



Antimicrobial and Antioxidative Activities in the Stem Extracts of *Derris trifoliata*, a Mangrove Shrub

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Authors' contributions

This work was carried out in collaboration between all authors. Author AS designed, analyzed, did experiments and wrote the first draft of the manuscript. Authors AG and SAG did experiments and contributed equally for this study. Author AR guided, designed, analyzed results and reviewed manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: An in-depth study on the phytochemical contents, antimicrobial and antioxidative activities of stem tissue of *Derris trifoliata* Lour. (Fabaceae), a mangrove shrub from Sundarban estuary, India.

Methodology: Phytochemical analyses were carried out using established methods for quantitative determination of phenolics, flavonoids, tannins, alkaloids and saponins. Antimicrobial potential of various extracts of *D. trifoliata* were evaluated by disc diffusion technique against two Gram-positive (*Bacillus subtilis* and *Bacillus coagulans*), two Gram-negative (*Escherichia coli* and *Proteus vulgaris*) bacteria and one fungus (*Saccharomyces cerevisiae*). The antioxidative efficacy was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. The tissue extracts were subjected to thin layer chromatography (TLC) separation and the fractions with antimicrobial and antioxidative properties were identified using TLC-bioautography.

Results: Phytochemical analyses showed the presence of appreciable amount of phenolics, flavonoids and alkaloids. All the extracts have shown activity against *B. subtilis* and *B. coagulans*, among which the methanolic has been found to be most effective. The antimicrobial activities of the

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extracts were found to be stable despite drastic pH and thermal treatments. The antioxidative property was also found to be quite appreciable and was considerably stable after thermal treatments. A number of the phytochemical fractions were found to possess antimicrobial/antioxidative properties when subjected to TLC-bioautography.

Conclusion: The study suggests *Derris trifoliata* stem as a potential source of bioactive compounds with stable antimicrobial and antioxidative properties and can be used as natural antimicrobial/antioxidative agents in clinical, pharmaceutical and food processing industries.

Keywords: *Derris trifoliata*; phytochemical; antimicrobial activity; antioxidant; mangrove plants; TLC-bioautography.

1. INTRODUCTION

The chemical compounds synthesized by plants as secondary metabolites commonly called as phytochemicals are of great interest in newer drug designing. Several of these plant derived compounds have different biological and other medicinal properties and are of increasing interest in therapeutic as well as other industrial applications. A lot of ethnobotanical information over the centuries has been recorded in traditional Indian, Chinese, Egyptian, European literatures. In India, usage of medicinal plants has been documented in ancient texts like *Rig-Veda* written between 4500-1600 BC reporting the use of herbs and minerals used in Ayurveda [1]. About 25% of the prescribed drugs are of plant origin of which 121 active compounds are reported to be in use [2]. World Health Organization has considered 252 drugs as the most essential drugs to be used commonly, 11% of which are from plant source and used as natural precursor to obtain a large number of synthetic drugs [2].

Mangroves are woody, specialized types of trees of the tropics that can live on the edge where rainforests meet oceans. This group of plants plays an important role in ethnobotany and can easily cope with changes in salinity (high salt content, lack of fresh water) by evolving both xeromorphic and halophytic characteristics. Mangroves are rich in secondary metabolites such as, phenolics, flavonoids, tannins, triterpenes, saponins and alkaloids [3]. These secondary metabolites are relatively stable as medicinally active agents and are used in dried forms (or as aqueous extracts) by traditional healers [4]. Our continued interest with regard to the biological properties of mangrove plants [5-10] has led us to examine the antimicrobial and antioxidant activities of *Derris trifoliata* in a greater detail.

