Preliminary Phytochemical Screening and *in vitro* Antioxidant Capacity of Some Traditional Medicinal Plants of Bangladesh

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Baccaurea motleyana Müll. Arg. (BO), *Psidium guajava* L (PG), *Zingiber officinale* L (ZO), *Dillenia indica* L (DI) and *Moringa oleifera* L (MO) are traditionally used in the treatment of many diseases in Bangladesh. BO fruits and peel are traditionally used as curative in stomachache and sore eye diseases.[1,2]

P. gujava has been claimed to possess antihepatic, antibacterial, antidiarrheal, antimicro-bacterial, antihyperglycemic, antimalarial, cytotoxic and antioxidant activities.[16] It contains phenolic compound (gallic acid, protocatechuie acid, caffeic acid, ferullic acid, chlorogenic aid, ellagic acid and gauvin b) and flavonoids (quercetin, leucocyanidin, kaempferol.[3,4]

G. officinale is a spice and has been extensively used as an important ingredient in herbal medicine (Chinese, Ayurvedic and Unani Tibb) since long. It has been used to prepare folklore-medicine for cold induced diseases, nausea, asthma, cough, colic, heart palpitation, swellings, dyspepsia, loss of appetite, and rheumatism widely in the Asian sub-continent.[5]

D. indica has been prevalently used in modern and herbal medicine for the treatment of many diseases such as indigestion, asthma, influenza, dysentery, jaundice, promeho, weakness and rheumatic pain. It is thought that the methanol extract of *D. indica* leaves contain phenolics and flavonoids which might be responsible for its marked antioxidant activity.[6]

M. oleifera is a good source of nutrition and exhibit several pharmacological activities like anti-tumor, anti-inflammatory, anti-ulcer, anti-atherosclerotic, antioxidant and anticonvulsant activities.[7] The leaves and seeds of *M. oleifera* contain high levels of phenolic compounds which may be key determinant of these medicinal activities.[8]

Previous scientific studies indicated that the plants under investigation exhibited various medicinal properties due to the presence of diverse phytochemicals (phenolics, flavonoids, and carotenoids, vitamin C, folate, and pro-vitamin A), minerals (potassium, calcium, and magnesium), and fibers.[9,10] Therefore, this study focused on preliminary phytochemical screening and assessment of antioxidant activity of 80% methanol and water extracts of *B. motleyana* (peel, leaves and seeds); water extract of *P. guajava* (leaves), 80% methanol extract of *D. indica* (fruits) and 50% ethanol extract of *Z. officinale* (pulp) and *M. oleifera* (leaves) following DPPH assay method.

Each peel, leaves and seeds of *B. motleyana* (BMP, BML and BMS, respectively)); fruits of *D.*

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indica (DIF), leaves of *P. guajava* (PGL), pulp of *Z. officinale* (ZOP) and leaves of *M. oleifera* (MOL) were collected from different places of Bangladesh. Then, all the dried samples were ground individually by a grinding machine. Then the powder of BMP, BML and BMS with 80% methanol and water; PGL with water; DIF with 80% methanol; ZOP and MOL with 50% ethanol were successively macerated. Dried powder of each sample was placed in a closed vessel and respective solvent was added and allowed to stand for 24 hours. After 24 hours the mixture was pressed and liquid was drained out. This process was repeated three times at 24 hours interval. Then the obtained liquid was filtered by Whatman No. 1 filter paper and the filtrate was concentrated.

For phytochemical study the presence of alkaloids, tannins, phenol, flavonoids, steroid, steroids, terpenoids and quinone were screened using different chemical tests.[11,12] Phytochemical constituent analysis and observations are listed in table 1 where '+' and '-' sign indicated the presence and absence of the particular, respectively.

For antioxidant activity of the extracts (BMPM, BMLM, BMSM, BMPW, BMLW, BMSW, PGLW, DIFM, ZOPE and MOLE) were studied following DPPH free radical scavenging assay method[13] with slight modification.

2 ml of sample solutions (concentrations 50, 100, 200, 300, 400 µg/ml) was added to 2 ml of DPPH solution (0.1 mM) was added to 2.0 ml of the each extract solution, mixed well and was kept in the dark at room temperature for 30 min. Then, the absorbance was measured at 517 nm against a blank with UV-1800 spectrophotometer (Shimadzu, Japan). Ascorbic acid was used as a reference substance.

