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# Significant Oxidative Stress Reduction by *Dioscorea alata* (Dioscoreaceae): Common Tuberous Vegetable in Bangladesh

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**Abstract**
Plants are the sources of variety of important secondary metabolites, in which some are often used as antioxidants. Hence, the objective of this study is to evaluate the free radical scavenging activity, total flavonoid and total phenolic content of underground tuber and aerial fruits (used as vegetable) of *Dioscorea alata* to get an idea about in-vitro oxidative stress reduction activity. Shade dried powders of tubers and fruits were extracted with solvents having different polarity for experimental assay procedure. Additionally, tuber and fruits were boiled with water to get hot water extract to make it simulation with traditional cooking method. Due to the different solvent polarity, antioxidant capacity ($IC_{50}$ values), total phenolic content and total flavonoid content varied significantly. It was observed that aqueous soluble fraction possessed highest antioxidant capacity (lowest $IC_{50}$ value= 31.89 µg/ml for tuber and 47.69 µg/ml for fruits) in comparable with standard BHT having $IC_{50}$ value 20.39 µg/ml); total phenolic content 37.80 and 29.15 mg of gallic acid equivalent/gm of extract for tuber and fruits respectively; total flavonoid content 10.56 and 9.67 mg of quercetin/gm of extract for tuber and fruits respectively. It is also mentionable that hot water extract revealed significant free radical scavenging activity ($IC_{50}$ value 27.65 µg/ml for tuber & 41.9 µg/ml for fruits), total phenolic content (43.56 and 37.85 mg of GAE/gm of extract for tuber and fruits) and total flavonoid content (15.78 & 13.56 mg of GAE/gm of extract for tuber and fruits). Antioxidant capacity, total phenolic and total flavonoid content of tuber and fruits were strongly positively correlated with significant P values ($P < 0.01$). Presence of significant phenolic compounds and flavonoids along with highest antioxidant capacity concluded that tubers and fruits of *D. alata* those are taken as vegetable can significantly reduce oxidative stress in human subject.

**Keywords:** *Dioscorea alata,* antioxidant capacity, total phenolic content, total flavonoid content, oxidative stress.

## Introduction
*Dioscorea alata* L. belongs to the family Dioscoreaceae which is also known as winged yam and used as tuberous vegetable in Bangladesh (Bangali name: mete alu) [1]. The underground edible tuber and areal fruits of *D. alata* are considered as functional food with high nutritive value and therapeutic potential. The tuber is known to possess anti-inflammatory properties in traditional medicine [2], contains diosgenin which is known as immunomodulatory agent activates the T cells on mice model to improve memory after orally treated with ethanol extract of purple yam tuber [3]. It is also reported that effects of folic acid of *D. alata* has improved neuroprotective capacity on different regions of the brain in HHcy-induced brain oxidative stress of rats with methionine as hyperhomocysteinemia (HHcy) is an oxidative stress inducer, has been implicated in several oxidative-related neurodegenerative diseases [4]. *D. alata* has reported for reducing the lipid peroxidation, brain pathological changes and the deterioration in the learning and memory ability in mice possibly because antioxidant compounds present in tuber [5]. It has also demonstrated that *D. alata* extract (methanol) has been significantly reduce serum triglycerides, phospholipid and to increase high density lipid (HDL) levels [6].

Plants are rich source of antioxidants that protect cells against the damaging effects of ROS (reactive oxygen species) such as superoxide, hydroxyl radicals, singlet oxygen, peroxy radicals etc. These active oxygen species and free radicals can attack molecules in biological membranes and tissues and thus inducing oxidative stress that further has been associated with cancer, ageing, inflammation, neurodegenerative diseases, hypertension, artherosclerosis [7, 8, 9, 10]. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. The main characteristic of an antioxidant is its ability to trap free radicals (Walton

NJ and Brown DE, 1999). It has been mentioned that antioxidant activity of plants might be due to their antioxidant compounds like vitamin C, vitamin E, carotenes, phenolic acids, phytoestrogen, phytate, tocophenols, benzoic acids, folic acid etc [11]. Additionally flavonoids are polyphenolic compounds that are also very common in plant sources. Phenolics are characterized by at least one aromatic rings (C6) bearing one or more hydroxyl groups [12]. Recently flavonoids and phenols have aroused considerable interest because of their beneficial effects on human health mainly antioxidant activity that depends on their molecular structure.

