Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [3] February 2021 : 20-28

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Phytochemicals, Antioxidant Activity in *Dioscorea oppositifolia* L. and Their Characterization by Thin Layer Chromatography

N. R. Anvitha and M. S. Nalini*
Department of Studies in Botany, University of Mysore, Manasagangothri, Mysore–570 006, Karnataka, India.
*Corresponding author's E-mail: nmsomaiah@gmail.com

ABSTRACT
Dioscorea oppositifolia is of family Dioscoreaceae and it is commonly known as yam. The tubers are well known source of triterpenoid compounds and is of economical and pharmacological interest. In the present study, the leaves and fruits collected from the natural forests of Western Ghats were investigated for the presence of phytochemicals in the solvent extracts of both parts. The extracts were also tested for the presence of total phenolic content and antioxidant activity by radical scavenging potentials and reducing power assay. The phytochemical screening conducted for the leaves and fruit solvent extracts revealed the presence of terpenoids in both the plant parts, whereas saponins in the leaf chloroform and methanolic extracts. The aqueous, ethanolic and methanolic extracts from leaves showed high total phenolic contents. The fruit aqueous, ethylacetate and methanolic extracts showed high total phenolic contents. The aqueous, ethylacetate, ethanolic and methanolic extracts showed high percentage of radical scavenging activity. The high percentage radical scavenging activity detected in ethanolic (89.4% µg/mL) and methanolic leaf extracts (87.4% µg/mL) with IC_{50} values ranging from31.5 and 43.3 µg/mL. In thin layer chromatography the leaf ethyl acetate extract showed high R_f value (0.93) and in fruit ethanolic extract showed high R_f value(0.92). All the solvent extracts of leaves showed good separation of bands for the presence of terpenoids and saponins, while the ethanolic extract of fruits showed good separation of bands for the presence of phenolic compounds. Results presented here envisage the presence of important phytochemicals in D. oppositifolia extracts.
Keywords: *Dioscorea oppositifolia, Phytochemicals, Antioxidant activity, Thin layer chromatography, terpenoids, saponins.*

Received 01.01.2021 Revised 22.01.2021 Accepted 11.02.2021

INTRODUCTION

Plants are universally recognized as a vital component of biodiversity and global sustainability [3]. Plants possess therapeutic properties or exert beneficial pharmacological effects on the human body or generally designated as medicinal plants [12]. The medicinal properties of plants are due to the presence of active components [20]. Medicinal plants have a promising future as there are about half million plants around the world, and most of medicinal activities have not been subjected to investigations [21]. The family Dioscoreaceae comprises of four genera and about 650 species and is distributed worldwide, but particularly in the tropical regions. With over 600 species, the genus *Dioscorea* has large number of species, and it is the most widely distributed. It is commonly known as yam consisting of large genus of annual twining herbs, distributed throughout the moist tropics of world and extending into warm temperate regions. About 50 species are found in India. *Dioscorea* species are distributed nearly throughout India except in the dry North-Western regions [26]. The genus name "*Dioscorea*" is derived from Dioscorides, a Greek physician and naturalist, while the species epithet "*oppositifolia*" refers to the opposite arrangement of its leaves. The common name of *D. oppositifolia* is "Vethalaivalli" [17, 18].

Yam is widely grown in many West African countries and manyspecies are eaten in various parts of the world [22]. Yams are edible starchy tubers and are of cultural, economic and nutritional importance in the tropical and subtropical regions of the world [24]. *Dioscorea* species especially from the tropical areas, are a very important source of secondary metabolites, and used in pharmaceutical industry and medicine [13]. The most predominant phytochemical characteristic of yam is the presence of dioscorine alkaloid and diosgenin saponin. Yams have been well respected by the herbalist community for generations due to their potency in enhancing fertility in males. This may be due to the presence of steroidal sapogenins such as diosgenin. It is used as precursor for the synthesis of hormones and are precursors for the semisynthesis of birth control pills as well as similar hormones and corticosteroids

[18]. Its roots contain diosgenin, which is a compound often used in the manufacture of progesterone and other steroid drugs. Leaf juice from *D. oppositifolia* can be used to treat snakebites and scorpion stings. *D. oppositifolia* has also been used traditionally as a contraceptive and in the treatment of various disorders of the genital organs as well as for asthma and arthritis [10]. Leaf paste is used as antiseptic for ulcers. The roots are chewed to cure toothache [14]. Yam has the high antioxidant activity but low phenolic content. Yam is ranked the seventh of highest antioxidant concentration among the 11 roots and tubers analyzed [5].

The plants of Dioscoreaceae, have many medicinal properties, due to the presence of phytochemical compounds. Plant parts have high nutritional value and known to exhibit antioxidant activity. Most of the research is focused on the tubers of *Dioscorea* species. The phytochemical screening and antioxidant evaluation as well as thin layer chromatography of leaf and fruit extracts of *D. oppositifolia* has not been reported. Therefore, the present study necessitates investigation on the above mentioned criteria.

MATERIALS AND METHODS

Phytochemical Screening

The leaves and fruits of *Dioscorea oppositifolia* were collected from Kodagu district, Western Ghats during December 2013. The plant was identified on the basis of flora [7] and the herbarium specimen has been maintained, in the DOS in Botany. The collected leaves and fruits of were dried separately and powdered by mixer. The powder was weighed and preserved in polythene covers and labelled. The leaf and fruit powder of 50g each were weighed and used for soxhlet extraction. Five solvents were used for the extraction based on their polarity *viz*., hexane, chloroform, ethylacetate, ethanol and methanol. The powdered materials were extracted with solvents in the soxhlet apparatus with 350mL of solvents. After extraction, the extracts were collected and poured into petriplate for drying. The extracts were scraped and transferred into pre-weighed Eppendorff tubes and stored until further use. For the preparation of leaf and fruit aqueous extracts, one gram of leaf and fruit powder were weighed and boiled with distilled water separately for 10 min. The extract was filtered through Whatman's filter paper into test tube, wrapped with aluminum foil and then used for phytochemical analysis.

