ORIGINAL ARTICLE

Investigation on antioxidant and antimicrobial properties of methanolic extract of *Combretum indicum* leaf

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Abstract

Aim: Study with the medicinal plant is growing to be familiar with the value in a healthy life. A traditional medicinal plant *Combretum indicum* leaf was selected to investigate its antioxidant and antimicrobial potential. **Materials and Methods:** The leaf extract was prepared with methanol solvent. Antioxidant potentiality was assessed by free radical scavenging assay with 2,2-diphenyl-1-picrylhydrazyl (DPPH) using ascorbic acid (AA) as standard and reducing power capacity was examined by the development of Perl's Prussian method with AA as standard. Antimicrobial activity was investigated by the method of disc diffusion with standard antibiotic Kanamycin. **Results:** The antioxidant activity in DPPH radical scavenging assay showed that the plant extract exhibits a dose-dependent scavenging of DPPH with IC_{50} 48.87 µg/ml while in standard AA showed an IC_{50} value of 21.14 µg/ml. In reducing power capacity assay, the extract was put on view moderate action in a dose-dependent way. In antimicrobial assessment, it was observed that the extract was active against a number of the Gram-positive microorganisms and Gram-negative microorganisms exercised in the study. Kanamycin was engaged as standard, which demonstrated very high action compared to the extract. **Conclusion:** The observed therapeutic potentiality encourages further in-depth investigation to understand and explain possible mechanisms of action of *C. indicum* extract as well as to consider this plant as a potential source of alternative medicines.

Key words: 2,2-diphenyl-1-picrylhydrazyl, antimicrobial activity, antioxidant, *Combretum indicum* leaf, methanolic extract

INTRODUCTION

The plant has been exercised for medicinal function long before the early period. Conventionally, plant parts are widely practiced on many accounts. Population rise, high-price of treatments, the small role of drugs, increasing of resistance to drugs for a particular disease, and side effects of certain synthetic drugs; these highlight on the use of plant parts considering as medicines. Treatment with the medicinal plant is well-thought-out very safe as there are no or minimal side effects. These remedies are found from nature, which is a principal benefit.[1] The primary fact is that the use of herbal treatments is independent of any age.[2] Medicinal plant is wealthy resources that can be used in therapy as either pharmacopeial, non-pharmacopeial, or synthetic drugs.[3]

C. indicum is known as the Basantilata in Bengali and Rangoon Creeper in English.[4,5] This plant is originated in many parts of the world, especially in Asia.[6-8] Conventionally, crude plant extracts are used as herbal medicine to treat infectious diseases.[9,10] The juice from the leaves is used to heal boils and ulcers and to treat ringworm infection and fever.[11] *C. indicum* is rich in a variety of phytochemicals, including

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quisqualic acid,[12,13] myristic acid, arachidonic acid,[14] flavonoids glycoside,[15] alkaloids, pelargonidin 3- glucoside, rutin,[16] tannins, palmitic acid, diphenylpropanoids, stearic acid, kaempferol, 1, 2, 4-oxadiazolidin-3, oleic acid, 5-dione derivative, methylursolate, α-xylofuranosyluracil, triterpenoids, arjunolic acid, dihydrocucurbitacin and 25-o-acetyl-23-24-dihydrocucurbitacin, and linoleic acid. These constituents function various pharmacological activities such as antibacterial, antifungal, antioxidant, and anti-inflammatory.[16,17]

MATERIALS AND METHODS

Collection and Identification of Plant

The fresh green leaves of *C. indicum* Linn. were collected from Dhaka, Bangladesh, during the month of April 2017. Immediately after collection, the plant was identified and authenticated by the expert taxonomist Dr. Syedur Rahman from Bangladesh National Herbarium, Dhaka, Bangladesh, and a specimen was preserved for future reference. The accession number of the plant is 45180.

