Polyphenols profile and antioxidant capacity of selected medicinal plants of Bangladesh

Bioresearch Communications
Volume 7, Issue 1, January 2021

Nazma Shaheen[1*], Avonti Basak Tukun[1], Saiful Islam[1], Kazi Turjaun Akhter[1], Md Sujan Hossen[1], and Thingnganing Longvah[2]

[1]Institute of Nutrition and Food Science, University of Dhaka, Dhaka-1000, Bangladesh. [2]National Institute of Nutrition, Hyderabad, Hyderabad-500 007, India.

ABSTRACT: Polyphenols in many plant foods have received a great deal of attention due to their biological potency and postulated protective role against certain non-communicable diseases like cancer, neurodegenerative disease, and cardiovascular disease. The current study focuses on 15 indigenous plants which are traditionally being used for medicinal and other purposes in Bangladesh. Specifically, this study aimed to determine the total phenolic content (TPC), profile the polyphenolic composition, and assess the antioxidant capacity (AC) of water and methanol extracts derived from these plants. The TPC was estimated using the Folin-Ciocalteu method, and DPPH inhibition was used to assess the AC. HPLC-centered simultaneous determination method of polyphenols was employed to identify and quantify individual polyphenolic compounds. TPC content (expressed as mg GAE/100 g FW) ranged from 15.5 (*Zanthoxylum rhetsa*) to 276.9 (*Moringa oleifera*). The lowest IC_{50} in the water and methanol extracts were shown by *Moringa oleifera* and *Zanthoxylum rhetsa* respectively and they also exhibited high free radical scavenging activity in the corresponding solvents (91.9% and 92.9% DPPH inhibition by *Moringa oleifera* and *Zanthoxylum rhetsa* respectively). The lowest DPPH inhibition was observed in *Alternanthera philoxeroides* (29.8%) and *Spilanthes calva* (24.5%) in the water and methanol extracts respectively. Ten polyphenols (coumaric acid, chlorogenic acid, caffeic acid, apigenin, apigenin-7-O-neohesperidoside, quercetin-3-β-D-glucoside, luteolin, quercetin-3-O-β-D-glucopyranoside, isorhamnetin and kaempferol) were initially identified and subsequently quantified in the plant extracts. *Spilanthes calva*, *Moringa oleifera*, *Oxalis corniculata*, and *Piper retrofractum* contained the highest amount of coumaric acid and chlorogenic acid, apigenin, caffeic acid and apigenin-7-O-neohesperidoside respectively. *Senna tora* had the greatest amount of kaempferol, quercetin-3-β-D-glucoside, and isorhamnetin whereas luteolin and quercetin-3-O-β-D-glucopyranoside were observed to be the maximum in *Alternanthera sessilis*. To conclude, *Moringa oleifera* and *Senna tora* with high percentage of DPPH inhibition and low IC_{50} were the most potent antioxidant sources among the analyzed samples.

KEYWORDS: Polyphenol profile, antioxidant capacity, DPPH, medicinal plant, Bangladesh.

RECEIVED: 15 September 2020, ACCEPTED: 12 December 2020

TYPE: Original Research

CORRESPONDING AUTHOR: Dr. Nazma Shaheen, Institute of Nutrition and Food Science, University of Dhaka, Dhaka-1000, Bangladesh.
Email: nzmshaheen58@gmail.com

Introduction

During the last few decades the world has been undergoing rapid dietary, nutrition and epidemiological transition due to industrialization and globalization. Chronic non-communicable diseases (NCDs) such as cardio-vascular diseases, chronic obstructive pulmonary diseases, Alzheimer's disease, cancer, and chronic kidney diseases are now surpassing the prevalence of contagious diseases worldwide which are even more expensive to cure, and majority of the patients die uncured. Interestingly, among the important determinants of NCDs, for instance socio-economic conditions, dietary practices, and lack of physical activity, association with dietary practice of consuming highly processed foods and minimal intake of vegetables and fruits is widely investigated and found to induce damage to organs affected by NCDs (Cencioni et al., 2013; Li et al., 2013; Reuter et al., 2010). Everyday human body produces several types of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical, and superoxide anions which are scavenged by body's scavenging enzyme system before causing significant oxidative stress and inflammation (Cheeseman and Slater, 1993). However, when these ROS are spontaneously produced and circulated in excess amount in our body, could cause damage to cellular components, particularly to lipids, DNA, and proteins, which, in turn, initiates a cascade of inflammation leading to the underlying pathogenesis of the NCDs (Cencioni et al., 2013; Li et al., 2013; Reuter et al., 2010). Vitamins and minerals with antioxidant capacities of commonly consumed foods are known to exhibit antioxidant activity against such free radicals to encounter oxidative damage of cellular components individually or as integral part of various free radical scavenging enzyme systems. Similarly, studies from different regions have provided evidences that many indigenous and underutilized herbaceous foods, traditionally known as medicinal plants, contain a special group of phenolic compounds of varied categories and concentrations which are proved to act as antioxidants through free radical inhibition (Aryal et al., 2019; Basak Tukun et al., 2014; Sukati and Khobjai, 2019; Aali et al., 2018). Traditionally, medicinal plants are also used to avert and manage NCDs, which inspired researchers to investigate this possibility scientifically (Mbikay, 2012). Several studies reported that the plants rich in polyphenols could be potent dietary sources to scavenge ROS, hence, prevent or delay development of NCDs (Koch, 2019). Polyphenols are the most widely studied secondary

