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PHYTOCHEMICAL, ANTIBACTERIAL AND ANTIOXIDANT SCREENING OF *Artemisia santolinifolia* VARIOUS PARTS

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Abstract

The current study was carried out to explore the phytochemical, antioxidant potential and antibacterial activities of the crude methanolic extract of *A. santolinifolia* Turcz. Ex Besser. The antioxidant activity was carried out by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay, while methanolic extract displayed the highest scavenging activity (DPPH) was 61.31µg/ml on *Artemisia santolinifolia* root and the lowest (51.05µg/ml) was record for their leaves. Similarly, in (ABTS) the highest activity (89.16µg/ml) was recorded for roots of *A. santolinifolia* followed by leaves (68.14µg/ml). In low 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 leaves extract of *A. santolinifolia* AsL showed MIC of 12.5µg/ml for *B. subtilis,* a gram-positive bacterium, 50µg/ml for gram positive bacteria *S. aureus* and 37.5 µg/ml for gram negative bacteria *P. aeruginosa* that is almost equal to the response of standard ciprofloxacin. Our current study revealed that *Artemisia santolinifolia* root (AsR) exhibited a significant antioxidant potential while AsL showed good antibacterial effect which is suggested to be used for treatment and management of different infectious diseases.

Keywords: *Artemisia santolinifolia*. Antioxidant Potential. Antibacterial Activity. Phytochemicals.

1. Introduction

Medicinal plants have intense effect on the health of animals and plants as well. The literature showed that medicinal plants are the main power of pharmaceutical companies. Most of the phytonutrients present in medicinal plants have antioxidant, antimicrobial, anti-inflammatory, phytotoxic and cytotoxic activities (Narayanaswamy and Balakrishn 2011; Balakrishn et al. 2013; Kotan et al. 2013). Richest source of crude drug are medicinal plants having high level of therapeutic agents. Traditional medicines are the most economical source of therapy to population.

Pakistan 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 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 family Asteraceae which is medicinally vital with many essential oils and secondary metabolites. 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 the presence of antimicrobial and anti-oxidative 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. santolinifolia* against gram positive and gram-negative bacteria to standard antibiotics e.g., ampicillin and ciprofloxacin at different concentration.

2. Material and Methods

The research study was carried out in the laboratory of Pharmacognosy, Department of Pharmacy, University of Malakand Chakdara, Dir Lower, Pakistan.

Collection and authentication

Different species of *Artemisia* (*Artemisia santolinifolia* Turcz. Ex Besser) were collected from district, Dir lower, and were identified by flora of Pakistan and taxonomist in herbarium and Botanical Garden, Department of Botany, University of Malakand. The specimens were deposited in herbarium of University of Malakand under voucher, As4/6/18. Artemisia santolinifolia.

Maceration

After collection and identification, leaves, stem, and roots of the 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 and reduced by rotary evaporator to produce a semisolid mass.

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.

Test for tannins: Tannins presence in various fractions were determined by the following method of DOSS (2009). In a test tube 20 ml of distilled water was boiled in 50 mg of each fraction and was filtered. In each test tube 0.1% ferric chloride few drops were added, and color change was observed, by brownish green or a blue-black coloration indicates the presence of tannins.

Phenolic test: The plant extract is treated by the presence of phenols, which is proven by ferric chloride solution few drops and bluish black color formation according to Silva et al. (2017) procedure.

Test for saponins: According to Mir et al. (2013) standard procedure saponins were carried out in various fractions determination. In 20 ml of distilled water boiled 20 mg of each sample for five minutes in a water bath and filtered. 10 ml of each filtrate were mixed with 5 ml of distilled water and vigorously shaken for froth formation. In the froth 3 drops of olive oil was mixed, vigorously shaken and the formation of emulsion was observed.

Terpenoids test: According to Mir et al. (2013) presence of terpenoids in various fractions was determined. 2 ml of chloroform was mixed with 5ml (1mg/ml) of each extract, and then to form a layer adds 3 ml of concentrated H_2SO_4 carefully. The presence of terpenoids shown by reddish brown coloration of the interface.

Flavonoid test: Take extract 25 mg and 50 ml of distal water and vigorously mix them and then add 2 to 3 ml NaOH solution and observed yellow coloration which indicate the flavonoids existence, according to standard protocol of Prabhavathi et al. (2016).

