

ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF LEAVES, BARK AND INFLORESCENCE OF *IPOMOEA ERIOCARPA*

MUHAMMAD AJAIB*¹, FAIZA SHAFI¹, SHAKEELA IQBAL¹, KHIZAR HAYAT BHATTI² AND MUHAMMAD FAHEEM SIDDIQUI³

¹Department of Botany, Mirpur University of Science and Technology (MUST), Mirpur-10250 (AJK), Pakistan ²Department of Botany, University of Karachi Pakistan ³Botany Department, Hafiz Hayat Campus, University of Gujrat, Gujrat-50700, Pakistan *Corresponding author:majaibchaudhry@yahoo.com

خلاصه

Abstract

In present research antimicrobial and antioxidant potential of leaves, bark and inflorescence of *Ipomoea eriocarpa* R. Br. was assessed and maceration method was used for the formation of extracts, by using ethanol as solvent. Antimicrobial potential of *I. eriocarpa* was determined against different bacterial and fungal strains. The antimicrobial potential of *I. eriocarpa* ranges from $9.6 \pm 1.2 \text{ mm}$ to $6 \pm 0.8 \text{ mm}$ with maximum activity showed by ethanolic extract of bark against *E. coli* and minimum efficacy by ethanolic extract of inflorescence against *B. subtilis* as compared to the zone of inhibition of standard antibacterial discs Methicillin, i.e. 9.4 ± 1.22 and Amoxicillin, i.e. $9.3 \pm 1.30 \text{ mm}$. The *A. oryzae* strain showed more resistance towards the plant extracts in comparison to *A. niger*. The leaves of *I. eriocarpa* had exhibited activity against *A. oryzae*, i.e. $14.8 \pm 0.3 \text{ mm}$ and bark demonstrated activity against *A. niger*, i.e. $12.6 \pm 1.2 \text{ mm}$. The inflorescence had showed maximum activity against *A. oryzae*, i.e. $10.3\pm0.8 \text{ mm}$ in comparison to antifungal disc Griseofulvin.

Antioxidant potential of *I. eriocarpa* was evaluated by three methods, viz., Total phenolic content (TPC), Total flavonoids content (TFC) and DPPH (1, 1- Diphenyl-2-Picrylhydrazyl) radical scavenging activity. The TPC was evaluated and expressed as Quercetin μg equiv. /mg of extract. The value of total flavonoid contents varied from 39.3 ± 0.85 to $1.14 \pm 0.13 \mu g/mg$. The highest amount of flavonoids was found in the ethanolic extracts of leaves ($39.3 \pm 0.85 \mu g/mg$). The value of % DPPH radical scavenging varied from 23.3 ± 0.03 to $76.5 \pm 0.09\%$ as compared to standard BHT, i.e. $87.7 \pm 0.12\%$. These results revealed reasonable antimicrobial and antioxidant activity, thus supporting its medicinal worth.

Keywords: Ipomoea eriocarpa, antimicrobial, antioxidants, crude extract

Introduction

To prevent the toxic effects of antibiotics and to enhance the multiple drug resistance the use of phytomedicines is increasing in this era (Shahzadi *et al.*, 2018). Throughout the world about 80 % of individuals obtain many effective and powerful drugs from herbal resources. To overcome the problem of multiple drug resistance the alternative antimicrobials drugs are discovered (Ajaib *et al.*, 2017; Maqbool *et al.*, 2021).

Antioxidants are natural or synthetic molecules that play a significant part in body defensing against free radicals that damage cells through oxidation reactions, they are being added as stabilizers in several food systems and cease the rate of lipid oxidation reactions and quench the free radicals (Ajaib *et al.*, 2016; Shahzadi *et al.*, 2018). During oxidation reactions, they interrupt the deterioration of substances caused by peroxides or oxygen. Antioxidants refer to organic enzymatic materials, for example β -carotene also called provitamin A or vitamin C & E, polyphenols, selenium, lycopene, lutein, and other carotenoids that are able to neutralize the destructive effects of oxidation in tissues (Aruoma, 1998; Shahzadi *et al.*, 2018). Few species of reactive oxygen are biologically important they help in production of energy, phagocytosis, intra cellular signaling, proper cell growth, etc. (Süleyman *et al.*, 2007). Some free radicals that are produced during metabolic reactions, ionizing radiations, UV light, sun light and chemical reactions have pathological effects (Yildirim *et al.*, 2001).

