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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF DIFFERENT EXTRACTS OF *TAMARIX APHYLLA* (L.) KARST. (ATHEL TAMARISK)

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ABSTRACT

The antibacterial activities of different solvents (methanol, n-hexane, n-butanol, ethyl acetate and aqueous) extracted samples of *Tamarix aphylla* (L.) Karst. were checked against different microbes i.e *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Xanthomonas compestris* and *Klebsiella pneumonia* using disc diffusion assay. Zone of inhibition (ZI) was calculated in percentage (%). In case of leaf samples, all extracts showed antibacterial activity against bacterial strains. The antimicrobial activity when the concentration of the extract was increased from 1 to 3 mg/disc. Aqueous phase, except for *P. aeruginosa, X. compestris* and *E. coli* at 3 mg/disc showed no activity against other microbes at all concentrations. The highest ZI was observed for ethyl acetate at 3 mg/disc concentration against *X. compestris* (86.0%) and *P. aeruginosa* (82.0%). In comparison to leaf extracts, the stem extracts showed comparatively high activities against all bacterial strains. The antimicrobial activity increased with increase in concentration (1-3 mg/disc). The aqueous phase except for *B. subtilis* at 3 mg/disc extract concentration showed no activity against other microbes at all concentration. In case of stem extracts, ethyl acetate showed maximum ZI (91.3%) against *X. compestris* and *K. pneumonia* (90.0%) at 3 mg/disc extract concentration. Higher concentration of phytochemicals i.e saponins, tannins, flavonoids, alkaloids and terpenoids were found in stem fractions as compared to leaf. The n-butanol and ethyl acetate fractions of both leaf and stem extracts showed high concentration of phytochemicals. The high antibacterial activities of n-butanol and ethyl acetate fractions may partly be attributed to the high concentrations of phytochemicals in these fractions.

Key Words: *Tamarix aphylla*, Antimicrobial activity, Phytochemicals, *Klebsiella pneumonia*

INTRODUCTION

Tamarix aphylla (L.) Karst., referred to as Khagal (Urdu) and Ghaz (Pashto) in Pakistan, is used as medicinal plant (Lefahal *et al*., 2010; Emad and Gamal, 2013). *T. aphylla* (Family, Tamaricaceae) is commonly planted on road side (Marwat *et al*., 2008). *T. aphylla* is a fast growing, moderate sized evergreen tree, up to 18 m high, with erect tapering trunk and many stout spreading purplish brown and smooth branches.

Ethno-botanically, its bark, leaves, stem and twigs are preferred for different diseases with no side effects by local practitioners in the country. Thus, leaves are considered as a source for curing different infectious diseases (Marwat *et al*., 2008; Marwat *et al*., 2009; Panhwar and Abro, 2007). The good antimicrobial activities of medicinal plants are due to the presence of secondary metabolites. Previously, different phyto-constituents like alkaloids, tannins and phenolic contents have been found in the leaves of *T. aphylla* (Achakzai *et al*., 2009).

The extracts of *Tamaricaceae* plants showed good antibacterial activities due to the presence of secondary metabolites against *Escherichia coli*, *Pseudomonas*, *Salmonella* and *Staphylococcus* species (Lefahal *et al*., 2010; Emad and Gamal, 2013). *Escherichia coli* are bacterial pathogens that causes diarrhea; similarly, *Pseudomonas aeruginosa* causes pulmonary infection and *Staphylococcus aureus* causes pneumonia and skin infections (De Victorica and Galván, 2001; Antai, 1987). Extracts from the tree are used in traditional medicine in Italy to get rid of warts, and as a remedy for curing the gastro-intestinal tract of worms in other parts of Europe. Extracts also proved to be potent chemo protective agent (Tahira *et al*., 2011). The small branches and leaves have astringent and diuretic properties and externally a compress of the leaves and twiglets will staunch bleeding. The flower galls are used in traditional medicine for their astringent properties and as a gargle. A decoction of the bark is used for eczema and other skin complaints (Adnan *et al*., 2015).

Keeping in view the importance of *T. aphylla* as a medicinal plant, its antimicrobial activity was under taken to be investigated using different plant parts such as leaves and stem. In the present study, we investigated the

antimicrobial activity and phytochemical analysis of various solvents extracts from leaves and stem of *T. aphylla* to create a background for future medicinal use of *T. aphylla*.

