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ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF FOUR UNDERUTILIZED FRUITS OF BANGLADESH

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Abstract

Antioxidant and antibacterial activities of four underutilized fruits namely Jujube (*Ziziphus mauritiana* Lam.), Olive (*Elaeocarpus serratus* L.), Roselle (*Hibiscus sabdariffa* L.), and Taikor (*Garcinia pedunculata* Roxb.) commonly found in Bangladesh were evaluated. The highest total phenolic content (685.08 ± 27.65 mg GAE/100 ml) and total flavonoid content (148.18 ± 0.40 mg QE/100 ml) were found in Taikor extract, which compliance with its highest DPPH scavenging activity and antibacterial properties. It exerted a significantly higher ($p \leq 0.05$) zone of inhibition against *Escerichia coli, Staphylococcus* spp. and *Pseudomonas* spp. than the other experimented fruit extracts. The antioxidant activity was found as Taikor (43.15 ± 1.61%)> Roselle (33.07 ± 1.61%) > Olive (28.68 ± 0.78%) > Jujube (24.81 ± 1.34%). *Bacillus* spp. are found to be more susceptible to the experimented sample extracts. Fruit samples studied may be utilized as potential sources of natural antioxidants and antibacterial compounds, and their extracts may be used as natural biopreservatives to extend the shelf life of foodstuffs.

Introduction

Underutilized fruits, potentially valuable resources to health and nutrition insecurity, are easier to grow and hardy in nature. They are rich sources of nutritional and medicinal values that have remarkable scope in different food and nutraceutical industries (Pal *et al.* 2019). The usage of various food additives, antioxidants, and antibacterial agents is rapidly increasing in Bangladesh. Application of synthetic compounds as food additives for various purposes affects public health including liver damages, carcinogenesis, and other toxicities (Kaur *et al.* 2020). Fruits and vegetables are natural sources of polyphenols and vitamins having antioxidant, antimicrobial, and anticarcinogenic properties (Koley *et al.* 2016). Nowadays, use of medicinal plants and fruit extracts having strong antibacterial and antioxidant potentials greatly increased (Shahbazi *et al.* 2016). Jujube (*Ziziphus mauritiana*) contains a good amount of compounds having antioxidant and antimicrobial properties (Afroz *et al.* 2014). In Bangladesh, about 12 varieties of jujubes are grown (Talukdar *et al.* 2014). Among them, the local sour variety is prevalent and commonly found all over the country. Olive (*Elaeocarpus serratus*) a tropical fruit is rich in flavonoids, condensed tannins, carotenoids, and Vitamin C (Fernando *et al.* 2019). Roselle (*Hibiscus sabdariffa*) is a fruit with dark red outer layer that gives it a distinct appearance. Utilization of its extract may include natural food colorants, emulsions for carbonated drinks, flavoring agents, and herbal medicine (Jung *et al.* 2013, Abdallah 2016). Taikor (*Garcinia pedunculata*) has a juicy interior with edible arils. Extract of this fruit has antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and cardioprotective properties (Mudoi *et al.* 2012, Islam *et al.* 2015). However, few studies have explored the antioxidants and antimicrobial properties of these four underutilized fruits. The knowledge regarding such fruits' antioxidant and

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antibacterial properties is crucial for improving their application to the food and pharmaceutical industries and in contributing to the creation of new products. Thus in the present study was conducted to evaluate the antioxidant and antibacterial activity against *Bacillus* spp., *Staphylococcus* spp., *Escherichia coli, Pseudomonas* spp. of underutilized fruits such as Jujube, Olive, Roselle, and Taikor.

