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Antibacterial and antifungal activity of *Dodonaea viscosa* (L.) Jacq., a wild plant of Azad Jammu and Kashmir

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Abstract

In present study, chloroform, ethanol and methanol crude extracts of stem bark and leaves of *Dodonaea viscosa* were investigated for their antibacterial and antifungal potential against two gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), one gram negative bacterium (*Escherichia coli*) and two yeast strains (*Candida albicans*, *Sccharomyces cervisiae*). Ethanol and methanol extracts were found to be active against the tested bacteria (gram positive and gram negative). None of the extracts inhibited the growth of *Candida albicans* and *Sccharomyces cervisiae*). All the tested microorganisms were resistant to chloroform extracts. Amongst the gram positive and gram negative bacteria, gram positive bacteria were more sensitive than gram negative bacterium used. Commercially available standard reference antibiotics discs Tetracycline (30 µg), Ciprofloxacin (5 µg) and Nystatin (100 units) were used to compare the zones of inhibition produced by the antibiotics with that of the extracts used.

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Introduction

Infectious diseases are difficult to control globally because of antimicrobial resistance. The potential of antibiotics action is being reduced by the appearance of multidrug-resistant (MDR) pathogens. The chief contributors of antimicrobial resistance problem are the hospitals (WHO, 2002a). The plants are found to be a good source of secondary metabolites which are chief source of valuable bioactive compounds and drugs (Dung and Loi, 1991). These plants are used in different regions of world for the treatment of microbial diseases. Microbes are closely associated with the health and welfare of human beings. Some microbes are found to be beneficial and some are harmful. The failure of chemotherapeutics and antibiotic resistance created by pathogens has opened the gate towards the utilization of medicinal plants as antimicrobial agents (Martins *et al.*, 2001).

Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants (Farnsworth and Loub, 1983).

Herbal medicine is useful all over the world and will become a major factor in the change of direction for health and the treatment of disease in the next century. Many of these plant medicines have been proven, while other are new and in the experimental stages. These newer plants may be addition to the timeless ones, share the potential to cure many of the illness are presently faced today (Biswas, 2006). In developing countries, over 80 % population of the world uses traditional medicines including plant extracts for their health care (WHO, 2002b).

It is a starting point for the discovery of antimicrobial drugs by the screening of plants for antimicrobial and phytochemicals potential (Cseke *et al.*, 2006). The bactericidal and fungicidal activity of plant extracts has been investigated by a very large number of scientists in different regions of the world. It is a need of hour to search new antimicrobial drugs of plants origin because commercial synthetic antibiotics are getting resistance against microbes (Iwu *et al.*, 1999; Wurochekker *et al.*, 2008; Krishnaiah *et al.*, 2009).

Dodonaea viscosa (L.) Jacq. Locally known as Sanatha belongs to family Sapindaceae, is a shrub that grows to a height of 2 m. It is used in folk medicine as a remedy for fever, rheumatism and goutd. Locally the leaves are found to be effective in the treatment of toothache if they are chewed without swallowing the juice. The crude extract has inhibitory effects against *Staphylococcus aureus, Streptococcus pyogenes, and Corynebacterium dephtherieae*, thereby suggesting potential against notable Gram positive organisms. It has antiviral potential as well as it inhibits the Coxsackie virus B3 and influenza A while it has no activity against yeast (Getie *et al.*, 2003). However, recently the antifungal property of *D. viscosa* against *C. albicans* was reported (Patel and Coogan, 2008). It has also shown anti-inflammatory effects in experimental animals (Khalil *et al.*, 2006).

Materials and methods

Stem bark and leaves of *Dodonaea viscosa* were selected for antibacterial and antifungal screening. Fresh plant parts were collected during the flowering stage. The plant material was shade dried at room temperature.

Extraction procedure

Under shade dried stem bark and leaves of *D. viscosa* were grinded into fine powder using electric grinder. About 50 g dried powder was put into conical flasks which were labeled with plant parts name and then soaked into 200 ml of chloroform, ethanol and methanol respectively.