Preparation of Plant Extract

The leaves were washed thoroughly with water, and then dried in the shade at 21–30°C for 10 days. This was done to avoid the damaging of heat labile components. Later, the materials were dried in an oven at a low temperature to facilitate grinding. Afterward, the dried leaves were crushed using a mechanical grinder and then passed through a size 100 mesh screen to obtain a coarse powder. The coarse powder of the leaves (500 g) was put in a clean flask (5L) and soaked in 4 L of methanol for 15 days at room temperature to get the methanolic extract. The soaking was accompanied by occasional shaking and stirring for uniform mixing. The mixture was then filtered through a cotton plug, followed by a Whatman no. 1 filter paper. Finally, the obtained filtrate was evaporated to dryness in a Heidolph rotary evaporator at 45°C to get a concentrated extract.[18]

Source of Microorganisms

This study was confined to eight bacterial strains, which include four Gram-positive and four Gram-negative bacteria. These were collected as pure culture from the Institution of Nutrition and Food Science, University of Dhaka. The bacterial strains were grown in nutrient broth medium at pH 7.0 and incubated overnight at 37°C.

Antioxidant Activity Assay

Determination of total antioxidant capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method

The DPPH free radical was used to evaluate the free radical scavenging capacity of antioxidant. DPPH free radical was reduced to the analogous hydrazine when it reacted with hydrogen donors.[19,20] Color changed from purple to yellow; then, the absorbance was decreased because of the DPPH scavenging by an antioxidant.[21] A hundred microliters of each fraction of extract solution and standard ascorbic acid (AA) in different concentrations were taken in test tubes.[22] Three milliliters of a 0.004% methanol solution of DPPH were added in each test tubes, then test tubes were put into an incubator for 30 min to complete reaction.[23,24] Then, at 517 nm, the absorbance was measured for the solutions using a spectrophotometer using methanol as blank.

The formula calculated the percentage (%) of inhibition activity-

$$\%I = \{(A_o - A_1)/A_o\} \times 100$$

A_0 is the absorbance of the blank, and A_1 is the absorbance of the sample.

Then, % inhibitions were plotted against concentration, and IC_{50} was calculated from the graph.[25,26]

Reducing Power capacity assessment

For the qualitative evaluation of the antioxidant capacity of plant extract, ferric reducing power capacity assay has been widely used in the phytochemical research. The test solution changes color from yellow to various shades of blue and green, depending on the antioxidant samples reducing power.[27] The presence of antioxidant substances as a reducing agent in the antioxidant samples causes the reduction where resulted in ferrous from Fe^{3+}/ferricyanide complex. Therefore, by measuring the formation of Perl's Prussian blue Fe^{2+} was monitored.[28] 2.0 ml of prepared each solution of different concentrations of plant extract or else standard were put in separate test tubes, and 2.5 ml of potassium ferricyanide 1% solution was mixed into each test tube.[29] The test tubes were moved to incubate for 10 min at 50°C to finish the reaction, and 2.5 ml of trichloroacetic acid, 10% solution was mixed into each test tubes. At 3000 rpm, the total mixtures were centrifuged for 10 min. 2.5 ml solution of supernatant was withdrawn from each of the mixtures followed by addition with 2.5 ml of distilled water. 0.5 ml of ferric chloride, 0.1% solution were mixed to each test tubes.[30] The absorbance of solutions was measured at 700 nm using a spectrophotometer against distilled water as blank. A typical blank solution contains the same solution mixer without standard and plant extract.[31] At 700 nm, the absorbance was measured of the blank solution against the solvent of the solution preparation. Increased absorbance of a reaction mixer specifies increased reducing power.[32]

Antimicrobial Activity Test

Disk diffusion test

The antibacterial activity of the crude leaf extracts (methanol) of *C. indicum* against four Gram-positive, as well as four

