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Phytochemical, antioxidant and antibacterial screening of *Artemisia Absinthium* from Dir Lower Pakistan

Tabinda Nowsheen[1*], Sayed Wadood Ali Shah[2], Ali Hazrat[1] , Gul Rahim[1], Abdul Khaliq[3], Ikram Ilahi[4] , Abdur Rahim[4], , Shah Zaman[1], Muhammad Asif Nawaz[5], Sidra Pervez[6], Zakia Ahmad[7], Muhammad Ibrar[2], Shabana Bibi[1], Jehan Zada[1], Amir Hassan Khan[8] , Muhammad Romman[9] and Afshan begum[10]

[1]Department of Botany University of Malakand, Chakdara, Dir Lower, KP, **Pakistan**
[2]Department of Pharmacy, University of Malakand, **Pakistan**
[3]Department of Chemistry Shaheed Benazir Bhutto University Sheringal Dir Upper, **KP, Pakistan**
[4]Department of Zoology University of Malakand, Chakdara, Dir Lower, KP, **Pakistan**
[5]Department of Biotechnology Shaheed Benazir Bhutto University Sheringal Dir Upper, KP, **Pakistan**
[6]Department of Biochemistry, Shaheed Benazir Bhutto Women University, Peshawar, **Pakistan**
[7]Department of Botany University of Swat, KP, **Pakistan**
[8]Department of Botany Shaheed Benazir Bhutto University Sheringal Dir Upper, KP, **Pakistan**
[9]Department of Botany University of Chitral, KP, **Pakistan**
[10]Department of Chemistry University of Malakand, KP, **Pakistan**

***Correspondence:** aliuom@gmail.com **Received 21-06-2020, Revised: 01-09-2020, Accepted: 20-09-2020 e-Published: 30-09-2020**

The current study was carried out, to explore the Antioxidant and antibacterial potential of the crude methanolic extract of selected species and estimation of antibacterial activities of the selected *Artemisia* species *(A. absinthium L.*). The antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay, the methanolic extract displayed the highest scavenging activity in (DPPH) was (89.58µg/ml) on *Artemisia absinthium L.* root and the lowest was 36.14µg/ml on *Artemisia absinthium L.* leaves, while in (ABTS) the highest activity was 93.35µg/ml on *Artemisia absinthium L.* stem and lowest was 46.31µg/ml on *Artemisia absinthium* leaves. In minimum inhibitory concentration assay the crude methanolic extracts showed significant inhibition against all tested microbes on different concentrations like 25 µg/ml, 50 µg/ml, and 100 µg/ml. The extract (*Artemisia absinthium* leaves) AaL showed MIC of 12.5µg/ml for *B. subtilis* which is gram positive bacteria, 12,5µg/ml for gram positive bacteria *S. aureus* and 6.25 µg/ml for gram negative bacteria *P. aeruginosa* that is almost equal to the response of standard ciprofloxacin. Our current study revealed that *Artemisia absinthium L.* root (AsR) exhibited a significant antioxidant potential while AaL showed good antibacterial effect which is suggested to be used for treatment and management of different infectious diseases.

Keywords: *Artemisia absinthium,* Antioxidant potential, antibacterial activity

INTRODUCTION

Medicinal plants have intense effect on the health of animals as well as plants. The literature showed that medicinal plants are the emerging power of pharmaceutical companies. Most of the phytonutrients present in medicinal plants have

antioxidant, antimicrobial, anti-inflammatory, phytotoxic and cytotoxic activities (Kotan et al. 2013, Narayanaswamy and Balakrishnan, 2011, Balakrishnan et al. 2013).