metabolites of plants, whose characteristic feature is the presence of multiple hydroxyl (OH) groups attached to the aromatic ring(s). This OH group plays an important role in neutralizing free radicals by donating its hydrogen atom or the electron. However, in the case of polyphenol, transferring a single electron is less common as it requires more energy in the process than transferring a hydrogen atom (Leopoldini et al., 2011). In Bangladesh, very few studies have been conducted regarding polyphenol profiles of locally grown wild and medicinal plants and their antioxidant capacity (Alam et al., 2020; Basak Tukun et al., 2014; Hasan et al., 2009; Jahan et al., 2014). Due to increasing interest in antioxidant capacity of the polyphenols, the present study investigated total polyphenols content (TPC) of fifteen indigenous medicinal plants. In addition, ten individual polyphenolic compounds were quantified in these plants' extracts followed by determination of their *in vitro* antioxidant capacity (AC).

Materials and Methods

Fifteen indigenous medicinal plant species were collected. The study samples with their English, local, and scientific names are provided in Table 1. All the analyzed samples were collected freshly and then processed at the Food Analysis Laboratory of Institute of Nutrition and Food Science, University of Dhaka for further analysis.

Table-1. Identification of the analyzed indigenous medicinal plant species

English name	Local name	Scientific name
Slender amaranth	Notay shak	*Amaranthus viridis*
Creeping wood-sorrel	Amrul shak	*Oxalis corniculata*
Spiny amaranth	Kata-nutia	*Amaranthus spinosus*
Pinkwood sorrel	Golapi Amrul	*Oxalis corymbosa*
Curry leaves	Curry pata	*Murraya koenigii*
Indian ivy-rue	Kantahorina	*Zanthoxylum rhetsa*
Sickle senna	Chakunda	*Senna tora*
Sessile joyweed	Chanchi shak	*Alternanthera sessilis*
Javanese long pepper	Choi	*Piper retrofractum*
Bengal dayflower	Dholpata	*Commelina benghalensis*
Gooseberry	Fotka	*Physalis angulata*
Alligator weed	Malancha shak	*Alternanthera philoxeroides*
Drumstick tree leaves	Sajna	*Moringa oleifera*
Paracress	Surja kannya	*Spilanthes calva*
Indian pennywort	Thankuni	*Centella asiatica*

Sample preparation

The identified edible portion of collected samples were separated immediately. Tender stem, shoots and leaves were taken for *A. viridis, A. philoxeroides, A. sessilis, A. spinosus* and *C. benghalensis* and tender leaves for *S. tora, M. koenigii, S. calva* and *M. oleifera*. Tender stem and leaves for *C. asiatica while the whole* plant except root were taken for *O. corniculata* and *O. corymbosa*. Only stem for *P. retrofractum,* only leaves for *Z. rhetsa* and tender stem, leaves and fruits for *P. angulata* were taken as edible portions. The edible portion of samples were then washed with running tap water followed by distilled water. They were drained completely and air dried over a paper towel. Each sample was then freeze-dried at -180°C, weighed, ground using homogenizer. The ground freeze-dried samples were stored in an air-tight packet at -20°C in the refrigerator until analysis.

