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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *COMMELINA NUDIFLORA* (COMMELINACEAE)

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ABSTRACT
The present study was carried out to study the antibacterial and antioxidant activities of *Commelina nudiflora* extracts and to analyze the extracts for secondary metabolites. Chloroform, acetone and ethanolic extracts of *Commelina nudiflora* were screened for tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides, and steroids. Antibacterial activity was carried out by Minimum Inhibitory Concentration (MIC) and antioxidant assay was carried out using 1,1-diphenyl picrylhydrazyl (DPPH) and total antioxidant activity. Flavonoids present in all extracts. Acetone and ethanolic extracts exhibited antibacterial activity. All extracts showed significant antioxidant activity. All extracts showed concentration dependent antioxidant activity. Ethanolic extract showed high antioxidant activity against DPPH with IC_{50} 11.25 mg/ml. The total antioxidant activity was high in chloroform extract with IC_{50} 0.1282 mg/ml. The results show *Commelina nudiflora* has potential antioxidants that can fight against life threatening diseases like inflammation, cancer etc.
Keywords: *Commelina nudiflora*, Phytochemicals, Antibacterial, MIC, Antioxidant, DPPH

INTRODUCTION

All living animals including human knowingly or unknowingly take plants to recover from their illness. Animals like sheep, goat, and cow eat medicinal plants when they have stomach problem or other illness. Similarly human race started using medicinal plants during their civilization period by trial and error. World Health Organization (WHO) estimated more than 80 % of people rely on medicinal plant for their primary healthcare[1]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds[2]. The compounds isolated from plants exhibit various activities like antibacterial, antifungal, antioxidant, anticancer, anti inflammatory, antiviral and analgesic. It is generally considered that compounds produced naturally rather than synthetically, are easily biodegradable and environmentally safe. Thus, natural antioxidants, antibacterial, cytotoxic, antiviral and fungicidal agents have gained popularity in recent years, and their use and positive image among consumers are spreading[3]. *Commelina nudiflora,* belongs to the family of commelinaceae is a weed. It is a mucilaginous, slender, creeping or ascending branched perennial herb, usually pubescent. It is native to Asia. Traditionally it is used as a febrifuge, rubefacient and diuretic agent. It is a blood coagulant, antifebrile and antidote, tonic for the heart. Folkloric uses of *C. nudiflora* are: In the Gold Coast, the leaves are used to relieve swellings of the groin. Sierra Leone people use this plant for dressing wound after circumcision. In China, decoction of whole plants used for defervescence and detoxification, for leucorrhea and health protection and dye from flowers used as paint. Carribean Indians have used the plant in medicinal baths and as tea to ward off influenza. In Mexico this plant used for treatment of conjunctivitis, dermatitis, and dysmenorrheal In India latex, leaf, and shoot used to stop bleeding of wounds and cuts· It also used for leprosy by Indians[4]. It also used for biliousness, hair loss, kidney disease and for cleansing of the wombs and tubes[5]. Previously this plant has been investigated for its antimicrobial[6], wound healing[7] and anti oxidant properties[8]. All works have been done with the aerial parts of *C. nudiflora*. There is no investigation on the whole plant of *C. nudiflora*. The present study was undertaken to investigate the antibacterial and antioxidant activities of *Commelina nudiflora* and the phytochemicals in the extracts were qualitatively screened.

MATERIALS AND METHODS

Plant material

Whole plants of *C. nudiflora* were collected from the banana field in Pongumoodu, Thiruvananthapuram (Kerala), India and verified by the taxonomist Dr. G. Jeya Jothi, Department of Plant biology and Biotechnology, Loyola College, Chennai, India. The herbarium voucher number is LCH 402. Plants were washed thoroughly and dried completely at room temperature under shade. Dried plants were ground into coarse powder and stored in air tight container for further works.

Macroscopic Observation of powder

The plant powder was examined for its taste, odor, shape, color, nature and texture based on the method described by Siddiqui *et al*. (2005)[9].

Preparation of plant extracts

Plant extracts were prepared by serial extraction method which involves successive extraction with solvents of increasing polarity from a low polar (chloroform) to more polar solvents (acetone and ethanol) to ensure that a wide polarity range of compound could be extracted. About 50 g of dried powder was soaked in 800 ml chloroform for 72 hours with intermittent shaking at 120 rpm in shaker. The extract was filtered through Whatman No. 1 filter paper. The filtrate was dried completely to get constant dry weight of extract. The remaining plant residue from chloroform extract was

dried completely and soaked in 800 ml of acetone and then ethanol successively as above mentioned and the extracts were collected. The percentage of yield was calculated using the following formula,

$$\text{Yield (\%)} = W1/ W2 \times 100$$

Where: W1 = the weight of the extract after evaporation of solvents, W2 = the dry weight of plant sample (powder)

The extracts were stored at 4°C for further use.

