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Evaluation of Antioxidant Potentiality of Methanolic and Aqueous Extracts of *Pandanus foetidus* R. Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Author Nurunnahar designed the study and performed the experimental analysis. Authors RZ and MBI managed the literature searches. Author MUH performed the statistical analysis, wrote the first draft of the manuscript and managed the final submission of the manuscript. Author AM managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Plants are the natural source of antioxidants take part a vital role in the treatment of many age-related diseases and improvement of public health. Therefore the present study was carried out to assess the antioxidant activity of the methanolic and aqueous extracts of *Pandanus foetidus* R. leaves.
Materials and Methods: Total phenolic and flavonoids content, total antioxidant activity and scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were used to evaluate antioxidant efficacy of methanolic and aqueous extracts of *Pandanus foetidus* R. leaves.
Results: The total phenolic contents of the methanol and aqueous leaf extracts (CME & CAE)

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were found to be 39.75±4.56 mg of GAE/g and 45.75±5.69 mg of GAE/g, whereas total flavonoid content in CME and CAE was 295.27±6.29 and 323.25±8.12 mg/g of catechin, respectively. In DPPH, CAE and CME showed moderate antioxidant potentiality with the IC_{50} value of 90.40 µg/ml and 115.60 µg/ml respectively. The total antioxidant capacity was found to be higher in CME (200.4 ± 8.96 mg/g of ascorbic acid) than in CAE (180.21 ± 7.38 mg/g of ascorbic acid).

Conclusion: This study reveals that, both methanolic and aqueous extracts of *Pandanus foetidus* leaves could be a potential source of natural antioxidant which could be used to prevent diseases associated with free radical.

Keywords: Antioxidant activity; free radical; DPPH; phenolic content; flavonoids content; Pandanus foetidus.

1. INTRODUCTION

Endogenous free radicals such as superoxide, nitric oxide and hydroxyl free radicals are produced in the human body during the metabolic process. However, oxidative stress is generated in individuals when these free radical generations is high as a result of a depletion of antioxidant levels; which is an important factor for various diseases such as inflammation, cancer, atherosclerosis and coronary heart disease [1,2]. This oxidative stress can be balanced with the supplementation of antioxidants. Medicinal plants could be an excellent source of natural antioxidants. Based on their diverse composition of various secondary metabolites, medicinal plants could be an excellent source of natural antioxidants which are used to prevent or cure disease, or to promote general health and well-being [3]. For a past few decades researchers have surveyed for potent and cost-effective natural antioxidants from various plant sources instead of synthetic antioxidants [4-7].

Pandanus foetidus Roxb. (Pandanaceae), locally known as kewa kata, Keora, Keurikanta or Kewakanta, a common hedge-plant with no proper stem, grows throughout Bangladesh, mainly in Sundarban known as mangrove forest [8] and Chittagong. Leaves of this plant are used in varieties of diseases such as leprosy, small pox, syphilis, scabies and heart and brain diseases [9,10]. Leaves and spadix are also used in diabetes [10]. The root is considered to be diuretic, depurative, and stimulating [10]. Essential oil of *P. foetidus* is also used as perfumery as well as medicinal sources [11]. Although the plant has promising biological activities but antioxidant activity of it has not been studied yet. So in this study we investigated the antioxidant potentiality of methanolic and aqueous extracts of *P. foetidus* leaves.

2. MATERIALS AND METHODS

2.1 Plant Materials

The leaves of *Pandanus foetidus* were collected from Rajshahi University campus, Bangladesh, in the month of December, 2012. The plant was authenticated by Prof. of Botany Department, University of Rajshahi and a voucher specimen no. 34912 was maintained in our laboratory for future reference.

2.2 Chemicals and Reagents

Ammonium molybdate, Folin-chiocaltu phenol reagent, Methanol, Sodium Phosphate and carrageenan were purchased from E. Merck (Germany). 1,1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, quercetin and potassium ferric cyanide were purchased from Sigma Co. (St. Louis, MO, USA). Diazepam and diclofenac-Na were collected from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals and reagents were of analytical grade.

