Antimicrobial and Cytotoxic Potential of *Anemone tetrasepala* Royle

Tahira shabir[1], Manzoor Hussain[1*], Saleha ishfaq[2], Saba Sultan[2], Naqash khan[3], Arman jan[4]

Zubair Ahmad[5*]

[1]*Departemt of Botany Hazara University Mansehra 21120, Khyber Pakhtunkhwa, Pakistan*

[2]*Department of Botany, University of Haripur, 22660, Khyber Pakhtunkhwa, Pakistan*

[3]*Department of Zoology, University of Haripur, 22660, Khyber Pakhtunkhwa, Pakistan*

[4]*Department of Botany, University of Swabi, Anbar, Khyber Pakhtunkhwa, 23561, Pakistan*

[5]*Department of Chemistry,University of Swabi, Anbar, Khyber Pakhtunkhwa, 23561, Pakistan*

Abstract

Anemone tetrasepala Royle is a medicinal herb commonly used in folk medicine to treat various diseases. In this study, we aimed to evaluate the antimicrobial and cytotoxic potential of the methanolic extract of *Anemone tetrasepala* Royle, a wild plant grown in Dunga Gali, district Abbottabad, against Brine Shrimp larvae and bacterial strains. Our results showed that the methanolic extract of the plant exhibited significant cytotoxic potential at concentrations of 10ppm, 100ppm, and 1000ppm. Notably, the extract had the most potent cytotoxic effect on the Brine Shrimp larvae, with significant mortality observed at 100ppm and 1000ppm of the leaves extract (91% and 100%), as compared to the root extract. Furthermore, the methanolic extract of *Anemone tetrasepala* Royle also exhibited significant antimicrobial activity against the tested bacterial strains. These findings suggest that *Anemone tetrasepala* Royle has the potential as a natural source of cytotoxic and antimicrobial agents.

Keywords: *Anemone tetrasepala*; organic extracts; phytochemicals; pharmacological effects

*Corresponding authors email:za3724364@gmail.com’ mhussain8pk@yahoo.com

1: Introduction

Plants have long been recognized as valuable sources of medicinal compounds, playing a crucial role in the development of therapeutic treatments worldwide [1, 2]. Traditional systems of medicine, such as folk medicine, have relied on the use of medicinal plants for centuries to treat various ailments [3]. These plants offer a vast array of chemical constituents with potential therapeutic effects, making them a valuable resource for drug discovery and development [4-6]. *Anemone tetrasepala* Royle, a perennial herb belonging to the family Ranunculaceae, is one such medicinal plant commonly used in traditional folk medicine. It is indigenous to specific regions, such as Dunga Gali in the district of Abbottabad, where it grows in the wild. The local inhabitants have long utilized *Anemone tetrasepala* Royle for its medicinal properties, employing different parts of the plant, particularly the roots, for various therapeutic purposes [7, 8]. Numerous studies have investigated the medicinal properties of plants belonging to the Ranunculaceae family, to which *Anemone tetrasepala* Royle belongs. a study conducted to examine the root extract of *Anemone tetrasepala* and reported its traditional use as a bronchitis and sedative agent [9]. Another related species, Anemone Genius, has been studied for its analgesic and fever-reducing properties, with the root extract being utilized for these purposes [10]. The antimicrobial potential of medicinal plants has gained significant attention due to the emergence of antibiotic resistance [11]. Several studies have explored the antimicrobial activity of plant extracts from the *Ranunculaceae* family [12]. Several studies have been performed to evaluate the antimicrobial properties of various plant species in this family and reported promising results, highlighting their potential as alternative antimicrobial agents [13].

Cytotoxicity studies provide crucial insights into the potential safety and efficacy of medicinal compounds. Brine Shrimp lethality assay is a commonly used method for evaluating the cytotoxicity of plant extracts. Various studies has been conducted to evaluate the root extract of *Anemone tetrasepala* Royle exhibited significant cytotoxic effects against Brine Shrimp larvae, further supporting its medicinal potential [14].

