



ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
JMPS 2018; 6(2): 152-158
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Received: 01-01-2018
Accepted: 04-02-2018

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Evaluation of phytochemicals, antioxidant and antibacterial potentials of *Alpinia calcarata*

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Abstract

Alpinia calcarata Roscoe (Zingiberaceae) has been widely used by indigenous communities in Sri Lanka, India, and Bangladesh as a traditional remedy for indigestion, impurities of blood and respiratory ailments. The present study was conducted to evaluate the phytochemicals, antioxidant and antibacterial activities of methanolic extract of leaves of *A. calcarata*. The total phenolic content (TPC), total flavonoid content (TFC), and total flavonol content (TFIC) were determined using spectrophotometric methods. Phytochemical screening revealed presence of alkaloid, steroid and anthraquinones with considerable amounts of phenolic compounds. To evaluate the antioxidant activities of the plant extract, 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, reducing power activity (RPA) assay and ferric-reducing antioxidant power (FRAP) assay were used. The leaves extract also exhibited remarkable antioxidant potentials as compared to the reference standard- ascorbic acid (AA) and butylated hydroxytoluene (BHT). The disc-diffusion method was conducted to evaluate the antibacterial property; methanolic extract of leaves had no antibacterial (gram-positive and gram-negative) activity. The results suggest that leaves extract of *A. calcarata* could be a potential source of natural antioxidants for use in food, cosmetics and pharmaceuticals industries.

Keywords: *Alpinia calcarata*, phytochemical screening, phenolic compounds, antioxidant activity, antibacterial activity

1. Introduction

Phytochemicals from medicinal plants is believed to be beneficial to human health and help to prevent various diseases such as infectious diseases, inflammation, cancer, diabetic and hypertension [1]. They serve as competitive weapons against bacteria, fungi, amoebae and insects. A number of secondary metabolites originated from medicinal plants are prominent sources of traditional and modern synthetic medicine. According to the World Health Organization (WHO), it is estimated that about 80% population in the developing countries primarily depends on the herbal medicine for primary health care due to a better cultural acceptability, better compatibility with human body and fewer side effects [2].

Phenolic compounds are important natural compounds which have been shown to have a range of bioactivities including antioxidant activity, anti-carcinogenic, anti-atherosclerotic, antibacterial, antiviral, anti-inflammatory activities, etc [3-5]. They also play an important role in the prevention of diabetes, osteoporosis, neurodegenerative diseases and major cardiovascular diseases such as hypertension [6, 7]. Free radicals and reactive oxygen species (super-oxide, hydroxyl and peroxy radicals) originating from endogenous (mitochondria, microsomes) and exogenous (radiation, toxic chemicals, dietary polyunsaturated fatty acids, cigarette smoking and alcohol consumption) sources may cause damage of important macromolecules (nucleic acids, proteins, lipids etc.) and initiate diseases (diabetes, cancer, alzheimers and cardiovascular diseases) [8]. Moreover, the uncertainties and side effects (liver damage and carcinogenesis) of synthetic antioxidant- butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate and *t*-butyl-hydroquinone have already been documented [9]. Antioxidants like flavonoid, phenols, terpenoids, polyphenols, etc., present in medicinal plants play a vital role in maintaining human health by preventing oxidative damage caused by uncontrolled production of reactive oxygen species (ROS) [10]. Therefore, at present strong emphasis has been given to find natural, effective, and safe antioxidants from dietary plants.

Recently, investigation of natural products has gained interest due to potential therapeutic effect against infectious diseases. Plant extracts and their components (alkaloids, tannins, flavonoids and phenolic compounds) have been known to exhibit antifungal and antibacterial properties [11]. Moreover, many researchers have reported that natural substances have shown little toxicity to host cells, whereas synthetic antibiotics have certain disadvantages and side effects [12]. Therefore, there has been increasing interest in finding novel drug candidates from medicinal plants for developing new antimicrobial drugs.