Extraction and determination of total phenolic content

Total phenol content (TPC) of indigenous plant samples was determined according to Georgé et al. (2005) using solid phase extraction (SPE) method. Samples were weighed and ground into a fine powder with 70% acetone, stirred for 30 min with a magnetic stirrer and supernatant (i.e., raw extract (RE)) was collected after centrifugation at 5000×g for 7 min. Raw extracts (3 mL) were passed into the SPE–HLB (hydrophilic-lipophilic balance) to recover the washing extract (WE). The different extracts (RE and WE), following addition to each of them of 3.5 mL of water diluted Folin – Ciocalteu reagent, were incubated for 2 min at room temperature. Then, each of the mixtures following addition 2.5 mL of sodium carbonate (75 g/L) was incubated again at 50ºC. After 15 minutes, absorbance was measured at 760 nm. The calibration curve of absorbance vs. concentration of standard (i.e., gallic acid) was used to quantify TPC. Results were expressed as mg gallic

acid equivalents per 100 g fresh weight (FW) of plant sample (mg GAE/ 100 g FW).

Extraction and quantification of *in vitro* AC

For measuring *in vitro* AC using the Blois (1958) method, methanol and water extracts were used. Each fraction (50 µL) was added to 150 µL of 62.5 µM DPPH (2,2-diphenyl-1-picrylhydrazyl) radical solution. After 30 minutes, absorbance was measured at 492 nm using a microplate reader (Multiskan 141 EX, Thermo Electron, USA). For positive control and blank control, ascorbate (vitamin C) and distilled water were used. For preparation of standard, 60% methanol was used to dissolve DPPH to have a final concentration of 0, 15.6, 31.25, 62.5, 125, 250, 500 and 1000 µM. The free radical scavenging capacity of the sample extracts were estimated against the calibration standard curve.

Extraction and quantification of individual phenolic compounds

The polyphenolic compound of the indigenous plants was estimated following the method described by Sakakibara et al., 2003. Before estimation of polyphenolic profile, an aliquot of methanolic extract was taken. After that the samples were evaporated to make dry using a rotary evaporator. Dry samples were then reconstituted using 1.5 and 3 mL of dimethyl sulfoxide (DMSO). This reconstituted sample extract was then centrifuged at 4200 rpm for 10 min and filtered through a Whatman 0.2 µm syringe filter (PTFE). In the HPLC analysis for quantification of polyphenolic profile, standards naming gallic acid, gallocatechin, protocatechuic acid, 3,4-dihydroxy benzoic acid, epigallocatechin, chlorogenic acid, catechin, syringic acid, vanillic acid, 3-OH benzoic acid, epicatechin, caffeic acid, sinapic acid, (-) catechin gallate, procyanidin B2, epigallocatechin gallate, ferulic acid, isoferulic acid, 4-coumaric acid, hesperdin, gallocatechin gallate, epicatechin galate, 2-coumaric acid, rosmaric acid, isovitexin, luteolin-7-O-glucoside, hespertin, quercetin-3-beta-galactoside, hyperoside, ellagic acid, rutin, physcion, daidzein, galangin, salicylic acid, rhoifolin, naringenin, flavonone (internal standard), quercetin, luteolin, chrysophanol, chalcone, rhein, isorhamnetin, myricetin, emodin, kaempferol, cinnamic acid and curcumin were used. Dilution of the stock samples was done with DMSO to keep the concentration of the standards 1 mg/mL then the calibration curve was made. The calibration curves showed peak area vs. concentration of standard in chromatograms. The HPLC system used was a Dionex HPLC series Ultimate 3000 (Germany) equipped with Dionex model with Chromeleon software, autosampler U-3000, and 3000 RS diode array detection system to monitor at all wavelengths from 200 to 600 nm. For the column, Dionex PA2 RSLC 120 A, Column C18 Acclaim RSLC, (100 mm x 2.1dm) i.e., 2.1µ Thermo Scientific Ltd was used at 35ºC. Solution A was composed of 50 mM sodium phosphate (pH 3.3) and 10% methanol while Solution B composed of 70% methanol. Analysis was done using flow rate of 0.47 mL/min. Initially flow rate was 100% of Solution A; for the next 0.03 min, 70% A; for another 2.65 min, 65% A; for another 7.9 min, 60% A; for another 11.5 min 50% A and finally 0% A for 13.1 min , again 17 min 100 % A and 20.25 min 100% A. The injection volume of the extract was kept 5µL (Sakakibara et al., 2003).

Results

Total phenol content (TPC)

Table 2 represents the TPC of the selected 15 medicinal plant samples. TPC was expressed as mg GAE/100 g FW. TPC ranged from 15.45 mg (*Z. rhetsa*) to 276.85 mg (*M. oleifera*) GAE/100 g. The TPC of the analyzed samples was found in the order as *M. oleifera* > *S. tora* > *P. angulata* > *A. sessilis* > *A. philoxeroides* > *A. viridis* > *C. benghalensis* > *S. calva* > *O. corniculata* > *C. asiatica* > *M. koenigii* > *A. spinosus* > *P. retrofractum* > *O. corymbosa* > *Z. rhetsa.*

***In vitro* AC**

The *in vitro* AC of the selected medicinal plants was estimated for both of their methanol and water extracts and the findings were presented in Table 2. The AC was determined as DPPH inhibition and was expressed as %DPPH inhibition and mg IC_{50}.

