Research Article

Evaluation of Phytochemicals, Antioxidant, and Antibacterial Potential of *Artemisia maritima* Various Parts from Lower Dir Pakistan

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Abstract | The use of herbs for medicinal purposes has trace back to ancient times. The current study was conducted with aim, to assess the phytochemicals, antibacterial and antioxidant potential of the methanolic crude extract of leaves, stem, and roots of *Artemisia maritime*. The phytochemical screening of *A. maritime* leaves, stem and roots extract indicates the existence of flavonoids, terpenoids, saponins, tannins, phenolics steroids and carbohydrates but deficient of proteins. In minimum inhibitory concentration assay, the crude methanolic extracts showed significant inhibition against all tested bacterial strains at25, 50 and 100 µg/ml. The methanolic crude extract of *A. maritime* various parts showed MIC of 37.5µg/ml for *S. aureus* which is gram-positive bacteria followed by 75µg/ml for *P. aeruginosa,* (gram negative), and *B. subtilis* (gram positive) that is nearly similar to the activity of ciprofloxacin (standard). The methanolic leaves extract of *A. maritime* displayed the highest scavenging activity (78.09µg/ml) in DPPH, while stem methanolic extract showed 61.81 µg/ml activities. In ABTS the highest activity (84.13µg/ml) was observed on leaves extract and lowest (69.18µg/ml) on stem extract. Our current study revealed that *A. maritime* root exhibited significant antioxidant potential and good antibacterial effect which suggested its usage for treatment and management of different contagious diseases. In the present study we concluded that *A. maritima* possess therapeutic effectiveness.

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Introduction

Plants have bioactive chemicals that are rich source of medicine for many ailments almost everywhere around the world (Khan *et al.*, 2022, 2023). Medicinal plants have significant health effects on animals as well as human and documented to have emerging influence on pharmaceutical industries (Sen and

Samanta, 2015). The medicinal value of plants has been utilized since ancient times, trace back to thousands of years (Bussmann and Sharon, 2006). The medicinal values of plants are attributed to the presence of various chemical constituents (Edeoga *et al.*, 2005). These constituents have a distinct physiological action on human's body. Most of the phytochemical constituents that medicinal plants possess have antioxidant, antimicrobial, anti-inflammatory, phytotoxic and cytotoxic potential (Kotan *et al.*, 2013). In many plants, a variety of bioactive molecules such as flavonoids, alkaloids, tannins, terpenoids, saponins phenolics etc. are presents (Shinwari *et al.*, 2013). Treatment of various ailments are carried out through plants possessing curative nature. The use of natural products and secondary metabolites originated from living system, mainly from plants had shown a boost to health care since ancient time. Furthermore, the modern medical science success rate is also dependent on the drugs that are acquired from natural resources (Rahman *et al.*, 2023). Plant extracts can improve the nutritional value by minimizing microbial growth and lipid oxidation (Zhang *et al.*, 2016). Many plant species and herbs preservative effect, recommends the presence of antimicrobial and anti-oxidative ingredients in their tissues. Plants having phenols and flavonoids are considered to have potent antioxidant potential to avoid the oxidative effect produced by oxygen and photons (Zhang *et al.*, 2013). In humans many pathogenic microorganisms had developed resistance to commercially available antimicrobial agents due to the unselective usage. Antibiotics that are effective against bacterial infections a decade ago had lost its effectiveness due to the emergence of bacterial resistance. The scientists are forced by this situation for finding novel antimicrobial compounds from many sources like plants. The medicinal nature of plants is consider a worthy source of innovative antimicrobial substances (Chassagne *et al.*, 2021). The numerous phytochemicals which have repressing effects on different types of pathogenic microorganism are gaining interest of scientists for drug development. The presence of diverse active chemical groups and the ability to combat infectious diseases reinforces the ongoing identification and study of medicinal plants for their antibacterial and antioxidant properties.