Derris trifoliata Lour. (Fabaceae), locally known as Pan Lata in Bengali, is a climbing mangrove

shrub and is traditionally used as stimulant, antispasmodic and counter-irritant [11]. Extracts from different parts of the plant are also used for treatment of wounds, rheumatism, asthma and dysmenorrhea [12]. Prior studies have reported the cytotoxic [13], analgesic [14], antidiarrhoeal [15], antimalarial, larvicidal [16,17,18], antimicrobial [19] and antioxidant [20] properties in *D. trifoliata* tissue extracts. Though, extensive work on the antimicrobial as well as antioxidative activities have been carried out on the other two species of *Derris* (*Derris elliptica* and *Derris indica*), not too much work have been reported on the biological properties from *D. trifoliata*, especially from the Indian parts of Sundarban mangrove forest. As the secondary metabolite profiles of plants are being dictated by environmental factors; therefore, differences in microenvironments and other biotic-abiotic stress factors in geographically distinct areas may lead to differential expression of activities. In the present study, we report on the major phytochemical contents, antimicrobial and antioxidant efficacies in the stem extracts of *D. trifoliata*, a mangrove associate from Indian Sundarban estuary. We have also identified components in the stem extracts that appear to be responsible for the antimicrobial and antioxidant activities using TLC linked assays which is likely to extend the current existing knowledge on this plant species.

2. MATERIALS AND METHODS

2.1 Plant Material

Derris trifoliata plant samples were collected from the Indian Sundarban forest, West Bengal. Stem of this species was used for preparation of the solvent extracts.

2.2 Preparation of Plant Extracts

The stem (500 g) from the plant was washed thoroughly under tap water and then with distilled water and air-dried in shade for three weeks. Using a commercial kitchen electric grinder

(Make: Philips), the dried plant tissues were then ground into fine powder.

2.2.1 For antimicrobial activity assay

The dried powder (40 g) was extracted with 400 mL of hexane, benzene, chloroform, methanol and water by sequential extraction, each for three days at room temperature. The extracts obtained were filtered with Whatman No. 1 filter paper and dried using a rotary evaporator. However, the aqueous extract was freeze-dried using a lyophilizer. The dried extracts were stored at -20°C until use.

2.2.2 For antioxidant activity assay

The dried powder (10 g) was extracted two times with 100 mL of absolute methanol at room temperature. The extract was then filtered using Whatman No. 1 filter paper and dried using a rotary evaporator. The dried extract was stored at -20°C until use.

2.3 Quantitative Phytochemical Analysis

The powdered stem of *D. trifoliata* was tested for quantitative estimation of phytochemical contents such as phenolics, flavonoids, tannins, alkaloids and saponins accordingly as described previously [5].

2.4 Antimicrobial Activity Assay

Four bacterial strains viz., *Bacillus subtilis* (MTCC 121), *Bacillus coagulans*, *Escherichia coli* (MTCC 484), *Proteus vulgaris* (MTCC 426) and one fungal strain i.e. *Saccharomyces cerevisiae* were used as test microorganisms to evaluate the antimicrobial activity. The growth and maintenance of these microbial strains and preparation of the inoculums were performed as described earlier [5].

Antimicrobial assay of the extracts was carried out by disc diffusion technique [21] as described previously [5]. Each of the paper discs was loaded with 6 µL of extract [250 mg/mL conc. in dimethylsulphoxide (DMSO) i.e. 1.5 mg/disc] in the present study. Minimum inhibitory concentration (MIC) of the stem extracts was determined as described previously [5] by spotting each disc with 6 µL extract of different concentrations. Ampicillin was used as positive control for *B. subtilis*, *B. coagulans* and *E. coli*, chloramphenicol for *P. vulgaris* and fluconazole was used in the case of *S. cerevisiae*.

2.5 Antioxidant Activity Determination by DPPH Assay

The free radical scavenging activity of methanolic extract of *D. trifoliata* stem was performed according to Liu et al. [22] with slight modification as described previously [23]. Briefly, a series of stocks (6.25, 12.5, 25, 50, 75 µg/mL) were prepared by dissolving the stem methanolic extracts in methanol. 200 µL of each of these stocks was added with 100 µL of 0.5 mM DPPH solution (also in methanol) and left in the dark at room temperature. After 30 min, the optical density was measured at 517 nm using a Molecular Devices SpectraMax M3 plate reader. The control value was obtained by replacing the sample solution with methanol. Quercetin was used as the standard antioxidant. The percentage of scavenging activity was calculated using the formula, DPPH radical scavenging activity (%) = [(A₅₁₇ of control – A₅₁₇ of sample) / A₅₁₇ of control] × 100. Lower IC₅₀ value indicated higher free radical scavenging activity.

