ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *ANDRACHNE CORDIFOLIA* (WALL. EX DECNE.) MUELL.

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Abstract

The antibacterial and antioxidant activity of *Andrachne cordifolia* was evaluated by preparing its extracts in petroleum ether, chloroform, methanol and water and using agar well diffusion and dilution method. Minimum inhibitory concentration (MIC) assay was performed. The maximum antibacterial activity was shown by leaf and bark extracts. The maximum potential was exhibited by petroleum ether extract of bark of *A. cordifolia* against *Pseudomonas aeruginosa*, i.e. 62.6 ± 1.5 mm. The least antibacterial activity was shown by aqueous extracts of both parts. The zones of inhibitions obtained against bacterial isolates were compared with zones obtained by standard antibiotic disc (positive control) to evaluate activity index (AI) of the respective plant and it was revealed that the highest AI was reported by petroleum ether extract of bark against *P. aeruginosa*. The MIC values for the leaf and bark were 0.122 ± 0.03 at 0.8 mg/mL concentration and 0.075 ± 0.02 at 0.6 mg/mL concentration respectively. The bark macerates of methanol of plant had exhibited best response to 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, i.e. 94.3 %. The leaf extracts of plant was potent for 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) and metal chelating activity. The aqueous extract of leaf and bark of *A. cordifolia* showed highest Total Flavonoid Contents (TFC) content on aqueous extracts i.e. 3723.45 ± 1.1 and 3723.45 ± 0.5 µg/mL.

Introduction

Plants as source for medicine can be traced back over five millennia to written documents of the early civilization in China, India and Near East. Neanderthals living 60,000 years back used plants which are now widely used in ethnomedicine around whole of the world (Mahesh and Satish, 2008). Benli *et al.,* (2008) reported a research conducted by WHO in 91 countries that the medicinal plants used for healing purposes are about 20,000. Although the certain poisonous compounds in most of the medicinal plants, had not been thoroughly evaluated. It is usually believed that medicines derived from plant products are safer than their synthetic counterparts (Ahmad *et al.,* 2015).

Any substance that is accountable for the inhibition of the pathogenic agent or its effect can be defined as antimicrobial (Cushnie and Lamb, 2005; Ajaib *et al.,* 2014). In past antimicrobial screening started with penicillin and streptomycin. Certainly the first large scale screening of green plants to determine their antibacterial activity was that of Osborn at Oxford University in 1943, who tested 2,300 species of plants and found 63 genera active against *Staphylococcus aureus* or *Escherichia coli*, or both (Nickell, 1959).

Throughout the world antibiotics are used to treat all microbial infections and for this instance strains are getting resistant to antibiotics and this become a serious issue in many areas of the world especially in underdeveloped countries. Due to regular use of conventional therapy it is apparent that pathogenic resistance to one or more antibiotics are emerging and spreading worldwide. Additionally unnecessary use of antibiotics has further stimulated this problem. Bacterial resistance toward antibiotics cause significant increase in the occurrence of infectious diseases. In addition other adverse effects associated with the use of antibiotics on the host includes are hypersensitivity, immuno-suppression and allergic reactions (Khan and Latif, 2014). Therefore, it is a need to develop alternative antibacterial drugs from medicinal plants and other natural extracts for the treatment of infectious diseases (Siddiqui *et al.,* 2015).

An antioxidant can be defined as a substance that can prevent or tends to prevent the oxidation reactions induced by oxygen or reactive oxygen species (ROS). Antioxidants are frequently used as food additives for the prevention of deterioration of lipid containing foods. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (PG) are commonly used synthetic antioxidants employed for extending the shelf-life of various foods. Previous literature has shown them to be the possible cause of carcinogenesis as well as other side effects. This has lead the scientists to search for safe and effective natural

antioxidants for use in medicines to fight against diseases caused by free radicals, shelf-foods to prevent their oxidation and deterioration, dietary supplements and as functional foods (Babri *et al.*, 2015).

