



Open Access



Date Received:
05/01/2015;
Date Revised:
09/02/2015;
Date Published:
25/02/2015:

Phytochemical Analyses for Antibacterial Activity and Therapeutic Compounds of *Convolvulus arvensis* L., Collected from the Salt Range of Pakistan

Muhammad Umair Khan, Noor-ul-Huda Ghori*, Muhammad Qasim Hayat

Abstract:

Background: Pharmaceutical world is currently facing a major issue of bacterial resistance against antibiotics. For the past few years, plants being an excellent source of phytochemicals have replaced many traditional antibacterial agents. Keeping this in view, the current study was conducted to detect the antibacterial activity and the presence of various phytochemicals in *Convolvulus arvensis* leaves and stem extracts.

Methods: Eight different organic and aqueous extracts of *Convolvulus arvensis* L. (*C. arvensis*) leaves and stem were prepared and their antimicrobial activity against 13 clinically important bacterial species was investigated using the disc diffusion assay. Phytochemical screening of the aqueous extracts of *C. arvensis* was performed using qualitative tests.

Results: All the thirteen bacterial species tested in this study were found sensitive to the stem and leaf extracts of *C. arvensis*. Highest antimicrobial activity was observed against *Escherichia coli* while among all the extracts butanol extract was the most effective antimicrobial agent. Phytochemical analyses using organic and aqueous extracts confirmed the presence of various secondary plant metabolites of therapeutic value i.e. saponins, flavonoids, steroids, tannins, phlobatannins, cardiac glycosides and coumarins.

Conclusion: Our results indicate that *C. arvensis* plant contains some phytochemicals with antimicrobial affects. These chemicals possess great potential to be used as advanced therapeutic compounds against broad range of pathogenic bacteria.

Authors' Affiliation:

Medicinal Plants
Research Group,
Department of Plant
Biotechnology, Atta-ur-
Rahman School of
Applied Biosciences
(ASAB), National
University of Sciences
and Technology
(NUST), H-12
Islamabad, Pakistan

*Corresponding Author:

Noor-ul-Huda Ghori
Email:
hudaghori@gmail.com

How to Cite:

Khan MU, Ghori NH,
Hayat MQ.
Phytochemical Analyses
for Antibacterial
Activity and
Therapeutic
Compounds of
Convolvulus arvensis L.,
Collected from the Salt
Range of Pakistan
(2015). Adv. Life Sci.
2(2). pp: 83-90.



Introduction

Convolvulus arvensis L. (Morning glory; family *Convolvulaceae*; figure 1) locally known as 'Leli' [1], is a persistent, herbaceous perennial weed that is commonly found in regions with temperate, tropical or mediterranean climates. It has been in use as a herbal food and as a traditional medicine since the eighteenth century. *C. arvensis* is commonly found in the salt ranges of Pakistan and the inhabitants of the area are using this plant as folk medicine for many generations [1].



Figure 1: *Convolvulus arvensis* L. growing on waste land in association with *Cynodon dactylon* (L.) Pers. Grass

In folk medicine it is employed as a medication to treat constipation, boils and inflammation. It has been found to cure cough, gonorrhoea, chronic ulcers, rheumatic pain and some skin diseases. It is also utilized as an aphrodisiac, a nervine tonic and a diuretic agent [2]. Whole plant is employed as a sedative to ease the pain in genital diseases as well as in nervous disorders e.g. hysteria and epilepsy. It has also been found useful as a blood purifier [3-5]. In veterinary medicine, helminthosis has been treated with the aerial parts of *C. arvensis* [6]. Therefore, many researchers have focused on the biological properties of *Convolvulus* species and its parts [7-10]. In the present study we analyzed the antimicrobial activity of different extracts of *C. arvensis* against some clinically important bacterial species. We also screened this plant for the potential presence of various compounds of therapeutic value.

Methods

Sample collection

Plants samples of *C. arvensis* were collected from Salt Range area of Islamabad and Rawalpindi districts of Pakistan in May 2012. The specimens were confirmed via visual examination at the Botanical Sciences Division (BSD) of the Pakistan Museum of Natural History (PMNH), Islamabad, Pakistan.