Artemisia is one of the diverse Genra of the Asteraceae which is medically important having essential oils and secondary metabolites. This Genra is widely spread in the northern frontier region, Karakoram, Himalaya, Hindukush region Gilgit, Skardu and Kashmir (Mannan *et al.*, 2010). The genus *Artemisia* known as "worm wood" a largest group comprising 800 species or more and are cosmopolitan distribution (Mirjalili *et al.*, 2007; Wright, 2002). Many species had been reported of genus *Artemisia* from various countries. India reported 34 species whereas 15 species were recognized in the flora of Lahaul and Spiti, (Aswal and Mehrotra, 1994). *A. meritima* known *as* sea worm wood is an important member of the Asteraceae. Due to its strong aromatic shrub nature, grow in dry and stony regions having intense cold habitat (Kumar *et al.*, 2011). The leaves powder was used as folk medicine for digestive disease treatment and stem for treating erythremia (Khan *et al.*, 2011; Gilani *et al.*, 2003). The goal of present work is the evaluation of antibacterial effect for *A. meritima* roots, stem and leaves against gram-negative and gram-positive bacterial strain relative to standard antibiotics ampicillin and ciprofloxacin at different concentration and antioxidant potential. The goal of present work is the evaluation of antibacterial effect for *A. maritime* roots, stem and leaves against gram-negative and gram-positive bacteria strain to standard antibiotics e.g., ampicillin at different concentration and antioxidant potential.

Materials and Methods

Study area

The aerial parts (roots, stem and leaves) of *A. maritima* were collected form Timergara, Dopa and, Chakdara Lower Dir, Pakistan and were identified by Plant taxonomist, Dr. Ali Hazrat, Lecturer, Department, of Botany, University of Malakand. The specimen was deposited in the herbarium of University of Malakand under voucher number Am2/6/18 (Figure 1).

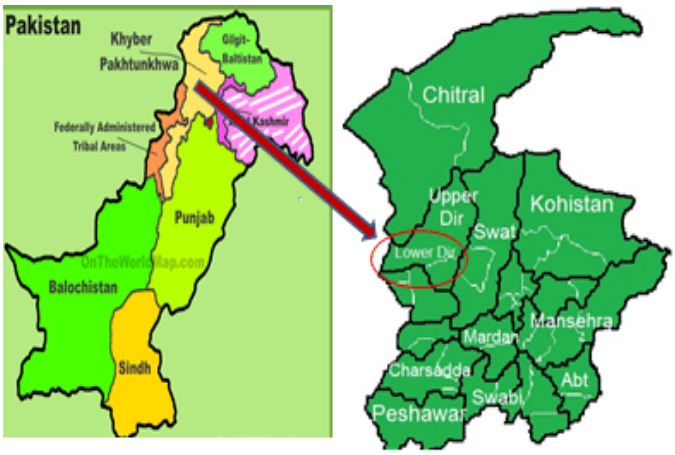

Figure 1: *Study map of lower Dir.*

Sample preparation

After collection and authentication, leaves, stem, and roots of selected plant specimen was rinse with tap water and then shade dried. After drying the specimen was pulverized and soaked in methanol for a prescribed period with occasional shaking (Ahmad *et al*., 2016). The specimen was filtered by a rotary evaporator to produce a semisolid mass and the crude drug was packed in a clean beaker.

Phytochemical analysis

For the presence of bioactive compounds, various chemical tests were carried out on each fraction of plant species by using standard procedures of for tannins test, (De Silva *et al*., 2017) for phenolic and protein test, (Mir *et al*., 2013) for saponins and terpenoid test, (Prabhavathi *et al*., 2016) for flavonoid and carbohydrate test and (Islam *et al*., 2016) for steroid test.

Antibacterial activity

The methanolic crude extract of *A. maritime* roots, stem, and leaves (AmR, AmS, AmL) were tested against *P. aeruginosa,* (gram negative), *S. aureus* and *B. subtilis* (gram positive), with the application of agar well diffusion technique (Jagessar *et al*., 2008). The prepared media (1000-ml distil water containing 28 g Nutrient agar) were sterilely poured onto the sterile Petri plates (20ml/plate) and allowed to solidification. The bacterial culture (10^6 to 10^8 CFU/ml) with sterile cotton swab was spread on media and then wells were bored in each plate with sterilized cork-borer (6mm diameter). 100µl drugs were added from each concentration (25, 50 and 100µg/ml) to different labeled well and allowed to diffuse by refrigerating for 30min. The plates were incubated for 24 hours at 37 ºC. Each treatment was performed in triplicate, and the zone of inhibition (ZOI) was measured in millimetres (mm). As a negative control, DMSO (dimethyl sulphonic acid) was used. The measurement was work out through the ZOI in millimeters (mm) and antibacterial potential was find out in comparison of ampicillin and ciprofloxacin (Shoaib *et al*., 2016).