The maceration of plants extract was carried out for 7 days in each solvent at room temperature (25±2 °C) with continuously shaking after each 24 hours. The solvent extracted material was filtered in separate labeled glass. A rotary evaporator at low temperature and pressure was used to evaporate the crude extracts.

Dilution

Ten mg/ml of dilution was prepared by dissolving 10 mg of crude extract in 1 ml of respective solvents.

Microorganisms used

All the tested organisms, Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus arureus*), Gram negative bacterium (*Escherichia coli*) and yeast strains (*Candida albicans*, *Sccharomyces cervisiae*) were obtained from microbiology Section University of Azad Jammu and Kashmir Muzaffarabad.

Culture media

The culture media for bacteria was prepared by dissolving 28 g dehydrated nutrient agar in 1000 ml distilled water, warmed and shaked. While the culture medium for yeasts was prepared by dissolving 65 g Sabouraud's dextrose agar in 1000 ml distilled water. The media was sterilized in autoclave at 121 °C for 15 minutes.

Antimicrobial assay

The antimicrobial assay of crude extracts was performed by disc diffusion method (Bauer *et al.*, 1966). Suspension or dilution of bacteria and yeasts were prepared by dipping a loop of bacterium and yeast in 10 ml distilled water taken in sterilized labeled test tube. One milliliter dilution was transferred from test tube to the respective sterilized Petri plates by using sterilized pipette. The Petri plates were gently rotated to mix the dilution and medium and allowed to solidify at room temperature.

For testing the antibacterial and antifungal activity of crude extracts, uniform filter paper discs (6 mm diameter) were formed, sterilized and dipped in chloroform, ethanol and methanol crude extracts of stem bark and leaves of *Dodonaea viscosa*. The filter paper discs were placed in Petri dishes at their labeled positions. In second step, commercially available antibiotics reference antibiotic discs were placed on the top of the media at the centre of Petri dishes. All the steps were performed in aseptic area.

Incubation of plates

The plates contained the bacterial culture were incubated at 37 °C for 24 hours and plates with yeasts suspension were incubated at 25 °C for 72 hrs. After the incubation time, all the plates were carefully examined for presence of zone of inhibiton as a property of antimicrobial activity. The experiment was performed in 3 replicates.

Statistical analysis

All values were expressed as means ±standard error of means. The means were also compared by using LSD at 5% (0.05) probability level (Steel and Torrie, 1980).

Results and discussion

Plant extracts have been studied against bacteria for years, but in a more intensified way in the last three decades. During this period, a lot of antimicrobial screening evaluations have been published based on the traditional use of Chinese, African and Asian plant drugs (Forestiere *et al.*, 1988; Vlietinck *et al.*, 1995). In present study different organic solvent extracts (chloroform, ethanol and methanol) of a stem bark and leaves of *Dodonaea viscosa* have been investigated for their antibacterial and antifungal properties. It is found in sub-tropical regions of Azad Jammu and Kashmir. It has not been investigated for antimicrobial activity previously from this region. The results are summarized in tables 1 and 2 while least significance difference was summarized in tables 3 and 4.

Previously it has been reported that *Dodonaea viscosa* does not show inhibitory activity against gram negative bacteria (Geitie *et al.*, 2003). However Khurram *et al.* (2009) reported the promising activity against gram –negative bacteria. In our case it was also observed that Stem bark and leaf extract of *Dodonaea viscosa* showed appreciate inhibitory activity against gram negative bacteria (Table 1 and 2). Moreover, Khurram *et al.* (2009) reported that gram negative bacteria are more sensitive than gram positive bacteria. While in our case it is opposite as gram positive bacteria were more sensitive than gram negative bacteria. *Staphylococcus aureus* was found to be highly sensitive against methanol extract of stem bark of *D. viscosa*. None of the extract inhibited

the growth of yeast strains (*Candida albicans* and *Sccharomyces cervisiae*). Previous work also showed that D. viscosa does not show activity against yeast (Geitie *et al*., 2003).

Table 1. Antimicrobial activity of D. viscosa (Stem bark) mean (mm) ± Standard Error Mean.

