

## ANTIOXIDANT, ANTIBACTERIAL POTENTIAL AND HPLC ANALYSIS OF *Dioscorea alata* BULB

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### ABSTRACT

The purpose of the present study was to evaluate antioxidant, antibacterial potential and HPLC analysis of methanol extract of *Dioscorea alata* bulb. Antioxidant activity investigated by DPPH free radical scavenging assay, the IC<sub>50</sub> value of plant extract was 24.99 µg/mL. Significant absorption of extract (1.317 of 1mg/mL) showed the ferric reducing activity. HPLC analysis indicated the presence of myricetin (471.53mg/100 g of dry extract) in the highest concentration. Extract showed significant zone of inhibition in antibacterial activity test. The presence of myricetin along with other detected polyphenols might contribute to antioxidant and antibacterial activities.

**Key words:** Total Phenolic content, Myricetin, Scavenging activity, Disc diffusion

### INTRODUCTION

Therapeutic uses of plants had in effect stored at the very beginning of human life on earth. The World Health Organization estimated that 80% of the populations of developing countries rely on traditional medicine. Also modern pharmacopoeia still contains at least 25% drugs derived from plants (Ghani, 2003). *Dioscorea alata* L. (Dioscoreaceae) locally known as mate alu is tuberous root vegetable. It is native to tropical regions throughout the world. It is well known for its high nutritional content (Osagie 1992). *D. alata* is a cheaper source of carbohydrate that contains more than 85% of the carbohydrate (Kochhar 1981), minerals of calcium, phosphorus, iron and vitamins such as riboflavin, thiamin etc. (Coursey 1967). Previous studies reported the presence of cyanidin 3,5-diglucoside; cyanidin-3-glucoside; cyanidin-3-rhamnoglucoside; (Lula *et al.*, 2015) hydro-Q chromene; gamma-tocopherol-9; RRR-alpha-tocopherol; coenzyme Q; 1-feruloylglycerol; (Cheng *et al.*,

2007) 4-Phenylbutan-2-one; (Gramshaw and Osinowo, 1982) alanins, cyaniding, peonidin (Moriya *et al.*, 2015) in *D. alata*. In Bangladesh traditionally it is used against leprosy and tumor. The present study was designed to evaluate antioxidant, antibacterial potential of methanol extract of *D. alata*. HPLC analysis was done to identify the existence of naturally occurring bioactive polyphenols.

### MATERIAL AND METHODS

#### Chemicals and reagents

Arbutin, gallic acid, hydroquinone, (+)-catechin hydrate, vanillic acid, caffeic acid, Syringic acid, (-)-epicatechin, vanillin, *p*-coumaric acid, *trans*-ferulic acid, rutin hydrate, ellagic acid, benzoic acid, rosmarinic acid, myricetin, quercetin, *trans*-cinnamic acid, kaempferol, 2, 2-Diphenyl-1-picrylhydrazyl, and butylated hydroxyl toluene were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC), methanol (HPLC), acetic acid (HPLC), and ethanol was obtained from Merck (Darmstadt, Germany).

### Microorganisms

Twelve pathogenic bacterial strains (Six gram positive and six gram negative) were collected from Microbiology Laboratory of Pharmacy Discipline, Khulna University, Bangladesh.

### Preparation of crude extract

*Dioscorea alata* bulb was collected from Khulna, Bangladesh and identified by the experts of Bangladesh National Herbarium, Dhaka (Accession no. DACB-37524). Cold extraction process in methanol was used get the extract. The crude extract was stored in a refrigerator at 4°C until experiment to commence.

### Phytochemical test

Different standard qualitative chemical test were carried out to identify different phytochemical constituents such as carbohydrates, alkaloids, glycosides, phenolic compounds, flavonoids, tannins, steroids, gum, saponins and acidic compounds (Ghani, 2005).

### DPPH free radical scavenging assay

Free radical scavenging activity was quantitatively estimated using DPPH (Islam *et al.*, 2014). From the stock solution of 1024µg/mL of extract, serial dilution was carried out to obtain the desired concentration series (512, 256, 128, 64, 32, 16, 8, 4, 2, 1µg/mL). In 1mL diluted extract solution from each concentration, 3mL of immediately prepared 0.004% w/v DPPH solution was added. After incubation for 30min in dark at room temperature, the presence of DPPH in each concentration of the sample was detected at 517nm. Ascorbic acid was used as standard. The antiradical activity of the extract and standard was calculated using the formula: % discoloration =  $[(Abs_0 - Abs_1) / Abs_0] \times 100$ ; where  $Abs_0$  is the absorbance of control and  $Abs_1$  is the absorbance of extract or standard. Concentration of extract providing 50% discoloration of DPPH solution ( $IC_{50}$  value), was estimated from the graph obtained by plotting the percent discoloration against concentration.

