Pharmacogn. Res., 2025; 17(1):272-276.
https://www.phcogres.com

Original Article

Phytochemical Screening and Antioxidant Activity of *Borassus flabellifer* L. Sprouts

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ABSTRACT

Background: Various plant parts are utilized in traditional medicine for their therapeutic capabilities. *Borassus flabellifer* is popularly referred to as double palm, Palmyra palm, or toddy palm. The tree's young roots have been utilized to serve as anti-parasitic and diuretic and root decoction serves to treat gastritis and respiratory conditions. **Objectives:** Qualitative phytochemical analysis of petroleum ether, ethanol and aqueous extracts of *Borassus flabellifer* sprouts, the quantification of phenol and flavonoid in petroleum ether, ethanol and aqueous extracts of *Borassus flabellifer* sprouts. Also, to determine the *in vitro* antioxidant activity of petroleum ether, ethanol and aqueous extracts of *Borassus flabellifer* sprouts. **Materials and Methods:** To find important phytoconstituents, freshly made plant extracts were put through both quantitative as well as qualitative phytochemical screening procedures. Antioxidant activity was analyzed using DPPH Radical Scavenging Assay to assess various extracts. **Results:** Numerous phytochemicals, including alkaloids, steroids, tannins, flavonoids, phenols, glycosides, coumarins and saponins are found in various *B. flabellifer* extractions. The largest quantity of phenol is found in aqueous extracts, also the highest concentration of flavonoids is found in aqueous extracts of *B. flabellifer. In vitro, B. flabellifer* ethanol extracts had noticeably higher antioxidant activity than other extracts. **Conclusion:** Nevertheless, presence of several phytochemicals gave all plant extracts antioxidant properties. The latest study makes it easier for researchers to find and separate novel bioactive substances with potential medical uses as well as how they will likely act against different biological processes.

Keywords: Antioxidant, *Borassus flabellifer*, Phytochemical, Radical scavenging, Rutin.

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Received: 05-12-2024;
Revised: 18-12-2024;
Accepted: 09-01-2025.

INTRODUCTION

Medicinal herbs are extensively utilized as traditional remedies in non-industrialized countries, mostly due to their accessibility and cost-effectiveness compared to contemporary pharmaceuticals. Around 80% of the global population continues to depend on phytopharmaceuticals; currently, numerous medications are derived from medicinal flora.[1] Herbal treatments have been utilized to treat, prevent, as well as cure ailments and diseases since antiquity. Nevertheless, the application of therapeutic plants may fail to satisfy standards of safety, quality and efficacy. During processes of processing, harvesting, storage, as well as distribution, medicinal plants are susceptible to contamination by various fungi, which may lead to deterioration and formation of mycotoxins.[2] Secondary metabolites, commonly referred to as plant constituents or natural chemicals, have substantial pharmacological and toxicological impacts on humans. Chemical molecules in plant resources are classified as primary as well as secondary metabolites according to their chemical structure and metabolic origin. Secondary metabolites have a wide array of therapeutic activities and directly engage with cell membranes, receptors and nucleic acids.[3]

Various plant parts are utilized in traditional medicine for their therapeutic capabilities. *B. flabellifer* L. represents a plant of therapeutic significance. Plants possess an array of phytochemicals which may exert an effect on the human body. Palmyra palm tree belongs to the 'Arecaceae' family. The Palmyra tree serves as state tree of Tamil Nadu in India. All components possess therapeutic effects. This advantageous tree contains albuminoids, gums, steroids, lipids, glycosides and carbohydrates such as sucrose, as well as saponins and steroids like borassosides and dioscin. The diverse components of the plant are utilized for numerous ailments, including antiperiodic conditions, secondary syphilis, heartburn and enlargement of the liver and spleen.[4] The

DOI: 10.5530/pres.20252040

B. flabellifer seed coat extract has antimicrobial action. Certain components have pharmacological and biological properties, such as diuretic, antibacterial and anthelmintic effects.[5] Present work was evaluated in *B. flabellifer* sprout to depict the antioxidant activity in various extracts and to understand the phytochemical constituents present in it.

