Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 11 | 2023
DOI: 10.5281/zenodo.10153074

PHYTOCHEMICAL AND ANTIBACTERIAL EVALUATION OF *CONVOLVULUS ARVENSIS* LINN

MUHAMMAD ASIF SHAHZAD *
Faculty of Pharmacy, Gomal University Dera Ismail Khan, Pakistan.
*Corresponding Author Email: asifshahzadmmd@gmail.com

HAROON KHAN
Faculty of Pharmacy, Gomal University Dera Ismail Khan, Pakistan.

MUHAMMAD AZAM TAHIR
Ripah Institute of Pharmaceutical Sciences, Sahiwal, Pakistan.

MURRYAM NAWAZ
Ripah Institute of Pharmaceutical Sciences, Sahiwal, Pakistan.

ESHWA DAR
Department of Pharmacology, Faculty of Pharmacy, University of Lahore, Lahore, Pakistan.

ANJUM KHURSHEED
Faculty of Pharmacy, Grand Asian University, Sialkot Pakistan.

SHABBIR AHMED
Fatima College of Health Sciences, Toba Tek Singh, Pakistan.

Abstract

Convolvulus is a genus of about 250 species of flowering plants widely distributed in Pakistan. The extract of its leaves has been prescribed in folk medicine in the treatment of various diseases. The present study aims to evaluate Convolvulus arvensis Linn whole plant both phytochemicals and for its antibacterial activity. The total phenolic contents in terms of µg gallic acid equivalent (GAE) per mg extract was found to be 96 µg GAE/mg while the flavonoid content in methanolic extract of Convolvulus arvensis Linn in terms of quercitin equivalent (QE) was found to be 12.9 µg QE/mg. The methanolic extract exhibited scavenging potential of 27.12% and the total antioxidant capacity was found to be 26.03 µg ascorbic acid equivalent/mg extract. The plant showed highest activity against subtilis aureus while least against Escherichia coli.

Keywords: Convolvulus arvensis, Total phenolic contents, Phytochemicals, Antibacterial activity.

1. INTRODUCTION

The folkloric medicinal herbs are generally considered as a key source of novel drugs. These herbs contain many ingredients which are used for treating different diseases. Peshawar, the capital of Khyber Pakhtunkhwa Pakistan possess rich medicinal plants. Some of these medicinally important plants are widely used for various diseases and disorders but they need to be evaluated scientifically. *Convolvulus arvensis* Linn is one of the plants among them. *Convolvulus* belongs to the family of *Convolvulaceae*. *Convolvulus arvensis* is very important plant medicinally and is widely used for its medicinal purposes in Pakistan, India, Saudi Arabia, Iran and Iraq [1]. The dried plant is

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 11 | 2023
DOI: 10.5281/zenodo.10153074

thought to be more effective than the fresh one. The plant is an important folkloric medicinal plant used in many parts of the world. The active secondary metabolites of the plant mainly include glycosides, bioflavonoids, lipids, alkaloids and caffeic acid. Phytochemical analysis of *Convolvulus arvensis* revealed the existence of glycosides, bioflavonoids, lipids, alkaloids and caffeic acid. The plant contains tropane alkaloids tropine and pseudotropine All parts of the plants are reported to be used for different diseases. The plant possesses hypertensive effect, it is a diuretic, cholagogue, cathartic and significant aperient. The alcoholic and aqueous extract showed moderate tranquilizing antihaemorrhagic activity

2. MATERIALS AND METHODS

2.1 Reagents:

Methanol, Folin-coicalteu, gallic acid, quercitin, DPPH (2,2 diphenyl-1-picrylhydrazyl), ascorbic acid, sodium phosphate, ammonium molybdate, ferric cyanide, dimethyl sulfoxide, potassium ferricyanide, phosphate buffer, different strains of bacteria (*P. aeruginosa, E. coli*, *K. pneumoniae, S. aureus, B. subtilis*) and cefixime.

2.2 Instruments:

Centrifuge, digital balance, grinder, magnetic stirrer, micro pipets, petri dishes, 96 well plates, UV spectrophotometer.

