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ANTIBACTERIAL AND FREE RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACT OF CRYSTELLA DENATATA (LEAVES)

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Abstract

Antibiotic resistance is getting global treat and also many diseases are associated with oxidative stress caused by free radicals. The present study evaluated the in vitro antioxidant and antibacterial activities of methanol extract of *Crystella denatata*. In vitro antioxidant activities of the plant extract were determined by DPPH scavenging method as compared with standard antioxidants. Extract showed significant radical scavenging activity as IC_{50}.25.27 µg/ml comparable IC_{50} 8.687 µg/ml of standard ascorbic acid. Mild antibacterial activities were comparable with standard drug, Kanamycin. The present study provides evidence that *Crystella denatata* prove to be potent natural antioxidants and could replace synthetic antioxidants. Plants can also be used against pathogenic bacterial strains.

Keywords: *Crystella denatata, antibacterial, free radical, ascorbic acid, kanamycin*

Introduction

In the past, ancient people greatly depended on local plants (flora) for their survival. They experimented on different part of the plants and sometimes some plant extract shows real and beneficial effect e.g. ancient Rhubarb plant which contained anthraquinones and it was used as a lead compound in the design of laxative drug named dantron[1]. A free radical is a molecules or molecular fragments which have one or more unpaired electrons in atomic or molecular orbital [2]. There is evidence that most of the degenerative disease which persecute humanity have their origin in detrimental free radical reaction. The disease caused by free radicals includes atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia, biological ageing [3]. Free radicals are produced in cells as part of normal cellular functions. Therefore excess production of free radical in the body might play a role in much disease. In this case, antioxidants prevent tissue damage caused by free radical through preventing the formation of free radical, scavenging them and promoting their decompositions [4]. Plant contains different compounds which possess antioxidant property. Plant antioxidant are composed of a variety of compounds like ascorbic acid, tocopherols, polyphenolic compounds which exert important function both in plant and human. Flavonoid is another polyphenolic compound which has a capacity to act as antioxidants [5-6]. Recently in "in vitro" test phenolics have been considered powerful antioxidants. It is proved to be more potent antioxidants than vitamin C and E and carotinoids [7-8]. Antibiotic resistant is considered as global health challenge. Globally 3.5% of new cases and 20.5% of previously treated cases are caused by tuberculosis and these strains are resistant to rifampicin and isoniazid [9]. Carbapenem resistant enterobacteriaceae species have isolated recently [10]. As day passes antibiotic resistant species of bacteria are increased. On the contrary new antibiotic are not developed. To face this great thread, plant source may be used to develop new antibiotic.

Methods

Sample collection and extraction

Leaves of *Christella dentata* were collected during March 2017 from Manikganj district, Bangladesh, and taxonomically identified at the Bangladesh National Herbarium (Accession Number43854). The undesirable materials were removed from the plants and sun dried for one week after cutting into small pieces. The dried plants were powdered with a mechanical grinder. The powder was extracted by using 98% methanol and the bottle was stand at room temperature and allowed for stand for 7 days. The extract was filtered off with vacuum filter to remove debris of plant. The filtrate (methanol extract) obtained was evaporated under ceiling fan and in water-bath until dried. After drying, the crude extract was stored at 4˚C in refrigerator.

Phytochemical Screening Methods

Phytochemical studied was carried out for identification of different chemical groups which present as described [11-13].

DPPH radical scavenging assay

Antioxidant activity of the methanol leaves extract was estimated by using stable free radical DPPH (1, 1- Diphenyl-2-Pycrylhydrazyl) quantitatively [14-17]. The potential activity of methanol extract was calculated on the basis of their scavenging activity of stable DPPH radical. At first 25 mg extract was mixed with 25 ml of methanol to prepare 1000 µg/ml solution of extract as stock solution. Then five concentration of sample were prepared by serial dilution method .these concentration were 500, 250, 125, 62.5, 31.25 µg/ml. After that 1 ml. of solution of each concentration was taken into test tubes designed for each concentration. 3 ml. of 0.004% DPPH solution was added to each concentration and kept for 30 minutes at dark place to allow any reaction that is to be occurred. After 30 minutes, absorbance was measured by UV-spectrophotometer at 517 nm. Ascorbic acid was used as reference standard. The percent of inhibition was calculated by using following formula-

$$\%\ of\ inhibition = \frac{blank\ absorbance - sample\ absorbance}{blank\ absorbance} \times 100$$

IC_{50} value was determined from % of inhibition vs. concentration graph which is discussed in result and discussions.

