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Research Article

Comparative Analysis of Chemical Attributes and Antioxidant Activity of *Dioscorea bulbifera* L. and *D. deltoidea* Wall. ex Griseb.

Raza Waris[1,2] Muhammad Arif [3], Manjoosha Srivastava[3], Amritesh C. Shukla[2*] & Priyanka Agnihotri[1*]

[1]*Plant Diversity, Systematics and Herbarium Division, CSIR-National Botanical Research Institute, Lucknow, 226001, Uttar Pradesh, India.*
[2]*Department of Botany, University of Lucknow, Lucknow, 226025, Uttar Pradesh, India.*
[3]*Phytochemistry Division, CSIR-National Botanical Research Institute, Lucknow, 226001, Uttar Pradesh, India.*

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ABSTRACT
Dioscorea L. is a member of the family Dioscoreaceae having many biological activities viz. anti-cancerous, anti-diabetic, anti-inflammatory, hepato-protective, antioxidant and many other activities. In the present study, we evaluated the phytochemical screening, extractive percent yield, total phenolic and flavonoids content, identification of secondary metabolites and antioxidant activity of different solvents based on their polarity. Results show that it is a good therapeutic plant. Phytochemical analysis revealed the presence of many secondary metabolites viz. carbohydrates, alkaloids, glycosides, sterols, tri-terpenoids, tannins, phenolic and flavonoids. The common secondary metabolites were identified at various Rf values in different solvent extracts of *Dioscorea* tubers. The maximum extractive yield was found in methanol extract of *D. bulbifera* leaves. The highest total phenolic content was estimated in methanolic extract of *D. deltoidea* leaves (384µg/m) while the total flavonoids content reported in *D. deltoidea* leaves (328µg/ml). The highest percent free l radical scavenging activity (84.62%) by DPPH assay at maximum concentration was recorded in methanolic extract of *D. bulbifera* leaves. The data prospects for exploration and utilization of leaves and tubers of *Dioscorea* species.
Keywords: *Dioscorea*; phytochemicals; secondary metabolites; antioxidant.

INTRODUCTION

Phytochemicals are found in natural products, particularly in plants. The most prevalent polyphenols in our diet are the flavonoids and phenolic compounds, which are found in a wide range of fruits, vegetables and cereals[1].Dietary polyphenols have received a lot on interest in recent years owing to its high antioxidant activities and their reliability in the treatment of many oxidative stress related disorders viz. cancer, diabetes, cardiovascular diseases and skin diseases etc.[2]. Medicinal plants contain various phytochemicals such as flavonoids, phenolic, tri-terpenoids and carotenoids having antioxidant activity, which is account for their health promoting properties [3]. Plant based antioxidants are more effective than synthetic antioxidants like butylatedhydroxy anisole (BHA) and butylatedhydroxy toluene (BHT) because of their natural origin and safer for utilization.

Dioscorea L. often called as Yam, is the type genus of the Dioscoreaceae family, consisting about 628 species in globally and 42 taxa (41 species and one variety) dispersed across India[4]. Dioscorea has long been valued as a food and medicinal source for different tribes and act as dietary staple for marginalized people in different locations due to the availability of nutraceutical and pharmaceutical compounds. The plant having several medicinally essential bio active compounds, making it one of the most valuable drug-producing plant. Dioscorea is known for its wide range of therapeutic characteristics, which are associated with presence of various natural phytochemicals such as flavonoids, glycosides, alkaloids, carotenoids, phenolic, steroidal saponins, tannins, triterpenoids and some volatile oils. Tubers and leaves of the plant are the most extensively utilized parts, with antioxidant, anti-bacterial, anti-inflammatory, anti-cancerous and anti-diabetic properties [5].

In our study, we focused on comparing different phytochemical parameters, identification of secondary metabolites, qualitative as well as quantitative estimation along with antioxidant activity of D. bulbifera and D. deltoidea (leaves and tubers).