Materials and Methods

Fresh and matured Jujube (local sour variety), Olive, Roselle, and Taikor fruits were collected from Bandar Bazar, Sylhet, Bangladesh. Total phenolic content was measured using the Folin-Ciocalteu's colorimetric assay (Amorim *et al.* 2008). Total flavonoid contents (TFC) was determined using the aluminum chloride colorimetric method (Meda *et al.* 2005). DPPH radical scavenging activity was evaluated according to Brand-Williams *et al*. (1995). Antibacterial activity of fruit extracts was determined by the agar disc-diffusion method followed by Mayachiew and Devahastin (2008). Two gram Positive (*Bacillus* spp. and *Staphylococcus* spp.) and two Gram negative (*Escherichia coli* and *Pseudomonas* spp.) bacteria were collected from the laboratory of Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh to investigate the potential activity of the extracts. Ciprofloxacin was used as the antibiotic for the blank test. The *in vitro* antibacterial property (Minimum Inhibitory Concentration and Minimum Bactericidal Concentration) of the samples was evaluated based on broth micro dilution test (Shahbazi *et al.* 2015). Methanol extracts were dissolved in Nutrient broth containing 5% (v/v) DMSO to reach final concentrations of 10 mg/ml. Next, selected dilutions were made in a concentration ranging between 1-10 mg/ml. Minitab statistical software was used to analyze the variance by the Tukey test. The $p \leq 0.05$ was regarded as statistically significant. Data were expressed as means ± standard deviation (SD) of three independent measurements.

Results and Discussion

Total phenolic content (TPC) and total flavonoid content (TFC), as well as antioxidant activity of Jujube (*Z. mauritiana*), Olive (*E. serratus*), Roselle (*H. sabdariffa*), and Taikor (*G. pedunculata*) are presented in Figs 1-3. Polyphenols are bio-metabolites and a critical key for determining the antioxidant capacity (Abdallah 2016, Fernando *et al.* 2019, Kaur *et al.* 2020). The TPC was found to be highest in Taikor extract (685.08 ± 27.65 mg GAE/100 ml) and lowest in Jujube (421.75 ± 17.5 mg GAE/100 ml) (Fig. 1). Roselle had comparatively higher TPC (539.25 ± 16.39 mg GAE/100 ml) than that of the Olive (440.08 ± 11.81 mg GAE/100 ml).

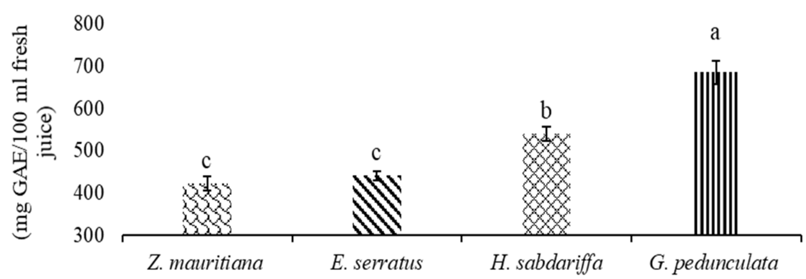

Fig. 1. Total phenolic content (TPC) *Z. mauritiana*, *E. serratus*, *H. sabdariffa*, and *G. pedunculata*. Values are given as mean ± SD. Different letters (a–c) between the columns are significantly different (Tukey's test, $p \leq 0.05$).

TPC of Bangladeshi native sour Jujube type is much higher than that of Indian Jujube and Bangladeshi Apple Kul variant (Afroz *et al.* 2014, Koley *et al.* 2016). These differences in the phenolic composition may depend on some factors such as variety, soil composition, climatic conditions, and light intensity (Krishna and Parashar 2013). Extraction procedure may also significantly influence the total phenolic content (Jiang *et al.* 2019).

Results presented in Fig. 2 showed that total flavonoid contents of the Taikor had a significantly ($p \leq 0.05$) higher amount of flavonoid (148.18 ± 0.40 mg QE/100 ml) than the Roselle (118.87 ± 1.09 mg QE/100 ml), Olive (92.30 ± 0.80 mg QE/100 ml), and Jujube (90.19 ± 0.30 mg QE/100 ml). All the fruits were found to contain higher amounts of flavonoids than another underutilized fruit Carambola (2.56 ± 0.40 mg QE/100 ml) as reported by Shourove *et al.* (2020). Mudoi *et al.* (2012) also reported that the methanolic extract of *G. pedenculata* had contained 71.4 ± 0.84 mg QE/100 g on dry weight basis. Fernando *et al.* (2019) found a significantly higher amount of flavonoids in Olive extract (120.49 QE mg/100 g). The higher amount of phenolic compounds in these fruits contributes to their antioxidant activity, nutritional value, and therapeutic benefits.