### Reducing power assay

The reducing power of extract was determined by the method of Oyaizu (Saha *et*

*al.*, 2013) with modifications. 2.5mL phosphate buffer (0.2mol/L; pH 6.6) and 2.5mL of 1% w/v potassium ferric cyanide were subsequently added to 1mL of various concentrations of extract solution. It was incubated for 20min at 50°C and then cooling at room temperature and 2.5mL of 10% w/v thiochloroacetic acid was added to it. The mixture was centrifuged at 3000rpm for 10min. 2.5mL aliquot of supernatant was mixed with 2.5mL distilled water and 0.50mL of 0.1%w/v ferric chloride with continuous shaking. Ten minutes later the absorbance was measured at 700nm against blank. Butylated hydroxy toluene (BHT) was used to compare the reducing power of the extract.

### Total phenol content

The total phenol content of the extract was determined by the modified Folin-Ciocalteu method (Hemayet *et al.*, 2013). Extract (1 mg/mL) was mixed with 5 mL of diluted Folin-Ciocalteu reagent. Then 4mL sodium carbonate (7.5% w/v) was added to the mixture and allowed to incubate at 40°C for 30min. Absorbance was measured at 765nm. The standard calibration curve was prepared using gallic acid (0-1mg/mL). Based on the absorbance of the extract, total phenol content was calculated and expressed in terms of mg of gallic acid equivalent per gram of dry extract.

### Total flavonoid Content

Aluminum chloride colorimetric method was used to determine the total flavonoid content in the extract (Shah *et al.*, 2012). Four milliliters distilled water and 0.3mL 5% w/v sodium nitrate was subsequently mixed into 1mg/mL extract. Five minutes later, 0.3mL 10% w/v aluminum chloride was added. At the sixth minute 2mL of 1M sodium hydroxide was added and the volume was adjusted to 10mL with distilled water. Absorbance was measured at 510nm against blank. Quercetin was used for standard calibration curve. After reading the quercetin equivalent from the calibration line, total content of flavonoid was expressed as mg quercetin equivalent (QE) per gram of dry plant extract.

### HPLC detection and quantification of polyphenolic compounds

Detection and quantification of selected phenolic compounds in the methanol extract were determined by HPLC-DAD analysis Ismet *et al.* (2014). It was carried out on a Dionex UltiMate 3000 system equipped with quaternary rapid separation pump (LPG-3400RS) and photodiode array detector (DAD-3000RS). Separation was performed using C<sub>18</sub> (5 $\mu$ m) Dionex column (4.6 x 250 mm) at 30°C with a flow rate of 1 mL/min and an injection volume of 20  $\mu$ l. The mobile phase consisted of solvent A, solvent B and solvent C with the gradient elution program of 5%A/95%B (0-5min), 10%A/90%B (6-9min), 15%A/75%B/10%C (11-15min), 20%A/65%B/15%C (16-19min), 30%A/50%B/20%C (20-29min), 40%A/30%B/30%C (30-35min) and 100%A (36-40min). In where solvent A is acetonitrile, solvent B is acetic acid solution (pH 3.0) and solvent C is methanol. The UV detector was set to 280 nm (0-22.0min), changed to 320nm (23-28.0min), again change to 280nm (29-35min) and finally to 380nm (36-40min) and held for the rest of the analysis period while the diode array detector was set at an acquisition range from 200 to 700nm. For the preparation of calibration curve, a standard stock solution was prepared in methanol containing arbutin, (-)-epicatechin (5 $\mu$ g/mL each), gallic acid, hydroquinone, vanillic acid, rosmarinic acid, myricetin (4 $\mu$ g/mL each), caffeic acid, Syringic acid, vanillin, *trans*-ferulic acid (3 $\mu$ g/mL each), *p*-coumaric acid, quercetin, kaempferol (2 $\mu$ g/mL each), (+)-catechin hydrate, ellagic acid (10 $\mu$ g/mL each), *trans*-cinnamic acid (1 $\mu$ g/mL), rutin hydrate (6 $\mu$ g/mL) and benzoic acid (8 $\mu$ g/mL). A solution of the extract was prepared in methanol having the concentration of 10mg/mL. Prior to HPLC analysis, all the solutions were filtered through 0.20 $\mu$ m syringe filter (Sartorius, Germany) and then degassed in an ultrasonic bath (Hwashin, Korea) for 15min.