The largest genus of the family Convolvulaceae is *Ipomoea* and consists upon more than 500 species (Wilkin, 1999; Noshad *et al.*, 2021). It includes mostly herbs, climbing veins, trailer, twiner, shrubs and small trees. The presence of whitish sap is special diagnostic feature of *Ipomoea* (Shukla, 1979). Plants belonging to this genus are used in medicinal, ritual, ornamental, agricultural purposes and as environmental indicators (Noshad *et al.*, 2020). Leaves of *I. aquatica* are used as a source of food on large scale in various countries of the world. Roots of some species of *Ipomoea* are used to treat constipation (Gill, 1988). *Ipomoea eriocarpa* R.Br. is annual and pserennial plant, also known as annual morning glories (Fig. 1). It is distributed all over the South American, African and Asian tropical areas and Northern Australia. *I. eriocarpa* is a prostrate or twinning herb, have butterfly shaped cotyledons. First true leaves are large, heart shaped, with deep lobes at the base, alternate, ovate-cordate to linear-oblong, having almost 1-4 cm long petiole and about 1-2 m long stems. Inflorescence axillary, flowers are small, funnel-shaped, regular, penta-merous, bisexual; pedicel about 3-5 mm long. Fruit are pods and seeds are released through slits (Austin and Ghazanfar, 1979). The plants are used in traditional medicines for treatment of illness, epilepsy, sores, rheumatism, fevers, Hansen's disease, seizers, headache, leprosy, cancer, eczema and ulcer (Ajaib *et al.*, 2021; Ishtiaq *et al.*, 2021; Kumar, 2013).



Fig.1. I. eriocarpa R. Br. in Natural Habitat

Materials and Methods

Collection of plant material

Fresh leaves, bark and inflorescence of *I. eriocarpa* belonging to the family to Convolvulaceae was collected from District Kotli Azad Kashmir in the month of August, 2017. The plant material was authenticated from MUST, Botany herbarium with a voucher specimen no. MUST.BOT. 5372

The leaves, bark and inflorescence of *I. eriocarpa* were separated from main plant body and dried at room temperature under optimum environmental conditions (shady condition) for 15- 20 days. The fully dried plant material was ground to powder by using mortar and pestle. Maceration procedure was selected due to the nature of biological activities to be performed. Maceration is a Latin word it means "To soak". The plant powder was soaked in ethanol for 7 days due to the extraction of potentially active bio-constituents present in it. Then the macerated plant material containing solvent was filtered and dried.

50 gm of bark powder, 500g powder of leaves and inflorescence of *I. eriocarpa* was weighed and soaked in ethanol. The plant material was then filtered by What man filter paper. Finally, the extracts were concentered at rotary evaporator. This extract was further used for evaluation of antimicrobial and antioxidant activities.

Evaluation of antimicrobial activity of I. eriocarpa

The microbes used for evaluation of antimicrobial activity of plant are both gram positive and gram negative bacteria were fresh cultured on the nutrient agar medium. The medium was prepared with the APHA standards and the ingredients were used according to Cruick-Shank *et al.*(1975) and Ajaib *et al.* (2014). The fungal strains were cultured on PDA medium using preparation of medium was given by Johansen (1940) and Maqbool *et al.*, (2021) in accordance to the APHA standards.

Determination of antioxidant activity of plant

The total phenol content was determined by the Folin Ciocalteu procedure by Makkar *et al.*,(1993) and Shahzadi *et al.*, (2018).The total flavonoid content of *I. eriocarpa* was determined by the Aluminium chloride calorimetric assay by using the Dewano *et al.*,(2002). 1, 1- Diphenyl-2-Picrylhydrazyl radical (DPPH) scavenging activity was carried out with the maximum value of its lambda, i.e. 515 nm. As it is mixed with sample its purple colour goes lighter and turns into yellow due to antioxidant property of sample. The sample was prepared as in the Total phenolic content.

