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Phytochemistry and Antibacterial Efficacy of Northeastern Pakistani *Artemisia rutifolia* Stephan ex Spreng. Extracts against Some Clinical and Phyto-pathogenic Bacterial strains

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ABSTRACT

Recently, most researches have focused on the biological activities of the extracts obtained from different *Artemisia* species due to the presence of essential compounds with strong activity against some gram-negative and gram-positive bacteria. In this study, five extracts of *Artemisia rutifolia* Stephan ex Spreng. from the northeastern Gilgit-Baltistan region of Pakistan were analyzed for total flavonoid and total phenolic contents and their antibacterial activities against some clinical and phyto-pathogenic bacterial strains were assessed with agar disk diffusion method. Results indicated that the methanol, ethanol, chloroform, ethyl acetate and *n*-hexane extracts of *A. rutifolia* are rich in flavonoids and phenols and all the tested extracts showed the broad spectrum growth inhibition of the tested gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacterial strains (*Escherichia coli* and *Pseudomonas aeruginosa*). Overall, methanol and ethyl acetate extracts showed better activities even at lower concentrations (5 mg/

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ml) where *B. subtilis* and *P. aeruginosa* were the most susceptible strains. Hence, the MICs of these two effective extracts (methanol and ethyl acetate extracts) were tested against most susceptible bacterial strains (*B. subtilis* and *P. aeruginosa*) at 1-4 mg/ml conc. Results of MICs showed that both the methanol and ethyle acetat extracts were effective against *B. subtilis* and *P. aeruginosa* at 3 and 4 mg/ml concentrations where ethyl acetate extract exhibited higher inhibitory effect than the methanol extract. Therefore, extracts of *A. rutifolia* could be used as operational sources against pathogenic bacterial diseases.

Keywords: *Artemisia rutifolia*, TFC, TPC, antibacterial activity, minimum inhibition concentrations.

INTRODUCTION

Artemisia L. is a noteworthy member of the Asteraceae family, which is a polymorphic genus and is important from both economic and therapeutic point of view. Species of this genus are mostly found in the northern hemisphere especially in the temperate zones, but few taxa are also present and reported from the southern hemisphere of the world[1]. There are ~500 species in the *Artemisia* genus including both shrubs and herbs[2] which are considered as a diverse genus from the Asteraceae family of the Anthemideae tribe[3]. In plants, there exist some organic and inorganic compounds and also individual elements are present which gives therapeutic effects against various infections.

For many years, the utilization of *Artemisia* species as medicine is a common exercise in traditional medicine and it is still continued in many communities. The extracts and essential oils from different *Artemisia* species are extensively used for a variety of medicinal purposes due to their pharmacological significance producing most of the medicinally significant secondary metabolites[4,5] with a sequence of biological activities including antioxidant and antimicrobial activities[6].

Artemisia rutifolia Stephan ex Spreng from the genus *Artemisia* is a shrub native to the northern Pakistan and called vernacular name is *Afsanteen*. It reaches the height of 20 to 80 cm[7] and is used traditionally in the North Pakistan for the treatment of asthma, cough, fever, inflammation, abdominal pain, cancer, and other ailments[8,9]. It has been showed that the essential oil from *A. rutifolia* possess compounds like thujone, germacranolide, eudesmanolide sesquiterpenoids and guaianolide[10] mainly responsible for the therapeutic effects against diseases.

Bacteria and viruses are the pathogens responsible for many health problems in humans and the occurrence and expansion of antibiotic resistance, as well

as the evolution of new disease causing bacterial and fungal strains are of great concern to the global health community. In this regard, the screening of antimicrobial potentials from plant extracts could be more helpful in monitoring phytopathogens and clinical uses as natural antimicrobials.

Frequently used medicinal plants of our community especially *Artemisia* plants are excellent drug sources to cope with problems posed by drug resistant microbes. While in the recent past, much focus has been given towards the pharmacological activities of *Asteraceae* plants[12,13]. There exist a knowledge gap about the antibacterial activity of some *Artemisia* species including *A. rutifolia* and the literature search also indicated no or very limited reported data availability on the antibacterial activity of this plant. Therefore, the present study aimed to report the TFC, TPC and the potential antibacterial activity of methanol, ethanol, ethyl acetate, chloroform and *n*-hexane extracts of *A. rutifolia* from the Northeast Gilgit-Baltistan region of Pakistan.

METHODOLOGY

The present study was conducted in the Biotechnology laboratory, Department of Biotechnology, University of Okara, Pakistan and Applied Microbiology and Biotechnology laboratory (AMBL), International Islamic University Islamabad Pakistan. *A. rutifolia* (Figure 1) was collected (Collectors, Adil hussain and Mujtaba Hassan), from the natural environment in the Ataabad Hunza-Nagar district of Gilgit-Baltistan region of Pakistan (Table 1). The study area (Gilgit-Baltistan) is situated in the northeast of Pakistan with diverse climate and the area is very much popular for its immense plants biodiversity[14]. It is situated in between the longitude latitude 35° to 37° east and 72° to 75° north having 7 major districts including Astore, Diamer, Baltistan, Ganche, Gilgit, Ghizar and Hunza-Nagar. The collected sample of *A. rutifolia* was first pressed with a wooden presser, dried up then mounted and labeled on the herbarium sheet (Figure 2). The prepared herbarium was submitted to the herbarium of Pakistan Museum of Natural History (PMNH) Islamabad, Pakistan to obtain herbarium specimen number[15] for future reference. The details collection, source and GPS locality details of *A. rutifolia* specimen are given in Table 1. The collected specimen was identified by assessing various morphological characteristics and by relating those characters with the already available herbarium specimen prior to the assessment of phytochemicals and antibacterial activity.