MATERIALS AND METHODS

Plant material

The current research work was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture, Peshawar. The plant material (Leaves and stem of *T. aphylla*) was collected from District Nowshera, Khyber Pukhtunkhwa. Plant material was washed to remove dust and was shade dried before crushing into powder form.

Crude extract preparation

The dried leaves and stem of *T. aphylla* were grinded using an electric grinder. The plant material was soaked in methanol for 7 to 8 days with regular shaking to dissolve the bioactive compounds followed by drying under reduced pressure using rotary evaporator. The process was repeated three times and extract was then transferred to another funnel filled with60-80 mL distilled. Specific amount of n-hexane was added, shaken and leave it to stand for 10-15 minutes

The organic layer was collected and the process was repeated by adding fresh organic solvents. Many of fractions were separated by adding n-hexane in water, ethyl acetate and n-butanol, one by one in this manner. All the organic layers were dried by using rotary evaporator.

Culture Media

Nutrient broth was used for shaking incubation and standardization of different microorganisms while nutrient agar media Bakht *et al*. (2011) was used for culturing and growth Bakht *et al.* (2011).

Microbial Activity

The major types of target microbes are from Kingdom Eubacteria; therefore antibacterial activities of the selected solvent extracts (leaves and stem) were tested against selected strains of bacteria.

Antibacterial Bioassay

The antibacterial were performed using disc diffusion assay according to Bakht *et al.* (2011) against selected strains of bacteria and fungi (Table 1). Microbial inoculums were subjected to the media plates (nutrient agar) for about 18-24 hours. The standard of inoculums used was McFarland Standard i.e. 1-2×107 CFU mL-1 0.5. Three discs of the filter paper of size of about 6 mm diameter were prepared and placed in the Petri dishes having media using sterile forceps. The plant extracts having a concentration of 0.5 and 1-mg were taken in the amounts in 6-µL and 12-µL volumes and used. The positive control antibiotics i.e., Ciprofloxacin 50-µg/6µL were used as 6-µL/disc while DMSO was used as a negative control with the same ratio. After inoculation, the plates were incubated at 37ºC for about 24 hours. Within a daytime period, inoculum has inhibited the microbial growth on the plates and was recorded as the ZI in mili-meter.

Phytochemical analysis

The aqueous crude extracts were used for the phytochemical analysis for alkaloids, terpenoids, saponins, flavonoids and tannins.

Test for saponins

About 0.5 g of crude extract was dissolved in 5 mL distilled water and filtered. Then, 0.01 g of $NaHCO_3$ was added. Shaking it, forms persistent foam indicating the presence of saponins (Khalid *et al.,* 2018).

Test for tannins

About 0.5 g of extract was taken in 1 mL distilled water. Then, 1-2 drops of 5% ferric chloride was added to solution and observed blue or greenish black color (Yadav and Agarwal, 2011).

Test for flavonoids (Alkaline Reagent Test)

Dilute NaOH was added to about 0.2 g of crude extract and few drops of diluted HCl were added. As a result, colorless solution is formed which showed the presence of flavonoids (Neelima *et al.,* 2011).

Test for alkaloids

The extract 0.05 g in weight was mixed with 10 mL of diluted HCl. Then filter it, few drops of Mayers reagent was added to the solution. White or creamy precipitates indicate alkaloids (Khalid *et al*., 2018).

Test for terpenoids

In 10 mL of methanol, 0.8 g of extract was dissolved and then filter. 1 mL of chloroform and 2 mL of H_2SO_4 was added. The formation of reddish brown color shows terpenoids (Astuti, 2011).

Data analysis

The data is presented in triplicate. Standard deviation and means were calculated by Microsoft excel (2010).

RESULTS

The current research study was performed to investigate the antimicrobial activities of leaves and stem of *Tamarix aphylla*. All the solvent extracted samples were tested against five bacterial strains.

Antimicrobial activities of different solvent extracted samples from leaves of *Tamarix aphylla*

Bacillus subtilis

The antimicrobial effect of ethyl acetate, n-butanol, n-hexane, methanolic, and aqueous (water) extracts from leaves of *Tamarix aphylla* against *B. subtilis* tabulated in (Table 2). The data showed inhibition of *B. subtilis* on all extracts except water where no zone of inhibition was seen at all extract concentrations. Among other extracts, crude (methanol), n-butanol and ethyl acetate showed maximum activities measured as zones of inhibition i.e. 73, 66 and 72%, respectively at 3 mg/disc extract concentration, respectively. N-hexane showed comparatively lower activity against *B. subtilis* at all three concentrations.

