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Phytochemical, mineral and vitamin analyses of *Boscia variabilis*

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ABSTRACT

People in rural areas of Myanmar often use wild plants as seasonal vegetables. The present study was focused on the analysis of phytoconstituents of the edible wild plant, *Boscia variabilis* Coll. & Hemsl. The phytochemical analysis of edible parts of this plant showed the various amount of major bioactive constituents such as flavonoids (26.72 mg/g), alkaloids (14.6 mg/g), tannins (24.9 mg/g), total phenols (31.75 mg/g) and saponins (26.5 mg/g). The studied plant is also rich in mineral elements; potassium (K) (18.466 mg/g), calcium (Ca) (1.691mg/g), magnesium (Mg) (0.96 mg/g), sodium (Na) (0.185 mg/g), iron (Fe) (0.006 mg/g), zinc (Zn) (0.003 mg/g) and cromium (Cr) (0.0004 mg/g). Analysis of vitamin constituents showed the presence of thiamin (0.004 mg/g), riboflavin (0.007mg/g) and niacin (0.026 mg/g) but ascorbic acid content was not detected. Futher investigations should be carried out to find out its biological activities.

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INTRODUCTION

Boscia variabilis Coll. & Hemsl is a tropical species belongs to family Capparaceae. It is indigenous to Myanmar and its local name is ‘Thamone’. It is a medium sized evergreen tree, which grows up to 18 m in height. During January to March, the trees bear pale green flowers. They are borne in clusters on short lateral shoots and are without petals. Its bark is dark brown to grayish brown, about 0.4 cm thick, smooth and slightly rough. The bark of *B. variabilis* is used as medicine for eye disease, and the flowers are famous for making salad. This species displayed high medicinal values. The leaves and roots are used in aching, sedema, cold extremities and also used as stomachic, expectorant, and counter irritant (San and Han, 1998). The gall of Thamone is used to relieve eye sore. It is believed that eating Thamone once a year can keep the good health throughout the lifespan. Wild edible plants are still consumed traditionally by different communities mainly in rural and suburban areas (Pinela et al., 2017). Fruits and vegetables are rich in minerals, vitamins and fibre and low in saturated fat (Batta, 2016). Since this plant is consumed as seasonal food by local people in Myanmar, it is required to investigate the nutritional and therapeutic value of this plant. The objective of present study is to evaluate phytochemicals, vitamins and minerals present in this plant.

MATERIALS AND METHODS

Plant materials

The edible parts of *B. variabilis* (the very young leaves and flowers) were collected from Mandalay Technological University Campus which is located in central Myanmar (22° 58′0” N 96° 11′0 ” E). The plant was identified by an authorized botanist from Department of Research and Innovative (DRI), Yangon, Myanmar. The plant materials were washed under running tap water, shade dried and homogenized to fine powder using electric grinder and used for futher analysis.

Determination of extractive values (w/w) of crude plant extracts in different solvents

The air-dried powder of edible parts of *B. variabilis* was

extracted by using different solvents (water, methanol, ethanol, ethyl acetate, acetone and petroleum ether) and the extraction was carried out by maceration in different solvents at room temperature. The extracts were then concentrated and the solvents were removed completely under reduced pressure (Azwanida, 2015). The extractive values were calculated in terms of percent in weight by weight of the dried powdered material.

Determination of ash, moisture, fibre and total fat content of dry plant materials

Ash, moisture, fibre and total fat content of the dried plant sample were analyzed by using the method of AOAC (2000).

Phytochemical analysis

Estimation of alkaloids

Alkaloids content was determined by using the method of Harborne (1998). 5 gm of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of flavonoids

Flavonoids content was determined by using the method of Boham and Kocipai (1994). 10 gm of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No. 42 (125 mm). The filtrate was transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Estimation of tannins

Tannins content was determined according to Atanassova and Christova-Bagdassarian (2009). 3 gm of plant sample was extracted with distilled deionized water into 250 ml volumetric flask during 4 h at room temperature and then the sample was filtered. 25 ml aliquot of the extract was mixed with 25 ml of indigo-carmine solution and 750 ml of distilled water. This mixture was titrated against 0.1 N $KMnO_4$ solution until the blue coloured solution passes through green to a final golden yellow.

Estimation of total phenols

Total phenols were determined according to the Folin-Ciocalteu method described by Singleton and Rossi (1965). 0.5 ml of dilute methanol extract was reacted with 2.5 ml of 0.2 mol/L Folin-Ciocalteu reagent for 4 min, and then 2 ml saturated sodium carbonate solution was added into the reaction mixture. The absorbance was taken at 760 nm after incubation at room temperature for 2 h. Gallic acid was used as a reference standard, and the results were expressed as milligram per gallic acid equivalent (mg GAE)/g dry weight of plant material.

