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Research Article

APPRAISAL OF THE *IN VITRO* ANTIBACTERIAL AND ANTIOXIDANT POTENTIAL OF THE LEAF EXTRACTS OF *CADABA FRUTICOSA*

B. UDHAYA LAVINYA, J.L.AKALYA, K.S.L. SRUJANA, EVAN PRINCE SABINA, V. DEVI RAJESWARI*

School of Biosciences and Technology VIT University Vellore, Tamilnadu, India. Email: vdevirajesari@vit.ac.in

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ABSTRACT

Objective: The aim of the present study is to evaluate the *in vitro* antimicrobial and antioxidant activities of the leaf extracts of the medicinal plant *C. fruticosa*.

Methods: The antimicrobial activities of the aqueous and methanolic extracts against different bacterial strains were determined by disc diffusion method and compared with that of the standard antibiotic cefotaxime. Determination of the reducing power and 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity were carried out on different concentrations of the methanolic extract in order to evaluate the antioxidant potential of *C. fruticosa*. The total phenolic content of the methanolic extract was measured.

Results: The antimicrobial activity of the aqueous extract was found to be lesser compared to that of the methanolic extract. The methanolic extract showed significant antimicrobial and antioxidant activities.

Conclusion: From the present study it is evident that the methanolic extract of the leaves of *C. fruticosa* possesses significant antimicrobial and antioxidant activities.

Keywords: Cadaba fruticosa, antimicrobial, antioxidant, DPPH assay

INTRODUCTION

The occurrence of bacterial infections and their severity has greatly increased in the last few decades. This has lead to increasing challenges in treating such infections, especially the Methicillin resistant *Staphylococcus aureus* (MRSA), Methicillin resistant *Staphylococcus epidermidis* (MRSE), Vancomycin resistant *Enterococci* (VRE) and other strains of Gram negative bacteria found in the cases of nosocomial infections that are multi drug resistant. Such organisms have evolved developing variations in their genetic basis for resistance to clinically used antibiotics. Inappropriate use of antibiotics has also contributed greatly to the emergence of such resistant strains [1]. Hence there is urgent need for new antimicrobial agents from natural sources. Several studies have explored the antimicrobial and antioxidant properties of herbal plants and plant based natural products and oils [2, 3].

Cadaba fruticosa (L.) or the Indian Cadaba is a shrub that grows up to 5m. It is a member of the Capparaceae family and commonly known as 'vizhuthi' in Tamil and 'Capper bush' in English. The shrub is widely distributed in the Indian subcontinent and is commonly used in Siddha medicine in the northern districts of Tamilnadu. It has been reported to possess hypoglycaemic activity [4] and the leaf juice is used internally to treat diarrhoea, dysentery and general weakness [5]. It is also used as an anti-allergic, antidote, antiscorbutic and anti-helminthic herbal drug [4, 5]. The leaf extracts also possesses antimicrobial activity [6] and is used in traditional medicine to treat syphilis and gonorrhoea [7]. The leaf extracts of *Andrographis echioides* and *Cadaba trifoliata* have shown synergistic larvicidal effect on *A. aegypti* (dengue causing mosquito) [8].

The deleterious effects of Reactive oxygen species (ROS) on cellular macromolecules causes loss of membrane integrity, cell injury and cell death. The generation of ROS plays a key role in accelerating the pathological effects and outcomes of many medical conditions like diabetes and its vascular complications [9], cancer [10], cardiovascular diseases [11], inflammatory diseases [12], toxicity by exogenous source of chemicals especially xenobiotics like bromobenzene, carbon tetrachloride (CCl_4), thioacetamide and D-Galactosamine inducing local inflammation and subsequent increase in oxidative stress [13].

The major bioactive components of most medicinal plants include flavonoids, phenolic compounds, tannins, alkaloids and saponins. The aqueous extract of the leaves of *C. fruticosa* had shown to contain terpenoids, flavonoids, proteins and furals while the alcoholic extract has shown to contain terpenoids, flavonoids, steroids, phenols, alkaloids, gums, sugars and saponins [4]. Therefore, the presence of these biologically active components in *C. fruticosa* may render antioxidant property to it.

The present study is aimed to evaluate the *in vitro* antibacterial potential of the aqueous and methanolic extracts of the leaves of *C. fruticosa* and also the *in vitro* antioxidant potential of methanolic extracts of the leaves of *C. fruticosa*.

MATERIALS AND METHODS

Plant material

The plant material was collected from a forest area near Tirupathi, Andhra Pradesh, India. The identification of the collected plant specimen was done and the same was confirmed with Dr. P. Jayaraman, Director of Plant Anatomy Research Centre (PARC), Tambaram, Chennai, Tamilnadu. A voucher specimen was deposited in the herbal garden, VIT University, India for reference.