The genus *Alpinia* were found to have analgesic, antimicrobial, and cytotoxic effects, which encouraged us to formulate our hypothesis on *A. calcarata*, presumably because of the interspecies similarities of the biological activities of different plant species. *A. calcarata* (family: Zingiberaceae) is widely distributed in different regions of Bangladesh and also in India and Sri Lanka. This plant is mainly used by various tribal communities (Murong, Chakma, and Tanchangya) of Bangladesh, for various ailments such as a remedy for bronchitis, cough, respiratory ailments, asthma and arthritis [13]. To the best of our knowledge, information concerning *in vitro* antioxidant and antibacterial features of *A. calcarata* has not been found in the literature. So, the present study was intended to examine the preliminary phytochemical screening and to evaluate the antioxidant and antibacterial potentials of the methanolic extract of *A. calcarata* leaves.

2. Methods

2.1. Collection of plant materials

The leaves of *Alpinia calcarata* were collected from the medicinal plant shed of Botanical Garden, Mirpur, Dhaka in the month of May, 2016. The plant was identified by Bangladesh National Herbarium, Mirpur, Dhaka with the accession number of DACB-73889.

2.2. Preparation of plant extract

The collected leaves were rinsed, cut into small pieces and dried under at room temperature for 8 to 10 days. The leaves were ground into a fine powder using electric blender. The powder was then stored in an airtight container and kept in a cool, dark and dry place until required. 30 gm sample of ground plant material was placed in a thimble and extracted with 250 mL of methanol in a Soxhlet apparatus for 6-8 h. The extracts were concentrated under reduced pressure at 40 °C by using a rotary evaporator. The crude extracts were then dried in a freeze dryer, weighed and stored in desiccators until further use.

2.3. Qualitative Phytochemical screening

Phytochemical screening on crude extracts of *A. calcarata* leaves was carried out to determine the presence of alkaloids, cardiac glycosides, steroids, saponins and anthraquinones, according to the methods described below. The detection was carried out based on visual observations of colour change or the formation of precipitates after the addition of specific reagents.

2.3.1. Tests for alkaloids

50 mg of freeze-dried extract of plant material was dissolved in 6 mL of 1% aqueous hydrochloric acid and then filtered. The filtrate was divided into three test tubes and tested with different alkaloidal reagents as follows:

A. Mayer's reagent

One or two drops of Mayer's Reagent were added by the side of the test tube. The formation of white or creamy precipitate shows the presence of alkaloids [14].

B. Dragendorff's reagent

Few drops of Dragendorff's reagent were added and the occurrence of orange-red precipitate indicates the presence of alkaloids [15].

C. Wagner's reagent

Wagner's reagent produces reddish brown precipitate confirming the presence of alkaloids [16].

2.3.2. Tests for cardiac glycosides (Keller-Killiani test)

100 mg of plant extract was dissolved in 2 mL of absolute methanol and this was followed by the addition of 2 mL of glacial acetic acid. Then, one drop of 5% ferric chloride solution and 1 mL of concentrated sulphuric acid were added. The appearance of reddish brown ring at the interface and a bluish green colour in the upper (acetic acid) layer indicate the presence of a deoxysugar characteristic of cardenolides [15].

2.3.3. Tests for Anthraquinone glycosides (Borntrager's test)

100 mg of plant extract was dissolved in 5 mL of chloroform and filtered. The filtrate was then shaken well with equal volume of 10% ammonia solution. The appearance of pink violet or red colour in the ammoniacal layer (lower) indicates the presence of anthraquinones [14].

2.3.4. Tests for Steroids

A. Liebermann-Burchard test

50 mg of the plant extract was dissolved in 2 mL of chloroform followed by the addition of 2 mL of acetic anhydride. The mixture was then boiled and cooled. A few drops of concentrated sulphuric acid was added from the side of the test tube. The development of greenish transient colour indicates the presence of steroids [17].

B. Salkowski Test

50 mg of each extract was dissolved in 2 mL of chloroform. Then, 2 mL of concentrated sulphuric acid was added carefully from the side of the test tube to form a layer. A colour development of reddish brown at the interface indicates the presence of steroids [17].

2.3.5. Test for saponins

100 mg of the plant extract was shaken vigorously in a test tube with 5 mL of distilled water. The formation of characteristic honeycomb like foam which persisted for ten minutes indicates the presence of saponins [14].