Qualitative Detection of Phytochemicals

Phytochemicals were tested according to the standard procedure for qualitative detection [8]

Tannins: Small quantity (1mg) was mixed with one ml of water and heated on water bath. The mixture was filtered and two drops of ferric chloride ($FeCl_3$) was added to the filtrate. Agreen color indicated the presence of tannins.

Saponins: One ml of aqueous extract of the plant parts were taken and 2 ml of distilled water and shaken vigorously, a stable persistent froth indicated the presence of saponins.

Flavonoids: A few drops of 1% ammonia solution were added to the aqueous extract of plant parts and concentrated sulphuric acid was also added, a yellow coloration indicated the presence of flavonoids.

Terpenoids: Fiveml of aqueous extract of plant parts was taken separately and mixed with2 ml of chloroform, to this mixture 3 ml of concentrated sulphuric acid was added carefully. The appearance of the reddish brown layer indicated the presence of terpenoids.

Steroids: Ten ml of chloroform was added to the 20 mg of plant parts and then filtered. 2 ml of acetic anhydride was added to this extract and then concentrated sodium hydroxide was added. A green ring indicated the presence of steroids.

Anthraquinones: Dried plant parts (0.5 g) were boiled with 10% HCl (v/v) for few minutes in a water bath, the contents were filtered cooled. To this filtrate equal amount of chloroform was added and a few drops of 10% NH_3were added to the mixture and heated. Formation of rose pink color indicated the presence of anthraquinones.

Phlobatannins: 1 ml of aqueous extract of plant parts was taken and boiled with 2% HCl solution which gives red precipitate, this indicated the presence of phlobatannins.

Cardiac Glycosides*:* Twoml of aqueous extract of plant parts was taken to this one ml of glacial acetic acid and $FeCl_3$ and concentrated sulphuric acid were added carefully which gives reddish blue coloration at the junction of two layers of solution and formation of the bluish green color at the upper layer, which indicates the presence of glycosides.

Reducing sugars: 1ml of aqueous extract of plant parts was boiled with a few drops of Fehling's solution A and B for a minute. An orange red precipitate indicated the presence of reducing sugars.

Alkaloids: About 0.2g of material was heated with 2% H_2SO_4 solution for a two minute and filtered. To this filtrate a few drops of Dragendorff's reagent were added. An orange red precipitate indicates the presence of alkaloids.

Evaluation of Antioxidant Activity in the Leaf and Fruit Extracts

The antioxidant activity of *D. oppositifolia* leaf and fruit extracts were evaluated by estimation of total phenolic content, radical scavenging activity and reducing power using standard procedures.

Estimation of Total Phenolic Content (TPC)

The total phenolic content of plant extracts were determined by the Folin-Ciocalteau (FC) method using Gallic acid as standard (5-25 µg/mL) by modifying the procedure of Volluri *et al*. [27]. One mg of hexane, chloroform, ethylacetate, ethanol and methanol extracts were weighed and dissolved in respective solvents up to one mL. Solutions of one mg/mL of different solvent and aqueous extracts were prepared in various concentrations ranging from 20–100 µg/mL. Different concentrations of standard as well as the extracts were taken in test tubes and one ml of FC reagent (1:1 dilution) was added, 3-5min later 2.0 ml of sodium carbonate (20%, w/v) was added and the mixture was allowed to stand for 45 min under dark condition. After the specified incubation period, the absorbance of standard and samples were read at 765 nm using aspectrophotometer. The concentration of total phenolics was expressed in terms of µg/ml GAE (Gallic acid equivalence).

Radical Scavenging Activity by 1,1-Diphenyl-2-Picrylhydrazyl (DPPH)

The antioxidant activity of the all six extracts were determined by the procedure of Pannangpetch *et al*. (2007) [16] using 1,1-diphenyl-2-picrylhydrazyl (DPPH). 0.004 g of DPPH was dissolved in 100 mL methanol. Different aliquots of standard ascorbic acid (5-25 µg/mL) and extracts of plant sources (20-100 µg/mL) were taken.

The per cent radical scavenging activity was calculated as follows:

Per cent radical scavenging activity= $\frac{Ac-As}{Ac} \times 100$

Where,A_c= absorbanceof control, A_s= absorbance of test sample.

Reducing Power Assay

The reducing power of the all six leaf and fruit extracts were determined by the procedure of Yen and Chen [29] with some modifications. Butaylated hydroxyl toluene (BHT) was taken as standard. For preparing a sample for reducing power, BHT, leaf and fruits extracts (1 mg each) were dissolved in one mL of methanol solvent.

Thin Layer Chromatography (TLC)

Thin layer chromatography was carried out to characterize the phytochemicals of *D. oppositifolia* found positive in the preliminary qualitative phytochemical analysis. The different solvent system indicating polarities were prepared and TLC studies were carried out to select the solvent system capable of showing better separation of phytochemicals.

Samples – 40 mg of leaf and fruit (hexane, chloroform, ethyacetate and ethanol) extracts were dissolved in one mL of methanolic solvent separately.

Solvent phase – Thin layer chromatography was performed using chloroform, cyclohexane and methanol solvents of 3:2:1 ratio [2].