Protein test: According to Silva et al. (2017) procedure, take 5mg extract mixed with 5ml water and then add million's reagent show a foggy ash color surface is the indication of the presence of protein.

Steroid test: According to standard procedure followed by Islam et al. (2016), 5 mg extract was mixed in 5ml chloroform and few drops of H_2SO_4 were added. The formation of reddish brown color indicates the presence of steroid.

Carbohydrate test: 5 mg extract was mixed with 10 ml distal water to form a solution then take 2 ml solution 5 ml H_2SO_4 and few drops of Molisch's reagent give purple color indicate carbohydrate, according to Prabhavathi et al. (2016).

Antioxidant activity

Antioxidant potential of plant extracts was screened against 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:

$$ABTS{\cdot}+\ scavenging\ effect\ (\%) = ((AB–AA)/ AB) \times 100\ (2)$$

Where, the absorbance of ABTS radical + methanol is AB; AA is absorbance of ABTS radical + sample extract/standard (Rajurkar and Hande 2011).

Antibacterial screening of AsL, AsR and AsS 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 well's 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 (Shoib 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 inhibitions was 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).

3. Results and Discussion

The research work was conducted in the Pharmacognosy lab, Pharmacy Department, university of Malakand. Phytochemical analysis, antioxidant and antibacterial activities worked out during the research study.

Phytochemical screening

Artemisia santolinifolia plant parts like root, leaves and stem possess metabolites like terpenoids, tannins, phenolics, flavonoids, carbohydrates, and steroids (Table 1). Proteins are absent in all parts (root, saponins leaves, and stem) of *A. santolinifolia* 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. Furthermore, special class of glycosides are saponins, considered as active antifungal agents and it have soapy characteristics (Salhi et al. 2017).

Table 1. Phytochemical screening of Artemisia santolinifolia.

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

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 shows that maximum response of AsL is 51.05% IC50.

The antioxidant DPPH activity also performed on different concentrations and the results shows different DPPH % inhibition in which the highest % inhibition is (80.45±1.84) on 1000 µg/ml in AsS while the other samples like AsL (77.11 ± 1.14) and AsR (77.23 ± 1.66) also show high DPPH activity at a concentration of 1000 µg/ml. At low concentration AsS at 62.5 µg/ml show the lowest DPPH activity which is (62.29±1.11) while on same concentration the highest DPPH % inhibition of AsR (49.19±1.32). While the ABTS percent inhibition also observed on different concentration in which the highest one in AsS (79.34±1.19) and AsL (78.23±1.09), and the lowest value is (45.69±1.11) in AsR. 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 (89.16 µg/ml, 70.56 µg/ml) and the lowest ABTS activity measured is (68.14 µg/ml) while DPPH highest activity is (61.31 µg/ml) and the lowest noted value is (51.05 µg/ml) (Figure 1).

Table 2. Antioxidant Assay of Artemisia santolinifolia with IC_{50} value.

Name	Code	Conc (µg/mL)	DPPH Percent inhibition	IC_{50} (µg/mL)	ABTS Percent inhibition	IC_{50} (µg/mL)
Artemisia santolinifolia	AsL	1000	77.11±1.14	51.05	78.23±1.09	68.14
		500	72.19±0.84		74.83±1.10	
		250	67.65±0.46		68.51±1.14	
		125	64.53±1.34		62.61±1.74	
		62.5	57.78±0.45		56.21±1.34	
Artemisia santolinifolia	AsR	1000	77.23±1.66	61.31	77.12±1.05	89.16
		500	70.39±1.01		71.34±1.34	
		250	66.52±1.41		63.45±1.87	
		125	59.21±1.57		54.78±1.21	
		62.5	49.19±1.35		45.69±1.11	
Artemisia santolinifolia	AsS	1000	80.45±1.84	53.61	79.34±1.19	70.56
		500	78.17±1.04		70.24±1.86	
		250	72.31±0.66		68.29±1.37	
		125	68.25±0.57		61.19±1.39	
		62.5	62.29±1.11		59.24±1.37	
Ascorbic acid	AsA	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	