The present study focuses on evaluating the antimicrobial and cytotoxic potential of the methanolic extract of *Anemone tetrasepala* Royle. The choice of methanolic extraction is

based on its ability to effectively extract a wide range of bioactive compounds from plant materials. This study aims to provide scientific validation for the traditional use of *Anemone tetrasepala* Royle as a medicinal herb and explore its potential for the development of novel therapeutic agents.

2. MATERIALS AND METHODS

2.1 Plant Collection

In uly 2020, Plant was collected, from dunga gali, district Abbottabad K.P.K, Pakistan. Plant identification was done by Prof. Dr. Manzoor Hussain. Plant was submitted in herbarium of Hazara University with voucher specimen number (T.S 01) and accession number 10301, 10302 and 10303.

2.2 Extract preparation

The dried plant powder was extracted by 70% methanol. The solvent was then evaporated at temperature of 40°C under reduced pressure. The residual were stored for further biological examination.

2.3 Cytotoxic effect assessment

For analyze the cytotoxic potential of research plant, Brine shrimp lethality was carried out by adopting the techniques as described by Atta-ur-Rehman [15].

2.3.1 Hatching of Brine Shrimp, Eggs

The Brine Shrimp eggs were stored in a cold environment at a temperature of approximately 4°C. The hatching tray had perforated walls that were distributed unevenly. A small amount of eggs was scattered over the solution and covered with black carbon paper to create darkness. The tray was positioned in front of a lamp. As the eggs hatched, the larvae swam quickly towards the illuminated portion of the tray.

To create artificial seawater, 38 grams of sea salt were dissolved in 1000ml of distilled water. This artificial seawater was then added to a plastic container with a hatching chamber divided into dark and light portions. The shrimp larvae were allowed to grow and develop into mature larvae over a period of two days after hatching. After 48 hours, 4ml of synthetic water was

added to each test tube containing the shrimp larvae. Ten brine shrimps were introduced into each tube, resulting in a total of 30 shrimps per dilution, and the volume was adjusted to 5ml with artificial seawater. The test tubes were exposed to light. After 24 hours, the number of dead shrimps was counted, and readings were recorded. The lethal dose (LD50) was calculated using Probit analysis at a 95% confidence level, following the method described by Kivcak et al [16]. The cytotoxic activity was illustrated in **Fig. 1.**

Fig. 1: The cytotoxic assay.

3. ANTIMICROBIAL ACTIVITY

Standard agar diffusion method of antimicrobial activity used by khalid *et al.*, 2013 [17]. Was used to find out the antimicrobial potential of *Anemone tetrasepala* Royle. Anti-bacterial activity was tested against six bacterial strains. Among them, five bacteria were gram negative while one was gram positive. The zone of inhibitions was measured in mm. Ager well diffusion method was employed to access the antimicrobial assay. Antibiotic Ciprofloxacin was used as control.

3.1 Stock solution preparation

One gram of the plant extract was used to prepare a stock solution, which was then diluted in ten ml of ethanol. To prepare the agar media, 38 grams of agar were added to one liter of distilled water. The mixture was heated on a heat plate, regulating the temperature. Once the medium started to boil, the heat plate was removed. Subsequently, the medium was autoclaved

for approximately 15 minutes. Sterilized petri dishes were prepared to hold the agar media. Approximately 25-30 ml of the medium was poured into each plate and allowed to solidify.

After the agar media in the petri dishes had fully solidified, a sterilized borer was used. The borer was sterilized using ethanol to eliminate any bacteria that may be present. The agar media was then poured into the sterilized petri dishes, and wells were created. Four wells were made: one for the root extract, two for the leaves, one for DMSO as a negative control, and one for the antibiotic.

Streaking was performed using a cotton swab. Once all the wells were filled, the petri plates were incubated for approximately 24 hours at a temperature of 37°C. At the end of the incubation period, the results were recorded. The preparation of media for antimicrobial activities has been shown in **Fig. 2**

Fig. 2: Preparation of antibacterial activity

4. RESULTS AND DISCUSSION

4.1. Cytotoxic Potential Evaluation

The current study demonstrated that the methanolic extract of the *Anemone tetrasepala* Royle plant exhibited significant cytotoxic potential at concentrations of 10ppm, 100ppm, and 1000ppm. Notably, the leaves extract of the plant showed the highest cytotoxic effect, with mortality rates of 91% and 100% observed at 100ppm and 1000ppm, respectively, compared to the root extract. The results are presented in **Table-1 and Fig. 3**. These findings suggest the presence of active constituents in the plant that possess cytotoxic properties. Further investigation is warranted to identify and characterize these specific active compounds responsible for the observed cytotoxic effects.

