Khulna University Studies 19(1): 66-73 : 2022

ORIGINAL ARTICLE **LIFE SCIENCE**

ANTIOXIDANT ACTIVITY AND CAPACITY OF SILVER NANOPARTICLES BIOSYNTHESIS OF COMMON FRUITS AQUEOUS EXTRACTS OF THE SUNDARBAN FOREST

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KUS: 22/08: 060222

Manuscript submitted: February 6, 2022 Accepted: May 30, 2022

Abstract

Fruits provide numerous health-promoting polyphenols and antioxidants. The Sundarbans mangrove forest of Bangladesh produces various fruits, and off them ten to twelve are known to be consumed or used as an ingredient in food preparations. These fruits were used in this study to evaluate the total polyphenols (TPH) contents following the method of Folin-Ciocalteu's; and the antioxidant activity by measuring 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging, reducing power and total antioxidant capacity. Additionally, capacity of silver nanoparticles (AgNPs) biosynthesis of the fruits was determined spectrophotometrically. *Sonneratia apetala* fruit showed the highest TPH content (45.7 mg of gallic acid equivalent (GAE)/g powder). The fruit also showed the largest antioxidant activity attributed to the DPPH free radicals scavenging, reducing power, and total antioxidant capacity. Additionally, *S. apetala* fruit showed the highest capacity in the AgNPs biosynthesis (OD, 0.41; 10 mg powder/mL). *Avicennia officinalis*, *Ceriops decandra*, *Heritiera fomes*, and *S. apetala* fruits showed scavenging of 50% DPPH free radicals at 81, 34.7, 61.2, and 33.5 µg powder/mL, respectively. Contents of total polyphenols in these fruits displayed strong positive correlations with reducing power (r^2 = 0.97), total antioxidant capacity (r^2 = 0.85) and scavenging DPPH free radicals (r^2 = 0.85) whereas that for the AgNPs biosynthesis capacity was small (r^2 = 0.32). Thus, the aqueous extract of *S. apetala* fruit is the most potential in antioxidant activity and biosynthesis of AgNPs.

Keywords: Antioxidant, mangrove fruits, polyphenols, silver nanoparticles, the Sundarbans

Introduction

Mangrove forest, the Sundarbans is located on the shoreline of the Bay of Bengal in the south-western region of Bangladesh. The forest is composed of diversified flora and fauna that can grow in the juncture of land and sea with numerous stressful conditions such as muddy soil, high salinity, low nutrients, tidal inundation, plenty of sunlight. The world's mangroves cover about 15 million hectares of land in 100 different countries (FAO, 2003). However, mangrove plants produce a variety of secondary metabolites as defense molecules to adapt to these stressful conditions. The metabolites produced in palatable parts of mangrove plants might be free from

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DOI: https://doi.org/10.53808/KUS.2022.19.01.2208-ls

toxicity with untapped potentials in therapeutic applications. Various recipes are also prepared using mangrove fruits (Brown, 2006). Accordingly, several mangrove fruits produced in the Sundarbans can be processed and consumed. For an instance, *S. apetala* fruits are extensively consumed in cooked, sauce, pickle, toic etc. Hossain et al., (2013; 2016; 2017) reported the phytochemicals and bioactivities of *S. apetala* fruits. Chen et al., (2009) reported that *S. caseolaris* fruit is non-toxic and popularly consumed. *S. caseolaris* fruits are processed to prepare syrup, pudding and cakes (Brown, 2006; Abeywickrama & Jayasooriya, 2011) because of specific flavors, taste and soft texture.

