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Original Article

Phytochemical Standardisation and Antimicrobial Effect of *Sida rhombifolia* Linn. Aerial Parts

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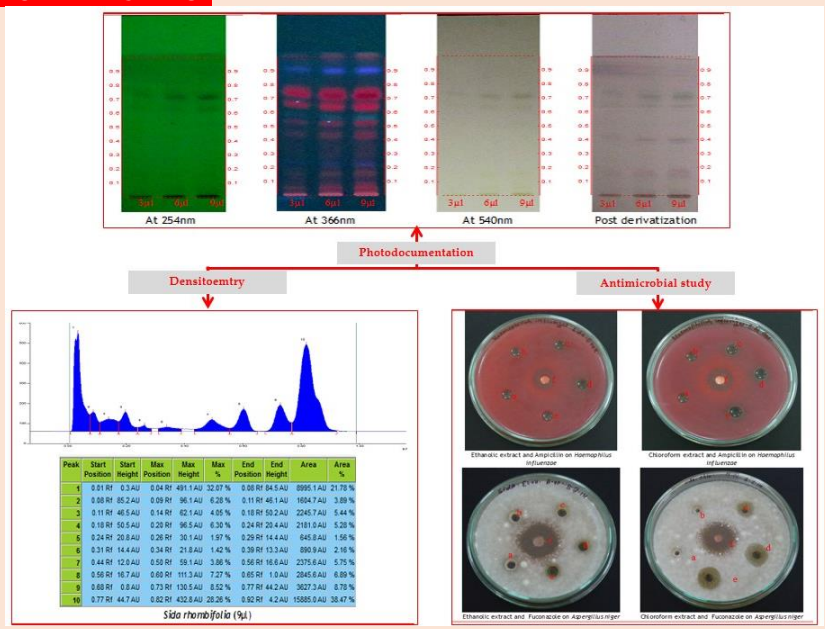
ABSTRACT

Introduction: *Sida rhombifolia* belonging to family Malvaceae is widely used in Ayurvedic practice for the treatment of infectious diseases, fever, diarrhea and diuretic. Several bioactive compounds are reported from the plant having medicinal activities. ***Methods:*** Standardization has been done to ensure the quality and purity of authentic specimen, Phytochemical test was carried out to explore its phyto-constituents. HPTLC fingerprinting profile was also been carried out. Further the ethanolic and chloroform extract of aerial parts was screened for antibacterial and antifungal activity by Agar well diffusion method. ***Results:*** The chloroform extract showed significant antibacterial and antifungal activity when compared to the ethanolic extract. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, tannin and coumarins. Distinct spots are also observed in the HPTLC prints. ***Conclusion:*** The present investigation provides useful information on antimicrobial activity of chloroform extract of *Sida rhombifolia* in treatment of various bacterial and fungal infections.

KEYWORDS

Aerial parts, antimicrobial, *Bala*, Sustainable harvesting

PICTORIAL ABSTRACT



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1. Introduction

Sida rhombifolia is a perennial or sometimes annual plant in the family Malvaceae. It is a cosmopolitan species, particularly in warmer regions. It is a small shrub or woody herbaceous plant with upright stems. It is used in stomach disorders like stomach pain, indigestion, flatulence, gastritis as emollient and demulcent. It is also found to have hepatoprotective and restorative activity[1,2]. Various biological activity studies have been reported from different morphological parts of *S. rhombifolia.* Aqueous extract of leaves was administered to hyperbilirubinemic rats, and showed potential of this plant as source new drugs for hyperbilirubinemic subjects[3]. In another report, it has been discussed that methanolic extract of the aerial part showed anti-inflammatory activity in animal model study[4]. Ethyl acetate and aqueous extracts of *Sida rhombifolia* was also reported to show marked antibacterial activity and significant antifungal activity[5]. The *in vitro* studies on antibacterial efficacy of different extracts of fruit also showed remarkable activity[6]. The present work is on phytochemical, antibacterial and antifungal activity of chloroform and ethanol extract of aerial parts of *S. rhombifolia* which can be used instead of root for sustainable supply as per demand.