Pseudomonas aeruginosa

Pseudomonas aeruginosa showed susceptibility to all extracts. Highest inhibition activities were showed by ethyl acetate (82.0%) followed by crude (70.7%) and n-butanol (64.7%) at 3 mg/disc extract concentration. The n-hexane extract showed comparatively lower activities at all three concentrations. The aqueous phase showed activity (32.5%) at 3 mg/disc concentration. However, no activity was observed at 1 and 2 mg/disc concentrations (Table 3).

Xanthomonas campestris

Xanthomonas campestris showed susceptibility to all fractions except aqueous extract. Higher inhibition activity was observed by ethyl acetate that measured 86.0% at 3 mg/disc concentrations (Table 4). Crude and n-butanol extracts also showed high activities i.e 69.1% and 68.1%, respectively at 3 mg/disc concentration. The n-hexane fraction showed comparatively lower activities at the three concentrations. The aqueous fraction showed activity only at 3 mg/disc concentration that was measured to be 28.5%.

Klebsiella pneumonia

Klebsiella pneumonia by all fractions except water where no activity was observed (Table 5). The highest activities were shown by ethyl acetate and crude extracts, measuring 68.6%, and 55.7% ZI, respectively at 3 mg/disc concentration. N-butanol and n-hexane fractions exhibited comparatively lower activities at all concentrations.

Escherichia coli

All fractions except water showed inhibition of *Escherichia coli* at all concentrations (Table 6). Maximum activity was shown by crude, ethyl acetate and n-butanol fractions measuring 66.4%, 69.7% and 61.0% ZI, respectively at 3 mg/disc concentration as compared to control. The n-hexane fraction exhibited comparatively lower activities at all the three concentrations. The aqueous fraction showed activity against *E. coli* only at 3 mg/disc concentration that measured as 24.5% ZI.

Antimicrobial activities of different solvent extracted samples from the stem of *Tamarix aphylla*

Bacillus subtilis

All fractions, except water showed activities against *B. subtilis* at all concentrations (Table 7). Analysis of data showed that n-butanol exhibited the highest activity (86%) followed by ethyl acetate (76%) and n-hexane (75%) at 3 mg/disc concentration. Crude methanols also showed good activity but slightly lower than the other fractions.

Aqueous fraction did not show any activity at 1 and 2 mg/disc concentrations; however, showed activity (30%) at 3 mg/disc concentration.

Pseudomonas aeruginosa

The data showed that all fractions showed high activities at all the three extract concentrations (Table 8). The highest ZI was recorded on ethyl acetate fraction (80.0%) followed n-butanol by (76.3%) at 3 mg/disc concentration. As in the case of previous results, aqueous fraction showed no activity all the three concentrations.

Table 1. List of bacterial strains used in the present study.

Microbial species/strains	**Strain Type (Gram)**	**Microbial strain sources**
Bacillus subtilis	Positive	Dept. of Microbiology, Quaid-I-Azam University Islamabad, Pakistan
Pseudomonas aeruginosa	Negative	ATCC # 9721
Xanthomonas compestris	Negative	ATCC # 33913
Klebsiella pneumonia	Negative	ATCC # 10231. Dept. of Plant Pathology, Uni. of Agric. Peshawar, Pakistan
Escherichia coli	Negative	ATCC # 25922

Table 2. Antimicrobial effect of different solvents extracted samples from leaves of *T. aphylla* on *B. subtilis*. Effect is shown as % zone of inhibition.

Plant Extract	**Concentration of extract (mg disc^{-1})**	**Mean ± SD**	**ZI (%)**	**Positive control Ciprofloxacin (mm)**	**Negative control (DMSO) (mm)**
Crude	1	12.3±0.6	41.0		-
	2	16.4±5.4	54.6		-
	3	22.0±6.6	73.0		-
n-hexane	1	10.3±1.2	34.3		-
	2	11.5±3.5	38.3		-
	3	13.6±3.8	45.3		-
n-butanol	1	13.3±1.5	44.3		-
	2	16.7±5.1	55.7	30	-
	3	19.8±5.6	66.0		-
Ethyl acetate	1	13.6±0.7	45.3		-
	2	17.2±5.4	57.3		-
	3	21.6±6.2	72.0		-
Aqueous	1	0.0±0.0	0.0		-
	2	0.0±0.0	0.0		-
	3	0.0±0.0	0.0		-