There are very few studies of reactive oxygen species in relation to different organic extracts as well as aqueous soluble fractions of underground tubers and areal fruits of *D. alata* against free radicals like DPPH and their relation to total phenolic and flavonoidal contents i.e. oxidative stress reduction Therefore, in this study an attempt has been made to investigate the total phenolic content (TPC) and total flavonoid content (TFC) and to evaluate the antioxidant activities of tuber and fruits of *D. alata* by using widely accepted protocol.

## Materials and Methods

### Collection of plant materials

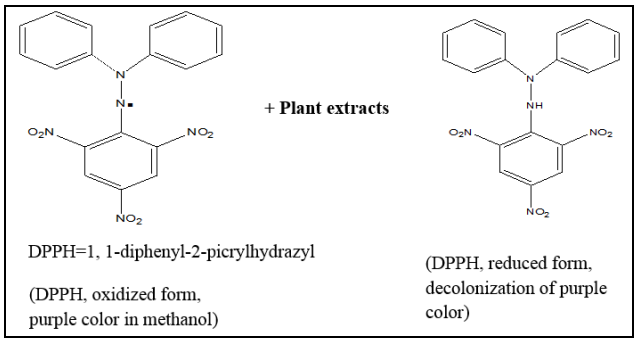
Underground tuber and aerial fruits of *D. alata* were collected from Savar region, Dhaka, Bangladesh in August, 2016 and identified by a botanist at Bangladesh National Herbarium. A voucher specimen (DACB 39908) for the plant sample was kept in Bangladesh National Herbarium for future reference.

### Preparation of plant extracts

Underground tuber and aerial fruits of *D. alata* were used for this study as separate experimental sample. After collection of the plant materials it was washed and dissected into small pieces for easy drying. After shade drying for several days, tuber and fruits slices were grinded into powder and kept in dark air tight container for further experimental purposes. Dried powder of tuber and fruits (150gm of each) were soaked into 500 ml of methanol, n-hexane, carbon tetrachloride, chloroform and aqueous media for cold solvent extraction respectively. After several days with occasional stirring plant materials were filtered (by cotton and Whatman filter paper) for clear solvent extracts. Additionally tuber and fruits powder were boiled separately with water and collected the filtrate to get hot water extract. Then the resulting filtrates were reduced in volume and made concentrated by using rotary evaporator [13]. Free radical scavenging activity, total flavonoid and total phenolic content were determined from prepared extracts.

### Assay for free radical scavenging activity: DPPH assay

In-vitro free radical scavenging activity (antioxidant capacity) of different solvent extracts along with hot water extracts of *D. alata* were estimated by reacting with stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Different solvent extracts of tuber and fruits having concentration of each 2.0 mg/ ml were diluted at different concentrations (2 ml each) mixed with 3.0 ml of a DPPH methanol solution (20 µg/ ml). The antioxidant capacity was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extracts as compared to standard antioxidant tert- butyl-1-hydroxytoluene (BHT) by UV spectrophotometer [14].




Antioxidant capacity or inhibition of free radical DPPH in percent (I %) was calculated as follows

$$\text{Percent of inhibition} = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph plotted inhibition percentage against extract concentration.

### Assay for total phenolic content (TPC)

Total phenolic content of different tuber and fruits extracts of *D. alata* (concentration 2 mg/ml of each 0.5 ml) were determined by reacting with 2.5 ml Folin-Ciocalteu reagent (10 times diluted) in alkaline solution of 2.0 ml $Na_2CO_3$ (7.5 % w/v solution). Folin-Ciocalteu reagent oxidized the ionized phenols present at plant extracts in alkaline solution and pertaining blue color due to oxidation of phenols from original yellow color of Folin-Ciocalteu reagent. The intensity of the color change was measured by spectrophotometer and resulting absorbance value will reflect the total phenolic content of the compound. The phenolic contents of the plant extracts were compared with standard gallic acid and expressed as mg of GAE (Gallic acid equivalent) / gm of the extract [15].

