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EXTRACTION, PHYTOCHEMICAL INVESTIGATION AND QUANTITATIVE STUDY OF TPC AND TPC BY UV VIS. SPECTROSCOPY IN EXTRACT OF *DIOSCOREA BULBIFERA*

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ABSTRACT

The present study focused on the extraction, phytochemical investigation, and quantitative analysis of total phenolic content (TPC) and total flavonoid content (TFC) of the hydroalcoholic extract of *Dioscorea bulbifera* leaves. The leaves were extracted using a hydroalcoholic solvent system by maceration, yielding 12.5% w/w of extract. Preliminary phytochemical screening revealed the presence of flavonoids, phenols, carbohydrates, saponins, and diterpenes. Thin-layer chromatography (TLC) further confirmed the presence of quercetin as one of the key flavonoid components. Quantification of TPC and TFC was carried out using UV-Visible spectrophotometry. The extract exhibited 0.461 mg GAE/100 mg of phenolic content and 0.875 mg QE/100 mg of flavonoid content, indicating strong antioxidant potential. The study provides a basis for the therapeutic application of *Dioscorea bulbifera* in oxidative stress and inflammatory disorders and supports its traditional medicinal use.

Keywords: *Dioscorea bulbifera*, hydroalcoholic extract, phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), TLC, UV-Vis spectrophotometry, antioxidant activity.

INTRODUCTION

Medicinal plants have been a cornerstone of traditional healthcare systems for centuries and continue to be a major source of novel therapeutic agents. One such plant is *Dioscorea bulbifera* L. (family: Dioscoreaceae), commonly known as air potato. It is widely distributed in tropical regions and is traditionally used for the treatment of various ailments such as tumors, inflammation, leprosy, and ulcers (Patil *et al.*, 2014).

Dioscorea bulbifera is known to possess a rich profile of phytochemicals, including flavonoids, phenolic compounds, saponins, and terpenoids, which contribute to its wide range of pharmacological activities (Sundaram *et al.*, 2012). Among these, total phenolic content (TPC) and total flavonoid content (TFC) are of particular interest due to their potent antioxidant and anti-inflammatory properties (Kumar & Pandey, 2013). Quantification of these compounds helps in correlating phytochemical content with biological activity and therapeutic potential.

The use of UV-Visible spectrophotometry is a reliable, rapid, and cost-effective method for the quantification of TPC and TFC in plant extracts. The method involves complexation reactions with specific reagents Folin-Ciocalteu reagent for TPC and aluminum chloride for TFC followed by absorbance

measurements at specific wavelengths (Singleton *et al.,* 1999; Chang *et al.,* 2002).

The current study is focused on the extraction of the hydroalcoholic extract of *Dioscorea bulbifera*, followed by qualitative phytochemical screening and quantitative estimation of TPC and TFC using UV-VIS spectrophotometry. This investigation aims to provide scientific validation for the traditional use of the plant and to support its potential for development into standardized herbal formulations.

MATERIALS AND METHODS

Materials

The study utilized various high-purity chemicals from reputed suppliers. Key reagents such as potassium mercuric iodide, picric acid, and ferric chloride were obtained from Thomas Baker, Mumbai**.** Chemicals like iodine, potassium iodide, sodium nitroprusside, lead acetate, and Folin–Ciocalteu reagent came from Loba Chemie Pvt. Ltd., Mumbai**.** Solvents including methanol, ethanol, and chloroform were procured from Qualigens Fine Chemicals, Mumbai**,** while pyridine, nitric acid, and copper acetate were sourced from S.D. Fine Chem. Ltd., Mumbai**.** Fehling's solution was obtained from Central Drug House Ltd., New Delhi**.** These materials supported phytochemical screening and UV-Vis-based estimation of phenolics and flavonoids.