2.3 Preparation of Plant Extract

The fresh leaves were thoroughly washed with water to remove all contaminants and dried in shade. The leaves were cut into small pieces to make them suitable for grinding and finally dried in an oven at 40-45℃ for 36 hrs. The materials were grinded into coarse powder with the help of a grinder, passing through sieve #40, and stored in a tight container. The dried powder material (3.6 kg) was refluxed with 6 L MeOH and water for three hours. The filtrates were concentrated to dryness, in vacuum at 40℃ to render the MeOH extract (2.1 gm) and aqueous extract (2.8 gm).

2.4 Phytochemical Analysis

Crude extract was subjected for preliminary phytochemical screening by various qualitative

chemical tests using standard procedures for several classes of natural products [12,13].

2.5 Free Radical Scavenging Activity Measured by DPPH

Free radical scavenging abilities of the test samples can be determined by measuring the change in absorbance of DPPH (1,1-Diphenyl-2-picrylhydrazyl radical) at 517 nm by the spectrophotometric method described by Braca et al. 2001 [14]. 0.1 ml of extracts, at various concentrations was added to 3 ml of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percentage inhibition activity was calculated from [(A0–A1)/A0]×100, where A0 is the absorbance of the control and A1 is the absorbance of the extract/ standard. IC_{50} value was calculated from the equation of line obtained by plotting a graph of concentration (µg/ml) versus % inhibition.

2.6 The Amount of Phenolic Compounds and Flavonoids

Plant polyphenols, a diverse group of phenolic compounds possess an ideal structural chemistry for free radical scavenging activity. Total phenolic content of two extracts of *P. foetidus* was determined employing the method as described by Hasan and Azam [15], involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard. The content of total flavonoid in two extracts of *Pandanus foetidus* leaves was determined by aluminum chloride colorimetric method. In this method aluminum chloride ($AlCl_3$) forms complex with hydroxyl groups of flavonoids present in the samples. This complex has the maximum absorbance at 510 nm.

2.7 Determination of Total Antioxidant Capacity

The total antioxidant activity of the extracts can be evaluated by the phosphomolybdenum method according to the procedure of Prieto et al [16] .The assay is based on the reduction of Mo (VI) – Mo (V) by the extract and subsequent formation of green PO_4/Mo (v) complex at acidic PH. Extracts (0.3 ml) were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95℃ for 90 min. Then, the absorbance of the solution was measured at 695 nm using a spectrophotometer (Shimadzu, UV-150-02) against blank after cooling to room temperature. Methanol (0.3 ml) is used as the blank experiment. The antioxidant activity is expressed as the number of equivalents of ascorbic acid using the following formula: C = (c×V)/m, where, C: total antioxidant activity, mg/g plant extract, in ascorbic acid; c: the concentration of ascorbic acid established from the calibration curve, mg/ml; V: the volume of extract, ml; m: the weight of pure plant extract, g.

2.8 Statistical Analysis

All values were expressed as the mean ± SEM of three replicate experiments. The analysis was performed using SPSS statistical package for WINDOWS (version 15.0; SPSS Inc, Chicago).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The crude methanolic and aqueous extracts were qualitatively tested for phytochemical constituents and the results revealed that both the extracts contained carbohydrates, saponins, tannins, glycosides, steroids, alkaloids and flavonoids.

3.2 *In vitro* Antioxidant Assays

3.2.1 DPPH radical scavenging assay

DPPH antioxidant assay is based on the ability of 1,1 diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The results of DPPH radical scavenging assays on different extracts and standard (ascorbic acid) are shown in Table 1 and IC_{50} value of the extracts are presented in Fig. 1. The IC_{50} values of CME and CAE were 115.60 µg/ml and 90.40 µg/ml, respectively. The IC_{50} value of standard (ascorbic acid) was 10.15 µg/ml. So between the two extracts, crude aqueous extract showed higher radical scavenging activity (90.40 µg/ml) which was also very low compare to standard (ascorbic acid). The smaller the IC_{50} values, the higher is the antioxidant activity of the plant extract.