Solvent Extraction

Before the extraction with organic solvents, the plant specimen was cleaned with deionized water and then shade dried for almost a week. The dried leaves

and aerial parts were grinded to fine powder with the help of mortar and pestle and the powder was filtered using gauss cloth. The powdered sample was stored in air tight containers at 4°C for further use. Five organic solvents like methanol, ethanol, chloroform, *n*-hexane and ethyl acetate were used to obtain extracts from the plants using soxhlet extraction procedures. Briefly, 10 grams of the powered samples were taken in the muslin cloth for continuous extraction process using soxhlet apparatus at a temperature below the boiling temperature of all solvents. A portion of the powdered samples of plants were soaked in the solvent in a conical flask, wrapped with aluminum foil and placed in shaker for 48 hrs at 120-130 rpm. After 48 hrs, the obtained extracts were filtered using whattman filter paper No: 1. Evaporation of the solvent from extract was done and the residue containing extract was dissolved in sterile dimethylsulfoxide (DMSO, 9:1) in 50 mg/ml concentration. The extract was then filtered with 0.22 micro filters (Type GV- Millipore) and then kept at 4°C for further study.

Total flavonoid content (TFC) in *A. rutifolia* extracts

The quantitative determination of total flavonoids content (TFC) was performed using the aluminum chloride colorimetric technique[16] with little modifications. Briefly, 20 µl test samples were taken from each stock solution, with the addition of 10 µl of aluminum chloride in 90 µl of water (w/v). 160 µl of water was added in 96 well plates along with 0.1 % of 10 µl potassium acetate. The solution was incubated for 30 minutes at ambient temperature. The absorbance was measured at 415 nm. The total flavonoids content was determined by using a microplate reader. The experiment was repeated thrice and results were expressed with unit µg QE/mg DW (micrograms equivalent to quercetin milligram dry weight).

Total phenolic content (TPC) in *A. rutifolia* extracts

The total phenolic content of *A. rutifolia* crude extract was estimated by using folin's ciocalteu's reagent[17]. 20 µl extract was taken and mixed with 90 µl of folin ciocalteu reagants (v/v) in 96 well plates. The solution was incubated for 5 minutes, and 90 µl of sodium carbonate solution was added. The assay plate reader absorbance was set at 630 nm, and the absorbance of 96 well plates was measured using a microplate reader. A calibration curve (R2= 0.98) was obtained by using gallic acid as a positive standard. The experiment was repeated thrice and results were noted, the expression of the result is mentioned with unit µg GAE/mg DW (as gallic acid equivalent milligram dry weight)[16].

Antibacterial activity of *A. rutifolia* extracts

For the antimicrobial activity of *A. rutifolia* extracts, both gram-positive and gram-negative pathogenic bacterial strains were used. The strains were *S. aureus, B. subtilis, E.coli*, and *P. aeruginosa* obtained from the Microbiology laboratory of Mirpur University of Science and Technology (MUST) AJK Pakistan. The stock cultures of the strains were maintained in nutrient agar slant at 4°C and were subcultured on monthly basis. Microscopic identification of the bacterial strains was done prior to the assessment of antibacterial activity of the plant extracts. For the antimicrobial activity of extracts, agar disk diffusion method was used[18]. Briefly, the plant extract residues (40 mg) were dissolved in the solvent which was sterilized with Millipore filter (0.22 mm) then loaded over sterile filter paper discs (8 mm in diameter) to get final concentration of 10 mg/disc. About 10 ml of Mueller-Hilton agar (MHA) medium was poured into sterile petri dishes as a basal layer followed with 15 ml of seeded medium previously inoculated with bacterial suspension (100 ml of medium/1 ml of 107 CFU) to attain CFU/ml of medium. Plant extract concentrations were loaded in sterile filter paper discs and were placed on the top of MHA plates. The standard antibiotic levofloxacin was used as a positive control and DMSO was used as negative control. The plates were kept in the fridge at 5°C for 2 hrs to allow diffusion of extracts then incubated at 35°C for 24 hrs. The measurement of inhibition zones was done by vernier caliper or zone reader scale and was considered as the indication for antibacterial activity.

Minimum inhibitory concentrations (MIC's) of *A. rutifolia* extracts

After assessing the susceptibility of the bacterial strains, the most effective extracts of *A. rutifolia* with strong antibacterial activity at 5 mg/ml were further assessed for MIC's against most susceptible bacterial strains at lower concentrations using disk diffusion method[18]. Different concentrations of the effective plant extracts (1-4 mg/ml) were arranged separately by dissolving 40 mg in 2 ml of the solvent. The standard antibiotic levofloxacin and DMSO were used as positive and negative controls. Inhibition zones were measured with a vernier caliper or zone reader scale for each concentration of the effective plant extracts.

Statistical analysis

Accuracy in measurement was obtained using the SPSS program (SPSS Inc. Chicago IL version 12.0). All readings were taken three times and 95% was the confidence interval for mean. Level of significance was ($P<0.05$).