Extract preparation

For the preparation of extracts, the plants' leaves and stems were dried under shade followed by grinding it into fine dust sized particles using a grinder. Ten grams of the ground material of leaves and stems was separately dissolved in eight different solvents (100 mL) to prepare crude plant extracts. The solvents used were Ethanol, Methanol, Chloroform, Acetone, n-Hexane, Butanol, Ethyle acetate and distilled water. These extracts were kept on a shaking incubator for 24 hrs at 28°C to assist the dissolution process and make a homogenous solution in the solvent. The solutions were then filtered using filter paper (Whatman no. 1) and a vacuumed rotary evaporator was used to concentrate the filtrate followed by its preservation at 4°C till further use.

In-vitro Antibacterial activity

Antibacterial activity of all prepared extracts was analyzed using disc diffusion method [11]. Agar cultures of the following bacteria were obtained from the National Institute of Health (NIH), Islamabad: *Staphylococcus aureus* IARS-4 (*S. aureus*), *Acinetobacter junii* IARS-2 (*A. junii*), *Klebsiella pneumoniae* IARS-11 (*K. pneumoniae*), *Acinetobacter baumannii* IARS-10 (*A. baumannii*), *Enterococcus faecalis* IARS-1 (*E. faecalis*), *Escherichia coli* IARS-3 (*E. coli*), *Pseudomonas aeruginosa* IARS-8 (*P. aeruginosa*), *Shigella dysenteriae* IARS-9 (*S. dysenteriae*), *Vibrio cholerae* IARS-13 (*V. cholerae*), *Proteus mirabilis* IARS-5 (*P. mirabilis*), *Salmonella paratyphi* IARS-12 (*S. paratyphi*), *Serratia marcescens* IARS-6 (*S. marcescens*) and *Enterobacter cloacae* IARS-7 (*E. cloacae*). Bacterial cultures were prepared on Mueller-Hinton agar. 40 µL from each extract with different concentrations 100 mg/mL, 50 mg/mL, 5 mg/mL were placed separately on 6 mm filter paper disc. Three filter paper discs of different concentrations of the same extract were placed in a single petri dish. For control, discs were impregnated with standard antibiotics (100 µg/mL) Amoxycillin and Gentamycin. The plates were incubated overnight at 36°C after which zones of inhibition was measured.

Phytochemical Screening

Organic extracts of *C. arvensis* were prepared as mentioned above for its preliminary quantitative phytochemical screening. Ground plant material was weighed and subjected to extraction in Soxhlet

Keywords:

Convolvulus arvensis,
Antimicrobial,
Phytochemical
Screening,
Organic Extracts,
Clinical Bacterial
Isolates,
Antimicrobial Disc
Diffusion Test,
Drug Resistance

apparatus at 60°C. Water extract was prepared by following the protocol [12]. The solution obtained was dried in a rotary evaporator at 50-60°C until all the solvents evaporated. The dried extract was subjected to various phytochemical tests to confirm their presence.

Steroids

To detect the presence of steroids 1 mL of the plant extract was dissolved in 10 mL of chloroform and equal volume of concentrated sulphuric acid (H₂SO₄). The indication of red upper layer and yellow H₂SO₄ layer green fluorescence confirmed the presence of steroids [13].

Terpenoids

Salkowski's Test was performed to confirm the presence of terpenoids in the extracts. Five mL of each aqueous and organic extracts were mixed in 2 mL of chloroform and then 3 mL of concentrated H₂SO₄ was carefully added so as to form a layer. Formation of reddish brown color at the interface confirmed the presence of terpenoids [14].

Flavonoids

To observe the presence of flavonoids 5 mL of dilute ammonia solution was added to a portion of aqueous filtrate of the plant extract followed by addition of concentrated H₂SO₄. Presence of flavonoids was shown by the appearance of yellow color [12, 15].