2. Material and methods

2.1 Collection of plant material and extraction

The aerial parts (stem and leaves) was collected from Kabbinale, Hebri in Udupi district of Karnataka, it was authenticated by referring to flora of Udupi[7].The plant material was dried and powdered and used for preparation of extract. About 10 gm of the powder was loaded into a thimble of Soxhlet extractor and successively extracted with chloroform and ethanol.

2.2 Standardization

Air dried powdered aerial part powder was standardized as per standard protocol[8].

2.3 Phytochemical screening

Total ethanol extract was tested for the presence of different phytoconstituents like alkaloid, steroid, flavonoid, tannin, glycoside etc[9].

2.4 HPTLC

2.4.1 Sample preparation

One gram of the powdered plant material which was previously dried and powdered was soaked in 10 ml ethanol for 24 hrs, filtered and filtrate was made up to 10 ml and used for sample preparation.

2.4.2 Development and documentation

3, 6 and 9µl of the sample was applied on aluminium plate pre-coated with silica gel 60 F_{254} of 0.2 mm thickness (Merck, Germany) using CAMAG LINOMAT 5 applicator[10]. The plate was developed in CAMAG glass twin trough chamber previously saturated with mobile phase toluene: ethyl acetate (6.0: 1.0). The plate was derivatized using vanillin- sulphuric acid (VS), and heated at 105 °C till the spots appeared[11,12]. The developed plates were visualized in CAMAG visualizing chamber and scanned in CAMAG SCANNER 4 under 254 nm, 366 nm and 540 nm (pre-derivatisation) with the help of CAMAG WinCATS software. R_f values and densitograms were recorded.

2.5 Antibacterial activity

2.5.1 Preparation of sample

Ethanolic and chloroform extract of *Sida rhombifolia* (500 mg) was dissolved in 5 ml of dimethyl sulphoxide (DMSO) and the stock solution was further diluted to required concentration.

2.5.2 Preparation of blood agar media

Dissolved proteose peptone (20g), dextrose (0.5g), sodium chloride (5g) and disodium hydrogen phosphate (5g) in 990 ml distilled water. The pH was adjusted to 7.2 ± 0.2 and volume was made up to 1000 ml. Finally, 15g of agar was added to the media and autoclave at 121°C for 20 minutes.

2.5.3 Agar well diffusion method

Work place was sterilized in laminar air flow using 70% ethanol and switch on the UV for 20 minutes. Inoculated one loop of *Haemophilus influenzae* from the culture into 10 ml of broth and mixed well. 15ml of the blood agar medium was poured uniformly over the sterile petri-dishes. 1 ml of broth containing the organism was added uniformly over petridish, mixed well and the media was allowed to solidify. Five equidistant wells were made on the plate. 100 µl of extract, standard (Ampicillin) and control was added to the wells. Test was conducted for different concentration of extract (1-100 mg/ml) separately. All the petridish were incubated at 37°C for 24 hrs. After the incubation period, the zone of inhibition was measured. Experiments were carried out in duplicate[13].

2.6 Antifungal activity

2.6.1 Preparation of sample

Ethanolic and chloroform extract of *S. rhombifolia* (500 mg) was dissolved in 5 ml of dimethyl sulphoxide (DMSO) and the stock solution was further diluted to required concentration.

2.6.2 Preparation of potato dextrose agar media

24g of potato dextrose broth was dissolved in 1000ml distilled water and 15g of agar was added to it. Media was autoclaved at 121°C for 20 minutes.