MATERIALS AND METHODS

Plant materials: The plants (D. bulbifera and D. deltoidea) were collected from two different regions of Jammu and Kashmir (32º59’26.11’’N 74º55’58.19’’E and 34º06’4.8’’N 74º25’12.1’’E) in July 2019 at an altitude of 1500 m and 2662 m above sea level respectively. The plants were taxonomically identified and authenticated by plant diversity, systematics and herbarium division, CSIR-NBRI, Lucknow with a voucher specimens number 110192 and 110191 respectively and has been deposited in the LWG herbarium, Lucknow.

Preparation of Extract: The plant material was collected, and shade dried for 15 days at room temperature before being pulverized. The coarse powder of 10gm were subjected to cold successive extraction with 200ml of organic solvents with increasing polarity viz. Hexane, Chloroform, Acetone, Methanol, Ethanol and Aqueous for 24 hrs. Whatman filter paper was used to filter the extracts and was allowed to dry using rotatory evaporator. The

**Author for Correspondence: amriteshcshukla@gmail.com*

extracts were stored in airtight bottle at 4℃ and were used for the further investigation.

Qualitative analysis: Qualitative analysis of various extracts for the presence of different phytoconstituents was carried out by standard qualitative methods. Each extracts were screened for secondary metabolites like alkaloids, glycosides, tri-terpenoids, flavonoids, steroids, carbohydrate and phenolic and glycosides etc.

Quantitative analysis

Total phenolic content was assessed using Folin-Ciocalteu method and total flavonoids content were estimated following aluminium chloride colorimetric method (Bray and Thorpe1954; Ordonez et al, 2006)

Total Phenolic Content

The total phenolic content in tubers as well as leaves was estimated by spectrophotometric method followed by Bray and Thorpe (1954) with slight modification. 1 ml methanol extract, 0.5 ml folin-ciocalteau reagent and 1.5 ml 20% sodium carbonate solution were mixed. The tubes were kept for 2 hours at room temperature and absorbance taken at 765 nm. The phenolic content in sample was read from reference curve prepared using gallic acid as standard and expressed in mg/g of sample.

Total Flavonoid Content

The method of Ordonez et al (2006) was used to estimate total flavonoids in the plant extracts (tubers and leaves) with minor modification. 1 ml of plant extract was mixed with 0.5 ml of 2% AlCl3 ethanol solution. The absorbance at 420nm was measured after one hour at room temperature. The standard was quercetin, and total flavonoids content of the sample was determined using the calibration curve and expressed in mg/g sample.

Identification of secondary metabolites

TLC is a technique for assessing mixtures and identifying how many different compounds are present. It can also be used to identify and test purity of a substance. The chromate grams were taken on precoated silica plates (Silica gel 60 F254, Merck). About 10 µlof plant extracts were loaded on precoated tlc plate and plate were analyze under UV 254 nm and 366 nm. Run 3 solvent system viz. Toluene: Ethylacetate: Formic acid (8:2:0.4) for both phenolic and flavonoids, Toluene: Ethyl acetate (9.3:0.7) for tri terpenoids, Petroleum Ether (4:1) for Fatty acid and Petroleum Ether: Ethylacetate: Methanol for Steroids but Toluene: Ethylacetate: Formic acid (8:2:0.4) was found best suited for identification of phenolic and flavonoids compounds in D. bulbifera and D. deltoidea. Anisaldehyde sulfuric acid reagent was used as a spraying agent and Rf value was calculated.

Determination of antioxidant activity though DPPH assay

Using a free radical DPPH assay, the scavenging activity of the methanol extract was assessed. (Liyana Pathirana et al., 2005). 0.1 mM DPPH solution was prepared by dissolving 3.94 mg DPPH in 100 ml methanol and was kept in dark for 2 hours before use. About 10mg ascorbic acid was dissolved in 10 ml methanol, to obtain a solution of 1mg/ml. from this stock solution, various working dilution were prepared to get concentration of 200, 400, 600, 800, 1000 µg/ml with distilled water. 1 ml DPPH was added to different concentration of sample solution and incubate for 30 minutes, absorbance was taken at 517nm. Ascorbic acid was used as standard. This experiment was performed in triplicate. The following equation was used to determine the Percentage DPPH radical scavenging activity.