Table-2. TPC and comparison between AC (DPPH inhibition) in selected medicinal plant by methanol and water extracts

Medicinal Plants	TPC (mg GAE/100 g FW)	DPPH Inhibition			
		Methanol Extract		Water Extract	
		%DPPH Inhibition	IC_{50} (mg)	%DPPH Inhibition	IC_{50} (mg)
A. viridis	100.85	70.12	5.35	75.35	4.98
O. corniculata	75.15	69.29	5.41	50.05	7.49
A. spinosus	46.20	37.34	10.05	41.17	9.11
O. corymbosa	16.15	84.80	4.42	78.73	4.76
M. koenigii	64.44	88.77	4.22	59.73	6.28
Z. rhetsa	15.45	92.86	4.04	81.36	4.61
S. tora	191.20	89.04	4.21	80.59	4.65
A. sessilis	120.20	86.31	4.35	85.29	4.40
P. retrofractum	19.65	48.29	7.77	34.43	10.91
C. benghalensis	97.55	32.03	11.71	50.36	7.45
P. angulata	174.10	60.54	6.20	52.06	7.20
A. philoxeroides	113.90	31.83	11.79	29.81	12.58
M. oleifera	276.85	73.72	5.09	91.87	4.08
S. calva	78.60	24.49	15.34	42.15	8.90
C. asiatica	72.60	27.05	13.87	78.50	4.88

Table 2 indicates that the studied samples have a diverse range of AC. The higher %DPPH inhibition and the lower IC_{50} values indicate higher AC for any sample extract. The %DPPH inhibition in the methanol and water extracts were the highest in *Z. rhetsa* (92.86) and *M. oleifera* (91.87) respectively and they also showed the lowest IC_{50} (4.04 mg and 4.08 mg, respectively) in the corresponding solvent system. The lowest %DPPH inhibition was found in *S. calva* (24.49) and *A. philoxeroides* (29.81) in the methanol and water extracts respectively which also showed the highest IC_{50} (15.34 mg and 12.58 mg, respectively) in the same solvent systems.

Individual polyphenols content

In this study, ten different polyphenols were identified and quantified from methanol extract of the selected medicinal plant samples. The polyphenols included chlorogenic acid (CHL), coumaric acid (CMR), caffeic acid (CFF), apigenin-7-O-neohesperidoside (PGN^1), apigenin (PGN^2), quercetin-3-β-D-glucoside (QRC^1), quercetin-3-O-β-D-glucopyranoside (QRC^2), kaempferol (KMP), isorhamnetin (SRM) and luteolin (LTL). The polyphenols content was expressed as mg/100 g FW and presented in Table 3.

Table-3. Individual polyphenols content in selected medicinal plant extracts

Medicinal Plants	Individual polyphenols content (mg/100 g FW)									
	CHL	CMR	CFF	PGN[1]	PGN[2]	QRC[1]	QRC[2]	KMP	SRM	LTL
A. viridis	17.47	1.26	0.33	ND	ND	ND	ND	ND	ND	ND
O. corniculata	12.53	1.45	1.16	1.88	2.66	ND	ND	ND	ND	ND
A. spinosus	1.56	1.56	0.41	ND	ND	ND	ND	ND	ND	ND
O. corymbosa	2.77	1.01	0.10	ND	ND	9.62	1.51	ND	ND	ND
M. koenigii	1.89	1.40	0.70	ND	ND	8.42	12.1	ND	ND	ND
Z. rhetsa	2.61	1.26	0.65	ND	ND	7.66	5.80	ND	ND	ND
S. tora	5.91	1.53	0.81	ND	ND	16.7	4.62	4.06	1.25	ND
A. sessilis	0.44	ND	0.08	ND	ND	4.08	13.5	ND	ND	3.28
P. retrofractum	ND	1.04	0.26	2.52	11.8	ND	ND	ND	ND	ND
C. benghalensis	2.04	ND	ND	1.47	1.86	ND	ND	ND	ND	ND
P. angulata	2.64	ND	0.26	ND	ND	6.99	7.15	ND	ND	1.26
A. philoxeroides	0.08	ND	ND	0.52	0.78	ND	ND	ND	ND	ND
M. oleifera	0.39	ND	ND	0.31	12.2	ND	ND	ND	ND	ND
S. calva	37.27	1.97	0.38	ND	ND	ND	ND	ND	ND	ND
C. asiatica	1.67	ND	ND	ND	ND	8.37	11.9	ND	ND	ND