Spraying reagent – Detection of terpenoids and saponins was achieved by acetic acid : sulphuric acid : P-anisaldehyde solutions (97:2:1) heated for approximately 5 min at 110°C to visualize terpenoid and saponin compounds. Green, brown and yellow colours are indicative of terpenoids, where as violet colour indicated the presence of saponins [6]. For the detection of phenolic compounds1% ferric chloride used as a spraying reagent. Blue colour bands indicated the presence of phenolics.

TLC plates – The plant extracts were loaded on pre-coated TLC plates using capillary tubes and developed in a TLC chamber using suitable mobile phase. After the movement of sample upto $3/4^{th}$ of the TLC plate, it was taken out from the TLC chamber and air dried. Detection of bands was achieved by spraying the respective reagents. The movement of the compound was expressed by its retention factor (R_f). Values were calculated for different samples and represented.

$$R_f = \frac{\text{distance traveled by solute}}{\text{distance traveled by solvent}}$$

RESULTS

Phytochemical Screening of *D. oppositifolia* extracts

The *D. oppositifolia* leaf and fruit extracts were dissolved in respective solvents and subjected to preliminary qualitative phytochemical screening for tannins, saponins, flavonoids, terpenoids, steroids, anthraquinones, phlobatannins, glycosides, reducing sugars and alkaloids using standard procedures. Phytochemical screening revealed the presence of terpenoids in all leaf and fruit extracts. Saponins were present in chloroform, ethyl acetate, ethanolic and methanolic fruit extracts. In leaves, saponins were present in chloroform and methanolic extracts (Table 1).

Table 1. Phytochemical screening for the solvent extracts of *D. oppositifolia* plant parts

Tests	Leaf						Fruit					
	Hex	Chl	EA	Eth	Meth	Aq	Hex	Chl	EA	Eth	Met	Aq
Tannins	–	–	–	–	–	–	–	–	–	–	–	–
Saponins	–	+	–	–	+	–	–	+	+	+	+	–
Flavonoids	–	–	–	–	–	–	–	–	–	–	–	–
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	–	–	–	–	–	–	–	–	–	–	–	–
Anthraquinones	–	–	–	–	–	–	–	–	–	–	–	–
Phlobatannins	–	–	–	–	–	–	–	–	–	–	–	–
Cardiac Glycosides	–	–	–	–	–	–	–	–	–	–	–	–
Reducing sugars	–	–	–	–	–	–	–	–	–	–	–	–
Alkaloids	–	–	–	–	–	–	–	–	–	–	–	–

"+" = presence **"–"** = absence of phytochemical. **Hex**-Hexane, **Chl**-Chloroform, **EA**-Ethylacetate, **Eth**-Ethanol, **Meth**-Methanol and **Aq**-Aqueous.

Evaluation of Antioxidant Activity in Leaf and Fruit Extracts

Estimation of TPC

D. oppositifolia leaf aqueous (262.9 µgGAE/g), ethanolic (197 µgGAE/g) and methanolic extracts (96 µgGAE/g) showed high total phenolic contents compared to other solvent extracts (Fig. 1 A). The fruit aqueous (128 µgGAE/g), ethylacetate (54.8 µgGAE/g) and methanolic extracts (64.3 µgGAE/g) showed high total phenolic contents compared to other solvent extracts (Fig. 1 B).

Evaluation of DPPH Radical Scavenging Activity

The high percentage radical scavenging activities were detected in the ethanolic (89.4% µg/mL) and methanolic leaf extracts (87.4% µg/mL)of *D. oppositifolia* (Fig. 2 A). The IC_{50} values of leaf aqueous, hexane, chloroform, ethylacetate, ethanolic and methanolic extracts were found to be 263.9, 129.8, 241.6, 122.4, 31.5 and 43.3 µg/mL respectively. The high percentage radical scavenging activity detected in aqueous (84.1% µg/mL), ethylacetate (59.4% µg/mL) and methanolic fruit extracts (66.3% µg/mL) (Fig. 2 B). The IC_{50} values of fruit aqueous, hexane, chloroform, ethylacetate, ethanolic and methanolic extracts were found to be 40.37, 308.9, 770, 77.1, 111.76 and 64.2 µg/mL respectively.

Evaluation of Reducing Power Assay

In leaves, high reducing power was found in ethanolic extract followed by methanol, aqueous, chloroform, ethylacetate and hexane extracts (Fig. 3 A). In fruits, high reducing power was detected in the methanolic extract followed by ethylacetate, aqueous, ethanol, chloroform and hexane extracts (Fig. 3 B). Butaylated Hydroxyl Toluene (BHT) was taken as the standard.