Table-1: Cytotoxic activity of leaves and root extract of *Anemone tetrasepala* Royle

Part of plant	Sample concentration	Total No Of Larva	Dead larva	Dead %
Leaves	10ppm	12	11	91.6%
	100ppm	12	11	91.6%
	1000ppm	12	12	100%
Root	10ppm	10	8	80%
	100ppm	10	9	90%
	1000ppm	10	9	90%

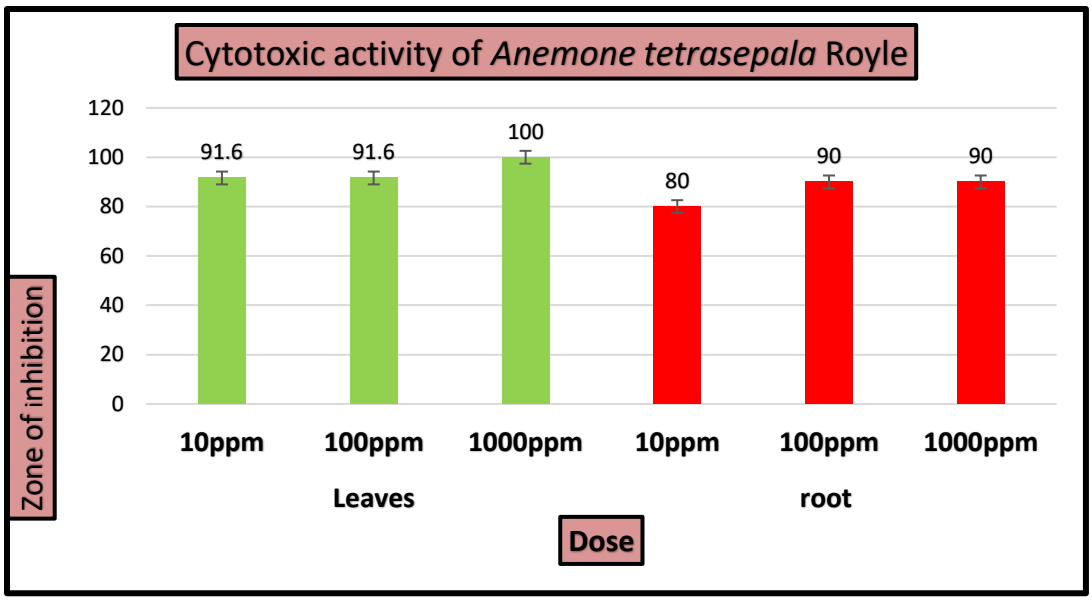

Fig. 3: Cytotoxic profile of the of leaves and root extract of *Anemone tetrasepala* Royle.

4.2 Antibacterial Activities

In the antibacterial assay, the crude extracts of the *Anemone tetrasepala* Royle plant's leaves and root, dissolved in methanol, were tested against different bacterial strains. The maximum zone of inhibition was observed against *E. coli*, measuring 20mm. On the other hand, *Staphylococcus* aureus exhibited the minimum zone of inhibition, measuring 13mm. The results are presented in **Table-2 and Fig. 4.** When the crude extract of the root of the studied plant was dissolved in methanol and tested against various bacterial strains, *Pseudomonas* showed the highest zone of inhibition (19mm), while the lowest zone of inhibition was recorded against *Staphylococcus* aureus (13mm)**.**

Table-2: Anti-bacterial activity of leaves and root extract of *Anemone tetrasepala* Royle

Bacterial strains	Antibiotic (ZOI)	Leaves (ZOI)	Mean±SD	Root (ZOI)	Mean±SD
E. coli	25mm	19mm	19.3±4.24	14mm	19.5±7.77
Staphylococcus aureus	18mm	18mm	18.5±0.5	13mm	15.5±3.53
K. pneumoniae	29mm	19mm	24.6±7.07	15mm	22±9.89
Salmonella. typhi	23mm	15mm	20.5±6.36	14mm	18.5±6.36
Acenitobacter	16mm	13mm	14.5±2.12	13mm	14.5±2.12
P . aeruginosa	19mm	16mm	17.5±2.12	18mm	18.5±0.70