ND: not detected

Statistical Analysis

To find out the relationship between TPC and *in vitro* AC Pearson correlation was carried out. However, there was no statistically significant association found between TPC, %DPPH inhibition, and IC_{50}. The %DPPH inhibition and IC_{50} were found negatively correlated for both methanol ($P < .0005$) and water extracts ($P = .005$). Moreover, %DPPH inhibition of water and methanol extracts were also positively correlated ($P < .0005$) which implies that irrespective of solvent the extract would exhibit similar *in vitro* AC. Unlike %DPPH inhibition and IC_{50}, there was no association found between individual polyphenols, TPC, and AC. It is also worth mentioning that in this study only ten individual polyphenols are screened, the presence of other polyphenols is unknown.

Discussion

The present study identified that *M. oleifera* with the highest TPC also exhibits the highest AC in water extract in terms of %DPPH inhibition with the smallest amount of IC_{50} (Figure 1 and 2). However, the highest AC in methanol extract is found in *Z. rhetsa* (Figure 1). While it was assumed that the AC of plant extract would be explained by the TPC, Pearson correlation indicates no such relation in this study. Similar results are observed for individual polyphenol. It could be, thus, construed that *in vitro* AC is not solely attributable to the TPC or to any specific polyphenol, rather a combined effect might be responsible.