DPPH free radical scavenging activity (%) = [Abs control-Abs sample/Abs control]*100

Abs sample is the absorbance of extract/standard, whereas Abs control is the absorbance of control.

RESULT AND DISCUSSION

Qualitative analysis

The qualitative analysis showed the presence of different phytochemicals viz. carbohydrates, glycosides, tannins, phenols, alkaloids, flavonoids, sterols and tri-terpenoids in various solvent extracts (Table 1, 2). Bioactivity and therapeutic value of the plant extract are attributed by the bio active compounds of the plant extract.

Extractive yield

Table 3 and Figure 1 revealed the extractive yield of the various extracts of tubers and leaves of *D. bulbifera* and *D. deltoidea*. The extractive yield of different solvents of *D. bulbifera* leaves was found to be in order methanol > ethanol > acetone > water > hexane > chloroform in an amount 10.56% > 5.68% > 4.54% > 2.03% > 1.06% > 0.84%. While in *D. deltoidea* leaves was found to be maximum in methanol (9.86%) followed ethanol (6.08%), acetone (4.68%), water (2.14%), hexane (1.96%) and chloroform (0.91%). in the case of tubers, methanol extract (9.45%) of *D. bulbifera* had maximum extractive yield followed by ethanol, acetone, water, hexane and chloroform in an amount of (4.99%), (3.08%), (1.96%), (0.92%) and (0.57%) respectively. On the other hand, the tubers of *D. deltoidea*, methanol extract has greater extractive yield (8.94%) followed by ethanol extract (5.06%) while the least extractive yield showed in the hexane extract (0.88%) followed by chloroform extract (0.54).

Total Phenolic content

The total phenolic content of methanol extracts of tubers and leaves of *D. bulbifera* and *D. deltoidea* depicted in table 4. The methanol extract (384 mg/gm) of *D. deltoidea* leaves showed maximum phenolic compound followed by *D. bulbifea* leaves (354.4 mg/gm). In *D. bulbifera* (216.9 mg/gm) tubers, the methanol extract had maximum quantity of phenolic content followed by *D. deltoidea* (178 mg/gm). The linear relationship between absorbance and concentration was used to validate the results, yielding an R^2 value of 0.9983 (Fig. 2).

Total flavonoids content

Table 5 shows the total flavonoids content of methanol extracts of D. bulbifera and D. deltoidea (leaves and tubers). The methanol extract (328 mg/gm) of the leaves of D. deltoidea having maximum amount of flavonoids content followed by D. bulbifera leaves (228 mg/gm). The total flavonoids content of methanol extract of tubers of D.

bulbifera (313 mg/gm) showed the highest flavonoids content than D. deltoidea tubers (145 mg/gm). The linear association between absorbance and concentration was used to validate the results, yielding an R2 value of 0.9975 (Figure.3).

Table 1: Qualitative analysis of the phytochemicals present in the extracts of *Dioscorea bulbifera* tubers and leaves

Extracts	**Hexane**		**Chloroform**		**Acetone**		**Ethanol**		**Methanol**		**Aqueous**	
Chemical tests	**DBT**	**DBL**	**DBT**	**DBL**	**DBT**	**DBL**	**DBT**	**DBL**	**DBT**	**DBL**	**DBT**	**DBL**
Carbohydrates												
Molish's test	-	-	-	+	-	+	+	+	+	+	+	+
Bendict's test	-	-	-	-	-	-	+	+	+	+	-+	-
Fehling's test	+	-	-	-	+	-	+	+	-	+	+	-
Glycosides												
Modified Brontrager's test	-	-	-	-	-	+	+	+	+	+	-	-
Froth formation test	-	-	-	-	+	-	+	+	+	+	+	+
Protiens												
Xanthoproteic test	-	-	+	-	-	+	+	+	+	-	-	+
Tanins												
Ferric Chloride test	-	-	-	-	+	+	-	+	-	+	-	+
Phenolics												
Lead acetate test	-	-	-	-	-	+	+	+	+	+	+	+
Alkaloids												
Dragendroff's test	-	-	-	+	-	-	+	-	+	-	+	-
Mayer's test	-	-	-	-	-	+	+	+	+	-	+	-
Flavonoids												
Zinc hydrochloride reduction test	-	-	-	-	+	+	+	+	+	+	+	-
Alkaline reagent test	-	-	-	-	+	+	+	+	+	+	+	+
Sterols and triterpenoids												
Libermann- burchard test	-	+	-	+	+	-	+	-	+	-	-	-
Salkowski test	-	+	-	+	-	+	+	-	+	-	+	-
Killer killani test	-	-	-	+	-	-	+	-	+	-	+	-