Estimation of saponin

20 gm of each plant samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h by continuous stirring at about 55°C. The mixture was filtered and the residue was reextracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated three times. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage (Obadoni and Ochuko, 2001).

Mineral analysis

The mineral contents such as Zn, Ca, Mg, Na, Fe, chromium (Cr) and K were determined using Perkin Elmer Optima 7300 DV ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy). 1 gm of dried plant sample was digested with 6 ml of concentrated HNO_3, 2 ml concentrated H_2O_2 in microwave digestion system. The temperature program was as follows: 2 min for 400 W, 5 min for 400 W, 5 min for 400 W, 8 min for 800 W and 8 min for 1000 W. The resulting solution was cooled and diluted to 10 ml with deionized water. The clear solution was used for the mineral analysis (Chernetsova et al., 2015; Kumaravel and Alagusundaram, 2014).

Table 1. Extractive values of the edible parts of *B. variabilis* on dry weight basis.

S/N	Solvents	Extractive value %
1	Water	36.8
2	Methanol	24
3	Ethanol	23.6
4	Ethyl acetate	1.2
5	Acetone	47.8
6	Petroleum ether	0.4

Vitamin analysis

Thiamine

Ten millitres (10 ml) of sample solution and 10 ml thiamine hydrochloride working standard solution were taken in two different dry separating funnels. Ten millitres (10 ml) of chloroform dye solution was added to both the standard and sample solution and then shaken for 2 min continuously. Then, they are allowed to stand for 5 min with occasional shaking. The chloroform layer was collected by passing through anhydrous sodium sulfate. The absorbance of the aliquots of the filtrate was read at 420 nm. The chloroform was used as blank (USP, 2000).

Niacin

Five grams (5 gm) of sample was treated with 50 ml of 1 N sulphuric acid for 30 min and 0.5 ml of ammonia solution was added to it. It was filtered and 5 ml of potassium cyanide was added to 10 ml of filtrate. Then, this filtrate was acidified with 5 ml of 0.02 NH_2SO_4. The absorbance of the resulting solution was recorded at 420 nm (Okwu, 2005)

Riboflavin

Five grams (5 gm) of sample was extracted with 100 ml of 50% ethanol solution and shaken for 1 h. 10 ml of 5% potassium permanganate and 10 ml of 30% H_2O_2 were added and allowed to stand over a hot water bath for about 30 min. Two millitres (2 ml) of 40% sodium sulphate was added. This was made up to 50 ml and the absorbance was measured at 510 nm (Okwu, 2005).

Ascorbic acid (vitamin C)

Vitamin C content was determined according to the method of Barakat et al. (1993). Five grams (5 gm) of the sample was mixed with 100 ml of EDTA\TCA (2:1) extracting solution and shaken for 30 min. This was centrifuged at 3000 rpm for about 20 min. Twenty millitres (20 ml) of the extract was mixed with 1% starch indicator and titrated with 20% $CuSO_4$ solution to get a dark end point.

Statistical analysis

Results of this research are expressed as the mean ± standard deviation of three independent experiments. Student *t* test was used for statistical analysis.

RESULTS AND DISCUSSION

Determination of bioactive phytoconstituents from plant material largely depends on the type of solvent used in the extraction procedure. The choice of the solvent will also depend on the targeted compounds to be extracted (Das et al., 2010). During extraction, solvents diffuse into solid plant material and solubilize compounds with their similar polarity (Cowan, 1999). According to Table 1, the extractive values are higher in polar solvents such as water, methanol, ethanol and acetone. Hence, the selected plant parts have potential to possess higher content of polar compounds such as phenols, flavonoids, alkaloids, tannins and saponins. The phytochemical concentrations of the same plant species can vary depending on the geographical locations, genetic variations and environmental factors (Hansen and Wold, 2008).

Moisture content of the plant is potential source of water and the studied plant sample contains moisture content of 6.788%. Moisture content is one of the factors that affect the efficacy of extracted plant phytochemicals (Ncube et al., 2008). The ash content of the plant sample is 5.856% and it is very important for mineral analysis. Ash contains minerals elements which are important for the physiological functions of the body. The edible parts of *B. variabilis* have fibre content of 1.795%. It was recommended by American Dietetic Association that intake of 20-30 gm of fibre in the diet are necessary for

Table 2. Ash, moisture, fibre and total fat content of edible parts of *B. variabilis*.

S/N	Parameters	Content %
1	Moisture content	6.788
2	Ash content	5.856
3	Total fat	0.043
4	Fibre	1.795

Table 3. Phytochemicals of the edible parts of *B. variabilis* on a dry weight basis.

S/N	Phytochemicals	Concentration in mg/g-dw[a]
1	Alkaloids	14.61±0.140
2	Flavonoids	26.72±0.056
3	Tannin	24.94±0.002
4	Total phenols	31.75 ± 0.027
5	Saponin	26.51±0.106

[a]Average concentration of phytochemicals ± standard deviation (n=3).