Antimicrobial Activity

Disc Diffusion Assay

Agar diffusion testing method was developed for assaying antimicrobial susceptibility [18]. Sterile blank discs (5mm) were saturated with the test extract at the doses of 500 µg/disc using micropipette. The desired concentration of extract was prepared by using methanol. Blank (control disc) were also prepared by using methanol. Both sample and control discs were dried. Sample containing discs, standard antibiotic discs (Kanamycin 30 µg/disc, Oxoid Ltd, UK) and control discs were placed in Petri dishes containing nutrient agar medium seeded with the test pathogens using sterile forceps. Then Petri dishes were transferred into incubator and incubated at 37 °C for 20 h. After incubation, zone of inhibition was measured using digital slide calliper [19-20].

Results

Phytochemical screening

DPPH scavenging assay

Anti-bacterial test

Discussion

The excessive use of antibiotic was contributed to the spread of antibiotic resistant bacteria in communities all over the world. Medicinal plant may be used to develop antibiotic against multidrug resistant bacteria [21]. Again antioxidant are prominent as prophylactic for certain disease like cancer, diabetes, cardiovascular disease, neurodegenerative disorders. These agents scavenge the reactive oxygenated species and prevent damage caused by them [22]. In the present investigation, extracts of *C. dentata* was evaluated for investigation of antimicrobial activity against some gram negative and gram positive bacteria.

The antioxidant activity of methanolic extract of *C. dentata* was evaluated by using standard DPPH free radical scavenging method. The IC_{50} of the *C. dentata* was 25.27µg/ml, whereas IC_{50} of Ascorbic Acid as a standard was 8.687 µg/ml. The extract showed antibacterial activity against all the tested pathogen at the doses of 500 µg/disc compare with standard kanamycin at the dose 30 µg/disc which is shown at the table 5 with zone of inhibition. Our findings confirm that significant antioxidant activity and modest antimicrobial activity.

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Table 1: phytochemical tests of *C.dentata*

Tested groups	**Methanol extract of *C. dentata***
Alkaloid	+
Reducing Sugar group	+
Flavonoids	+
Saponin	+
Tannin	+

(+) indicates the presence.

Table 2: % of scavenging by Ascorbic acid and extract *C. dentata*.

Concentration	% of DPPH scavenging by Ascorbic acid	% of DPPH scavenging by *C. dentata*
500	86.86	90.87
250	80.60	82.80
125	75.78	74.21
62.5	69.25	63.157
31.25	60.55	51.75

Table 3: IC_{50} values of the extracts of *C.dentata* and standard.

Test sample	Regression line	R^2 value	IC_{50} (µg/ml)
Ascorbic acid	y = 9.2289ln(x) + 30.048	0.9891	8.687
Crystella dentata	y = 14.123ln(x) + 4.3679	0.9936	25.27

Table 4: Antibacterial activity of *C. dentata* in disk diffusion assay.

Bacterial strains	Type of bacteria	Diameter of zone of inhibition		
		Blank	Kanamycin(30µg/disc)	Extract (500 µg/disc)
Escherichia coli	Gram (-)	-	34 mm	8 mm
Salmonella typhi	Gram (-)	-	40 mm	8 mm
Staphylococcus epidermis	Gram (+)	-	32 mm	-
Bacillus subtilis	Gram (+)	-	38 mm	9 mm

Figure 1: Anti-oxidant activity of ascorbic acid and extract.