Methods

Selection and Collection of plant material

Ethnobotanical surveys were conducted in different tribal localities of Madhya Pradesh. The method adopted for collection of data was interview with tribals, local medicine men and one to one discussion about therapeutic use of local plants in the treatment of various diseases. Present work carried out on plant species *Dioscorea bulbifera*. Leaves of *Dioscorea bulbifera* were collected from rural area of Bhopal (M.P), in the months of February, 2025.

Extraction procedure

Following procedure was adopted for the preparation of hydroalcoholic extract from the shade dried and powdered herbs (Mukherjee, 2007).

Extraction by maceration process

50 gm dried powdered leaves of *Dioscorea bulbifera* has been extracted with hydroalcoholic solvent (ethanol: water; 80:20) using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40ºC.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. The percentage yield of each extract was calculated by using following formula:

$$\% \text{ yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical Screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids

etc.). The chemical tests were performed for testing different chemical groups present in extracts (Kokate, 1994).

A. Alkaloids To the extract dilute hydrochloric acid was added. Then it was boiled and filtered.

i. ***Hager's test***

To 2-3 ml of filtrate, few drops of Hager's reagent were added. Formation of yellow precipitate indicated the presence of alkaloids.

B. Carbohydrates

i. ***Fehling's test*** (Reducing sugars): To 2 ml of extract, equal volume of mixture of equal parts of Fehling's solution A and B were added and boiled for few minutes in boiling water bath. Formation of red or brick red coloured precipitate indicated the presence of reducing sugars.

C. Flavonoids

i. ***Lead acetate test***

Test solution with few drops of Lead acetate solution shows intense yellow precipitate colour.

ii. ***Alkaline reagent test:*** To 2 ml of test solution add 2 ml alkali, gives yellow color, which disappears on addition of dil. HCl it disappears, which indicates presence of flavonoids.

D. Proteins

i. ***Biuret's test*** (General test)

To 1 ml of test extract, 4% of sodium hydroxide solution and few drops of 1% copper sulphate solution were added. Formation of a violet red colour indicated the presence of proteins.

E. Saponins

*i. **Foam test***

The extract was shaken vigorously with water in a test tube. Formation of persistent foam indicated the presence of saponins.

F. Detection of proteins and amino acids

*i. **Xanthoproteic Test***

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

G. Glycosides

*i. **Legals test***

To 2 ml of test solution, 1 ml of pyridine and 1 ml of sodium nitroprusside was added. Change in color to pink or red indicated presence of cardiac glycosides.

H. Phenol

*i. **Ferric chloride test***

Extract solutions were treated with 5% ferric chloride solution. Formation of blue colours indicated the presence of hydrolysable tannins and formation of green colour indicated the presence of condensed Phenol.

I. Diterpenes

i. ***Copper acetate Test***

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Qualitative chromatographic analysis by thin layer chromatography

Thin-layer chromatography (TLC) plates were prepared by mixing silica gel with water to form a slurry, which was uniformly spread onto clean plates and air-dried, followed by activation at 100–110°C for 30 minutes to ensure effective solute migration. The development chamber was saturated using the mobile phase and filter paper to maintain solvent vapors. Samples were applied onto the activated plates using a capillary tube, and the plates were placed in the chamber with the solvent at a depth of 0.5 cm. After development, the solvent front was marked,

and residual solvent was evaporated in an oven to prepare the plates for analysis.

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Mishra *et al.,* 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 2 ml (1mg/ml) of this extract was for the estimation of Phenol. 2 ml of each extract or standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min at 40℃ for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Mishra *et al.,* 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 3 ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

RESULTS AND DISCUSSION

The hydroalcoholic extract of *Dioscorea bulbifera* leaves showed a moderate percentage yield of 12.5%**,** indicating effective extraction efficiency using the selected solvent system. Phytochemical screening (Table 2) confirmed the presence of several important bioactive compounds. Notably, flavonoids**,** phenols**,** carbohydrates**,** saponins**,** and diterpenes were found to be present, while alkaloids and proteins/amino acids were absent. These findings support the therapeutic potential of the plant, particularly due to the presence of flavonoids and phenolics, which are known for their strong antioxidant and anti-inflammatory properties.