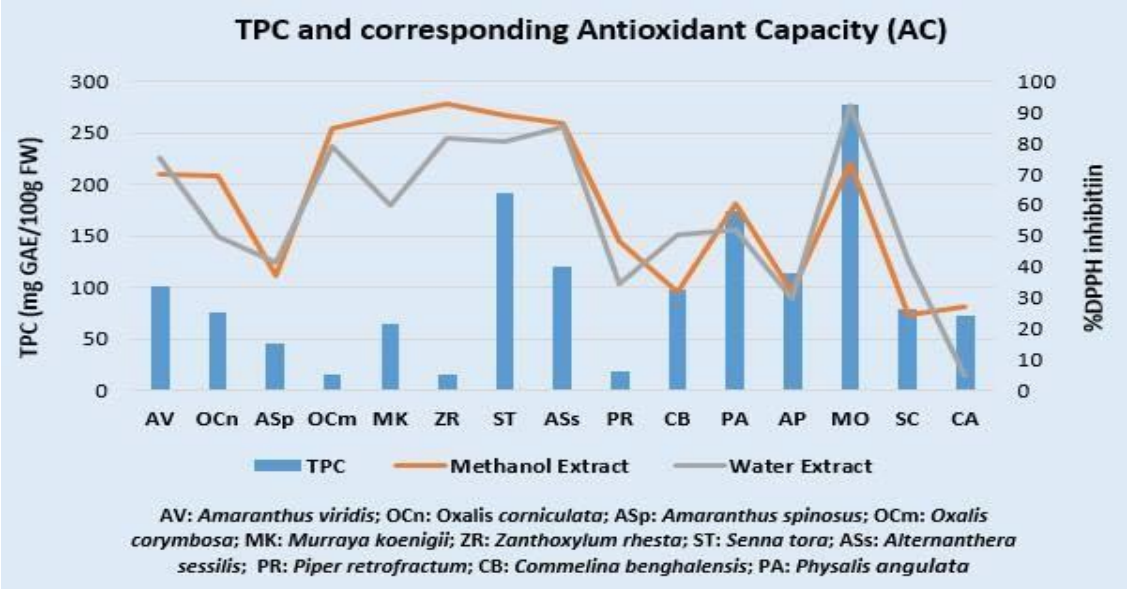

Figure-1. TPC and corresponding AC of the 15 indigenous medicinal plants

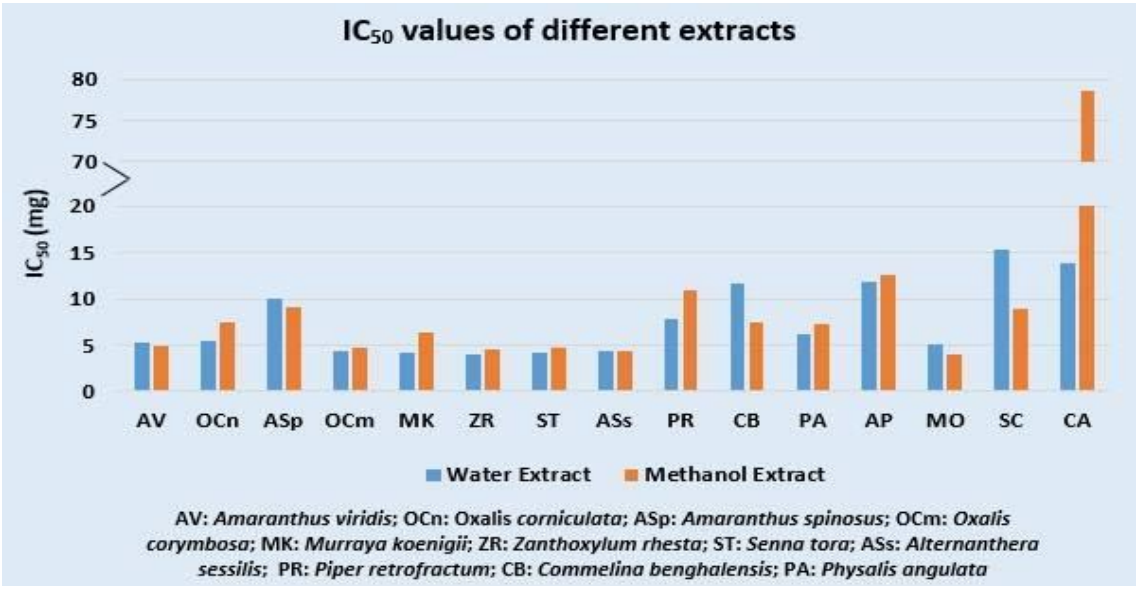

Figure-2. Comparison of IC_{50} values of water and methanol extracts of 15 indigenous medicinal plants.

It is observed that plants extracts in the 4th quartile of the %DPPH inhibition and in 1st quartile of IC_{50} contain good amounts of QRC^1 and QRC^2 in the analyzed medicinal plants. That is plant extract with QRC^1 and QRC^2 exhibits high AC, except *M. oleifera* and *C. asiatica*. In health perspective, samples containing quercetin (QRC^1 and QRC^2), also having high AC are important, because quercetin defends cells from oxidative stress, inflammation, and DNA damage through its antioxidant activities and modulates growth of many cancer cell lines by blocking cell cycle progression and tumor cell proliferation and by inducing apoptosis. Quercetin, the abundant flavonoid polyphenol in onion has been shown to inhibit the expression of metalloproteinase 1 (MMP1) and to impede atherosclerotic plaques, thus reducing the risk of coronary heart disease mortality (García-Lafuente et al., 2009). Evidence from epidemiological studies showed that consumption of food rich in quercetin could lower the risk of developing gastric cancer by 43%, colon cancer by 32% and lung cancer by 51% and in heavy smokers by 65% (Niedzwiecki et al., 2016). Research study reported the use of quercetin prolonged the lifespan of yeast model through its capacity to combat oxidative stress and this finding may be extrapolated to human cells (Belinha et al., 2007). In another study, quercetin was found to show significant antioxidant effect in protecting erythrocytes from *tert*-butylhydroperoxide induced oxidative changes suggesting that quercetin rich diet may have some protective effect against developing complications among patient having diabetes mellitus for several years (Rizvi and Mishra, 2009). Considering these empirical evidences, *Z. rhetsa*, *O. corymbosa*, *M. koenigii*, *C. asiatica*, *A. sessilis*, *P. angulata*, and *S. tora* could be

investigated further for health benefits by assessing the effect on relevant biomarkers.

Extracts from *M. oleifera* in this study though contain no QRC^1 and QRC^2, show the highest AC in water extract. What is found in *M. oleifera* is the highest amount of apigenin (PGN^2) among analyzed samples and a small amount of apigenin-7-O-neohesperidoside (PGN^1) and chlorogenic acid (CHL). Apigenin, a widely distributed flavonoid in onion, orange, oregano and tea leaves, has been found to prevent and protect against diabetic complications, amnesia, Alzheimer's and Parkinson's diseases, depression and insomnia, inflammation and cancer in both animal models and human clinical trials (Nabavi et al., 2018; Salehi et al., 2019). Traditionally *M. oleifera* has been known long for its hyperglycemic effects and recently a review on it found tangible evidence on beneficial effects of this plant against hyperglycemia and dyslipidemia (Mbikay, 2012). In the present study, only *in vitro* AC and 10 individual polyphenols were analyzed, this could be construed that PGN^2 is responsible for its highest AC. Another study found numerous polyphenols in *M. oleifera* especially in high amounts in leaves (Prabakaran et al., 2018). Prabakaran and his colleagues found CHL, KMP, quercetin, o-coumaric acids in *M. oleifera* leaves along with other polyphenols. They also reported significant AC and anti-microbial activity of polyphenol extracts of *M. oleifera* leaves.

