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Research Article

PHYTOCHEMISTRY AND BIO-EFFICACY OF A WEED, *DODONAEA VISCOSA*

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ABSTRACT

The plant *Dodonaea viscosa* is distributed as a weed from coast to the elevation of more than 2000 meters. The weed is distributed in tropical as well as subtropical regions of the world. The widely spread weed is potentially used as a folklore medicine in the state of Tamil Nadu, India for various reasons. The study on its phytochemistry, antibacterial, antifungal and larvicidal potency of all the plant parts, i.e. leaf, stem and root of the plant using different solvent were not studied so far. In this study, the study on phytochemistry of aqueous extract of the plant, antimicrobial, antibacterial and larvicidal activity of the leaf, stem and root of the plant using aqueous, methanol and chloroform (organic solvents) were conducted. The results showed that methanol extract of the leaf possess maximum antibacterial activity against more number of bacteria. The methanolic extract of all plant parts against the fungi and Aqueous extract of the leaf against the larvae, *Artemia salina*. Tannins, Saponins, Flavanoids and Terpenoids were detected from the aqueous extract of all the plant parts.

Keywords: *Dodonaea viscosa*, Phytochemical analysis, Antibacterial, Antifungal, Larvicidal, *Artemia salina*.

INTRODUCTION

Weeds have been a part of civilization and many ancient documents speak of humans battling weeds in the crops they grow. A weed is commonly defined as a plant that grows out of place and is competitive, persistent and pernicious[1]. Weeds are also found to be resistant to most of the microbial and pesticidal disease when compared to the crops which shows disease symptoms[2]. The plant *Dodonaea viscosa* belonging to the family Sapindaceae is distributed as a weed from coast to the elevation of more than 2000 meters[3]. The weed is distributed in tropical as well as subtropical regions of the world. The widely spread weed is potentially used as a folklore medicine in the state of Tamil Nadu, India for various reasons. The study on its phytochemistry, antibacterial, antifungal and larvicidal potency of all the plant parts, i.e. leaf, stem and root of the plant using different solvent were not studied so far. In this study, the study on phytochemistry of aqueous extract of the plant, antimicrobial, antibacterial and larvicidal activity of the leaf, stem and root of the plant using aqueous, methanol and chloroform (organic solvents) were conducted.

MATERIALS AND METHODS

Source

The plant was samples of *Dodonaea viscosa* of family Sapindaceae were collected from Guduvanchery, Chennai, India. The leaves, stem and root of the plant were separated and examined for their infection and physical damage. Healthy plant parts were used for the study. The Bacterial cultures of *Bacillus subtilis* (MTCC 121), *Vibrio cholerae* (MTCC 1068), *Staphylococcus aureus* (MTCC 96), *Proteus mirabilis* (MTCC 425) and *Escherichia coli* (MTCC 443) were procured from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. Fungal cultures of *Aspergillus flavus* (MLRDAF03), *Aspergillus niger* (MLRDAN08), *Penicillium citrinum* (MLRDPC06), *Fusarium oxysporum* (MLRDFS07) and *Curvularia lunata* (MLRDCL02) belonging to the culture collection of Research and Development, Marina Labs, Chennai were used for the study. The seed of Artemia salina was bought from Ocean Star International, Chennai.

Preparation of Plant Extract

The extracts were prepared by hot percolation method. The plant parts were separated as leaf, stem and root and washed thoroughly in running tap water, dried under shade and grounded into uniform powder. Around 10g of each sample and 150ml of solvents (aqueous, methanol and chloroform) was used to extract the crude. The aqueous extract is obtained by boiling the soaked sample in the distilled water whereas other solvent extracts are obtained using soxhlet apparatus. The plant extract were filtered through Whatman no.1 filter paper into pill vials. The extracts were dried using rotary evaporator and the dried extract was stored in a refrigerator till further use. The stored product was reconstituted again using a same solvent for required concentration when used.

Antibacterial Activity

The assay is carried out by disc diffusion method. The pooled extracts were concentrated and extracts were loaded into sterile readymade discs (Hi-media, Bombay) in different volumes of 15µl, 20µl and 25µl / disc respectively and allowed to dry for 24 hours at room temperature. Mueller Hinton agar plates were spread with 100µl of actively growing broth cultures of the respective bacteria and are allowed to dry for 10 min. The sterile readymade discs loaded with each extract individually (15µl, 20µl and 25µl / disc respectively) were imposed on the inoculated plates. The plates were then incubated at 37°C for 36 hours. The development of the inhibition of zone around the extract loaded disc was recorded.

Antifungal Activity

The assay is carried out by well diffusion method. Potato Dextrose agar plates were spread with respective fungi using a swab and three wells are bored in each plate at the diameter of 5mm each. The wells are filled with varying capacities of extracts (15µl, 20µl and 25µl / well respectively). The plates are kept undisturbed and incubated at 28°C for 72 hours. Negative control is also setup for all the concentrations and solvents and incubated similarly. The development of the inhibition of zone around the extract loaded well was compared with the plates kept for negative control and the differences were recorded.