Table 4. Mineral composition of the edible parts of *B. variabilis* on a dry weight basis.

S/N	Minerals	Concentration in mg/g-dw[b]
1	Potassium (K)	18.466 ± 1.52
2	Calcium (Ca)	1.691±0.07
3	Magnesium (Mg)	0.96 ± 0.18
4	Sodium (Na)	0.185 ±0.05
5	Iron (Fe)	0.006 ± 0.00
6	Zinc (Zn)	0.003 ± 0.00
7	Chromium (Cr)	0.0004± 0.00

[b]Average concentration of element ± standard deviation (n=3).

Table 5. Vitamin contents of the edible parts of *B. variabilis* on a dry weight basis.

S/N	Vitamins	Concentration in mg/g-dw[c]
1	Niacin	0.026 ± 0.00
2	Riboflavin	0.007 ± 0.00
3	Thiamine	0.004 ± 0.00
4	Ascorbic acid	ND

[c]Average concentration of vitamins ± standard deviation (n=3); ND, not detected.

digestion and for effective elimination of wastes (Vadival, 2005). However, the edible parts of this plant have low level of total fat content. It is only 0.04% in this selected plant parts (Table 2).

The phytochemicals of edible part of this plant are shown in Table 3. According to these results, the selected plant parts have significant amount of bioactive constituents such as phenols (31.75 mg/g), flavonoids (26.72 mg/g), tannins (24.94 mg/g), saponins (26.51 mg/g) and alkaloids (14.61 mg/g). All these bioactive constituents have distinct structural features and various pharmacological activities: antimicrobial, anti-helmintic and anti-diarrhoea activities (Tiwari et al., 2011). Flavonoids are known to be the potent antioxidants and anti-proliferative agents (Lin and Weng, 2006).

Elemental and vitamin analysis indicates the nutritional value of edible parts of this plant (Table 4 and 5). The edible parts of this plant recorded the concentration of K

(18.466 mg/g). The K is most abundant among the minerals quantified. Potassium is an essential nutrient used to maintain fluid and electrolyte balance in the body. Daily value for dietary supplement of K is 4700 mg (FDA, 2018). Ca is the most common mineral in the human body which helps in strengthening bones and teeth, regulating muscle and heart functions (FAO/WHO, 2001). Daily value for dietary supplement of Ca is 1300 mg. The concentration of Ca in edible parts of studied plant is recorded as 1.69 mg/g. Mg functions as enzyme cofactors in energy metabolism, protein synthesis, RNA and DNA synthesis and maintenance of electrical potential of nervous tissues and cell membrane (FAO/WHO, 2001). Food and Drug Administration regulations recommended that 450 mg/day is required for normal human health. Mg concentration of edible parts of this plant is found to be 0.96 mg/g. The concentration of micro elements such as Fe, Zn and Cr for edible parts of the studied plant is found to be (0.006 mg/g), (0.003 mg/g) and (0.0004 mg/g) respectively. These micro elements also have vital functions in the human body. Fe serves as a carrier of oxygen to the tissues from the lungs by red blood cells haemoglobin; as a transport medium for electrons within the cells and as an integrated part of important enzymes systems in various tissues. Zn occurs as a natural constituent in all plant and animal tissues and function as an integral part of several enzymes systems. It is an essential trace element, the requirement for Zn changes throughout life and health effects associated with Zn deficiency are numerous (Nair, 1997). Cr occurs as an essential trace element in both plant and animal origins, taking part in various metabolic processes (Reczajska et al., 2005). According to FDA, the daily values for dietary supplement of Fe, Zn and Cr are 18 mg, 11 mg and 35 µg, receptively (FDA, 2018).

The concentration of thiamin, riboflavin and niacin for edible parts of studied plant are found to be 0.004, 0.007 and 0.026 mg/g receptively. Plant-derived vitamins are of great interest because of their impact on human health. Thiamin serves as co-enzyme functions in metabolism of carbohydrates and branched chain amino acids. Thiamin deficiency causes the disease Beri-beri (FAO/WHO, 2001). Riboflavin also serves as co-enzymefunction in numerous oxidation and reduction reactions (FAO/WHO, 2001). Niacin serves as co-substrate/co-enzyme for oxygen transfer with numerous dehydrogenases. Niacin deficiency causes Pellagra with diarrhea, dermatitis and dementia (FAO/WHO, 2001). FDA approved that daily values for dietary supplement of these micro elements are 1.2, 1.3 and 16 mg receptively (FDA, 2018).

Conclusion

The results of the present study revealed that this edible plant has the potential to provide the essential nutrients to human beings. More works need to be carried out to investigate the biological activities of *B. variabilis*. The physicochemical properties determined in this study will be useful for further research.

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