Various diseases in humans are due to the lack of proper amount of phytochemicals in dietary substances. Dietary phytochemicals generate synergistic effects on health promotion. Among the phytochemicals, the leading molecules are antioxidants, which are involved greatly in preventing various diseases. Earlier reports showed antiamylase, antiglucosidase and antiallergic effects of edible fruits in Bangladesh (Hossain et al*.,* 2008). Also antihyperglycemic effects of (+)-catechins were also reported (Hossain et al., 2002). Therefore, it is important to identify potential mangrove fruit(s) as a source of antioxidants for maintaining the public health of coastal people. Moreover, antioxidants are reducing agents, enable them to give up their electrons, and therefore, are used for the biosynthesis of metallic nanoparticles such as silver, gold etc. Biosynthesized silver nanoparticles (AgNPs) showed superior effects to chemically synthesize ones (Ahmed et al., 2016). Tran et al., (2013) showed the uses AgNPs in different fields including environmental, chemical, agricultural and medicinal industries.

This study differs from the previous work of Hosen et al. (2020) in terms of using aqueous extracts of edible mangrove fruits for assaying antioxidant activity. The aqueous extracts were also used to evaluate the biosynthesis potentiality of AgNPs. Then, the relationships between total polyphenols content to antioxidant activity, and also to the ability in AgNPs biosynthesis of the fruits were elucidated.

Materials and Methods

Fruit samples

The mature fruits namely *Aegiceras corniculatum* (L.) Blanco. (black mangrove), *Avicennia officinalis* L. (gray mangrove), *Bruguiera gymnorrhiza* (L.) Lam. (oriental mangrove), *Ceriops decandra* (Griff.) Ding Hou (spurred mangrove), *Heritiera fomes* Buch.-Ham. (sunder), *Nypa fruticans* Wurmb. (nypa palm), *Phoenix paludosa* Roxb. (mangrove date palm), *Sarcolobus globosus* Wall. (sarcolobus), *Sonneratia apetala* Buch.-Ham. (mangrove apple), *S. caseolaris* (L.) Engl. (crabapple mangrove), and *Xylocarpus mekongensis* Pierre (cedar mangrove) were collected from the Sundarbans forest, Bangladesh, from July to September of 2017. After being cut into small pieces, each fruit was shade-dried and then ground into fine powder. The powder was stored tightly in a container at room temperature.

Extraction

Fine powder of each fruit (10 g) was extracted with distilled water (100 mL). The mixture was kept overnight at 30 ^{o}C, 150 rpm, and 20 h. The supernatant of the mixture was collected after centrifugation at 3000 rpm for 10 min. The prepared extract (supernatant) was stored at 4^{o}C in a refrigerator.

Measurement of total polyphenol (TPH)

The quantity of total polyphenol (TPH) in the extracts of the fruits was measured following the method of Folin-Ciocalteu (Ough and Amerine, 1988). Diluted extract (1 mL) was added to 50% Folin-Ciocalteu's reagent (1 mL). Then the mixture was mixed with 1 mL sodium carbonate solution (10% w/v) and incubated at room temperature for 1 h. At 700 nm, the spectrophotometric measurement was done.

Determination of DPPH free radicals scavenging

The extract was mixed with 0.5 mL acetic acid buffer (0.5 M, pH 5.5). Then 1 mL of DPPH in ethanol (0.2 mM) and 1.5 mL ethanol aqueous solution (50%, v/v) were added (Blois, 1958). After mixing, the preparation was incubated at room temperature for 40 min in dark. The remaining DPPH was measured spectrophotometrically (Hach DR6000) at 517 nm.

Determination of the reducing power

The extracts were used to determine the reducing power as described (Oyaizu, 1986). Phosphate buffer (0.2 M, pH 6.6, 2.5 mL) and potassium ferricyanide solution (1%, 2.5 mL) were mixed with the extract. The mixture was incubated at 50^{o}C, 20 min. Then the preparation was mixed with 2.5 mL trichloroacetic acid (10%). The supernatant (2.5 mL) was collected after centrifugation (650 g, 10 min), and mixed with 2.5 mL distilled water followed by 0.5 mL ferric chloride (1%). The spectrophotometric measurement was performed at 700 nm. Ascorbic acid was used as a positive control.