Minimum inhibitory concentration (MIC)

The MIC values were calculated by using the methanol crude extract from selected parts of *A. maritime* against tested microorganisms. Broth dilution technique was used for the determination of MIC values. The tubes were incubated for turbidity at 37ºC for 24 hours and examined. No antimicrobial agent was added to a control tube and ciprofloxacin was used as standard. The lowest concentration was regarded as MIC on which the growth and activity of bacteria cease (Shoaib *et al*., 2016).

Antioxidant activities

Antioxidant potential of plant extracts was screened through DPPH and ABTS free radicals.

DPPH assay

1,1-diphenyl-2-picrylhydrazyl assay (DPPH) assay was used for the determination of antioxidant potential of crude extract of *A. maritima* leaves, stem, and roots. 5 ml of 0.004% (w/v) methanolic solution of DPPH and 50 µL of 2mg/mL leaf extract was added with reference to 80% methanol as blank. After 30 min of incubation, absorbance was measured at 517 nm. The free radical scavenging activity of DPPH (%) was calculated using the following formula.

$$\text{DPPH scavenging activity \%} = A_0 - A_1 A_0 \times 100$$

Where the absorbance of the plant sample is A_1, and the control absorbance is A_0. The scavenging percentage of *A. maritima* different parts extract (roots, stem and leaves) were compared with positive controls i.e., Vitamin C (Oktay *et al*., 2003).

ABTS assay

The antioxidant capacity of plant extract was estimated using the 2,2'-Azino-Bis-3-ethylbenzthiazoline-6-sulphonic acid radical cation depolarization assay. ABTSradical cation was created in water by the reaction between 07mM ABTS and 2.45mM potassium persulfate (1:1) and then placed in dark at normal temperature for a period of 12-16 hours. After the initial mixing, 5µl of crude drug was added to 3.995ml of diluted $ABTS^{\cdot+}$ solution and the absorbance was measured after 30 min. In each assay methanol (solvent) blank was run as a reference. The whole process was performed several times for accurate value. By using the formula described by (Rajurkar and Hande, 2011), percent inhibition of absorbance at 734 nm was calculated.

ABTS+ scavenging effect (%) = ((AB –AA)/AB) ×100 (2), where AB is absorbance of ABTS radical + methanol; AA is absorbance of ABTS radical + sample extract/standard.

Results and Discussion

Phytochemical screening

Different tests were conducted for the detection of various metabolites like saponin, tannins, terpenoids, protein, carbohydrates, phenolic compounds, steroids, and flavonoids in extract of *A. maritima*. The screening for phytochemicals showed the existence of carbohydrates, terpenoids, tannins steroids, saponins and flavonoids while proteins were absent. Results were displayed in the (Table 1) indicate that *A. maritima* leaves, roots and stem contain various phytochemicals which suggests the medicinal value of the selected species. A study explored that the presence of different phytochemical constituents (alkaloids, flavonoids, saponins, tannins, and steroids) in the extracts are responsible for antifungal and antibacterial activities (Salhi *et al*., 2017). Our analysis showed the presence of phenolic and flavonoid contents which are known as "biological response modifiers" because of its role in modifying virus and allergens and their role as an antioxidant and antimicrobial. In accordance to our data (Umamaheswari and Sangeetha, 2015) showed that presence of phenolic and flavonoid content can induce antibacterial response. The medicinal virtue of *A. maritima*can be attributed to the presence of a variety of phytochemicals and need to be further elucidated.

Table 1: *Phytochemical screening of A. maritima roots, stem, and leaves.*

Phytochemicals	Leaves	Stem	Root
Carbohydrates	++	++	++
Proteins	– –	– –	– –
Phenolics	++	++	++
Saponins	++	++	++
Flavonoids	++	++	++
Tannins	++	++	++
Terpenoids	++	++	++
Steroids	++	++	++