3.2.2 Total phenolic and flavonoid content

The total phenolic content of methanol and aqueous extract of *Pandanus foetidus* leaves was determined using Folin-Ciocalteu reagent.

Phenolic content of the samples were calculated on the basis of the standard curve for gallic acid as shown in Table 2. The results were expressed as mg of gallic acid equivalent (GAE/gm) of extract. CAE possesses the higher phenolic content between two extracts, at 200 µg/ml. Phenols are the most important plant constituents because of their scavenging ability due to their hydroxyl group. Phenolic content in extract varies according to the polarity of the solvents used in this study. Therefore, higher phenolic components present in CAE may contribute directly to its antioxidant activity.

Table 1. DPPH radical scavenging activity of ascorbic acid, CAE and CME of *Pandanus foetidus* leaves

Name of sample	% of scavenging mean ± STD	IC_{50} (µg/ml)
Ascorbic acid	18.12 ± 2.88 74.45 ± 2.5 85.75 ± 3.93 93.66 ± 5.06 15.01± 2.96	10.15
Aqueous extract	25.12±3.15 36.45 ± 3.33 54.46 ± 4.07 85.99 ± 2.01	90.40
Methanol extract	24.45 ± 3.16 24.25 ± 3.33 25.12 ± 1.30 42.11 ± 1.21 75.58 ±2.5	115.60

Table 2. Determination of total phenolic content of the crude methanol and aqueous extracts of (CME and CAE) of *Pandanus foetidus* leaves

Sample	Dose	Total phenol mg/g equivalent of Gallic acid
Methanol extract	200	39.75±4.56
Aqueous extract	200	45.75±5.69

Flavonoids are large class of benzo-pyrone derivatives, ubiquitous in plants exhibit antioxidant activities. Total flavonoids content of crude methanol extract and aqueous extract were determined using much known aluminum chloride colorimetric method. Flavonoid content of the samples was calculated on the basis of the standard curve for catechin as shown in Table 3. The results are expressed as mg of catechin equivalent (CE)/gm of extracts. The total flavonoid content in CME and CAE were 295.27 ± 6.29 and 323.25 ± 8.12 mg/g of catechin, respectively. The result represent that CAE contain higher amount of flavonoids between the extracts. The antiradical property of flavonoids is directed mostly toward hydroxyl superoxide as well as peroxyl and alkoxyl radicals.

Table 3. Determination of total flavonoids content of the crude methanol and aqueous extracts of *Pandanus foetidus*

Sample	Dose µg/ml	Total flavonoid mg/g equivalent of catechin
Methanol extract	250	295.27±6.29
Aqueous extract	250	323.25±8.12

3.2.3 Total antioxidant capacity

The antioxidative effect is mainly due to phenolic components, such as phenolic acids, and phenolic diterpenes [17]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching single and triplet oxygen, or decomposing peroxides [18].

The phosphomolybdenum method is based on the reduction of Mo (V1) to Mo (v) by the antioxidant compound and the formation of green phosphate/ Mo (v) complex with a maximum absorption at 695 nm. Total antioxidant capacity of the plant extracts expressed as the number of gram equivalent of ascorbic acid shown in Table 4. Total antioxidant capacity of CME and CAE were 200.4 ± 8.96 mg/g and 180.21 ± 7.38 mg/g, ascorbic acid equivalent, respectively. So, CME has higher total antioxidant capacity between the tested samples.