Phenols + $Na_2CO_3$ $\xrightarrow{\text{Complete ionization}}$ Ionized phenols

Ionized phenols + Folin-Ciocalteu reagent (Yellow) $\xrightarrow{\text{Oxidation}}$ Folin-Ciocalteu reagent complex (Blue)

### Assay for total flavonoidal content (TFC)

Total flavonoid content of different extracts of *D. alata* was determined by using aluminium chloride ($AlCl_3$) colorimetric method [16, 17] with slight modifications using quercetin as standard. One ml of each solvent extracts (methanol, n-hexane, carbontetrachloride, chloroform and aqueous soluble partitionates) were added to 4ml of water and subsequently 0.3ml of 5% $NaNO_2$ in a 10 ml volumetric flask. After 5 minutes resulting mixtures of each extracts were reacted with 0.3ml of 10% $AlCl_3$ and subsequently 2ml of 1M NaOH, and total volume was made up to 10ml with distilled water. The absorbance of the reaction mixtures were measured and compared with standard quercetin. Total flavonoidal content of the extracts were expressed in milligram of quercetin equivalents/gm of extract.

Total flavonoidal content can be calculated from the formula:

$$T = \frac{C_1 V}{M}$$

Where,
T=Total flavonoidal concentration
C= Concentration of quercetin from caliberation curve
V= Volume of extract
M= Weight of different plant extracts

**Statistical analysis**
Experimental results were expressed as means ± standard deviation (SD) and all measurements were replicated three times. Free radical scavenging activity ($IC_{50}$) values were also calculated by linear regression analysis Obtained results were analyzed for Pearson correlation coefficient(r) between total phenolic, flavonoid and antioxidant capacity using the Microsoft Excel 2010 software The values were considered to be significantly different at $P < 0.01$.

**Results**
Underground tuber and aeriall fruits of *D. alata* were partitioned into methanol, n-hexane, carbon-tetrachloride, chloroform and aqueous soluble fractions along with hot water extracts. The resultant partitions were subjected for assay of antioxidant capacity, total phenolic and total flavonoid content by following different standard protocols.
Antioxidant capacity, total phenolic and total flavonid content of tuber and fruits of *D. alata* were showed in table 1 and table 2 respectively. Here antioxidant capacity was expressed in terms of $IC_{50}$ value where total phenolic and total flavonoid content were expressed in terms of mg of gallic acid equivalent (GAE) and mg of quercetin equivalent (QE) per gram of the dry weight basis. The results revealed that antioxidant capacity, total phenolic and total flavonoid content vary in different solvent extracts of *D. alata*.
It was observed that hot water soluble extract of underground tuber exhibited the highest free radical-scavenging activity (lowest $IC_{50}$ value= 27.65 µg/ml in comparable with standard BHT having $IC_{50}$ value 20.39 µg/ml) as well as highest total phenolic content (43.56 mg of gallic acid equivalent/ gm of extract) and total flavonoid content (15.78 mg of quercetin equivalent/ gm of extract). Additionally, among all other partitionates obtained by cold solvent extraction process of tuber, aqueous soluble extract and methanolic extract showed significant antioxidant capacity; total phenolic and total flavonoid content. (Table 1).

**Table 1:** $IC_{50}$ values, total phenolic and total flavonoid content of different partitionates of tuber of *D. alata*

| Extracts of underground tuber of *D. alata* | Antioxidant capacity ($IC_{50}$ value, µg/ml) | Total phenolic content (mg of gallic acid equivalent/ gm of extract) | Total flavonoid content (mg of quercetin equivalent/ gm of extract) |
|---|---|---|---|
| Methanolic extract | 33.75 ± 0.651 | 39.89 ± 0.121 | 11.15 ± 0.221 |
| n-hexane soluble extract | 51.90 ± 0. 426 | 16.80 ± 0.357 | 6.45 ± 3.51 |
| Carbon-tetrachloride soluble extract | 47.73 ± 0.730 | 19.70 ± 0.750 | 7.25 ± 2.31 |
| Chloroform soluble extract | 60.48 ± 1.02 | 11.5 ± 2.89 | 3.56 ± 0.360 |
| Aqueous soluble extract | 31.89 ± 0.312 | 37.80 ± 0.346 | 10.56 ± 0.435 |
| Hot water extract | 27.65 ± 0.035 | 43.56 ± 0.221 | 15.78 ± 0.530 |
| Standard BHT | 20.39 ± 0.089 | - | - |