2.3 Collection and identification of plant:

Plant material was collected from Peshawar, the capital of Khyber Pakhtunkhwa Pakistan and authenticated by Dr. Haroon Khan, Faculty of Pharmacy, Gomal University, Dera Ismail Khan, Pakistan.

2.4 Preparation of extract:

The plant was shade dried for 4 weeks at room temperature. The dried plant material (leaves) was comminuted by using electric grinder with final dry weight of 750 g. The extract was prepared by macerating 100 g of powder in methanol. Plant material was soaked in separate volumetric flask for three days and occasionally mixed six times a day. Afterward the extract was filtered and the residue was again dipped in the solvent. The process was repeated thrice. In this way crude extract was obtained.

2.5 Phytochemical analysis:

a. Total phenolic content determination*:*

The phenolic contents were determined as describe previously by Haq et al. [2]. Gallic acid was employed as positive standard while Folin-coicalteu as reagent. The reaction mixture which was composed of 20 µl sample 90 µl Folin-coicalteu reagent was incubated for 30 minutes followed by the addition of 90 µl of sodium bicarbonate. Absorbance of this reaction mixture was measured at 630 nm using microplate reader employing reagent as blank. This procedure was also followed for the standard and the experiment was

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 11 | 2023
DOI: 10.5281/zenodo.10153074

performed in triplicate. Calibration curve was obtained in parallel employing gallic acid as a positive control. The resultant phenolics were expressed as µg gallic acid equivalent (GAE) per mg extract.

b. Total flavonoid content determination

Total flavonoid contents were found by employing the procedure describe previously by Haq et al [3] Quercitin was employed as positive control. A mixture of 20 µl sample (4mg/ml DMSO), 10 µl of 10% aluminium chloride, 160 µl of distilled water and 10 µl of potassium acetate was incubated. Afterward absorbance was measured at 415 nm by using microplate reader. The calibration curve was drawn by employing quercitin at final concentration of 3.12, 6.25, 12.5, 25 and 50µg/ml. TFC were expressed as µg QE/ mg of extract after triplicate analysis.

2.6 Antioxidant potential evaluation:

i. Free radical scavenging assay:

The method is also called DPPH assay. The method is based on the discoloration of purple colored DPPH (2, 2 diphenyl-1-picrylhydrazyl free radical, 3.92 mg/100 ml methanol) solution. Antioxidant activity of the plant extract was found by following the standard procedure by Bibi *et al* [4]. 180 µl of DPPH aliquots was added to each well of 96 well plates followed by the addition of 20 µl sample solution in each respective well. Vitamin c was employed as reference standard. The absorbance was measured at 517 nm of the reaction mixture and the standard. The percentage radical scavenging was found by the formula

$$\% \text{ scavenging activity} = (Abc - abs)/Abc \times 100$$

Where Abc is the absorbance of negative control containing the reagent solution without sample and abs indicated the absorbance the absorbance of DPPH solution with samples.

ii. Phosphomolybdenum based total antioxidant potential determination:

Total antioxidant activity of the sample was assessed by incubating 100 µl of 4mg/ml DMSO stock solution with reagent solution which was composed of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate at 90 °C for 90 minutes [5]. Absorbance of reaction mixture was measured at 645nm by using spectrophotometer and compared with ascorbic acid which was employed as positive control and reagent except sample (negative control). The procedure was performed in triplicate.

2.7 Total reducing power assay:

The reducing power was determined by following the procedure of Jafri *et al* [6] with minor modifications. Ascorbic acid was employed as standard. A mixture of 100 µl sample, 1 % potassium ferricyanide and 0.2 ml phosphate buffer (pH 6.6) was incubated for 30 minutes at 50 C. 10 % trichloroacetic acid was added to the mixture and the mixture was centrifuged at 3000 rpm for about 10 minutes. The supernatant of reaction mixture was

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 11 | 2023
DOI: 10.5281/zenodo.10153074

transferred to 96 well plate followed by the addition of 0.1% ferric cyanide and distilled water. Phosphate buffer was employed as blank. Spectrophotometric absorbance of reaction mixture, blank and standard was measured at 760 nm. The results were expressed as µd Ascorbic acid equivalent per mg of extract after triplicate analysis.