Andrachne cordifolia is a potent medicinal plant, commonly found in low hills of Northern hilly areas of Pakistan and Himalayan foot hills including Azad Jammu & Kashmir. It is a small shrub, about a meter tall with ovate to elliptic leaves (Fig. 1). It is distributed in Kashmir, Pakistan, India and Nepal, on plains as well as on rocks, cliffs, waste lands and on bank of streams. The powder of the leaves of *A. cordifolia* is used for healing wounds (Ajaib, 2012).

Materials and Methods

Plant material: *Andrachne cordifolia* was collected from Khuiratta, District Kotli, Azad Jammu and Kashmir (AJK) for current studies. It belongs to family Euphorbiaceae (Spurge family). The plant was identified with the authenticated voucher number GC.BOT.HERB.2951 from GC University, Lahore and plant sample was deposited to the herbarium of GC University.

Maceration of the plant material: Leaf and bark of the plant dried under shade and macerated in different solvents according to their polarity index (Petroleum ether, Chloroform, Methanol, Aqueous). The resultant extracts were dried on rotary evaporator to get final concentrated form of extracts.

Antibacterial activity

Zone of inhibition assay: Investigation of antimicrobial activity was carried out by using Agar well diffusion method (Bauer *et al.* 1966). For the investigation of antimicrobial activity of bark and leaf of *A. cordifolia*, 2 gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and 2 gram positive *(Bacillus subtilis* and *Staphylococcus aureus*) bacteria were used, following the method described by Cruick-shank *et al*. (1975). Autoclaved nutrient-agar media was used to check antibacterial activity following Johansen (1940).

Measurement of Activity index: The activity index (AI) was estimated by comparing the zone of inhibition of the extract with standard antimicrobial agents employing the following formula:

$$\text{Activity index} = \frac{\text{Zone of inhibition by extract}}{\text{Zone of inhibition by standard antimicrobial agent}}$$

Evaluation of Minimum Inhibitory Concentration (MIC): Antimicrobial potential of plant with lower constancy have evaluated by using Broth-dilution method following by Murray *et al*., (1999).

Antioxidant assays: 1000µL of (500, 250, 125, 60µg/mL) extract of respective 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 DPPH (%) radical remaining was calculated using following formula:

$$\text{DPPH (\%)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Total Flavonoid Contents (TFC): Total Flavonoid Contents of *A. cordifolia* was evaluated using the methodology employed by Dewanto *et al*., (2002).

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

Results and Discussion

To confirm that the antibacterial potential was exhibited purely by *A. cordifolia* and solvents were not responsible, respective solvents, i.e. petroleum ether, chloroform; methanol and distilled water were used as negative control and they possess no results against pathogenic bacteria. In the present study gram positive bacteria *S. aureus* and *Bacillus subtilis*, and gram negative bacteria *E. coli* and *P. aeruginosa* were used to evaluate the antibacterial potential of bark and leaf extracts of the selected plant parts (Fig. 6a-f).

The petroleum ether extracts of *Andrachne cordifolia* exhibited inhibitory potential against *S. aureus*. The chloroform extracts of bark had exhibited more significant results as compared to the leaf. The methanolic extracts of both parts had displayed somewhat similar potential, *i.e.* 24.3 ± 0.5 mm and 24 ± 1.5 mm against *S. aureus* (Table 1). The potential exhibited by both parts was almost greater as compared to standard discs i.e. Cefotaxime (35 ± 2.5) (Fig. 2). The overall minimum potency was exhibited by the aqueous extract of both leaf and bark (Table 1). The bark extract had exhibited tremendous potential against *E. coli* as compared to standard

discs (Fig. 3). The chloroform extract of bark showed maximum potential, i.e. 31.3 ± 1.5 mm. The petroleum ether and chloroform extract of leaf had showed similar activity i.e. 20.9 ± 1.5mm and 21 ± 1.0mm respectively. The methanol extract of leaf had showed little activity i.e. 17 ± 0.3 while the methanol extract of bark had displayed much activity compared to leaf (Table 1).