Accurately weigh 0.024 g of DPPH was added in flask and then mixed with small amount of ethanol. The final volume of solution is 1000 ml. The solution can be stored at 4 °C. BHT was used as standard solution. Same as of DPPH the different concentrations of BHT were prepared by serial dilution method. 0.1 g of BHT was weighed and mixed with ethanol to raise final volume of solution up to 100 mL. The % radical scavenging potential of plant extracts was determined by using following method. By using pipette 0.1 ml of plant part added in test tube then 3.9 mL of DPPH. The mixture in test tube was fully shaken and incubated for half an hour in the dark at 25 0c. The absorbance was measured at 517 nm through spectrophotometer. As a control / blank only 3.9ml of DPPH was used. The % DPPH value of samples was found by using following formula:

% **DPPH scavenging activity =** Abs. of control - <u>Abs. of sample x 100</u>

Absorbance of control

Results and Discussion

The current investigation was carried out to evaluate the antimicrobial efficacy and antioxidant significance of leaves, bark and inflorescence of *I pomoeaeriocarpa*. Now a day, there has been a rebirth of scientific investigations in the use of plants in our daily life matters. This study was also carried out to assess the active, safe, low-cost and best quality phytomedicine. The plant was collected; different parts of plant were separated, dried and ground to powder. The maceration method was adopted because of its low cost and to yield best quality extracts in comparison to other methodologies. Ethanol was used as solvent in maceration method.

The highest yield obtained from the ethanolic extract of the bark of *I. eriocarpa i.e.* (6.2 %)which was nearly similar to the yield extract recorded by Daud *et al.* (2011) while evaluating *Donaxgrandis* antioxidant potential and with Parekh and Chanda (2008) during the research on some Indian plants to determine their antifungal properties. Whereas, the minimum yield was obtained from the ethanolic extract of leaves of *I. eriocarpa* i.e. 4.3 %. Which was in accordance to the yield extract obtained from the leaves extract *Menthalongifolia* (L) Huds. reported by Khond *et al.* (2009) during screening of *in-vitro* antimicrobial activity of some selected medicinal plants extracts. The ethanolic extract of the inflorescence of *I. eriocarpa* shown yield about 6.16%. The difference in percentage yield might be due to different parts of plants as well as chemical nature of plant parts (Priya *et al.*, 2012).

Physical appearance of plant extracts

The preliminary study of plant extracts was carried out by noticing their physical features such as colour, texture and physical appearance are mentioned in table below (Table 1).

Plant Part	Color	Appearance	Texture
Leaves	Blackish Green	Smooth	Sticky
Bark	Dark brown	Smooth	Sticky
Inflorescence	Dark Green	Smooth	Sticky

Antimicrobial Screening

Antimicrobial screening of the *I. eriocarpa* extracts that were prepared through maceration method was used to find out the zone of inhibition.

Antibacterial activity

Antibacterial activity was evaluated using four bacterial strains i.e. E. coli, S. aureus, B. subtili sand P. aeruginosa by using agar well diffusion technique. The antibacterial standard discs Azithromycin, Ampicillin, and Amikacin were used as positive control aids. Maximum activity was revealed by Azithromycin against E. *coli* that is 14.2 \pm 1.0 mm and minimum activity was showed by Ampicillin against *E. coli* i.e. 7.6 \pm 2.12 mm.Ethanol wasused as negative control, all strains of bacteria used as test organisms showed negligible response against ethanol which was used as solvent for maceration of plant material. The antimicrobial potential of *I. eriocarpa* ranges from 9.6 ± 1.2 mm to 6 ± 0.8 mm with maximum activity showed by ethanolic extract of bark against E. coli and minimum efficacy by ethanolic extract of inflorescence against B. subtilis. The antimicrobial effect of *I. eriocarpa* was determine by calculating zone of inhibition against bacterial and fungal strains and results were compared with Iqbal et al. (2012). The results of antifungal findings were relevant to the findings of Ajaib et al. (2013 and Magbool et al. (2021). The leaves of I. eriocarpa had showed activity within the range of 0 ± 0 to 9.2 ± 0.8 mm. The value of maximum Zone of Inhibition produced by ethanol extract of leaves i.e. 9.2 ± 0.8 mm against S. *aureus*. Bark had displayed the potential within the range of 0 ± 0 to $9.6\pm$ 1.2mm. Whereas, maximum value of Zone of Inhibition of ethanol extract of bark i.e. 9.6 + 1.2 mm against E. *coli*. The Inflorescence of *I. eriocarpa* had showed activity within the range of 0 ± 0 to 8.7 ± 1.4 mm the maximum value of zone of inhibition of Inflorescence is 8.7 ± 1.4 against E. coli (Table 2) also observed by Ajaib et al. (2015) while investigation of the phyto-chemicals of P. undulata. The standard discs (Azithromycin, Amoxicillin & Amikacin) were used to compare the results of zone of inhibition.