Richest source of drug is medicinal plants by carrying new therapeutic agents. Economical source of therapy to population is traditional medicines. Our country has a rich store of undiscovered Phyto medicinal flora (Ahmad et al. 2015). Herbal medicines extensive use for health care has been noted and many natural products with novel healing properties are commercialized (Riaz and Rahman, 2015). Plants medicinal properties have been investigated in the light of conversant scientific expansion all over the world, because of their low toxicity and rich pharmacological applications (Vaquero et al. 2010). Different medicinal plants are used for the treatment of different diseases. Plant extracts and their varied formulations in the therapeutic utilization of many diseases in traditional remedy goes back to ancient time (Kamal et al. 2016). Artemisia is one of the diverse genera of Asteraceae family which is medicinally vital with many essential oils and secondary metabolites. Different species of Artemisia are categorizing in to different groups based on their biological activities (Ahameethunisa and Hopper, 2010). Natural extract of different synthetic compounds are phenolic rich compounds obtained from plants can boost the overall quality of food by reducing microbial growth and lipid oxidation (Zhang et al. 2016).

Many plant species and herbs preservative effect recommends the presence of antimicrobial and antioxidative ingredients in their tissues. It is knowledgeable that between antioxidant status and incidence of human diseases, there is an inverse relationship such as neurodegenerative disease, atherosclerosis, aging and cancer (Morales et al. 2008). The goal of this work is the evaluation of antibacterial effect for A.absinthium against gram positive and gram negative bacteria to standard antibiotics e,g ampicillin and ciprofloxacin at different concentration.

MATERIALS AND METHODS

The research study was carried out in the pharmacognosy lab, department of Pharmacy, University of Malakand.

Collection and authentication

Different species of Artemisia (*Artemisia absinthium*L.) were collected from district Dir lower and were identified by Dr Ali Hazrat,Dept of botany,University of Malakand . *The specimens were deposited in herbarium of University of Malakand under voucher,* Aa3/6/18*. Artemisia absinthium.*

Maceration

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

Phytochemical analysis

For the presence of bioactive compounds, various chemical tests were carried out in each portion of all plant species by using standard procedures of Doss (2009) 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.

Antioxidant activity

Antioxidant potential of plant extracts of *Artemisia absinthium* leaves, roots and stem was screened through DPPH and ABTS free radicals

DPPH Activity

All parts (root, flower, leaf and stem) antioxidant activity was determined *via* 1,1-diphenyl-2-picrylhydrazyl assay (DPPH). 5 ml of 0.004% (w/v) solution of DPPH in methanol 50 µL of 2.0 mg/mL leaf extract (or 80% methanol as blank) was added. By using dark box, it was kept for 30 min of incubation. Plant parts which were leaves (2.0 mg/mL), roots (2.0 mg/mL) and stem (2.0 mg/mL) used for the same procedure. Ascorbic acid was used as standard. After 30 min of incubation, absorbance was measured at 517 nm.. The scavenging DPPH activity (%) was calculated using the following formula.

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. All the plant extracts (root, leaf and stem) scavenging percentage was compared with positive controls (Oktay et al. 2003).

ABTS activity

On plant samples free radical scavenging activity was determined by ABTS via 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid radical cation decolorization assay. Before use stored in the dark at room temperature for 12-16 h, ABTS·cation radical was produced in water by the reaction between 7 mM ABTS and 2.45 mM potassium persulfate (1:1). With methanol ABTS·+ solution was diluted to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µl of plant extract to 3.995 ml of diluted ABTS·+ solution, after the initial mixing the absorbance was measured at 30 min. In each assay an appropriate solvent blank was run. All the measurements were carried out five times. By using the formula calculated percent inhibition of absorbance at 734 nm, ABTS·+ scavenging effect (%) = ((AB–AA)/ AB) ×100 (2), where, the absorbance of ABTS radical + methanol is AB; AA is absorbance of ABTS radical + sample extract/standard (Rajurkar and Hande, 2011)**.**