Table 1. Collection details of *A. rutifolia* from the Gilgit-Baltistan region of Pakistan with voucher specimen number

Artemisia Sp.	**Location**	**Latitude**	**Longitude**	**Altitude (m a.s.l.)**	**Herbarium specimen no**	**Collectors**
Artemisia rutifolia Stephan ex Spreng.	Ata abad Hunza-Nagar	N-36'20.35	E-74'52.15	2419	PMNH-00046359	Adil Hussain and Mujtaba Hassan

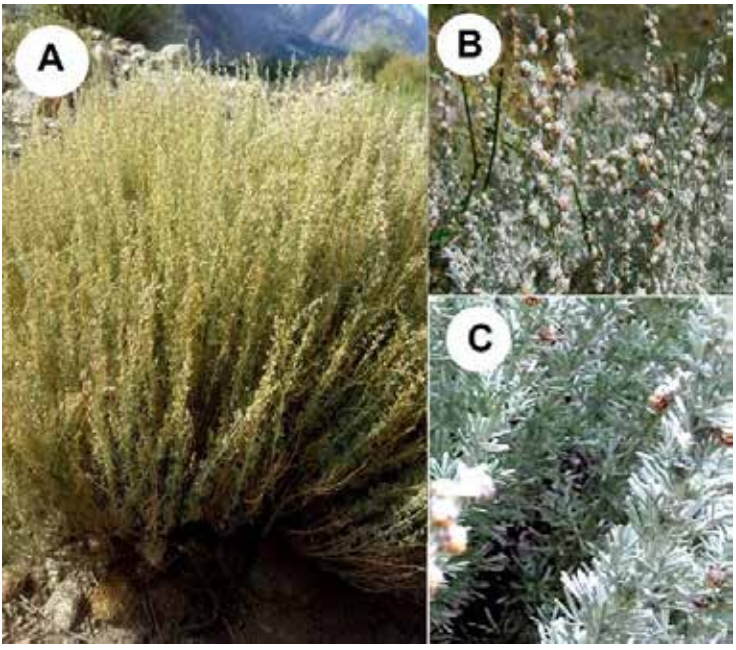

Figure 1. Morphology of *A. rutifolia* collected from Gilgit-Baltistan Pakistan A) Habit, B) Aerial part with synflorescence, C) Middle cauline leaves

Figure 2. Herbarium specimen (PMNH-00046359) of *A. rutifolia* deposited in the Pakistan Museum of Natural History (PMNH) Islamabad, Pakistan

RESULTS and DISCUSSION

Plants extraction yield (%)

The percentage yields of plant extract obtained from *A. rutifolia* using different solvents are given in Table 2. The extract from 40 g dried plant material with methanol yielded plant extract residue of 3.83 g (9.58 %), ethanol yielded plant extract residue of 4.12 g (10.31%), ethyl acetate yielded 1.73 g (4.32 %), chloroform yielded 1.56 g (3.92 %) and *n*-hexane yielded 0.50 g (1.25 %) respectively.

Table 2. Percentage yield (w/v) of *A. rutifolia* extracts obtained using different solvents

Sr. No	Solvent	Plant biomass	Extract obtained	% Yield (w/v)
1	Methanol	40g	3.83g	9.58%
2	Ethanol	40g	4.12g	10.31%
3	Ethyl Acetate	40g	1.73g	4.32%
4	Chloroform	40g	1.56g	3.92%
5	n-Hexane	40g	0.50g	1.25%

TPC and TFC of *A. rutifolia* extracts

The quantitative estimation of TFC and TPC of the *A. rutifolia* confirmed higher phenol and flavonoid contents in its extracts. The maximum amount of phenols and flavonoids were recorded for ethanol extract and in comparison, ethyl acetate, chloroform, *n*-hexane, and methanol exhibited slightly lower TPC and TFC values respectively (Figure 3 and 4). The amount of TPC for *A. rutifolia* extracts was in the range between 31 μgGAE/mg to 57 μgGAE/mg (Figure 3). Ethanol extract showed a greater extent of TPC (57 μgGAE/mg) and *n*-hexane displayed minimum TPC values (31 μg GAE/mg).

TFC of *A. rutifolia* extracts recorded were in the range between 57.21μgQE/mg to 93.75μgQE/mg (Figure 4) where the ethanol extract showed maximum TFC (93.75 μgQE/mg) and *n*-hexane displayed minimum TFC (57.21 μgQE/mg). The overall pattern of the amount of flavonoids and phenols recorded in *A. rutifolia* extracts from highest to lowest is as follow: Ethanol > methanol > ethyl acetate > chloroform > *n*-hexane.