Among 15 samples, *S. calva*, *A. viridis*, and *O. corniculata* contain high amounts of chlorogenic acid (CHL). However, high content of CHL has not shown any association with the *in vitro* AC. Review studies on phytochemicals ameliorating diabetes mellitus showed that CHL rich fruits and beverages are effective in managing postprandial hyperglycemia by reducing S-Glut-1 mediated intestinal transport of glucose (Dembinska-Kiec et al., 2008). *S. tora* contains high amounts of both coumaric acid (CMR) and caffeic acid (CFF). Both polyphenols have been shown to inhibit microbial growth in human food systems. *p*-Coumaric acid has been indicated to fight against tumor, stomach and colon cancer as well as heart diseases through its free radical scavenging potential (Stojković et al., 2013; Boz, 2015). In our analysis, luteolin (LTL) is not detected in *S. tora*, but other studies show that phytotoxic activity of this plant is attributed by several polyphenols including LTL (Ravi et al., 2018). This phytotoxic activity was found to exhibit beneficiary potential to treat Parkinson's disease *in vitro* by averting neuronal cell death (Ravi et al., 2018).

In the present study, 15 indigenous medicinal plants of Bangladesh were analyzed to evaluate their polyphenolics profile as well as their *in vitro* antioxidant capacity. These plants are underutilized, hence understudied, but they contain a diverse range of different polyphenol compounds with promising health beneficial effects. Especially, based on the findings of this study, presence of other polyphenols, their content, and their health beneficial potentiality need to be investigated for *S. tora* and *M. oleifera*, and *Z. rhetsa* to confirm their health effect through mechanism of action *in vivo*.

References

Aali, M.H., Ismaiel, N.J. and Majid, F.A.A. 2018. Antioxidant, and Antimicrobial Activities of Phenolic and Flavonoid Rich Medicinal Plants (Fritillaria zagrica and Tulipa kurdica) Bulbs Collected in Kurdistan Region of Iraq. *Zanco Journal of Pure and Applied Sciences.* **30**(5), pp.1-16.

Alam, M.K., Rana, Z.H., Islam, S.N. and Akhtaruzzaman, M. 2020. Comparative assessment of nutritional composition, polyphenol profile, antidiabetic and antioxidative properties of selected edible wild plant species of Bangladesh. *Food Chemistry.* p126646.

Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R. and Koirala, N. 2019. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants.* **8**(4), p96.

Basak Tukun, A., Shaheen, N., Banu, C.P., Mohiduzzaman, M., Islam, S. and Begum, M. 2014. Antioxidant capacity and total phenolic contents in hydrophilic extracts of selected Bangladeshi medicinal plants. *Asian Pacific journal of tropical medicine.* **7**, pp.S568-S573.

Belinha, I., Amorim, M.A., Rodrigues, P., de Freitas, V., Moradas-Ferreira, P., Mateus, N. and Costa, V. 2007. Quercetin increases oxidative stress resistance and longevity in Saccharomyces cerevisiae. *Journal of agricultural and food chemistry.* **55**(6), pp.2446-2451.

Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. *Nature*. **181**(4617), pp.1199-1200.

Boz, H. 2015. p-Coumaric acid in cereals: presence, antioxidant and antimicrobial effects. *International Journal of Food Science & Technology.* **50**(11), pp.2323-2328.

Cencioni, C., Spallotta, F., Martelli, F., Valente, S., Mai, A., Zeiher, A.M. and Gaetano, C. 2013. Oxidative stress and epigenetic regulation in ageing and age-related diseases. *International journal of molecular sciences.* **14**(9), pp.17643-17663.

Cheeseman, K. and Slater, T. 1993. An introduction to free radical biochemistry. *British medical bulletin.* **49**(3), pp.481-493.

Dembinska-Kiec, A., Mykkänen, O., Kiec-Wilk, B. and Mykkänen, H. 2008. Antioxidant phytochemicals against type 2 diabetes. *British Journal of Nutrition.* **99**(E-S1), pp.ES109-ES117.