**Each value is expressed as mean ± S.E. (Standard Error, where n=3)**
Furthermore, hot water extract of aerial fruits revealed highest antioxidant capacity among all solvent partitionates (lowest $IC_{50}$ value= 41.9 µg/ml in comparable with standard BHT having $IC_{50}$ value 20.39 µg/ml) as well as highest total phenolic content (37.85 mg of gallic acid equivalent/ gm of extract) and total flavonoid content (13.56 mg of quercetin equivalent/ gm of extract). Results also showed that aqueous soluble extract and methanolic extract possessed moderate antioxidant capacity; total phenolic content and total flavonoid content. (Table 2)

**Table 2:** $IC_{50}$ values, total phenolic and total flavonoid content of different partitionates of fruits of *D. alata*

| Extracts of aerial fruits of *D. alata* | Antioxidant capacity ($IC_{50}$ value, µg/ml) | Total phenolic content (mg of gallic acid equivalent/ gm of extract) | Total flavonoid content (mg of quercetin equivalent/ gm of extract) |
|---|---|---|---|
| Methanolic extract | 59.85 ± 0.165 | 21.92 ± 0.340 | 8.05 ± 0.750 |
| n-hexane soluble extract | 74.56 ± 0.359 | 13.35 ± 2.67 | 6.75 ± 0.805 |
| Carbon-tetrachloride soluble extract | 67.70 ± 0.291 | 17.45 ± 0.875 | 7.25 ± 0.446 |
| Chloroform soluble extract | 109.4 ± 0.095 | 8.95 ± 0.643 | 5.45 ± 0.332 |
| Aqueous soluble extract | 47.69 ± 0.097 | 29.15 ± 0.371 | 9.67 ± 3.60 |
| Hot water soluble extract | 41.90 ± 0.597 | 37.85 ± 1.73 | 13.56 ± 0.235 |
| Standard BHT | 20.39 ± 0.089 | - | - |

**Each value is expressed as mean ± S.E. (Standard Error, where n=3)**
Correlation coefficient between total phenolic, total flavonoid content and antioxidant capacity of different fractions for both tuber and fruits was determined in order to establish the relation between them and it was found that most of the partitionates revealed strong positive correlation with significant p values where $P< 0.01$ (Table 3 & 4). Among all of the fractions, n-hexane soluble fraction of tuber (Table 3) and chloroform & n-hexane soluble fractions of fruit showed moderate positive correlation with non-significant P values (Table 4).

**Table 3:** Correlations between total phenolic, total flavonoids content and antioxidant capacity ($IC_{50}$ values) of underground tuber of *D. alata*

| Extracts of underground tuber of *D. alata* | Total phenolic content and Antioxidant Capacity ($IC_{50}$ values) | | | Total flavonoid content and Antioxidant Capacity ($IC_{50}$ values) | | |
|---|---|---|---|---|---|---|
| | Correlation (r) | P value (<0.01) | Remarks | Correlation (r) | P value (<0.01) | Remarks |
| Methanolic extract | 0.9615 | 0.009016 | Significant | 0.9619 | 0.008876 | Significant |
| n-hexane soluble extract | 0.0784 | 0.90028. | Not significant | 0.5006 | 0.391002 | Not significant |
| Carbon-tetrachloride soluble extract | 0.8937 | 0.040934 | Significant | 0.8847 | 0.0875 | Significant |
| Chloroform soluble extract | 0.9089 | 0.032553 | Significant | 0.8903 | 0.0987 | Significant |
| Aqueous soluble extract | 0.9826 | 0.002289 | Significant | 0.9027 | 0.0562 | Significant |
| Hot water extract | 0.9956 | 0.0022 | Significant | 0.9810 | 0.00231 | Significant |