Table 1. Zone of Inhibition produced by Bark and leaves extracts of *Andrachne cordifolia* against bacterial strains.

Plant Parts	Extracts	Zone of Inhibition (mm)			
		E.coli	***S.aureus***	***P.aeruginosa***	***B.subtils***
Bark	**Petroleum ether**	12.6 ± 0.5	17.3 ± 0.5	62.6 ± 1.5	21.0 ± 1.1
	Chloroform	31.3 ± 1.5	18 ± 0.3	20.6 ± 0.5	25 ± 0.5
	Methanol	24 ± 1.0	24 ± 1.5	24.3 ± 0.5	28.6 ± 1.5
	Aqueous	12 ± 0.7	11.1 ± 0.5	12.3 ± 0.5	16.6 ± 1.5
Leaf	**Petroleum ether**	20.9 ± 1.5	12.3 ± 0.5	39.6 ± 2.0	41.0 ± 1.0
	Chloroform	21 ± 1.0	13 ± 1.5	31.3 ± 1.5	24 ± 1.5
	Methanol	17.3 ± 0.5	24.3 ± 0.5	24 ± 1.0	25.3 ± 1.1
	Aqueous	17 ± 1.0	14.9 ± 3.0	12.3 ± 0.5	12.0 ± 1.0

Table 2. Activity Index (AI) of *A. cordifolia* against bacterial test organisms.

Plant Part	Solvents	Zone of inhibition (mm)			
		S. aureus	***E. coli***	***P. aeruginosa***	***B. subtilis***
Leaf	Petroleum ether	0.75	1.40	*3.20	1.82
	Chloroform	0.81	1.40	*2.58	1.09
	Methanol	1.50	1.13	1.92	1.13
	Aqueous	0.87	1.06	1.00	0.54
Bark	Petroleum ether	1.06	0.86	**5.20	1.00
	Chloroform	1.13	2.00	1.61	1.13
	Methanol	1.51	1.62	2.00	1.31
	Aqueous	0.68	0.81	1.00	0.73

Note: The Zones of Inhibition produced by Azithromycin against bacterial isolates were treated as standard one * while two ** indicates the significance of the plant macerates as antibacterial agent.

Table 3. MIC values (mg/mL) exhibited by leaf and bark of *Andrachne cordifolia* 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	0.7	0.202 ± 0.01	0.8	0.122 ± 0.03	0.8	0.114 ± 0.02	0.9	0.150 ± 0.02
Bark	0.2	0.011 ± 0.04	0.6	0.075 ± 0.02	1	0.084 ± 0.04	1	0.021 ± 0.01

Table 4. Total Phenolic content (TPC), Total Flavonoid content (TFC), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging potential (%), bound iron (%) and Trolox-Equivalent Antioxidant Capacity (TEAC) value of *Andrachne cordifolia.*

Plant Parts	Extracts	TPC µg/mL	TFC µg/mL	DPPH (%)	Bound iron (%)	TEAC value
Bark	Petroleum ether	634.25 ± 1.6	3009.82 ± 0.4	80.55	-60.32 ± 1.0	1.85 ± 0.7
	Chloroform	534.25 ± 0.5	681.64 ± 0.7	84.43	39.56 ± 1.3	3.26 ± 0.3
	Methanol	2721.75 ± 0.6	1390.72 ± 1.6	94.3	14.76 ± 1.7	9.19 ± 1.4
	Aqueous	2301.75 ± 0.9	3723.45 ± 0.5	65.26	4.79 ± 0.7	4.61 ± 0.1
Leaf	Petroleum ether	676.75 ± 0.5	2736.18 ± 0.7	82.23	40.31 ± 0.5	3.54 ± 0.2
	Chloroform	271.75 ± 0.7	763.45 ± 0.3	87.06	16.78 ± 0.9	3.77 ± 0.5
	Methanol	1746.74 ± 1.5	1365.27 ± 0.5	93.4	8.53 ± 0.8	10.4 ± 0.3
	Aqueous	2346.75 ± 0.1	3723.45 ± 1.1	56.2	-60.67 ± 1.4	8.22 ± 1.9

