

## ***In vitro* Bioactivities of Aerial Parts of *Dioscorea alata***

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### **Abstract**

The methanol extract of aerial parts of *Dioscorea alata* and its organic and aqueous soluble materials were subjected to screenings for antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities. The polyphenol content and DPPH free radical scavenging assay were conducted to evaluate the antioxidant activity. The total phenolic content was found to vary for different test samples ranging from 23.69±0.25 to 26.60±0.22 mg of GAE/gm of dried extract. In DPPH method the chloroform soluble fraction (CSF) of *D. alata* revealed the highest free radical scavenging activity with IC<sub>50</sub> 25.33±0.05 µg/ml. The petroleum ether soluble fraction (PESF) exhibited highest cytotoxic potential having LC<sub>50</sub> value of 6.1 µg/ml. During the membrane stabilizing assay, the aqueous soluble fraction (AQSF) inhibited 31.91% and 47.55% haemolysis of RBC in hypotonic solution- and heat-induced method respectively. In thrombolytic assay, the chloroform soluble fraction (CSF) displayed 40.45% clot lysis. On the other hand the carbon tetra chloride (CTCSF) and chloroform soluble fraction (CSF) revealed mild to moderate antimicrobial activity with zone of inhibition ranging from 8 to 12 mm.

**Key words:** *Dioscoreaalata*, antioxidant, cytotoxicity, membrane stabilizing, thrombolytic, antimicrobial.

### **Introduction**

The genus *Dioscorea* belonging to the family Dioscoreaceae, commonly known as yam, comprises of about 600 species distributed throughout the world, but mostly in tropical regions. Most species contain steroidal saponins and sapogenins, such as diosgenin, dioscorin, dioscin which are the starting materials for synthesis of many steroidal hormones used as anti-inflammatory agents and contraceptive drugs. *Dioscorea alata* commonly known as 'greater yam' and locally known as 'Kathaloo', are climbing perennial vines with heart-shaped leaves (Dattuet *et al.*, 2015). Among various yams, *D. alata* is also known for its high nutritional values with crude protein. Traditionally tuber paste is applied on cancerous wounds, leprosy, gonorrhoea and skin diseases. About 2-3 gm of paste of the tuber is tied on the infected part to heal. The root contains phyosterols, alkaloids, tannins, carbohydrates and other substances like aluminium, ascorbic acid, beta-carotene, calcium,

chromium, cobalt, iron, magnesium, manganese, niacin, potassium, phosphorus, protein, riboflavin, selenium, silicon, sodium, thiamine, tin, zinc etc (Chowdhury *et al.*, 2008). *D. alata* has numerous edible and medical uses, due to its high carbohydrate content in the form of starch. The root is considered to be the ideal source for protein and energy. Recent pharmacognostic studies have demonstrated potent immunomodulatory activities of *D. alata* extracts (Dey and Chaudhuri, 2015). Much works have been done on various medicinal and clinical aspects of the *D. alata* tubers (Daset *et al.*, 2012; Chenet *et al.*, 2003), but no such substantial evidence exists on the study with aerial parts of this plant. So, an attempt was made to assess the *in vitro* bioactivities of the aerial parts of *D. alata*.

### **Materials and Method**

*Collection of plant materials:* Aerial parts of *D. alata* were collected from Dhaka, Bangladesh in

October, 2015. The plant was authenticated by an expert botanist of Bangladesh National Herbarium (DACB), Mirpur, Dhaka (Accession Number: 40388) and a voucher specimen has been submitted for future reference.

**Extraction:** After proper washing, the aerial parts were sun dried and ground to a coarse powder. Methanolic extract was prepared by 400 gm of powder soaked in 2.5 liters of methanol. The concentrated methanolic extract was partitioned using petroleum ether, chloroform, carbon tetrachloride and aqueous by modified Kupchan partitioning protocol and the resultant partitionates were evaporated to dryness to yield petroleum ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble fractions. The residues were then stored in a refrigerator until further use.

**Table 1. Kupchanpartitionates of *D. alata***

Crude extract/ fraction	<i>D. alata</i> (g)
ME	5.53
PESF	0.57
CTCSF	0.63
CSF	0.42
AQSF	1.68

ME= methanolic crude extract; PESF= pet ether soluble fraction; CTCSF= carbon tetrachloride soluble fraction; CSF= chloroform soluble fraction; AQSF= aqueous soluble fraction.