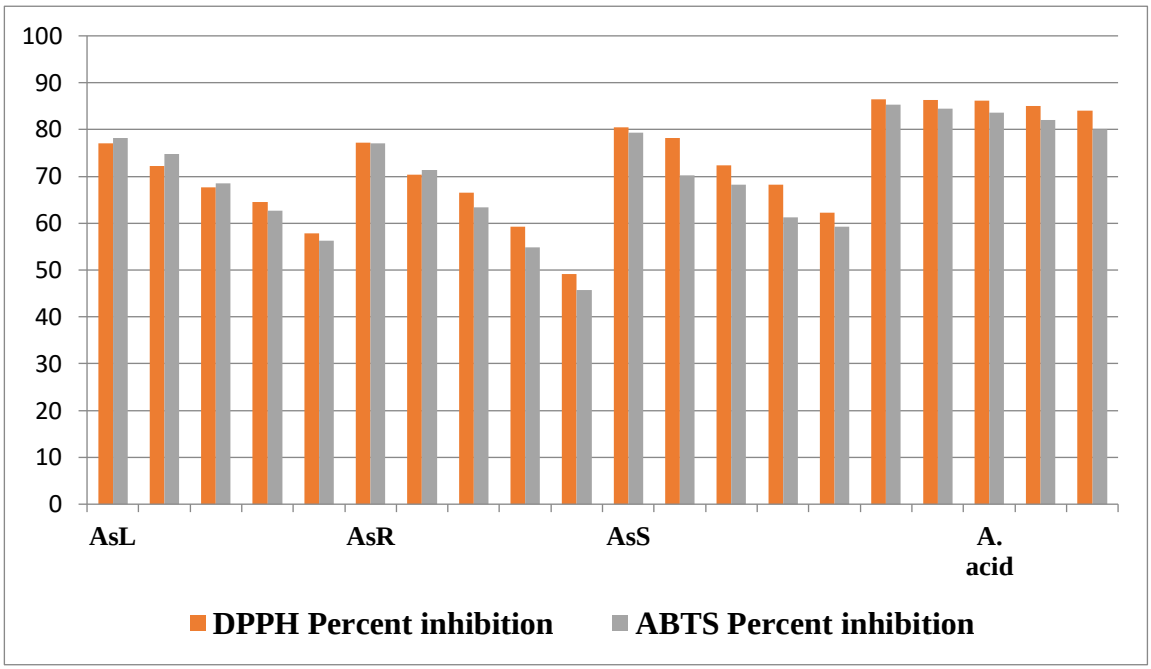

Figure 1. Antioxidant activities of *Artemisia santolinifolia.*

Antibacterial 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 AsL zone of inhibition is (23.21 ± 1.14) and (29.13 ± 1.18) towards gram-positive bacteria *B. subtalis* and *S. aureus* respectively, while at same concentration gram-negative bacteria *P. aeruginosa* inhibition zone is (25.35±1.33), the same gram-negative bacteria zone of inhibition at 100 µg/ml is (29.18±1.72). The bacterial response which may be gram-positive or gram-negative the inhibition zone towards standard Ampicillin and Ciprofloxacin means 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 200µg/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-negative and gram-positive 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 (Table 3).

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 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 AsL possess inhibitory potentials at low concentration against all tested bacteria. The extract AsL showed MIC of 12.5µg/ml for *B. subtilis* which is gram positive bacteria, 50µg/ml for gram positive bacteria *S. aureus* and 37.5 µg/ml for gram negative bacteria *P. aeruginosa.*

Table 3. Antibacterial activities of *Artemisia santolinifolia.*

Crude samples	Concentration (µg/ml)	Zone of inhibition (mm)		
		Gram-positive Bacteria		Gram-negative Bacteria
		B. subtilis	*S. aureus*	*P. aeruginosa*
AsL	25	19.32±2.61	25.11±2.61	23.09±0.91
	50	23.21±1.14	29.13±1.18	25.35±1.33
	100	21.09±1.82	28.22±1.71	29.18±1.72
AsR	25	13.15±1.71	16.19±1.48	15.28±1.41
	50	15.13±2.11	16.71±1.72	16.09±2.08
	100	19.11±1.51	18.19±1.51	21.38±1.14
AsS	25	10.81±1.71	6.18±1.84	7.09±2.13
	50	13.39±2.21	8.90±0.58	8.27±1.04
	100	14.65±1.91	8.27±1.41	8.77±1.26
Ampicillin	10	29.09±1.78	38.15±1.51	33.12±0.96
Ciprofloxacin	10	31.35±2.01	35.12±1.39	32.01±1.05

All values are taken as mean ±SEM (n=3).