Table 4. Determination of total antioxidant capacity of the methanol and aqueous extracts of *Pandanus foetidus*

Sample	Dose µg/ml	Total antioxidant mg/g equivalent of ascorbic acid
Methanol extract	100	200.4 ± 8.96
Aqueous extract	100	180.21 ± 7.38

Free radicals are known to play a definite role in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, alzheimer,

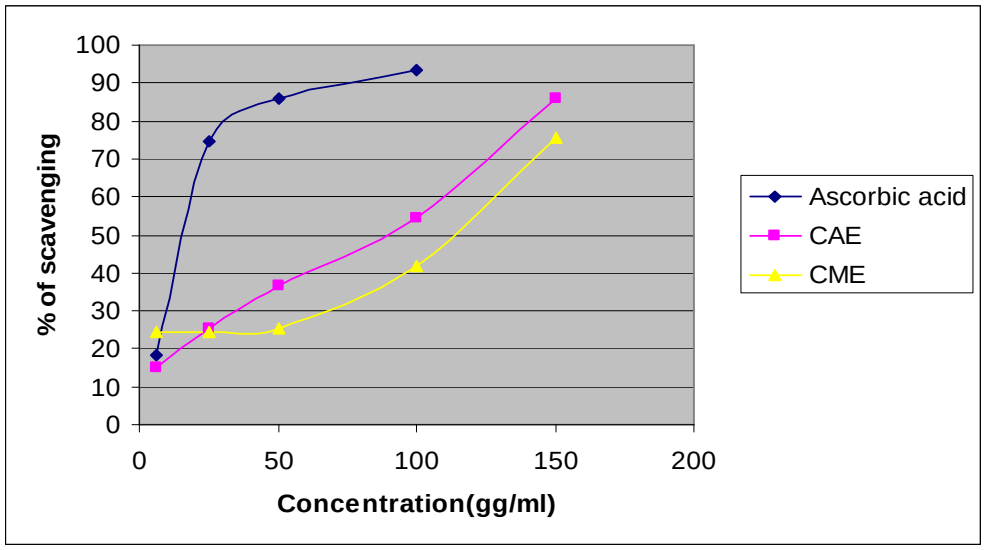

Fig. 1. DPPH radical scavenging activity of *Pandanus foetidus* and ascorbic acid

hepatic damage etc. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. The methanolic and aqueous extracts of *Pandanus foetidus* showed mild 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and total antioxidant capacity. Both the extracts also have considerable tannin, polyphenol and flavonoid content which were responsible for the antioxidant property of the extracts. They are known to hasten the healing of wounds, inflamed mucous membranes and helps in managing oxidative stress [19,20].

In our study we found that the IC_{50} value of the ascorbic acid (standard) and samples CME and CAE were 10.15 µg/ml, 115.60 µg/ml and 90.40 µg/ml respectively. So, after comparison with the ascorbic acid (standard), it is clear that plant extracts possess very mild antiradical activity. Our findings agree with previous studies on scavenging of free radicals [21,22]. Between the two extracts, CAE showed larger radical scavenging activity with IC_{50} value 90.40 µg/ml. CAE possesses the higher phenolic content between the extracts. At 200 µg/ml concentration, the phenolic content was found to be (45.75 ± 5.69 mg/g) in CAE followed by CME (39.75 ± 4.56 mg/g). The total flavonoid contents in CME and CAE were 295.27 ± 6.29 mg/g and 323.25 ± 8.12 mg/g of catechin, respectively. So, it was found that CAE had the higher flavonoid content between the extracts. Total antioxidant capacity of CME and CAE were 200.4 ± 8.96 mg/g and 180.21 ± 7.38 mg/g, ascorbic acid equivalents, respectively. So, CME showed higher total antioxidant capacity between the extracts.

4. CONCLUSION

The present results suggest that all the tested plant extracts have moderate antioxidant activity. The phytochemical tests indicated the presence of polyphenol and flavonoids in the crude methanolic and aqueous extracts. Compounds of such classes are known to possess potent antioxidant activity. However, the chemical constituents present in the extract, which are responsible for this activity still have not been reported and need to be investigated.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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