**Table 4:** Correlations between total phenolic, total flavonoid content and antioxidant capacity ($IC_{50}$ values) of aerial fruits of *D. alata*

| Extracts of aerial fruits of *D. alata* | Total phenolic content and Antioxidant Capacity ($IC_{50}$ values) | | | Total flavonoid content and Antioxidant Capacity ($IC_{50}$ values) | | |
|---|---|---|---|---|---|---|
| | Correlation coefficient (r) | P value (<0.01) | Remarks | Correlation coefficient (r) | P value (<0.01) | Remarks |
| Methanolic extract | 0.9115 | 0.08916 | Significant | 0.9019 | 0.008576 | Significant |
| n-hexane soluble extract | 0.6084 | 0.85028. | Not significant | 0.7506 | 0.91802 | Not significant |
| Carbon-tetrachloride soluble extract | 0.8631 | 0.050932 | Significant | 0.8741 | 0.08609 | Significant |
| Chloroform soluble extract | 0.3089 | 0.79953 | Not significant | 0.4953 | 0.4987 | Not significant |
| Aqueous soluble extract | 0.9026 | 0.005689 | Significant | 0.9521 | 0.0079 | Significant |
| Hot water extract | 0.9786 | 0.00449 | Significant | 0.9613 | 0.00732 | Significant |

Antioxidant capacity, total phenolic and total flavonoid content of same solvent extracts between tuber and fruits varied significantly. A comparison of antioxidant capacity, total phenolic and total flavonoid content between same solvent extracts showed in figure 1, 2 & 3 respectively.

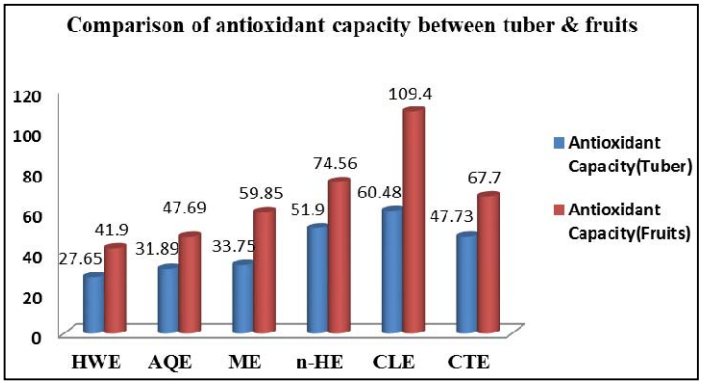



**Fig 1:** Comparison of antioxidant capacity of different solvent extracts between tuber & fruits

HWE= Hot water extract, AQE= Aqueous soluble extract, n-HE= n-Hexane soluble extract, CLE= Chloroform soluble extract and CTE= Carbon-tetrachloride soluble extract

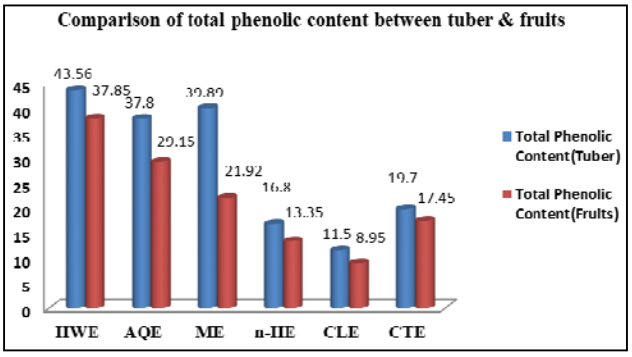



**Fig 2:** Comparison of total phenolic content of different solvent extracts between tuber & fruits

HWE= Hot water extract, AQE= Aqueous soluble extract, n-HE= n-Hexane soluble extract, CLE= Chloroform soluble extract and CTE= Carbon-tetrachloride soluble extract

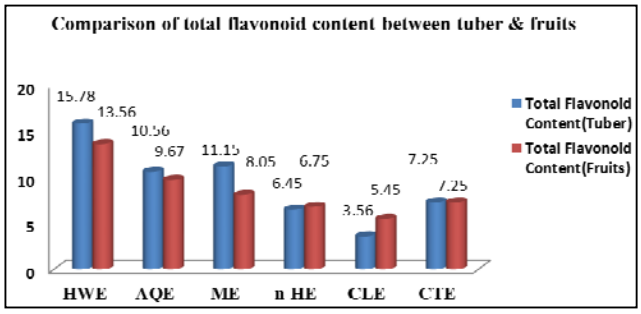



**Fig 3:** Comparison of total flavonoid content of different solvent extracts between tuber & fruits

HWE= Hot water extract, AQE= Aqueous soluble extract, n-HE= n-Hexane soluble extract, CLE= Chloroform soluble extract and CTE= Carbon-tetrachloride soluble extract

Percent of antioxidant capacity, total phenolic and total flavonoid content of hot water extract was compared with other solvent extracts for both tuber and fruits. It was observed that hot water extract possessed highest antioxidant capacity, total phenolic and total flavonoid content than all other solvent extracts (Figure 4, 5 & 6).