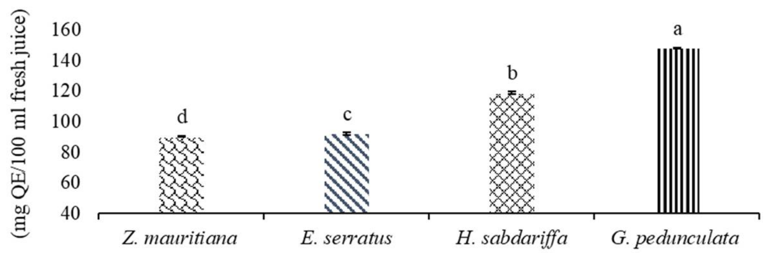

Fig. 2. Total flavonoid content (TFC) of *Z. mauritiana*, *E. serratus*, *H. sabdariffa* and *G. pedunculata*. Values are given as mean± SD. Different letters (a–d) between the columns are significantly different (Tukey's test, $p \leq 0.05$).

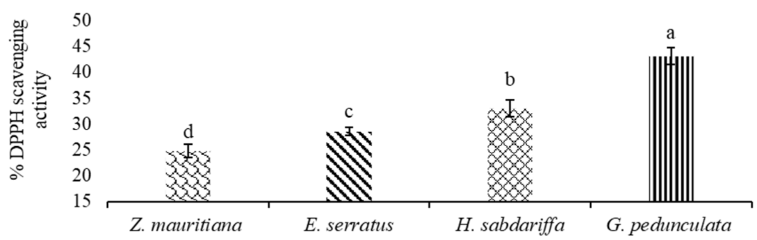

Fig. 3. DPPH radical scavenging activity of *Z. mauritiana*, *E. serratus*, *H. sabdariffa* and *G. pedunculata*. Values are given as mean ± SD. Different letters (a–d) between the columns are significantly different (Tukey's test, $p \leq 0.05$).

Results of DPPH radical scavenging activity of four examined underutilized fruits are presented in Fig. 3 which revealed that the highest and lowest amount of DPPH radical scavenging activity was found in Taikor (43.15 ± 1.61%), and in Jujube (24.81 ± 1.34%), respectively. Taikor exhibited the highest DPPH free radical scavenging activity among all the tested samples supporting its TPC and TFC values. Jayaprakasha *et al.* (2006) reported that the DPPH scavenging activity of Taikor was 60 ± 4.1 and 67 ± 5.1% for hexane and chloroform extract, respectively.

Figure 4 showed the antibacterial activity of the samples that were tested. Maximum zone of inhibition (17.33 ± 1.52 mm) was measured in case of methanolic extract of Taikor among all four fruit samples against the Gram-negative bacteria *E. coli*. The Gram-positive bacteria was found to be more sensitive than the Gram-negative bacteria. This might be due to the difference in their cell wall composition. A controversial result was found from the study of Beg *et al.* (2016), where the higher zone of inhibition of methanolic extract of Jujube was reported against *E. coli.* (ZOI= 11 mm) than the *S. aureus* (ZOI= 8 mm).