García-Lafuente, A., Guillamón, E., Villares, A., Rostagno, M.A. and Martínez, J.A. 2009. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflammation Research.* **58**(9), pp.537-552.

Georgé, S., Brat, P., Alter, P. and Amiot, M.J. 2005. Rapid determination of polyphenols and vitamin C in plant-derived products. *Journal of Agricultural and food chemistry.* **53**(5), pp.1370-1373.

Hasan, S.R., Hossain, M.M., Akter, R., Jamila, M., Mazumder, M.E.H. and Rahman, S. 2009. DPPH free radical scavenging activity of some Bangladeshi medicinal plants. *Journal of Medicinal plants research.* **3**(11), pp.875-879.

Jahan, I.A., Hossain, H., Akbar, P.N., Rahman, M.M., Khan, T.A., Rahman, S.E. and Siraj, M.A. 2014. Antioxidant properties and HPLC assay of bioactive polyphenols of the ethanol extract of

Excoecaria agallocha stem bark growing in Bangladesh. *Journal of Pharmaceutical Research International.* pp.2116-2125.

Koch, W. 2019. Dietary Polyphenols—Important Non-Nutrients in the Prevention of Chronic Noncommunicable Diseases. A Systematic Review. *Nutrients.* **11**(5), p1039.

Leopoldini, M., Russo, N. and Toscano, M. 2011. The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry.* **125**(2), pp.288-306.

Li, H., Horke, S. and Förstermann, U. 2013. Oxidative stress in vascular disease and its pharmacological prevention. *Trends in pharmacological sciences.* **34**(6), pp.313-319.

Mbikay, M. 2012. Therapeutic Potential of Moringa oleifera Leaves in Chronic Hyperglycemia and Dyslipidemia: A Review. *Frontiers in Pharmacology.* **3**(24).

Nabavi, S.F., Khan, H., D'onofrio, G., Šamec, D., Shirooie, S., Dehpour, A.R., Argüelles, S., Habtemariam, S. and Sobarzo-Sanchez, E. 2018. Apigenin as neuroprotective agent: Of mice and men. *Pharmacological Research.* **128**, pp.359-365.

Niedzwiecki, A., Roomi, M.W., Kalinovsky, T. and Rath, M. 2016. Anticancer efficacy of polyphenols and their combinations. *Nutrients.* **8**(9), p552.

Prabakaran, M., Kim, S.-H., Sasireka, A., Chandrasekaran, M. and Chung, I.-M. 2018. Polyphenol composition and antimicrobial activity of various solvent extracts from different plant parts of Moringa oleifera. *Food Bioscience.* **26**, pp.23-29.

Ravi, S.K., Narasingappa, R.B., Joshi, C.G., Girish, T.K. and Vincent, B. 2018. Neuroprotective effects of Cassia tora against paraquat-induced neurodegeneration: relevance for Parkinson's disease. *Natural Product Research.* **32**(12), pp.1476-1480.

Reuter, S., Gupta, S.C., Chaturvedi, M.M. and Aggarwal, B.B. 2010. Oxidative stress, inflammation, and cancer: how are they linked? *Free radical biology and medicine.* **49**(11), pp.1603-1616.

Rizvi, S.I. and Mishra, N. 2009. Anti-oxidant effects of quercetin on type 2 diabetic erythrocytes. *Journal of food biochemistry.* **33**(3), pp.404-415.

Sakakibara, H., Honda, Y., Nakagawa, S., Ashida, H. and Kanazawa, K. 2003. Simultaneous determination of all polyphenols in vegetables, fruits, and teas. *Journal of Agricultural and Food Chemistry.* **51**(3), pp.571-581.

Salehi, B., Venditti, A., Sharifi-Rad, M., Kręgiel, D., Sharifi-Rad, J., Durazzo, A., Lucarini, M., Santini, A., Souto, E.B. and Novellino, E. 2019. The therapeutic potential of apigenin. *International journal of molecular sciences.* **20**(6), p1305.

Stojković, D., Petrović, J., Soković, M., Glamočlija, J., Kukić-Marković, J. and Petrović, S. 2013. In situ antioxidant and antimicrobial activities of naturally occurring caffeic acid, p-coumaric acid and rutin, using food systems. *Journal of the Science of Food and Agriculture.* **93**(13), pp.3205-3208.

Sukati, S. and Khobjai, W. 2019. Total Phenolic Content and DPPH Free Radical Scavenging Activity of Young Turmeric Grown in Southern Thailand. In: *Applied Mechanics and Materials*: Trans Tech Publ, pp.61-69.