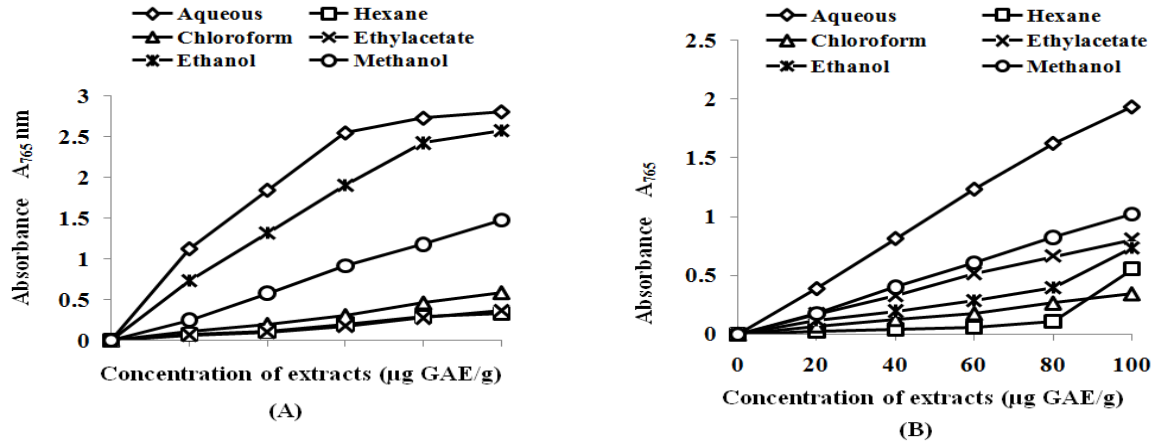


Figure 1: Total phenolic content in the solvent extracts of *D. oppositifolia* leaves and fruits

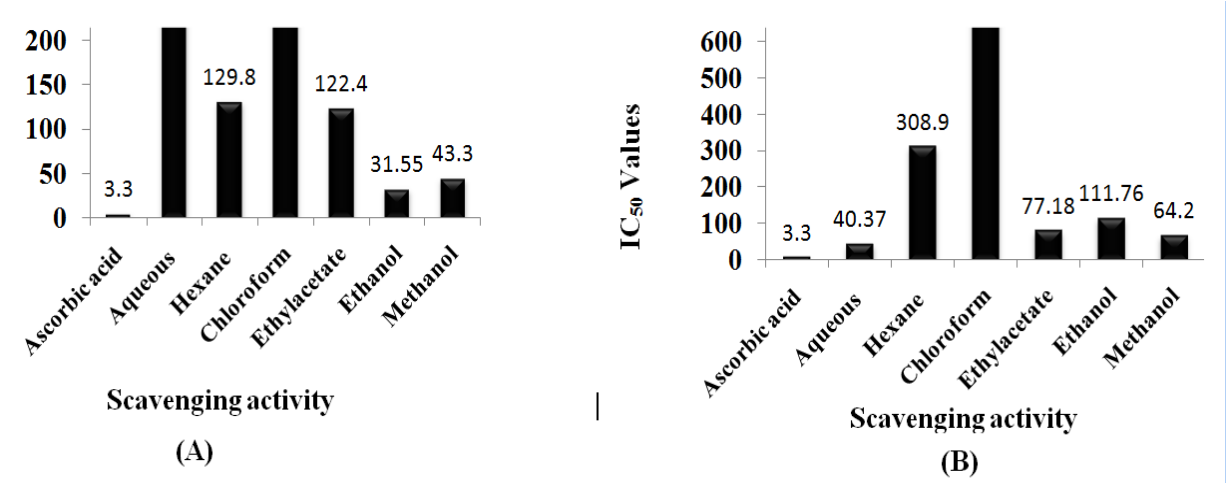


Figure 2: IC_{50} values of solvent extracts in *D. oppositifolia* leaves and fruits

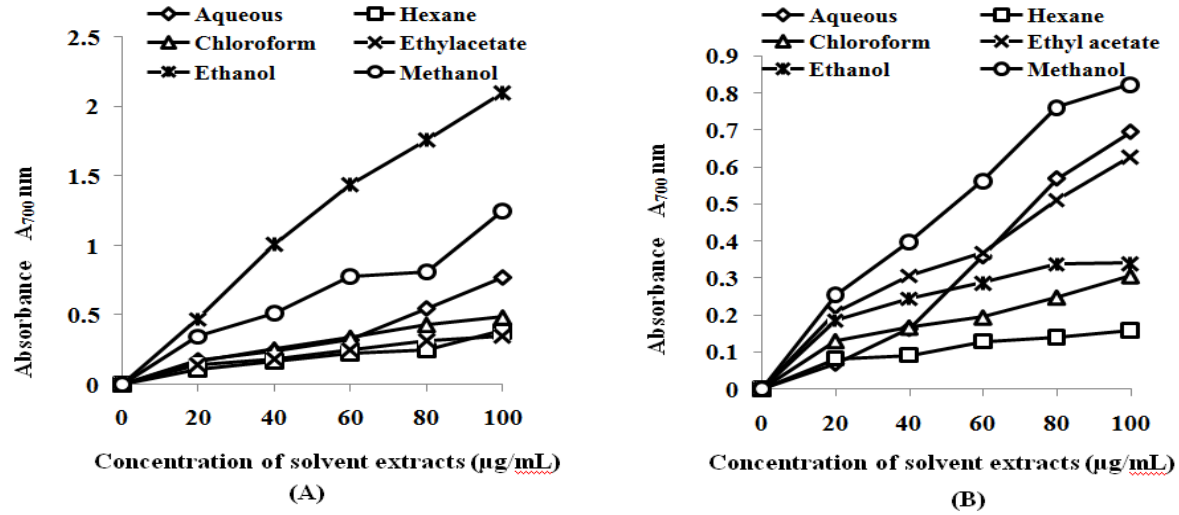


Figure 3: Reducing power of *D. oppositifolia* leaves and fruits solvent extracts

TLC for the Separation of Terpenoids and Saponins

Hexane extracts: The leaf hexane extract revealed the presence of three compounds having R_f values of 0.89 (dark green colour), 0.81 (yellow) and 0.76 (light green). The fruit hexane extract revealed the presence of two compounds having R_f values of 0.85 (light green) and 0.76 (light brown) (Fig.4 A).

