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PHYTOCHEMICAL SCREENING AND BIOLOGICAL EFFICACY OF EXTRACTS FROM *CADABA FRUTICOSA* L. AGAINST HUMAN PATHOGENIC BACTERIA

[1]S. Surabi and [2]V. Krishnan

Department of Plant Biology and Plant Biotechnology, Presidency College, Chennai - 600 005.

***Corresponding Author Email: dheena2it@gmail.com**

ABSTRACT

Medicinal plants are proving as an important source of potentially therapeutic drugs which are curing all kinds of infectious diseases throughout the world since the tradition of mankind, are still broadly used and have considerable importance in international trade. The present study deals with the phytochemical screening and biological activities of methanol extract of Cadaba fruticosa L. (Capparidaceae family). Varying amount of alkaloids, phenols, flavonoids and phytosterols were detected. Methanol extracts were showed excellent antimicrobial and antibiofilm activities. Based on the results this can take it further research to identify the active principles of above activities.

KEY WORDS

Phytochemical analysis, antimicrobial activity, antibiofilm.

INTRODUCTION

The medicinal flora in the tropical eco-region has a preponderance of plants that provide raw material for addressing a range of medical disorders and pharmaceutical requirements. Collectively, plants produce a remarkably diverse array of over 500,000 low molecular mass natural products also known as secondary metabolites [1]. The medicinal value of these secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these include: alkaloids, glucosides, glycosides, steroids, flavanoids, fatty oils, phenols, resins, phosphorus and calcium for cell growth, replacement, and body building [2]. *Cadaba fruticosa* L. (*Capparaceae* family) is a shrub commonly found in tropical countries especially in lower altitude of India. This plant grows up to 3 metres in height, bearing cylindrical stems. The leaves are ovate-oblong with glabrous and fully margined [3]. The leaves and roots are used as deobstruent, anthel- mintic, emmenagogue and uterine obstructions. The leaves and fruits are used to treat worm infestation, swellings, eczema and constipation [4,5]. The juice of the leaves are especially used to cure gonorrhoea and ver- mifuge [6,7]. The active principles, Stachydrine and 3-hydroxystachydrine isolated from the stemand roots of *C. fruticosa* [8,9]. The leaves contain cadabine [8,10] and terpenoids, flavones, sugar and proteins [11]. In its natural range, *C. fruticosa* provides cattle fodder, edible and cosmetic oils, medicinal products, shade and shelter. There is increasing interest in the use of plant extracts as therapeutic agents, particularly the capacity for these extracts to inhibit the growth of pathogenic microorganisms. Recent finding indicate that phenol and natural phenolic compound have an antibiofauling effect on biofilm formation [12]. The main objective of the study is to evaluate phytochemical composition, biological activities such as antimicrobial and anti-biofilm activities against human pathogenic bacteria.

MATERIALS AND METHODS

Plant collection

The plant leaves of *Cadaba fruticosa* L. was used for the investigation was collected from Thiruvallur district, Tamilnadu, India. The voucher specimen was deposited at Presidency College, Department of Plant Biology and Plant Biotechnology, Tamilnadu, India.

Preparation of methanol

The leaves were separated, washed thoroughly with tap water to remove adhered dirt, shade dried and stored in air tight container. The shade dried leaves of the plant were pulverized in a mechanical grinder to obtain coarse powder. The dried powdered leaf material (1kg) was extracted with methanol for 3 times at room temperature. Following filtration, the extract was concentrated by rotor vapor under reduced pressure at 45°C to give a gummy mass. It was preserved in a refrigerator at 4˚C for further use.

Phytochemical analysis

Phytochemical screening of plant extract was carried out according to the method adopted [13,14,15,16].