2.4. Quantitative determination of phenolic compounds of crude extract

2.4.1. Determination of total phenolic content (TPC)

The TPC of the plant extract was determined using the Folin-Ciocalteu method as described by Meda *et al.*, 2005 [18]. An aliquot 0.5 mL of plant extract (1 mg/mL solution) was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7.5% (w/v) sodium carbonate. After vortexing for 15 seconds, the reaction mixture was incubated at room temperature in the dark for 20 minutes and the absorbance was measured at 765 nm using a UV-Visible spectrophotometer. A standard curve

was plotted using gallic acid as a standard (0-300 ppm). The TPC was calculated using the regression equation from the calibration curve and the results were expressed in mg of gallic acid/g (GAE) of dry weight of samples.

2.4.2. Determination of total flavonoid content

The total flavonoid content was determined according to the method described by Lin and Tang, 2007 [19]. The test samples were dissolved in methanol and 500 µL of this sample solution was mixed with 1.5 mL methanol, 0.1 mL 10% aluminum chloride hexahydrate, 0.1 mL 1M potassium acetate and 2.8 mL distilled water. After 40 min of incubation at room temperature, the absorbance was measured at 415 nm using a UV-visible spectrophotometer. The results were expressed as mg of quercetin equivalents per gram of dry weight of the extract (mg QE/g DW). These values were calculated from a standard calibration curve of quercetin in the concentration range from 0 to 50 µg/mL.

2.4.3. Determination of total flavonol content

The total flavonol content was determined using the method of Kumaran and Karunakaran, 2007 [19]. An aliquot of 1 mL of the plant extract (1 mg/mL) was mixed with 1 mL of aluminium trichloride (20 mg/mL in methanol) and 3 mL of 50 g/L sodium acetate solution. After incubation for 2.5 hours at room temperature, the absorbance was measured at 440 nm. Quercetin, treated in the same manner as the sample, was used to produce a standard calibration curve in the range of 0 to 50 µg/mL. The results were expressed as mg of quercetin equivalent (QE) per gram of dry weight of extract (mg QE/g DW).

2.5. Antioxidant activities of extracts

2.5.1. DPPH radical scavenging activity assay

The DPPH radical scavenging activity of test samples was carried out according to the procedures as described by Ud-Daula *et al.*, 2016 [20]. A solution of DPPH (0.1 mM) was prepared using methanol, and 3 mL of this solution was added to 300 µL of various concentrations (5-500 µg/mL) of methanolic extracts. After 30 min of incubation at ambient temperature, the absorbance was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid and BHT were used as positive controls. The inhibitory concentration (IC) was calculated according to the following equation: $IC (\%) = (A_0 - A_t)/A_0 \times 100$, where A_0 is the absorbance value of blank sample and A_t is the absorbance value of the test samples. Percentage (%) inhibition of both standards and test samples were calculated for each concentration and graphs of % inhibition against concentration were plotted. From these graphs, the concentrations that reduce the absorption of DPPH solution by 50% (IC_{50}) were calculated. A lower IC_{50} value indicates a greater scavenging or antioxidant activity.

2.5.2. Reducing power activity assay

The reducing power of the plant extracts and standards (ascorbic acid and BHT) were measured following the procedure described by Oyaizu, 1986 [21]. An aliquot of various concentrations of plant extract (40-500 µg/mL) was mixed with 2.5 mL of sodium phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was then incubated in a water bath at 50 °C for 20 minutes and then cooled. Reaction was then terminated by adding 2.5 mL of 10% (w/v) trichloroacetic acid. The mixture was centrifuged at 3000 rpm for 10 minutes. 2.5 mL of the supernatant was mixed with 2.5 mL distilled water and 0.5

mL freshly prepared 0.1% (w/v) anhydrous ferric chloride solution. The absorbance was measured at 700 nm against the blank. Higher absorbance values indicate stronger reducing power. The results were expressed as the effective concentration (EC_{50}) values, the concentration of sample which produced an absorbance value of 0.5 and obtained from a linear regression graph of absorbance against extract concentration.