IC_{50} is a measure of the efficacy of a substance and the lowest IC_{50} value indicates the highest activity of antioxidant molecule, was calculated using equation of log dose inhibition curve.[14] The percentage of inhibition was calculated against blank: $I\% = [(Abs_{blank} - Abs_{sample})/Abs_{blank}]\times 100$; where, Abs_{blank} is the absorbance of the 0.1 mM DPPH solution and Abs_{sample} is the absorbance of the reaction mixture.

Result was presented as IC_{50} and also in terms of ascorbic acid equivalent antioxidant capacities (AEAC) which indicates the ratio of I% of the sample to I% of Ascorbic acid at the same concentration. The AEAC was calculated as follows: AEAC (g AA/100 g) = IC_{50} (ascorbic acid)/IC_{50} sample x 100.

Table 1 shows the results of the preliminary phytochemical screening of different parts of the studied plants. The results revealed the presence of phenols and saponins in every sample. Alkaloids were present in BMPW, BMPM and BMSW. Tannins were present in BMPM, BMLW, BMSM, PGLW and DIFM. All of the samples contain flavonoids except BMSW, BMSM. Steroid was present in BMLW, ZOPE and MOLE extracts. Terpenoids were present in BMLW, PGLW and ZOPE. It was also revealed that quinone were absent in all extracts except MOLE. The results in table 1 revealed that due to the presence of alkaloids, tannins and terpenoid and high quantity of phenols and flavonoids in different plants extracts materials may exert different pharmacological activities including antioxidant activity with promising therapeutic potential.

In antioxidant activity ascorbic acid is a superior inhibitor of $DPPH^{\cdot}$ compared to different plant extracts. For all the antioxidants, with higher the concentrations, the bleaching ability of the $DPPH^{\cdot}$ solution was nearly complete.[15] Ascorbic acid was substantially more active whatever the concentrations used in comparison with different extracts of different plants. With high concentration of ascorbic acid, it was possible to observe nearly a 100% free radical scavenging effect. In this study, ascorbic acid showed high percentage inhibition of 109.2% at100 µg/mL and as expected all the extracts under investigation showed less inhibition for all concentrations compared to ascorbic acid. Among all the extracts, *D. indica* fruits 80% methanol extract showed highest percentage inhibition and *B. motleyana* water extract showed lowest percentage inhibition at 400 µg/mL in Table 2.

Table 1. Presence or absence of phytochemicals in the plants extracts.

Sample name	Part of sample	Extract	Phytochemicals							
			Alkaloids	Tannins	Phenols	Flavonoids	Saponins	Steroids	Terpenoids	Quinone
B. motleyana	Peel	Water	+	-	+	-	+	-	-	-
B. motleyana	Peel	80% methanol	+	+	+	+	+	-	-	-
B. motleyana	Leaves	water	-	+	+	+	+	+	+	-
B. motleyana	Leaves	80% methanol	-	-	+	+	+	-	-	-
B. motleyana	Seeds	Water	+	-	+	-	+	-	-	-
B. motleyana	Seeds	80% methanol	-	+	+	-	+	-	-	-
P. guajava	Leaves	Water	-	+	+	+	+	-	+	-
D. indica	Fruit	80% methanol	-	+	+	+	+	-	-	-
Z. officinale	Pulp	50% ethanol	-	-	+	+	+	+	+	-
M. oleifera	Leaves	50% ethanol	-	+	+	+	+	+	-	+

Present (+), Absent (-)

Table 2. Percentage (%) inhibition of the different extracts at 400 µg/ml,

Plant name	Part of plant	Extraction solvent	Inhibition (%)
B. motleyana.	Peel	Water	54.02
B. motleyana.	Peel	80% methanol	80.11
B. motleyana	Leaves	Water	80.68
B. motleyana	Leaves	80% methanol	78.52
B. motleyana	Seeds	water	24.17
B. motleyana	Seeds	80% methanol	31.74
P. guajava	Leaves	water	69.94
D. indica	Fruit	80% methanol	89.71
Z. officinale	Pulp	50% ethanol	73.95
M. oleifera	Leaves	50% ethanol	80.49

Table 3. IC_{50} and Ascorbic acid equivalent antioxidant capacity (AEAC) values of different extracts of the studied plants.