Measurement of total antioxidant capacity (TAC)

The antioxidant capacity of the extracts was measured as described (Prieto et al., 1999). For this, a reagent solution was prepared by mixing 28 mM sodium phosphate, 4 mM ammonium molybdate and 0.6 M sulfuric acid. The extract was added to the reagent solution and incubated for 90 min at 90^{o}C. Then the mixture was cool down to room temperature, and optical density (OD) was examined at 695 nm. The TAC was expressed as mg equivalent of ascorbic acid (mg AAE/g powder) and gallic acid (mg GAE/g powder).

Biosynthesis of silver nanoparticles (AgNPs)

Silver nitrate (1 mM) solution was mixed with the extract and the volume was adjusted to 10 mL. Then the mixture was incubated at 30^{o}C for 20 h at 150 rpm. The colour of the reaction mixture changed to yellowish-brown indicating the production of AgNPs (Lukman et al., 2011). Earlier report showed distinctive character of AgNPs at around 440 nm (Lee & Jun, 2019). Hence, the optical density (OD) of the mixture was taken at 440 nm using a spectrophotometer (Hach DR6000). Each experiment was carried out at least three times and the mean values were calculated.

Statistical analysis

The results were expressed as mean ± SD where n = 3-5. Using SPSS, the analysis of variance (ANOVA) was performed to determine the statistical difference. Significant differences were considered when P values < 0.05.

Results and Discussion

Total polyphenols

The quantity of total polyphenols in the aqueous extracts of the fruits in the Sundarbans ranged from 3.1 to 45.7 mg of GAE/g powder (**Table 1**). Fruits of *S. apetala* showed the highest amount of polyphenols (45.7 mg of GAE/g powder) followed by *C. decandra* (40.7 mg of GAE/g powder) and *A. officinalis* (26.8 mg of GAE/g powder). Among the dietary bioactive components, polyphenols are the largest group that exert various health benefits. Scalbert and Williamson (2000) estimated around 1 g of polyphenols is consumed in a day from fruits, vegetables and beverages. These consumed polyphenols probably have synergistic effects both for physical and mental health. Health-promoting anti-microbial, anti-cancer, anti-diabetic, anti-aging and cardio-protective effects of polyphenols were reported (Pandey & Rizvi, 2009; Daglia, 2012). However among the

edible Bangladeshi fruits, non-mangrove fruit of *Emblica officinalis* showed the highest content of polyphenols (89 mg GAE/g powder) (Alam et al., 2021), which is larger than mangrove fruit of *S. apetala* (46 mg GAE/g powder).

Table 1. Total polyphenols, antioxidant activity and AgNPs biosynthesis of the fruits

Fruits name	Polyphenol (mg GAE/g powder)	DPPH scavenging (% at 0.1 mg powder /mL)	Reducing power (OD) at 1 mg powder/mL	Total antioxidant capacity		AgNPs (OD) at 10 mg powder/mL
				mg GAE/g powder	mg AAE/g powder	
A. corniculatum	18.3±0.9^{f}	60.5±0.9^{e}	0.29±0.03^{c}	24.6±1.2^{d}	9.5±0.4^{d}	0.05±0.01^{b}
A. officinalis	26.8±0.6^{h}	77.8±0.9^{d}	0.47±0.05^{d}	22.9±0.5^{d}	8.9±0.2^{d}	0.02±0.01^{a}
B. gymnorrhiza	5.7±0.1^{b}	21.4±0.7^{a}	0.13±0.01^{b}	7.3±0.3^{a}	2.8±0.1^{a}	0.06±0.03^{b}
C. decandra	40.7±0.7^{i}	91.1±0.6^{f}	0.79±0.02^{e}	29.3±1.7^{e}	11.3±0.6^{e}	0.32±0.02^{e}
H. fomes	22.4±0.6^{g}	85.7±1.2^{e}	0.47±0.01^{d}	25.2±1.4^{d}	9.7±0.5^{d}	0.23±0.02^{d}
N. fruticans	3.1±0.5^{a}	24.4±0.3^{a}	0.04±0.01^{a}	8.3±0.7^{b}	3.2±0.2^{b}	0.11±0.01^{c}
P. paludosa	15.4±0.7^{e}	48.1±1.5^{b}	0.26±0.01^{c}	14.9±0.4^{c}	5.8±0.2^{c}	0.15±0.02^{c}
S. globosus	6.2±0.2^{b}	45.2±2.1^{b}	0.21±0.02^{c}	6.1±0.5^{a}	2.3±0.2^{a}	0.04±0.01^{b}
S. apetala	45.7±0.9^{j}	91.9±0.5^{f}	0.92±0.04^{f}	31.6±0.9^{f}	12.2±0.3^{f}	0.41±0.01^{f}
S. caseolaris	12.1±0.8^{d}	46.4±2.3^{b}	0.21±0.01^{c}	15.4±0.7^{c}	5.9±0.3^{c}	0.33±0.02^{e}
X. mekongensis	9.2±0.8^{c}	24.6±0.8^{a}	0.09±0.01^{a}	7.1±0.2^{a}	2.7±0.1^{a}	0.09±0.01^{c}