Tannins

Developed protocol was followed to detect the presence of tannins. 2 drops of ferric chloride were added to 1 mL of each extract. Development of a transient greenish to black color revealed the presence of tannins [16].

Saponins

Presence of saponins was detected by diluting 5 mL of the extract with 20 mL of distilled water, followed by agitation for 15 minutes in a graduated cylinder. Formation of foam pointed to the presence of saponins [17].

Anthocyanins

Anthocyanins detection test was performed by adding 2 mL of aqueous extract to equal volumes of 2N hydrochloric acid (HCl) and ammonia. The appearance of pinkish red color that turned bluish violet indicated the presence of anthocyanins.

Leucoanthocyanins

To observe the presence of leucoanthocyanins, 5 mL of the aqueous extract was added to an equal volume of isoamyl alcohol. Supernatant turned red in color pointing the presence of leucoanthocyanins [18].

Coumarins

Presence of coumarins was detected by adding 3 mL of 10% sodium hydroxide (NaOH) to 2 mL of aqueous extracts. Formation of yellow color indicated the presence of coumarins [19].

Phlobatannins

For the presence of phlobatannins, the deposition of a red precipitate when an aqueous extract of plant was boiled with 1% aqueous HCl, was taken as evidence [14].

Cardiac glycosides

For detection of cardiac glycosides we performed the Keller-Killani's [14] test. 5 mL of extract was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 mL of concentrated H₂SO₄. Development of a brown ring at the interface showed a deoxysugar characteristic of cardenolides.