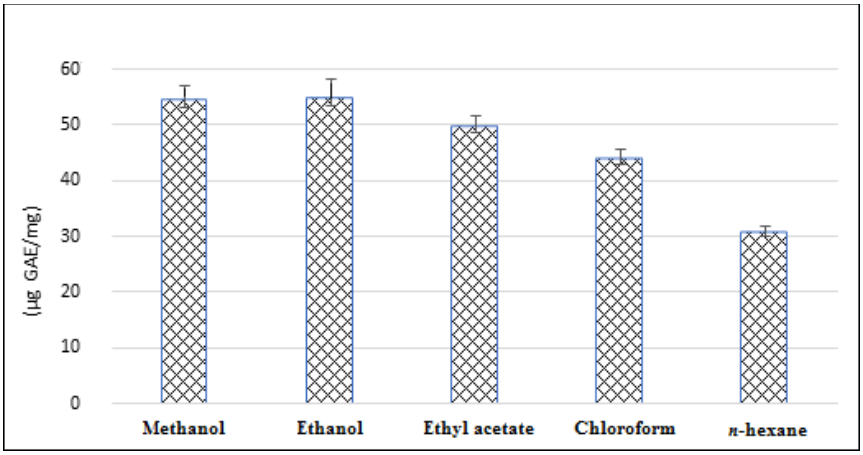

Figure 3. Total phenolic content (TPC) in different *A. rutifolia* extracts

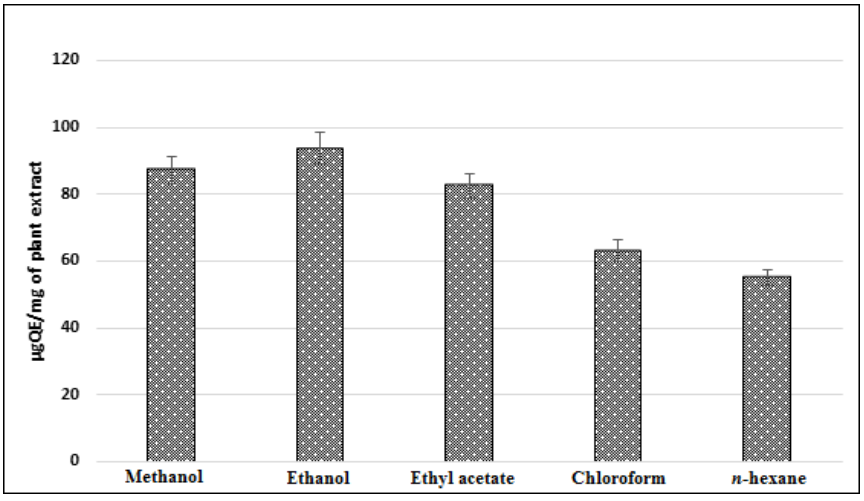

Figure 4. Total flavonoid content (TFC) in different *A. rutifolia* extracts

Antimicrobial activity of *A. rutifolia* extracts

Antibacterial activity of *A. rutifolia* extracts against two strains of gram-positive bacteria (*B. subtilis* and *S. aureus*) and gram negative bacteria (*E. coli,* and *P. aeruginosa*) using disc diffusion method displayed a very noteworthy outcomes. The antibacterial activity of organic solvent extracts displayed changing magnitudes of inhibition configurations with standard positive and negative controls depending on the tested strains susceptibility. Growth of all the tested bacterial strains was inhibited by all extracts of *A. rutifolia*. The mean inhibi-

tory zones of extracts against tested bacterial strains are summarized in Table 3 and illustrated in Figures 5-9. All extracts of *A. rutifolia* maximally retarded the microbial growth at the concentrations of 50, 25 mg/ml while slightly lower growth inhibition was recorded at 10 and 5 mg/ml concentrations for all extracts (Tables 3).

The methanol extract of *A. rutifolia* exhibited inhibitory effects (zones of inhibition) against the pathogenic strains at different concentrations ranges from 10.11 to 19.21 mm as shown in Table 3 and illustrated in Figure 5. At 50 mg/ml concentration, highest inhibitory effect of methanol extract was recorded against *B. subtilis* (19.21 mm), these are followed by *S. aureus* (15.23 mm). While minimum inhibitory effect of 14.05 mm was observed in *P. aeruginosa* and *E. coli*. At 25 mg/ml, the methanol extract displayed higher inhibitory effect against *B. subtilis* (18.73 mm) and minimum effects were noted for *S. aureus* (13.76 mm), *P. aeruginosa* (12.18 mm) and *E. coli* (12.33 mm). At 10 mg/ml, methanol extract displayed higher inhibitory effect against *B. subtilis* (15.21 mm) and minimum effects at 10 mg/ml methanol extract were noticed against *S. aureus* (10.01 mm), *E. coli* (11.44 mm) and *P. aeruginosa* (12.33 mm). At 5 mg/ml concentration, maximum inhibitory effect was shown against *B. subtilis* (14.45 mm) and lower effects were recorded for *E. coli* (10.11 mm) and *P. aeruginosa* (10.56 mm). Overall, the *S. aureus* strain was the most resistant to the methanol extract of *A. rutifolia,* at 5 mg/ml concentration, while other tested strains showed more susceptibility to the methanol extract at different concentrations respectively.

A. rutifolia ethanol extract demonstrated zones of inhibition range from 9 to 17 mm against the tested bacterial strains at different concentrations as shown in Table 3 and illustrated in Figure 6. The ethanol extract when taken 50 mg/ml, displayed maximum inhibitory effects against *P. aeruginosa* (17 mm), *B. subtilis* (16 mm) and *S. aureus* (16 mm) while lower effect (14 mm) was observed for the *E. coli* strain. At 25 mg/ml concentration, ethanol extract displayed maximum inhibitory effects against *P. aeruginosa* (16 mm) and *S. aureus* (15 mm) and slightly lower effects were observed for *B. subtilis* (13 mm) and *E. coli* (11 mm). At 10 mg/ml concentration, higher inhibitory effects (13 mm) were noticed against *S. aureus* and *P. aeruginosa* and low inhibitory effects were recorded against *B. subtilis* (12 mm) and *E. coli* (11 mm). When 5 mg/ml concentration of ethanol extract used, a greater inhibitory effect was observed against *P. aeruginosa* (11 mm) and *B. subtilis* (10 mm) and lower effect was noticed against *E. coli* (9 mm). Overall at 5 mg/ml concentration of *A. rutifolia* ethanol extract*, S. aureus* was the most resistant strain while all tested bacterial strains were most susceptible to the ethanol extract at different concentrations.

