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IN VITRO EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF METHANOL EXTRACTS OF *JASMINUM CUSPIDATUM* (L.)

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ABSTRACT

Objective: To evaluate in vitro antimicrobial and antioxidant activities of methanolic extract of *Jasminum cuspidatum* leaves extract. **Method:** Methanolic extract of *J. cuspidatum* was evaluated for its antimicrobial activity by well diffusion method and their possible antioxidant assay by DPPH scavenging activity. **Result:** In the DPPH scavenging activity, the IC_{50} value of methanol extract was 131.46 µg/mL. Further, the extract showed inhibitory activity for Gram-positive and negative bacteria at different concentration. **Conclusion:** These results clearly indicate that *J. cuspidatum* is effective in scavenging free radicals and has the potential to be a powerful antioxidant. Thus, the results obtained in the present study indicate that *J. cuspidatum* leaves extract could be considered as a potential source of natural antioxidants and that could be used as an effective source against bacterial diseases.

KEYWORDS: Antimicrobial, antioxidant activities, TLC, *Jasminum cuspidatum.*

INTRODUCTION

The global interest in the medicinal potential of plants during the last few decades is therefore quite logical. Promising potential of antimicrobial plant extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals alternative medicine and natural therapies. On screening plant extracts for antimicrobial activity, it has been shown that higher plants represent a promising source of new antimicrobial agents (OJala *et al.*, 2000). There is urge to identify the potent antimicrobial active compounds from

natural sources such as plant, animals, microorganisms, which is not creating the side effect or less side effect to the consumers. *Tecoma capensis*, *Sonchus oleraceus*, *Pityriasis alba*, *Pinus nigra*, *Jasminum sambac*, *Rosmarinus officinalis*, *Lavandula angustifolia* and *Laurus nobilis* are great sources of antimicrobial compounds and quorum sensing inhibitors (Al-Hussaini and Mahasneh, 2009). *Jasminum officinale* used as a urinary anti-infective in folk medicine. *In vitro* anti-bacterial activity of ethanolic extracts of different parts (flowers, stems, leaves and roots) of *J. officinale* growing in local gardens was evaluated against four reference bacteria by broth dilution assay and agar diffusion assay. The MIC value of the ethanolic extracts of flowers and stems plus leaves against all bacteria was 2 mg/mL and the MIC value of roots against *S. aureus*, *E.faecalis* and *E. coli* was 4 mg/mL and the MIC value of roots against *P. aeruginosa* was 2 mg/mL. In agar well diffusion assay, the ethanolic extracts of all parts of the plant showed substantial activity against all bacteria (Shahbaa, 2015). Foods rich in natural antioxidants such as polyphenols, flavonoids are related to reduced risk of incidence of cardiovascular and other chronic diseases and certain types of cancer, which has led to a revival of interest in plant-based foods (Choi *et al*., 2007). In the recent years interest in the study of antioxidant activity of plant extracts and isolation from plants has grown due to the fact that the free radicals have been related to degenerative diseases. Antioxidants are compounds which act as radical scavengers when added to the food products and prevent the radical chain reaction of oxidation delay or inhibit the oxidation process and increase shelf life by retarding the processes of lipid peroxidation. The main objective of the study is to evaluate biological activities such as *in vitro* DPPH antioxidant, antimicrobial activities against human pathogenic bacteria.

MATERIALS AND METHODS

Plant collection

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

Preparation of methanol extract

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.

Estimation of secondary metabolites of total phenol and flavanoid

Total phenol of methanol extracts were estimated using Folin phenol reagent based on the reaction between phenol and oxidizing agent phosphomolybdate according to Slinkard and Singleton (1977). Aluminum chloride colorimetric method was used for determination of flavonoids (Chang *et al.,* 2002). Gallic acid and Quercetin were used as standard for phenol and flavanoid respectively. The total phenol contents are expressed mg GAE/g DW and flavonoids are expressed as mg QE/ g DW of methanol extracts.

Determination of *in vitro* antimicrobial activities using methanolic extracts

Well diffusion method

The *in vitro* antimicrobial activities of *Jasminum cuspidatum extracts* of leaves were determined by the well diffusion method as described by Perez *et al*., (1990). The well diffusion test was performed using Mueller Hinton Agar (MHA) medium for bacteria and potato dextrose agar (PDA) for fungi. The medium was prepared and autoclaved at 15 lbs pressure (121ºC) for 5 min. The medium was cooled to 50-55º 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,

$$\text{Percentage of inhibition (\%)} = \text{[Zone of inhibition/ Dia. of the pertiplate] x 100 }\}\text{(mm)]}\times 100$$

Minimum inhibitory concentration (MIC) by broth dilution method

Methanolic extracts of *Jasminum cuspidatum* of leaves which showed significant zones of inhibition were chosen to assay for minimum inhibitory concentration. MIC was determined

by the standard method of Wariso and Ebong (1996). 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.