Preliminary phytochemical screening

Preliminary phytochemical screening was done for chloroform, acetone and ethanol extracts of *C. nudiflora* using standard protocols of Harborne *et al*. (1973)[10].

Test for Tannins

To 3 ml of plant extracts few drops of 0.1 % ferric chloride solution was added. Formation of brownish green or a blue-black coloration indicated the presence of tannins.

Test for Phlobatanins

To 3 ml of extracts few drops of 1 % hydrochloric acid was added and heated in boiling water bath. Deposition of red precipitate indicates the presence of phlobatanins.

Test for Saponins (Foam Test)

The 3 ml extracts was added with few ml of distilled water to make the volume to 10 ml. This was agitated for 10 minutes. Formation of foam, up to 3 cm indicates presence of saponin(s).

Test for Flavonoids (Action of Alkali and Acid)

Extract were separately treated with alkali (1 % ammonium solution). Formation of yellow color solution; which on addition of acid (con. H_2SO_4) becomes colorless indicate presence of flavonoid(s).

Test for terpenoids (Salkowski's Test)

Few drops of concentrated sulfuric acid were added to the chloroform solution of the extracts. The solution was changed to brownish red color indicate the presence of phytosterol(s).

Test for Cardiac Glycosides -Keller killiani test [test for Deoxy sugars]

To the extract 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added. 0.5 ml of concentrated sulphuric acid was added to the sides of the test tube. Appearance of blue colour in the acetic acid layer indicates the presence of cardiac glycosides.

Test for Sterols

To the extracts 10 ml of chloroform was added and filtered. To 2 ml of filtrate, 2 ml of acetic anhydride and concentrated H_2SO_4 was added. Blue-green ring indicate the presence of sterols.

Bacterial strains

The antibacterial activity of the extracts were tested for *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 1320), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 96) and *Streptococcus epidermidis* (MTCC 435) procured from Microbial Type Culture Collection and Gene Bank (Chandigarh, India) by Minimum Inhibitory Concentration Method using 96 well plates.

Minimum Inhibitory Concentration

Plant extract at 100 mg/ml was used as the initial concentration and it was serially diluted (Sule *et al*., 2008)[11] by transferring 5 ml of extract in to 5 ml of nutrient broth. This will give 50 mg/ml concentration. Serial dilution was performed to obtain the concentrations of 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml and 1.6 mg/ml. Streptomycin was used as positive control. Sterile distilled water was used as a negative control. In each well 0.1 ml of 24 hours bacterial suspension was added. The plates were kept for incubation for 24 h at room temperature. Turbidity on the wells indicated the growth of bacteria. The lowest concentration of plant dilution which inhibits the bacterial growth by showing clear well was taken as Minimum Inhibitory Concentration (MIC).

Free radical scavenging by DPPH

The free radical scavenging by DPPH was performed, described by Manzocco *et al* (1998)[12]. Plant extract with different concentration (5, 10, 15, 20, 25 mg/ml) was taken in test tube and diluted with methanol. To this 0.5 ml of DPPH was added. The methanolic solution of extract without DPPH was taken as control, whereas BHA was used as reference. It was incubated at room temperature for 30 minutes and the absorbance was read at 517 nm. Percentage inhibition was calculated using the formula

$$\text{\% Inhibition of DPPH} = [(\text{Control absorbance} - \text{Test absorbance})/ \text{Control absorbance}] \times 100$$

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration in mg of dry material per ml (mg / ml) that inhibits the formation of DPPH radicals by 50 %[13]. All samples were analyzed in triplicate.

Determination of Total antioxidant activity

The total antioxidant activity was determined by thiocyanate method[14]. Various concentrations of plant extracts (5, 10, 15, 20, 25 mg/ml) were prepared in methanol and added to the linoleic acid emulsion (2.5 ml, 40 mM, pH 7.0) and phosphate buffer (2 ml, 40 mM, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g linoleic acid with 0.2804 g Tween-20 as emulsifier in 50 ml of 40 mM phosphate buffer. The mixture was then homogenized. The final volume was adjusted to 5 ml with 40 mM phosphate buffer (pH 7.0). The samples were incubated at room temperature for 60 h. One milliliter of the incubated sample was removed at 12 h interval and 0.1 ml of 20 mM $FeCl_2$ and 0.1 ml of 30 % ammonium thiocyanate were added. The absorbance was read at 500 nm using BHA as reference. The control contained same amount of the solvent added to the linoleic acid emulsion in plant extract with BHA. The percentage inhibition of lipid peroxide generation was calculated using the following formula:

$$\text{\% Inhibition of lipid peroxide generation} = [(\text{Control absorbance} - \text{Test absorbance})/ \text{Control absorbance}] \times 100$$

All samples were analyzed in triplicate.