Table 2. The Zone of Inhibition produced by the leaves, bark and inflorescence of <i>I. eriocarpa</i> against the		
bacterial strains was documented in mm		

Plant Parts/standard disc		Zone of Inhibition (mm)		
	S. aureus	E. coli	P. aeruginosa	B. subtilis
Leaves	8.6±1.4	8.9 ± 0.65	9.2 ± 0.8	6.3 ± 1.1
Bark	9.2 ± 1.3	9.6 ± 1.2	8.9 ± 1.1	7.4 ± 0.9
Inflorescence	8.7 ± 0.8	8.7 ± 1.4	8.2 ± 0.6	6 ± 0.8
Azithromycin	16 ±2.5	15±0.8	-	8±0.3
Amoxicillin	12±1.2	25±0.3	-	10±0.4
Amikacin	18±0.6	-	21±0.5	-

All results were run in triplicates and written as Mean \pm Standard error.

Antifungal activity

The fungal specimens showed negligible response against ethanol that was used as negative control. The maximum potential against the respective fungal strain was exhibited by the ethanolic extract of leaves within the range of 0 ± 0 to 14.8 ± 0.3 mm and bark had demonstrated the efficacy within the range of 0 ± 0 to 12.6 ± 1.2 mm. The inflorescence had showed activity within the range of 0 ± 0 to 10.3 ± 0.8 mm (Table 3).

of gainshis		
Plant Part/standard disc	Zone of Inhibition	
	A. niger	A. oryzae
Leaves	10 ± 0.6	14.8 ± 0.3
Bark	12.6 ± 1.2	12 ± 0.5
Inflorescence	9.9 ± 0.3	10.3 ± 0.8
Nystatin	24±0.9	32±0.1
Griseofluvin	22±0.4	27±0.6

 Table 3. Zone of Inhibition exhibited by leaves, bark and inflorescence of *I. eriocarpa*against fungal test organisms

All results were run in triplicates and stated as Mean \pm Standard error.

Antioxidant activity

Now a day, the interest for the search of natural source of antioxidants is increasing rapidly due to their importance in the field of apothecary and medication. The bioactive compounds of medicinal plants are effective source of novel drugs. These biological drugs are very effective against various harmful diseases. Here the methods used for the study of antioxidant potential of *I. eriocarpa* are Total phenolic content, Total flavonoids content and 1, 1- Diphenyl-2-Picrylhydrazyl radical scavenging potential.

Determination of total phenolic content

The total phenolic content of *I. eriocarpa* was assessed by Folin-Ciocalteau (FC) reagent. During this method due to metal oxide reduction blue colour appears, that displays an extreme broad light absorption at 760 nm. The results are showed in Gallic acid equivalents (GAE). The value of Total phenolic content (mg gallic acid equivalent per gram dry extract weight) of plant extracts was shown in Table 4. The plant extracts which displayed the value within the range 48.6 ± 0.01 to $1.96 \pm 0.6 \mu g/ml$ GAE. The Total phenolic content of leaves the ethanolic extract of the *I. eriocarpa* leaves range from 48.6 ± 0.01 to $3.116 \pm 1.4 \mu g/ml$ GAE. Bark extract had to 40.7 ± 0.05 to $1.96 \pm 0.6 \mu g/ml$ GAE and Inflorescence extract had 39.63 ± 0.04 to $2.21 \pm 0.93 \mu g/ml$ GAE. As whole maximum activity was showed by leaves extracts (Table 4), followed by bark and inflorescence.

Plant part	Concentration (µg/ml)	Absorbance at 760 nm	GAE (µg/ mL gallic acid)
Leaves	500	0.62 ± 0.12	48.6 ± 0.01
	250	0.42 ± 0.02	24.16 ± 0.25
	125	0.29 ± 0.05	11.416 ± 0.9
	62.5	0.212 ± 0.3	6.05 ± 1.23
	31.5	0.184 ± 1.21	3.116 ± 1.4
Bark	500	0.43 ± 0.02	40.7 ± 0.05
	250	0.37 ± 0.6	21.24 ± 0.1
	125	0.21 ± 1.3	9.24 ± 1.65
	62.5	0.17 ± 0.05	5.916 ± 0.85
	31.5	0.06 ± 0.13	1.96 ± 0.6
Inflorescence	500	0.42 ± 0.05	39.63 ± 0.04
	250	0.31 ± 0.12	18.21 ± 0.61
	125	0.24 ± 2.3	9.79 ± 0.03
	62.5	0.188 ± 0.5	5.621 ± 0.01
-	31.5	0.09 ± 0.1	2.21 ± 0.93