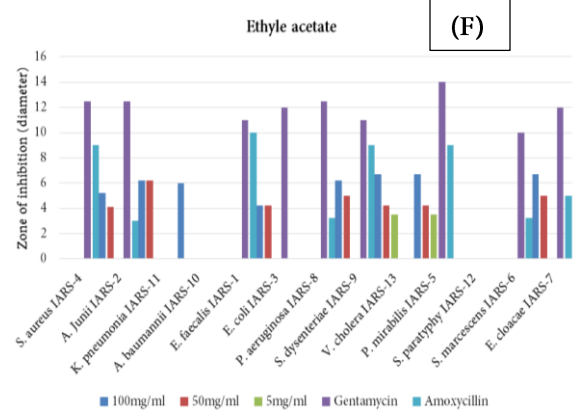
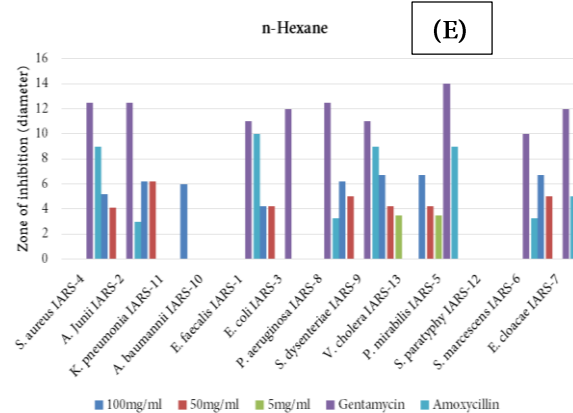
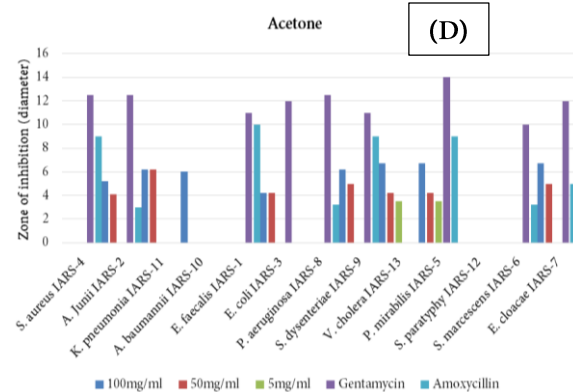
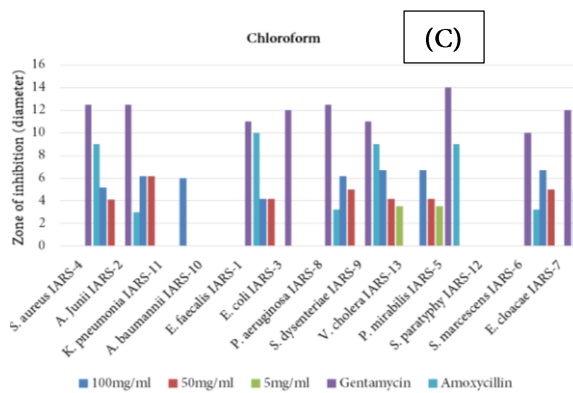
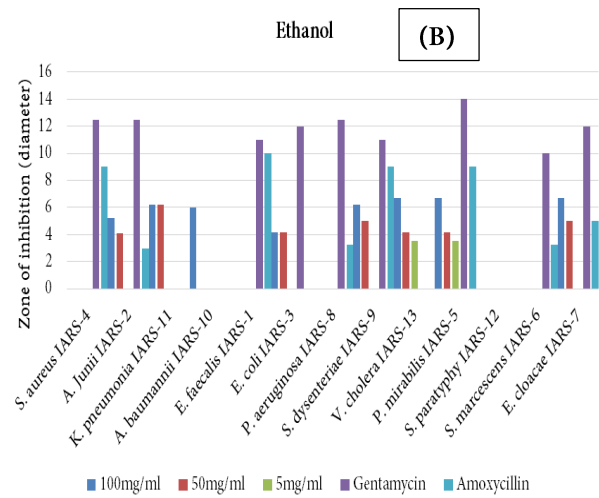
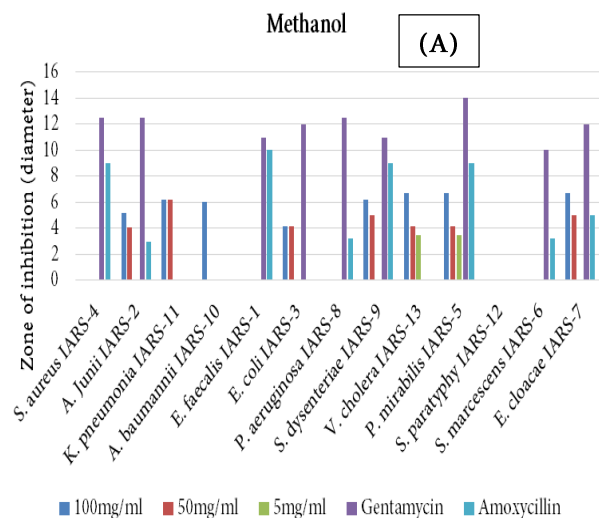
Results

The antibacterial activity of eight different extracts including aqueous extracts of *C. arvensis* was examined against 13 bacterial strains. All extracts of different concentrations showed high degree of antimicrobial activity against all pathogenic bacterial strains used in the study. The antibacterial activity of *C. arvensis* leaves and stem is summarized in Figure 2 and 3.

The tested butanolic extracts from *C. arvensis* showed relatively high level of antimicrobial activity against all the microorganisms. Among all the tested bacterial strains, *E. coli* was found to be the most sensitive. *A. junii*, showed an average sensitivity to different extracts. The minimum inhibitory concentration (MIC) for Butanol was 5 mg/mL for stem and 50 mg/mL for leaves against these bacterial strains.

K. pneumonia, *A. baumannii*, *E. faecalis*, *S. paratyphi* and *S. marcescens* remained resistant to most of the stem extracts of *C. arvensis* whereas, *K. pneumonia*, *E. cloacae*, *S. paratyphi*, *V. cholera* and *S. dysenteriae* showed resistance to leaves extracts of *C. arvensis* except for showing activity for butanol extract.