Ascorbic acid equivalent, AAE; gallic acid equivalent, GAE; 2, 2-diphenyl-1-picrylhydrazyl, DPPH.
Values represent the mean±SD (n=3-5).
Values with different letters (a-j) within the same column differ significantly (P<0.05).

Antioxidant activity

Aqueous extracts of the mangrove fruits were used to determine the antioxidant activity following the scavenging DPPH free radical, reducing power and TAC (Table 1). At the dose of 0.1 mg powder/mL, the highest (92%) scavenging of DPPH radicals was shown by the fruit, *S. apetala* followed by *C. decandra* (91%), *H. fomes* (85.7%) and *A. officinalis* (77.8%) (Table 1). The increase of DPPH scavenging activity with increasing the concentration of *A. officinalis*, *C. decandra*, *H. fomes* and *S. apetala* were also studied (Figure 1) where positive control was ascorbic acid (AA). Depending on the concentration-dependent curves, the inhibitory concentrations 50 (IC_{50}) of *A. officinalis*, *C. decandra*, *H. fomes* and *S. apetala* fruits for DPPH radicals scavenging were calculated. Fruits of *S. apetala* showed the strongest DPPH scavenging activity with IC_{50} 33.5 µg powder/mL followed by *C. decandra* (IC_{50}, 34.7 µg powder/mL), *H. fomes* (IC_{50}, 61.2 µg powder/mL), and *A. officinalis* (IC_{50}, 81 µg powder/mL) whereas standard ascorbic acid had IC_{50} of 2.2 µg/mL. The IC_{50} means the concentration of fruit (µg powder/mL) at which 50% of total DPPH free radicals is scavenged. Therefore, the smaller IC_{50} value the larger scavenging of DPPH free radicals or antioxidant activity. It has been reported that the hydrogen donating ability of various biomolecules such as cysteine, ascorbic acid, glutathione, tocopherol, aromatic amines and polyhydroxy aromatic compounds reduce and decolorize DPPH (Blois, 1958).

The oxidized ferric iron (Fe^{3+}) is reduced to ferrous iron (Fe^{2+}) with the presence of antioxidant compounds. The potassium ferricyanide reduction method was followed to determine the reducing power of the fruits. It ranged from an optical density (OD) 0.92 to 0.04 at 1 mg powder/mL (Table 1). At that concentration, fruits of *S. apetala* demonstrated the highest reducing power (OD, 0.92) following *C. decandra* (OD, 0.79), and *H. fomes* (OD, 0.47). It is due to their high polyphenols contents, and hydrogen donating capacity as well. Table 1 showed the total antioxidant capacity (TAC) of the fruits as expressed in mg equivalent of ascorbic acid (AAE/g powder), and gallic acid (GAE/g powder). *S. apetala* fruits had the highest (31.6 mg GAE/g powder; 12.2 mg AAE/g powder) antioxidant capacity, which is followed by *C. decandra*. Reportedly, health benefits of dietary substances are associated with TAC. In addition, measurement of TAC is considered to assess diet quality as well as healthy eating because diets with high quality have larger dietary TAC (Salari-Moghaddam et al., 2022).

