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Evaluation of the Antibacterial Activity of the Solvent Fractions of the Leaves of *Dodonaea angustifolia*

Bereket Dessalegn[1], Seleshe Nigatu[2] and Ambaye Kinubeh[3]*

[1]*Unit of Biomedical Science, College of Veterinary Medicine and Animal Science, University of Gondar, Gondar, Ethiopia*

[2]*Department of Veterinary Epidemiology and Public Health, College of Veterinary Medicine and Animal Science, University of Gondar, Gondar, Ethiopia*

[3]*Department of Paraclinical Studies, College of Veterinary Medicine and Animal Science, University of Gondar, Gondar, Ethiopia*

***Corresponding Author:** Ambaye Kenubih, Department of Paraclinical Studies, College of Veterinary Medicine and Animal Science, University of Gondar, Gondar, Ethiopia.

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Abstract

Background: As a result of the growth of antibiotic resistance microbes, infectious illnesses have become one of the world's most serious challenges. Medicinal plants are a vast species of plant that are being used to treat a wide variety of infections. To treat bacterial infections, *Dodonaea angustifolia* (DA) is employed. *Dodonaea angustifolia* (DA) is used for the treatment of bacterial infections. This study was undertaken from November 2018-April 2019 to determine the active compounds from the leaves of DA and antibacterial effects of fractions.

Methods: Using the agar-well diffusion technique, the antibacterial activity of the solvent fractions (chloroform and ethyl acetate) was investigated against six disease-causing bacteria at varied concentrations. The solvent fractions' minimum inhibitory concentration (MIC) was evaluated using the broth micro-dilution method and tetrazolium chloride.

Result: The sensitive bacterial species were *Escherichia coli*, *Salmonella typhi*, and *C. freundii*. Ethyl acetate and chloroform fractions showed antibacterial activity against the development of harmful bacteria with variable degrees of antibacterial impact. When compared to other bacterial strains, *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains were not susceptible to the fraction.

Conclusion: When compared to the chloroform fraction, the ethyl acetate fraction showed substantial antibacterial efficacy against pathogenic bacteria growth. Further research should be carried out to isolate and characterize the bioactive components, as a recommendation.

Keywords: *Antibacterial; Dodonaea angustifolia; Test Bacteria; Solvent Fractions*

Background

Infectious diseases prove to be a significant cause of illness and mortality in livestock of Ethiopia. Inadequate supply of contemporary pharmaceuticals, which is driven by the government's lack of investment in procuring those medications, has exacerbated the crisis. Use of a small number of antimicrobial is incriminated as a cause for the growth of antibiotic resistance infections (Duguma, 2013). Due to these circumstances, the majority of livestock owners in Ethiopia have been compelled to rely on traditional animal health techniques to

manage and control prevalent livestock health problems. In addition, ethnoveterinary drugs provide low-cost options to the impoverished societies of underdeveloped countries (Admasu, 2011; Medihin and Abebe, 1998). More than 80% of the people in undeveloped countries including Ethiopia rely heavily on traditional medicine (Awas, 2007). As a result, research into the development of low-cost, synthetic-resistant, free, and locally accessible antibiotics is currently ongoing [1].

Plants offer greater than 95 percent of the components traditionally used in prepared remedies. Markets are stocked with medicinal herbs that may be used for fumigation, vermifuge, pain alleviation, and the treatment of ill animals.

Among thus the plants that used for traditional medicine are *Dodonaea angustifolia* (sand olive), locally called Kitkita in Amharic, a shrub which belongs to the family Sapindaceae and genus *Dodonaea*. It grows at altitudes between 800 and 2650m above sea level. Although found in different parts of the world, it is a popular hedge plant in East Africa including Ethiopia, Kenya, and Uganda (Avato., *et al.* 2006). The wood is extremely hard and used in Ethiopia as a fuel wood and for construction materials [2,3]. In folk medicine of Ethiopia, the leaves of *D. angustifolia* are used to treat wound [4] sudden diarrhea, wring worm and scabies [5], ectoparasite, lymphatic swelling [6] and bloat [7] in animal. While in human it is used to treat herpes zoster [8], parasitic worm [3,9] wound dressing [10], intestinal disease, unidentified swelling and sever dysentery [10].