2.6 pH and Thermal Stability of Bioactivities

2.6.1 pH treatment

The pH treatment and evaluation of residual antimicrobial activities of these treated samples were carried out as described earlier [5].

2.6.2 Heat treatment

The thermal treatment and evaluation of residual antimicrobial activities of these treated samples were performed as stated before [5]. To determine the antioxidative stability, the methanolic extract was heated in water-bath at 80 and 100°C for 30 min [9] and the residual antioxidative activity was evaluated as described before.

2.7 Thin Layer Chromatography (TLC) and TLC-Bioautography

TLC of the extract exhibiting the maximum antimicrobial activity was performed as described previously [5] using toluene: ethyl acetate: formic acid (60:40:1) (TEaF) as mobile phase.

The bioautography technique was carried out as described previously [5,24] for detection of the antimicrobial constituents present in the extract. The antioxidative bioautography was performed

as stated earlier [23] to identify the fractions having radical scavenging properties, using the mobile phase TEaF for TLC separation.

2.8 Statistical Analysis

The computation of mean, standard deviation (SD), IC₅₀ values and analysis of variance (ANOVA) were done using Microsoft Office Excel 2007. ANOVA was carried out to study the differences at 5% level of significance among the results of the antimicrobial activities, antioxidant activities and residual bioactivities after the pH and thermal treatments.

3. RESULTS AND DISCUSSION

3.1 Quantitative Phytochemical Analysis

The biological activities with significant medicinal properties, exhibited by plants, have been widely attributed to the nature of their phytochemical contents. These phytochemicals have been evolved to play an important role in the plants' defensive mechanism against the destructive elements of nature, in resisting pathogenic microorganisms as well as the grazing herbivores [25,26]. Phytochemicals like phenolics, flavonoids and tannins play important roles in defending plants against pathogenic microorganisms [27]. These include membrane destabilization leading to increase in permeability, interference with bacterial virulence factors like β -lactamase inhibition, efflux pump inactivation, topoisomerase inhibition, electron transport system disruption, deficiency of essential metal ions etc. [28-30]. Saponins form complex with the cholesterol in the protozoal cell membranes resulting in cell lysis [31]. Also, plants can be good source of natural dietary antioxidants. Reports have been made on the protective effect of phytochemicals like flavonoids, tannin and other phenolics against the oxidative damages by free radicals [32]. The conjugated ring structures and hydroxyl groups of phenolics scavenges superoxide anion, singlet oxygen and lipid peroxy radicals thereby stabilizing free radicals either by hydrogenation or forming complex with oxidizing agents [22]. Presence in highly hostile and stressful condition has evolved the mangrove plants to synthesize a wide range of such secondary metabolites [7]. Therefore, investigation on the phytochemical profile can reflect the biological potential of the experimental plant species. The result of phytochemical analysis of stem of *D. trifoliata* is represented in Table 1.

The assay results show the stem to be a rich source of flavonoids (118.50 ± 3.04 QE/g dry weight). Presence of appreciable amount of alkaloids (57.20 ± 5.03 mg/g dry weight) and phenolics (48.85 ± 1.78 mg GAE/g dry weight) has also been observed in the stem of the species. However, the tannin content (9.57 ± 0.31 mg TAE/g dry weight) as well as the saponin content (6.00 ± 1.41 mg/g dry weight) has been found to be relatively low in *D. trifoliata* stem. Therefore, the analysis has revealed the presence of considerable amount of major phytochemicals in *D. trifoliata* stem that are generally responsible for the antimicrobial and antioxidant efficacy of a plant species.

3.2 Antimicrobial Activities

The determination of antimicrobial activities have been carried out using hexane, benzene, chloroform, methanol and aqueous extracts of *D. trifoliata* stem against five microbial strains including Gram-positive bacteria (*B. subtilis*, *B. coagulans*), Gram-negative bacteria (*E. coli*, *P. vulgaris*) and fungus (*S. cerevisiae*). The findings are represented in Table 2.