A. rutifolia ethyle acetate extract exhibited inhibitory effects against the pathogenic strains at different concentrations ranges from 10 to 19 mm as shown in Table 3 and illustrated in Figure 7. At 50 mg/ml, highest inhibitory effect of *A. rutifolia* ethyle acetate extract was noticed against *B. subtilis* (19 mm) and *P. aeruginosa* (18 mm) and minimum (16 mm and 15 mm) for *E. coli* and *S. aureus* were observed. At 25 mg/ml concentration, ethyle acetate extract displayed higher effects against *B. subtilis* (17 mm) and *P. aeruginosa* (16 mm) and low inhibitory effects at 25 mg/ml were perceived for *B. aureus* (15 mm) and *E. coli* (15 mm). At 10 mg/ml concentration, ethyle acetate extract exhibited higher inhibitory effects (16 mm) against *B. subtilis* and *P. aeruginosa* (15 mm), while lower inhibitory effects at this concentration were seen for *E. coli* (11 mm) and *S. aureus* (13) mm). At 5 mg/ml, higher inhibitory effects of 14 mm against *P. aeruginosa* and *B. subtilis* and lower effects against *E. coli* (10 mm) and *S. aureus* (11) were recorded for the ethyle acetate extract. When 5 mg/ml concentration of ethyle acetate extract used, none of the tested bacterial strains displayed resistance but all were most susceptible.

A. rutifolia chloroform extract displayed inhibitory effects against the pathogenic strains at different concentrations ranges from 7 to 19 mm (Table 3, Figure 8). At 50 mg/ml, maximum inhibitory effects of chloroform extract were perceived against *P. aeruginosa* (18 mm), *B. subtilis* (16 mm) and *S. aureus* (14) while lower effect (9 mm) was perceived for *E. coli*. At a concentration of 25 mg/ml, chloroform extract displayed greater inhibitory effects against *P. aeruginosa* (19 mm) and *B. subtilis* (14 mm) while lower inhibitory effect was shownby *S. aureus* (12 mm) and *E. coli* (7 mm). At 10 mg/ml concentration, maximum inhibitory effects were detected against *P. aeruginosa* (16 mm) and *B. subtilis* (13 mm), while minimum effects of the chloroform extract were observed against *S. aureus* (12 mm). The chloroform extract of *A. rutifolia* showed that *E.coli* was the most resistant strain at 10 mg/ml concentration with no zone of inhibition. At 5 mg/ml concentration chloroform extract showed maximum inhibitory effects of 15 mm against *P. aeruginosa* and 13 mm against *B. subtilis* while lower was noticed against *S. aureus* (11). At 5 mg/ml concentration, *A. rutifolia* chloroform extract displayed that *E. coli* was the most resistant strain while the rest of the tested strains were susceptible to the chloroform extract of *A. rutifolia* at different concentrations.

A. rutifolia n-hexane extract also executed inhibitory effects for the tested strains at different concentrations with zones of inhibition range from 11 to 19 mm (Table 3, Figure 9). At 50 mg/ml, maximum growth inhibitions by *A. rutifolia n*-hexane extract were noted for *P. aeruginosa* (19 mm), *B. subtilis* (15 mm) and *E. coli* (14) and minimum inhibition (13 mm) was observed for *S. au-*

reus. *n*-hexane extract at 25 mg/ml concentration, displayed higher inhibitions against *P. aeruginosa* (17 mm) and *B. subtilis* (14 mm) while lower retardation in growth at 25 mg/ml concentration were discerned against *E. coli* (13 mm) and *S. aureus* (12.5 mm). At 10 mg/ml concentration, *n*-hexane extract indicated higher growth inhibition (15 mm) for *P. aeruginosa* and *B. subtilis* (14 mm), while lower retardations in microbial growth at 10 mg/ml were perceived for *S. aureus* (12 mm) and *E. coli* (11 mm). At 5 mg/ml concentration n-hexane extract of *A. rutifolia* showed greater growth inhibition (14 mm) for *P. aeruginosa* and while lower inhibition (11 mm) was noticed for *B. subtilis* as shown in Table 3 and illustrated in Figure 9. At 5 mg/ml concentration of *A. rutifolia* *n*-hexane extract, *E. coli* and *S. aureus* were the most resistant strains with no zones of inhibition, while other strains were most susceptible to the *n*-hexane extract.

Table 3. Antibacterial activity of *A. rutifolia* extracts with different solvents against pathogenic bacterial strains

Sr. No	Solvents	Concentration (mg/ml)	Zone of inhibition (mm) for bacterial strains			
			E. coli (Mean ± S.D)	*B. subtilis* (Mean ± S.D)	*S. aureus* (Mean ± S.D)	*P. aeruginosa* (Mean ± S.D)
1	Methanol	5	10.11±0.88	14.45±1.43	0±0.00	10.56±0.22
		10	11.44±1.81	15.21±3.12	10.01±1.11	12.33±1.97
		25	12.23±1.95	18.73±4.97	13.76±2.15	12.18±1.10
		50	14.04±3.39	19.21±5.77	15.23±2.87	14.19±3.12
2	Ethanol	5	9.44±0.50	10.33±0.78	0±0.00	11.17±0.87
		10	11.67±1.11	12.06±1.90	13.15±2.19	13.56±2.21
		25	11.70±1.20	13.11±2.03	15.45±2.66	16.88±3.55
		50	14.07±2.21	16.12±4.05	16.32±4.15	17.05±4.96
3	Ethyl acetate	5	10.91±1.09	14.56±3.65	11.67±1.68	14.12±3.24
		10	13.45±2.16	16.22±3.87	13.54±2.24	15.11±3.33
		25	15.14±3.40	17.03±5.12	15.08±3.66	16.22±4.08
		50	15.76±3.98	19.83±5.34	16.66±4.72	18.78±5.41
4	Chloroform	5	0±0.00	13.08±1.98	11.34±1.23	15.56±3.51
		10	0±0.00	13.11±2.05	12.11±1.43	16.32±4.55
		25	7.12±0.15	14.32±3.08	12.45±1.67	19.11±5.60
		50	9.49±0.56	16.65±3.88	14.11±4.11	18.02±5.10
5	n-Hexane	5	0±0.00	11.78±1.15	0±0.00	14.39±2.19
		10	11.44±1.70	14.41±3.44	12.21±1.63	15.11±4.21
		25	13.21±2.79	14.65±3.48	12.55±1.70	17.12±5.67
		50	14.34±3.11	15.12±2.50	13.01±2.01	19.19±5.61