Anti-bacterial activities

The crude methanolic extract from selected parts of *A. maritima* was screened against gram-negative and gram-positive bacteria species through agar well diffusion method. The data obtained was presented in Table 2. The results showed that highest ZOI was observed by using AmRat 100µg/ml against gram-negative bacteria *P. aeruginosa* (23.78±1.31mm), followed by gram-positive bacteria *B. subtills* and *S. aureus* (21.51±1.71mm) and (19.26±2.71mm), respectively. The methanolic extract of AmL showed the highest activity against gram-negative bacteria *P. aeruginosa* with 18.15±1.73mm ZOI at 50µg/ml and 17.39±0.87 at 100µg/ml. The lowest ZOI was recorded at 25µg/ml for AmS against Gram positive *S. aureus* (8.91±1.19) followed by gram negative *P. aeruginosa* (9.34±1.66) and gram-positive bacteria *B. subtilis* (10.23±2.08). Ampicillin and Ciprofloxacin was used as a standard antibiotic disc in each plate and both gram positive and negative bacterial strain showed higher inhibition zone (Table 2). Among the methanolic extract of different parts of *A. maritima,* AmR showed higher antibacterial activity compared to AmL and AmS (Table 2). A study reported that essential oils in *A. maritima* attributed to inhibition activity both in gram positive and negative bacterial strains (Sharma *et al*., 2014). Further investigation on *A. maritima* will help to explore the types of oils present in different extract isolated from various parts. A previously reported study on *A. douglasiana* showed the presence of camphor which perform bacteriostatic activity (Tirillini *et al*., 1996). Based on the previous research on *Artemisia* genus and current investigation on *A. maritima* concluded that this high-altitude medicinal plant showed antibacterial potential against a broad range of Gram positive and negative bacteria.

Table 2: *Antibacterial activities of A. maritima leaves, roots, and stem.*

Crude samples	Concentration (µg/ml)	Zone of inhibition (ZOI) (mm)		
		Gram-positive bacteria		Gram-negative bacteria
		B. subtilis	*S. aureus*	*P. aeruginosa*
AmL	25	12.59±1.71	10.67±1.51	13.29±1.54
	50	15.18±1.91	13.22±1.25	18.15±1.73
	100	16.65±1.18	15.91±1.73	17.39±0.87
AmR	25	18.17±2.17	20.67±1.13	21.02±1.47
	50	19.11±1.81	18.58±1.10	19.36±1.07
	100	21.51±1.71	19.26±2.71	23.78±1.31
AmS	25	10.23±2.08	8.91±1.19	9.34±1.66
	50	13.76±2.15	10.12±1.21	11.06±0.83
	100	13.09±1.71	10.67±1.45	11.54±1.24
Ciprofloxacin		31.35±2.01	35.12±1.39	32.01±1.05
Ampicillin		29.09±1.78	38.15±1.51	33.12±0.96

The MIC (µg/ml) of crude drugs for various parts of the selected plant against Gram-negative and Gram-positive bacteria were calculated (Table 3). It

is observed that AmR and AmL possess inhibitory capacities at low concentrations against tested bacteria. The AmR extract showed MIC of 37.5µg/ml for gram-positive bacteria*S. aureus*, 87.5µg/ml for bacteria *B. subtilis* and 75µg/ml for*P. aeruginosa.* Similarly, AmS showedMIC of 75µg/ml for *P. aeruginosa.*In accordance to the current investigation (Al-Moghazy *et al.*, 2017) reported the antibacterial potential of artemisia and portulaca plant extracts against, *S. aureus, Streptococci, B. dysenteriae, E. coli, B. subtilis, B. typhi,* and Pseudomonas. Study reported that the essential oil (EO) present in the plant extract showed antibacterial potential against antibiotic resistant *E. coli* dhpα-pUCl8 strain with MIC of 6.25mg/mL (Petrosyan *et al.*, 2018). The EO obtained from *A. dracunculus* can be used as antimicrobial in cosmetics, medicine, and food (Petrosyan *et al.*, 2018). The current investigation revealed that the methanolic extract of various parts of *A. maritima* showed more effective inhibition of Gram-positive bacteria with lower MIC (Table 3).

Table 3: The antibacterial activity MIC of selected *Artemisia* species crude extract.

Crude extract	MIC (µg/ml)		
	Gram-positive bacteria		Gram-negative bacteria
	B. subtilis	***S. aureus***	***P. aeruginosa***
AmL	75	75	87.5
AmR	87.5	37.5	75
AmS	100	87.5	75
Ciprofloxacin	6.25	6.25	6.25

Antioxidant activities

The antioxidant potential of leaves, stem, and roots of the selected plant was determined *via* DPPH and ABTS assay. Ascorbic acid was taken as a standard. The result of antioxidant activity against DPPH and ABTS are presented in Table 4. The antioxidant DPPH activity checked on different concentrations showed a concentration dependent % inhibition. The highest % inhibition (81.61±0.84) was observed in the methanolic extract of AmR at 1000µg/ml. At the same concentration AmL showed 74.14±0.86 % inhibition whereas AmS showed 81.22±1.23.