Abbreviation: DBT- *Dioscorea bulbifera* tuber; DBL- *Dioscorea bulbifera* leaves; DDT- *Dioscorea deltoidea* tuber; DDL- *Dioscorea deltoidea* leaves

Table 2: Qualitative analysis of the phytochemicals present in the extracts of *Dioscorea deltoidea* tubers and leaves**.**

Extracts	Hexane		Chloroform		Acetone		Ethanol		Methanol		Aqueous	
Chemical tests	**DDT**	**DDL**	**DDT**	**DDL**	**DDT**	**DDL**	**DDT**	**DDL**	**DDT**	**DDL**	**DDT**	**DDL**
Carbohydrates												
Molish's test	-	-	-	+	-	-	+	+	-	-	+	+
Bendict's test	-	-	+	-	-	-	+	-	+	-	+	-
Fehling's test	-	-	+	+	-	-	+	+	+	+	+	+
Glycosides												
Modified Brontrager's test	+	-	+	-	-	+	+	+	+	+	+	-
Froth formation test	-	-	-	-	+	-	+	+	+	+	+	+
Protiens												
Xanthoproteic test	-	-	+	-	+	+	+	+	+	-	-	+
Tanins												
Ferric Chloride test	-	-	-	-	+	+	+	+	+	+	-	+
Phenolics												
Lead acetate test	-	-	-	-	+	+	+	+	+	+	+	+
Alkaloids												

Dragendroff's test	-	-	-	-	-	-	+	+	+	+	+	-
Mayer's test	-	-	-	-	+	-	+	-	+	+	+	+
Flavonoids												
Zinc hydrochloride reduction test	-	-	-	-	+	+	+	+	+	+	-	-
Alkaline reagent test	-	-	-	-	+	-	+	+	+	+	+	+
Sterols and triterpenoids												
Libermann-burchard test	+	+	+	+	+	+	-	-	-	-	-	-
Salkowski test	-	-	+	+	+	+	-	+	-	+	-	-
Killer killani test	-	-	-	+	-	-	+	-	+	-	+	-

Abbreviation: DBT- *Dioscorea bulbifera* tuber; DBL- *Dioscorea bulbifera* leaves; DDT- *Dioscorea deltoidea* tuber; DDL- *Dioscorea deltoidea* leaves

Table 3: Percentage yield of different extracts of the Species of *D. bulbifera* and *D. deltoidea*

S.NO	Solvent Extracts	Percent Yield of different solvent extracts			
		DBT	DBL	DDT	DDL
1.	Hexane	0.92	1.06	0.88	1.96
2.	Chloroform	0.57	0.84	0.54	0.91
3.	Acetone	3.08	4.54	3.54	4.68
4.	Methanol	9.45	10.56	8.94	9.86
5.	Ethanol	4.99	5.68	5.06	6.08
6.	Water	1.96	2.03	1.98	2.14