Antibacterial Activity

Antibacterial screening of AaL, AaR and AaS from various parts of selected species, gram-positive and gram-negative bacterial strains were tested against, using method of agar well diffusion, about 20ml in sterile petri plates Mueller-Hinton Agar was poured and allowed to solidification. In the bacterial culture (10^6 to 10^8CFU/ml) the sterile cotton swab was dipped, and the agar plates were evenly inoculated by swabbing followed by the wells formation using sterilized cork-borer (6mm diameter). Pre labeled each well was filled with 100 µl of various concentrations of flavones derivatives and allowed to diffuse by refrigerating for 30min. the plates were incubated at 37ºC for 24 hrs. Each treatment was prepared by triplicate plates and excluding well average zone of inhibition was recorded. As a negative control DMSO (dimethyl sulphonic acid) was used. In zone of inhibition in millimeters (mm) antibacterial potential was compared with standard antibiotic ampicillin and ciprofloxacin (Shoaib et al. 2016).

Minimum inhibitory concentration (MIC)

Crude extract from various parts of selected species for MIC values MIC inhibiting growth of one or more of the above microorganism's inhibition were again tested. Broth dilution technique determined the MIC values. Each compound stock solution was organized in dimethyl sulfoxide (DMSO) and a serially diluted to achieve the desire concentrations range. To each of pre-identified sterile test tube containing specific concentration of test compound, a standard volume was added of nutrient broth medium. To each tube was added the inoculum consisting of an overnight broth culture of microorganisms. The tubes were incubated for turbidity at 37ºC for 24 hrs and examined. No antimicrobial agent was added to a control tube and ciprofloxacin was used as standard. To stop the growth of bacteria the lowest concentration was regarded as MIC (Shoaib et al. 2016).

RESULTS AND DISCUSSION

Phytochemical Screening

Artemisia absinthium plant parts like root, leaves and stem having metabolites like terpenoids, saponins, flavonoids, carbohydrates and steroids while proteins, tannins and phenolics are absent in all parts (root, leaves, and stem) of A.absinthium which suggest medicinal value of the specie. Some chemical compounds, revealed by phytochemical characterization of the composition of the aqueous extracts (alkaloids, flavonoids, saponins, tannins, and steroids) for the required antifungal activities these are responsible (Salhi et al. 2017). Leaves of barley have maximum number of flavonoids sand saponin, and both composites have strong antioxidant activities. It also comprises magnesium which is important essential element, for glucose metabolism and insulin level optimization it serves as a co-factor (Qasim et al. 2016). In medicinal plants synthesized universally flavonoids are phenolic compounds due to the existence of carbonyl group that can induce antibacterial response (Umamaheswari and Sangeetha, 2015) Phenolic compounds are present in Artemisia species proved by phenolic test currently (Silva-Alves et al. 2013). Pharmacological importance may be assumed, from the medicinal plant tannins were isolated against fungi and bacteria it is possessing remarkable toxic activity.

Table 1: Phytochemical screening of methanolic crude extracts of *Artemisia absinthium* various parts

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

Furthermore, special class of glycosides are saponins, considered as active antifungal agents and it have soapy characteristics (Salhi et al. 2017).

2. Antioxidant activities

The antioxidant activity of all parts (root, leaf and stem) was determined *via* DPPH assay and ABTS assay. Ascorbic acid was taken as a standard. The result of antioxidant activity against DPPH and ABTS are given in table 2. The table show that maximum response of AaL is 36.14% IC50.

Table.2: Antioxidant assay of methanolic crude extracts of *Artemisia absinthium* various parts