In vitro and *in vivo* studies revealed that extracts showed an effect on Herpes Simplex [11], anti-inflammatory effect [12], antibacterial, antioxidant effect [13,14] and anticholinesterase effect [15], antimalarial effect [16-18].

Objective of the Study

The objective of this study was to assess the *in vitro* antibacterial effect of solvent fractions of the leaves of *D. angustifolia* against pathogenic bacteria.

Materials and Methods

Drugs, reagents, and bacteriological media

The solvents used for extracting the plant material were distilled water, ethyl acetate, and chloroform. The standard antibiotic discs that were used in the antibacterial activity are chloramphenicol 30 µg/disc and Gentamicin 10 µg/disc. In addition, other reagents like 0.5% McFarland standard, Dimethyl Sulfoxide (DMSO), sterile physiological saline, iodine, ferric chloride, and tetrazolium chloride were used during the study. Media that were used in the study include Mueller Hinton agar (MHA), Mueller Hinton broth (MHB), Plate count agar (Tryptone glucose yeast extract agar), nutrient agar and nutrient broth. All chemicals and reagents used during this study were laboratory grade chemicals.

Collection, identification, and preparation of plant materials

The fresh leaves of *D. angustifolia* was collected from its natural habitat in Tara Gedam, Libo kemekem district, south Gondar zone, Amhara regional state, northwestern Ethiopia (12°04.351′-12°10.926′N and 37°44.266′- 37°50.057′E) in November, 2018 using a clean scalpel blades. The plant specimen was identified and voucher specimen was deposited in Ethiopian National Herbarium (ENH), with voucher specimen number (AK 007).

Plant extraction

Approximately 1 kg of dry powder leaves were macerated and extracted in 80% methanol (1:5) for 48hrs three times, then the crude extracts were filtered using Whatman filter paper No. 1. The crude extracts were kept at 4°C and all three extracts were mixed and subjected to evaporation by a rota-vaporizer (Buchi, England) set at 50°C. Next, they were dried using a freeze drier (Ningbo, China). The crude extract was further fractionated a on separatory funnel by using nonpolar solvents (n-hexane followed by dichloromethane fol-

lowed by ethyl acetate) for 4hrs three times and further subjected to evaporation by rota-vaporizer set at 50°C. Finally, it was dried with a freeze drier.

Phytochemical screening tests

The qualitative phytochemical tests were carried out using standard tests [19,20]. Briefly, the following were applied: the Salkowski test for terpenoids, the Liebermann-Burchard test for steroids, the frothing test for saponins, the ferric chloride test for tannins, the Keller-Killiani test for cardiac glycosides, the Dragendorff's and the Mayer's tests for alkaloids, and the ammonia test for detection of flavonoids.

Thin layer chromatography

Ten milligrams of the different fractions were diluted in 1 ml of solvent and spotted on a line made with a pencil at one end of the silica gel plate, according to [21,22]. After that, the TLC plates were generated using various polarity solvent solutions including the mobile phases, such as benzene/ethanol/ammonia (18:2:0.2). In this manner, thin layer chromatography plates were mounted in closed tanks. Thin layer chromatography plates were put in closed tanks with their ends in contact with the mobile phase near the sample application region. After permitting the chromatogram to run for 15 minutes, it was dried with a cold air stream. For best color development, the dried TLC plates were sprayed with ferric chloride ($FeCl_3$), dried, and heated in an oven at 100°C. The retention factor (Rf) was calculated by comparing the distance traveled by each band to the distance traveled by the solvent [23].

Antimicrobial assay

Inoculum preparation and standardization

Standard bacterial strains including *E. coli* (American Type Culture Collection (ATCC 25922)), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 29213), *S. typhi* (ATCC 6539), *K. pneumoniae* (ATCC. 700603) and *C. freundii* (ATCC 43864*)* were obtained from Ethiopian Public Health Institution (EPHI).

Agar well diffusion method

The antibacterial activities of fractions were evaluated using the agar well diffusion technique [24]. The inoculum turbidity was adjusted by comparing with 0.5 McFarland standard, which is expected to contain 1 x 10^8 CFU/ml.