Fig. 1. *Andrachne cordifolia.*

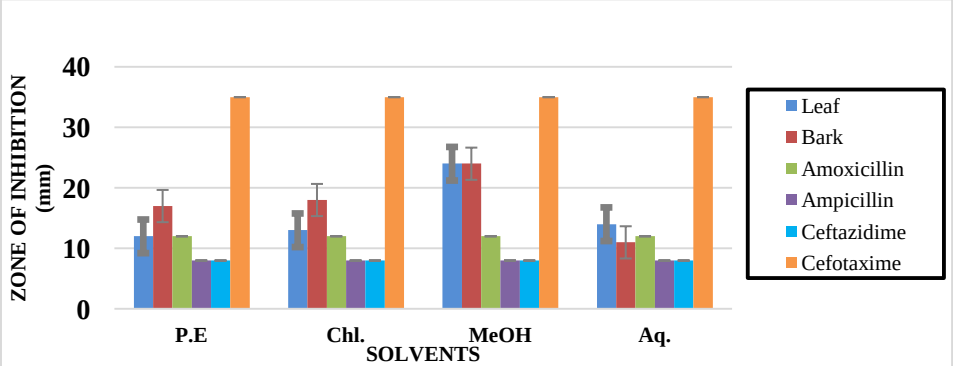

Fig. 2. Zone of Inhibition produced by extracts of *A. cordifolia* against *S. aureus.*

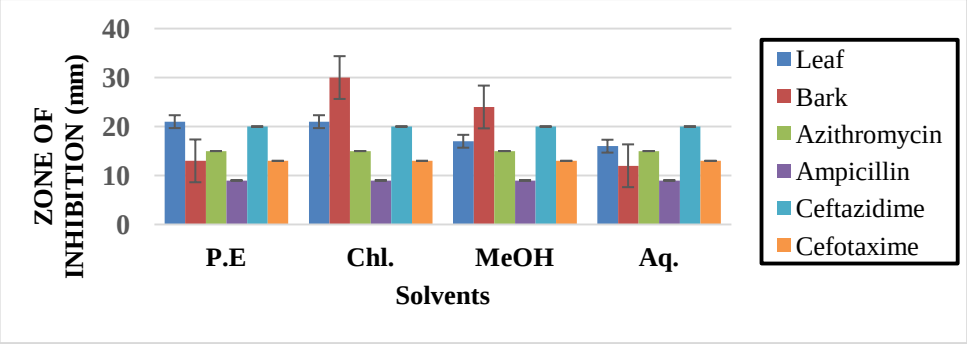

Fig. 3. Zone of Inhibition of extracts of *A. cordifolia* against *E. coli.*

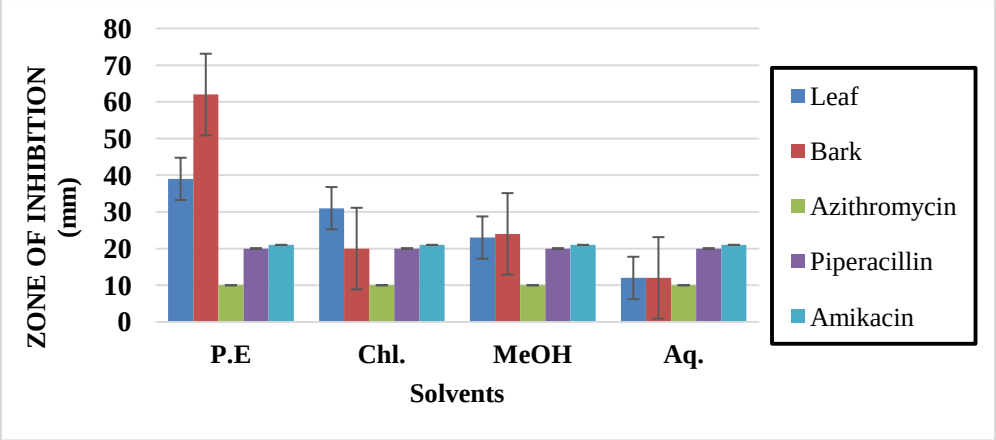

Fig. 4. Zone of Inhibition of extracts of *A. cordifolia* against *P. aeruginosa.*