Thin Layer Chromatography (TLC) results further confirmed the presence of quercetin**,** a known flavonoid, in the extract, with matching Rf values under normal light, short UV, and long UV conditions. This supports the reliability of the extract's composition as observed in preliminary phytochemical tests.

The quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) (Table 3) using UV-Vis spectroscopy revealed significant levels: 0.461 mg/100 mg GAE for phenolics and 0.875 mg/100 mg QE for flavonoids. These results indicate a rich presence of natural antioxidants in the extract, validating its potential use in formulations targeting oxidative stress-related diseases such as arthritis, inflammation, and metabolic disorders. Overall, the study provides a strong phytochemical basis for the therapeutic evaluation of *Dioscorea bulbifera* extract.

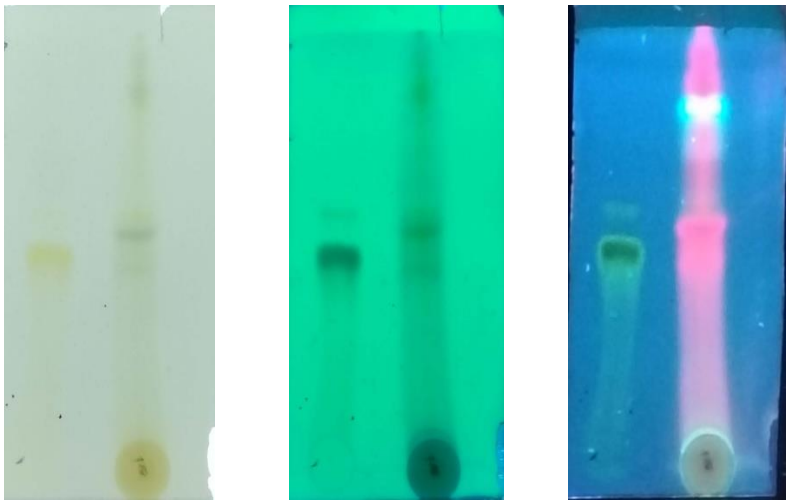
Table 1: % Yield of hydroalcoholic extract of *Dioscorea bulbifera*

S. No.	Part	% Yield (w/w)
1.	Leaves	12.5%

Table 2: Phytochemical screening of hydroalcoholic extract of *Dioscorea bulbifera*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Dragendroff's test	-ve
	Hager's test	-ve
2.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
3.	**Phenol**	
	$FeCl_3$	+ve
4.	**Proteins and Amino acids**	
	Xanthoproteic test	-ve
5.	**Carbohydrates**	
	Fehling's test	+ve
6.	**Saponins**	
	Foam test	+ve
7.	**Diterpenes**	
	Copper acetate test	+ve

[+ve=Positive; -ve= Negative]



Normal light **Short UV** **Long UV**

Figure 1: TLC of *Dioscorea bulbifera* extracts (Quercetin)

Table 3: Total phenolic and total flavonoid content of *Dioscorea bulbifera*

S. No.	Extract	Total phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	Hydroalcoholic extract	0.461	0.875

CONCLUSION

The present investigation successfully demonstrated that the hydroalcoholic extract of *Dioscorea bulbifera* leaves possesses valuable phytochemicals, including flavonoids, phenolics, carbohydrates, saponins, and diterpenes. The percentage yield of 12.5% indicates efficient extraction using the selected solvent system. Phytochemical screening and TLC analysis confirmed the presence of bioactive constituents like quercetin. Quantitative estimation using UV-Vis spectroscopy revealed considerable amounts of total phenolic (0.461 mg GAE/100 mg) and flavonoid content (0.875 mg QE/100 mg), suggesting strong antioxidant potential. These findings support the traditional use of *Dioscorea bulbifera* in herbal medicine and provide a scientific foundation for its potential application in managing oxidative stress-related and inflammatory disorders such as arthritis. Further pharmacological and clinical studies are recommended to validate and explore its therapeutic efficacy.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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