections, venereal diseases, toothache, rheumatism, cough, ulcer and wounds while *Flueggea virosa* (Roxb. ex Willd.) Voigt belong to this family which is also commonly called Spurge family. *F. virosa* occurs naturally throughout tropical Africa and extending through Asia. It is a dioecious, profusely branched shrub with some small thorne-like branches. The bark is reddish brown to brown and leaves are green crowded along branchlets. Flowers creamy green in umbellate cymes. Fruits globose, white and fleshy and appear in December-March. It is used for healing wounds whereas members of the genus *Flueggea* have antiproliferative and analgesic properties (Smith, 1968).

MATERIALS AND METHODS

Plant material

The plant, i.e. *F. virosa* (Roxb. ex Willd.) Voigt was collected from District Kotli, Azad Jammu & Kashmir (AJK). The plant specimen was identified with the authenticated voucher no. (SAH. 2952) and deposited to the Dr. Sultan Ahmed herbarium, Department of Botany, GC University, Lahore.

Maceration of the plant material

Leaf and bark of selected plant dried under shade and macerated in different solvents, i.e. Petroleum ether, Chloroform, Methanol and Aqueous. The resultant extracts were dried on rotary evaporator to get final concentrated form of extracts.

Antimicrobial activity

Zone of inhibition assay

Antimicrobial activity of plant was investigated by using agar well diffusion method (Bauer et al. 1966). For the investigation of antimicrobial activity of respective plant, i.e. bark and leaf of *F. virosa*, 2 gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*), 2 gram positive *(Bacillus subtilis* & *Staphylococcus aureus*), and 2 fungal strain *Aspergillus niger* & *Fusarium solani*) were used following (Cruick-shank et al., 1975) by autoclaved prepared nutrient-agar media for antibacterial activity and Potato Dextrose Agar media for antifungal activity following Johansen (1940).

Evaluation of Minimum Inhibitory Concentration (MIC)

Plant sample having antimicrobial potential with lower constancy evaluated by using Broth-dilution method following by Murray et al. (1999).

Antioxidant assays:

1000µL of (500, 250, 125, 60µg/mL) extract of selected plants were taken followed by 2.5mL of freshly prepared DPPH solution, shaken vigorously and incubated at room temperature for 45-60 minutes. Then absorbance was taken at 515nm against methanol as a blank in the spectrophotometer. The %age of DPPH radical remaining was calculated using following formula:

$$\%age\ DPPH = \frac{Abs.\ of\ sample}{\text{Abs. of control}} \times 100$$

Total Flavonoid Contents (TFC)

Total Flavonoid Contents of *F. virosa* was evaluated using the methodology applied by Dewanto et al. (2002).

Total Phenolic Contents (TPC)

For the estimation of Total Phenolic Contents of *F. virosa*, the procedure of Makkar et al. (1993) was followed.

RESULTS AND DISCUSSION

In the present study gram positive bacteria i.e. *Staphylococcus aureus*, *Bacillus subtilis,* gram negative bacteria i.e. *Escherichia coli, Pseudomonas aeruginosa*, and fungal strains, i.e. *A.niger, A. oryzae* were used to evaluate the antibacterial and antifungal potential of leaf and bark extracts of the *Flueggea virosa*.

Maximum activity against *S. aureus* had reported by the aqueous extract of leaf of *F. virosa*. The bark extract had exhibited greater potential as compared to leaf against respective strain whereas the chloroform and methanol extract of bark had showed similar potential i.e. 22±0.6 mm and 22±2.5 mm respectively (Table I; Fig. 1).

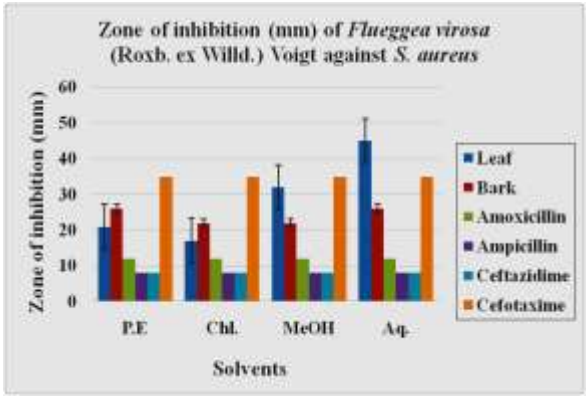

Figure1: Zone of Inhibition produced by extracts of *F. virosa* against *S. aureus*

The extracts of bark had exhibited the maximum potential as compared to leaf against *E. coli*. The chloroform extract of both parts had exhibited almost similar potential i.e. 23±2.0 mm and 23.6±1.5 mm (Table I). The methanol extract of bark had shown significant potential as compared to leaf (Fig. 2).