RESULTS

Macroscopic observation of powder

After grinding the powder of *C. nudiflora* was slightly sour in taste, olive green in color with characteristic odor, rough texture, light weighted and fibrous in nature.

The percentage of yield

The high percentage of yield was obtained from ethanol extraction (1.9 %) and chloroform yielded 1.7 % extract. Acetone showed less yield of 1.0 %.

Phytochemical analysis

All extracts showed the presence of flavonoids. Cardiac glycosides were present in acetone extract. Chloroform extract showed the presence of sterols. Terpenoids present in chloroform and acetone extract. Tannins present in ethanolic extract. The results are shown in Table 1, Figure 1, 2 and 3.

Table 1: Preliminary phytochemical screening of *Commelina nudiflora*

Extract	Tannin	Phlobatannin	Saponin	Flavonoids	Terpenoids	Cardiac glycosides	Sterols
Chloroform	-	-	-	+	+	-	+
Acetone	-	-	-	+	+	+	-
Ethanol	+	-	-	+	-	-	-

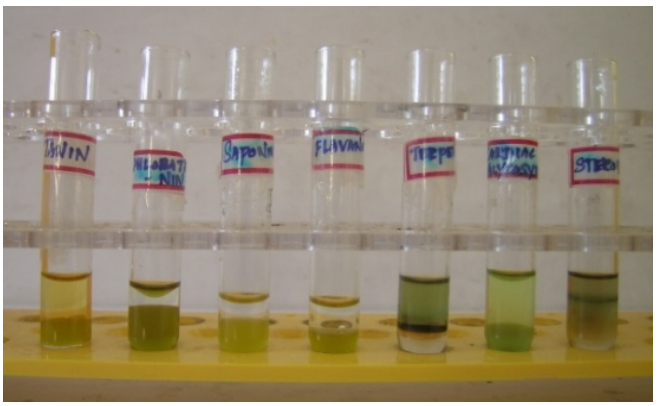

Figure 1: Preliminary phytochemical screening of chloroform extract of *Commelina nudiflora*

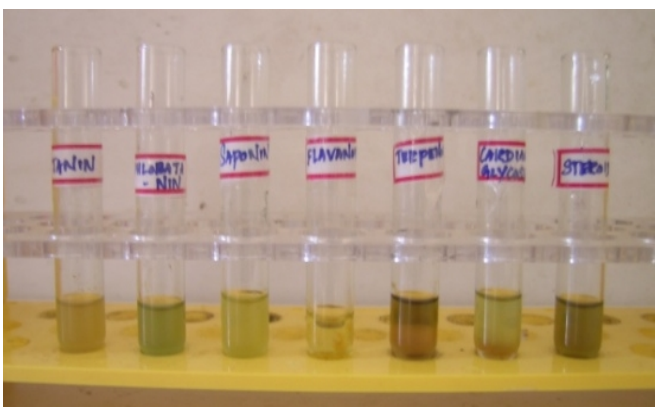

Figure 2: Preliminary phytochemical screening of acetone extract of *Commelina nudiflora*

Figure 3: Preliminary phytochemical screening of ethanolic extract of *Commelina nudiflora*

Table 2: Minimum Inhibitory Concentration of *Commelina nudiflora* against bacteria

Bacteria	Minimum Inhibitory Concentration (mg/ml)			
	Chloroform	Acetone	Ethanol	Streptomycin
Escherichia coli	-	50	100	6.25
Staphylococcus aureus	-	50	50	50
Staphylococcus epidermidis	-	50	50	25
Bacillus subtilis	-	50	50	12.5
Klebsiella pneumoniae	-	50	50	50
Vibrio parahaemolyticus	-	100	50	25