Phytochemical screening was performed to check the presence of steroids, tannins, terpenoids, flavanoids, anthocyanins, leucoanthocyanins, coumarins, cardiac glycosides, saponins, phlobatannins. The results of phytochemical analyses are summarized in Table 1 for organic and aqueous extracts of *C. arvensis*. The table shows the presence of different phytochemicals when extracted using different solvents. Methanol extracts were rich in steroids, flavonoids and cardiac glycosides. Ethanol extracted tannins, flavonoids, coumarins and saponins from the plant. Presence of cardiac glycosides and flavonoids were confirmed in chloroform extracts whereas, saponins, cardiac glycosides and tannins were also common in ethyl acetate and aqueous extracts. Anthocyanins, Leucoanthocyanins, Phlobatannins were not found in any of the extracts.



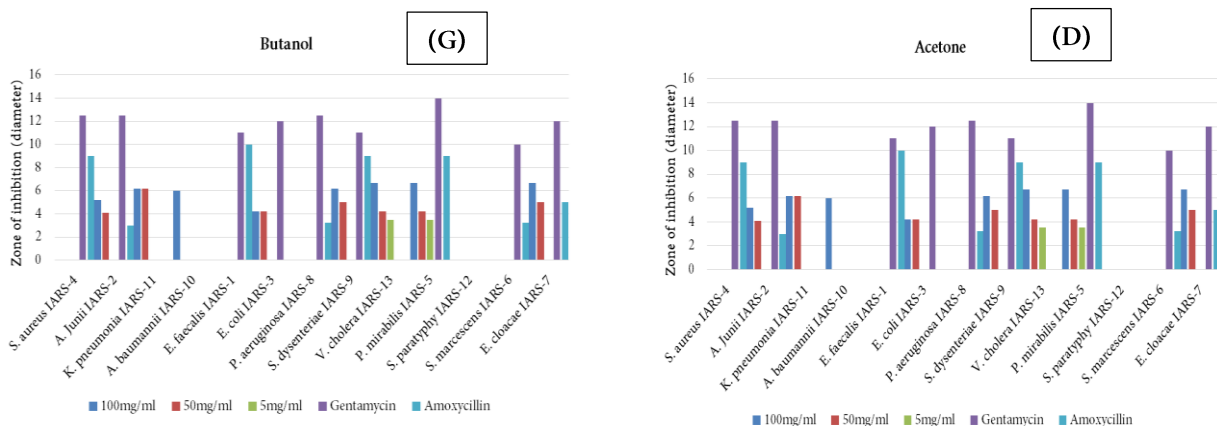


Figure 2 (A- G): Graphical representation of Bacterial zone of inhibition for the leaves extracts of *C. arvensis*