In vitro* DPPH scavenging assay on TLC by dot blot and spectrophotometricaly of methanol extract of leaves of *Jasminum cuspidatum

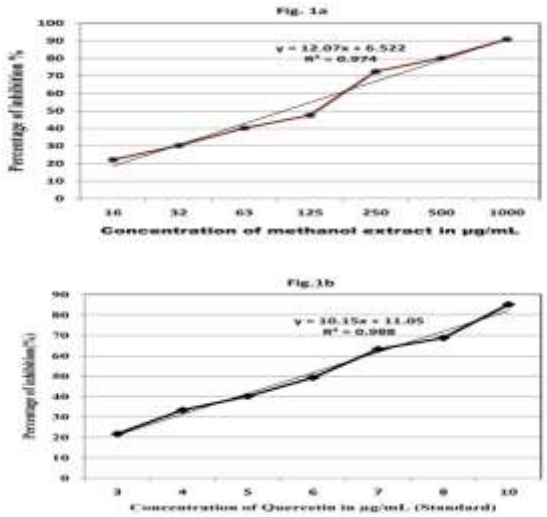
The ability of methanol extracts of *Jasminum cuspidatum* to scavenge the DPPH radical was investigated in a rapid dot-plot screening method. The rapid screening assay was performed by the method proposed by Soler-Rivas *et al*. (2000) with slight modification. Ten microlitres of extract (mg/mL) in methanol were spotted carefully on the TLC plate and dried for 5 min. Then, 0.4 mM DPPH solution was spotted onto the spots of extract; the layers were dried for 2 min. Quercetin was used as the standard reference. The stained silica gel layer with purple backgrounds and yellow spots revealed radical scavenging capacity. DPPH radical reacts with an antioxidant compounds that can donate hydrogen and get reduced. DPPH when acted upon by non-radical, conversion of diphenypicryl hydrazyl to diphenylpicryl hydrazine occurs. This can be identified by the conversion of purple to light yellow colour.

According to Blois (1958) DPPH in methanol (0.1 mM) was prepared and 1.0 mL of this solution was added to 1 mL of extract solution in methanol at different concentrations. After incubation for 30 min, all the reaction mixtures were read with spectrophotometer at 517 nm. A blank was prepared without extracts. Quercetin at various concentrations was used as the standard. The capability of scavenge activity was calculated by the formula.

Scavenging activity (%) = [(Control OD - sample OD)/Control OD] × 100

RESULTS

In this communication we report the antioxidant, antimicrobial activities of the methanol extracts of J. cuspidatum leaves. The total phenol content of methanol extracts of leaves was recorded 289 mg/g Dry Weight of Gallic Acid Equivalence and flavanoid content was 357 mg/g Dry Weight of Quercetin Equivalence. *In vitro* Dot blots DPPH activity of methanol extracts of leaves of *Jasminum cuspidatum* showed the purple backgrounds and yellow spots**.** *In vitro* DPPH activity spectrophotometric analysis of methanol extracts of all parts of *Jasminum cuspidatum* showed various ranges of percentage of inhibitions. The methanol extracts were selected and the concentrations 16-1000 µg/mL was tested and maximum percentage of inhibition was determined **Figure 1a and 1b**.



The maximum percentages of inhibition by different methanol extracts were 90.25% at 1000 µg/mL, whereas standard quercetin was 85.15 at the concentration of 10 µg/mL. IC_{50} (µg/mL) values was 131.46, while for standard quercetin it was (6.08 µg/mL). Table 1 and 2

Table 1. *In vitro* DPPH radical scavenging activities of methanol extracts *of Jasminum cuspidatum*.

S. No	Conc. of methanol Extract (µg/mL)	Percentage of inhibitions
1	16	22.18±1.55
2	32	30.26±2.12
3	63	40.23±2.82
4	125	47.54±3.33
5	250	72.49±5.07
6	500	80.16±5.61
7	1000	90.84±6.36
IC 50 value (µg/mL)		**131.46**

Table 2: *In vitro* DPPH radical scavenging activities of quercetin (Standard).