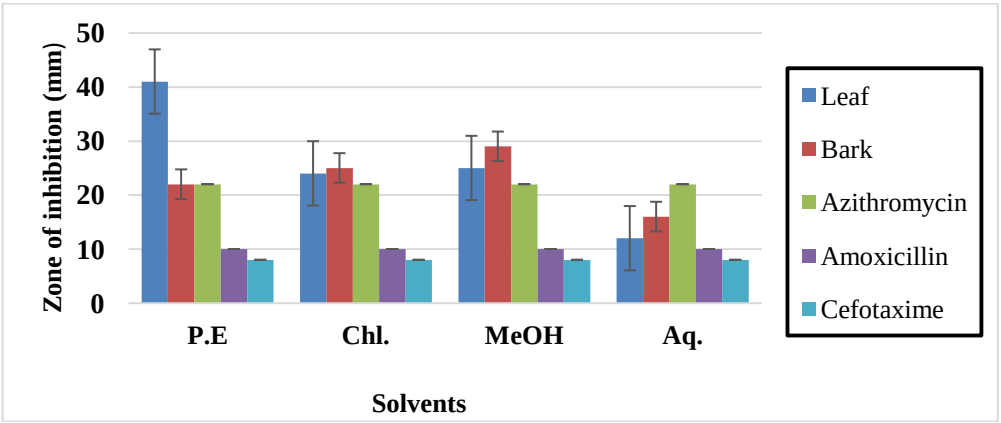

Fig. 5. Zone of Inhibition of extracts of *A. cordifolia* against *B. subtilis.*

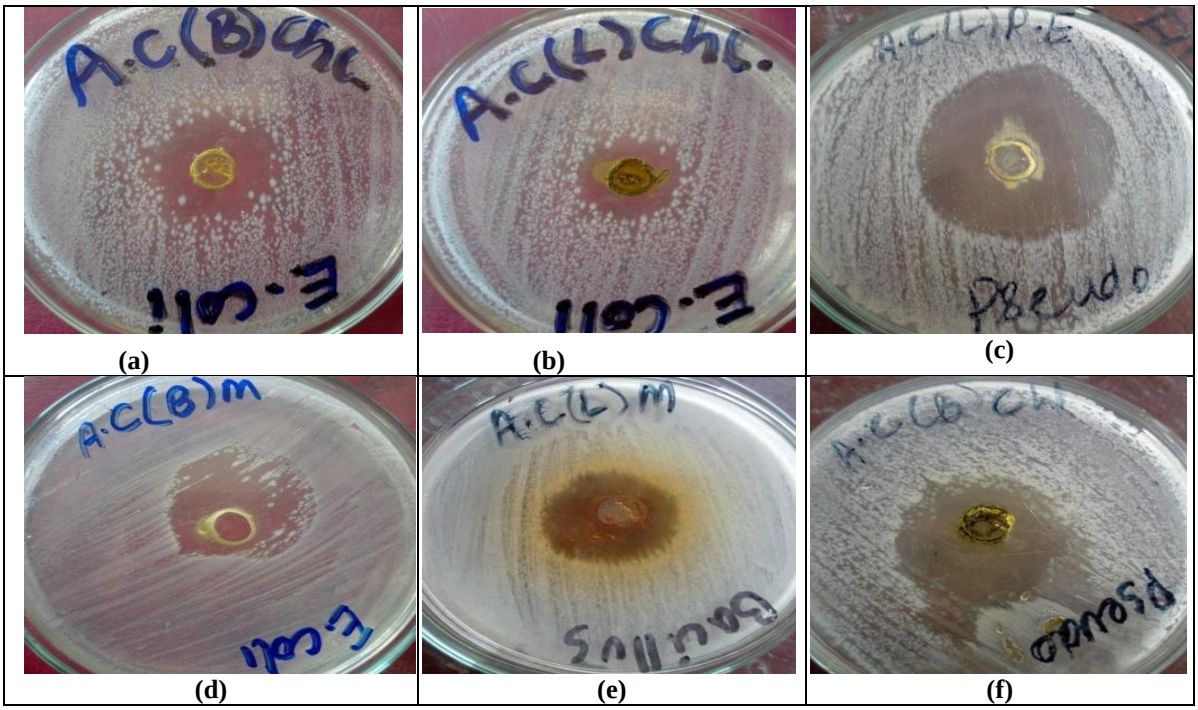

Fig. 6. Zone of inhibition produced by: Leaf, bark extracts against (a) *E. coli* (Chloroform extract), (b) *E. coli* (Chloroform extract), (c) *E. coli* (Methanol extract), (d) *P. aeruginosa* (Petroleum ether extract) (e) *B. subtilis* (Methanol extract) (f). *P. aeruginosa* (Chloroform extract).

The bark extract had showed the maximum potential against *P. aeruginosa* as well with the petroleum ether extract being the most effective. The petroleum ether extracts of both parts had shown good to satisfactory results. The chloroform extract of leaf had exhibited significant potential as compared to bark. The methanol extracts of both parts had shown significant potential. The aqueous extracts of both leaf and bark had exhibited minimal and almost similar potential i.e. 12.3 ± 0.5 mm and 12.3 ± 0.5 mm (Table 1). The potential exhibited by both parts was greater as compared to antibiotic standard discs (Fig. 4).

The petroleum ether extract of leaf was exception, where the maximum activity was reported by the leaf extract against *B. subtilis* i.e. 41.0 ± 1.0 (Fig. 5). The chloroform extract of leaf had exhibited almost similar potential. The methanol extract of both leaf and bark had shown good potential *i.e* 25.3 ± 1.1 and 28.6 ± 1.5 respectively. The aqueous extracts had shown minimal potential. The potential exhibited by both parts was greater as compared to standard discs except aqueous extracts of leaf and bark (Table 1).

The zones of inhibitions obtained by *A. cordifolia* against bacterial isolates were compared with zones obtained by standard antibiotic disc (positive control) to evaluated activity index (AI) of the respective plant (Table 2). The highest AI was reported by petroleum ether extract of bark against *P. aeruginosa.