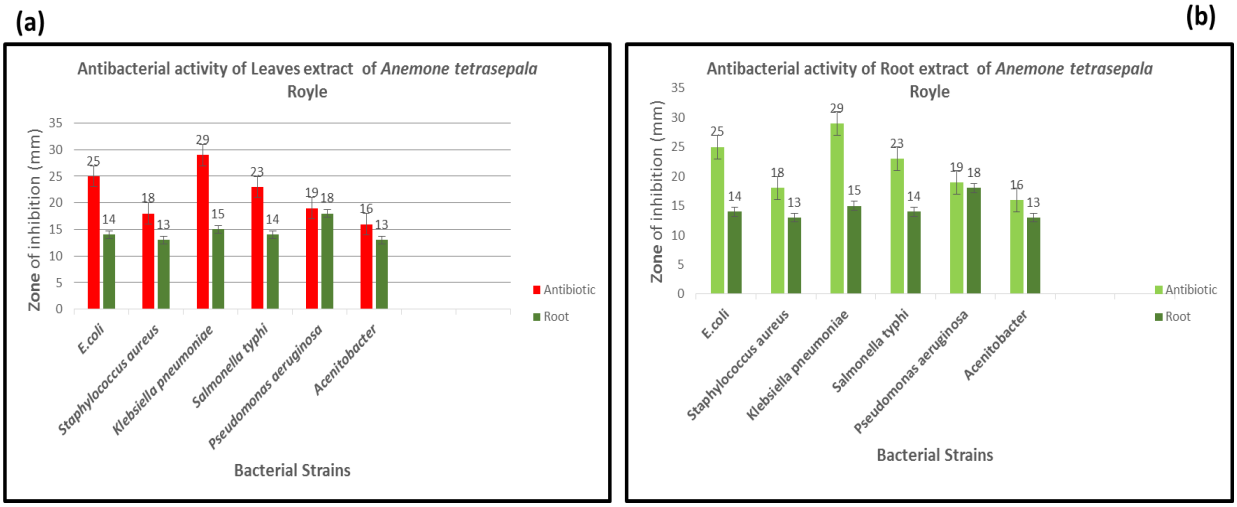

Fig. 4: Antibacterial activity of *Anemone tetrasepala* royle leaves (a) and roots extract (b).

5.0 DISCUSSION

The observed cytotoxic and antimicrobial effects of *Anemone tetrasepala* Royle align with previous studies on other Anemone species. For instance, Anemone cathayensis has been reported to exhibit antimicrobial, antioxidant, and anticancer activities. The wide range of pharmacological actions displayed by various Anemone species highlights their potential as valuable sources of natural compounds with diverse medicinal properties. The present study provides important insights into the therapeutic potential of *Anemone tetrasepala* Royle. The cytotoxic activity of the plant extract suggests its possible use in cancer treatment or as a source for developing new cytotoxic agents. Additionally, the demonstrated antimicrobial activity against bacterial strains indicates the potential use of *Anemone tetrasepala* Royle in combating bacterial infections. Further research is necessary to identify the specific active compounds responsible for the observed cytotoxic and antimicrobial effects of *Anemone tetrasepala* Royle. Isolation and characterization of these bioactive constituents can contribute to the development of novel drugs or therapeutic agents with enhanced efficacy and reduced side effects.

5.0 CONCLUSIONS AND RECOMMENDATIONS

Our finding revealed that plant has significant antimicrobial and cytotoxic activity. On the basis of these result plant appear to be good and safe natural microbial agent. Further studies should be done to search new compound from it. According to the cytotoxic assay, there may be certain active constituents present in these plants that have the capacity to demonstrate cytotoxic potential, and a further investigation may be carried out for the limitation of particular active cytotoxic substances. Plant extract's antimicrobial activity indicated that it may be used to treat various illnesses as well as a source of novel antibiotics.