2.5.3. Ferric reducing antioxidant potential

The ferric reducing ability of the plant extracts were determined according to the method described by Benzie & Strain, 1996 [22]. An aliquot of 0.3 mL of various concentrations of plant extract (0-500 µg/mL) was mixed with 3 mL of FRAP reagent. After vortexing for 15 seconds, the reaction mixture was incubated at room temperature in the dark for 5 minutes and the absorbance was measured at 593 nm using a UV-Vis spectrophotometer. The increase in absorbance due to the reduction of ferric to ferrous tripyridyltriazine complex (Fe^{3+} -TPTZ \rightarrow Fe^{2+} -TPTZ) in the presence of the test sample was recorded at 593 nm. The antioxidant potential (EC_{50}) of the sample was calculated from a linear regression analysis and compared with those of the standards, ascorbic acid and BHT.

2.6. Antibacterial activity of extracts

2.6.1. Test microorganisms

A total of four different microorganisms were used in the determination of antibacterial activities of methanolic extracts of *A. calcarata* leaves. Two species of Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 15422) and two species of Gram-positive bacteria (*Micrococcus luteus* ATCC 4698 and *Staphylococcus aureus* ATCC 2592) were used as test microorganisms for antibacterial assays. All of these bacteria were purchased from BioMérieux, Inc. (Durham, North Carolina, USA).

2.6.2. Disc-diffusion assay

Methanolic extracts of *A. calcarata* leaves were subjected to antibacterial test using the disc-diffusion method as described by Sacchetti *et al.*, 2005 [23]. Bacterial stock cultures were prepared by inoculating Mueller Hinton Agar (MHA) plates with commercial bacteriological loops containing the test organisms, and then incubating the plates at 37 °C for 24 h. Microbial suspensions were prepared in nutrient broth for bacteria and incubated at 37 °C for 24 h. The turbidity of each culture was then adjusted to a similar optical density to that of McFarland 0.5. The standardized cultures were inoculated onto agar plates by swabbing uniformly over the entire surface of the medium. Sterile paper discs (6 mm diameter, made from Whatman No. 1 filter) impregnated with 10 µL of plant extract (10-500 µg/mL) or a negative control (methanol or distilled water) were then placed carefully on the surface of the inoculated agar plate with slight pressure. Standard antimicrobial disks (Amicasin, 3µg/disc) were used as positive control. The plates were then incubated at 37 °C for 24 h for bacterial strains. The zone of inhibition (including the disc) was measured in mm as an indication of the antibacterial activity.

2.7. Statistics and calculations

In this study, the results were expressed as mean \pm standard deviation of at least three replicates. Statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS, IBM Corp., Armonk, NY, USA). Tukey's pairwise

comparison (HSD) and least significant difference (LSD) tests were performed to analyze variance and determine significant differences among mean values ($P < 0.05$).

3. Result and Discussion

3.1. Phytochemical screening

The results of qualitative phytochemical screening are summarized in Table 1. These chemical tests indicated the presence of alkaloids, steroids, saponin and anthraquinone. Similar compositions of secondary metabolites have been reported for leaves of *Alpinia nigra* [24], *Alpinia purpurata* [25] and rhizomes of *Alpinia officinarum* [26].

Table 1: Phytochemical screening of methanolic extracts of *Alpinia calcarata* leaves.

Phytoconstituent	Test performed	Methanolic extract of Leaves
Alkaloids	Mayer's reagent	+
	Wagner's reagent	+
	Dragendoff's reagent	+
Cardiac glycosides	Keller-Killiani Test	-
Steroids	Salkowski's Test	+
	Lieberman-Burchard test	+
Saponins	Frothing test	-
Anthraquinones	Borntrager's test	+

'+' indicates presence of the constituents and '-' indicates absence.

The secondary metabolites which detected in the leaves

extract of *A. calcarata* are used in various industrial and medicinal applications. For example, alkaloids derived from plant sources have a wide range of medicinal properties such as analgesic, antiarrhythmic, antibacterial, antiasthma, cholinomimetic, and vasodilatory [27]. Steroids also have a wide variety of effects that affect the development, activity, and plasticity of the nervous system and modulate pain [28]. Saponins are natural detergents that prevent cholesterol reabsorption and thus reduces blood cholesterol levels in humans. They also have anti-inflammatory, expectorant, immune stimulating, and antineoplastic effects [29]. Anthraquinones are another important plant class of secondary metabolites and their respective glycoside derivatives have been shown to have various biological activities including antioxidant, antitumor, antidiabetic, antiproliferative, cytotoxic, antimicrobial, antifungal and immune-enhancing properties [30, 31].