* The significant values were also reported by methanol and chloroform extract of bark against *E.coli* and *P. aeruginosa*, in addition to chloroform and petroleum ether extract of leaf against *P. aeruginosa.* None of the extract had displayed insignificant potential.

The MIC values for the leaf and bark were 0.202 ± 0.01 at 0.7 mg/mL concentration and 0.011 ± 0.04 at 0.2 mg/mL concentration respectively. The bark extract showed the significant potential against the *E.coli.* The bark extracts were more effective than leaf extracts. The MIC values for the leaf and bark were 0.122 ± 0.03 at 0.8 mg/mL concentration and 0.075 ± 0.02 at 0.6 mg/mL concentration respectively (Table 3).

The techniques employed for the determination of antioxidant potential of *A. cordifolia* were Total Phenolic Contents (TPC), Total Flavonoid Contents (TFC), DPPH radical scavenging action, Metal chelating activity and ABTS Assay. *A. cordifolia* had displayed wide range of phenol concentration that is 271.75 ± 0.7-2346.75 ± 0.1 GAE µg/mL (Table 4). The highest concentration of phenol was measured in methanolic and aqueous extracts. The chloroform and petroleum ether exhibited considerably smaller concentration of phenols (Table 4). The total flavonoid content of the *A. cordifolia* had reported to be in the range of 681.64 ± 0.7-3723.45 ± 0.5 catchecin equivalent µg/mL (CE µg/mL) (Table 4). The maximum potential showed by the aqueous macerate of both leaf and bark of respective plant. The least potential was being exhibited by the chloroform extract of bark. The methanol and petroleum ether also exhibited good flavonoid content. The % DPPH radical scavenging potential of selected plant was reported to be in the range of 56.2% - 94.3% (Table 4). 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. The minimum capacity to inhibit DPPH radicals was determined for aqueous extract of leaf.