All the extracts have shown activity against *B. subtilis* and *B. coagulans*, among which the methanolic has been found to be most effective. This methanolic extract of *D. trifoliata* stem has exhibited the highest mean activity for both *B. subtilis* (11.67 ± 0.58 mm) and *B. coagulans* (10 mm). The Gram-negative bacterium, *P. vulgaris* has been found to be slightly sensitive to the aqueous extract (6.83 ± 0.29 mm). None of the extracts have shown any detectable activity against *E. coli* and *S. cerevisiae* at the concentration used. The antimicrobial activities exhibited by different extracts have been found to possess significant differences ($P = .000$) when analyzed. Ampicillin, chloramphenicol and fluconazole were used as positive controls while, DMSO, the stem tissue extract solubiliser was used as negative control. The phytochemical investigation revealed the presence of considerable amount of phenolics, flavonoids, alkaloids and to some extent of tannin as well as saponins which may be linked with the observed activities of the extracts.

According to Andrews [33], "MIC is defined as the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation". It has been referred as the 'gold standard' for evaluation of the sensitivity of organisms to growth inhibitory agents and used to crosscheck

the performance of all other sensitivity testing procedures [33]. The *D. trifoliata* stem extracts showing maximum activities against the tested microbial strains (Table 2) have been selected further to determine their MIC values. The findings are represented in Table 3. The MIC values of the extracts have been found to vary with the strains used. The hexane extract has significant MIC value of 0.98 mg/mL against *B. coagulans* whereas in case of the methanol

extract the same has been found to be 1.47 mg/mL. The MIC values of hexane and methanol extracts against *B. subtilis* have been found to be 4.88 mg/mL and 7.81 mg/mL, respectively. Whereas, the aqueous extract has revealed MIC value of 3.91 mg/mL against *P. vulgaris*. The MIC values revealed by *D. trifoliata* stem extracts are quite appreciable as only 6 μ L of the said concentrations have been spotted on the discs for the assay.

Table 1. Quantitative estimation of phytochemicals from *D. trifoliata* stem extracts

Sl. No.	Phytochemicals	Amount
1.	Total phenolics	048.85 \pm 1.78 mg GAE/g dry weight
2.	Total flavonoids	118.50 \pm 3.04 mg QE/g dry weight
3.	Total tannin	009.57 \pm 0.31 mg TAE/g dry weight
4.	Total alkaloid	057.20 \pm 5.03 mg/g dry weight
5.	Total saponin	006.00 \pm 1.41 mg/g dry weight

Each value is the mean \pm standard deviation from three replicates

Table 2. Antimicrobial activities of *D. trifoliata* stem extracts against test microorganisms by disc diffusion method

Sl. No.	Extract	Inhibition zone diameter (mm) ^a				
		Microorganisms				
		<i>B. subtilis</i>	<i>B. coagulans</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>S. cerevisiae</i>
1.	Hexane	08.83 \pm 1.26	09.17 \pm 0.76	-	-	-
2.	Benzene	07.00 \pm 1.00	08.67 \pm 0.58	-	-	-
3.	Chloroform	07.17 \pm 0.76	06.67 \pm 0.58	-	-	-
4.	Methanol	11.67 \pm 0.58	10.00 \pm 0.00	-	-	-
5.	Aqueous	07.67 \pm 0.58	07.33 \pm 0.58	-	06.83 \pm 0.29	-
Controls						
6.	Ampicillin ^b	23.00 \pm 1.73	28.67 \pm 1.15	11.00 \pm 1.00	-	-
7.	Chloram ^c	-	-	-	17.17 \pm 1.26	-
8.	Fluconazole ^d	-	-	-	-	24.67 \pm 1.15
9.	DMSO	-	-	-	-	-

Each value is the mean \pm standard deviation from three replicates. All the extracts used were 1.5 mg/disc.

^a Inhibition zone diameter including disc diameter of 5.5 mm; ^b Ampicillin used was 3 μ L/disc of conc. 125 μ g/mL against Gram-positive bacteria; and 3 μ L/disc of conc. 500 μ g/mL against *E. coli*; ^c Chloramphenicol used was 3 μ L/disc of conc. 10 mg/mL against *P. vulgaris*; ^d Fluconazole used was 3 μ L/disc of conc. 10 mg/mL against *S. cerevisiae*; DMSO served as negative control

Table 3. MIC values of *D. trifoliata* stem extracts against test microorganisms

Extracts	MIC values in mg/mL		
	Microorganisms		
	<i>B. subtilis</i>	<i>B. coagulans</i>	<i>P. vulgaris</i>
Hexane	4.88	0.98	-
Methanol	7.81	1.47	-
Aqueous	-	-	3.91