Determination of *in vitro* antimicrobial and antibiofilm activities using methanolic extracts

Well diffusion method

The *in vitro* antimicrobial activities of *Cadaba fruticosa* L. extracts of leaves were determined by the well diffusion method as described by [17]. The well diffusion test was performed using Muller Hinton Agar (MHA) medium for bacteria and Potato Dextrose Agar (PDA) for fungi. The medium was prepared and autoclaved at 15 lbs pressure (121°C) for 5 min. The medium was cooled to 50-55°C and poured into sterile petri plates to a uniform depth of 4 mm which is equivalent to approximately 25-30 mL in a 90 mm plate. Once the medium was solidified, standardized (0.5 McFarland standards such that final inoculum would contain 5 x 10^5 CFU/mL) bacterial suspension was swabbed on the medium within 15 min of adjusting the density of the inoculum. The plates were undisturbed for 3 to 5 min to absorb the excess moisture. Sterilized 9 mm cork borer was used to make agar wells; sample extracts of concentrations 250 µg/mL, 500 µg/mL, 1000 µg/mL from the stock solution was dispensed into each well and 100% DMSO as a control. Kanamycin (30 µg) for bacteria and fluconazole (30 µg) for fungi suspended in sterile glass distilled water were used as positive control. Zone of inhibition (ZI) were measured by 1 mm accuracy caliber and percentage of inhibition was calculated by the formula,

Percentage of inhibition (%) = [Zone of inhibition/ Dia. of the pertiplate] x 100} (mm)] ×100

Minimum inhibitory concentration (MIC) by broth dilution method

Methanolic extracts of *Cadaba fruticosa L.* of leaves which showed significant zones of inhibition were chosen to assay for minimum inhibitory concentration. MIC was determined by the standard method of [18]. Muller Hinton broth was prepared and sterilized using autoclave. One mL of the prepared broth was dispensed into the test tubes numbered 1-9 using sterile micropipette. Then, 1 mL sample from stock solution containing mg/mL of the extract was dispensed into tube numbered 1. Subsequently, from tube 1, serial dilution was carried out and 1 mL from tube 1 was transferred up to tube number 7 and 1 mL from the tube 7 was discarded. Tube 8 was control to assess sterility of the medium and tube 9 to assess viability of the organisms. The density of bacterial/fungal inoculum cultures were adjusted with 0.5 McFarland standards and final inoculum contains 5 x 10^5 CFU/mL. One mL of the inoculum was transferred into each tube from tube 1 to tube 9 with exception of tube 8, to which another 1 mL of sterile nutrient broth was added. The final concentration (500, 250, 125, 62.5, 31.25, 15.625 and 7.8125 µg/mL) of the methanol extract in each of the test tubes numbered 1-7 after dilution were incubated at 37°C for 24 h and examined for growth. The last tube in which growth failed to occur was MIC tube. Kannamycin and flucanoazole were used as standard for bacteria and fungi respectively.

Antibiofilm activities of methanol extract of leaves of *Cadaba fruticosa L.*

Methanolic extract of *Cadaba fruticosa* of leaves which showed significant zones of inhibition were chosen to assay for antibiofilm by slight modification [18]. Muller Hinton broth for *bacteria and* Sabouraud Dextrose Broth (SDB*)* for *Candida albicans* was prepared and sterilized using autoclave. One mL of the prepared broth was dispensed into the test tubes numbered 1-5 using sterile micropipette. Then, 1 mL of methanolic extract (dissolved in phosphate buffer saline) was dispensed

into tube numbered 1. Subsequently, from tube 1, serial dilution was carried out and 1 mL from tube 1 was transferred up to tube number 3 and 1 mL from the tube 3 was discarded. Tube 4 was control to assess sterility of the medium and tube 5 to assess viability of the organisms. The density of bacterial/fungal inoculum cultures were adjusted with 0.5 McFarland standards and final inoculum contains 5 x 10^5 CFU/mL. One mL of the inoculum was transferred into each tube from tube 1 to tube 5 with exception of tube 4, to which another 1 mL of sterile nutrient broth was added. The final concentration (500, 250, 125 µg/mL) of the methanolic extract in each of the test tubes numbered 1-5 after dilution were incubated at 37°C for 8 h in static condition and examined for antibiofilm activity. Then, the surface pellicles and the cultures were carefully removed from the tested tubes. Each tube was gently rinsed twice with distilled water and the remaining cells and matrices were stained with 1.5 mL of a 1% crystal violet solution for 25 min at room temperature. After washing twice with distilled water, the crystal violet attached to the biofilm was solubilized in 1.5 mL DMSO, and quantified by slight modification [19]., measuring its absorbance at 570 nm. Percentage of antibiofilm activity was measured using following formula.