Gram-negative bacterial strains, was evaluated by the disk diffusion method.[33,34] The bacterium was cultured in individual Petri dishes using a test tube containing nutrient agar. The crude was dissolved in methanol, and the disks were placed in the watch glass to absorb the extract. The test sample disk and standard disk were placed with a space in each petri dish containing individual microbe. Petri dishes were placed in the 18–22°C for 2 h; then, all Petri dishes were placed in the incubator for the next 24 h. After 24 h, the results were measured.[35]

RESULTS

Determination of Total Antioxidant Capacity (DPPH)

The crude methanolic extract of *C. indicum* indeed functioned antioxidant property by scavenging of DPPH radical. The percentage of inhibition was gradually increased with concentration for both standard and extract [Figure 1]. In the present study, IC_{50} of the extract is 48.87 µg/ml, whereas the value is 21.14 µg/ml for standard [Table 1].

Determination of reducing power capacity assay

Ferric reducing power capacity assay measures the reducing potential of an antioxidant reaction evaluating Fe^{3+}/Fe^{2+}conversion.[36] In general, the reducing properties are associated with the presence of compounds that exert their action by breaking the free radical chain by donating a hydrogen atom.[37] In the present study, the trend for ferric ion reducing activities of *C. indicum* and the standard (AA) are shown in Figure 2. The absorbance of *C. indicum* increased with increasing concentration. Notably, the absorbance of the extract is higher than the standard in every concentration. It indicates a strong antioxidant capacity of the plant extract in a dose-dependent manner [Table 2].

Evaluation of Antimicrobial Activity

The results of the antimicrobial screening of extract of *C. indicum* have been presented in Table 3. The extract of *C. indicum* showed moderate efficacy against all the microorganisms selected.

DISCUSSION

There has been a dynamic equilibrium between the production of reactive oxygen species (ROS) and the neutralization of these ROS by antioxidants in a biological system. Oxidative

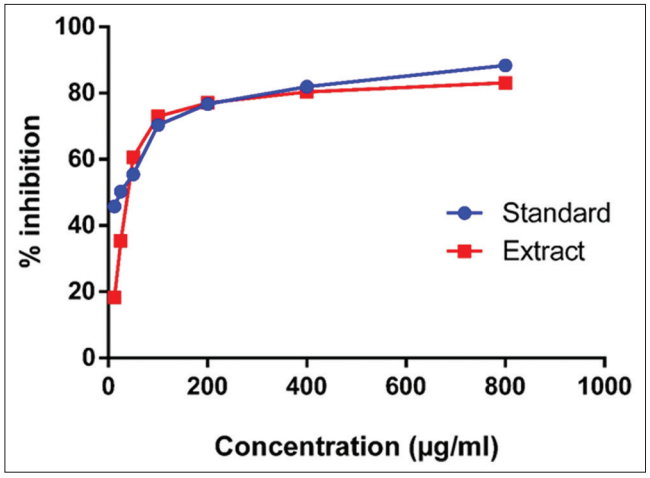

Figure 1: Comparison between % inhibition of standard ascorbic acid and methanolic extract of *Combretum indicum*

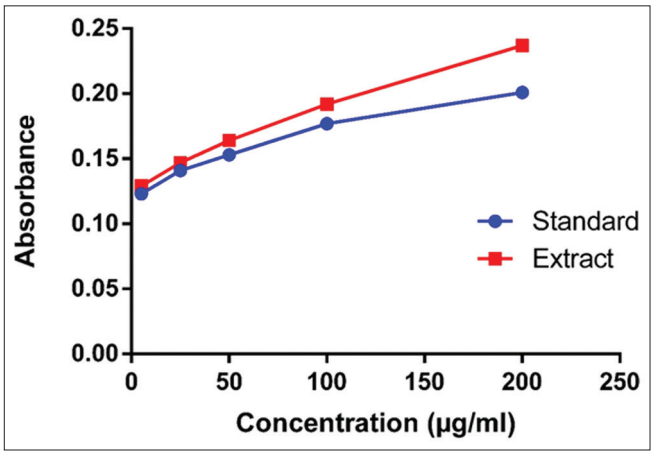

Figure 2: Comparative absorbance between standard (ascorbic acid) versus extract of *C. indicum*