Sample name	Part of sample	Extract	IC_{50} (µg/mL)	AEAC (g/100g)
Ascorbic acid	NA	NA	41.44	NA
B. motleyana.	Peel	Water	404.8	10.23
B. motleyana.	Peel	80% methanol	234.88	17.64
B. motleyana	Leaves	Water	245.72	16.86
B. motleyana	Leaves	80% methanol	284.42	14.57
B. motleyana	Seeds	water	778.41	5.32
B. motleyana	Seeds	80% methanol	616.18	6.72
P. guajava	Leaves	water	325.10	12.75
D. indica	Fruit	80% methanol	215.18	19.26
Z. officinale	Pulp	50% ethanol	267.16	15.51
M. oleifera	Leaves	50% ethanol	231.61	17.89

The lowest IC_{50} value indicates the highest activity as antioxidant molecule. Present study demonstrated that the extracts of the studied plants had strong antioxidant capacity with IC_{50} value in (Table 3). Additionally, the ascorbic acid equivalent antioxidant capacity of the extracts ranged from 5.05 g/100g to 19.26 g/100g. Overall the results suggested that all the extracts except methanol (80%) extract

and water extract of *B. moyetleana* seeds have strong antioxidant capacity enriched with phytochemicals as expected.

Figure 1 elucidates that ascorbic acid is a superior inhibitor of DPPH˙ compared to different plant extracts. Ascorbic acid showed highest percentage inhibition of 109.2% at100 µg/mL, while the other plant extracts showed decreased inhibitory effect for all concentrations. The percentage inhibition of different extracts of the plants in this study revealed the highest percent inhibition was found to DIFM (89.71%) and lowest for BALM (23.62%) at 400 µg/ml. For the other extracts different value of percent inhibition were found.

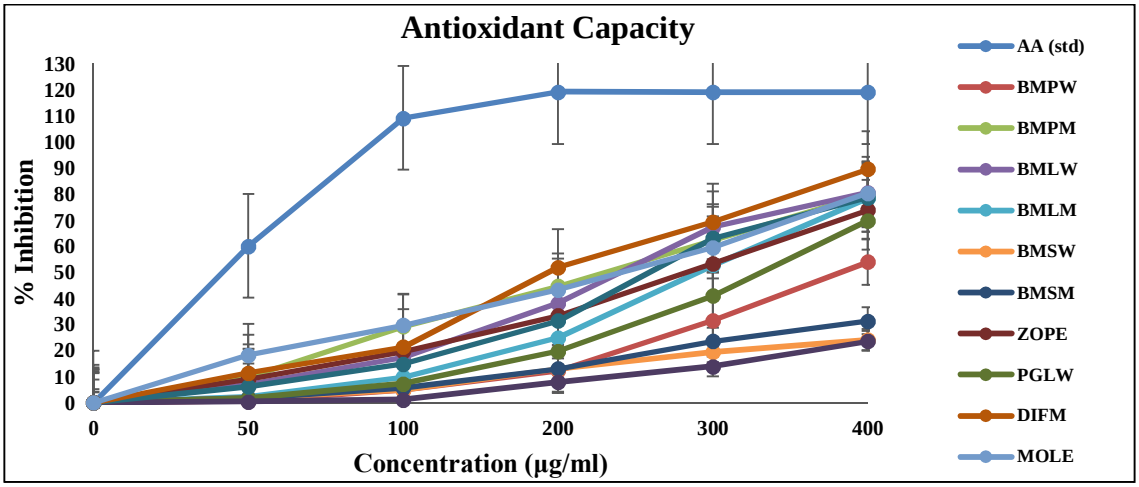

Figure 1. I% of Ascorbic acid and different extracts of different plants following DPPH method. [Ascorbic acid= AA; 80% methanol extract of B. motleyana (peel= BMPM, leaves= BMLM, seeds= BMSM), water extract of B. motleyana (peel= BMPW, leaves= BMLW, seeds= BMSW), P. guajava leaves water extract= PGLW, 80% methanol extract of (D. indica fruit= DIFM) and 50% ethanol extract of (Z. officinale pulp (ZOPE), M. oleifera leaves= MOLE)]

The parameter IC_{50} was calculated for each type of sample (Table 3). The lowest IC_{50} value were found for DIFM (215.18 µg/ml) and highest for BMSW (778.41 µg/ml) of ten samples.

As evident in table 3 AEAC of different extracts of different plants ranging from 5.05 g/100g to 19.26 g/100g where DIFM showed highest AEAC and BMSW showed lowest AEAC at same concentration.

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