Values are the average of at least three readings (±SD)

Minimum inhibitory concentrations (MIC's) of the effective extracts of *A. rutifolia*

Results of antimicrobial activity of the *A. rutifolia* extracts corroborated that at 5 mg/ml concentration, few strains were resistant, while most of the strains were susceptible at all concentrations (5, 10, 25 and 50 mg/ml) respectively (Table 4). Moreover, in the methanol and ethyl acetate extracts, all the tested bacterial strains were susceptible and these two extracts showed a strong activity against the tested strains even at lowest concentration of 5 mg/ml. Among the strains, *B. subtilis* and *P. aeruginosa* bacterial strains were most susceptible at low concentration of 5 mg/ml of all extracts. Hence, experiments were conducted to check the MIC's of the most effective plant extracts (methanol and ethyl acetate) against the most susceptible bacterial strains (*B. subtilis* and *P. aeruginosa)* at lower concentrations (1-4 mg/ml). The results of MICs are given in Table 4 (Figures not shown). The MIC effect of *A. rutifolia* methanol extract started at 3 mg/ml with inhibition zones of 4 mm and 5 mm against *B. subtilis* and *P. aeruginosa* and the inhibitory effects of ethyl acetate extract also started at 3 mg/ml with inhibition zones of 5 mm and 6 mm against *B. subtilis* and *P. aeruginosa*. Overall, both the methanol and ethyl acetate extracts of *A. rutifolia* displayed higher inhibitory effects against *P. aeruginosa* as compared to *B. subtilis* at lower concentrations.

Table 4. MIC's of the most effective extracts of *A. rutifolia* against the most susceptible bacterial strains

Solvent used	Conc. mg/ml	Inhibition zones (mm)	
		Gram + *B. subtilis* (Mean ± S.D)	Gram - *P. aeruginosa* (Mean ± S.D)
Methanol	1	0±0.00	0±0.00
	2	0±0.00	0±0.00
	3	4.83±0.65	5.66±0.85
	4	7.33±1.55	9.67±2.31
Ethyl acetate	1	0±0.00	0±0.00
	2	0±0.00	0±0.00
	3	5.83±0.67	6.66±1.02
	4	9.12±2.23	10.18±3.01

Values are the average of at least three readings (±SD)

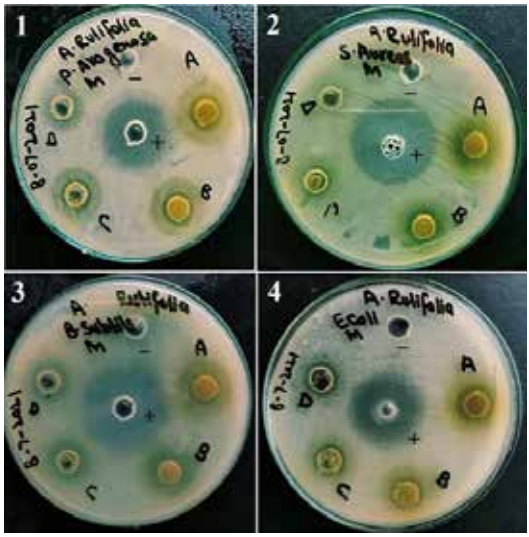

Figure 5. Growth inhibition of pathogenic bacteria by methanolic extract of *A. rutifolia. 1= P. aeruginosa, 2= S. aureus, 3= B. subtilis, 4= E. coli.* A-D are the extract concentrations used against the tested bacterial strains, A= 50 mg/ml, B = 25 mg/ml, C = 10 mg/ml, D = 5 mg/ml, - = Negative control (DMSO), + = Positive control (Levofloxacin)

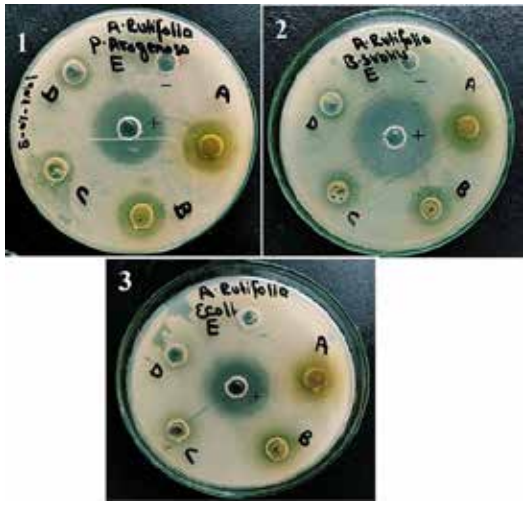

Figure 6. Growth inhibition of pathogenic bacteria by ethanol extract of *A. rutifolia. 1= P. aeruginosa, 2= B. subtilis, 3= E. coli.* A-D are the extract concentrations used against the tested bacterial strains, A= 50 mg/ml, B = 25 mg/ml, C = 10 mg/ml, D = 5 mg/ml, - = Negative control (DMSO), + = Positive control (Levofloxacin)