Name	Code	Conc (µg/mL)	DPPH Percent inhibition	IC_{50} (µg/mL)	ABTS Percent inhibition	IC_{50} (µg/mL)
Artemisia absinthium	AaS	1000 500 250 125 62.5	74.41±1.11 68.51±1.41 62.65±1.47 56.21±1.43 50.71±1.72	68.11	74.78±1.21 66.45±1.33 58.67±1.11 53.31±1.67 47.88±1.71	93.35
Artemisia absinthium	AaR	1000 500 250 125 62.5	73.51±1.14 66.09±1.31 59.46±1.13 54.39±1.54 46.67±1.39	89.58	75.56±1.08 68.31±1.33 61.45±1.13 55.66±1.67 48.98±1.17	77.43
Artemisia absinthium	AaL	1000 500 250 125 62.5	81.12±1.14 75.92±1.54 69.23±1.31 64.71±1.43 55.09±1.72	36.14	77.21±1.02 71.67±1.34 65.34±1.23 59.88±1.03 53.11±1.11	46.31
Ascorbic acid		1000 500 250 125 62.5	86.50±0.00 86.33±0.16 86.23±0.14 85.00±0.28 84.00±0.28	<1	85.37±0.87 84.52±0.22 83.67±1.39 82.09±1.31 80.11±1.01	<1

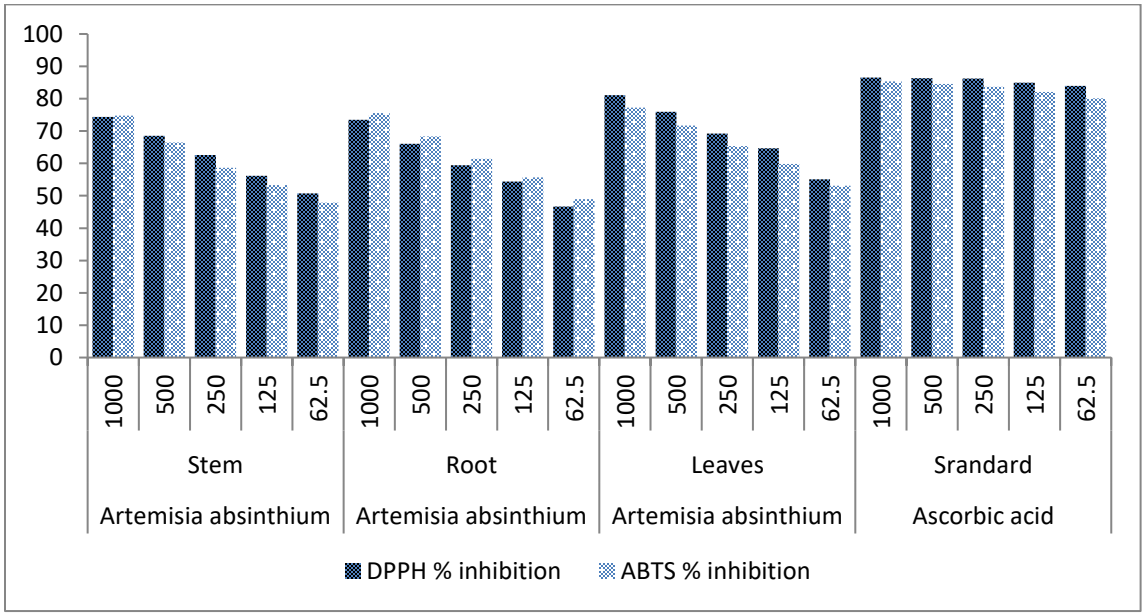

Figure1: Antioxidant assay of methanolic crude extracts of *Artemisia absinthium* various parts

The antioxidant DPPH activity also performed on different concentrations and the results shows different DPPH % inhibition in which the highest % inhibition is (81.12±1.14) on 1000 µg/ml in AaL while the other samples like AaS (74.41 ± 1.11) and AaR (73.51 ± 1.14) also show high DPPH activity at a concentration of 1000 µg/ml. At low concentration AaR at 62.5 µg/ml show the lowest DPPH activity which is (46.67±1.39) while on same concentration the highest DPPH % inhibition of AsR (55.09±1.72). While the ABTS percent inhibition also observed on different concentration in which the highest one in AaL (77.21±1.02) and AaR (75.56±1.08), and the lowest value is (47.88±1.71) in AaS. It is to be noted by IC_{50} calculated for all plant species obtained by ABTS assay were higher than those obtained by DPPH assay like ABTS highest mean value is (93.35 µg/ml, 77.43 µg/ml) and the lowest ABTS activity measured is (46.31 µg/ml) while DPPH highest activity is (89.58 µg/ml) and the lowest noted value is (36.14 µg/ml).