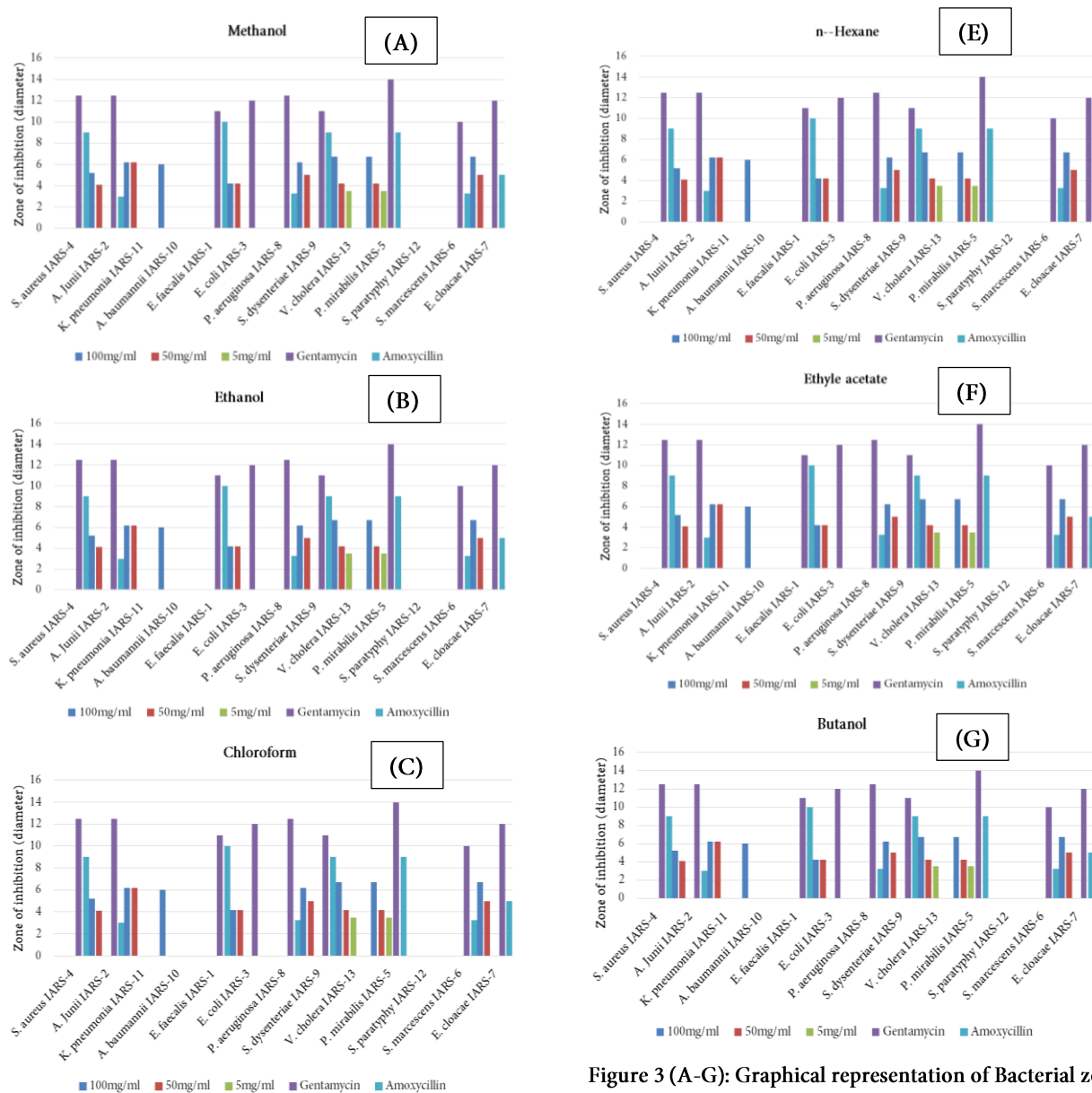


Figure 3 (A-G): Graphical representation of Bacterial zone of inhibition for the stem extracts of *C. arvensis*

	Methanol	Ethanol	Chloroform	Ethyl acetate	Acetone	Aqueous	Butanol	Hexane
Steroids	+	-	-	-	-	++	-	+
Tannins	-	+++	-	++	+++	---	+++	+
Terpenoids	-	-	---	-	-	-	---	-
Flavanoids	+	++	+	-	-	+	-	-
Anthocyanins	-	-	-	-	-	-	-	-
Leucoanthocyanins	-	-	-	-	-	-	-	-
Coumarins	-	+	-	-	-	-	-	+
Cardiac Glycosides	+	-	++	++	-	++	-	-
Saponins	--	++	-	+	+	+++	+	-
Phlobatannins	-	-	-	-	-	+	-	-

+ (present), - (not present)

Table 1: Showing the results of phytochemical analyses of *C. arvensis*

Discussion

Plants are a rich source of phytochemicals that have been used as chemotherapeutic agents for ages. In the current study, antibacterial activity of organic and aqueous extracts of *C. arvensis* leaves and stems in comparison with standard drugs Amoxycillin and Gentamycin was investigated. The activity was found to proceed in a dose dependent manner against major pathogenic isolates. Strong positive activity was observed in Butanol extracts against all bacterial strains under study. Among all the tested bacterial strains, *E. coli* was found to be the most sensitive. These results however, are contradictory to previous study on *C. arvensis* collected from Khyber Pakhtunkhwa region [20]. The difference in results is inferred to be effected by changes in the environment of the plant sample under study. Plants growing in different environmental conditions have varying secondary metabolites and thus, varying bioactivities [21, 22]. The difference in bioactivities and composition of secondary metabolites is also dependent upon the genetic lineage of a plant [23].