± SD: standard deviation; ZI: zone of inhibition; mm: millimeter

Xanthomonas campestris

Data indicated that almost all extracted samples were exhibited to be potent against *Xanthomonas campestris* and showed highest ZI. In comparison to other fractions, ethyl acetate exhibited the highest zone of inhibition (91.3%) at 3 mg/disc concentration, followed by n-hexane (84%). The aqueous phase did not show any activity at all the three concentrations (Table 9).

Klebciela pneumonia

Data showed that all extracted samples were effective to counter *K. pneumonia* except aqueous extract (Table 10). Maximum ZI i.e 90.0% was exhibited by ethyl acetate followed by n-hexane and n-butanol with 79 and 78% ZI

respectively at 3 mg/disc concentrations. However aqueous fraction showed no activity on all the three concentrations.

Table 3. Antimicrobial effect of different solvents extracted samples from leaves of *T. aphylla* on *P. aeruginosa.* Effect is shown as % zone of inhibition.

Plant Extract	Concentration of extract (mg disc^{-1})	Mean ± SD	ZI (%)	Positive control Ciprofloxacin (mm)	Negative control DMSO (mm)
	1	12.2±4.7	40.7		-
Crude	2	16.5±5.0	55.0		-
	3	21.2±6.0	70.7		-
	1	8.8±0.4	29.3		-
n-hexane	2	10.5±3.4	35.0		-
	3	14.2±4.1	47.3		-
	1	13.5±2.7	45.0		-
n-butanol	2	14.6±4.4	48.7	30	-
	3	19.4±5.4	64.7		-
	1	14.3±3.0	47.7		-
Ethyl acetate	2	20.9±6.2	69.7		-
	3	24.6±7.9	82.0		-
	1	0.0±0.0	0.0		-
Aqueous	2	0.0±0.0	0.0		-
	3	7.6±5.0	32.5		-

± SD: standard deviation; ZI: zone of inhibition; mm: millimeter

Table 4. Antimicrobial effect of different solvents extracted samples from leaves of *T. aphylla* on X. *compestris.* Effect is shown as % zone of inhibition.

Plant Extract	Concentration (mg disc^{-1})	Mean ± SD	ZI (%)	Positive control Ciprofloxacin (mm)	Negative control DMSO (mm)
	1	10.7±1.2	33.4		-
Crude	2	16.9±5.4	52.8		-
	3	22.1±6.6	69.1		-
	1	10.2±1.1	31.9		-
n-hexane	2	10.1±3.3	31.6		-
	3	12.1±3.4	37.8		-
	1	12.9±0.8	40.3		-
n-butanol	2	16.8±5.3	52.5	32	-
	3	21.8±6.5	68.1		-
	1	16.1±2.4	50.3		-
Ethyl acetate	2	24.3±7.5	75.9		-
	3	25.8±8.6	86.0		-
	1	0.0±0.0	0.0		-
Aqueous	2	0.0±0.0	0.0		-
	3	7.2±2.8	28.5		-

± SD: standard deviation; ZI: zone of inhibition; mm: millimeter

Escherichia coli

Data indicated that *E. coli* were highly susceptible to fractions obtained from ethyl acetate and crude showing 80 and 77% ZI, respectively at 3 mg/disc concentrations. Aqueous fractions showed no activities at all concentrations (Table 11).

In comparison to the leaves of *T. aphylla*, stem samples were comparatively higher inhibition activities against all bacterial strains. It means, the fractions from the stem samples may contain higher number or concentrations of phytochemicals than those in the leaves samples.

Table 5. Antimicrobial effect of different solvents extracted samples from leaves of *T. aphylla* on *K. pneumonia.* Effect is shown as % zone of inhibition.