3. Anti-bacterial activities

Crude extract from various parts of selected species by using agar well diffusion method was tested against gram-negative and gram-positive bacterial strains. For each treatment plates were prepared and excluding well average zone of inhibition was recorded by using gram- negative and gram-positive bacteria at different concentration (25ml, 50ml and 100ml). At 50 µg/ml AaL zone of inhibition is (24.01 ± 1.71) and (29.19 ± 1.71) towards gram-positive bacteria *B. subtalis* and *S. aureus* respectively, while at same concentration gram-negative bacteria *P. aeruginosa* inhibition zone is (25.91±1.23), the same gram-negative bacteria zone of inhibition at 100 µg/ml is (28.74±1.07). The bacterial response which may be gram-positive or gram-negative the inhibition zone towards standard Ampicillin and Ciprofloxacin mean values (n=3) (29.09± 1.78), (38.15± 1.51), (33.12± 0.96), (31.35± 2.01), (35.12± 1.39) and (32.01± 1.05) respectively shows bacterial activity against different concentrations.

Up to the level of 200 µg/ml all the organisms are inactive in the organic solvents, it is shown by L. elasticus extracts. At a concentration of 25µg/ml activity in the ethanol and methanol extracts is shown by both gram-negative and gram-positive organisms. All the tested organism, ethanol and methanol shows positive results in comparison with others (Krishnaveni et al., 2016).

During anti-microbial activity, the higher quantity of essential oil (80µL/well) is needed for maximum zone of inhibition against all the microorganisms. Against Pseudomonas fluorescence (MTCC-664) and Bacillus subtilis (MTCC-2451) bacterial strains essential oils shown maximum and minimum zone of inhibition concentration, which indicate that essential oil of *A. maritima* Linn has inhibiting capacity to growth of both gram –ve and gram-positive +ve bacterial strains. Because of Artemisia genus previous studies and present results it can be concluded that *A.maritima* Linn is an aromatic and higher altitude medicinal plants, many grams positive and gram-negative bacterial strains acts as a significant anti-microbial agent (Sharma et al., 2014).MIC of *Artemisia. dracunculus* Essential Oil was 6.25 mg/mL against *S. aureus* and *B. subtilis*. MIC value against *E. coli* VKPM-M17 was 50 mg/mL, but *P*. aeruginosa was less susceptible to EO components and MIC value reached 150 mg/mL. The antibiotic-resistant *E. coli* dhpα-pUCl8 strain possessed high sensitivity against the EO with 6.25 mg/mL MIC value. The action of EO was bactericidal. Tested yeasts were more susceptible against oil component, MIC=1.56 mg/mL. The obtained results show that A. dracunculus EO can be useful for cosmetics, medicine and food as antimicrobial natural agent. The present investigation revealed that gram-positive bacteria were more sensitive to it. The MIC of *A. dracunculus* EO was 6.25 mg/mL against *S. aureus* and *B. subtilis*. MIC values of oil under investigation against E. coli were 50 mg/mL, but P. aeruginosa was less susceptible to EO components and MIC value reached 150 mg/mL. The antibiotic- resistant *E. coli* was the most sensitive gram-negative microorganism against the investigated oil with 6.25 mg/mL MIC value. Tested fungi were more susceptible against oil components: MIC = 1.56 mg/mL against both tested yeasts. Thus, the MIC values determined are acceptable, effective, and the action of essential oils in this study was evaluated to be bactericidal (Petrosyan et al. 2018).