2.6.3 Agar well diffusion method

Work place was sterilized in laminar air flow using 70% ethanol and UV was switched on for 20 minutes. One loop of *Aspergillus niger* was inoculated from culture into 10ml of broth and mixed well. 15ml of the Potato dextrose agar medium was poured uniformly over the sterile petridish and 1ml of broth containing the fungus was added uniformly over petridish. It was mixed well and the media was allowed to solidify. Five equidistant wells were made on the plate, 100µl of the extract, standard (Fluconazole) and control were added to the wells. Test were conducted for different concentration of extract (1-100mg/ml) separately. All the petridishes were incubated at 25°C for 5 days. After the incubation period, the zone of inhibition was measured. Experiments were carried out in duplicate[13].

3. Results and discussion

Physicochemical test performed as per WHO guidelines parameters are presented in (Table 1).

Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, tannin and coumarins. The phytochemical constituents present in the extract can be held responsible for different medicinal activities of the plant (Table 2).

Table 1. Physicochemical constants of aerial parts of *Sida rhombifolia*

Parameter	Results n=3 %w/w
Loss on drying	6.6776
Total ash	6.175
Acid insoluble ash	0.498
Water soluble ash	1.585
Alcohol soluble extractive	1.8244
Water soluble extractive	3.49

Table 2. Results of preliminary phytochemical tests for ethanolic extract of *Sida rhombifolia* aerial parts

Tests	Colour if positive	*Sida rhombifolia*	Inference
Alkaloids			+
Dragendrof's test	Orange precipitate	Orange precipitate	
Wagners test	Red precipitate	Red precipitate	
Mayers test	Dull white precipitate	Dull white precipitate	
Hagers test	Yellow precipitate	Yellow precipitate	
Steroids			-
Liebermann- buchard test	Bluish green	Red color	
Salkowski test	Bluish red to cherry red	Reddish brown color	
Carbohydrate			+
Molish test	Violet ring	Violet ring	
Fehlings test	Brick red precipitate	Brick red precipitate	
Benedicts test	Red precipitate	Red precipitate	
Tannin			+
With $FeCl_3$	Dark blue or green or brown	Brown color	
Flavanoids			-
Shinoda's test	Red to pink	Brown solution	
Saponins			-
With $NaHCO_3$	Stable froth	No stable froth	
Triterpenoids			-
Tin and thionyl chloride test	Pink	Brown solution	
Coumarins			+
With 2 N NaOH	Dark Yellow color	Dark Yellow color	
Phenols			-
With alcoholic ferric chloride	Blue to blue black	Brown solution	
Carboxylic acid			–
With water and $NaHCO_3$	Brisk effervescence	No brisk effervescence	
Resin			–
With aqueous acetone	Turbidity	Yellow clear solution	
Quinone			–
5% NaOH	Pink/purple/red	Brown solution	

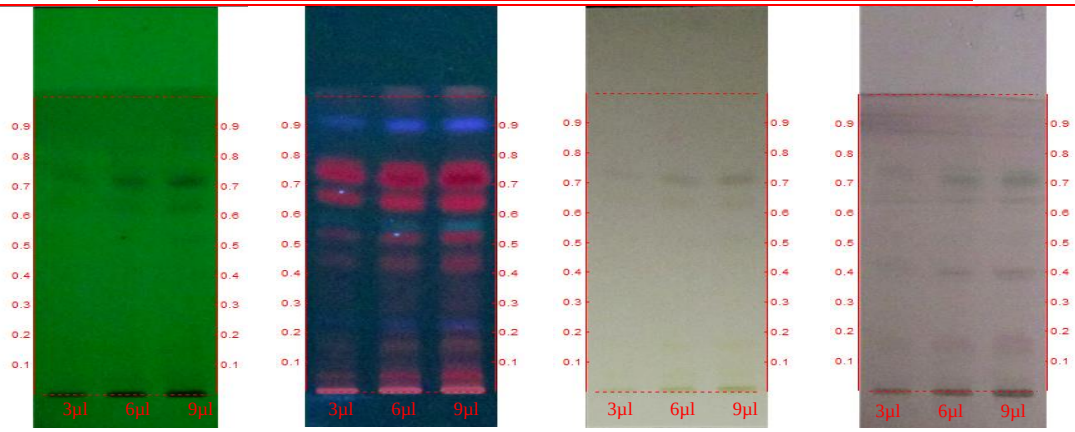
HPTLC finger printing profiles of *Sida rhombifolia* under 254nm showed the presence of 3 spots (all in green) at Rf of 0.53, 0.64 and 0.73. Under 366nm there were 9 prominent spots (fluorescent) at Rf of 0.05, 0.16, 0.24, 0.44, 0.53, 0.57, 0.64, 0.73, 0.91. When scanned under white light 540nm 2 spots were present at Rf of 0.64 and 0.73. Following post derivitisation with vanillin sulphuric acid, spots (in different colors) were evident at Rf 0.16, 0.17, 0.40, 0.64, 0.73 and 0.76. Among these the spots at Rf of 0.64 and 0.73 were common in different color intensities (Table 3, Figure 1). The densitograms at 254 nm, 366 nm, and 620 nm post derivatisation are represented in Figure 2.1-2.3.