**Total phenolic content:** The total phenolic content of the extractives was determined with Folin-Ciocalteu reagent by using the method developed by Harbertson and Spayd (2006).

**DPPH free radical scavenging assay:** Following the method developed by Brand-Williams *et al.*, (1995), the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxyl toluene (BHT) and ascorbic acid as positive controls.

**Brine shrimp lethality bioassay:** This technique was applied for the determination of general toxic properties of the dimethylsulfoxide (DMSO) solution

of plant extractives against *Artemia salina* in a single day assay (Meyer *et al.*, 1982) by using vincristine sulphate as positive control.

**Membrane stabilizing activity:** The membrane stabilizing activity of the extractives was evaluated by the inhibition of heat- and hypotonic solution-induced haemolysis of human erythrocytes following the method developed by Omale *et al.* (2008).

**Thrombolytic activity:** The method developed by Prasad *et al.*, (2007) and Harbertson *et al.* (2006) was used to determine the thrombolytic activity by using lyophilised streptokinase (SK) as positive control.

**Antimicrobial screening:** Antimicrobial activity was determined by the disc diffusion method (Bauer *et al.*, 1966). Five strains of Gram-positive bacteria and eight Gram-negative bacteria were used to evaluate the antimicrobial activity.

**Statistical analysis:** For all bioassays, three replicates of each sample were used for statistical analysis and the values are reported as mean  $\pm$  SD.

## Results and discussion

The crude methanol extracts of aerial parts of *D. alata* as well as their partitionates were evaluated for the total phenolic content, free radical scavenging, cytotoxic, membrane stabilizing, thrombolytic and antimicrobial activities. The total phenolic content of the extractives of *D. alata* was found in the range of  $23.687 \pm 0.25$  to  $26.603 \pm 0.22$  mg of GAE/gm of extractives, with the highest amount of phenolics ( $26.603 \pm 0.22$  mg) being observed in the chloroform (CSF) soluble fraction (Table 2).

In the DPPH free radical scavenging assay, the chloroform (CSF) soluble fraction of *D. alata* revealed maximum free radical scavenging activity ( $IC_{50} = 25.325 \pm 0.05$   $\mu$ g/ml) when compared to ascorbic acid ( $IC_{50} = 5.80$   $\mu$ g/ml) (Table 2).

In the brine shrimp lethality bioassay, the pet ether (PESF) soluble fraction of *D. alata* displayed the highest cytotoxic potential with  $LC_{50}$  value  $6.10 \pm 0.43$   $\mu$ g/ml as compared to  $0.45$   $\mu$ g/ml for vincristine sulphate. This suggested the presence of potent

bioactive components in the above mentioned extractives (Table 2).

The extractives of *D. alata* were assayed for thrombolysis to determine the ability of clot lysis. Addition of 100µl streptokinase (SK), a positive control (30,000 I.U.) to the clots of human blood and subsequent incubation for 90 minutes at 37°C displayed 65.16% lysis of the clot as compared to distilled water which displaying a negligible lysis of clot (3.09%). In this study, different extractives of *D. alata* demonstrated significant clot lysis from 11.37% to 40.45% (Table 3).

The membrane stabilizing activity of the extractives of *D. alata* was also determined. They significantly protected the lysis of human erythrocyte membrane induced by heat and hypotonic solution, as compared to the standard acetyl salicylic acid. In hypotonic solution-induced condition, the aqueous extract of *D. alata* (2.0mg/ml) inhibited 31.91% haemolysis of RBCs as compared to 43.74% revealed by acetyl salicylic acid (0.10mg/ml) and in heat-induced condition, the aqueous extract inhibited 47.55% haemolysis of RBCs as compared to 24.97% shown by standard acetyl salicylic acid (0.10mg/ml) (Table 3).