Traditionally, *C. arvensis* is used for treating jaundice [24] and its ethanol extract is said to be rich in flavonoids including quercetin and Kaempferol [25]. The quercetin flavonoid is particularly responsible for the hepatoprotective activity of *C. arvensis* whereas, the phytochemical analysis of *C. arvensis* in this study has shown the richness of saponins, terpenoids, tannins, cardiac glycosides and coumarins in the plant. The phytochemicals are responsible for various scientific effects such as anti-angiogenesis, cytotoxicity as well as

immune stimulatory [25-27].

We have presented the antibacterial activity of the plant against most common bacterial strains involved in human pathogenesis. The Butanol extract of the plant has shown great tendency of antibacterial activity and it is inferred that the activity is due to tannins and saponins. Tannins have been observed to form complexes with proteins by forming hydrogen or covalent bonds or through hydrophobic forces. Through these forces they act as antibacterial agents and inactivate microbial adhesins, cell envelope transport proteins and enzymes [28]. Thus, the study confirms the presence of antibacterial activity in *C. arvensis* that is inferred to be due to presence of Tannins. Further investigations to exploit bioactive compounds of the plant can lead to identification of many pharmaceutically important compounds.

Adaption of bacterial resistance against antibiotics is a great challenge faced by pharmaceutical industry today. The results of this study shows that *C. arvensis* leaves and stem extracts have strong antibacterial activity against most of the human pathogenic bacteria. In addition, phytochemical analysis has also shown the presence of various bioactive compounds such as tannins, cardiac glycosides and saponins that can be evaluated for their potential activities against other diseases.

Acknowledgements

The authors would like to thank Dr. Muhammad Faraz Bhatti, Dr. Syed Aneel Gilani and Dr. Nasar-um-Minullah (National University of Sciences and Technology) for their constructive feedback during this

work and also for reviewing this document. They are also thankful to the Atta-ur-Rahman School of Applied Biosciences of the National University of Science and Technology for funding this research from its sources.