A sterile Muller Hinton Agar (MHA) plate is uniformly streaked using a sterile cotton swab. The five equally spaced wells were made with a sterile cork borer (6 mm diameter). The labeled wells were filled with 100 µL of 500 mg/mL, 250 mg/mL, 125 mg/mL and 62.5 mg/mL of each of the solvent fractions [25]. The fifth well was filled with 100 µL DMSO used as a negative control. While commercial antibiotic discs of Chloramphenicol 0.005 mg/disc and gentamicin 0.01 mg/disc was used as a positive control. The fraction and standard antibiotic were allowed to diffuse for 30 minutes into the plates before being inverted and incubated at 37°C for 18 hours. The zone of growth inhibition (mm) around the wells was measured to get the result. Each test was carried out three times.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of fractions was determined according to Molla., *et al.* [26] and Belay., *et al.* [25]. Briefly, 100 µl of Muller Hinton Broth (MHB) was aseptically added to each well of a 96-well microtiter plate. Then, 100 µl fraction stock solutions containing 500 mg/ml fraction was dispensed aseptically to the first columns of the micro-titer plate. A serial two-fold dilution of the solvent fraction was conducted from the second to the tenth rows. The bacterial strains were prepared by diluting 0.1 ml of 0.5 McFarland turbidity standard bacteria (10^8 CFU/ml) with 20 ml MHB in the ratio of 1:20 to get a final dilution of 5 x 10^6 CFU/ml. Twenty microliter of the adjusted inoculum was dispensed aseptically to each well micro-titer plate up to except 11^{th} wells (negative control 5% DMSO). Plates were rapped with parafilm to prevent desiccation and incubated for 24 hours at 37°C. The MIC was recognized by adding 30µl of

0.2% tetrazolium chloride and further incubated at 37°C for 30 minutes. The bacterial growth indicator tetrazolium chloride has been used; bacteria metabolize it and become pink. The bacteria had not thrived in the wells that did not change color after adding tetrazolium chloride, hence they were accepted as MIC values. Three times the tests were carried out.

Determination of minimum bactericidal concentration

It was determined by identifying wells which had no bacterial growth during the MIC determination, then taking 3 µl of the contents and evenly spreading it across the whole surface of plate count agar [25]. The plates were incubator for 24 hours at 37°C. The lowest concentration of the fractions which showed no bacterial growth after incubation was observed for each triplicate and noted as the MBC.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 24.0 software was used to analyze the data. Using one-way analysis of variance (ANOVA) and Tukey Post Hoc Multiple Comparison tests at a significance level of $P < 0.05$, the statistical difference of the mean zone of inhibition of solvent fractions and commercial antibiotics.

Results

Phytochemical screening

Phytochemical analysis of the left extract revealed the presence of bioactive substances (Table 1). Results showed that the ethyl acetate extract of the leaves of *D. angustifolia* revealed that it contains tannins, saponins, alkaloids, flavonoids, terpenoids, steroids and phenols. The chloroform extracts of the leaves were found to have flavonoids, coumarins, phenolics, saponins and tannins. Cardiac glycosides were absent in the fractions. Alkaloids were detected only in the ethyl acetate fraction. Anthraquinones were absent in both ethyl acetate and chloroform extraction. The presence of pharmacologically useful substances such as tannins, flavonoids, saponins, phenolics and steroids in the leaves of *D. angustifolia* has revealed by phytochemical screening test confirming the diverse claims and application of the leaves of the plant in the treatment of various ailments.

Phytochemicals	Chloroform fraction	Ethyl acetate fraction
Flavonoids	+	+
Alkaloids	-	+
Steroids	_	+
Terpenoids	_	+
Cardiac glycosides	_	_
Coumarins	+	_
Phenol	+	+
Anthraquinones	_	_
Tannins	+	+
Saponins	+	+

***Table 1**: Phytochemical investigation of D. angustifolia leaves fractions.*

Remark: += Present, -= Absent.

Thin layer chromatography fingerprinting

The dried chloroform and ethyl acetate fractions were reconstituted to 10 mg/ml, spotted and run on a silica gel plates to separate the secondary bioactive compounds found on DA using a combination of solvents (benzene: ethanol: ammonia) (Table 2).