Larvicidal Activity

Artemia salina cysts were incubated in saline water and the eggs were hatched in 24 hours. The hatched nauplii (larvae) were then used (48h growth) for larvicidal activity assay. One gram of dried extract is dissolved in 10ml of saline water (master dilution). Four different concentrations (0.5, 1, 1.5 and 2) were prepared from the master dilution. Ten nauplii were transferred to each test sample and incubated in room temperature for 24h. After 24 hours the susceptibility of the nauplii were observed.

Phytochemical Analysis

The test for tannins, Phlobatannins, Saponins, Flavanoids, Terpenoids and Cardiac Glycosides were carried out to detect the bioactive compounds using qualitative test[4].

RESULTS

Antibacterial Activity

The zone of inhibition studied against the bacteria showed, that *Vibrio cholerae* was controlled by all parts of *Dodonaea viscosa* extracted through all the three types of solvent. Maximum zone of inhibition was recorded by the methanol extract of stem against the bacteria. Similarly, the bacteria *Bacillus subtilis* was controlled by all the extracts except that of methanol extract of root. The root extract of the weed showed no efficacy against the bacteria, *Escherichia coli* and *Proteus mirabilis.* The zones of inhibition recorded by different parts of the weed plant using different solvent against the bacteria studied are presented in Table 1.

Antifungal Activity

Among the solvents studied for their antifungal efficacy of different parts of the plant, maximum efficacy was recorded for the methanol extract. Other solvents like Aqueous and Chloroform extract showed poor zone of inhibition or of any significance. The methanol extract of leaf of the plant showed maximum activity against the fungi, *Curvularia lunata* and *Fusarium oxysporum.* The methanol extract of root of the plant showed maximum activity against the fungus, *Aspergillus flavus.* Similarly, it was the methanolic extract of stem of the plant which showed maximum activity against the fungus, *Penicillium citrinum.* However no significant level of activity was recorded against the fungus *Aspergillus niger* by any of the extracts studied. The zone of inhibition recorded for each organism against different solvent of different plant parts of the weed, *Dodonaea viscosa* is presented in Table 2.

Larvicidal Activity

Among different parts of the plant, *Dodonaea viscosa* studied for its bioactivity against the nauplii larvae of *Artemia salina* the leaf extract alone showed significant level of lethality. The aqueous extract of leaf showed 100 % lethality against the larvae at the concentration of 1 %. Similarly aqueous extract of root extract of the plant showed 100 % lethality of larvae at the concentration of 1.5 %. The stem is concerned only the methanol extract of stem at the concentration of 2 % showed 100 % lethality of larvae. The mortality rate recorded for different plant parts of *Dodonaea viscosa* using different solvents is presented in Table 3.

Phytochemical Analysis

Freshly prepared plant extracts of *D. viscosa* were studied for their presence of chemicals like Tannin, Phlobatannin, Saponin, Flavanoids, Terpenoids and Cardiac glycosides. The leaf, stem and root of the plant showed the presence of Tannins, Saponins, Flavanoids and Terpenoids (Table 4).

Table 1: Antibacterial activity of *Dodonaea viscos*

S. No.	Test Organisms	Aqueous			Methanol			Chloroform		
		15µl	20µl	25µl	15µl	20µl	25µl	15µl	20µl	25µl
Leaf										
1	*Bacillus subtilis*	9	10	13	8	9	9	11	12	13
2	*Escherichia coli*	7	8	9	-	-	-	9	12	12
3	*Proteus mirabilis*	-	-	-	10	11	13	-	-	-
4	*Staphylococcus aureus*	10	12	12	11	11	13	-	-	-
5	*Vibrio cholerae*	10	11	13	9	10	12	11	12	13
Stem										
1	*Bacillus subtilis*	10	12	12	9	9	10	10	10	11
2	*Escherichia coli*	-	-	-	12	14	16	-	-	-
3	*Proteus mirabilis*	-	-	-	-	-	-	9	10	10
4	*Staphylococcus aureus*	10	12	17	-	-	-	7	7	10
5	*Vibrio cholerae*	9	11	12	23	25	26	14	14	16
Root										
1	*Bacillus subtilis*	10	10	12	-	-	-	12	14	17
2	*Escherichia coli*	-	-	-	-	-	-	-	-	-
3	*Proteus mirabilis*	-	-	-	-	-	-	-	-	-
4	*Staphylococcus aureus*	-	-	-	8	8	8	14	14	18
5	*Vibrio cholerae*	10	11	12	7	7	7	14	15	17