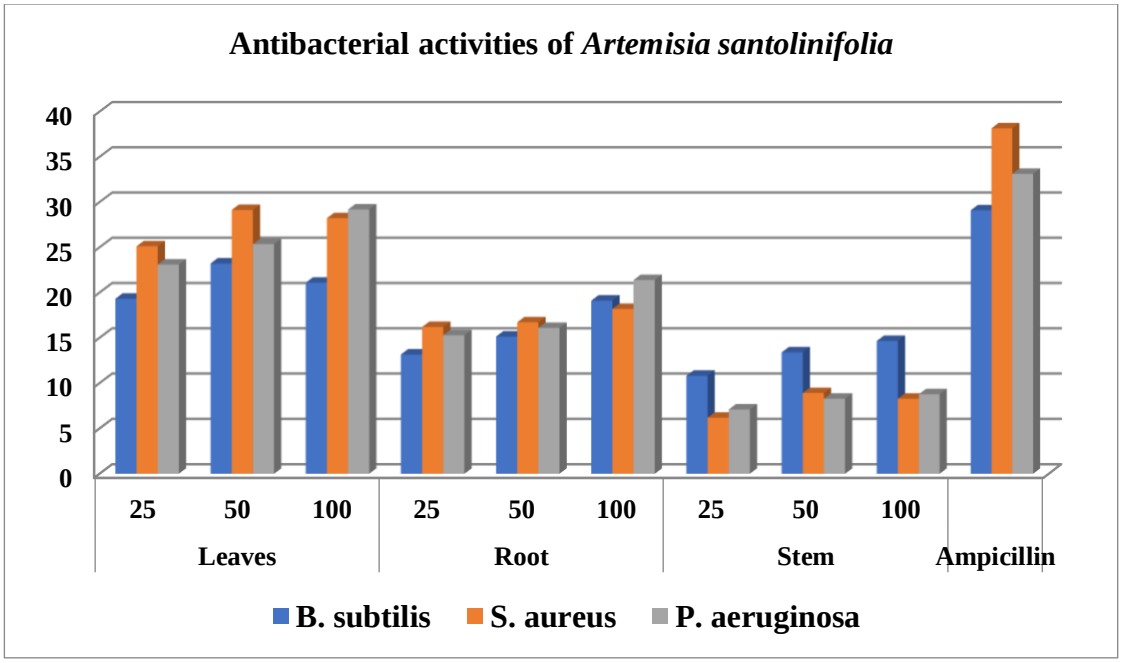

Figure 2. Antibacterial activities of *Artemisia santolinifolia.*

Table 4. The antibacterial activity MIC of selected Artemisia species crude extract.

Crude extract samples from various parts of selected species	MIC (µg/ml)		
	Gram-positive Bacteria		Gram-negative bacteria
	B.subtilis	*S.aureus*	*P. aeruginosa*
AsL	12.5	50	37.5
AsR	62.5	50	25
AsS	100	100	>125
Ciprofloxacin	6.25	6.25	6.25

4. Conclusions

This study concluded that even through the availability of western medicines for simple and complicated diseases is available. Many people in the study area continue to depend on medicinal plants, atleast for the treatment of some simple diseases like cough, cold, fever, bites, headache, skin diseases and tooth infection. The experts of medicinal plants (Hakims) have good interaction with patients and this improves the quality of healthcare. Today traditional system of treatment was very old. Due to lack of interest among the younger generation as well as their tendency to migrate cities for beneficial jobs, there is a possibility of losing this wealth of knowledge soon. It is necessary to get hold of and preserve this traditional system of medicine by proper documentation. The present project, results offer a scientific basis for the traditional use of medicinal plants. However, further studies on medicinal plants are necessary to determine their active constituents. The antibacterial activity may be enhanced if active components of Artemisia species are purified. After phytochemical analysis different valuable chemicals are also identified, which also showed the importance of medicinal plants.

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