Table.3: Antibacterial activity of methanolic crude extracts of *Artemisia absinthium* various parts

Crude samples	Concentration (µg/ml)	Zone of inhibition (mm)		
		Gram-positive Bacteria		Gram-negative Bacteria
		B. subtilis	*S. aureus*	*P. aeruginosa*
AaS	25	15.28±2.17	22.39±1.78	19.19±1.17
	50	17.19±1.19	15.19±1.31	17.31±0.86
	100	18.87±2.08	23.79±1.52	21.67±1.21
AaR	25	13.18±2.71	20.19±1.81	20.26±1.71
	50	16.91±2.09	14.22±1.13	19.18±1.16
	100	19.07±1.18	21.91±2.21	23.51±1.67
AaL	25	24.01±1.71	29.19±1.71	25.91±1.23
	50	26.10±1.41	30.19±1.15	28.14±1.41
	100	25.91±1.87	32.72±1.08	28.74±1.07
Ciprofloxacin		31.35±2.01	35.12±1.39	32.01±1.05

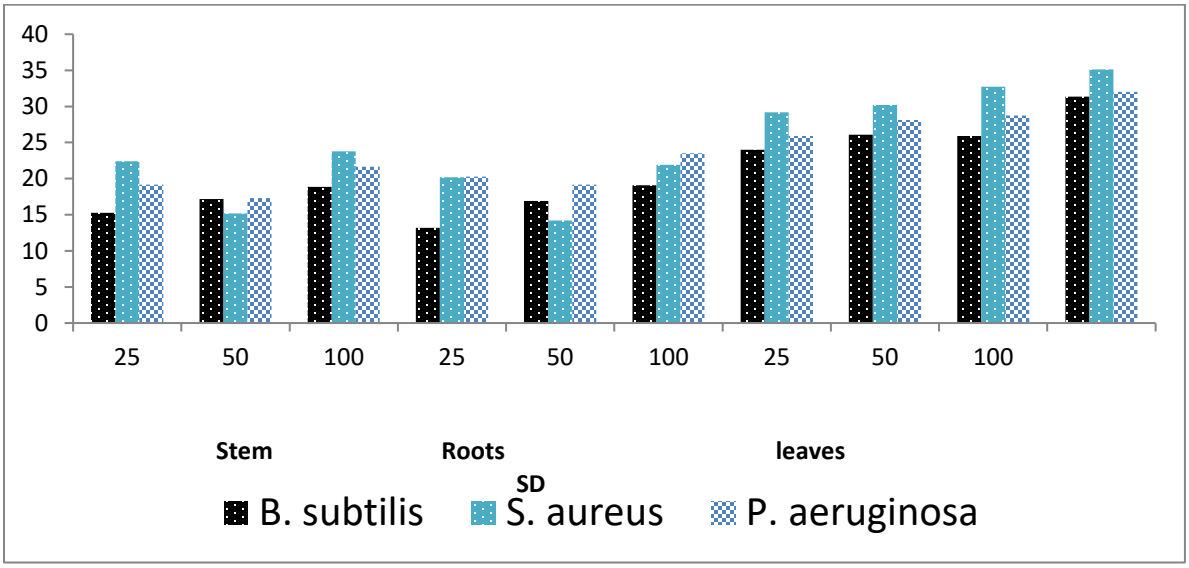

Figure 2: Antibacterial activity of methanolic crude extracts of *Artemisia absinthium* various parts

Table 4: The antibacterial activity MIC methanolic crude extracts of *Artemisia absinthium* various parts

Crude extract samples from various parts of selected species	MIC (µg/ml)		
	Gram-positive Bacteria		Gram-negative bacteria
	B. subtilis	*S. aureus*	*P. aeruginosa*
AaS	37.5	25	255
AaR	25	37.5	50
AaL	12.5	12.5	6.25
Ciprofloxacin	6.25	6.25	6.25