Stem bark of *Dodonaea viscosa* Zone of inhibition mean (mm) ± Standard Error Mean					
Extracts	*B. subtilis*	*S. aureus*	*E. coli*	*C. albicans*	*S. cervisiae*
Chloroform	-	-	-	-	-
Ethanol	15.00±0.58	13.67±1.33	13.67±0.88	-	-
Methanol	11.67±0.88	18.67±0.33	10.67±0.33	-	-
Tetracycline	35.00±0.58	33.00±0.58	30.00±0.58	-	-
Ciprofloxacin	32.00±0.58	31.00±0.58	34.00±0.58	-	-
Nystatin	-	-	-	17.00±0.58	16.00±0.58

Table 2. Antimicrobial activity of D. viscosa (Leaves) mean (mm) ± Standard Error Mean.

Leaves of *Dodonaea viscosa* Zone of inhibition mean (mm) ± Standard Error Mean					
Extracts	B. subtilis	*S. aureus*	*E. coli*	*C. albicans*	*S. cervisiae*
Chloroform	-	-	-	-	-
Ethanol	12.33±0.33	12.33±0.33	10.67±0.67	-	-
Methanol	13.00±0.58	12.00±0.58	12.00±0.58	-	-
Tetracycline	35.00±0.58	33.00±0.58	30.00±0.58	-	-
Ciprofloxacin	32.00±0.58	31.00±0.58	34.00±0.58	-	-
Nystatin	-	-	-	17.00±0.58	16.00±0.58

Fig. 1. showed the comparative zones of inhibition of stem bark crude extracts (chloroform, ethanol and methanol) of *D. viscosa* with reference antibiotics. It was observed that chloroform extract did not show any activity against all the tested microorganisms. Ethanol extract iduced high antibacterial activity against *Bacillus substilis*, *S. aureus* and *E. coli* (15.00±0.58, 13.67±1.33 and 13.67±0.88 mm) while both the tested yeasts were resistant. In case of methanol extract, moderate antibacterial activity was noted against *Bacillus subtilis* and *E. coli* (11.67±0.58 mm and 10.67±0.33 mm). *S. aureus* was found to be strongly inhibited (18.67±0.33 mm) by methanol extract of stem bark. Methanol extract was also inactive against both yeast strains.

The antibacterial and antifungal activity of leaf extract of *D. viscosa* was shown in Figure 2. Chloroform leaf extract was inactive against all the tested microorganisms. High antibacterial activity of ethanol leaf extract was noted against *B. subtilis* and *S. aureus* (12.33±0.33, 12.33±0.33 mm) while *E. coli* was moderately inhibited (10.67±0.67 mm). Methanol leaf extract was found to be highly active against *B. subtilis*, *S. aureus* and *E. coli* (13.00±0.58, 12.00±0.58, 12.00±0.58 mm). Both ethanol and methanol extract of leaves of *D. viscosa* were also inactive against tested yeasts strains. Avato *et al.* (1997) reported that extracts from Bellis perennis have a high antimicrobial activity against bacteria than fungus. In our research, it has also observed that plant extracts show more activity against bacteria than fungi used.

Table 3. Antibacterial and antifungal activity of *D. viscosa* (Stem Bark) extracts Least Significance Difference (LSD) at 5 % significance level.

Extracts	Concentration	*B. subtilis*	*S. aureus*	*E. coli*	*C. albicans*	*S. cervisiae*
Chloroform	10 mg/ml	0.00e	0.00d	0.00e	0.00b	0.00b
Ethanol	10 mg/ml	15.00c	13.67c	13.67c	0.00b	0.00b
Methanol	10 mg/ml	11.67d	18.67b	10.67d	0.00b	0.00b
Tetracycline	30 µg	35.00a	33.00a	30.00b	0.00b	0.00b
Ciprofloxacin	5 µg	32.00b	31.00a	34.00a	0.00b	0.00b
Nystatin	100 units	0.00e	0.00d	0.00e	17.00a	16.00a

Mean with same letter shows no significance difference.

Table 4. Antibacterial and antifungal activity of *D. viscosa* (Leaves) extracts Least Significance Difference (LSD) at 5 % significance level.