MATERIALS AND METHODS

Chemicals

All chemicals used in this experiment are analytical grade.

Collection and Extraction of Plant Materials

Fresh plant tubers had been rinsed with distilled water after being thoroughly cleaned with tap water. Tubers were cut and dried in a microwave oven for 48 hr. The dried tubers are powdered well. 20 g of powder weighed in 3 beakers and 100 mL of different solvents such as petroleum ether, ethanol, as well as distilled water were added. The extracts were filtered and the residue of each extract was kept in a glass beaker that was wrapped using aluminum foil and muslin cloth. The filtered extract is evaporated to dryness by being kept in a water bath at 50 °C.

Qualitative Phytochemical Screening of Secondary Metabolites

Freshly prepared plant extracts were diluted with petroleum ether, ethanol and aqueous and then analyzed using standard phytochemical detection protocols to ascertain the existence of phytochemical elements such as steroids, alkaloids, tannins, phenols, flavonoids, glycosides, coumarins and saponins.[6]

Quantitative Estimation of Phenol

200 µL samples had been in addition to 1.4 mL distilled water and 100 µL Folin-Ciocalteu reagent.[7] After 30 sec (not exceeding 8 min), 300 µL of 20% Na_2CO_3 solution was introduced, and the combination was permitted to stand for 1 hr. Absorbance had been calculated at 765 nm utilizing a UV-Vis Spectrophotometer (Labtronics LT-290). Standard gallic acid solutions (1-10 µL Concentration) were prepared for the construction of a standard curve. The data were presented as the gram equivalent of the dry gallic acid sample. The test was run in and analysis of the sample was run in triplicate and averaged.

Quantitative Estimation of Flavonoid

The technique has been employed to estimate each plant extract's total flavonoid content.[8] According to this approach, each sample (1.0 mL) was mixed with 4 mL of distilled water and then with 0.3 mL of a 10% $NaNO_2$ solution. After 5 min, 0.3 mL of 10% $AlCl_3$ solution was added, followed by 2 mL of 1% NaOH solution to combination. The mixture was promptly homogenized, then absorbance had been subsequently measured at 510 nm against blank. A standard curve for Rutin was established and equivalents of Rutin were determined (gram equivalents of Rutin).

Determination of *in vitro* Antioxidant Activity by DPPH Radical Scavenging Activity

Ability to scavenge free radicals *B. flabellifer* was assessed utilizing 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) method.[9] A total of 24 mg of DPPH has been dissolved in 100 mL methanol for preparing stock solution. Methanol filtration of DPPH stock solution resulted in viable combination exhibiting absorbance for around 0.973 at 517 nm. In many test tubes, 3 mL of DPPH working solutions were mixed with 100 µL of leaf extract and ascorbic acid. A standard solution typically comprises 3 mL of DPPH within 100 mL methanol. After that, tubes had been kept completely dark for half an hour. The absorption had consequently measured at 517 nm. The subsequent formula was employed to calculate proportion of antioxidants.

$$\text{Percentage of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

Where, Ac-absorbance of Control, As–absorbance of testing solution.

RESULTS

Phytochemical Analysis of Secondary Metabolites in Petroleum Ether, Ethanol and Aqueous Extracts of *B. flabellifer* Sprouts

A preliminary phytochemical investigation of secondary metabolites was performed utilizing ethanol, petroleum ether and aqueous extracts of *B. flabellifer* sprouts. During initial screening, all extracts of *B. flabellifer* sprouts demonstrated the presence of secondary metabolites, including flavonoids, coumarins, glycosides, saponins, phenols, steroids, tannins, as well as alkaloids (Table 1).

Quantification of Phenol and Flavonoid in Petroleum Ether, Ethanol and Aqueous Extracts of *B. flabellifer* Sprouts

In the spectrophotometric method, Folin-Ciocalteu (F-C) is generally employed, consisting of a mixture of sodium molybdate, sodium tungstate and other reagents. They undergo a rapid oxidation reaction of phenol in the presence of alkali forming phenolate ions. Gallic acid serves as a reference in this procedure. The largest quantity of phenol is found in aqueous extract than other extracts (Table 2).