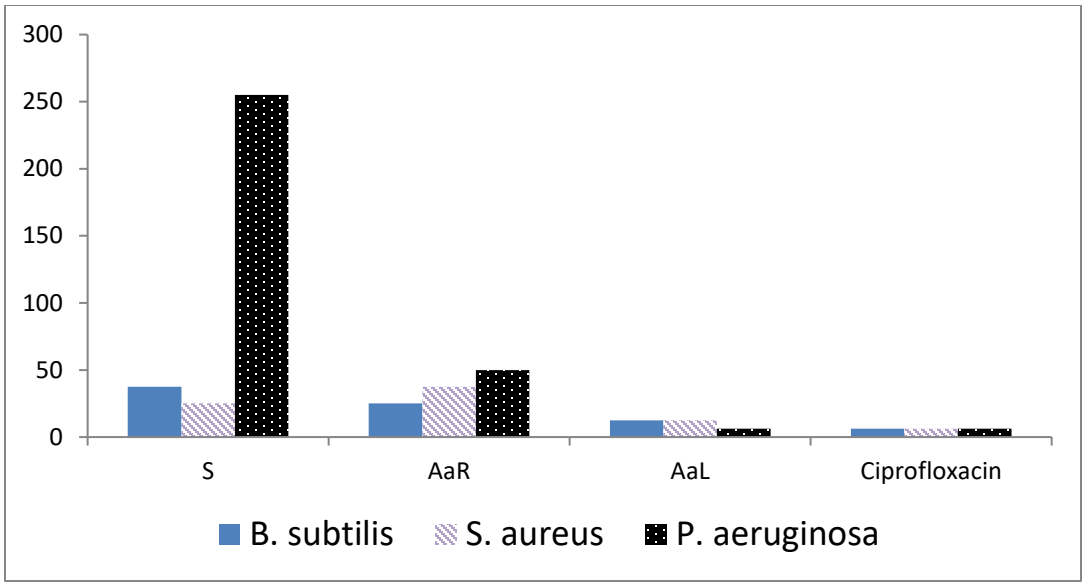

Figure3: The antibacterial activity MIC of selected *Artemisia* species crude extract.

MIC

Table 4 illustrates the MIC (µg/ml) of crude extract from various parts of selected species against Gram-negative and Gram-positive bacteria. It is experiential that AaL possess inhibitory potentials at low concentration against all tested bacteria. The extract AaL showed MIC of 12.5µg/ml for *B. subtilis* which is gram positive bacteria, 12.5µg/ml for gram positive bacteria *S. aureus* and 6.25 µg/ml for gram negative bacteria *P. aeruginosa.*

CONCLUSION

It was concluded that genus Artemisia species possess therapeutic potential and are still part of the culture of Pakistan. Many people in the studied area of Dir lower continue to depend on medicinal plants, at least for the treatment of some simple diseases such as cough cold, fever, headache, skin diseases, bites and tooth infections. Artemisia species are a good source of bioactive chemical compounds include tannins, saponins, terpenoids, terpenes, flavonoids, phenolics that showed potential anti-oxidant activity compared with ascorbic acid and it could be useful source of natural antioxidants and anti-bacterial. This antioxidant capability may open a channel to explore its efficacy against disorders related to oxidative stress and may serve as potential candidates for antioxidant enzymes like catalase and superoxide dismutase. Artemisia extract showed the broad spectrum of antibacterial activity on the tested microorganism. The antibacterial activities may be enhanced if active components of Artemisia species are purified, further more natural anti-bacterial development will help to decrease the negative effect of synthetic drugs. These agents may serve as an alternate to microorganisms that have developed resistance like in many multidrug resistance microorganisms. Characterization and fractionation of these active compounds will be the future work to investigate.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All the authors contributed in this research workTN, SWAS, AH, GR designed and performed the experiments and also wrote the manuscript. AK, II, AR, and SZ performed experiments and data analysis. MAN, SP, ZA and MI designed experiments and SB, JZ, AHK and MR reviewed the manuscript and identification of plant specimens. All authors read and approved the final version

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