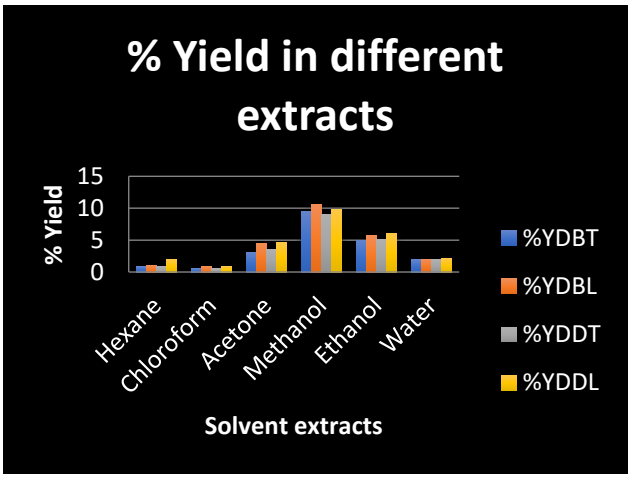

Figure.1: Percent yield of different solvents

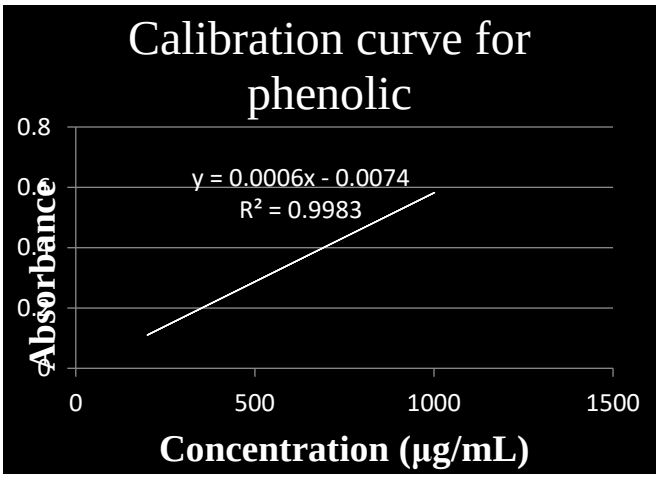

Figure.2: Standard curve for phenolic

Thin Layer chromatograph (TLC)

The TLC profile of methanolic extracts for *D. bulbifera and D. deltoidea* is summarized in Table 6 with their Rf values. Hexane extract showed the presence of Triterpenoids (Rf 0.625) and Fatty acid (Rf 0.712); Chloroform showed presence of Phenolic (Rf 0.687) and Steroids (Rf 0.829) followed by Acetone indicate the presence of Steroids (Rf 0.817) and Triterpenoids (Rf 0.6) while in methanol extract revealed the presence of Phenolic (Rf 0.375); Flavonoids (Rf 0.304); Triterpenoids (Rf 0.325) and Steroids (Rf 0.451). As a result, more research is needed to isolate, purify and characterized the molecules responsible for antioxidant activity.

In methanol extract, the solvent system Toluene:Ethylacetate:Formic acid (10:1.1:1.1:2.6) provided the best resolution and maximum number of components. As depicted in the table 6, different components were separated and identified.

DPPH radical scavenging activity

Table 7 and Figure 7 showed the DPPH radical scavenging ability of the leaves and tuber extracts of *D. bulbidera* and *D. deltoidea*. Methanol extract of leaves of *D. deltoidea* leaves at maximum concentration had the highest scavenging activity (84.62%) followed by *D. bulbifera* leaves (82.50%), *D. bulbifera* tubers (81.15%). The least radical scavenging activity was found in *D. deltoidea* tubers (79.97%). The methanol extract (leaves and tubers) has a free radical scavenging activity comparable to the ascorbic acid (77.35%).

Table 4: Determination of Total Phenolic content of *D. bulbifera* and *D. deltoidea* tubers and leaves

Plant sample	Sample solution (µl)	Wt.of dry extract/ml (M) (gm)	Absorbance (mean)	GAE Conc. (C) (µg/ml)	GAE Conc. (C) (mg/ml)	TPC as GAE, A=C*V/M (mg/gm)
DBT	1000	0.001	1.294	216.9	0.219	216.9
	500	0.001	0.563	95.06	0.095	47.5
DBL	1000	0.001	2.119	354.4	0.354	354.4
	500	0.001	1.163	195.06	0.195	97.5
UKDT	1000	0.001	1.061	178.06	0.178	178
	500	0.001	0.511	86.4	0.086	43.2
UKDDL	1000	0.001	2.302	384.9	0.384	384
	500	0.001	1.506	252.2	0.252	252.2