Acknowledgements

We would like to acknowledge University of Swabi, Pakistan, University of Haripur, Pakistan and Hazara University Pakistan for joint collaboration and providing research facilities.

Conflict of interest

None declared.

References

[1] R.E. Schultes, The future of plants as sources of new biodynamic compounds, in: Plants in the development of modern medicine, Harvard University Press, 1972, pp. 103-124.
[2] F. Stéphane, B. Jules, G. Batiha, I. Ali, L.N. Bruno, Extraction of bioactive compounds from medicinal plants and herbs, Nat Med Plants, (2021).
[3] H. Rahman, A. Rauf, S.A. Khan, Z. Ahmad, A. Alshammari, M. Alharbi, A. Alam, H.A.R. Suleria, Green Synthesis of Silver Nanoparticles Using Rhazya stricta Decne Extracts and Their Anti-Microbial and Anti-Oxidant Activities, Crystals, 13 (2023) 398.
[4] A.K. Shakya, Medicinal plants: Future source of new drugs, International journal of herbal medicine, 4 (2016) 59-64.
[5] E. Christaki, E. Bonos, I. Giannenas, P. Florou-Paneri, Aromatic plants as a source of bioactive compounds, Agriculture, 2 (2012) 228-243.
[6] H. Chandran, M. Meena, T. Barupal, K. Sharma, Plant tissue culture as a perpetual source for production of industrially important bioactive compounds, Biotechnology reports, 26 (2020) e00450.
[7] X. Liao, B. Li, L. Ding, Y. Pan, Y. Chen, Triterpenoid saponins from Anemone tetrasepala, Chinese Journal of Organic Chemistry, 21 (2001) 299.
[8] H. Sher, I. Inamuddin, Z. Khan, R.W. Bussmann, I.U. Rahman, Medicinal plant diversity of Hindubaig Mountain, Lalku Valley, District Swat, Pakistan, Ethnobotany Research and Applications, 20 (2020) 1-13.
[9] D.-C. Hao, X. Gu, P. Xiao, Anemone medicinal plants: ethnopharmacology, phytochemistry and biology, Acta pharmaceutica sinica B, 7 (2017) 146-158.
[10] F. Bhat, D. Mahajan, M. Sayyed, A. Bhat, Ethno-Medicinal survey of North Kashmir Himalaya-a case study of Lolab valley (J&K), India, Ecology, Environment and Conservation, 20 (2014) 59-71.
[11] M. Tariq, Z. Ahmad, S.A. Shah, Z. Gul, S.A. Khan, Phytochemical Analysis and Antibacterial Activity of Nicotiana tabacum and Nicotiana rustica, RADS Journal of Biological Research & Applied Sciences, 12 (2021) 60-65.
[12] H. Da-Cheng, X. Pei-Gen, M. Hong-Ying, P. Yong, H. Chun-Nian, Mining chemodiversity from biodiversity: pharmacophylogeny of medicinal plants of Ranunculaceae, Chinese journal of natural medicines, 13 (2015) 507-520.
[13] Y.-K. Goo, Therapeutic Potential of Ranunculus Species (Ranunculaceae): A Literature Review on Traditional Medicinal Herbs, Plants, 11 (2022) 1599.
[14] P.K. Rana, P. Kumar, V.K. Singhal, Spindle irregularities, chromatin transfer, and chromatin stickiness during male meiosis in Anemone tetrasepala (Ranunculaceae), Turkish Journal of Botany, 37 (2013) 167-176.
[15] M.I. Choudhary, W.J. Thomsen, Bioassay techniques for drug development, CRC Press, 2001.
[16] B. Kivçak, T. MERT, H.T. ÖZTÜRK, Antimicrobial and cytotoxic activities of Ceratonia siliqua L. extracts, Turkish Journal of Biology, 26 (2002) 197-200.
[17] A. Khalid, U.-u.-. Rehman, A. Sethi, S. Khilji, U. Fatima, M.I. Khan, M.K. Waqas, Q. Saqib, K. Farzana, M. Asad, Antimicrobial activity analysis of extracts of Acacia modesta, Artimisia absinthium, Nigella sativa and Saussurea lappa against Gram positive and Gram negative microorganisms, African Journal of Biotechnology, 10 (2011) 4574-4580.