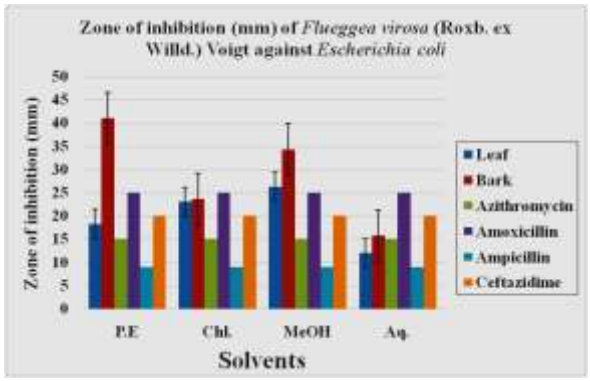

Figure. 2: Zone of Inhibition of extracts of F. virosa against E. coli.

The bark extract of *F. virosa* had documented the maximum potential against *P. aeruginosa* well with the petroleum ether extract being the most effective. The petroleum ether extracts of leaf and bark had shown maximum results, i.e. 33.3±2.5 and 39.6±2.0 respectively (Table 1). Minimum potency was observed by the aqueous extract of both parts (Fig. 3).

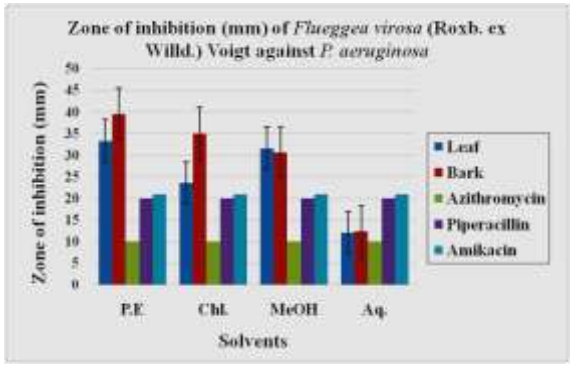

Figure. *3: Zone of Inhibition of extracts of F.virosa* against *P. aeruginosa*

The petroleum ether extract of leaf and methanol extract of bark showed significant activity was against *B. subtilis.* The petroleum ether extract of bark had also displayed significant activity, i.e. 35±2.6 mm against the respective strain (Table 1). The chloroform extract of both leaf and bark had displayed satisfactory potential (Fig. 4).

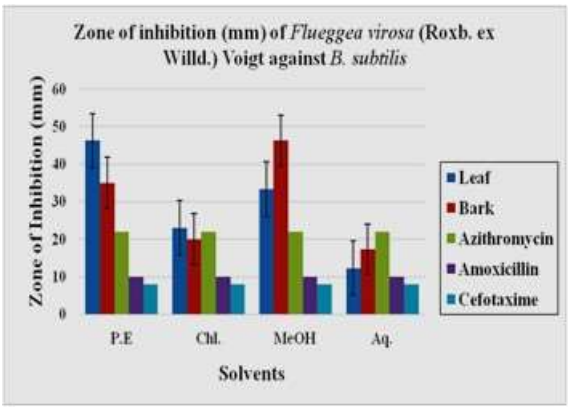

Figure. 4: Zone of Inhibition of extracts of F. virosa against *B. subtilis.*

The methanolic extracts were only employed for the estimation of MIC. The *S. aureus* was reported to be the most resistant against the methanolic extract of bark. The MIC values for the leaf and bark were 0.108±0.05 at 0.8 mg/ml concentration and 0.089±0.03 at 0.5 mg/ml concentration respectively (Table 2).

The *E. coli* was reported to be most sensitive against the bark extract when all methanolic extracts under investigation were taken to investigation. The MIC values for the leaf and bark were 0.090±0.02 at 0.1 mg/ml concentration and 0.029±0.01 at 0.6 mg/ml concentration respectively (Table 2).

The methanolic extracts inhibited the growth of *B. subtilis* effectively. The leaf extracts were more effective than the bark extracts. The MIC values estimated for leaf and bark with this particular strain were 0.052±0.05 at 0.1 mg/mL and 0.090±0.01 at 0.3 mg/mL respectively (Table 2).