The antioxidant potential through ABTS assay showed AmS at 1000µg/ml had higher % inhibition (78.39±1.03) followed by AmR (77.21±1.09), and the lowest activity was observed in AmL (75.87±1.20). By comparing the IC50 value for both assays, DPPH showed reduction in IC50 value relative to ABTS. Another study (Mak *et al.*, 2013) reported that the ethanolic and aqueous extract of *Cassia* and *Hibiscus* showed a higher scavenging effect on DPPH.

Table 4: *Antioxidant activities of Artemisia maritima leaves, roots, and stem.*

Name	Code	Concentration (µg/mL)	DPPH % inhibition	IC_{50} (µg/mL)	ABTS % inhibition	IC_{50} (µg/mL)
Artemisia maritima	AmL	1000	74.14±0.86	78.09	75.87±1.20	
		500	66.22±1.37		67.21±1.39	
		250	62.09±1.33		61.34±1.21	84.13
		125	57.48±1.36		54.76±1.65	
		62.5	47.91±1.42		47.44±1.31	
Artemisia maritima	AmR	1000	81.61±0.84	60.34	77.21±1.09	77.92
		500	77.09±1.04		75.41±1.18	
		250	73.08±0.57		67.29±1.37	
		125	67.21±1.11		63.09±1.33	
		62.5	59.17±0.66		57.41±1.36	
Artemisia maritima	AmS	1000	81.22±1.23	61.81	78.39±1.03	69.18
		500	77.74±0.22		74.38±1.17	
		250	76.82±1.07		70.45±1.64	
		125	64.59±0.32		64.61±1.21	
		62.5	58.31±0.16		56.41±1.32	
Ascorbic acid		1000	86.50±0.00	<1	85.37±0.87	<1
		500	86.33±0.16		84.52±0.22	
		250	86.23±0.14		83.67±1.39	
		125	85.00±0.28		82.09±1.31	
		62.5	84.00±0.28		80.11±1.01	

The natural antioxidants got much more attention because of their health benefits in recent years. Drug formulations based on antioxidant are very important both for preventing and management of many diseases. Antioxidant work by scavenging free radicals and prevent oxidation which may be caused by reactive oxygen species (ROS) overproduction in the body (Ali *et al.*, 2008). The ROS are extremely harmful due to its attack on macromolecules like DNA, proteins, lipids and resulting in cancer, genotoxicity, arthritis, arteriosclerosis, diabetes, inflammation, and neurological diseases like AD (Shah *et al.*, 2015). It is reported that plant having medicinal properties are rich source of phenolic compounds such as flavonoids, saponin, tannins, terpenoids that have many biological functions like antioxidants (Rubio *et al.*, 2013; Stankovic *et al.*, 2016). The current study evaluated the methanolic extract of different parts of *A. maritima* demonstrated antioxidant characteristics. Our results are in accordance with Kiran *et al.* (2018) which showed the presence of flavonoid and phenolic

in seven kind of roses and their high antioxidant potential. The various phytochemicals like alkaloids, tannins, terpenoids, flavonoids, vitamins, and anthocyanins can also affect DPPH scavenging activity due to structure and biological assets (Kiran *et al*., 2018).

Conclusions and Recommendations

In current study we attempted to evaluate the phytochemical constituents and efficacy of methanolic extract of various parts of *A. maritima against* bacterial infections and oxidative stress. To the best of our knowledge and search this is the first study evaluating the antibacterial and antioxidant potential and it was concluded that *A. maritima* possess therapeutic effectiveness. The diversified phytochemical constituents like flavonoids, phenolics, saponin, tannins present in *A. maritima* were speculated to attribute for the antibacterial and antioxidant efficacy. Further studies on the antioxidant capability will open a new area of research to explore its efficacy against disorders related to oxidative stress and may serve as potential candidates for antioxidant enzymes like catalase and superoxide dismutase. The antibacterial potential of *A. maritima* will be enhanced by further investigation and isolating active compounds. It is speculated that active compounds may serve as an alternate to microorganisms that have developed resistance to many other antimicrobial agents.

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Novelty Statement

To the best of our knowledge and search this is the first study evaluating the antibacterial and antioxidant potential and it was concluded that *A. maritima* possess therapeutic effectiveness.

Author's Contribution

Tabinda Nowsheen, Ali Hazarat: Conception and design.
Tabinda Nowsheen, Sayed Wadood Ali Shah, Muhammad Ajmal Khan: Development of methodology.
Tabinda Nowsheen, Muhammad Yahya, Muhammad Mukhtiar: Acquisition of the data.
Sayed Wadood Ali Shah, Ali Hazarat, Muhammad Ajmal Khan, Gul Rahim: Writing, review, and/or revision of the manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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