Chemicals

1,1-diphenyl-2-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma chemical Co. The culture media and antibiotic discs used in the study were purchased from Himedia. All other chemicals used in this study were of analytical grade.

Preparation of the methanolic extract of the leaves of *Cadaba fruticosa*

The dried leaves of *C. fruticosa* were finely powdered and the 5g of the powder was extracted with 50ml of methanol for 24 hours in a glass conical flask at 25ºC using a shaker. This was then filtered

using Whatmann filter paper no.1 and the filtrate thus obtained was concentrated using a rotary vaporiser at 40ºC under low pressure. The residue was evaporated to dryness in a hot air oven and stored at 4ºC until further use.

Fig. 1: ***C. fruticosa*** **shrub**

Preparation of aqueous extract of the leaves of *Cadaba fruticosa*

5g of finely powdered leaves of *C. fruticosa* was dissolved in 50ml of sterile distilled water and extracted for 48 hours in a glass conical flask. This was filtered using Whatmann filter paper no.1 and the filtrate thus obtained was concentrated using a rotary vaporiser at 40ºC under low pressure. The residue was allowed to dry in a hot air oven and stored at 4ºC until further use.

Antimicrobial potential

Preparation of the test organisms

Staphylococcus aureus, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Pseudomonas aeruginosa* obtained from MTTC were used in the present study to determine the antibacterial activity of the aqueous and methanolic extracts of *C. fruticosa* by the method described by Kirby Bauer (Disc diffusion method). All organisms were sub-cultured onto Nutrient agar from their respective stock cultures except Streptococcus pyogenes which was sub-cultured onto Blood agar. For confirmation purpose the colonies were subjected to biochemical testing by MMTPC (mannitol fermentation and motility test in mannitol motility medium, triple sugar iron agar for fermentation of three sugars, indole production in peptone water and citrate utilization in Simon's citrate medium) in the case of Gram negative bacteria and coagulase test in the case of *Staphylococcus aureus*.

Evaluation of antibacterial activity by disc diffusion method

About 3-5 colonies of the 6 different organisms were inoculated onto 1ml of sterile nutrient broth (1ml of serum was added for enrichment in the case of *Streptococcus*) each using a loop and incubated at 37ºC for 2 hours. In each case the inoculum was spread evenly on Muller Hinton agar using sterile swabs. Muller Hinton Blood agar was used for *Streptococcus*. 6mm diameter sterile filter paper discs were impregnated with loopful of extract and placed on the agar. This was done with both aqueous and methanolic extract for each organism and Cefotaxime was used for comparison in each case. All procedures were done with sterile precautions in a laminar air flow hood. The plates were incubated at 37ºC overnight. The clear zones of inhibition indicate antimicrobial activity. All strains were tested in triplicate.

Antioxidant potential

Estimation of total phenolic content

The total phenolic content of the methanolic extract of C. fruticosa was determined by the method of Singleton et al., 1999 [14]. 0.05g of methanolic extract was dissolved in 50ml of methanol from which 1ml was taken and mixed with Folin-Ciocalteau reagent. 1ml of sodium carbonate (100mg/ml) was added after 2 minutes and incubated for 2hrs at room temperature. The intensity of the blue colour formed was measured spectrophotometrically at 765nm. The total phenolic content of the methanolic extract solution of C. fruticosa was expressed as mg gallic acid equivalent (GAE)/g dry basis.

DPPH assay

The DPPH scavenging activity of the methanolic extract was determined by the method of Blois, 1958 [15]. Different concentrations of the extract (200, 100, 80, 60, 40, 20, 10 and 5µg/ml) were prepared and used as sample solutions. 2ml of each sample solution was mixed with 1ml of methanolic solution of DPPH (0.2mM DPPH) by vigorous shaking and incubated in dark condition for 30min. In the control tube, 2ml of methanol replaced the sample solution and quercetin was used as standard reference in the assay. All sample solutions were tested in triplicate. The absorbance was measured spectrophotometrically at 517nm. The following formula was used to calculate the free radical scavenging activity:

Scavenging activity (%)

= [(Absorbance of control-Absorbance of sample)/ Absorbance of control] x 100

Determination of reducing power

The reducing power of the methanolic extract of *C. fruticosa* was determined by the method of Oyaizu, 1988 [16]. 2ml of each sample solution was mixed with 2ml of phosphate buffered saline (0.2M, pH 6.6) and 2ml of potassium ferricyanide (10mg/ml) and incubated for 20min at 50ºC. 2ml of TCA (100mg/ml) was added to each tube and mixed well. 2ml of each of the above mixtures were mixed with 2ml of distilled water and 0.4ml of 0.1% ferric chloride in a fresh set of test tubes. This mixture was allowed to react at room temperature for 10min and the absorbance was measured at 700nm. All sample solutions were tested in triplicate.