The bark extract of *F. virosa* had documented the maximum potential against *Aspergillus niger.* The aqueous extracts had not shown any activity against the particular organism. The overall minimum potency was displayed by the petroleum ether extracts of both parts (Table 3; Fig. 5). The chloroform extracts of both leaf and bark showed maximum activity against *A. oryzae,* i.e. 30.0±0.5mm. The petroleum ether and methanol extracts of both parts had exhibited minimum potential (Table 3).

The MIC values for the leaf and bark *A. niger* were 0.201±0.01 at 0.3 mg/ml concentration and 0.052±0.02 at 0.1 mg/ml concentration respectively. The MIC values for the leaf and bark against *A. oryzae* were 0.203±0.03 at 0.2 mg/ml concentration and 0.023±0.02 at 0.3 mg/ml concentration respectively (Table 4).

The techniques employed for the determination of antioxidant potential of *Flueggea virosa* were Total Phenolic Contents (TPC), Total Flavonoid Contents (TFC), DPPH radical scavenging action, Metal chelating activity and ABTS Assay.

Table 1: Zone of Inhibition produced by Bark and leaves extracts of *F. virosa* against bacterial strains

Plant Parts	Extracts	Zone of Inhibition (mm)			
		E. coli	***S.aureus***	***P.aeruginosa***	***B.subtils***
Bark	**Petroleum ether**	41±1.5	26±0.5	39.6±2.0	35±2.6
	Chloroform	23.6±1.5	22±0.6	35±2.6	20±0.2
	Methanol	34.3±2.5	22±2.5	30.6±1.1	46.3±1.5
	Aqueous	15.7±1.5	26±1.9	12.3±1.5	17.3±0.6
Leaf	**Petroleum ether**	18.3±2.0	21±0.2	33.3±2.5	46.3±1.5
	Chloroform	23±2.0	17±0.5	23.6±1.5	23±1.9
	Methanol	26.3±2.0	32±1.7	31.6±2.0	33.3±2.5
	Aqueous	12±1.0	45±1.0	12±1.0	12.3±0.1

Table 2: MIC values (mg/mL) exhibited by leaf and bark of *F. virosa* against Gram-negative and Gram-positive bacterial strains

Plant Part	***S. aureus***		***E. coli***		***P.aureginosa***		***B. subtilis***	
	Conc.	**MIC**	**Conc.**	**MIC**	**Conc.**	**MIC**	**Conc.**	**MIC**
Leaf	0.8	0.108±0.05	0.1	0.090±0.02	0.9	0.072±0.04	0.1	0.052±0.05
Bark	0.5	0.089±0.03	0.6	0.029±0.01	0.3	0.099±0.08	0.3	0.090±0.01

Table 3: Zone of Inhibition produced by Bark and leaves extracts of *F. virosa* against fungal strains

Plant Parts	Extracts	Zone of Inhibition (mm)	
		Aspergillus niger	*Aspergillus oryzae*
Bark	Petroleum ether	12.0±1.0	12.0±1.0
	Chloroform	21.0±1.5	30.0±0.5
	Methanol	14.6±0.5	12.0±1.5
	Aqueous	-	-
Leaf	Petroleum ether	13.0 ±0.1	12.6 ±0.2
	Chloroform	15.6±1.3	29.0±0.2
	Methanol	16.6±1.5	13.0±1.0
	Aqueous	-	-

Table 4: MIC (mg/mL) exhibited by leaf and bark of *F. virosa* against fungal agents

Plant Part	*A. niger*		*A. oryzae*	
	Conc.	MIC	Conc.	MIC
Leaf	0.3	0.201±0.01	0.2	0.203±0.03
Bark	0.1	0.052±0.02	0.3	0.023±0.02

Table 5: Total Phenolic content, Total Flavonoid content, %DPPH radical scavenging potential, %age bound iron and TEAC value of *F. virosa*