Sample	Sample travelling (cm)	Retention factor value
Chloroform	1.1	0.15
	1.8	0.25
	2.6	0.36
	3.6	0.49
	4.5	0.62
	6.5	0.89
	7.1	0.97
Ethyl acetate	1.5	0.2
	2.3	0.31
	3.4	0.47
	4	0.55
	4.8	0.66
	5.1	0.69
	6.5	0.89
	6.8	0.93

Table 2*: Shows that the TLC constituents of Dodonaea angustifolia extracted from the solvent of benzene, ethanol, and ammonia (18: 2: 0.2) mixture.*

BEA solvent combination was able to separate ethyl acetate fractions by two different bands with Rf values ranging from 0.2 to 0.93. Fractions of chloroform were separated into bands with Rf values ranging from 0.15 to 0.97 (Table 2). Most of the bands were visible in daylight. Those not visible in daylight were viewed under ultraviolet light at 256 nm and 365 nm.

Antibacterial activity

The growths of test bacterial strains were inhibited by the tested concentrations of ethyl acetate and chloroform fraction of *D. angustifolia* plants. The observed zones of inhibition of the ethyl acetate and chloroform fractions at the tested concentrations were statistically significant different as compared to that of positive controls ($p < 0.05$) (Table 3).

Among the test bacteria, gram negative bacterial species of *K. pneumonia,* and *S. typhi* were more susceptible than that of the gram positive bacterial species at the corresponding tested concentrations of the chloroform fraction. As depicted in table 3, the most susceptible bacterium at 500 mg/ml was *K. pneumonia* and *S. typhi* with equal mean zone of inhibition of 17 mm and the most susceptible bacterium at 250 mg/ml was *C. freundii*, *K. pneumonia* and *S. typhi* with a mean zone of inhibition of 14.33 mm, 14 mm and 14 mm respectively. Moreover, the zone of inhibition of chloroform fraction at 62.5 mg/ml was significantly different ($p < 0.05$) compared to that of its zone of inhibition at 500 mg/ml and 250 mg/ml against the growth of each test bacteria (Table 3).

Similarly, gram-negative bacteria were more susceptible than that of the gram-positive ones at equal concentrations of ethyl acetate fraction. The most susceptible bacterium against ethyl acetate fraction at 500 mg/ml concentration was *K. pneumonia* followed by *S. typhi* with a maximum mean zone of inhibition of 20.67 mm and 17.67mm, respectively. While at 250 mg/ml concentration *K. pneumonia* and *S. typhi* have showed a mean zone of inhibition of 17.33 mm and 15.33 mm, respectively (Table 3).

Category of test	Concentration	Bacteria		
		E. coli	***S. typhi***	***S. aureus***
Chloroform	62.5 mg/ml	8.0 ± 0.57^{a3c1d2e2}	9.0 ± 0.57^{a3c1d2e2}	8.33 ± 0.33^{a3c2d2e3}
	125 mg/ml	10.67 ± 0.33^{a3d1e2}	12.0 ± 0.57^{a2e1}	11.0 ± 0.00^{a3d1e2}
	250 mg/ml	12.33 ± 0.33^{a3e1}	14.0 ± 0.57^{a2}	12.33 ± 0.33^{a3e1}
	500 mg/ml	15.00 ± 0.57^{a2}	17.0 ± 1.00^{a1}	14.33 ± 0.33^{a3}
	Chlora 30µg/disk	20.0 ± 0.57	20.33 ± 0.88	22.33 ± 0.33
Ethyl acetate	62.5 mg/ml	9.67 ± 0.88^{a2d2e2}	9.67 ± 0.3^{a3c2d3e3}	8.33 ± 0.33^{a3c2d3e3}
	125 mg/ml	11.67 ± 0.33^{a3d2e2}	13.33 ± 0.33^{a3d1e2}	11.33 ± 0.33^{a3d2e2}
	250 mg/ml	14.33 ± 0.33^{a2}	15.33 ± 0.33^{a3e2}	13.67 ± 0.33^{a3e2}
	500 mg/ml	16.00 ± 0.57^{a1}	17.67 ± 0.33^{a2}	17.00 ± 0.57^{a2}
	CAF	19.67 ± 0.67	20.33 ± 0.33	22.33 ± 0.33
Category of test	**Concentration**	**Bacteria**		
		C. freundii	***K. pneumonia***	***P. aeruginosa***
Chloroform	62.5 mg/ml	9.67 ± 0.3^{a3c2d3e3}	9.00 ± 1.000^{a2d1e2}	$8.67 \pm 0.333^{a3c2d2e3}$
	125 mg/ml	$10.00 \pm 0.577^{a3c1d2e2}$	10.67 ± 1.202^{a2e2}	11.67 ± 0.333^{a3d1e2}
	250 mg/ml	12.33 ± 0.333^{a3d1e2}	14.00 ± 0.577^{a2e1}	13.33 ± 0.333^{a2e2}
	500 mg/ml	14.33 ± 0.333^{a2e1}	17.00 ± 0.577^{a1}	15.67 ± 0.333^{a2}
	Chlora 30 µg/disk	20.00 ± 0.577	20.67 ± 1.202	20.33 ± 0.667
Ethyl acetate	62.5 mg/ml	$8.67 \pm 0.333^{a3c1d2e2}$	9.00 ± 1.000^{a3d1e2}	$7.33 \pm 0.882^{a3c1d2e2}$
	125 mg/ml	10.67 ± 1.202^{a2e2}	13.00 ± 1.732^{a1e1}	12.33 ± 0.333^{a3d1e2}
	250 mg/ml	11.00 ± 0.577^{a3e2}	15.00 ± 0.577^{a2}	13.33 ± 0.333^{a3e1}
	500 mg/ml	13.00 ± 0.577^{a2}	17.33 ± 1.856^{a1}	16.00 ± 0.577^{a2}
	CAF	20.3 ± 0.667	20.67 ± 0.333	18.33 ± 0.333