RESULTS AND DISCUSSION

***Antimicrobial potential of the leaf extracts of* C. fruticosa**

Most of the bacterial test strains used in this study were inhibited by the extracts of C. fruticosa. The methanolic extract was more effective compared with that of the aqueous extract as shown in Table.1. The results were compared with cefotaxime which was used as the standard reference antibiotic. From the results obtained it is evident that the methanolic extract of *C. fruticosa* has significant amount of antimicrobial activity.

Table 1: Antimicrobial activity of the leaf extracts of C. fruticosa and Cefotaxime on the selected bacterial strains

Microorganism	Aqueous extract (µg/ml per disc)		Methanolic extract (µg/ml per disc)		Cefotaxime
	100	200	100	200	
Staphylococcus aureus	-	9	10	14	23
Streptococcus pyogenes	-	8	9	13	28
Escherichia coli	8	11	11	14	25
Klebsiella pneumonia	-	9	9	12	24
Salmonella typhi	9	11	11	15	25
Pseudomonas aeruginosa	8	12	7	11	26

Data reported are the means of the tests carried out in triplicate in each case. The diameter zones of inhibition are reported in mm; (-) indicates resistance

Antioxidant potential of the leaf extracts of C. Fruticosa

Total phenolic content of methanolic extract of the leaves of *C. fruticosa*

The total phenolic content of methanolic extract of the leaves of *C. fruticosa* was found to be 39.8±1.92 mg GAE/g (dry basis). Phenolic compounds are a large group of plant secondary metabolites that are essential components of human diet and gain significance as they possess antioxidant properties. They contain aromatic ring with one or more hydroxyl groups. Their ability to act as hydrogen donors

makes them good antioxidants [17]. Flavonoids are the major components of phenolic content in plants. The phenolic content of many medicinal plants has shown to contribute to their antioxidant activity [18].

DPPH scavenging activity

The DPPH free radical scavenging activity depends on the reduction of the purple coloured DPPH on reaction with an antioxidant forming a yellow coloured stable compound [15]. In the present study a decrease in the absorbance of DPPH was found with increasing concentration of the extract as in Figure.1. This may be due to the presence of antioxidants in the methanolic extract of *C. fruticosa*. The DPPH scavenging activity of the methanolic extract of *C. fruticosa* was lesser than that of quercetin.

The DPPH assay results in this study show that the leaves of *C. fruticosa* are a source of natural antioxidants. The identification and isolation of the active components involved in this needs further investigation.

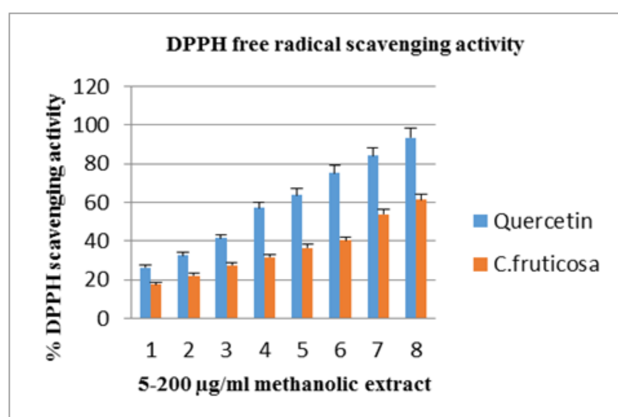

Fig. 2: DPPH free radical scavenging activity of methanolic extracts of the leaves of *C. fruticosa* and quercetin

Reducing power of the methanolic extract of the leaves of *C. fruticosa*

Reducing power is an important measure of the antioxidant potential of many medicinal plants [19]. In the present study the methanolic extract of *C. fruticosa* showed dose-dependent reducing power (Table.2). This indicates that the methanolic extract of *C. fruticosa* possesses antioxidant property.

Table 2: Reducing power of the methanolic leaf extract of C. fruticosa

Concentration of extract(µg/ml)	Absorbance at 700nm
100	0.091
200	0.122
300	0.155
400	0.214
500	0.386

CONCLUSION

From the results obtained in the present study it is evident that the methanolic extract of the leaves of *C. fruticosa* possess significant antimicrobial and antioxidant activities. The antioxidant potential may be due to the phenolic content, free radical scavenging activity and reducing power. However further *in vitro* and *in vivo* studies should reveal the individual active components of *C. fruticosa* leaf and their use as potent pharmacotherapeutic agents in microbial infections, oxidative stress and inflammatory diseases like rheumatoid arthritis.

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