Plant Extract	Concentration of extract (mg disc^{-1})	Mean ± SD	ZI (%)	Positive control Ciprofloxacin (mm)	Negative control DMSO (mm)
	1	11.1±2.3	31.7		-
Crude	2	16.5±5.0	47.1		-
	3	19.5±5.7	55.7		-
	1	9.2±1.0	26.3		-
n-hexane	2	12.3±3.8	35.1	35	-
	3	14.0±4.1	40.0		-
	1	11.5±1.0	32.8		-
n-butanol	2	14.4±4.6	41.1		-
	3	17.0±5.1	48.6		-
	1	16.0±2.6	45.7		-
Ethyl acetate	2	19.3±6.0	55.1		-
	3	24.0±6.9	68.6		-
	1	0.0±0.0	0.0		-
Aqueous	2	0.0±0.0	0.0		-
	3	0.0±0.0	0.0		-

± SD: standard deviation; ZI: zone of inhibition; mm: millimeter

Table 6. Antimicrobial effect of different solvents extracted samples from leaves of *T. aphylla* on *E. coli.* Effect is shown as % zone of inhibition.

Plant Extract	Concentration of extract (mg disc^{-1})	Mean ± SD	ZI (%)	Positive control Ciprofloxacin (mm)	Negative control DMSO (mm)
	1	11.9±1.0	36.1		-
Crude	2	14.8±4.6	44.8		-
	3	21.9±6.4	66.4		-
	1	11.0±1.3	33.3		-
n-hexane	2	12.8±3.9	38.8	33	-
	3	15.3±4.3	46.4		-
	1	12.3±0.9	37.3		-
n-butanol	2	16.1±5.1	48.8		-
	3	20.1±5.9	61.0		-
	1	12.4±3.0	37.6		-
Ethyl acetate	2	15.9±4.5	48.2		-
	3	23.0±6.6	69.7		-
	1	0	0		-
Aqueous	2	0	0		-
	3	8.1±2.6	24.5		-

± SD: standard deviation; ZI: zone of inhibition; mm: millimeter

Phytochemical analysis

Phytochemicals such as saponins, tannins, alkaloids, flavonoids and terpenoids are extremely important agents to confer resistance and tolerance against a number of biotic and abiotic stresses. Analysis of the phytochemicals in the various fractions of the leaves and stem samples of *T. aphylla* are summarized (Tables 12). Concentrations of the phytochemicals were analyzed based on changing of coloration upon addition of chemicals required for detection.

The color strength was linked with the concentration of the phytochemical. Low, moderate and high concentrations were denoted by symbols as +, ++, and +++, respectively. According to the data in Table 12 n-butanol and ethyl acetate fractions from the leaves samples showed high concentrations of these compounds. These fractions were also found with high inhibition activities against all bacterial strains. Therefore, this data may reveal that the high activities of these fractions might be due to the presence of comparatively higher phytochemicals concentrations. However, the concentrations of these phytochemicals increased in the same fractions from the stem samples (Table 12). These results confirm that the stem samples of *T. aphylla* are more effective than those of the leaves samples to counteract the growth of bacterial strains. Also, the aqueous fraction contained no or very low concentrations of the phytochemicals.

Table 7. Antimicrobial effects of different solvent-extracted samples from the stem of *T. aphylla* against *B. subtilis*. Effect is shown as % zone of inhibition.

Plant Extract	Concentration (mg disc^{-1})	Mean ± SD	ZI (%)	Ciprofloxacin (millimeter)	DMSO (mm)
Crude	1	12.6 ± 1.4	42.0		-
	2	18.7 ± 1.8	62.3		-
	3	20.3 ± 2.8	68.0		-
n-hexane	1	12.7 ± 2.9	42.3		-
	2	19.1 ± 1.5	64.0		-
	3	22.4 ± 2.0	75.0		-
n-butanol	1	17.3 ± 2.0	58.0		-
	2	22.4 ± 1.0	75.0	30	-
	3	25.8 ± 1.3	86.0		-
Ethyl acetate	1	11.0 ± 1.2	37.0		-
	2	20.7 ± 1.8	69.0		-
	3	22.7 ± 1.3	76.0		-
Aqueous	1	0.0 ± 0.0	0.0		-
	2	0.0 ± 0.0	0.0		-
	3	9.0 ± 1.7	30.0		-

± SD: standard deviation; ZI: zone of inhibition; mm: millimeter

Table 8. Antimicrobial effects of different solvent-extracted samples from the stem of *T. aphylla* against *P. aeruginosa*. Effect shown as % zone of inhibition.