The % age bound iron of *A. cordifolia* was reported to be in the range of -60.67 ± 1.4% to 40.31 ± 0.5 (Table 4). The largest capacity was exhibited by the petroleum ether extract of leaf. A moderate capacity was observed for chloroform extract and methanol extracts of both parts. The minimum capacity was shown by petroleum ether, chloroform and methanol extract of leaf. *A. cordifolia* had showed wide range of antioxidant potential as they all have scavenged the ABTS radical cation. The antioxidant potential varies from 1.85 ± 0.7-10.4 ± 0.3 mM of TE (Table 4). The highest potential was being displayed by the methanolic extract of leaf. The petroleum ether of bark had displayed minimum potential. All other macerates had good potential for scavenging the ABTS radical cation.

Discussion

Comparatively satisfactory activity by the chloroform extracts of *Andrachne cordifolia* leaf and bark against the bacterial strains was observed, same extract had exhibited similar and greater potential against gram negative strain. While leaf extract of the respective plant showed the minimum potential against the *S. aureus* which was in accordance to the potential exhibited by the *L. pyrotechnica* against the similar strain documented by Munazir *et al.,* (2012) and Mazhar *et al.,* (2015). Leaves of *Kalanchoe crenata* were also showed similar potential (Aibinu *et al.,* 2007). 25 ± 0.5 mm potential exhibited by the chloroform extract of bark of the respective plant against the *B. subtilis* which was in accordance to the potential exhibited by the extracts of *Coleus vettiveroids* (Kamal *et al.,* 2014). The overall activity of the extracts of the plant was ranging from 13 ± 1.5-31.3 ± 1.5 mm.

Methanolic extracts of plant had exhibited satisfactory results among the solvents against both gram positive and gram negative bacteria. The maximum potential was exhibited by the *A. cordifolia* bark extract, *i.e.* 28.6 ± 1.5 mm against *B. subtilis.* The leaf and bark showed similar potential against *S. aureus*, the same was observed in *A. nilotica* and *Ziziphus mauritiana* against *S. aureus* (Mahesh and Satish, 2008). Minimum potential had shown by leaf extract i.e. 17.3 ± 0.5 mm against *E.coli* which was similar to the potential by *B.*

monosperma against similar strain. The plant had shown activity in the range of 17.3 ± 0.5-28.6 ± 1.5 mm. The potential of the plant against bacterial strain was *B. subtilis* > *P. aeruginosa* = *S. aureus* > *E. coli*.

Results of MIC and Zone of Inhibition were relevant to each other except the results of *S. aureus*. The difference might be due to the temperature and the nature of the medium on the susceptibility of the bacteria as the MIC was carried in the liquid medium while the zone of inhibition was measured into solid medium. This point was brought to observation by Rodkhum *et al.,* (2011) while evaluating the impact of water, temperature on the susceptibility of *Streptococcus agalactiae* that was responsible for causing infection within the fishes indicated in river. The detailed analysis of total phenolic and total flavonoid content had indicated that both these components were present in different ratios and their results were not associated. The same findings were reported by Iqbal *et al.,* (2012) arguing that the differences in the amount of TPC and TFC may be due to the varied efficacy of the extracting solvents to dissolve endogenous compounds.

Conclusion

Plants serve as good source of medication for various diseases. Plants produce secondary metabolites due to which these are used as medicine. The current study was an effort to investigate the ethnopharmacological potential of *Andrachne cordifolia*. The results indicated that the leaf and bark extracts had good to satisfactory antibacterial and antifungal potential. Aqueous extract of both parts exhibited minimum potential in all the macerates against bacterial strains. The antioxidant evaluation had put forward that the bark extracts of *A. cordifolia* had provided best response to TPC, TFC and DPPH activity. The leaf extracts of this plant were potent for ABTS and metal chelating activity.