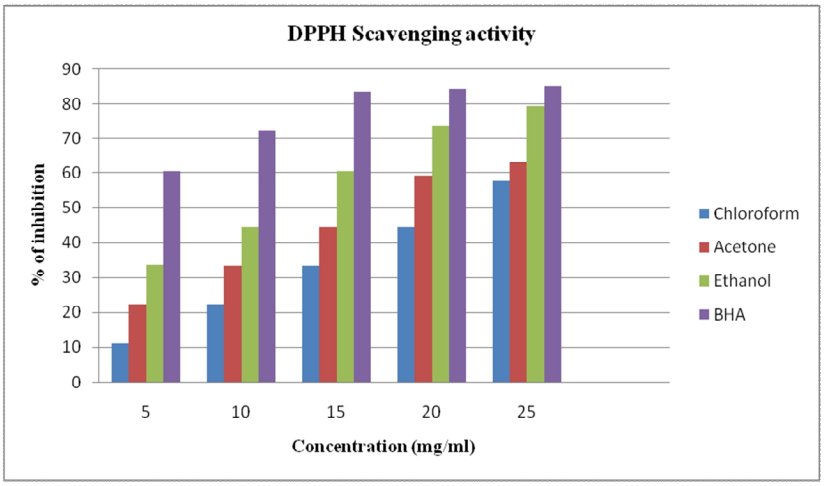

Figure 4: DPPH Scavenging effect of *Commelina nudiflora* extracts

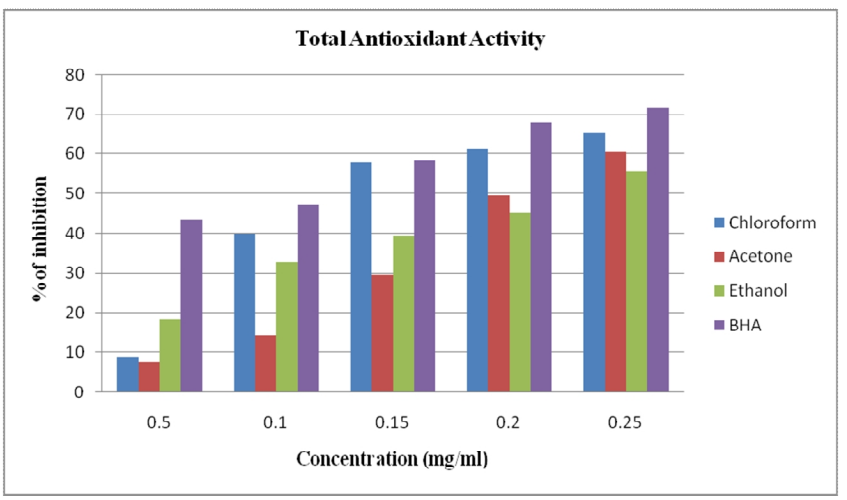

Figure 5: Total antioxidant activity of *Commelina nudiflora* extracts

Antibacterial assay (MIC)

The acetone and ethanolic extracts showed antibacterial activity. There was no inhibition with chloroform extract. The Minimum Inhibitory Concentration of various extracts of *C. nudiflora* is shown in Table 2.

Antioxidant Assay

The evaluation of anti-radical property of *C. nudiflora* was performed by DPPH radical scavenging assay. The 50 % inhibition of DPPH radical (IC_{50}) by the different plant materials was determined, a lower value would reflect greater antioxidant activity of the sample[13]. The plant C. nudiflora showed antioxidant activity in all extract. The antioxidant activity increased with increase in concentration. Antioxidant activity was decreased in the order of ethanolic extract > acetone extract > chloroform extract. The IC_{50} value of ethanolic extract was 11.25 mg/ml. Acetone extract showed IC50 value of 17.5 mg/ml. Chloroform extract showed comparatively less IC_{50} value of 21.25 mg/ml. DPPH scavenging activity is shown in Figure 4.

Total antioxidant activity

The total antioxidant activity was determined by ammonium thiocyanate method. The chloroform extract showed highest total antioxidant activity. It showed the IC_{50} value of 0.1282 mg/ml. Next to chloroform extract, the acetone extract of *C. nudiflora* showed high total antioxidant activity. It showed IC_{50} value of 0.2037 mg/ml which is greater than ethanolic extract. The ethanolic extract showed IC_{50} value of 0.2219 mg/ ml. The total antioxidant activity is shown in Figure 5.

DISCUSSION

Plants are nature's chemical factories. The present study was investigated the Phytochemicals, antibacterial screening and antioxidant activity of the whole plant of *C. nudiflora*. Sequential extraction method with three different solvents (chloroform, acetone and ethanol extracts) of various polarities were used. All extracts of whole plant of *C. nudiflora* showed the presence of flavonoids. Flavonoids play a major role in many degenerative diseases. This plant also has significant antioxidant activity in all extracts. The ethanolic extract of *C. nudiflora* is potentially a good source of antioxidant compounds. High antioxidant activity might be due to the presence of flavonoids in the extracts. This clearly states that *Commelina nudiflora* has potential antioxidant bio molecule that can act as a drug or therapeutic agent for various dreadful diseases. Further investigation is needed to isolate and identify the antioxidant compounds present in *Commelina nudiflora*.

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