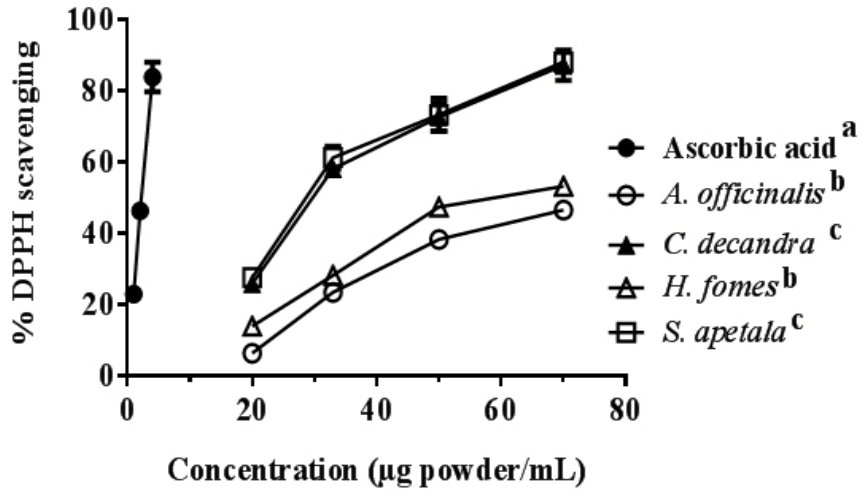

Figure 1. Concentration-dependent DPPH free radicals scavenging activity of the potential fruits.

Biosynthesis of silver nanoparticles (AgNPs)

The Ag^+ in the reaction mixture reduced to AgNPs, which turned the color to yellowish-brown because of surface plasmon resonance (SPR) excitation in metal nanoparticles (Rastogi & Arunachalam, 2011). The formation of yellowish-brown color of the reaction mixture was the indicator of the production of AgNPs. It was recorded by measuring the UV-VIS absorption spectra at 440 nm. Lee & Jun (2019) reported unique character of AgNPs at around 440 nm. The results were expressed in optical density (OD). Among the fruits, *S. apetala* had the highest biosynthesis potentiality of AgNPs (OD, 0.41) at 10 mg powder/mL followed by *S. caseolaris* (OD, 0.33), *C. decandra* (OD, 0.32), *H. fomes* (OD, 0.23) (Table 1). Since *S. apetala* fruit had high reducing power as well as antioxidant activity, it also showed the highest potentiality in the reduction of Ag^+ ions.

Pearson's correlation coefficient

In this study, the highest correlation ($r^2 = 0.97$) was observed between TPH and the reducing power of the fruits. Similar results were also reported for common fruits (Alam et al., 2021) and leafy vegetables (Hossain et al., 2015) in Bangladesh. The total polyphenol of the fruits also exhibited a strong correlation ($r^2 = 0.85$) with total antioxidant capacity, and also with DPPH radical scavenging ($r^2 = 0.82$) (Figure 2). Therefore, the

antioxidant activity of these fruits is highly dependent on the content of total polyphenols. The biosynthesis potential of AgNPs showed a small correlation with total polyphenols ($r^2 = 0.32$), and the reducing power ($r^2 = 0.44$) of the fruits. Hence, not the quantity but the specific type(s) of polyphenols in these fruits was involved in the reduction of Ag for the formation of AgNPs.