3.2. Total phenolic, flavonoid, and flavonol contents

The total phenolic content of the leaves extract of *A. calcarata* is expressed as gallic acid equivalent and the total flavonoid content and the total flavonol content of the extract is expressed as quercetin equivalent. The results (Table 2) revealed that the extract of *A. calcarata* leaves contained considerable amount of phenolic compounds (102.33 mg GAE/g DW). However, more total flavonoid content (66.25 mg QE/g DW) was measured than the total flavonol content (34.246 mg QE/g DW) in the leaves extract of *A. calcarata*.

Table 2: Total phenolic, flavonoid, and flavonol contents of methanolic extracts of leaves of *Alpinia calcarata*.

Plant extract	Total phenolic content (mg GAE/g DW) ^a	Total flavonoid content (mg QE/g DW) ^b	Total flavonol content (mg QE/g DW) ^b
Leaves	102.33 ± 0.25	66.25 ± 0.12	34.24 ± 0.16

Values are means ± SD ($n=3$). Within rows, mean values were significantly different ($P < 0.01$; Tukey's HSD and LSD test)

^a Total phenolic content is expressed as mg gallic acid equivalents per g dry weight (mg GAE/g DW).

^b Total flavonoid and flavonol contents are expressed as mg quercetin equivalents per g DW (mg QE/g DW).

Phenolic compounds are a unique category of phytochemicals especially in terms of their health benefiting properties. They have multiple biological effects such as prevention of platelet aggregation and damage of red blood cells [32]. They play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. It has been reported that many medicinal plants show antioxidant properties due to the presence of phenolic compounds which may become a natural remedy for cancer.

Flavonoids (quercetin, kaempferol, catechins, and anthocyanidins) are the most common group of polyphenolic compounds thought to provide health benefits through cell signaling pathways and antioxidant effects [33]. They act as chemical messengers, physiological regulators, and cell cycle inhibitors [3]. They present as coloring pigments may function as protective antioxidants at various levels and also prevents oxidation of lipids. They also prevent various life style and neurodegenerative diseases by acting as strong free radicals scavenger [33]. Flavonols are a class of flavonoids present in a wide variety of fruits and vegetables are also prominent for their antioxidant properties [33].

3.3. Antioxidant activities

Antioxidant activity represents a complex process and cannot be evaluated satisfactorily by a simple antioxidant test.

Therefore, in this study, three different test methods (DPPH, RPA and FRAP) were conducted in order to evaluate the antioxidant activity of the methanolic extract of leaves of *A. calcarata*.

3.3.1. DPPH radical scavenging activity

The DPPH shows their antioxidant activity based on the principle of decolorizing in the presence of free radical scavenger. The color turns purple to yellow is an indication of the reaction in which DPPH radical serves as the oxidizing radical to be reduced by the antioxidant. The change of color is due to the presence of odd electron in DPPH and is also responsible for the absorbance at 517 nm. The methanolic extract of the *A. calcarata* showed antioxidant activity by reacting with DPPH which was reduced to DPPH-H. The degree of antioxidant activity was determined by the discoloration tendency.

In the DPPH assay, radical scavenging potential of the methanolic extract of leaves of *A. calcarata* and two reference standards: ascorbic acid (AA) and BHT are presented in Figure 1A demonstrating a concentration dependent radical scavenging effect. The activity of ascorbic acid (96.74%) was the highest, followed by BHT (93.51%) and leaves (93.37%) (Fig 1A).