Inhibition percentage = Control OD – sample OD / Control OD X 100.

RESULT AND DISCUSSION

The phytochemicals screening of *Cadaba fruticosa L.* of methanol extracts revealed the presence of different secondary metabolites such as alkaloids, phenols, flavanoids, phytosterols, fixed oil, glycosides, phenolic and carbohydrates. **Table 1.** Methanol extracts of *Cadaba fruticosa L.* showed various ranges of antibacterial activity at concentrations of 250 µg, 500 µg and 1000 µg. Antibacterial activity of methanol extracts revealed that the zone of inhibition (percentage of inhibition) against both gram positive and negative bacteria ranges between 10 mm (11.11%) to 18 mm (20%). Methanol extract of *Cadaba fruticosa L.* showed zone of clearance and percentage of inhibition at higher concentration (1000 µg) against human pathogen. Maximum zone of inhibition were recorded against *Streptococcus mutans* (18 mm), *Pseudomonas aeruginosa and Staphylococcus aureus* (17 mm), *E. coli and Bacillus subtilis* (15 mm), *Klebsiella pneumonia, Vibrio cholerae and Enterococcus faecalis* (14mm), *Salmonella typhi and Shigella flexneri* (13 mm). Similarly, methanol extracts of *Cadaba fruticosa L.* exhibited an effective antifungal activity against *Candida albicans* (Table 2). Similarly, antifungal (*Candida albicans*) activities of methanol extract of *Cadaba fruticosa L.* showed maximum zone of inhibition 16 mm.

Based on the zone of inhibition, concentrations of methanol extracts were selected for minimum inhibitory concentration of *Cadaba fruticosa L.* against ten bacterial pathogens and one fungal pathogen (*Candida albicans*). The concentrations used for MIC were 500, 250, 125, 62.5, 31.25, 15.62 and 7.81µg/mL. Methanol extract of leaves showed minimum inhibitory concentrations ranging between 125 µg/mL and 1000 µg/mL while, standard kanamycin showed MIC at 15.62 µg/mL for all the pathogens. Similarly, minimum inhibitory concentrations of leaves was 125 µg/mL against fungi (*Candida albicans)* while, it was 31.25 µg/mL for standard fluconazole (**Table 2**).

Table 1. Phytochemical screening of methanol extract of leaves of *Cadaba fruticosa* L.

S.No	Phytochemical test	Methanol extract of Leaves
	Alkaloids	
I	1. Mayer's Test	+
	2. Wagner's Test	+
	3. Hager's test	+
	4. Dragendorff' s test	+
II	**Flavonoids** Alkaline reagent test	+
III	**Fixed oil test** Spot test	+
	Carbohydrate	
IV	1. Fehling's test	+
	2. Benedict's test	+

V	**Glycosides**	
	Borntrages's test	**+**
VI	**Saponins:**	
	Foam test	**-**
VII	**Phytosterols:**	
	Libermann-Burhard's test	**+**
VIII	**Phenols**	
	1. Ferric chloride test	+
	2. Gelatin test	+
	3. Lead acetate test	+

Table 2. *In vitro* antimicrobial activities of methanol extracts of *Cadaba fruticosa* L.