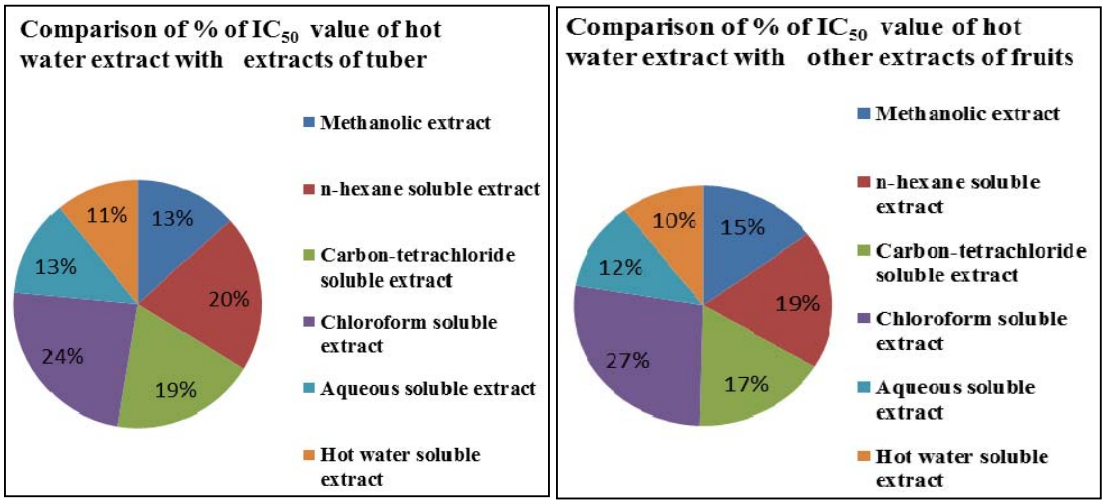



**Fig 4:** Comparison of % of antioxidant capacity of hot water extract with other solvent extracts of *D. alata*

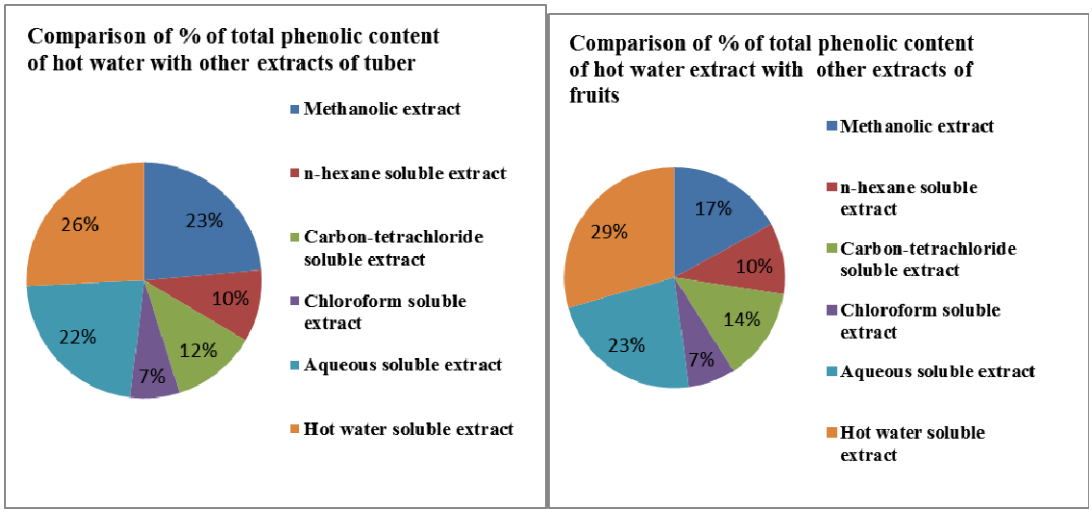



**Fig 5:** Comparison of % of total phenolic content of hot water extract with other solvent extracts of *D. alata*

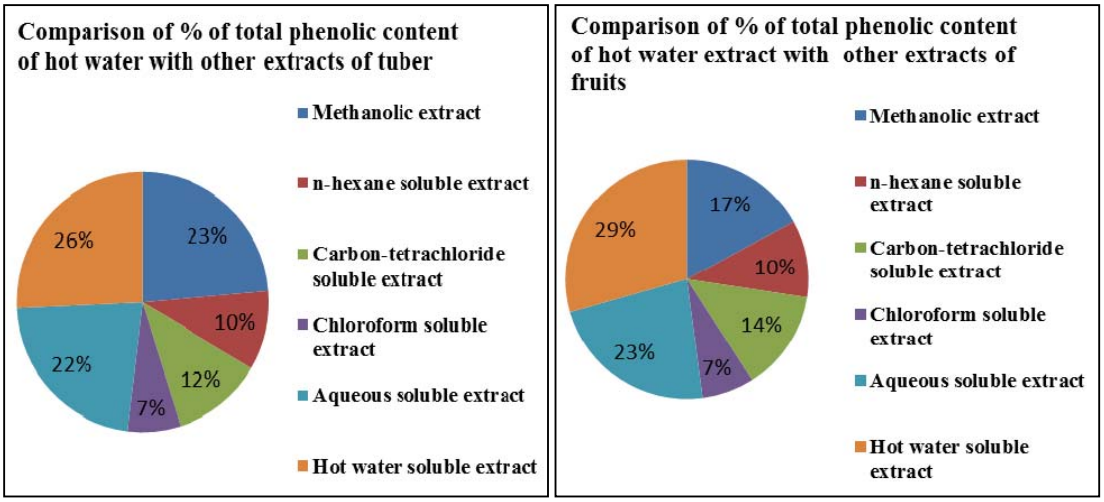



**Fig 6:** Comparison of % of total flavonoid content of hot water extract with other solvent extracts of *D. alata*

## Discussion

Different organic and aqueous solvent extracts of both tuber and fruits were subjected for estimation of antioxidant capacity, total phenolic and total flavonoid content and to establish a relationship between them. Experimental results varied significantly among different solvent extracts and same solvent extracts of tuber and fruits respectively.

Antioxidant compounds are usually in the phenolic form and Phenols & polyphenols are the most common and abundant compounds in plants. The antioxidant properties of phenolic compounds originate from their properties of proton loss, chelate formation, and dismutation of radicals because they

contain hydroxyl groups and can form stable phenoxyl radicals by giving up hydrogen ion [18, 19]. Therefore, determination of the quantity of phenolic compounds is very important in order to determine the antioxidant capacity of plant extracts. So it is necessary to explore more safe compounds from natural (plant) sources which can be used against oxidative stress. In this study aqueous soluble extract obtained from cold solvent extraction process possessed significant total phenolic, total flavonoid and thus highest free radical scavenging activity. The results also exhibited that hot water