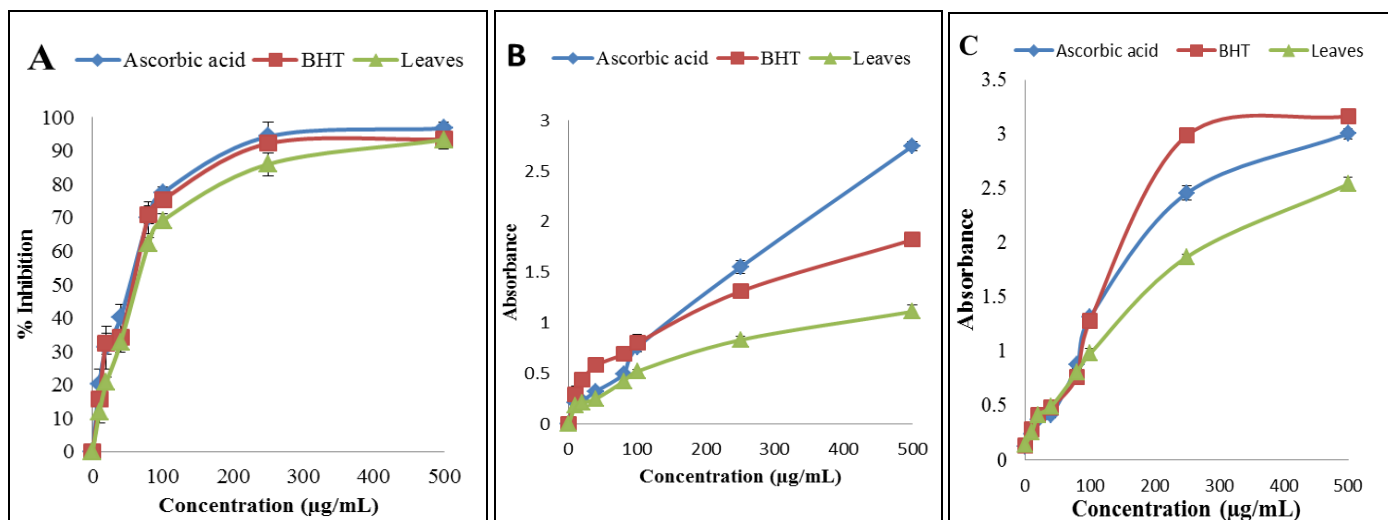


Fig 1: Antioxidant activities of methanolic extracts of *Alpinia calcarata* leaves. (A) DPPH radical scavenging activity, (B) Reducing power activity, and (C) Ferric-reducing antioxidant power.

The IC_{50} values of leaves extract, AA and BHT are shown in Table 3. The leaf extract of *A. calcarata* ($IC_{50} = 142.84 \mu\text{g/mL}$) showed moderate antioxidant activity as compared with those of the standards, ascorbic acid ($IC_{50} = 102.12$

$\mu\text{g/mL}$) and BHT ($IC_{50} = 114.05 \mu\text{g/mL}$). A lower IC_{50} value indicates stronger DPPH scavenging activity, while a higher IC_{50} value indicates a lower scavenging activity.

Table 3: Antioxidant activities of methanolic extract of leaves of *Alpinia calcarata* in DPPH, RPA, and FRAP assays.

Plant part	IC_{50} in DPPH radical scavenging assay ($\mu\text{g/mL}$)	EC_{50} in reducing power assay ($\mu\text{g/mL}$)	EC_{50} in ferric reducing assay ($\mu\text{g/mL}$)
Leaves	142.84 ± 1.28	$150.95^a \pm 5.09$	34.95 ± 0.09
Ascorbic Acid	$102.12^a \pm 2.40$	$70.74^a \pm 3.44$	$25.25^a \pm 0.70$
BHT	$114.05^a \pm 0.63$	$51.56^a \pm 2.03$	$20.30^a \pm 1.69$

Values are mean \pm SD ($n=3$). Within each column and row, means followed by the same superscript letter and number of asterisks are not statistically different ($P < 0.05$; Tukey's HSD).

3.3.2. Reducing power activity assay

The reduction of Fe^{3+} is often used as an indicator of electron-donating activity where the yellow colour of the solution changes to different shades of blue and green depending on the power of a compound to reduce the free radicals. The basic principle is based on the conversion of ferric-ferricyanide complex to prussian blue ferrocyanide complex by the phenols.