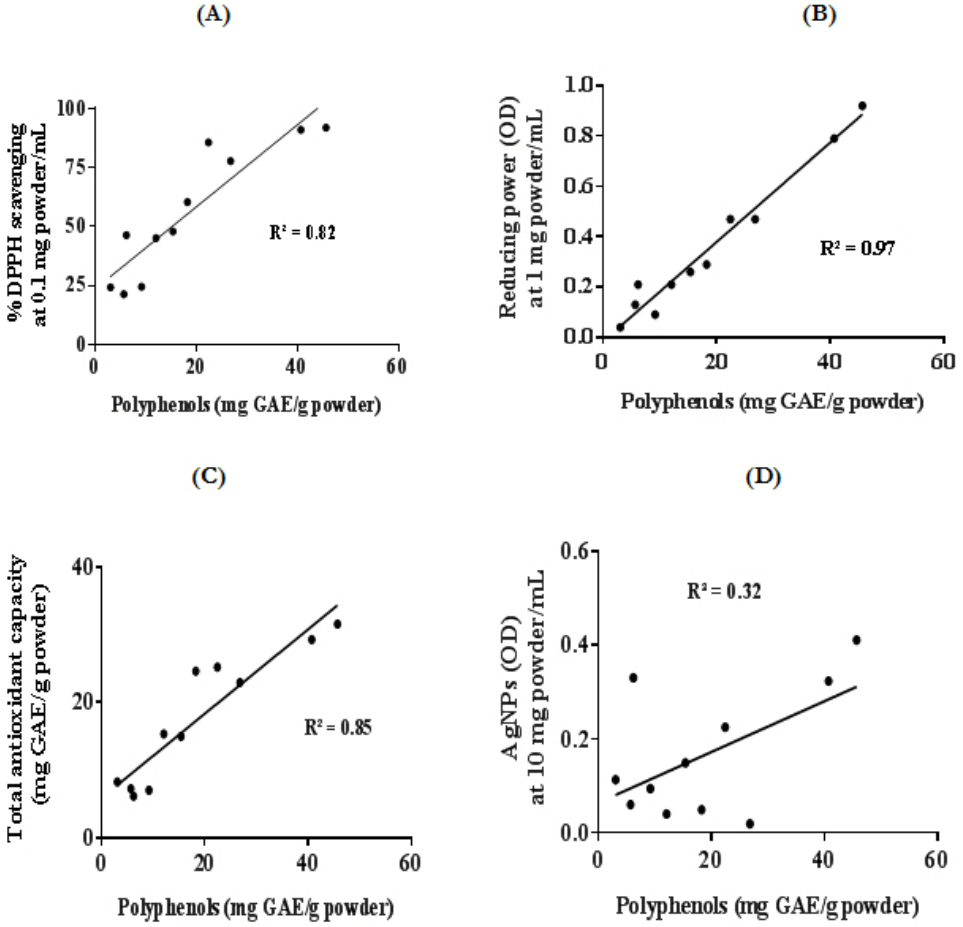

Figure 2. Pearson's correlation of total polyphenols to (A) DPPH scavenging, (B) reducing power, (C) total antioxidant capacity (mg GAE) and (D) AgNPs biosynthesis of the fruits. GAE, gallic acid equivalent.

Conclusion

The present research reveals the high polyphenols content and antioxidant activity of *S. apetala* fruits. The fruit is highly consumed in various forms among the coastal people of Bangladesh without showing any toxicity. Polyphenols as well as antioxidants are known to involve in preventing pathogenesis of various diseases in humans. Therefore, necessary steps should be taken to cultivate the fruit in the salinity prone areas of Bangladesh to promote public health and the environment. In addition, biocompatible AgNPs can be

produced through an eco-friendly, rapid and easy process using the aqueous extract of *S. apetala* fruit. The fruit has the potential to be exploited in nanobiotechnological usages in the future.

Acknowledgment

University Grants Commission of Bangladesh provided the financial support of the research work through grant no. 6(75)/Bio-36/2015, which is acknowledged thankfully.

Author disclosure statement

There is no conflict of interest among the authors.

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