Plant Parts	Extracts	TPC	TFC	%DPPH	%age bound iron	TEAC value
Bark	Petroleum ether	1176.75±0.3	1079.82±1.1	82.96	18.17±1.1	5.79±1.0
	Chloroform	161.75±1.5	688±1.2	81.3	9.57±0.2	5.79±0.1
	Methanol	2719.25±0.5	1319.82±0.5	94.3	56.75±1.0	9.90±0.6
	Aqueous	2579.25±0.4	1714.36±0.6	91.0	13.32±0.7	10.56±0.7
Leaf	Petroleum ether	1176.75±2.4	2631.64±2.5	89.73	-31.31±0.7	7.53±0.1
	Chloroform	334.25±0.1	858±1.6	87.8	-60.61±0.2	7.62±0.5
	Methanol	3029.25±0.2	2116.18±0.9	92.23	-60.84±0.9	10.20±0.8
	Aqueous	2066.75±2.3	3370.73±0.8	26.8	44.63±1.6	10.16±0.6

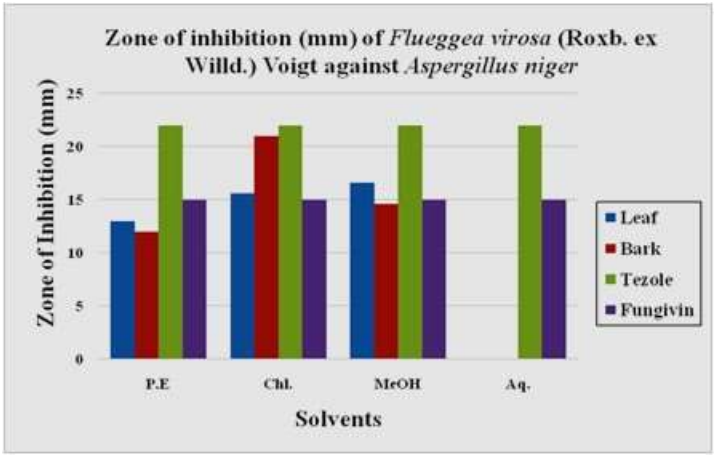

Figure 5: Zone *of Inhibition of extracts of F. virosa against A. nige*

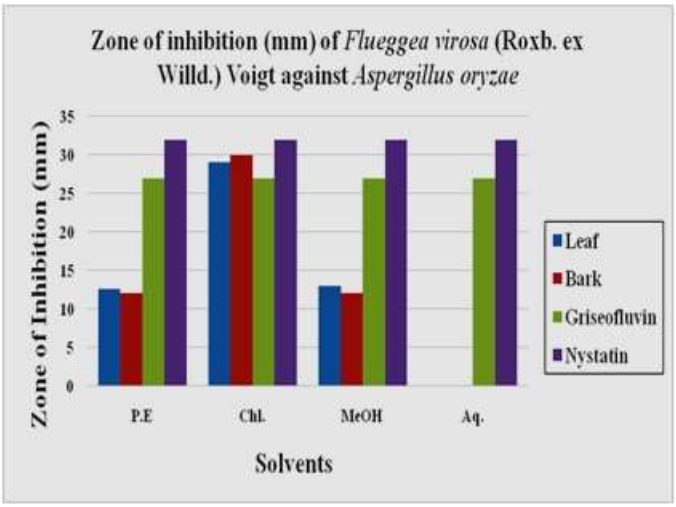

Figure 6: Zone of Inhibition of extracts of *F. virosa against A. oryzae*

The phenolic content of *F. virosa* was reported to be in the range of 161.75±1.5-3029.25±0.2 Gallic acid equivalent (GAE µg/mL) (Table 5) with maximum potential exhibited by the methanol extract of leaf while least content was reported from chloroform extract of bark.

F. virosa had displayed wide range of flavonoid content that is 688±1.2-3370.73±0.8 CE µg/mL (Table V). Maximum concentration was present in aqueous extract of leaf and minimum potential was exhibited by chloroform bark extract.

F. virosa had displayed much capacity to neutralize DPPH radicals. The obtained values varied from 26.8-94.3%. The largest capacity to neutralize DPPH radicals was exhibited by methanolic extract of bark. A moderate capacity was observed for chloroform and petroleum ether extracts. (Table 5).

F. virosa had also displayed much capacity of percentage of bound iron ranging from -60.84±0.9 to 56.75±1.0 (Table V). The maximum capacity was being displayed by methanol extract of bark. The aqueous extract of both parts and petroleum ether extract of bark had provided moderate potential.

The capacity for scavenging the ABTS radical cation was reported to be in the range 5.79±1.0-10.56±0.7 mM of TE (Table 5). The maximum capacity was displayed by the aqueous extract of bark. The petroleum ether and chloroform extract of bark had displayed minimum potential. The potential exhibiting by the respective plant was Aqueous > Methanol > Chlorofrom > Petroleum ether.