Chloroform extracts: The chloroform extract of leaves revealed the presence of five compounds having R_f values of 0.92 (yellow), 0.87 (violet), 0.80 (light green), 0.75 (dark green) and 0.67 (dark green). In fruit, the chloroform extract revealed the presence of four compounds having R_f values of 0.82 (dark green), 0.79 (violet), 0.75 (light green) and 0.72 (light brown) (Fig. 4 B).

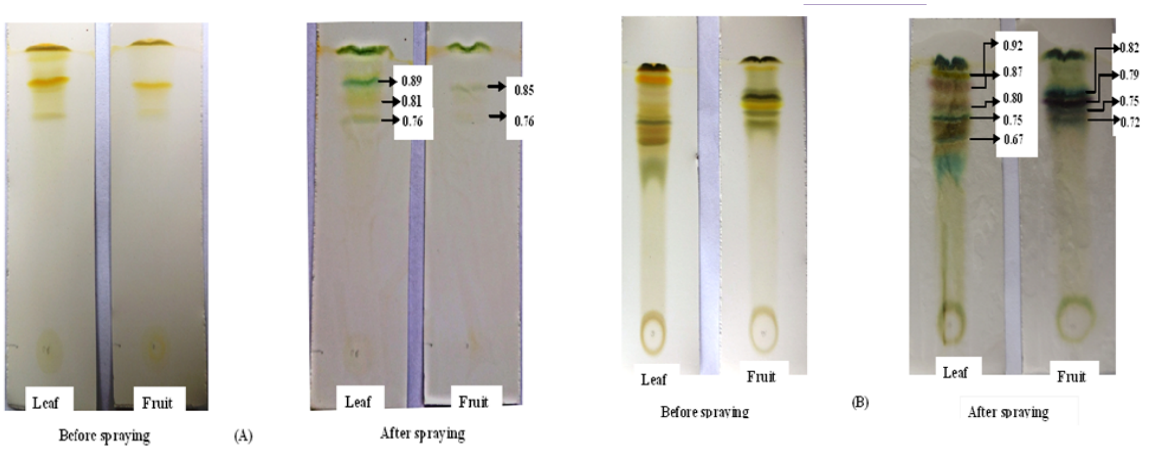
Ethylacetate extracts: The leaf ethylacetate extract revealed the presence of three compounds having R_f values of 0.93 (dark green), 0.88 (light green) and 0.80 (light brown). The fruit ethylacetate extract revealed the presence of five compounds having R_f values of 0.87 (blue), 0.75 (yelow), 0.59 (violet), 0.57 (green) and 0.50 (pink) (Fig. 5 A).

Ethanolic extracts: The leaf ethanolic extract revealed the presence of two compounds having R_f values of 0.38 (light green) and 0.34 (light green). Ethanolic fruit extract revealed the presence of five compounds having R_f values of 0.92 (brown), 0.87 (violet), 0.7 (green), 0.68 (yellow), and 0.63 (pink) (Fig. 5 B).

Methanolic extracts: The leaf methanolic extract revealed the presence of four compounds having R_f values of 0.81 (violet), 0.74 (violet), 0.7 (yellow) and 0.67 (light green). The fruit methanolic extract revealed the presence of five compounds having R_f values of 0.77 (yellow), 0.71 (light green), 0.64 (yellow), 0.49 (pink) and 0.31 (yellow) (Fig. 6).

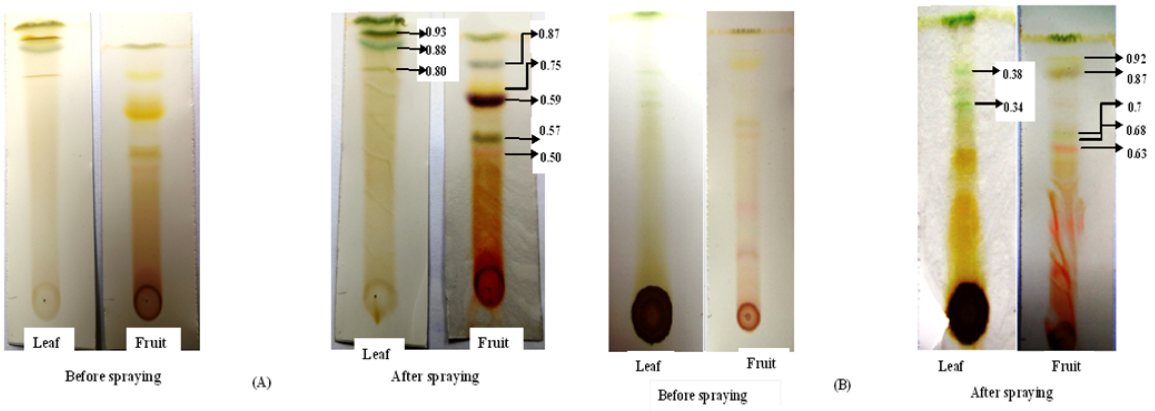
TLC for the Separation of Phenolic Compounds

The TLC profile of Gallic acid was taken as standard for phenolic compounds and it showed the presence of one compound having value R_f 0.288 (Fig. 7 A). The fruit ethanolic extract showed eight phenolic compounds having R_f values 0.88, 0.83, 0.80, 0.78, 0.45, 0.42, 0.37 and 0.34. After spraying the reagent phenolic compounds appeared as blue color bands (Fig. 8 B).