### Antibacterial Activity

Disk diffusion assay was applied to assess antibacterial activity of extract against both gram-positive and gram-negative bacterial strains. Second subculture of each bacterial strain was prepared. Each

subculture was transferred into vials containing nutrient broth and incubated at 37°C for 2-4h. Then seeded nutrient agar medium was poured into petri-dishes and let to solidify at room temperature. Discs impregnated with extract (200, 300 and 500 $\mu$ g/disk), standard antibiotic disc (Kanamycin 30 $\mu$ g/disk) and blank discs containing ethanol (control) were placed on the petri-dishes and incubated at 37°C for 18-24h and the zone of inhibition was measured in millimeters (Pervin *et al.*, 2013).

### RESULTS AND DISCUSSION

In traditional societies nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes. In recent years much attention has been devoted to natural antioxidant and their association with health benefits (Arnous *et al.*, 2001). All these activities are due to the phytochemicals present in the plant. Preliminary phytochemical screening of the methanol extract of *D. alata* indicated the presence of carbohydrate, alkaloids, glycosides, phenolic compounds, flavonoids, tannins, steroids and gum but absence of protein and saponin. Phenolic compounds are wide spread constituents of edible and non-edible plants. It has recently showed that phenolic antioxidants in herbs have capacities of quenching lipid peroxidation, preventing DNA oxidative damage and scavenging reactive oxygen species (Ksouri *et al.*, 2009). The antioxidant effect is mainly due to the hydroxyl groups present in their structure (Rice-Evans *et al.*, 1997). Flavonoids are well-known antioxidant constituents of plants and possess a broad spectrum of chemical and biological activity, including radical scavenging and antibacterial properties (Miliauskas *et al.*, 2004). The total phenol content of the extract was found to be 222.99mg GAE/g of dry extract. Meanwhile the total flavonoid content was 98.95mg QE/g of dried plant extract. Identification and quantification of individual phenolic compounds in the methanol extract of *D. alata* were analysed by HPLC. The chromatographic separations of polyphenols in standard and methanol extract are shown in figure 1 and 2, respectively.

Table I. Composition of polymeric matrices for the different formulation

	F1	F2	F3	F4
Timolol maleate (mg)	60	60	60	60
Guar gum (% w/v)	0.25%	0.50%	0.75%	1.0%
Dibutyl phthalate (w/w of polymer)	30%	30%	30%	30%
Purified water (mL)	100	100	100	100

Table II. Evaluation of ocular inserts

Code	Thickness ( $\mu\text{m}$ )	Weight (mg)	Surface pH	% drug content
Batch A	41.12 $\pm$ 0.04	0.84 $\pm$ 0.07	6.5-7.0	98.69 $\pm$ 0.58
Batch B	54.52 $\pm$ 0.03	1.07 $\pm$ 0.04	6.5-7.0	99.66 $\pm$ 0.88
Batch C	69.09 $\pm$ 0.05	1.68 $\pm$ 0.10	6.5-7.0	97.92 $\pm$ 0.67
Batch D	79.90 $\pm$ 0.03	2.11 $\pm$ 0.09	6.5-7.0	96.37 $\pm$ 0.58

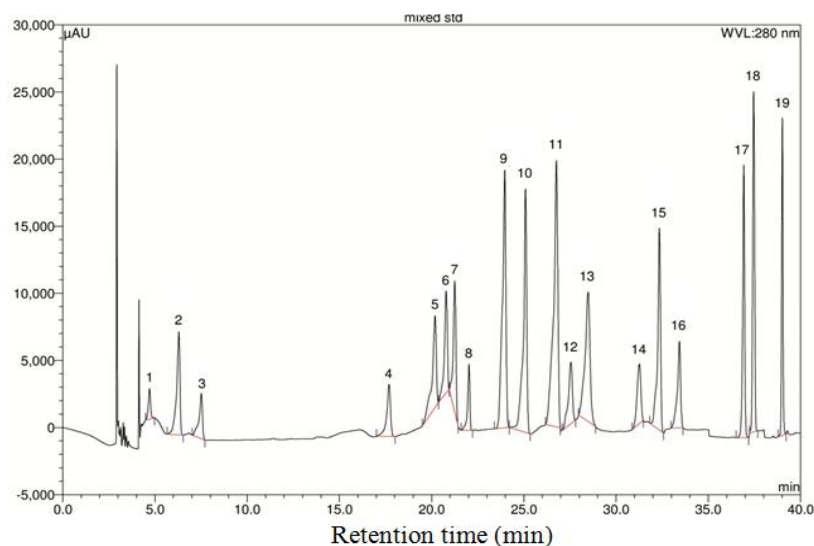


Figure 1: HPLC chromatogram of a standard mixture of polyphenolic compounds. Peaks 1: arbutin, 2: gallic acid, 3: hydroquinone, 4: (+)-catechin, 5: vanillic acid 6: caffeic acid, 7: syringic acid, 8: (-)-epicatechin, 9: vanillin, 10: *p*-coumaric acid, 11: *trans*-ferulic acid, 12: rutin hydrate, 13: ellagic acid. 14: benzoic acid, 15: rosmarinic acid, 16: myricetin, 17: quercetin, 18: *trans*-cinnamic acid, 19: kaempferol.