Table 4. Total Phenolic Contents (expressed as µg/ mL of gallic acid) of *I.eriocarpa*

*Results were carried out in triplicates and written as Mean \pm Standard error

Estimation of total flavonoid content

The total content of flavonoids was evaluated and expressed as μg Quercetin equivalents (QE) /mg of extract. The value of total flavonoid contents varied from 39.3 ± 0.85 to $1.14 \pm 0.13 \mu g/mg$. The highest amount of flavonoids was found in the ethanolic extracts of leaves ($39.3 \pm 0.85 \mu g/mg$). Overall activity of plant extracts exhibited as leaves > bark > inflorescence. Results (Table 5) revealed that leaves extracts possessed highest flavonoid contents as compare to bark and inflorescence.

	Table 5: Total Flavonoi	l Contents (ex	pressed as (Juercetin ec	uivalent) of	I.eriocarpa.
--	-------------------------	----------------	--------------	--------------	--------------	--------------

Plant part	Concentration (µg/ml)	Absorbance at 510 nm	QUE (µg/ mL of Quercetin)
Leaves	<u>400</u>	0.82 ± 0.13	39.3 ± 0.85
	200	0.69 ± 0.03	19.53 ± 0.02
_	100	0.432 ± 0.35	9.5 ± 0.1
	50	0.33 ± 0.02	4.79 ± 1.5
	25	0.209 ± 1.2	2.34 ± 0.09
Bark	400	0.61 ± 0.34	37.31 ± 0.05
	200	0.45 ± 0.04	17.3 ± 1.02
	100	0.36 ± 0.5	3.52 ± 0.18
	50	0.27 ± 0.01	3.29 ± 0.52
	25	0.134 ± 0.2	1.94 ± 1.09
Inflorescence	400	0.52 ± 0.01	35.13 ± 1.03
	200	0.37 ± 0.13	12.32 ± 0.12
	100	0.29 ± 0.14	6.9 ± 0.53
	50	0.21 ± 0.05	9.8 ± 1.23
	25	0.192 ± 0.02	1.14 ± 0.13

*Results reported were done in triplicates and written as Mean ± Standard error

1, 1- Diphenyl-2-Picrylhydrazyl radical scavenging property

The % DPPH radical scavenging activity was assessed and expressed against Butylated hydroxyl toluene as standard. The control (standard BHT) showed maximum activity about (87.7 \pm 0.12%). The value of % DPPH radical scavenging varied from 23.3 \pm 0.03 to 76.5 \pm 0.09% (Table 6). The results revealed that the ethanolic extract of the *I. eriocarpa* leaves exhibited the highest radical scavenging activity with 76.5 \pm 0.09 % followed by the Bark extract of 74.4 \pm 0.2 and 73.2 \pm 0.14% of Inflorescence extract and minimum % DPPH radical scavenging activity i.e. 23.3 \pm 0.03 % that was shown by ethanolic extract of leaf when conc. is 2 ppm.

As whole maximum activity was showed by leaves extracts (Table 6), followed by bark and inflorescence. Overall activity of plant extracts exhibited as leaves> bark > inflorescence.

Plant part	Concentration (ppm)	Absorbance	% DPPH remaining
Leaves	10	0.211 ± 0.016	76.5 ± 0.09
	8	0.313 ± 0.01	65.2 ± 0.23
	6	0.444 ± 0.02	50.6 ± 0.01
	4	0.61 ± 0.011	32.2 ± 0.5
	2	0.69 ± 0.012	23.3 ± 0.03
Bark	10	0.23 ± 0.02	74.4 ± 0.2
	8	0.33 ± 0.04	63.3 ± 0.13
	6	0.39 ± 0.01	56.7 ± 0.02
	4	0.471 ± 0.04	47.8 ±0.12
	2	0.51 ± 0.01	43.3 ± 0.03
Inflorescence	10	0.241 ± 0.01	73.2 ± 0.14
	8	0.29 ± 0.017	67.7 ± 0.03
	6	0.36 ± 0.011	60.2 ± 0.05
	4	0.467 ± 0.012	53.1 ± 0.06
	2	0.48 ± 0.02	48.3 ±0.4
BHT	10	$0.11 \pm .56$	87.7 ± 0.12
	8	0.16 ± 0.23	82.3 ± 0.5
-	6	0.184 ± 0.14	79.6 ± 0.12
	4	0.179 ± 0.12	75.2 ± 0.62
	2	0.168 ± 0.01	70.2 ± 0.14