Hexane extract **Chloroform extract**

Figure 4 TLC profile of *D. oppositifolia* extracts for the separation of saponins and terpenoids



Ethylacetate extract **Ethanolic extract**

Figure 6 TLC profile of *D. oppositifolia* extracts for the separation of saponins and terpenoids

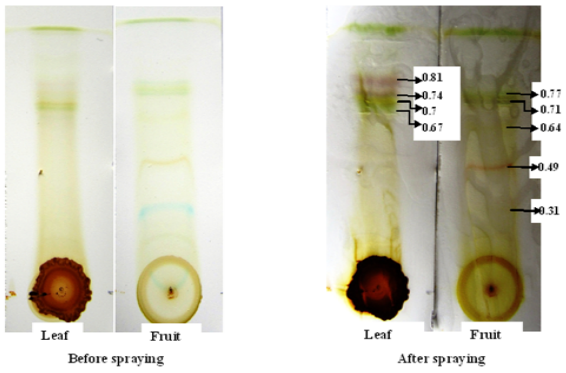


Figure 7 TLC profile of *D. oppositifolia* methanolic extracts for the separation of saponins and terpenoids

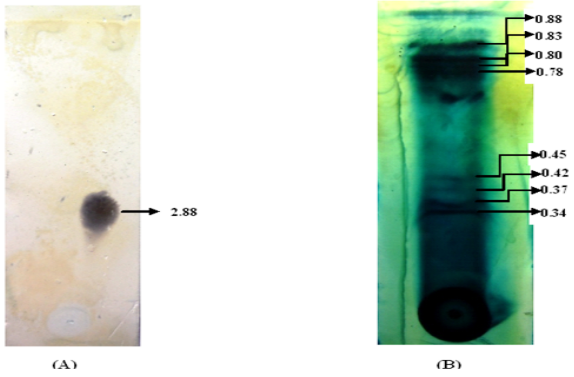


Figure 8 TLC profile of *D. oppositifolia* ethanolic fruit extract for the separation of phenolic compounds

DISCUSSION

The current investigation is aimed at the evaluation of phytochemicals, antioxidative and TLC separation of three important class of phytochemicals in the leaf and fruit extracts of *D. oppositifolia*, one of the tuberous medicinal plant species collected from the Western Ghats of southern India. Our study revealed the presence of terpenoids in hexane, chloroform, ethylacetate, ethanol, methanol and aqueous leaf and fruit extracts of *D.oppositifolia*. Saponins were present in chloroform, ethylacetate, ethanol, methanol fruit extracts and chloroform leaf extract. Similarly, phytochemical screening of *D. domentorum* tuber showed presence of saponins and terpenoids [9]. Nilofer *et al*. (2013)[15] reported the phytochemical screening of root tubers of *D. pentaphylla,D. alata, D. oppositifolia, D. bulbifera, D. glabra*and *D. pubera*.Powdered tubers were treated with methanol and ethyl acetate. Phytochemical screening revealed that, in the ethyl acetate extracts, terpenoids and saponins were present in *D. pentaphylla. D. alata, D. bulbifera.* Terpenoids were present in all methanolic extracts, except *D. glabra*. Saponins were present in all methanolic extracts except *D. alata.*

D. oppositifolia is a well known source oftriterpenoid compounds in the tubers. These are sometimes used as an herbaltonic. Terpenoids have antihepatotoxic properties, thus helping to prevent liver damage (cirrhosis).They equally have antimicrobial or antiseptic properties. Saponins are expectorants, cough suppressants and administered for hemolytic activities[11]. *D. villosa* have five steroidal saponins; Dioscin, Protodioscin, Meprotodioscin, Perrisaponin and Progenin II. It also has a spirostanol glycoside.Other constituents include phytosterols (beta-sitosterol), alkaloids and tannins that make this plant useful as an antiinflammatory, diuretic, antispasmodic, cholagogue, diaphoretic and vasodilator. A decoction of the root is used to alleviate many of the symptoms of menopause. It is also used to treat irritable bowel syndrome, gastritis, painful menstruation, and in small doses is especially helpful in treating the nausea of pregnant women [1]. *Dioscorea* attributes antimicrobial activities to the presence of secondary metabolites, Diosgenyl saponins the most abundant steroid saponins, reported to exert a large variety of biological functions [19].

The present study revealed the presence of high total phenolic content in leaf aqueous (262.9 µgGAE/g), ethanolic (197 µgGAE/g) and methanolic extracts (96 µgGAE/g) and fruit, ethylacetate and methanolic extracts showed high total phenolic content. The *D. oppositifolia* methanolic tuberextract was found to contain 0.56 g/100g total phenolics [17]. The total phenolic contents of methanolic extract of *D. alata* tubers were found to be 0.68 g/100 g. The methanolic extracts of *D. alata* leaf showed strong antioxidant activity in various in vitro assays [22]. Phenolic compounds are the principal antioxidant constituents of natural plant products are composed of phenolic acid and flavonoids. These compounds are potent radical terminators by donating a hydrogen atom to the radical and preventing lipid oxidation at initial step. These compounds have multiple biological effects like anticancer, anti-proliferative, antimicrobial, wound healing and antibacterial activities including antioxidant activity[14].

In present study, the high percentage radical scavenging activity detected in ethanolic and methanolic leaf extracts and aqueous, ethylacetate and methanolic fruit extracts. The DPPH test provided information on the reactivity of test compounds with a stable free radical. Because of its odd electron, DPPH gives a strong absorption band at 517 nm in visible spectroscopy. The procedure involves measurement of decrease in absorbance of DPPH at 517 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. DPPH is a stable, nitrogen-centered free radical which produces violet color in methanol solution. It was reduced to yellow colored product, diphneylpicryl hydrazine, with the addition of extracts in a concentration manner. Murugan and Mohan [22] reported the DPPH radical scavenging activity of *D. esculenta* tuber methanolic extract which showed 79.33% scavenging activity at 1000 µg/mL.The reducing power of methanolic extract was very potent in *D. alata* tubers [22] and methanolic extract of *D. esculenta* tubers showed high reducing power[14].