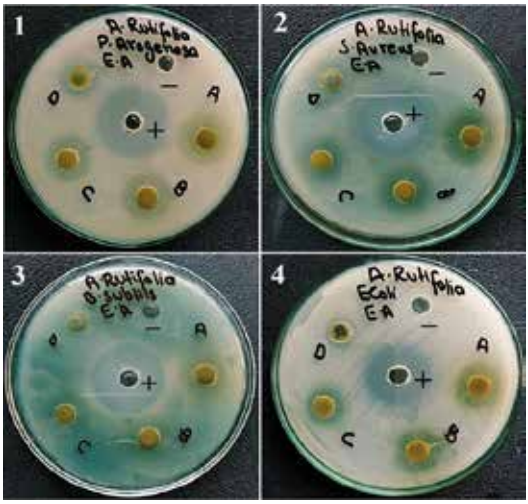

Figure 7. Growth inhibition of pathogenic bacteria by the ethyl acetate extract of *A. rutifolia. 1= P. aeruginosa, 2= S. aureus, 3= B. subtilis, 4= E. coli.* A-D are the extract concentrations used against the tested strains, A= 50 mg/ml, B = 25 mg/ml, C = 10 mg/ml, D = 5 mg/ml, - = Negative control (DMSO), + = Positive control (Levofloxacin)

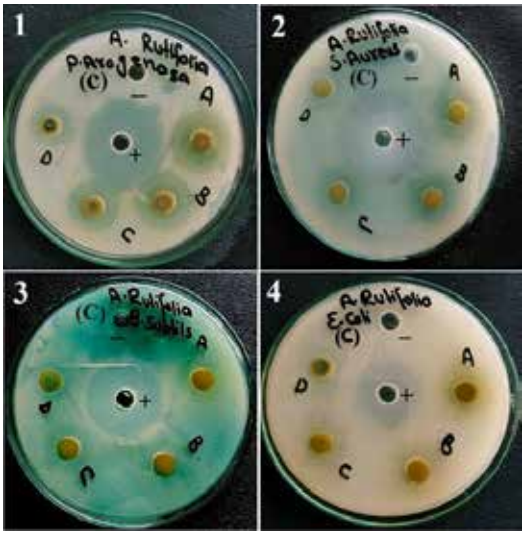

Figure 8. Growth inhibition of pathogenic bacteria by chloroform extract of *A. rutifolia. 1 P. aeruginosa, 2= S. aureus, 3= B. subtilis, 4= E. coli.* A-D are the extract concentrations used against the tested strains, A= 50 mg/ml, B = 25 mg/ml, C = 10 mg/ml, D = 5 mg/ml, - = Negative control (DMSO), + = Positive control (Levofloxacin)

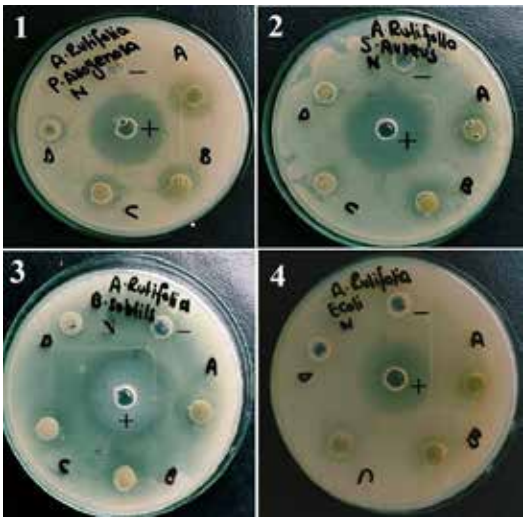

Figure 9. Growth inhibition of pathogenic bacteria by n-hexane extract of *A. rutifolia. 1= P. aeruginosa, 2= S. aureus, 3= B. subtilis, 4= E. coli.* A-D are the extract concentrations used against the tested strains, A= 50 mg/ml, B = 25 mg/ml, C = 10 mg/ml, D = 5 mg/ml, - = Negative control (DMSO), + = Positive control (Levofloxacin)

In this study, the antibacterial activities of A. rutifolia extracts were assessed against clinical and phytopathogens initiating human diseases and damaging most important crops. We adapted two approaches before selecting A. rutifolia plant for its TFC, TPC and potential antimicrobial activity. Firstly, we selected *A. rutifolia* on the basis of its local occurrence and its extensive folk traditional uses in the studied area. Secondly, a very scarce availability of data on the phytochemistry and biological activates of *A. rutifolia*. The findings regarding TPC and TFC of *A. rutifolia* confirmed the presence of phenols and flavonoids in its extracts. The maximum amount of phenols and flavonoids were recorded for ethanol extract and minimum for *n*-hexane extract as shown in Figures 3 and 4. Plants are rich in significant phytochemicals and their utilization could be very significant in enhancing the therapeutic approaches to cure pathogenic as well as genetic diseases**.** This milestone could be easily achieved if the phytochemical profile of plant species is well understood. A lot of studies globally reported the presence of significant phytochemicals in the extracts of different *Artemisia* species[19-39] proposing *Artemisia* species a very rich source of essential chemical constituents with potential biological activities including antioxidant,[40-42] antimicrobial,[40,41,43-47] antiviral,[48-53] antimalarial,[54-58] anticancerous,[59-62] antidiabetic/hypoglycemic,[63-68] anti-inflammatory,[61,69,70] and anthelmintic activities[71-73].