Table 3*: Zone of inhibition (in mm) of the different concentrations of chloroform and ethyl acetate fractions of the leaves of D. angustifolia against the tested bacteria.*

Remark: *Values are expressed as Mean ± S.E.M (n = 3), analysis was performed with One-Way ANOVA followed by Tukey test; as compared to positive control, b to 62.5 mg/ml, c to 125 mg/ml, d to 250 mg/ml, e to 500 mg/ml; $^{2}P < 0.01$, $^{3}P < 0.001$. The negative control has shown no antibacterial activity. CAF: Chloramphenicol.*

Minimum inhibitory concentration (MIC) of solvent fractions

As indicated in table 4, the MIC value of the chloroform fraction ranged from 13.89 mg/ml to 69.44 mg/ml in all bacterial species for which it was active with lowest MIC (against *C. freundii*) and maximum MIC (against *K. pneumoniae*) respectively. The highest MIC value of ethyl acetate fraction was 69.44 mg/ml (against *P. aeruginosa*) and the lowest MIC value was 7.71 mg/ml (against *E. coli*). When compared the MIC value of solvent fractions; ethyl acetate fraction was more effective against all tested bacteria with minimum concentration except *S. aureus*.

Minimum bactericidal concentration (MBC) of solvent fractions

As presented in table 5, the corresponding mean MIC value and MBC value of the ethyl acetate and chloroform fractions were equal against the growth of the majority of the bacterial strains except *E. coli* and *K. pneumoniae*. As depicted in table 5, the maximum mean MBC

Bacteria	Extraction chemicals	
	Chloroform fraction	Ethyl acetate fraction
	MIC (Mean ± SE)	MIC (Mean ± SE)
E. coli	20.063 ± 11.13	7.71 ± 3.08
S. typhi	60.18 ± 33.38	10.80 ± 3.08
S. aureus	20.06 ± 11.13	32.41 ± 9.26
C. freundii	13.89 ± 0.000	13.89 ± 0.000
K. pneumoniae	69.44 ± 27.77	13.89 ± 0.000
P. aeruginosa	41.67 ± 0.000	69.44 ± 27.77

Table 4*: The MIC (mg/ml) of D. angustifolia leave fractions against tested bacteria.*

Remark: MIC: Minimum Inhibitory Concentration, the values are the average of triplicate tests.

Note: Where ciprofloxacin was used as a positive control.

(least dilution) was 69.44 mg/ml (against *P. aeruginosa*) and the minimum mean MBC (highest dilution) of the ethyl acetate fraction of the study plant was 10.80 mg/ml (against *S. typhi*). Similarly, the range for the mean MBC values of the chloroform fraction was ranged from 60.18 mg/ml to 13.89 mg/ml with the growth of the bacterial species which were susceptible in its antibacterial activity testing experiment. Taken together, the ethyl acetate fraction was more potent and killed the bacteria at lower concentration compared to that of the chloroform fractions except *S. aureus* and *P. aeruginosa*.