Plant Extract	Concentration (mg disc^{-1})	Mean ± SD	ZI (%)	Ciprofloxacin (millimeter)	DMSO (mm)
Crude	1	15.5 ± 1.6	52.0		-
	2	18.7 ± 2.3	62.3		-
	3	21.8 ± 2.3	73.0		-
n-hexane	1	15.3 ± 2.3	51.0		-
	2	21.3 ± 2.7	71.0		-
	3	21.3 ± 2.5	71.0		-
n-butanol	1	17.3 ± 2.3	58.0		-
	2	21.3 ± 2.3	71.0	30	-
	3	22.9 ± 1.7	76.3		-
Ethyl acetate	1	16.4 ± 2.3	53.0		-
	2	21.3 ± 2.6	71.0		-
	3	24.1 ± 1.0	80.3		-
Aqueous	1	0.0 ± 0.0	0.0		-
	2	0.0 ± 0.0	0.0		-
	3	0.0 ± 0.0	0.0		-

± SD: standard deviation; ZI: zone of inhibition; mm: millimeter

Table 9. Antimicrobial effects of different solvent extracted samples from the stem of *T. aphylla* against *X. campestris*. Effect shown as % zone of inhibition.

Plant Extract	Concentration (mg disc^{-1})	Mean ± SD	ZI (%)	Ciprofloxacin (mm)	DMSO (mm)
	1	14.4 ± 1.6	48.0		-
Crude	2	20.8 ± 2.8	69.3		-
	3	23.4 ± 2.8	78.0		-
	1	15.6 ± 2.8	52.0		-
n-hexane	2	24.1 ± 1.7	80.3		-
	3	25.2 ± 1.2	84.0		-
	1	17.4 ± 1.1	58.0		-
n-butanol	2	22.2 ± 2.0	74.0	30	-
	3	23.9 ± 2.3	80.0		-
	1	19.1 ± 3.3	64.0		-
Ethyl acetate	2	25.9 ± 3.2	86.3		-
	3	27.4 ± 2.4	91.3		-
	1	0.0 ± 0.0	0.0		-
Aqueous	2	0.0 ± 0.0	0.0		-
	3	0.0 ±0.0	0.0		-

± SD: standard deviation; ZI: zone of inhibition; mm: millimeter

Table 10. Antimicrobial effects of different solvent extracted samples from the stem of *T. aphylla* against *K. pneumonia*. Effect is shown as % zone of inhibition.

Plant Extract	Concentration (mg disc^{-1})	Mean ± SD	ZI (%)	Ciprofloxacin (mm))	DMSO (mm)
	1	13.2 ± 2.0	44.0		-
Crude	2	20.1 ± 2.2	67.3		-
	3	22.0 ± 3.4	73.3		-
	1	13.3 ± 2.0	44.3		-
n-hexane	2	18.3 ± 2.0	61.0		-
	3	23.6 ± 3.0	79.0		-
	1	16.0 ± 2.3	53.3		-
n-butanol	2	23.0 ± 3.6	76.3	30	-
	3	23.4 ± 3.0	78.0		-
	1	14.1 ± 1.6	47.0		-
Ethyl acetate	2	20.5 ± 2.6	68.3		-
	3	27.2 ± 2.5	90.0		-
	1	0.0 ± 0.0	0.0		-
Aqueous	2	0. 0± 0.0	0.0		-
	3	0.0 ± 0.0	0.0		-

± SD: standard deviation; ZI: zone of inhibition; mm: millimeter

Table 11. Antimicrobial effects of different solvent extracted samples from the stem of *T. aphylla* against *E. coli* from different solvents extracted samples.

Plant Extract	Concentration (mg disc^{-1})	Mean ± SD	ZI (%)	Ciprofloxacin (mili-meter)	DMSO (mm)
	1	16.9 ± 1.4	56.3		-
Crude	2	20.2 ± 2.0	67.3		-
	3	23.0 ± 1.7	77.0		-
	1	13.8 ± 1.7	46.0		-
n-hexane	2	17.6 ± 2.3	59.0		-
	3	20.8 ± 3.3	69.3		-
	1	14.5 ± 2.5	48.3		-
n-butanol	2	20.9 ± 2.8	70.3	30	-
	3	21.5 ± 1.8	72.0		-
	1	15.0 ± 2.0	50.0		-
Ethyl acetate	2	19.5 ± 2.4	65.0		-
	3	24.0 ± 3.1	80.0		-
	1	0.0 ± 0.0	0.0		-
Aqueous	2	0.0 ± 0.0	0.0		-
	3	0.0 ± 0.0	0.0		-

± SD: standard deviation; ZI: zone of inhibition; mm: millimeter

Table 12. Phytochemical constituents of *T. aphylla* leaves extracts.