The results obtained from the TLC showed more number of terpenoid bands in both leaf and fruit extracts. The leaf hexane extract revealed the presence of three compounds having R_f values of 0.89, 0.81 and 0.76. The fruit hexane extract revealed the presence of two compounds having R_f values of 0.85 and 0.76. The chloroform extract of leaves revealed the presence of five compounds having R_f values of 0.92, 0.87, 0.80, 0.75 and 0.67. In fruits, the chloroform extract revealed the presence of four compounds having R_f values of 0.82, 0.79, 0.75 and 0.72. The leaf ethylacetate extract revealed the presence of three compounds having R_f values of 0.93, 0.88 and 0.80. The fruit ethylacetate extract revealed the presence of five compounds having R_f values of 0.87, 0.75, 0.59, 0.57 and 0.50. The leaf ethanolic extract revealed the presence of two compounds having R_f values of 3.8 and 3.4. Ethanolic fruit extract revealed the presence of five compounds having R_f values of 0.92, 0.87, 0.7, 0.68, and 0.63. The leaf methanolic extract revealed the presence of four compounds having R_f values of 0.81, 0.74, 0.7 and 0.67. The fruit methanolic extract revealed the presence of five compounds having R_f values of 0.77, 0.71, 0.64, 0.49 and 0.31. Sarla *et al*. (2013) [24]] reported the TLC profiling of *D. alata* tubers. The chloroform, methanolic and ethanolic

extracts were subjected to different combination of Water: Chloroform: Methanol solvents for TLC. Chloroform, methanolic and ethanolic extracts showed 0.80-0.92, 0.19-0.68 and 0.19-0.74 ranged R_f values respectively. TLC of leaf extract of *Centella asiatica* revealed the presence of five compounds having R*f* values of 0.08 when a solvent phase of Chloroform: glacial acetic acid: methanol: water (6:2:1:1)) was used. TLC of leaf extract of *C. asiatica* revealed the presence of five compounds with R*f* values of 0.36, 0.40, 0.46, 0.73, 0.86 respectively when a solvent phase of Benzene: Ethylacetate (1:1) was used [23].

Wahab *et al*. [28] screened three Loranthaceae plants (*Phragmathera* species, *Tapinanthus* species and *Globimetula* species) for secondary metabolites such as alkaloids, anthraquinones, ketones and terpenoids. Two solvent systems were used for identification of terpenoids such as ethyl acetate: toluene (8:4) and ethyl acetate: toluene : acetic acid (8 :4 : 1). Anisaldehyde in sulphuric acid used as spraying reagent for terpenoids. TLC was performed for *Plectranthes hadiensis* by Darsan *et al*. [4], to detect terpene rich compounds, ethyl acetate: chloroform: methanol (4:3:3) and methanol: chloroform (9:11) were selected as solvent system and sulphuric acid. Bluish-green colored five bands were visualized.

D. oppositifolia is a medicinal plant of immense importance. The phytochemical screening indeed has revealed the presence of terpenoids in all leaf and fruit extracts. The extracts of both fruits and leaves have indicated radical scavenging potentials. The overall results of the present study haveprovided evidence that *D. oppositifolia* contain terpenoids and saponin compounds by TLC. Leaf extracts revealed the presence of high antioxidant activity. Therefore, *D*. *oppositifolia* extracts are good sources of phytochemicals.

AKNOWLEDGEMENT

NRA is thankful to the University of Mysore for providing non-net fellowship as a financial support during the course of M.Phil degree in Botany. The authors thank the Chairman, DOS in Botany for providing all necessary facilities and encouragement for the present work.