S. No	Conc. of Methanol extracts	Percentage of inhibitions
1	3	21.7±1.52
2	4	33.37±2.34
3	5	40.12±2.81
4	6	49.31±3.45
5	7	63.19±4.42
6	8	68.78±4.81
7	10	85.15±5.96
IC 50 value (µg/mL)		**6.08**

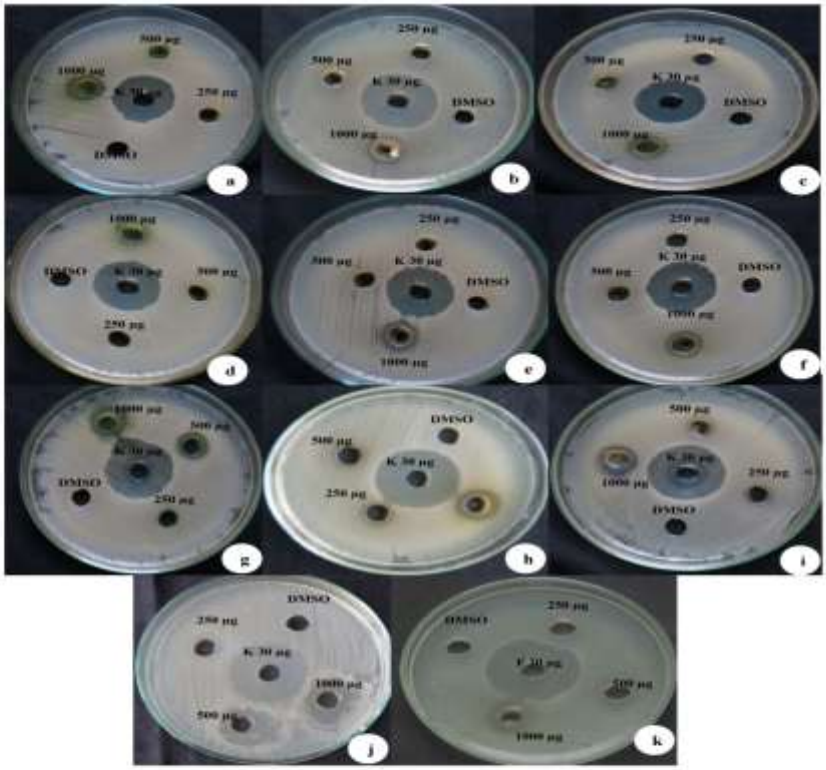
Methanol extracts of *Jasminum cuspidatum* showed various ranges of antibacterial activity at concentrations of 250 µg, 500 µg and 1000 µg. Antibacterial activity of methanol extracts revealed that the zone of inhibition (percentage of inhibition) against both gram positive and negative bacteria ranges between 10 mm (11.11%) to 18 mm (20%). Methanol extract of *J. cuspidatum* 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 *J. cuspidatum* exhibited an effective antifungal activity against *Candida albicans* (Table 3 Fig. 2). Similarly, antifungal (*Candida albicans*) activities of methanol extract of *J. cuspidatum* showed maximum zone of inhibition 16 mm.

Based on the zone of inhibition, concentrations of methanol extracts were selected for minimum inhibitory concentration of *J. cuspidatum* 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 3 and Figure 2).

DISCUSSION

Phenolic compounds have attracted a great attention in relation to their potential of antimicrobial and antioxidant activities for beneficial effects on health (Narayana *et al*., 2001). Flower of *Jasminum humile* of different extracts showed the various concentration of

phenol content (mg/g)in different extracts on dry weight basis in ethyl acetate (173 mg), butanol (129 mg) hexane (84 mg) (Afsar *et al*., 2014). Similarly methanol extracts *J. cuspidatum* revealed the presence of 289 mg/g DWGAE and flavanoid content was 357 mg/g DWQE. The stable DPPH is a widely used method to evaluate antioxidant activities in relatively short time compare to other methods. The decrease in absorbance of DPPH caused by antioxidant is due to the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation. This is visualized as a discoloration from purple to yellow. in vitro antioxidant activities of DPPH radical scavenging activities of methanol extracts showed the 131.46 µg/mL, which revealed that potentiality of *J. cuspidatum*. Methanol extract of *J. cuspidatum* revealed the antimicrobial activities MIC ≤ 1000 µg/mL. This suggests that extracts of these plants are broad spectrum in their activities.



Antimicrobial activity of methanol extract of *Jasminum cuspidatum* against human pathogens.

a. Escherichia coli *b. Pseudomonas aeruginosa,*
c. Salmonella typhi, *d. Shigella flexneri,*
e. Vibrio cholerae, *f. Klebsiella pneumoniae*
g. *Bacillus subtilis,* h. *Staphylococcus aureus,*
i. *Enterococcus faecalis,* j. *Streptococcus mutans*
h. Candida albicans

K 30 µg- Kanamycin 30 µg; F-30µg- Fluconazole 30µg

Figure 2.

Table 3: *In vitro* antimicrobial activities of methanol extracts of *Jasminum cuspidatum*.

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.

CONCLUSION

The study has shown that, methanol extract from *Jasminum cuspidatum* have in vitro antimicrobial and antioxidant activities which could support the use of the plant by traditional ealers to treat various infective diseases.

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