Table 2: Antifungal activity of *Dodonaea viscose*

S. No.	Test Organisms	Aqueous			Methanol			Chloroform		
		15µl	20µl	25µl	15µl	20µl	25µl	15µl	20µl	25µl
Leaf										
1	*Curvularia lunata*	6	6	-	18	19	23	-	-	-
2	*Fusarium oxysporum*	7	11	12	16	19	19	7	11	11
3	*Aspergillus flavus*	6	6	7	12	13	19	-	-	-
4	*Aspergillus niger*	7	8	8	8	10	11	6	7	7
5	*Pencillium citrinum*	-	-	-	11	12	14	6	7	8
Stem										
1	*Curvularia lunata*	6	7	7	10	11	13	-	-	-
2	*Fusarium oxysporum*	7	9	11	11	16	17	-	-	-
3	*Aspergillus flavus*	-	6	-	11	15	16	6	6	7
4	*Aspergillus niger*	6	7	9	9	12	13	7	7	8
5	*Pencillium citrinum*	-	-	-	9	10	16	7	7	8
Root										
1	*Curvularia lunata*	6	8	9	7	9	11	-	-	-
2	*Fusarium oxysporum*	7	8	8	10	14	16	-	-	-
3	*Aspergillus flavus*	6	7	7	17	18	24	6	7	8
4	*Aspergillus niger*	-	6	7	10	11	14	8	8	9
5	*Pencillium citrinum*	-	-	-	10	10	12	6	6	7

Table 3: Larvicidal activity of *Dodonaea viscosa* against *Artemia salin.* (Percentage of organisms killed)

S. No.	Concentration	Aqueous			Methanol			Chloroform		
	In Percentage	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
1	0.5	20	60	70	30	30	20	20	0	0
2	1	100	70	80	40	50	30	30	20	10
3	1.5	100	80	100	40	90	30	30	20	20
4	2	100	90	100	50	100	70	40	30	20

Table 4: Qualitative analysis of Phytochemicals of *Dodonaea viscosa*

Phytoconstituents	Leaf	Stem	Root
Tannins	+	+	+
Phlobatannins	-	-	-
Saponins	+	+	+
Flavanoids	+	+	+
Terpenoids	+	+	+
Cardiac Glycosides	-	-	-

DISCUSSION

In our study, *Bacillus subtilis* and *Vibrio cholerae* showed higher response for the extracts of the plant and this report is similar to that of Rani *et al*[5]. Khurram *et al*[6], reported that maximum zone of inhibition was recorded for the bacteria, *Bacillus subtilis* from the shoot part of the plant. In evidence with Aslam *et al*[7] stating that *D. viscosa* has high antifungal property among some medicinal plants, the aqueous, methanol and chloroform extracts of *D. viscosa* showed efficacy against the fungi studied and the methanol extract showed better activity against the fungi when compared to aqueous and chloroform. Maximum zone of inhibition against bacteria was recorded by methanolic extract of the members of Myrtaceae was previously reported[8].

Artemia salina nauplii was used as a reference organism to study the larvicidal activity for various reasons such as availability of the cyst throughout the year, suitable test species to evaluate the relative toxicity for a broad range of chemical compounds and mainly the early larval stage of *Artemia* can survive several days without food, which prove them to be suitable for acute toxicity tests[9]. *Bioactive* compounds of *D. viscosa* have been found to be toxic in high doses. It is clearly evident that the highest bioactivity resides in the aqueous extracts of the plant. Also, leaf and stem extracts showed good result when compared with root. Similar result was recorded[10]. This part of work is first of its kind on the study related to larvicidal activity of *Dodonaea viscosa.* Phytochemistry results are in relevance to the work of Venkatesh *et al*[11]. Phytochemical analysis of common weeds of northern districts of Tamil Nadu was studied by Udayaprakash *et al*[12].

Although, different kinds of bioactivity of the plant *Dodonaea viscosa* was studied by different authors, i.e. biopesticide activity[3], antidiabetic, hypolipidaemic and antioxidant activity[13], Antihyperglycemic activity[14], Antimicrobial activity[15], Anticandidal activity[16], Antibacterial activity[5], Antifungal activity[17] and Antidiarrheal activity[18]. The studies conducted by previous authors are confined to any one part of the plant or with different type of solvent. The present study has an advantage and of first of its kind in that all parts of the weed, *Dodonaea viscosa* was studied for their antibacterial, antifungal and larvicidal activity using 3 different solvent systems.

CONCLUSION

From the work done, it is concluded that *Dodonaea viscosa*, a common weed has many bioactivities. Good anti bacterial activity is observed against *Bacillus subtilis, Vibrio cholerae, Escherichia coli* and *Staphylococcus aureus. D. viscosa* also showed antifungal activity against *Fusarium oxysporum, Aspergillus niger and Aspergillus flavus, Curvularia lunata and Pencillium citrinum.* The results of larvicidal activity were also found positive especially high in leaf extract. Further, it is found that different part of the plant possess different kind of bio-property and using suitable plant part with suitable solvent for the desirable efficacy is utmost important. The phytochemical tests revealed the presence of saponins, tannins, terpenoids and flavanoids. Hence the biopotency of the weed is determined.

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