Table 6. DPPH scavenging activity of I. eriocarpa, con	ompared with the standard antioxidant
--	---------------------------------------

*The results reported were run in triplicates and stated as Mean \pm Standard error

Oxidative stress occurs in both plants and animals. In plants the oxidative stress is caused by photosystem. In humans, about 1-3% of the peroxisomes, mitochondria, microsomes and others the ROS formation might occur (Schemp, 2005). Many antioxidants are obtained from natural resources, that are used as free radical and active oxygen scavengers. Now a day, the use of natural antioxidants is increasing to replace the synthetic antioxidants. Antioxidants also play role in retard the progress of diseases as well as lipid oxidative process in foods (Lia and Chou 2001).

Conclusion

Medicinal plants signify a significant source of biological agents that are source of potent drugs. Though thousands of plant species have been verified for antimicrobial activity. The different parts of medicinal plants such as root, stem, leaves, flower and fruits are used to extract raw drugs that have diverse properties. It is a crucial requirement to regulate antimicrobial resistance by enhanced antibiotic drugs. It is necessary to develop of new drugs to sustain the antimicrobial management. The present study indicates that the ethanol extract shows antimicrobial activity against four bacterial strains i.e. *S. aureus, P. aeruginosa, B. subtilis and E. coli* and two fungal strains i.e. *A. oryzae A. niger.* The results showed that the plant can be studied further to assess efficacy as an antimicrobial agent by clinical trials. The antioxidant potential of *I. eriocarpa* lowers the concentration of free radicals as results comparable with those of standard antioxidant, i.e. BHT. This research data more support the opinion that this plant is auspicious source of natural antioxidants and biologically useful medications.

Acknowledgement

The authors are thankful to Department of Botany, MUST, Bhimber Campus for providing laboratory facility including test organisms (bacteria and fungi) and chemicals.