REFERENCES

1. Anitha, R., Geetha, R. V.and Lakshmi, T. (2012).Evaluation of the antibacterial activity of ethanolic extract of *Dioscorea villosa* tubers - An *in vitro* study.International Journal of Pharmacy and Pharmaceutical Sciences,4(1): 314-316.
2. Barbara, K. and Daniel, M. (2005). Terpenoids and sterols from *Nepeta cataria* L. *var*. *citriodora* (Lamiaceae). Acta Poloniae Pharmaceutica - Drug Research,62 (3): 231-235.
3. Convention on Biological Diversity Plant Conservation Report: A Review of Progress in Implementing the Global Strategy of Plant Conservation (GSPC). (2009). Secretariat of the Convention on Biological Diversity. Pp: 2-48.
4. Darsan, B. M. Sasikumar, J. M. and Gopalakrishnan, V. K. (2013). Analysis of the methanolic extract of the shoot of *Plectranthus hadiensis*. International Research Journal of Pharmacy, 4(5): 233-237.
5. Djanna, F. C., Rowena, G. O. R. and Inacrist, M. G.(2011). Philippine Yam (*Dioscorea* spp.) tubers phenolic content and antioxidant capacity.Philippine Journal of Science, 140 (2): 145-152.
6. Elisabeth, A. D. and Lacey, J. L. (2006). Comparative terpenoid metabolism in *Artemisia annua* seedlings. Degree of Bachelor of Science, Worcester polytechnic institute. Pp: 19.
7. Gamble, J. S. (1921). Flora of the Presidency of Madras. Published under the authority of the secretary of state for India in council, Vol:III, Pp:1506-1509.
8. Harborne A.J.**(**1998). Phytochemical Methods: A guide to modern techniques of plant analysis. Springer, Pp. 320.
9. Luka, C. D., Saleh, B. and Mohammed, A. (2012). Effect of aqueous extract of *Dioscorea domentorum* on some biochemical parameters in alloxan-induced diabetic rats. Asian Journal of Experimental Biological Sciences, 3(2): 450-453.
10. Tu, M.(2002). The nature conservancy's wildland invasive species team. In: Element stewardship abstract for *Dioscorea oppositifolia* L. *syn. Dioscorea batatas* (Decne) (John, M. R. and Tunyalee, M. Eds.), Department of Vegetable Crops and Weed Sciences, University of California.
11. Mensah, J. K., Ihenyen, J. O. and Okhiure, M. O. (2013). Nutritional, phytochemical and antimicrobial properties of two wild aromatic vegetables. Journal of Natural Products and Plant Resources, 3(1):8-14.
12. Motaleb, M. A., Istiak, S., Khairul, A., Niaz, A. K. and Remeen, F. (2011). Selected medicinalplants of Chittagong hill tracts. IUCN (International Union for Conservation of Nature), Dhaka, Bangladesh. Progressive Printers Pvt. Ltd. Pp: 1-116.
13. Muhamad, M. A. W., Nashriyah, M. and Mohd, H. R. (2012). A review on the effect of irrigation in *Dioscorea hispida* Dennst. Growth and phytochemical. International Journal of Agriculture: Research and Review, 2(2):39-46.
14. Murugan, M.and Mohan, V. R. (2012). *In vitro*antioxidant studies of *Dioscorea esculenta*(Lour). Burkill. Asian Pacific Journal of Tropical Biomedicine,2(3): S1620- S1624.
15. Nilofer, S., Yogendra, K., Misra, A.K. and Lokho, P. (2013). Phytochemical screening to validate the ethnobotanical importance of root tubers of *Dioscorea* species of Meghalaya, North East India. Journal of Medicinal Plants Studies,1(6): 62-69.

16. Pannangpetch, P., Laupatarakasem, P., Kukonviriyapan, V., Kukongriyapa, C., Kongyinayoes, B. and Aromdu, C. (2007). Antioxidant activity and protective effect against oxidative hemolysis of *Clinacanthus nutans* (Burm, f), London. Sonlgklanakarin Journal of Science and Technology,29(1): 1-9.
17. Paulpriya, K. and Mohan, V. R. (2012). *In vitro* antioxidant potential of methanol extract of *Dioscorea oppositifolia*. Journal of Science Research Reporter, 2(3): 239-245.
18. Poornima, G. N. and Ravishankar, R. V. (2009). Evaluation of phytonutrients and vitamin contents in a wild yam, *Dioscorea belophylla* (Prain) Haines. African Journal of Biotechnology, 8(6): 971-973.
19. Prakash, G. and Hosetti, B. B. (2010). Investigation of antimicrobial properties of *Dioscorea pentaphylla* from mid Western Ghats, India.Scientific World,8(8): 91-96.
20. Ramawat, K.G., Dass, S. and Meeta, M. (2009).Herbal drugs: Ethnomedicine to modern medicine. In: The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants (Ramawat, K.G. Ed.), Springer-Verlag Berlin Heidelberg, Pp: 7-32.
21. Rasool, H. A. B. (2012). Medicinal Plants (Importance and Uses). Pharmaceutica Analytica Acta,3(10): 1000e139.
22. Sakthidevi, G. and Mohan, V. R. (2013). Total phenolic, flavonoid contents and *in vitro* antioxidant activity of *Dioscorea alata* tuber. Journal of Pharmaceutical Sciences and Research, 5(5): 115 – 119.
23. Sanjay, R. B. and Bhagyashri, D. R. (2013). Extraction of some secondary metabolites and thin layer chromatography from different parts of *Centella asiatica* L. (URB). American Journal of Life Sciences, 1(6): 243-247.
24. Sarla, S., Subhash, C. and Abhay, P. M. (2013). Nutritional profile, antinutritional profile and phytochemical screening of Garhwal Himalaya medicinal plant *Dioscorea alata* tuber. International Journal of Pharmaceutical Sciences Review Research,23(2): 42-46.
25. Shajeela, P. S., Mohan, V. R., Louis, J. L. and Tresina, S. P. (2011). Nutritional and antinutritional evaluation of wild yam (*Dioscorea* spp.). (Evaluation del valor nutritional factors antinuticionales de *Dioscorea* spp. silvestre). Tropical and Subtropical Agroecosystems, 14(2): 723-730.
26. Subasini, U., Thenmozhi, S., Sathyamurthy, D., Vetriselvan, S., Victor, R. G. and Dubey, G. P. (2013). Pharmacognostic and phytochemical investigations of *Dioscorea bulbifera* L. International Journal of Pharmacy and Life Sciences,4(5): 2693-2700.
27. Volluri, S. S., Bammidi, R. S., Chippada, C. S. and Vangalpati, M. (2011). *In vitro* antioxidant artirts and estimation of total phenolic content in methanolic extract of *Bacopa monniera*. Molecules,4(2): 381-386.
28. Wahab, O. M., Ayodele, A. E. and Moody, J. O. (2010). TLC phytochemical screening in some Nigerian Loranthaceae. Journal of Pharmacognosy and Phytotherapy,2(5): 64-70.
29. Yen, G. C. and Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry,43(1):27-32.

CITATION OF THIS ARTICLE

N. R. Anvitha and M. S. Nalini. Phytochemicals, Antioxidant Activity in *Dioscorea oppositifolia* L. and Their Characterization by Thin Layer Chromatography. Bull. Env.Pharmacol. Life Sci., Vol10 [3] February 2021 : 20-28