Plant part	Solvents	Saponins	Tannins	Alkaloids	Flavonoids	Terpenoids
Leaves	Water	+	+	_	+	+
	n-butanol	+++	+++	++	++	+++
	n-Hexane	++	++	+	+	++
	Ethyl-Acetate	+++	+++	+	++	+++
Stem	Water	+	++	++	+	+
	n-butanol	+++	+++	++	++	+++
	n-Hexane	++	+++	++	++	+++
	Ethyl-Acetate	+++	+++	++	+++	+++

Legend: += Low concentration, ++= Moderate concentration, +++= High Concentration, - = Absent

DISCUSSION

All fractions, except aqueous one from the leaves and stem of *T. aphylla* revealed maximum activity against the tested bacterial strains at all concentrations when measured through disc diffusion susceptibility assay. However, ethyl acetate, n-butanol and crude extracts showed comparatively higher activities against all microbes at the higher substrate concentration. N-hexane revealed lower activities than those of ethyl acetate, n-butanol and crude extracts. Similar results have been reported previously by Bakht *et al.* (2014). Sautron and Cock (2014) and Shakeri *et al.*, (2015) also supported the findings of the present research. The higher activities by the ethyl acetate fraction as compared to other against all bacterial strains may be attributed to the high concentration of phytochemicals in this fraction.

The data also described that crude extracted samples had effectively decreased the growth of all the tested microbial strains at all concentrations. The results showed that crude extracted samples from leaves and stem of *T. aphylla* exhibited good activity against the tested microbial strains at all the three concentrations. The data further indicated that crude extracts from leaves and stems of *T. aphylla* were effective against *Pseudomonas aeruginosa* and *E. coli* at the all concentrations upon comparing with other solvent samples and controls. Similar results were also demonstrated by Prakash *et al.* (2016) which reported that methanolic leaf extract was found to be more effective against selected pathogenic bacterial spp. as compared to acetone leaf extract. Furthermore, the leaf extracts more effectively inhibited the growth of gram-positive bacterial strains than gram-negative bacteria. Khanam *et al.* (2015) also reported similar activities for crude extracts of *Eurycoma longifolia* showing high levels of bioactive compounds.

Results concerning the antimicrobial activity of n-butanol fractions from leaves and stem of *T. aphylla* commendably restricted the growth of the tested microbes at all concentrations. Among the tested samples, n-butanol extracted fractions showed the highest activity against all microbes except *K. pneumonia* at all concentrations compared to control sample. Furthermore, n-butanol fractions of stem exhibited higher activity than leaves. This may be due to bioactive compounds present in leaves compared to stem. The results are similar to findings in previous studies where high activities were observed for the n-butanol fraction (Armatu *et al.*, 2011; Bakht *et al.*, 2013).

N-hexane extracted fractions from leaves of *T. aphylla* showed comparatively lower activities than the other fractions. However, the hexane fraction from the stem sample showed high activities than those leaves extracts. These results are somehow in partial agreement with those of Vogt *et al.* (2010) that reported antifungal activity mainly in methanol extract against *Fusarium graminearum* and *Macrophomina phaseolina*. Some other studies also showed lower activities for hexane fraction (Sautron and Cock, 2014).

All microbial strains showed resistance against the aqueous fraction from both leaves and stem samples. Activities were observed for some microbes only at higher substrate concentration. This might be due to the lower concentration of phytochemicals in the aqueous fraction as revealed by the phytochemical analysis. At all concentrations of our tests, all the bacteria strains were resistant to the aqueous samples from leaves and stem of *T. aphylla* showing that bioactive compounds were less soluble in water. These results are in agreement with Armatu *et al*. (2011) and Bakht *et al*. (2014).

The stem extracted fractions showed higher inhibitory activities than those of the leaves extracted fractions. It may be conclude that the stem fractions particularly, ethyl acetate, n-butanol and crude are highly effective against all bacterial strains. These fractions also contained higher concentrations of phytochemicals. The stem extracted

fractions should be further analyzed for phytochemicals using advanced analytical tools which will pave the way for the industrial application of *T. aphylla* for more cheap and safe therapeutic products and drugs.

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