Table 3. Rf values of ethanolic extract of *Sida rhombifolia* (9µl)

At 254nm	At 366nm	At 540nm	Post derivatization
-	0.05 (FD red)	-	-
-	0.16 (FL pink)	-	0.16 (L pink)
-	-	-	0.17 (L pink)
-	0.24 (FD blue)	-	-
-	-	-	0.40 (L purple)
-	0.44 (FD red)	-	-
0.53 (L green)	0.53 (FD red)	-	-
-	0.57 (FL blue)	-	-
0.64 (L green)	0.64 (FD red)	0.64 (L green)	0.64 (L green)
0.73 (D green)	0.73 (FD red)	0.73 (L green)	0.73 (D green)
-	-	-	0.76 (L green)
-	0.91(FD blue)	-	-

*F-Fluorescent; D-Dark; L-Light

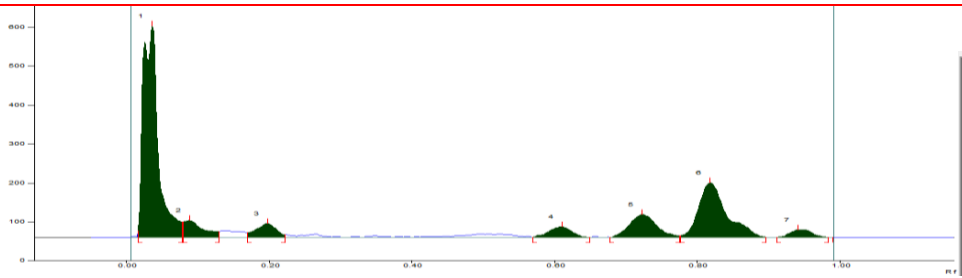
Figure 1. TLC photodocumentation of ethanolic extract of *Sida rhombifolia* aerial parts



1.1 At 254nm **1.2** At 366nm **1.3** At 540nm **1.4** Post derivatization

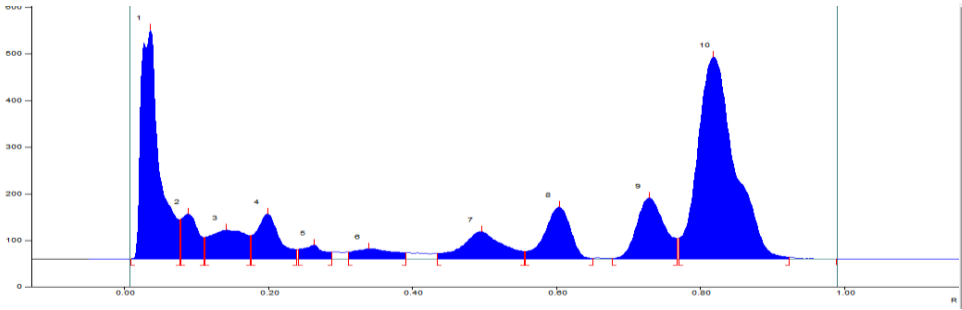
Solvent system - Toluene : ethyl acetate (6:1)