Table 1: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of standard ascorbic acid and the extract

Concentration µg/ml	Ascorbic acid		Methanolic extract of *Combretum indicum*	
	% of inhibition	IC_{50} (µg/ml)	% of inhibition	IC_{50} (µg/ml)
12.5	45.77	21.14	18.25	48.87
25	50.31		35.36	
50	55.46		60.62	
100	70.41		72.99	
200	76.70		77.01	
400	81.96		80.41	
800	88.35		83.09	

Table 2: Reducing power capacity of standard ascorbic acid and methanolic extract of leaf of *Combretum indicum* at different concentrations

Concentration µg/ml	Absorbance of control	Ascorbic acid		Methanolic extract of *Combretum indicum*	
		Absorbance	Corrected value	Absorbance	Corrected value
5	0.122	0.123	0.001	0.129	0.007
25		0.141	0.019	0.147	0.025
50		0.153	0.031	0.164	0.042
100		0.177	0.055	0.192	0.07
200		0.201	0.079	0.237	0.115

Table 3: Antimicrobial screening of extracts of *Combretum indicum*

Name of bacteria	Zone of inhibition (mm)		
	Combretum indicum extract (200 µg/ml)	*Combretum indicum* extract (400 µg/ml)	Kanamycin (50 µg/ml)
Gram positive (+)			
Bacillus subtilis	11	15	26
Bacillus cereus	9	13	21
Sarcina lutea	10	17	28
Staphylococcus aureus	3	4	25
Gram negative (–)			
Shigella dysenteriae	9	14	23
Escherichia coli	13	19	27
Shigella boydii	4	5	22
Vibrio parahaemolyticus	14	18	26

stress occurs when this balance is hampered. Further production of other reactive compounds such as aldehydes and ketones may be initiated by excessive intermediates.[38,39] Antioxidants may work through the enzymatic or non-enzymatic activity. They significantly impede oxidation of oxidizable substrates when present at lower concentrations than the substrate.[32] Plants, however, have been considered and used as a rich source of dietary antioxidants. The observed antioxidant activities can be characterized by the different mechanisms exhibited by different polyphenolic compounds. Polyphenolic compounds include flavonoids, tocopherols, tannins, and other organic acids. It has been well established that these compounds can exert synergistic effects on scavenging free radicals.[40-42] Hence, we could conclude that the presence of flavonoids, including the flavonoids glycoside and tannins or any other polyphenols, could play a key role in the observed antioxidant activity in this study.

The antimicrobial potency of the sample was assessed by their activity to prevent the microbial growth in the media surrounding the discs giving a clear, distinct area defined as a zone of inhibition. It has been well established that crude methanolic extracts are generally predominant with terpenoids, tannins, flavones, phenones, polyphenols, saponins, etc.[43-45] Antimicrobial activity can be obtained through multiple mechanisms of action, including through membrane distortion or separation, enzymatic inhibition during protein synthesis, complexion with a metal ion, complex with cell wall, or binding to adhesion.[46,47] It is expected that the observed antimicrobial activity is the result of the single or combination of these processes.

CONCLUSION

In this study, the methanolic leaf extract of *C. indicum* showed strong dose-dependent antioxidant activity and mild antimicrobial activity. Earlier reports on the antioxidant activity of *C. indicum* are infrequent in the literature. Therefore, it is tough to compare the current results with that of previous studies. Hence, this can be a novel pharmacological effect of this plant that was not explored before. It also showed the moderate antimicrobial property. Still, more studies are recommended to explore the total pharmacological activity of this plant in various *in vitro* and *in vivo* models. In this current analysis, we carried out established tests to find out whether this plant can be used as a source of alternative medicine. With the antioxidant and antimicrobial properties, *C. indicum* leaf may be an excellent alternative medicine that can be used to cure diseases.

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