Figure 1B shows the dose-response curves for the reducing power of methanolic extracts of *A. calcarata* and reference standards. Both the plant extracts and standards were able to reduce the ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) and the activities of the extracts varied significantly. Reducing power of the plant extracts increased with increasing concentration of the extract which is evident from the absorbance of the reaction mixture at 700 nm. The extract showed some degree of electron donation but lower than that of reference standard, ascorbic acid and BHT (Fig 1B). In this assay, the standards and plant extract could be ranked based on their EC_{50} values, from strongest reducing power to weakest, as follows: BHT ($EC_{50} = 51.56 \mu\text{g/mL}$) > ascorbic acid ($EC_{50} = 70.74 \mu\text{g/mL}$) > leaves ($EC_{50} = 150.94 \mu\text{g/mL}$).

3.3.3. Ferric reducing antioxidant power

The FRAP assay is one of most widely accepted method for evaluating the antioxidant capacity of plant extract. The principle of this method based on the conversion of ferric

tripirydyltriazine (Fe^{3+} -TPTZ) complex to coloured ferrous tripyridyltriazine (Fe^{2+} -TPTZ). The results of FRAP assay for methanolic extracts of leaves of *A. calcarata* and standards are shown in Figure 1C. It was observed that the FRAP activity of extracts of leaves and standard antioxidants (AA and BHT) increased with increasing concentration. However, they were significantly different from each other. In this assay, BHT ($EC_{50} = 20.30 \mu\text{g/mL}$) showed the highest activity followed by ascorbic acid ($EC_{50} = 25.25 \mu\text{g/mL}$) and leaves extract ($EC_{50} = 34.95 \mu\text{g/mL}$) of *A. calcarata*.

3.4. Antimicrobial activities

The disc-diffusion method was used to evaluate the antibacterial activity of the methanolic extract of *A. calcarata* leaves against two Gram-positive and two Gram-negative bacteria. Results of antibacterial activity of crude extracts of *A. calcarata* are shown in Table 4. None of the concentrations of methanolic extract of *A. calcarata* showed antibacterial activity against Gram-positive (*S. aureus* and *Micrococcus luteus*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Similar observation was also reported by [34], where the leaf extracts and fractions of *A. galanga* did not show any antibacterial activity against *S. aureus* and *M. luteus* [35]. *Alpinia katsumadai* seeds also showed resistance against *S. aureus* [36]. Rhizome extracts and fractions of *A. galanga* also had no inhibitory effect on *M. luteus* and *S. aureus* [35].

Table 4: Antimicrobial activity of the methanolic extract of leaves of *Alpinia calcarata*.

Microorganism	Mean inhibition zone (mm)				
	Standard (Amikacin)	Leaves extract			
	3 mg/disc	5 mg/disc	1 mg/disc	0.5 mg/disc	0.25 mg/disc
Gram Negative					
<i>Pseudomonas aeruginosa</i>	23 ± 0.0	-	-	-	-
<i>Escherichia coli</i>	23 ± 0.0	-	-	-	-
Gram Positive					
<i>Micrococcus luteus</i>	19 ± 0.50	-	-	-	-
<i>Staphylococcus aureus</i>	24 ± 0.29	-	-	-	-

Values are means ± SD (n=3).

In contrast to present study, methanolic extract of *Alpinia nigra* leaf showed mild anti-bacterial activity compared to tetracycline (50 mcg/disc) against *S. aureus* and *E. coli* [24]. Hydroalcoholic and methanolic extract of *Alpinia officinarum* rhizome flowers also showed moderate to potent antibacterial activity against the *B. cereus*, *S. aureus*, *P. aeruginosa* and *E. coli* [26]. Methanolic Extracts from *A. galanga* and *A. purpurata* flowers also to have strong activity against *M. luteus* [37].

4. Conclusion

The result of this study shows the presence of potent phytochemicals such as alkaloids, steroids, saponin and anthraquinones in leaves methanolic extracts of *A. calcarata*. The leaves extract of *A. calcarata* also possesses high content of phenolic compounds. This study also revealed that the leaves extracts have moderate antioxidant activity in comparison with reference standard. However, the extract didn't demonstrate any antibacterial activity. Thus, it can be concluded from the above study that its antioxidant properties might be due to the presence of high content of phenolic compounds and other phytochemicals present in this plant. So, the isolation of bioactive constituents from this plant might be a potential alternative for synthetic antioxidant.

5. Acknowledgements

We thank the Research Cell of Noakhali Science and Technology University, Bangladesh, for providing the research grant to perform this research.

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