The content of each phenolic compound was calculated from the corresponding calibration curve and presented as the mean of five determinations as shown in table I.

The experimental results indicated presence of myricetin, gallic acid, ellagic acid, vanillic acid, syringic acid, epicatechin, vanillin, *p*-coumaric acid, *trans*-cinnamic acid and kaempferol. Among the compounds myricitene, a member of flavonoids possess strong

antioxidant. Antioxidant activity of HPLC detected compounds is recognized in previous studies (Emira *et al.*, 2014). The investigated extract showed a free radical scavenging activity in the DPPH assay ( $\text{IC}_{50} = 14.68\mu\text{g/mL}$ ) which is comparable to that of standard antioxidant ascorbic acid ( $\text{IC}_{50} = 24.95\mu\text{g/mL}$ ). Also reducing power of extract was comparable to that of standard. Extract showed maximum absorption of 1.317 and 0.942 in where

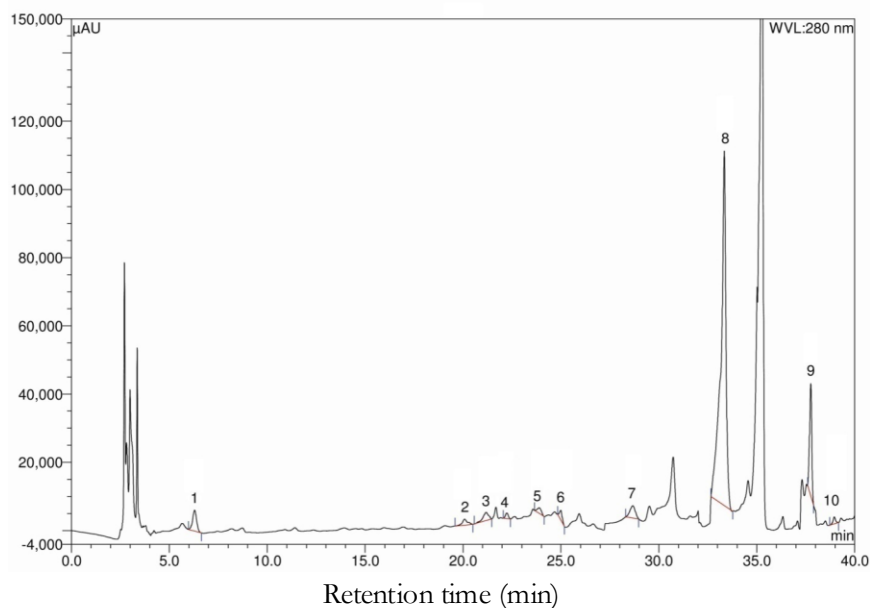


Figure 2: HPLC chromatogram of *D. alata*. Peaks 1: gallic acid, 2: vanillic acid, 3: syringic acid, 4: epicatechin, 5: vanillin, 6: *p*-coumaric acid, 7: ellagic acid, 8: myricetin, 9: *trans*-cinnamic acid, 10: kaempferol.

standard BHT showed 1.472 and 1.242 at 1mg/mL and 0.5mg/mL respectively. Strong DPPH free radical and reducing power activity of the extract may be due to the presence of higher content of myricetin along with other detected polyphenols that change DPPH free radical and Fe (III) in their reduced form.

The extract also showed antibacterial activity against all experimental bacterial strains except *Staphylococcus epidermidis*. At the concentration of 500 $\mu$ g/disc extract showed maximum zone of inhibition of 17.16mm against *Shigella dysenteriae* and at lower dose 200 $\mu$ g/disc maximum zone of inhibition was 8.52mm against *Shigella flexneri* (table II). Antibacterial activity of plants can be detected by observing the growth response of various microorganisms to those plant extracts that are placed in contact with them. Medicinal plants containing phenolic and flavonoids have been reported to possess antibacterial activity in previous studies (Emira *et al.*, 2014). Antimicrobial activity of myricetin, gallic acid, vanillic acid, kaempferol, *p*-coumaric acid, *trans*-cinnamic acid, epicatechine, vanillin has been reported (Monika *et al.*, 2014; Emira *et al.*, 2014). In the present study antibacterial activity

of *D. alata* might be due to the natural polyphenols present in the extract.

## CONCLUSION

Present study showed that methanol extract of *D. alata* exerts antioxidant and antibacterial activities. The presence of myricetin along with other detected polyphenols might contribute to these activities.

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