Abbreviation: DBT- *Dioscorea bulbifera* tuber; DBL- *Dioscorea bulbifera* leaves; DDT- *Dioscorea deltoidea* tuber; DDL- *Dioscorea deltoidea* leaves; GAE- Gallic acid equivalent; TPC- Total phenolic content

Table 5: Determination of total flavonoid content of *D. bulbifera* and *D. deltoidea* tubers and leaves

Plant sample	Sample solution (µl)	Wt. of dry extract/ml (M) (gm)	Absorbance (mean)	QE Conc. (C) (µg/ml)	QE Conc. (C) (mg/ml)	TFC as QE, A=C*V/M (mg/gm)
DBT	1000	0.001	0.308	313.33	0.313	313
	500	0.001	0.162	216	0.216	108
DBL	1000	0.001	0.181	228.66	0.228	228
	500	0.001	0.103	176.66	0.176	88
DDT	1000	0.001	0.056	145.33	0.145	145
	500	0.001	0.035	131.33	0.131	65.5
DDL	1000	0.001	0.331	328.66	0.328	328
	500	0.001	0.213	250	0.25	125

Abbreviation: DBT- *Dioscorea bulbifera* tuber; DBL- *Dioscorea bulbifera* leaves; DDT- *Dioscorea deltoidea* tuber; DDL- *Dioscorea deltoidea* leaves; QE- Quercetin equivalent; TFC- Total Flavonoids content

Table 6: Comparison between Total Phenolic and flavonoids

S. No	Extracts	Total Phenolic	Total Flavonoids
01.	DBT	216.9 (µg/mL)	198.66 (µg/mL)
02.	DBL	354.4 (µg/mL)	228.66 (µg/mL)
03.	UKDT	178.06(µg/mL)	145.33 (µg/mL)
04.	UKDDL	384.9 (µg/mL)	328.66 (µg/mL)

Table: 7. Rf values for different phytocompounds in various extracts

S.No	Phytoconstituents	Solvent system	Spot Color	Extracts	Rf value (366nm)
1.	Phenolic	T: EA: FA 8:2:0.2	Green	Chloroform	0.687
			L. Orange	Methanol	0.375
2.	Flavonoids	T: EA: FA 8:2:0.2	Green	Methanol	0.304
3.	Triterpenoids	T: EA 9.3:0.7	Blue	Hexane	0.625
			Blue	Chloroform	0.612
			Purple	Acetone	0.6
			Green	Methanol	0.325
4.	Fatty acid	PE:DE 4:1	Blue	Hexane	0.712
5.	Steroids	PE: EA:M 9:5:5	L. Purple	Acetone	0.817
			Blue	Chloroform	0.829
			Green	Methanol	0.451

Table.8: Percent Inhibition of DPPH mediated free radical scavenging activity of *D. bulbifera* and *D. deltoidea* (Tuber, Leaves) of methanol extract at various concentrations

Concentration (µg/ml)	Standard (% inhibition)	**DDT** (% inhibition)	**DDL** (% inhibition)	**DBT** (% inhibition)	**DBL** (% inhibition)
200	26.83	27.22	32.85	28.14	30.10
400	40.57	44.82	49.86	45.48	47.44
600	53.40	56.21	62.82	58.18	59.48
800	63.61	68.39	73.69	71.66	72.97
1000	77.35	79.97	84.62	81.15	82.52