2.8 Antibacterial assay:

The antibacterial activity of the plant extract was determined by disc diffusion method by Bibi et al [7] with minor modifications. Before the sensitivity determination the bacterial strains *Staphylococcus aureus, bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Klibesella pneumoniae* were inoculated from stored strains (4°C) into nutrient broth. The prepared inocula were incubated for 24 hours at 37°C. 100 µl of bacterial inocula were swabbed onto the petri dishes which contains fresh nutrient agar. The test sample (5 µl of 20mg/ml DMSO) infused filter paper discs were placed over the swabbed surface. Similarly discs impregnated with 5 µl of reference standards (4 mg/ml DMSO) served as positive control (cefixime, standard antibiotic) DMSO impregnated discs were used as negative control. The plates were incubated for 24 hours at 37°C. Then the zone of inhibition around the sample and standard treated discs was measured through callipers and were recorded.

2.8 Data Analysis:

Each assay in this experiment was performed in triplicates. The data is presented as mean ± standard deviation. Mean values of different assays were subjected to analysis of variance.

3. RESULTS AND DISCUSSIONS

3.1 Total phenolic and flavonoid content:

The phenolic content in methanolic extract of *Convolvulus arvensis* Linn in terms of gallic acid equivalent (GAE) was found to be 96 µg GAE/mg. While the flavonoid content in methanolic extract of *Convolvulus arvensis* Linn in terms of quercitin equivalent (QE) was found to be 12.9 µg QE/mg. Flavonoid and phenolic contents have been reported to be associated with antioxidant potential in biological systems, quenching singlet oxygen and free radicals. Antioxidant capacity of phenols is due to the methoxy, hydroxyl and ketonic group in a molecule that contains phenols. The antioxidant activity of phenols may be due to the fact that these contain methoxy, hydroxyl, double bond conjugation or ketonic group. Afshar et al [8]

3.2 Antioxidant potential evaluation:

Antioxidant activity of plant extract cannot be evaluated by single method [Yildirim et al 9] so in the current study following two methods were employed for finding antioxidant activity.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 11 | 2023
DOI: 10.5281/zenodo.10153074

i: Result of free radical scavenging Assay:

The DPPH method is preferred over other methods because it is easy, reliable and fast. The basic mechanism for DPPH antioxidant capacity is based on the ability of antioxidants to decolorize 2, 2-diphenul-1-picryl-hydrazyl. An electron from the donor antioxidant (in the sample) is accepted which leads to discolorations. This can be measured quantitatively from changes in absorbance. In this study the methanolic extract of *Convolvulus arvensis* Linn showed DPPH scavenging activity of 27.12%. Antioxidant compounds are usually in phenolic form. Phenols have the ability to destroy the radicals due to the presence of hydroxyl group. *Convolvulus arvensis* Linn whole plant has good pharmacological importance and its isolation, purification, and characterization is strongly suggested to be investigated.

ii: Total antioxidant capacity:

The total antioxidant capacity was determined by Phosphomolybdenum method. The basic mechanism of this method is based on the formation of green coloured phosphate molybdate complex in acidic environment which gives absorbance at 645 nm (Prieto) et al. [5]. The total antioxidant capacity of methanolic extract of *Convolvulus arvensis* Linn was found to be 26.03 µg AAE/mg. A positive correlation was confirmed between flavonoids, phenolics and antioxidant activity in the current study. This meets the earlier work done by many other authors who also have documented the positive correlation between TFC, TAC and radical scavenging activity (Erel et al.) [10]