Extracts showing maximum activity against each tested microorganisms have been considered for MIC. 6 μ L of each of the stated concentration have been used for MIC

3.3 DPPH Radical Scavenging Activity

A wide range of cellular damages caused due to the generation of oxygen radicals, such as superoxide radical, hydroxyl radical and non-free radical species like hydrogen peroxide and singlet oxygen leads to severe health issues [34,35]. Pathogenic progression of some serious ailments like atherosclerosis, liver cirrhosis, cataracts, cardiovascular diseases, cancer, neurodegenerative disorders, diabetes etc. gets triggered as a result of the oxidative stress caused due to the imbalance of oxidants and natural antioxidants in the body [36]. The antioxidative potential as indicated by free radical scavenging activity of the methanolic extract of *D. trifoliata* stem has been determined by DPPH radical scavenging assay. DPPH is a stable radical which when dissolved in methanol produces violet colour due to the unpaired nitrogen electron at the center and gets reduced by either hydrogen- or electron-donation forming yellow coloured DPPH-H in presence of antioxidant compound [22,37]. The findings from the DPPH radical scavenging activity assay of *D. trifoliata* stem are represented in Table 4.

The methanolic extract of *D. trifoliata* stem has revealed considerable free radical scavenging activity and the percentage of the same has been found to be >85%. The IC₅₀ value of the sample has been found to be 40.28 µg/mL. The same has been found to be 10.69 µg/mL for ascorbic acid, a standard antioxidant compound. Though the antioxidant activity of ascorbic acid has been found to be better compared to the methanolic extract of *D. trifoliata* stem, further purification of antioxidative compounds from the crude extract of the species might show better activity with respect to the crude one.

3.4 Effect of pH and Thermal Treatments on Bioactivities

The *D. trifoliata* stem extracts showing maximum antimicrobial activities have been considered further to determine the effect of pH and temperature on the activities of these extracts. The results obtained are represented in Table 5.

No significant change in the antimicrobial activities has been observed before and after the pH and thermal treatment of the samples ($P > .05$). Thus, the *D. trifoliata* stem extracts tested for pH and thermal stability have been found to be stable and retained their antimicrobial activities against the respective microbial strains despite drastic treatments. Therefore, the study

suggests the activities of the extracts are tolerant to temperature and pH change. pH adjusted DMSO solutions (pH 3.0, 6.0 and 9.0) and normal DMSO showed no inhibitory activity against the strains tested (negative controls; data not shown). The antioxidant activity exhibited by the methanolic extract of *D. trifoliata* stem has also been found to be stable despite the thermal treatment (80°C and 100°C for 30 min) (Table 4). Minor differences as observed has been found to be statistically insignificant ($P = .44$). The IC₅₀ value of the 80°C treated sample (40.84 µg/mL) is slightly higher, whereas the same for the 100°C treated sample has been found to be bit lower (40.08 µg/mL) than the untreated one. A study by Kaur and Kapoor [38] suggests that heat treatment can trigger the development of certain compounds with antioxidative potential like Maillard reaction products or enhance the activity of natural antioxidants, augmenting the total antioxidant activity in turn. The study also reveals that, thermal treatment results in augmenting the bioavailability of β-carotene which might be responsible for increasing the antioxidative activity. The stable nature of both antimicrobial and antioxidative properties of *D. trifoliata* extracts may find its applications in food-industry as well as drug discovery domain. Thermostable compounds derived from plant extracts possess potential role as natural preservatives in food processing sectors. Whereas, in the drug discovery domain, pH tolerance has remained one of the major challenges [39] as the pH value of gastrointestinal tract varies from low pH in the stomach (pH 1.2) to high pH (pH 8.0) in the small and large intestine. Alteration in molecular structure or charge of the compound at certain pH values can lead to loss of activity and can limit oral exposure of the intended drugs.

3.5 TLC and Bioautography

TLC is a fast and reliable technique widely used to analyze mixture of organic compounds through separation, based on their molecular weight and polarity [40]. The method has extensive applications in determining compositions of biological and chemical samples and is ideal for natural products' separation [41]. When it is used along with bioautography, the hyphenated procedure helps in identification of the active fractions on the TLC plate possessing biological activities [42]. Annegowda et al. [43] described TLC-bioautography as an efficient technique in determining the contribution of

phytoconstituents in the extract for the observed pharmacological activity.