extract for both of tuber and fruits possessed highest antioxidant capacity due to presence of high contents of phenolics and flavonoids. Hot water extract basically resemblance the traditional cooking method in our country through which tuber of *D. alata* mainly eaten here. It has been reported that the antioxidant activity of many botanicals were proportional to their phenolic content, suggesting a causative relationship between them [20].

To find the relationship between the antioxidant activity, phenolics and flavonoid contents, a linear regression and correlation analyses has performed and the correlations of total phenolic and total flavonoid content against the antioxidant activity based on the $IC_{50}$ values obtained DPPH assay was strongly positive correlation with significant P values ($P<0.01$). Hot water extract showed good correlation with phenolics compared to flavonoids for both tuber and fruits show that phenols and polyphenols act through scavenging or chelating process for significant antioxidant capacity.

## Conclusion

Tuber and fruits of *D. alata* possess significant antioxidant capacity because of having significant amount of phenols, polyphenols and flvaonoid type of compounds. It is recommended that further investigation to isolate, purify and characterize important antioxidant type secondary metabolites for therapeutic application. To the best of our knowledge, the present study is the first report on tuber and fruits of *D. alata* having significant antioxidant capacity with strongly positive correlation between total phenolic and total flavonoid based on antioxidant capacity ($IC_{50}$ values) having significant P values.

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