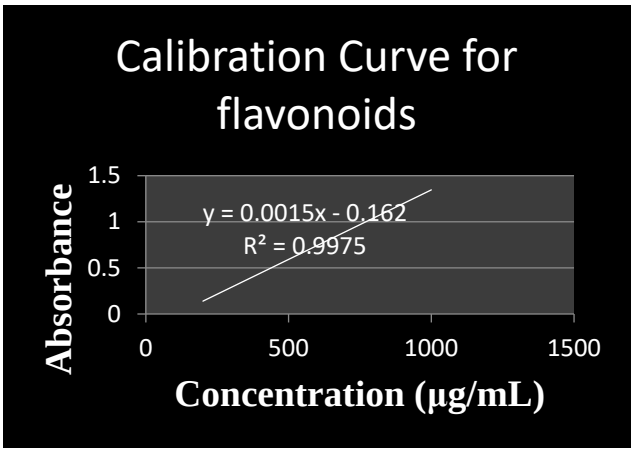

Fig.3: Standard curve for Flavonoids

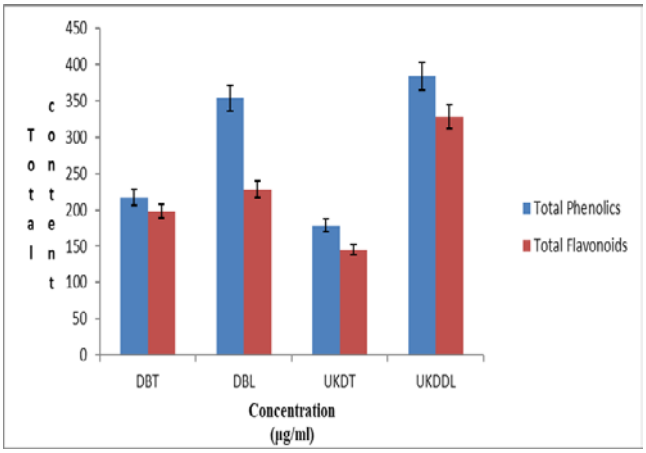

Fig.4: Comparison between total phenolic and total flavonoids content

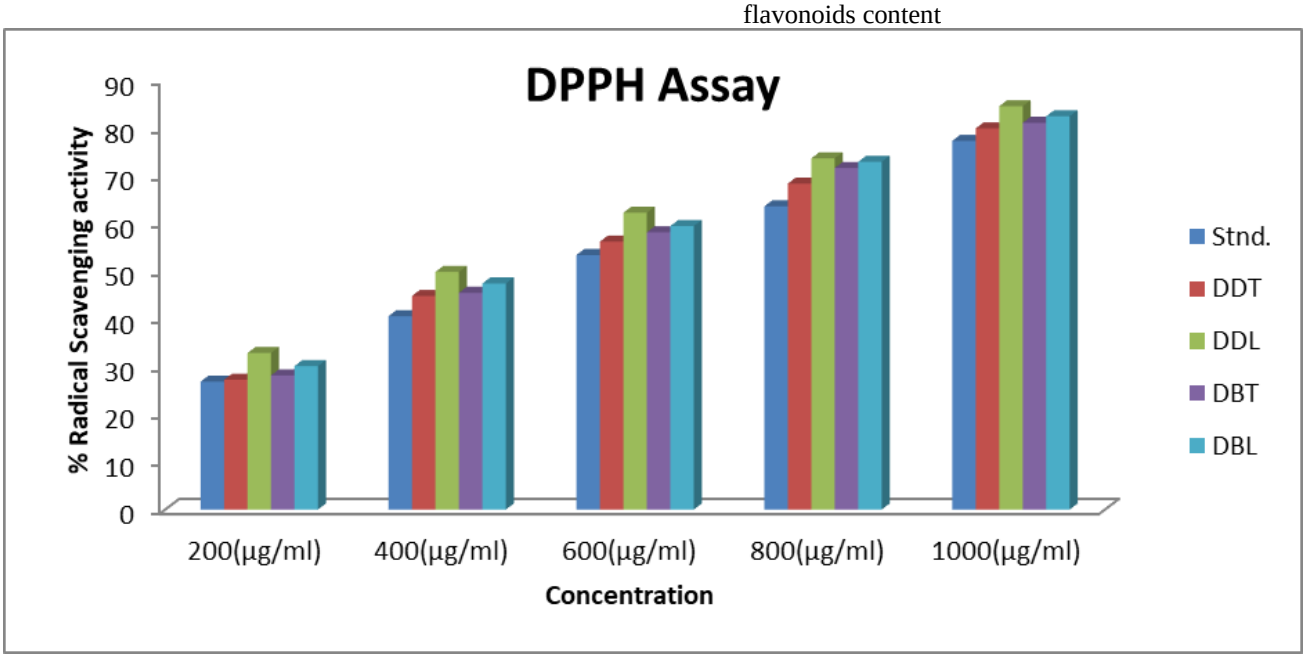

Fig.5: Percent Inhibition of DPPH free radial scavenging activity of *D. bulbifera* and *D. deltoidea* (tubers, leaves) of methanolic extract.