Figure 2. HPTLC Densitometric scan of ethanolic extract of *Sida rhombifolia* aerial parts



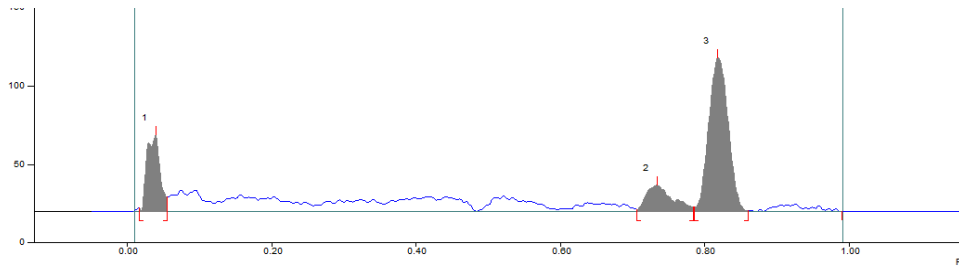
Peak	Max Position	Area %
1	0.04 Rf	50.61 %
2	0.09 Rf	5.21 %
3	0.20 Rf	4.32 %
4	0.61 Rf	3.78 %
5	0.72 Rf	9.72 %
6	0.82 Rf	23.75 %
7	0.94 Rf	2.61 %

2.1 At 254 nm (9µl)



Peak	Max Position	Area %
1	0.04 Rf	21.78 %
2	0.09 Rf	3.89 %
3	0.14 Rf	5.44 %
4	0.20 Rf	5.28 %
5	0.26 Rf	1.56 %
6	0.34 Rf	2.16 %
7	0.50 Rf	5.75 %
8	0.60 Rf	6.89 %
9	0.73 Rf	8.78 %
10	0.82 Rf	38.47 %

2.2 At 366 nm (9µl)



Peak	Max Position	Area %
1	0.04 Rf	21.52 %
2	0.74 Rf	14.78 %
3	0.82 Rf	63.70 %

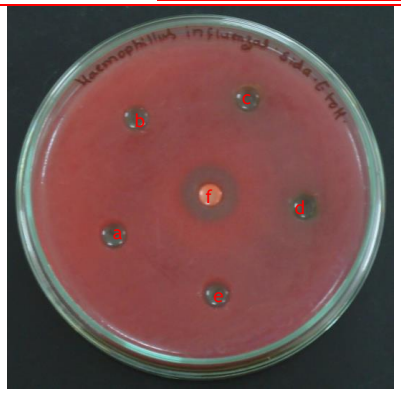
2.3 At 540 nm (9µl)

Table 4. Zone of inhibition (mm) of extracts of *Sida rhombifolia* aerial parts

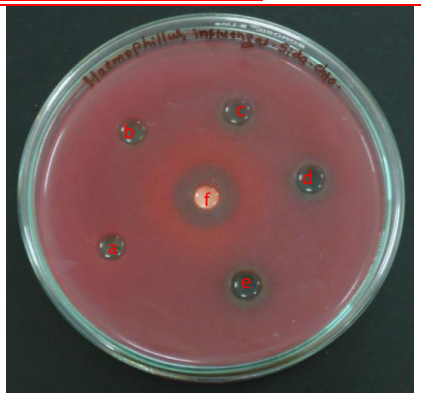
Haemophilus influenzae			*Aspergillus niger*	
Ampicillin - 08 mm at 500 mg/ml concentration*			Fluconazole - 13 mm at 100 mg/ml concentration*	
Conc. (mg/ml)	ZoI (mm) of ethanolic extract	ZoI (mm) of choloroform extract	ZoI (mm) of ethanolic extract	ZoI (mm) of choloroform extract
1	0	0	0	0
	0	0	0	0
10	0	05	0	10
	0	05	0	10
50	0	06	0	12
	0	06	0	12
100	0	06	0	13
	0	06	0	13