Bacteria	Extraction chemical	
	Chloroform fraction	Ethyl acetate fraction
	MBC (Mean ± SE)	MBC (Mean ± SE)
E. coli	20.06 ± 11.12	13.89 ± 0.000
S. typhi	60.18 ± 33.38	10.80 ± 3.08
S. aureus	20.06 ± 11.13	32.41 ± 9.26
C. freundii	13.89 ± 0.000	13.89 ± 0.000
K. pneumoniae	41.67 ± 0.000	13.89 ± 0.000
P. aeruginosa	41.67 ± 0.000	69.44 ± 27.77

Table 5*: The MBC (mg/ml) of D. angustifolia leave fractions against tested bacteria.*

Remark: MBC=Minimum Bactericidal Concentration, the values are the average of triplicate tests.

Note: Where ciprofloxacin was used as a positive control.

Discussion

Dodonaea angustifolia is one of the most popular herb used for the treatment of various disorders including bacterial infections [4,27,28]. The present study was done to determine the antimicrobial active effects of solvent fractions of *D. angustifolia* leaves and qualitative assessment of secondary metabolites in the fraction.

The chloroform and ethyl acetate fractions of *D*. *angustifolia* showed a varying degree of antimicrobial activity against the tested organisms. According to Getie., *et al.* [29] tested the methanol extract of *D. viscosa* against Gram negative organisms and bacteriostatic activity against Gram positive bacteria. Although they tested 100mg/ml methanol extract, it showed no effect on gram negative bacteria. However, in this study we used 500mg/ml fraction (Table 3) and it showed a promising antimicrobial activities against both gram positive and gram negative bacteria. However, a TLC bio-autography technique revealed an inhibitory potential of different solvent extracts against Gram negative and Gram positive organisms [13].

The n-hexane, ethyl acetate, and n-butanol fractions of *Dodonaea viscosa* produced prominent inhibitory effects against *B. subtilis*. *S. aureus*, which produced a 12.0 ± 0.3 mm zone of inhibition, failed to produce any zone of inhibition in the contact bio-autography, which is most likely due to the synergistic action of the compounds present in the extract [13]. While *S*. *aureus* were the least susceptible bacteria for the chloroform and ethyl acetate fractions at equal concentrations compared to the other test bacteria [13].

The methanol extract of *D. angustifolia* produced a prominent zone of inhibition against *E.* coli (13.1 ± 0.37 mm), *K. pneumoniae* (10.9 ± 0.3 mm) and *S. typhi* (9.7 ± 0.64 mm). The methanolic extracts exhibited the least inhibitory activity against *S. aureus* with zone of inhibition (7.7 ± 0.45 mm). The ethanol extract of *D. angustifolia* against *Shigella dysenteriae* was 14.4 ± 0.45 mm followed by *K. pneumoniae* (13.9 ± 0.35 mm), *S. typhi* (12.9 ± 0.51 mm) and 11.5 ± 0.51 mm against *E. coli*. Moderate inhibitory activity was noticed against *S. aureus* (10 ± 0.15 mm) and *P. aeruginosa* (8.5 ± 0.55 mm) The diethyl ether extract produced a maximum zone size of 12.1 ± 0.3 mm against *P. aeruginosa* and a moderate activity of 8 ± 0.2 mm against *S. dysenteriae* and 7.9 ± 0.35 mm against *K. pneumoniae* and minimum inhibitory activity against *E. coli* with a zone size of 5.6 ± 0.52 mm. Acetone extract of *D. angustifolia* inhibited *S. aureus* with a highest zone of inhibition 11.9 ± 0.25 mm and minimal inhibition was 6.8 ± 0.2 mm and 5.7 ± 0.32 mm against *S. dysenteriae* and *P. aeruginosa*. No good antibacterial activity was excreted by the Hexane extracts [30].