Human pathogens	Conc. of extract (µg)	Methanol extracts		Kannamycin and Fluconazole (30)	Minimum inhibitory concentration (µg/mL)	
		Zone of inhibition in mm	Percentage of inhibition (%)	Zone of inhibition in mm (Percentage of inhibition)	Methanol extracts	Kannamycin and Fluconazole
Escherichia coli	250	-	-	26±1.82 (28.89±2.02)	1000	15.62
	500	-	-			
	1000	15±1.05	16.67±1.17			
Pseudomonas aeruginosa	250	-	-	30±2.1 (33.33±2.33)	500	15.62
	500	13±0.91	14.44±1.01			
	1000	17±1.19	18.89±1.32			
Salmonella typhi	250	-	-	27±1.89 (30.00±2.10)	500	15.62
	500	10±0.7	11.11±0.78			
	1000	13±0.91	14.44±1.01			
Shigella flexneri	250	-	-	28±1.96 (31.11±2.18)	1000	15.62
	500	-	-			
	1000	13±0.91	14.44±1.01			
Vibrio cholerae	250	-	-	28±1.96 (31.11±2.18)	1000	15.62
	500	-	-			
	1000	14±0.98	15.56±1.09			
Klebsiella pneumoniae	250	-	-	25±1.75 (27.78±1.94)	1000	15.62
	500	-	-			
	1000	14±0.98	15.56±1.09			
Bacillus subtilis	250	-	-	26±1.82 (28.89±2.02)	500	15.62
	500	13±0.91	14.44±1.01			
	1000	15±1.05	16.67±1.17			
Staphylococcus aureus	250	10±0.7	11.11±0.78	26±1.82 (28.89±2.02)	125	15.62
	500	13±0.91	14.44±1.01			
	1000	17±1.19	18.89±1.32			
Enterococcus faecalis	250	10±0.70	11.11±0.78	27±1.89 (30.00±2.10)	125	15.62
	500	11±0.77	12.22±0.86			
	1000	14±0.98	15.56±1.09			
Streptococcus mutans	250	10±0.7	11.11±0.78	31±2.17 (34.44±2.41)	125	15.62
	500	14±0.98	15.56±1.09			
	1000	18±1.26	20.00±1.40			
Candida albicans	250	10±0.70	11.11±0.78	18±1.26 (20.00±1.40)	125	31.25
	500	13±0.91	14.44±1.01			
	1000	16±1.12	17.78±1.24			

Note: '-'= Activity is absent; Values are mean of triplicates ± standard deviation.

Methanol extract was tested for antibiofilm activity on ten bacteria and one fungi with the concentration ranging from 125µg/mL to 500 µg/mL. All the concentration showed different ranges of antibiofilm activity in dose dependent manner (**Table 3**). The methanol extract at the maximum concentrations of (500 µg/mL), the antibiofilm activities were recorded in *Shigella flexneri* (78.29%), *Escherichia coli* (77.46), *Pseudomonas aeruginosa* (77.22), *Vibrio cholera* (76.28), *Bacillus subtilis* (74.97), *Staphylococcus aureus* (73.55), *Candida albicans* (66.43), *Enterococcus faecalis* (61.80), *Salmonella typhi* (59.31), *Klebsiella pneumonia* (59.07) and *Streptococcus mutans* (54.57).

Table 3. Anti-biofilm assay of methanolic extracts of *Cadaba fruticosa L.*

S.No	Human pathogens	Inhibition percentage of Bio-film formation		
		Concentration of methanol extracts		
		125 µg/mL	250 µg/mL	500 µg/mL
1	*Escherichia coli*	49.35±3.45	64.41±4.51	77.46±5.42
2	*Klebsiella pneumoniae*	40.57±2.84	53.14±3.72	59.07±4.14
3	*Pseudomonas aeruginosa*	49.70±3.48	63.23±4.43	77.22±5.41
4	*Salmonella typhi*	51.13±3.58	51.25±3.59	59.31±4.15
5	*Shigella flexneri*	52.43±3.67	55.28±3.87	78.29±5.48
6	*Vibrio cholera*	50.53±3.54	63.11±4.42	76.28±5.34
7	*Bacillus subtilis*	52.43±3.67	55.52±3.89	74.97±5.25
8	*Staphylococcus aureus*	41.76±2.92	64.53±4.52	73.55±5.15
9	*Enterococcus faecalis*	37.60±2.63	49.23±3.45	61.80±4.33
10	*Streptococcus mutans*	16.84±1.18	34.52±2.42	54.57±3.82
11	*Candida albicans*	28.71±2.01	46.38±3.25	66.43±4.65

Values are mean of triplicates ± standard deviation

CONCLUSION

Cadaba fruticosa L. leaf extract exhibited broad spectrum antimicrobial activity against the test bacterial and fungal isolates. Several relevant phytochemical constituents such as alkaloids, flavonoids and phenols that can be used as components of new antimicrobial agents were also present in different amounts. There is a need to conduct further studies aimed at determining the percentage yield of antimicrobial compounds and the antibacterial activity of the leaf extract on multiple drug resistant bacteria.

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***Corresponding Author:**
S.Surabi*
Email: **dheena2it@gmail.com**