*no zone of inhibition for control

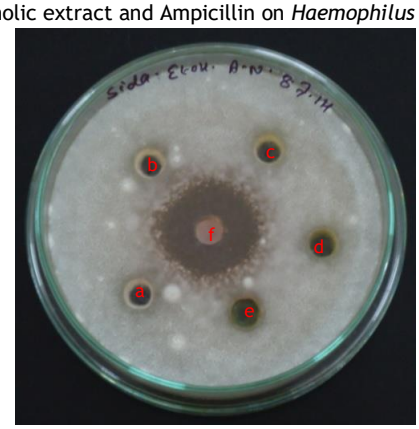
Figure 3. Antimicrobial activity of extracts of *Sida rhombifolia* aerial parts



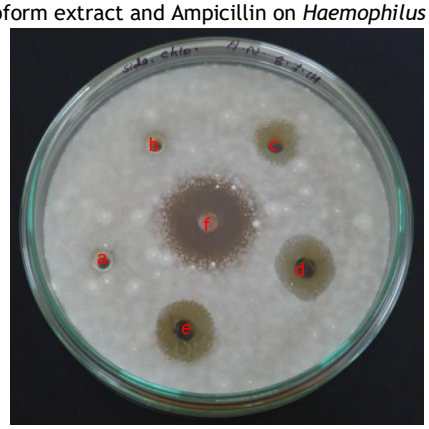
3.1 Ethanolic extract and Ampicillin on *Haemophilus influenzae*



3.2 Chloroform extract and Ampicillin on *Haemophilus influenzae*



3.3 Ethanolic extract and Fuconazole on *Aspergillus niger*



3.4 Chloroform extract and Fuconazole on *Aspergillus niger*

The antimicrobial activity of the ethanolic and chloroform extracts of *Sida rhombifolia* were studied in different concentrations (1, 10, 50, and 100µg/ml) against pathogenic bacterial strain (*Haemophilus influenzae*) and a fungal strain (*Aspergillus niger*). These strains have been selected on the basis of its application purpose for further formulation study.

H. influenzae seems to occur in humans especially in infants and young children *H. influenzae* type b (Hib) causes bacteremia, pneumonia, epiglottitis and acute bacterial meningitis. Occasionally it causes cellulitis, osteomyelitis and infectious arthritis. In this study the plant extract showed inhibition towards *H. influenzae*.[14]

Aspergillus niger has been associated with otomycosis[15], cutaneous infections[16] and pulmonary disease. There were reports of *A. niger* causing pneumonia. In some case *A. niger* pulmonary infections were fatal.

Antibacterial and antifungal potential of extracts of aerial parts were assessed in terms of zone of inhibition of bacterial and fungal growth. The results of the antibacterial activity and antifungal activity of ethanolic and chloroform extracts respectively are represented in Table 4 and Figure 3. The antibacterial and antifungal sensitivity was significant in chloroform extract where as its sensitivity observed to be none in ethanol extract.

4. Conclusion

There was no antibacterial as well as antifungal activity for ethanolic extract of *Sida rhombifolia* where as it was fairly significant in chloroform extract. The present investigation provides useful information on antimicrobial activity of chloroform extract of *Sida rhombifolia* aerial parts in treatment of various bacterial and fungal infections. As aerial parts of *Sida spp* may be used in place of root alone as it goes waste while harvesting of roots. As industries are already using whole plant instead of roots of these herbs the study provides important insight on standards for aerial parts of *Sida rhombifoila.*

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SOURCE OF SUPPORT Nil

CONFLICT OF INTEREST Authors declare no conflict of Interest

CONTRIBUTORS Miss. Ananya Rai and Mrs. Suchitra performed all experimental work in phytochemical parameter, TLC and any other laboratory work. Dr Vishwanatha contributed to the antimicrobial study. Dr KN Sunil Kumar and Dr. B Ravishankar contributed to planning and execution of research work, literature survey for article, drafting and finalization of article as per the format.

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