Here, all the *A. rutifolia* extracts showed effective growth retardation against two gram positive (*B. subtilis* and *S. aureus*) and two gram negative bacterial strains (*E. coli,* and *P. aeruginosa*) at concentrations of 50 and 25 mg/ml while low growth retardation was observed against the tested strains at 10 and 5 mg/ml concentrations in all extracts of *A. rutifolia*. Among the 5 tested extracts of *A. rutifolia,* the methanol and ethyle acetate exhibited better antibacterial activity even at lowest concentration of 5 mg/ml where *B. subtilis* and *P. aeruginosa* were the most susceptible strains. It is assumed that the *Artemisia* species possess significant secondary metabolites which give therapeutic effect5 against diseases and a lot of studies on antimicrobial and antioxidant activities of *Artemisia* species around the world have been reported[22,41,46,74-84].

In a study, antimicrobial activity of methanolic extracts of the aerial parts of *A. oliveriana, A. diffusa*, *A. turanica* and *A. scoparia* against *S. aureus, B. subtilis, E. coli, C. albicans* and *P. aeruginosa* were documented[77] against pathogenic bacteria.

Suresh *et al.*[46] studied antimicrobial activity of ethanolic extracts of *A. pallens* and *A. abrotanum* that showed maximum activity at 30 mg/ml against *B. stearothermophilus* and *P. cepacia*. Two flavones from *A. giraldii* were found to be effective against *S. lutea, S. aureus, E. coli, Proteus* sp.*, P. aeruginosa, T. viride* and *A. flavus*[75].

Ahameethunisa and Hopper[22] showed six organic solvent extracts of *A. nilagirica* from India with inhibitory effect against gram-positive and gram-negative bacteria except for *E. faecalis, K. pneumonia* and *S.aureus*.

Akrout *et al.*[80] investigated the antiradical and antimicrobial activities of *A. campestris* essential oil from Tunisia where its essential oil displayed a strong inhibitory effect on *E. coli* bacterial strain. The methanol extracts of *A. campestris* were also scrutinized for antibacterial activity by Naili *et al.*[81] and the extract was found to have a sturdy inhibitory effect on *B. subtilis* and *S. aureus* strains. The essential oils and ethanolic extracts of *A. santonicum* from Tekirdağ and *A. absinthium*, *A. scoparia* and *A. vulgaris* from Erzurum were evaluated for antimicrobial activity against 4 bacteria and *C. albicans*. Only *A. scoparia* oil was reported to have an inhibitory effect against *C. albicans* and *E. col*[74].

In another study, *A. scoparia* was also reported with antimicrobial activity against 15 oral bacteria using the minimum inhibitory concentration (MIC) method by Cha *et al.*[78]. Dulger *et al.*[85] investigated *A. absinthium* extracts and showed inhibitory effect against *Salmonella* and *Bacillus* strains.

In a study, *A. arborescen, A. absinthium, A. scoparia, A. campestris, A. vulgaris* and *A. santonicum* from Turkey were examined for their antimicrobial activity against eight bacterial and one fungal strain where the studied *Artemisia* species displayed a better antimicrobial activity[41]. In another study, antibacterial activity of methanol extracts of aerial part of *A. sieberi* against *E. cloacae P. aeruginosa, E.coli* and *P. mirabilis* were found to have better inhibitory action[82].

The essential oil and compounds of *A. annua* flowering part were tested against *S. Enteritidis, E. coli* O157, *S. Typhi, L. monocytogenes* and *Y. enterocolitica,* where all the extracts showed great effect against foodborne pathogens83. Study of Javid *et al.*[84] showed chloroform, ethyl acetate and butanol extracts of *A. indica* with high inhibitory effect between 15-20 mm against *S. aureus, P. aeruginosa* and *E. coli.*

It is believed that these reported antimicrobial activities of different species related to Asteraceae including the species of genus *Artemisia* are primarily accredited to its most active ingredients like the alkaloids and polyphenols[86,87]. Other crucial group of compounds like flavonoids from plant extracts has been found to possess antioxidants and antimicrobial actions[88-90]. Antibacterial results of the current investigation validate that *A. rutifolia* extracts screened are proven to be operative antimicrobials which might be due to the presence of phenols and flavonoids which are validated to be conceivably active in controlling disease causing bacteria.

Conclusively, all the extracts (Methanolic, ethanolic, chloroform, ethyl acetate and *n*-hexane) of *A. rutifolia* are rich in flavonoids and phenols and exhibited potential antimicrobial activity against the tested pathogenic bacterial strains at different concentrations (5 mg/ml, 10 mg/ml, 25 mg/ml and 50 mg/ml). MICs results showed that the methanol and ethyl acetate extracts are effective against *B. subtilis* and *P. aeruginosa* with low concentrations of 3 and 4 mg/ml and the ethyl acetate extract possess a higher 392 inhibition activity against *P. aeruginosa* and *B. subtilis* as compared to the methanol extract. Hence, the isolation and purification of therapeutic phenols and flavonoids from *A. rutifolia* extracts could be used as an operational source against human and plant bacterial infections. It is recommended that, more detailed phytochemical and pharmacological studies are needed on *A. rutifolia* extracts in order to find out active compounds against clinical and phyto-pathogenic bacterial strains.

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CONFLICT OF INTEREST

Nothing to declare

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