3.3 Total reducing power assay:

The reducing power of the sample extract is associated with antioxidant activity and may serve as significant reflection of the antioxidant activity. The total reducing power of the methanolic extract of *Convolvulus arvensis* Linn was found to be 16.60 µg AAE/mg. There is a direct relationship between total reducing power and free radical scavenging activity of many plant extracts. The main mechanism of reducing power is that a hydrogen atom is donated by a reductant and reducing free radical into a non-reductive species thereby employing antioxidant action or potential. Reductants are associated with reducing power potential thereby donating a hydrogen atom and reducing free radical into non-reactive species and hence anti-oxidant action is produced. (Wangetal *et al*) [11]

Table 1: Summary of results of phytochemical analysis

TPC	TFC	Antioxidant potential		TRP
GAE/mg	µg QE/mg.	DPPH assay (%)	TAC (µg AAE/mg)	µg AAE/mg
96	12.9	27.12	26.03	16.60

3.4 Antibacterial assay:

The extracts showed different growth inhibition against different strains of bacteria. Both gram positive and gram negative bacteria were employed in the assay. The methanolic extract of *Convolvulus arvensis* Linn showed highest activity against the given strains of *S. aureus* (25 mm) while the least activity of both the extracts was shown by *E.coli* i.e. 0

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 11 | 2023
DOI: 10.5281/zenodo.10153074

mm or no activity. DMSO was nontoxic as there were no growth inhibition zones around DMSO impregnated discs. The growth inhibition of different bacterial strains by extract of *Convolvulus arvensis* Linn is given in Table 2.

Table 2: Growth inhibition of different bacterial strains by extract of *Convolvulus arvensis* Linn

Extract	**Gram Negative**		**Gram Positive**		
Bacterial strain	*P. aeruginosa*	*K. pneumonia*	*E.Coli*	*S. aureus*	*B. subtillis*
Growth inhibition (mm)	7	11	_	25	5
Negative control (DMSO)	-	-	-	-	-

4. CONCLUSION

The methanolic extract of medicinal plant *Convolvulus arvensis* Linn whole plant proved to have antibacterial activities based on disc diffusion method. It also shows that the plant species has excellent antioxidant potential. The plant species contains phenols and flavonoids. It has been used against different diseases but still more work needs to be done regarding its medicinal importance for the betterment of human health.

References

1) Manbir K., A. N Kalia., 2012.Convolvulus arvensis-a useful weed. International journal of Pharmacy and Pharmaceutical Sciences 4, (1). 38-40...

2) Haq, I.U., B. Mirza, S. Kanwal, M.S. Ahmad, B. Mirza (2012). *Iran. J. Pharm. Res.* 11**:** 241-49

3) Bibi, G., I.U. Haq, N. Ullah, A. Mannan, B. Mirza (2011) *Afric. J. Pharm. Pharmacol.* 5**:** 252-58.

4) Prieto, P., M. Pineda, M. Aguilar (1999). *Analy. Biochem.* 269: 337-41

5) Jafri, L., S. Saleem, I.U. Haq, N. Ullah, B. Mirza (2014) *Arab. J. Chem*.

6) Bibi, G., I.U. Haq, N. Ullah, A. Mannan, B. Mirza (2011). *Afric. J. Pharm. Pharmacol.* **5:** 252-58.

7) Afshar F, Delazar A, Nazemiyeh H, Esnaashari S, Moghadam S ,2012. Comparison of the total phenol, flavonoid contents and antioxidant activity of methanolic extracts of *Artemisia spicigera* and *A. splendens* growing in Iran. Pharmaceutical Science 18, 165–170.

8) Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V, 2000.Comparison of antioxidant and antimicrobial activities of tilia (Tilia argenta DesfEx DC), sage (*Salvia triloba* L.) and black tea (*Camellia sinensis*) extracts. Journal of Agricultural and Food Chemistry 48, 503.

9) Erel SB, Reznicek G, S¸enol SG, Nefi NUK, Konyalioglu S, Zeybek AU, 2012. Antimicrobial and antioxidant properties of *Artemisia* L. species from western Anatolia. Turkish Journal of Biology 36, 75–84

10) Wang H,Gao XD,Zhou GC,Cai L,Yao WB , 2008.Invitro and in vivo antioxidant activity of aqueous extract from *Choerospondias axillaris* fruit. Food Chemistry 106, 888-895.