During our preliminary investigation, we have found that the hexane and methanolic extracts of *D. trifoliata* stem exhibit appreciable antimicrobial as well as antioxidant (only methanolic) activities in different assay systems. Therefore, both of these extracts have been considered for further detailed studies. TLC of these extracts have been developed using toluene: ethyl acetate: formic acid (60:40:1) as the mobile phase. The developed chromatograms, when visualized under UV light at 254 nm (Fig. 1: Lanes A & B), a number of bands or spots were observed at different *Rf* points representing the separated phytochemicals. The chromatogram of the methanolic extract (Fig. 1: Lane A) under 254 nm has been found to contain spots at *Rf* 0.64 and 0.77. But none of the bioautograms revealed any detectable activity with regard to the antimicrobial and antioxidant properties for the

visible or invisible spots of constituents in methanolic extract (data not shown). The chromatogram of the hexane extract (Fig. 1: Lane B) under 254 nm shows a number of spots at *Rf* 0.44, 0.53, 0.58, 0.64, 0.76, 0.85 and 0.93. To detect the spots with antimicrobial efficacy, bioautography was carried out using the chromatogram of the hexane extract against *B. coagulans*. Clear zones of inhibition seen at *Rf* 0.48, 0.55, 0.64, 0.70 and 0.76 on the bioautogram implying the presence of active antimicrobial substances at these locations inhibiting the growth of *B. coagulans* (Fig. 1: Lane C). Among these, the fractions at *Rf* 0.55, 0.64 and 0.70 have been found to exhibit potent antimicrobial efficacy.

Hostettmann [44] has described the DPPH bioautography technique as a means of discovering new antioxidants from plants by spraying DPPH radical on the TLC plate. This produces white spots on a purple background

Table 4. *In vitro* free radical scavenging activity by DPPH method

Sample	DPPH radical scavenging activity (%) ^a					
	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	75 µg/mL	IC ₅₀ (µg/mL)
Untreated	07.29 ± 4.42	16.17 ± 4.65	33.68 ± 4.30	69.91 ± 9.69	85.61 ± 0.88	40.28
80°C	08.93 ± 0.91	17.69 ± 0.92	33.67 ± 1.17	63.91 ± 0.54	86.87 ± 0.85	40.84
100°C	10.42 ± 1.20	18.76 ± 1.24	35.54 ± 1.56	64.76 ± 0.36	87.22 ± 0.58	40.08
	5 µg/mL	10 µg/mL	15 µg/mL	20 µg/mL		
Ascorbic acid	23.17 ± 2.99	45.56 ± 1.38	72.62 ± 0.48	92.35 ± 0.44		10.69

^a Each value is the mean ± standard deviation from three replicates

Table 5. Effect of pH and thermal treatment of *D. trifoliata* stem extracts^a on antimicrobial activities^b (mm) against bacteria

Organisms	Tissue extract	Positive control	pH				Temperature (°C)			
			3.0	6.0	9.0	40	60	80	100	
<i>B. subtilis</i>	Hexane	09.12 ± 0.75	08.75 ± 0.35	09.50 ± 0.70	09.50 ± 0.70	09.50 ± 0.70	09.00 ± 0.00	09.00 ± 0.00	08.50 ± 0.70	
	Methanol	09.62 ± 1.10	09.50 ± 0.70	09.25 ± 0.35	10.00 ± 0.00	09.50 ± 0.70	09.25 ± 1.06	09.50 ± 0.70	09.50 ± 0.70	
<i>B. coagulans</i>	Hexane	08.00 ± 1.22	08.75 ± 0.35	07.75 ± 0.35	08.50 ± 0.70	08.00 ± 0.70	07.50 ± 0.70	07.50 ± 0.70	07.50 ± 0.70	
	Methanol	07.75 ± 0.50	07.50 ± 0.70	07.25 ± 0.35	07.50 ± 0.70	07.75 ± 0.35	07.75 ± 0.35	08.00 ± 0.00	08.00 ± 0.00	
<i>P. vulgaris</i>	Aqueous	07.12 ± 0.62	07.75 ± 0.35	06.50 ± 0.70	07.25 ± 0.35	06.75 ± 0.35	07.25 ± 0.35	07.00 ± 0.00	07.25 ± 0.35	

^a Extracts exhibiting maximum activity against respective microbial strain have been considered; All the extracts used at 1.5 mg/disc.