The methanol extract of *D. angustifolia* showed MIC activity at 6.25 mg/ml concentration against *E. coli* and *S. typhi* followed by *S. dysenteriae* and *K. pneumoniae* at 12.5 mg/ml concentration. The ethanol extracts showed strong MIC activity at 1.56 mg/ml against *S. dysenteriae* and against *S. typhi* at 6.25 mg/ml concentration followed by *S. aureus* and *P. aeruginosa* at 12.5 mg/ml. The acetone extract of *D. angustifolia* exhibited a MIC at12.5 mg/ml against *S. dysenteriae* followed by *S. aureus* at 25 mg/ml and at 50 mg/ml against *P. aeruginosa* and *K. pneumonia* [30].

The ethyl acetate extract showed the highest zone of inhibition against *Streptococcus spp*. isolated from high vaginal swab and *Streptococcus* group B isolated from high vaginal swab microbial strains at 2 mg/ml concentration [31]. The other three concentrations (1, 0.5 and 0.25 mg/ml) of ethyl acetate extract also showed significant potency against *Streptococcus spp*. isolated from high vaginal swab and *Streptococcus* group B isolated from high vaginal swab microbial strains. Similarly, the chloroform crude extract showed potency against *Streptococcus spp*. isolated from high vaginal swab at the concentrations of 2 and 1 mg/ml with a zone of inhibition of 10 and 9 mm, respectively. On the other hand, the chloroform extract also showed very good potency against *Streptococcus* group B isolated from high vaginal swab with the inhibition zones of 0 - 15 mm. The methanol extract did not show activity against *E. anisi*. In addition, methanol extract also showed significant potency against gram-positive microbial *S. aureus.* The hexane extract showed good potency against *Streptococcus* group B isolated from high vaginal swab at all applied concentrations. However, the extract gave moderate potency *Streptococcus spp*. isolated from high vaginal swab and *E. anisi* only at the highest concentration (2 mg/ml), while other concentrations of hexane extract did not show any potency [31]. The water crude extract did not show any potency at any selected microbial strains except 2 mg/ml against *Streptococcus spp*. isolated from high vaginal swab[31]. Phytochemical screening reported by Veerapur., *et al*. [32], showed that the selected species have several bioactive compounds, i.e. alkaloids, tannins, and saponin, flavonoids, terpenoids, and they are highly responsible for biological activities. Previously, it has been reported that *D. viscose* does not show inhibitory potency against gram-negative microbial strains [29]. However, other studies reported that the extracts showed promising potency against gram-negative microbial strains [13]. In

our case, it was also observed that the leaves extract of *D. viscose* showed low potency against gram-negative microbial strains. Therefore, our result supports what has been reported by Khurram., *et al.* [13] that gram-negative microbial were less sensitive than gram-positive microbial strains. Mehmood., *et al.* [33] reported that *S. aureus* was showed high potency against methanol extract of *D. viscose* leaves.

Phytochemical compounds commonly associated with combating microbial resistance and having antimicrobial activity in medicinal plants are flavonoids, alkaloids, tannins, terpenoids, essential oils, saponins, and phenols. Kaempferol methyl ethers isolated from *Dodonaea viscosa* Jacq. var. *angustifolia* leaf extracts have showed a good antimicrobial effect against *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa* varied from 16 µg/ml to more than 250 µg/ml [14].

As per the thin layer chromatography (TLC) analysis, this study showed the bands (Table 2) and the Rf value (Table 2) indicating the presence of phytochemicals in the ethyl acetate and chloroform fractions. As compared with the chloroform fractions, ethyl acetate has the highest bands that may be due to the combination of additional different constituents.

Conclusion

The present study indicated that the chloroform and ethyl acetate fractions have antibacterial effect against the growth of selected pathogenic bacteria with varying antibacterial spectra. Therefore, the study offers a scientific basis for the claimed practice of the plant for the treatment bacterial infection both in human and animals. Further studies should be conducted to isolate, purify and identify bioactive principles responsible for the antibacterial activities and toxicological studies should be done for the safety of the extracts of the *D. angustifolia*.

Competing Interests

The authors declare that there is no any direct or indirect conflict of interests, which may call into question the validity of this study.

Consent for Publication

All authors read and approved the final manuscript.

Availability of Data and Material

All the data is contained in the manuscript.

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Authors' Contributions

The authors reported no conflicts of interest for this work and declare that there is no direct or indirect conflict of interest which may call into question the validity of this study.

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