In recent years there has been a resurgence of scientific interest in the use of medicinal plant for the development of new parmacotherapeutic agents. The present study was conducted to evaluate the effective, safe and cheap medicinal agents from plants as potential alternative for controlling microbial agents.

Flueggea virosa (Roxb. ex Willd.) Voigt had exhibited maximum potential with the methanolic extracts of bark, i.e. 46.3±1.5 mm against *B. subtilis.* Gram negative strains were more resistant than gram positive strains. This was in agreement with the Rabe and Staden (1997) during investigation of water and methanolic extracts from 21 South African plant species. The leaf extract of the respective plant exhibited potential 26.3±2.0 mm against *E. coli* which was in agreement to the potential exhibited by the *Stevia rebaudiaria* methanol extract investigated by Fazal et al. (2011). The methanolic extracts inhibited the growth of both gram positive and gram negative, it was similar to the findings achieved by Grierson and Afolayan (1998). Both leaf and bark had shown marked potential against *B. subtilis, P. aeruginosa* and *S. aureus*.

The leaf extract of petroleum ether of *F. virosa* had exhibited greater activity as compared to bark (46.3±1.5 mm) which was similar to the potential exhibited by the petroleum ether extract of leaf of *C. splendens*. The zone exhibited by the leaf against *S. aureus* was similar with the petroleum ether extract of *Coleus vettiveroids* against the same strain documented by Kamal et al. (2014).

The maximum activity was exhibited by chloroform extract of bark against *A. oryzae,* i.e.

30.0±0.5mm. The extract exhibited by the chloroform extract of leaf was 15.6±1.3 mm which was in agreement with the potential exhibited by the *Plaguochasma appendiculatum* against *A. niger* Singh et al. (2006). The petroleum ether extract of both parts of the respective plant had exhibited minimum activity against both organisms. The potential exhibited by the plant against the organisms was *A. oryzae* > *A. niger.*

The results reported during the DPPH radical scavenging analysis had established that highest radical scavenging potential was present in the methanol extracts of both plants. The same findings were revealed by Bokhari et al. (2013). The antioxidant %DPPH potency of the petroleum ether extract of the leaf of *Flueggea virosa* (Roxb. ex Willd.) Voigt was in accordance to the result obtained by Ajaib et al. (2013) and Siddiqui et al. (2016) during investigation of antioxidants in medicinal plants.

CONCLUSION

From our study and with previous literature survey we can come to conclusion that the *Flueggea virosa* leaves and bark are rich of phytochemicals which has free radicals scavenging activity and significant antimicrobial activity. Hence, *F. virosa* leaf and bark could be used as an easy accessible source of natural antioxidants and antimicrobial agent. Further purification of the active compounds and *in vivo* evaluation of antioxidant and antimicrobial activity along with toxicity studies of the extracts from *F. virosa* are therefore, suggested for further studies.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

MA designed and SQW performleed the experiments and both also wrote the manuscript. FS, MTZ, MFS and TA reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Ajaib M, Fatima S, Khan KM, Perveen S and Shah S, 2016. *Nicotiana plumbaginifolia*: A Rich Antimicrobial and Antioxidant Source. J. Chem. Soc. Pak. 38(1):143-149.

Ajaib M, Khan KM, Perveen S and Shah S, 2015. Antioxidant and Antimicrobial Activities of *Helinus lanceolatus.* J. Chem. Soc. Pak. 37(1):139-143.

Ajaib M, M Ishtiaq, Shafi F, Bhatti KH and Zahid MT, 2020. Antimicrobial and antioxidant analysis of *Strobilanthes glutinous*: an unexplored medicinal plant. Bioscience Research 17(2): 1521-1534.

Ajaib M, Wahla SQ, Wahla UG, Khan KM, Perveen S and Shah S, 2017. Phytochemical Screening and Anthelmintic Activity of *Flueggea virosa.* J. Chem. Soc. Pak. 40(4): 702-706.

Ajaib M, Zikrea A, Khan KM, Perveen S, Shah S and Karim A, 2013. *Rivina humilis*: A Potential Antimicrobial and Antioxidant Source. J. Chem. Soc. Pak. 35(5): 1384-1398.