Abbreviation: DBT- *Dioscorea bulbifera* tuber; DBL- *Dioscorea bulbifera* leaves; DDT- *Dioscorea deltoidea* tuber; DDL- *Dioscorea deltoidea* leaves

DISCUSSION

The herbal medication should be pure and unadulterated to preserve the standard and reproducibility. Pharmacological investigations can help to assure this. As a result, in recent years, the standardization of medicinal plants has been received a lot of attention. Pharmacological research aids in the correct identification and evaluation of plant medications [8].

The qualitative analysis could help identify secondary metabolites, which could lead to the therapeutic discovery and development. Moreover, present work makes it easier to separate pharmacologically active chemical substances quantitatively and qualitatively. This study has been revealed the presence of carbohydrates, glycosides, tannins, phenols, alkaloids, flavonoids, sterols and tri-terpenoids in the tubers and leaves in various extracts of D. bulbifera and D. deltoidea. Phenolic and flavonoids were

best identified in methanol, acetone and ethanol extracts of Dioscorea species in both parts (leaves and tubers) while sterols and tri-terpenoids in non-polar solvents viz. hexane, chloroform and acetone. Furthermore, therapeutic properties of Dioscorea may be due to the presence of many different phytoconstituents in these extracts.

The chemical compounds extracted from a certain amount of dried material are expressed as an extractive value. To determine the exhausted and falsified medicines, extractive values of plant component can be calculated using a variety of solvents [9]. In the present study, extractive yield was maximum in methanol extract of D. bulbifera leaves and D. bulbifera tubers.

All secondary metabolites have therapeutic characteristics and have distinct healing capabilities, beneficial action and non-toxic effects[10]. It is generally known that have a wide range of actions that may aid in the prevention of certain chronic diseases [11]. The data showed the selection of specific solvent systems for identification of secondary metabolites. The present work depicted flavonoids and phenolic were identified in same solvent system (T: EA:FA:8:2:0.2). Many beneficial qualities of flavonoids and phenolic have been identified, including anti-inflammatory, estrogenic, antibacterial, anti-allergic, antioxidant, vascular and cytotoxic anticancer activity[12]. The methanol extract of D. bulbifera leaves containing high amount of flavonoids and phenolic showed with higher free radical scavenging activity. Plotting percent scavenging activity (μg/ml) against TFC and TPC (mg/g) revealed a link between antioxidant activity and total flavonoids and phenolic. The high flavonoids and phenolic content of the sample revealed a clear relationship between radical scavenging activity and TFC, TPC, indicating that the DPPH free radical scavenging activity of the methanol extracts is due to the flavonoids and phenolic compounds.

CONCLUSION

Flavonoids and phenolic substances, which are antioxidants, have long been known to be effective in the cure of cardiovascular disorders[13, 14]. The objective of this research is to determine the possible antioxidant function of Dioscorea plant. The findings reveal that the methanol extract of D. bulbifera leaves has a significant antioxidant function with scavenging activity against DPPH, making it a good source of natural antioxidants and can be used to treat a variety of ailments. It is crucial to use herbal drugs for disease bio control as a non-toxic alternative to medicine and also ecologically acceptable. From this study, it is concluded that phytochemicals were observed in the methanol extract of the plant is highly valuable in medicinal uses for the treatment of various disorders and leaves may have better therapeutic values than tubers due to high content of phenolic and flavonoids which are used to combat the oxidative stress disease. Hence, it can better utilize for product development. However, more research is required before they can be recommended for the use in healthcare system.

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CONFLICTS OF INTEREST

There are no conflicts of interest declared by the authors.

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