^b Inhibition zone diameter including disc diameter of 5.5 mm and is the mean ± standard deviation from two replicates except control; Positive control value is the mean ± standard deviation from four replicates. Dried extracts dissolved in DMSO served as positive controls in both pH and thermal stability experiments

after DPPH is reduced by the bioactive fractions possessing antioxidant or radical scavenging properties. When the hexane extract chromatogram sprayed with 0.02% (w/v) DPPH solution in methanol to identify the bioactive fractions having radical scavenging properties, the chromatogram exhibited convincing radical scavenging activities at R_f 0.76 and 0.93. Therefore, the hexane extract fraction at R_f 0.76 has been found to possess both antimicrobial and antioxidative properties.

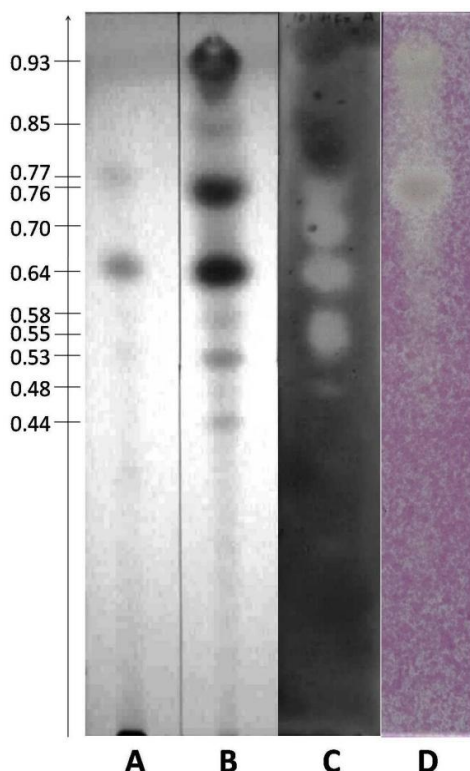


Fig. 1. TLC/TLC-bioautography of the methanolic extract (lane A) and hexane extract (lanes B-D) of *D. trifoliata* stem

Lanes A & B as visualized under short wave UV light (254 nm); Lane C is the bioautography plate with *B. coagulans* showing antimicrobial activities and lane D is the bioautography plate after DPPH spray exhibiting radical scavenging activities by separated fractions of the hexane extract

Many tissue extracts have been found to lose their activity upon separation, therefore making the purification of their bioactive principles quite difficult. Our report on the bioactive fractions of the extracts may provide further lead(s) for isolation and purification of the compound(s) with antimicrobial/antioxidative properties from this species.

4. CONCLUSION

The present study was undertaken to investigate the antimicrobial and antioxidant potential of *D. trifoliata* stem of Sundarban origin. The tissue has been found to be a good source of flavonoids, phenolics, alkaloids with low tannin and saponin content. The presence of these phytochemicals can be linked with different bioactivities exhibited by plant species, as discussed earlier. The methanol extract has revealed highest antimicrobial activities against the Gram-positive bacteria followed by the hexane extract of the species. The aqueous extract has shown a moderate broad spectrum activity against both Gram-positive and Gram-negative bacteria. Though, the microbial strains used in the current study are non-pathogenic in nature, they have been used as representative organisms and the extracts are expected to retain their activity against some other harmful microbes, too. The methanolic extract has revealed considerable free radical scavenging activity thus proving the antioxidative potential of the species. The extracts have retained their antimicrobial as well as antioxidative properties despite drastic treatments. The TLC-bioautography study has revealed a number of phytochemical fractions possessing antimicrobial properties of which one has been found to have both antimicrobial as well as free radical scavenging properties. Therefore, further studies with respect to the isolation and purification of these bioactive compounds from *D. trifoliata* should be undertaken, which may have application as natural preservatives in food processing industries and in the newer drug discovery process.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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