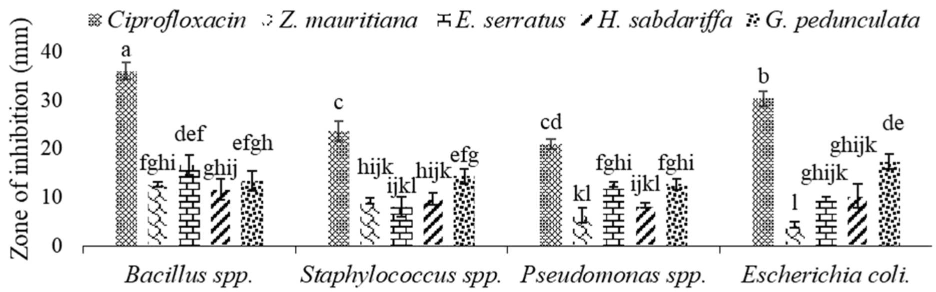

Fig. 4. Zone of inhibition of ciprofloxacin, *Z. mauritiana, E. serratus, H. sabdariffa,* and *G. pedenculata* extract against two Gram-positive and two Gram-negative bacteria. Values are given as mean ± SD. Different letters (a–l) between the columns are significantly different (Tukey's test, $p \leq 0.05$).

The highest inhibition zone was found in case of the methanolic extract of both Olive (ZOI= 16.67 ± 2.08 mm) and Roselle (ZOI = 11.67 ± 2.08 mm) against *Bacillus* spp. The zone of inhibition was found by the Roselle extract against *Bacillus* spp. (ZOI = 11.67 ± 2.08 mm), *E. coli* (ZOI=10.33 ± 2.51 mm), *S. aureus* (9.67 ± 1.15 mm), *Pseudomonas* spp. (8.33 ± 0.57 mm). Jung *et al.* (2013) reported that ethanolic extract of Roselle showed the potent antimicrobial compound at 25 mg/ml concentration, where the zone of inhibition against *B. subtilis* (10.70 ± 0.87 mm), *S. aureus* (18.37 ± 0.60 mm) and *E. coli* (11.73 ± 0.61 mm). Taikor has the maximum antibacterial property showing the highest inhibition zone in Gram-negative bacteria *E. coli.* The highest antibacterial activity (36 ± 1.73 mm) of Ciprofloxacin was observed against *Bacillus* spp.while it was lowest (21±1.00 mm) against *Pseudomonas* spp.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values are presented in Table 1. Sample extracts of different concentrations ranging from 1-10 mg/ml were used to evaluate the MIC and MBC values. Taikor extract showed the highest antibacterial effect against all the experimented bacterial species at lower MIC (6-8 mg/ml) and MBC (7-9 mg/ml) values. The lowest MIC value (5 mg/ml) of the Olive extract was found against Gram-positive bacteria *Bacillus* spp. Roselle showed the highest antimicrobial effect against *Staphylococcus spp.*, where MIC (8 mg/ml) and MBC (9 mg/ml). Fernando *et al.* (2019) reported that the ethanolic extract of Olive had the minimum inhibitory concentration against *E. coli* 1.3 mg/ml and *S. aureus* 2.0 mg/ml. Taikor extract had shown the highest TPC, TFC and antioxidant activity, which may be responsible for its higher antibacterial properties. Potential antimicrobial action of flavonoids is due to the complexation with the soluble extracellular proteins of the bacterial cell wall, which results in the breakage of the cell wall (Fernando *et al.* 2019). The differences in microorganism's susceptibility to antimicrobial agents may be attributed to the outer cytoplasmic membrane surrounding the thin peptidoglycan structure of Gram-negative bacteria (Lv *et al.* 2011).

Table 1. Antibacterial effect of *Z. mauritiana, E. serratus, H. sabdariffa,* and *G. pedunculata* extracts indicated as Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) mg/ml.

Organisms	*Z. mauritiana*		*E. serratus*		*H. sabdariffa*		*G. pedunculata*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Bacillus spp.	10	NA	5	6	NA	NA	7	8
Staphylococcus spp.	NA	NA	10	NA	8	9	6	7
Pseudomonas spp.	NA	NA	10	NA	10	NA	6	7
Escherichia coli	NA	NA	10	NA	NA	NA	8	9

NA: No antibacterial activity was found with the concentrations used in this work.

The highest antioxidant and antimicrobial activity were found in Taikor among all the experimented fruit extracts. All of the fruit extracts are suggested as natural bio preservatives for extending the shelf life of stored food commodities and may be used in lieu of synthetic food additives in various food processing sectors. Further study is needed to optimize the antioxidant and antimicrobial activity of the sample extracts by using novel technology.

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