References

- Ajaib, M., Ashraf, Z. and Siddiqui, M.F. (2017). *Cocculuslaurifolius*: A rich antimicrobial, antioxidant and phytochemical source. Pak. J. Bot. 49(1): 337-344.
- Ajaib, M., Boota, F., Khan, K.M. Perveen,S. and Shah, S. (2014). Clerodendrumsplendens: A Potential Source of Antimicrobials. J. Chem. Soc. Pak. 36(4): 763-770.
- Ajaib, M., Ishtiaq, M., Bhatti, K.H., Ahmed, K.S., Maqbool, M. and Hussain, T. (2021). Traditional ethnobotanical knowledge of wild plants of TillaJogian district Jhelum, Pakistan. Pak. J. Bot. 53(4): 1303-1406.
- Ajaib, M., Mati-ur-Rehman, A., Parveen, S. and Shah, S. (2015). *Pulicariaundulata*; A Potential Phytochemical, antimicrobial and antioxidant source. J. Chem. Soc. Pak. 37(3): 559-566.
- Ajaib, M., Wahla, S.Q., Siddiqui, M.F. and Khan, I.A. (2016). Antibacterial and Antioxidant Activities of *AndrachneCordifolia* (Wall. ex Decne.) Muell. *FUUAST J. Biol.*, 6(1): 127-134.
- Ajaib, M., Zikrea, A., Khan, K.M, Perveen, S., Shah, S. and Karim, A. (2013). *Rivinahumilis*: A Potential Antimicrobial and Antioxidant Source. J. Chem. Soc. Pak. 35(5): 1384-1398.
- Aruoma, O. I. (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. Journal of the American Oil Chemists' Society, 75(2), 199-212.
- Austin, D.F. and Ghazanfar, S. (1979). Flora of West Pakistan. Convolvulaceae. No. 126. (Nasir, E. and Ali, S. I. eds.) Department of Botany, University of Karachi.
- Cruick-Shank, R., Dugid, J.P., Marinionon, B.P. and Swain, R.H.A. (1975). Screening of Some Greek Aromatic Plants for antioxidant Activity. Phytother. Res. 17(2): 194-195.
- Daud, J. M., Hassan, H.H.M. and Tahir, M. (2011). Phytochemicals Screening and Antioxidant Activities of Malaysian Donax Grandis Extracts. European Journal of Scientific Research 61(4): 572–577.
- Dewanto, V., Wu X., Adom, K.K. and Liu, R.H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem. 50: 3010–3014.
- Gill, L.S. (1988). Taxonomy of Flowering Plants. Africana-Fep Publishers Limited, 116-119p.
- Iqbal, M.J., Hanif, S., Mahmood, Z., Anwar, F. and Jamil, A. (2012). Antioxidant and antimicrobial activities of Chowlai (*Amaranthusviridis* L.) leaf and seed extracts. J. Med. Plants Res. 6(27): 4450-4455.
- Ishtiaq, M., Maqbool, M., Ajaib, M., Ahmed, M., Hussain, I., Khanam, H., Mushtaq, W., Hussain, T., Azam, S., Bhatti, K.H., Ghani. A. (2021). Ethnomedicinal and folklore inventory of wild plants used by rural communities of valley Samahni, District Bhimber Azad Jammu and Kashmir, Pakistan. PLoS ONE 16(1): e0243151.
- Johansen, D.A. (1940). Plant Microtechnique. MC-Graw-Hill Book Company, Inc. New York.
- Khond, M., Bhosale, J.D., Arif, T., Mandal, T.K., Padhi, M.M. and Dabur, R. (2009). Screening of Some Selected Medicinal Plants Extracts for *In Vitro* Antimicrobial Activity. Middle –East. J. Sci. Res. 4(4):271-278.
- Kumar, V. and Akhtar, M. (2013). Medicinal Convolvulaceous plants of Eastern Uttar Pradesh. Indian J. L. Sci. 2(2), 63-65.
- Lia, L.S. and Chou, S.T. (2001). Studies on the antioxidative activities of *Mesonaprocumbens*leaf gum. J. Agric. Food Chem.49: 963-968).
- Makkar, H.P.S., Blummel, M., Borowy, N.K. and Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agri. 61: 161–165.
- Maqbool, M., Ajaib, M. Ishtiaq, M., Mushtaq, W., Hussain, T. and Ahmed, W. (2021). Investigation of Antimicrobial and Antioxidant Activity of *Aervasanguinolenta*(L.) Blume from District Bhimber (AJK), Pakistan. Bioscience Research 18(1): 640-652.
- Maqbool, M., Ajaib, M., Ishtiaq, M., Mushtaq, W., Hussain, T. and Ahmed, W. (2021). Investigation of Antimicrobial and Antioxidant Activity of *Aervasanguinolenta*(L.) Blume from District Bhimber (AJK), Pakistan. Bioscience Research 18(1): 640-652.
- Noshad, Q., Ajaib, M. and Kiran, A. (2020). Comparative investigation of palynological characters of *Cuscutaeeflexa* and few members of Convolvulaceae. J. Anim. Plant Sci. 30(5): 1215-1223.
- Noshad, Q., Ajaib, M., Kiran, A., Ishtiaq, M., Bashir, T. and Siddiqui, M.F. (2021). Study On Genetic Diversity of *Cuscutareflexa*Roxb. and few members of Convolvulaceae on the basis of RAPD and SDS-Page. Pak. J. Bot. 53(3): 959-965.
- Priya, G.S., Phadika, R. and Siddhuraju, P. (2012). Antioxidant and antimicrobial activity of traditional Indian leafy vegetables. International J. Pharm. Pharm. Sci. 4(2): 513-521.

- Schemp, H., Hippeli, S. and Elstner, E.F. (2005). Plant stress; Avoidance, adaptation, defense. In: Hock, B. and Elstner, E. F. Plant Toxicology87-129.
- Shahzadi, T., Riaz, T., Abbasi, M.A., Mazhar, F., Shahid, M. and Ajaib, M. (2018). *Wendlandiaexserta*: a pertinent source of antioxidant and antimicrobial agent. Turk. J. Biochem.43(4): 456-463.
- Suleiman, M.S., Singh, R.J. and Stewart, C.E. (2007) Apoptosis and the cardiac action of insulin-like growth factor I. Pharmacol. Ther. 114(3):278–294.
- Wilkin, P. (1999). A morphological cladistic analysis of the *Ipomoea* (Convolvulaceae). Kew Bulletin 54: 853 876.
- Yildirim, A., Mavi, A., Kara, A.A. (2001). Determination of antioxidant and antimicrobial activities of *Rumexcrispus* L. extracts. J. Agric. Food Chem. 49(8): 4083-4089.