**Table 2. Total phenolic content, free radical scavenging and cytotoxic activity of *D. alata*.**

Test sample	Total phenolic content (mg of GAE/gm of extractive)	DPPH Free radical scavenging activity IC <sub>50</sub> (µg/ml)	Cytotoxicity LC <sub>50</sub> (µg/ml)
ME	23.687±0.25	26.716± 0.85	26.57±0.18
PESF	25.080±0.05	27.479±0.22	06.10 ±0.43
CTCSF	25.599±0.18	25.937±0.09	11.33±0.09
CSF	26.603±0.22	25.325±0.05	14.04 ±0.21
AQSF	24.027±0.32	25.403±0.14	57.62±0.05
VS			0.45
BHT		23.50	
Ascorbic acid		5.8	

ME = methanolic extract, PESF = pet ether soluble fraction, CTCSF = carbon tetrachloride soluble fraction, CSF = chloroform soluble fraction and AQSF = aqueous soluble fraction.

**Table 3. Percentage inhibition of hypotonic solution and heat-induced hemolysis of erythrocyte membrane and thrombolytic activity of *D. alata*.**

Standard/ Sample	Concentration	% clot lysis	%inhibition of hemolysis	
			hypotonic solution-induced	heat-induced
ME	2.0 mg/ml	27.50±0.14	15.20±0.26	17.29±0.34
PESF	2.0 mg/ml	31.61±0.34	12.27±0.58	32.18±0.48
CTCSF	2.0 mg/ml	11.37±0.56	19.95±0.87	18.78±0.24
CSF	2.0 mg/ml	40.45±0.21	12.59±0.21	29.07±0.19
AQSF	2.0 mg/ml	25.84±0.11	31.91±0.53	47.55±0.12
ASA	0.1 mg/ml		43.74±0.18	24.97±0.23
Blank		3.09±0.25		
Streptokinase		65.16±0.90		

ME = methanolic extract, PESF = pet ether soluble fraction, CTCSF = carbon tetrachloride soluble fraction, CSF = chloroform soluble fraction and AQSF = aqueous soluble fraction. ASA = acetyl salicylic acid.

The extractives of *D. alata* when screened for antibacterial activity against five gram positive and eight gram negative bacteria at a concentration of 400µg/disc, the chloroform (CSF) and the carbon tetra chloride (CTCSF) soluble fractions of *D. alata* revealed mild to moderate inhibitory activity against the test pathogens having zone of inhibition ranging from 08.0-12.0 mm. The highest inhibition of bacterial growth of the chloroform soluble fraction

(CSF)(12.0 mm) against *Salmonella paratyphi*. Same fraction displayed (11.0 mm) against *Shigella dysenteriae* and *Sarcinalutea* (Table 4). On the other hand, the methanolic (ME), pet ether (PESF) and aqueous (AQSF) soluble fractions of *D. alata* exhibited no antimicrobial activity. The inhibitory activity of the extractives were compared with ciprofloxacin (30µg/disc) as standard.

**Table 4. Antimicrobial activity of *D. alata*.**

Test microorganism	Test sample ( <i>Dioscorea alata</i> ) diameter of zone of inhibition (mm)					Ciprofloxacin
	ME	PESF	CTCSF	CSF	AQSF	
<i>Bacillus cereus</i>	-	-	10.0	10.0	-	41.0
<i>Bacillus megaterium</i>	-	-	7.0	7.0	-	40.0
<i>Bacillus subtilis</i>	-	-	7.0	7.0	-	35.0
<i>Staphylococcus aureus</i>	-	-	7.0	7.0	-	38.0
<i>Sarcinalutea</i>	-	-	8.0	11.0	-	39.0
<i>Salmonella paratyphi</i>	-	-	10.0	12.0	-	36.0
<i>Salmonella typhi</i>	-	-	-	7.0	-	30.0
<i>Vibrio parahemolyticus</i>	-	-	-	-	-	34.0
<i>Escherichia coli</i>	-	-	8.0	8.0	-	38.0
<i>Vibrio mimicus</i>	-	-	8.0	8.0	-	35.0
<i>Shigelladysenteriae</i>	-	-	11.0	11.0	-	28.0
<i>Pseudomonas aeruginosa</i>	-	-	8.0	7.0	-	40.0
<i>Shigellaboydil</i>	-	-	10.0	10.0	-	40.0

## Conclusion

It is clearly evident from the above findings that different fractionates of the aerial parts of *D. alata* displayed mild to moderate free radical scavenging, cytotoxic, thrombolytic potential and membrane stabilizing properties. Our findings justify the traditional uses of this plant species. Therefore, the plant is good candidates for further chemical investigations to isolate the active constituents.

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