Extracts	Concentration	*B. subtilis*	*S. aureus*	*E. coli*	*C. albicans*	*S. cervisiae*
Chloroform	10 mg/ml	0.00e	0.00d	0.00e	0.00b	0.00b
Ethanol	10 mg/ml	12.33c	12.33c	10.67c	0.00b	0.00b
Methanol	10 mg/ml	13.00c	12.00c	12.00c	0.00b	0.00b
Tetracycline	30 µg	35.00a	33.00a	30.00b	0.00b	0.00b
Ciprofloxacin	5 µg	32.00b	31.00b	34.00a	0.00b	0.00b
Nystatin	100 units	0.00d	0.00d	0.00d	17.00a	16.00a

Mean with same letter shows no significance difference.

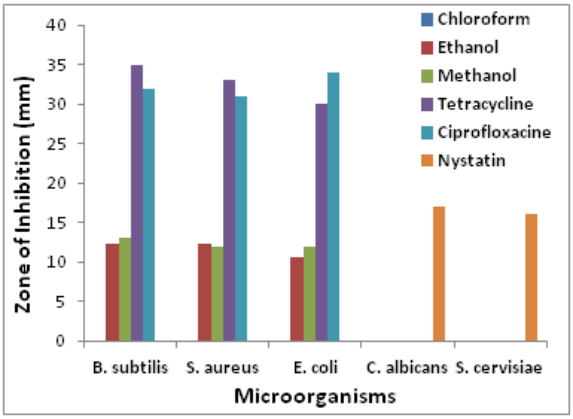

Fig. 1. Comparative zones of inhibition of crude extracts of stem bark of *D. viscosa*.

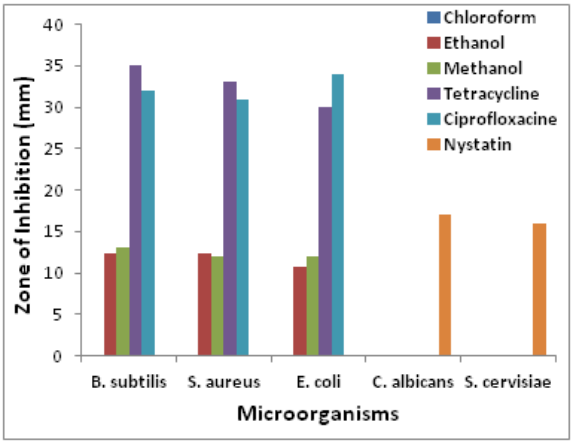

Fig. 2. Comparative zones of inhibition of crude extracts of leaf of *D. viscosa*.

The results of the present study correspond with the findings of Jankovsky and Landa (2002) that the methanolic extract of *Hyssopus officinalis* showed broad spectrum antibacterial activity against *B. subtillus* and *Pseudomonas aeruginosa*. The activity of plant extracts against the microorganisms may be due to presence of different bioactive compounds in them. Plant extracts usually contain polyphenols and flavonoids which could be the antibacterial agents. The biological activity of plant extracts is associated to phytochemicals present in them. The antiviral (Mehrangiz *et al.*, 2011), antimicrobial (Maria *et al.*, 2009) and spasmolytic (Julianeli *et al.*, 2011) activity of flavonoids have been reported. Similarly plants extracted alkaloids have also found to be antimicrobial (Ahamed *et al.*, 2010). The antibacterial activities of these compounds might be due to their ability to complex with bacterial cell wall and therefore, inhibiting the microbial growth.

Conclusion

Many medicinal plants have been found effective in the cure of bacterial diseases. From the present study it can be concluded that there exists a grand prospective in the exploration of innovative and more effective antimicrobial substances from the natural sources. The prospective for developing antimicrobials from plant extracts emerges satisfying as it will lead to the development of phytomedicines to act against microorganisms. It was found that methanol extracts of bark and leaf extract of *D. viscosa* showed prominent antibacterial activity followed by ethanol extract. This plant is not found effective against yeast strains. Future research can be conducted to isolate the phytochemicals from methanol extract which have great antibacterial potential.

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