References

Ahmad, N., Z.K. Shinwari, J. Hussain and R. Perveen. (2015). Phytochemicals, antibacterial and antioxidant investigation of *Alhagi maurorum* Medik. *Pak. J. Bot.,* 47(1):121-124.

Aibinu, I.E., O.R. Akinsulire, T. Adenipekun, T. Adelowotan and T. Odugbemi. (2007). *In vitro* Antimicrobial Activity of Crude Extracts from Plants *Bryophyllum pinnatum* and *Kalanchoe crenata*. *Afr. J. Trad. Comp. Med*., 4(3): 338 344.

Ajaib, M., (2012). *Exploration of Floral Diversity of District Kotli (Azad Jammu & Kashmir) and evaluation of Ethnopharmacological Effects of some Medicinal Plants of the Area*. Ph. D. thesis submitted in Department of Botany, GC University, Lahore.

Ajaib, M., F. Boota, K.M. Khan, S. Parveen and S. Shah. (2014). *Clerodendrum splendens*: A Potential source of Antimicrobials. *J. Chem. Soc. Pak*., 36(4): 763-770.

Babri, R.A., I. Khokhar and S. Mahmud. (2015). Antioxidant potential and iron chelating activities of some bioactive compounds. *J. Chem. Soc. Pak.,* 37(3).

Bauer A, Kirby W, Sherris J, and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45:493-496.

Benli, M., Bingol, U.M. , Geven, F., Guney, K. and Yigit, N. (2008). An investigation on the antimicrobial activity of some endemic plant species from Turkey. *Afr. J. Biotech*, 7(1):001-005.

Cruick-Shank, R.,J.P. Dugid, B.P. Marinionon and R.H.A. Swain. (1975). Screening of Some Greek Aromatic Plants for antioxidant Activity. *Phytother. Res.,* 17(2): 194-195.

Cushnie, T.P.T. and Lamb, A.J. (2005). Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agr.,* 26: 343-356.

Dewanto, V., X. Wu, K.K. Adom and R.H Liu. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem*., 50: 3010–3014.

Iqbal, M.J., S. Hanif, Z. Mahmood, F. Anwar and A. Jamil. (2012). Antioxidant and antimicrobial activities of Chowlai (*Amaranthus viridis* L.) leaf and seed extracts. *J. Med. Plants Res.,* 6(27): 4450-4455.

Johansen, D.A. (1940). *Plant Microtechnique*. MC-Graw-Hill Book Company, Inc. New York.

Kamal, B.S. and V. Padmaja. (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. Pharmac. Sci.,* 6(1): 638-640.

Khan, A.Y. and Z. Latif. (2014). Screening of medicinal natural extracts for their antibacterial activity against *Salmonella* species. *Pak. J. Bot.,* 46(6): 2269-2275.

Mahesh, B. and S. Satish. (2008). Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. *World J. Agri. Sci.,* 4:839-843.

Makkar, H.P.S., M. Blummel, N.K. Borowy and K. Becker. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J. Sci. Food Agri*., 61: 161–165.

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

Munazir, M., R. Qureshi, M. Arshad and M. Gulfraz. (2012). Antimicrobial Activity of Root and Fruit Extracts of *Leptadenia pyrotechnica* (Asclepidaceae) from *Pakistan. J.Bot.*, 44(4):1209-1213.

Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover and R. H. Yolke. (1999). *Manual of Clinical Microbiology*. 7th ed. American Society for Microbiology (ASM), Washington DC: 1527-1539.

Nickell, L.G. (1959). Antimicrobial activity of Vascular Plants. *Economic Bot.*, 13(4):281-318.

Rodkhum, C., P. Kayansamruaj and Pirart. N. (2011). Effect of the water Temperature on Susceptibility to Streptococcus agalactiae Serotype la infection in Nile Tilapia (*Oreochromis niloticus). Thai. J. Vet. Med.*, 41(3): 309-314.

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