References

- Iqbal H, Sher Z, Khan ZU. Medicinal plants from salt range Pind Dadan Khan, district Jhelum, Punjab. Pakistan. *Journal of Medicinal Plants Research*, (2011); 5(11): 2157-2168.
- Murad W, Ahmad A, Gilani SA, Khan MA. Indigenous knowledge and folk use of medicinal plants by the tribal communities of Hazar Nao Forest, Malakand District, North Pakistan. *Journal of Medicinal Plants Research*, (2011); 5(7): 1072-1086.
- Singh PK, Kumar V, Tiwari RK, Sharma A, Rao CV, *et al.* Medico-ethnobotany of 'Chatara' Block of district Sonbhadra, Uttar Pradesh, India. *Advances in Biological Research*, (2010); 4(1): 65-80.
- Asad Ullah, Rashid A. Weeds and livelihood in Mankial valley, Hindukush range, Pakistan. *Pakistan Journal of Weed Research*, (2007); 13(1-2): 27-32.
- Akhtar N, Begum S. Ethnopharmacological important plants of Jalala, district Mardan, Pakistan. *Pakistan Journal of Science*, (2009); 15(2): 95-100.
- Hussain A, Khan MN, Iqbal Z, Sajid MS. An account of the botanical anthelmintics used in traditional veterinary practices in Sahiwal district of Punjab. *Pakistan Journal of Ethnopharmacology*, (2008); 119: 185-190.
- Oudhia P, Tripathi RS. Medicinal weeds of kharif crops in the plains of Chhattisgarh. *Bhartiya Krishi Anusandhan Patrika*, (1998); 13(1-2): 33-38.
- Calvino N. Anti angiogenesis properties of a common weed *Convolvulus arvensis*. *Journal of Chiropractic Medicine*, (2002); 1(3): 116.
- Meng XL, Riordan NH, Casciari JJ, Zhu Y, Zhong J, *et al.* Effects of a high molecular mass *Convolvulus arvensis* extract on tumor growth and angiogenesis. *Puerto Rico Health Sciences Journal*, (2002); 21: 323-328.
- Riordan NH, Menh X, Taylor P, Riordan HD. Anti-angiogenic, anti-tumor and immunostimulatory effects of a nontoxic plant extracts (PMG). *Comprehensive Cancer Care*, (2000).
- Malika N, Mohamed F, Chakib A. Antimicrobial Activities of Natural Honey from Aromatic and Medicinal Plants on Antibioresistant Strains of Bacteria. *International Journal of Agriculture and Biology*, (2004); 6: 289-293.
- Harborne JB. *Photochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 1973; 279. Chapman A. & Hall.
- Gibbs RD. *Chemotaxonomy of flowering plants*. 1974; Vols I-IV: 1-680. McGill-Queen's univ. Press: Montreal & London.
- Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, (2005); 4(7):685-688.
- Sofowora A. *Medicinal Plants and Traditional Medicines in Africa*. (1993); 97-145. Chichester John Wiley & Sons New York.
- Kumar A, Ilavarasn R, Jayachandran T, Decaraman M, Aravindhnan P, *et al.* Phytochemicals investigation on a tropical plant, *Syzygium cumini* from Kattuppalayam, Erode district, Tamil Nadu, South India. *Pakistan Journal of Nutrition*, (2009); 8(1): 83-85.
- Mojab F, Kamalinejad M, Ghaderi N, Vahidipour HR. Phytochemical screening of some species of Iranian plants. *Iranian Journal of Pharmaceutical Research*, (2010); 2(2): 77-82.
- Paris R, Moyses H. *Precis de matiere medicinale*. (1969); Paris: Masson.
- Rizk AM. Constituents of plants growing in Qatar. *Fitoterapia*, (1982); 52: 35-42.
- Raza M, Fozia, Rehman A, Wahab A, Iqbal H, *et al.* Comparative antibacterial study of *Convolvulus arvensis* collected from different areas of Khyber Pakhtunkhwa, Pakistan. *International research journal of Pharmacy*, (2012); 3(10): 220-222.
- Zobayed SMA, Afreen F, Kozai T. Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's wort. *Plant Physiology and Biochemistry*, (2005); 43(10): 977-984.
- Kirakosyan A, Seymour E, Kaufman PB, Warber S, Bolling S, Chang SC. Antioxidant capacity of polyphenolic extracts from leaves of *Crataegus laevigata* and *Crataegus monogyna* (hawthorn) subjected to drought and cold stress. *Journal of Agricultural and Food Chemistry*, (2003); 51(14): 3973-3976.
- Pichersky E, David RG. Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends in Plant Science*, (2000); 5(10): 439-445.

24. Thakral J, Borar S, Kalia JAR. Antioxidant potential fractionation from methanol extract of aerial parts of *Convolvulus arvensis* Linn. (*Convolvulaceae*). *International Journal of Pharmaceutical Science and Drug Research*, (2010); 2(3): 219-23.
25. Kaur M, Kalia A. *Convolvulus arvensis*: A useful weed. *International Journal of Pharmacy and Pharmaceutical Sciences*, (2012); 4(1): 38-40.
26. Elzaawely AA, Tawata S. Antioxident Activity of Phenolic Rich Fraction obtained from *Convolvulus arvensis* L. leaves grown in Egypt. *Asian Journal of Crop Science*, (2012); 4(1): 32-40.
27. Mahmoudi M, Zamani TRS, Zamani TR, S, Emami SA. A study to investigate the biological activity of proteoglycan mixture extract from *Convolvulus arvensis*. *Journal of Complementary and Integrative Medicine*, (2014); 11(4): 265-272.
28. Hashem FM, El-Kiey MA. *Nigella sativa* seeds of Egypt. *Journal of Pharmaceutical Sciences*, (1962); 3(1): 121-133.