Balandrin MF, Kjocke AJ and Wurtele E, 1985. Natural plant chemicals: sources of industrial and mechanical materials. Science, 228: 1154-1160.

Benoit-Vical F, Grellier P, Abdoulaye A, Moussa I, Ousmane A and Berry A, (2006). In vitro and *in vivo* Antiplasmodial Activity of *Momordica balsamina* Alone or in a Traditional Mixture. Chemotherapy 52(6) :288-292.

Bokhari TH, Hussain M, Hina A, Zubair M, Rasool N, Riaz M, Iqbal M, Bukhari IH, Hussain AI, Shafiq M, and Shahid M, 2013. Antioxidant, antimicrobial, cytotoxic studies of methanolic extract, fractions and essential oil of curry patta (Chalcas koeinigii) from Pakistan. J. Chem. Soc. Pak. 36(2): 468-475.

Fazal H, Ahmad H, Ullah I, Inayat H, Khan L and Abbasi BH, 2011. Antibacterial Potential in *Parthenium hysterophorus*, *Stevia rebaudiana* and *Ginkgo biloba.* Pak. J. Bot. 43(2): 1307-1313.

Grierson DS and Afolayan AJ, 1998. Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape, South Arica. J. Ethnopharmacol. 66: 103-106.

Kamal BS and Padmaja V, 2014. Evaluation of

antimicrobial activity of various and volatile oil from the whole plant of *Coleus vettiveroids* K.C. Jacob: An *in vitro* study. Inter. J. Pharm. Sci. 6(1): 638-640.

Kamran SH, Ahmad M, Shahwar D and Ajaib M, 2016. Anti-diabetic and anti-oxidant status of *Loranthus pulverulentus* obtained from two different hosts. Bangl. J. Pharmacol. 11: 181-189.

Lu Y, Zhao YP, Wang ZC, Chen SY and Fu CX, 2007. Composition and Antimicrobial Activity of the Essential Oil of *Actinidia Macrosperma* from China. Natural Product Research, 21(3): 227-233.

Mazhar F, Khanum R, Ajaib M and Jahangir M, 2015. Potent AChE enzyme inhibition activity of *Zizyphus oxyphylla*: A new source of antioxidant compounds. Pak. J. Pharm. Sci. 28(6): 2053-2059.

Mbwambo ZH, Moshi MJ, Masimba PJ, Kapingu MC and Nondo RS, 2007. Antimicrobial Activity and Brine Shrimp Toxicity of Extracts of Terminalia brownie Roots and Stem. BMC Complementary and Alternative Medicine. 7 (9): 1-5.

N'guessan JD, Dinzedi MR, Guessennd N, Coulibaly A, Dosso M, Djaman AJ and Guede-Guina F, 2007. Antibacterial Activity of the Aqueous Extract of *Thonningia sanguinea* Against Extended-Spectrum-Lactamases (ESBL) Producing *Escherichia coli* and *Klebsiella pneumoniae* Strains. Tropical J. Pharm. Res. (6) (3): 779-783.

Rabe T and Staden J, 1997. Antibacterial activity of South African plant used for medicinal purposes. J. Ethnopharmacol. 56:81-87.

Senatore F, Rigano D, Formisano C, Grassia A, Basile A and Sorbo S, 2007. Phytogrowth-Inhibitory and Antibacterial Activity of *Verbascum sinuatum.* Fitoterapia 78(3): 244-247.

Siddiqui SZ, Ali, S, Aziz-ur-Rehman, Rubab K, Abbasi MA, Ajaib M and Rasool ZG, 2015. *Pyrus pashia*: A persuasive source of natural antioxidants. Pak. J. Pharm. Sci. 28(5):1763-1772.

Singh G, Maurya S, Lampasona MP and Catalan CA, 2007. A Comparison of Chemical, Antioxidant and Antimicrobial Studies of Cinnamon Leaf and Bark Volatile Oils, Oleoresins and Their Constituents. Food and Chemical Toxicology 45(9): 1650-1661.

Singh M, Govindarajan R, Nath V, Rawat AKS and Mehrotra S, 2006. Antimicrobial, wound healing and antioxidant activity of *Plagiochasma appendiculatum* Lehm. Lind. J. Ethnopharmacol. 107: 67-72.

Smith AR, 1968. *Flora of Pakistan.* Euphorbiaceae. No.172. (Nasir & Ali, eds.). Department of Botany, University of Karachi.