The quantification of flavonoid content in ethanol, petroleum ether and aqueous extract of *B. flabellifer* sprouts was done using the Aluminium Chloride colorimetric method. The C4 keto group and the C3 or C5 hydroxyl group of flavones and flavanols combine with aluminum chloride to form complexes that are acid-stable. Rutin serves as a benchmark. The amounts of flavonoids in ethanol, petroleum ether, as well as water-based

extracts of *B. flabellifer* sprouts, are determined to be 250, 270 and 880 gm/Equivalent of Flavonoids respectively (Table 2).

In vitro Antioxidant Activity in Petroleum Ether, Ethanol and Aqueous Extracts of *B. flabellifer* Sprouts

Utilizing the DPPH radical scavenging experiment, the antioxidant properties of *B. flabellifer* sprout have been studied. DPPH is a stable free radical capable of accepting hydrogen or electrons from antioxidants, leading to the formation of a stable molecule. Ascorbic acid was used as a standard in this procedure. *B. flabellifer* sprouts showed variation in their antioxidant activity in petroleum ether, ethanol and aqueous extracts. The findings demonstrated that at 25 µg/mL, the ethanol extract of *B. flabellifer* exhibited the maximum antioxidant activity, with an inhibition percentage of 48% (Figure 1).

DISCUSSION

Herbal remedies are useful in both preventing and curing human illnesses. Since ancient times, people have been applying plants as a kind of traditional medicine.[10] Conventional phytotherapy has been employed globally for the management of diabetes mellitus. Numerous herbs have been recognized for their efficacy in treating and managing diabetes among various meditative practices and alternative therapies. Moreover, they exhibit no adverse effects.[11] These herbal herbs possess hypoglycemic and additional advantageous qualities. Secondary metabolites, commonly referred to as plant constituents or natural chemicals, have considerable pharmacological and toxicological impacts on humans. Key secondary metabolites encompass phenols, terpenoids, flavonoids, alkaloids, as well as glycosides.[3]

This study examines ethanol, petroleum ether and aqueous extracts of *B. flabellifer* sprouts are evaluated to know their phytochemical importance and therapeutic properties like antioxidant activity. Initial phytochemical analysis of many extracts of *B. flabellifer* noticeable appearance of secondary metabolites like saponins, coumarins, tannins, alkaloids, phenols, terpenoids, steroids and flavonoids. According to phenol and flavonoid quantifications, all extracts show significant content of them. The petroleum ether, ethanol and aqueous extracts are detected to have phenol and flavonoids respectively. Aqueous possessed the highest quantity of phenol as well as flavonoids. One method for evaluating antioxidant properties *in vitro* includes the DPPH radical scavenging experiment. By this technique, *B. flabellifer* sprout various extracts showed a significant level of antioxidant activity. Ethanol extracts had the highest *in vitro* antioxidant activity (48%).

Certain phytochemicals, including flavonoids, tannins, saponins, glycosides and terpenoids, were found in aqueous, ethanolic and methanolic extracts of seed coat of *B. flabellifer* after preliminary phytochemical screening.[12] Phytochemical analysis of *B. flabellifer* fruit indicated the existence of several bioactive phytoconstituents, including phenol, alkaloids, flavonoids, saponins, tannin, as well as terpenoids.[13]

Strong evidence of a connection between plant's total phenolic contents and their consequent antioxidant capabilities has been

Table 1: Phytochemical Analysis of Secondary Metabolites in Petroleum Ether, Ethanol and Aqueous Extracts of *B. flabellifer* Sprouts.

Sl. No.	Phytochemical Constituents	Test	Petroleum Ether	Ethanol	Aqueous
1	Coumarin	NaOH+Chloroform	+	+	+
2	Flavonoids	Lead acetate	+	+	+
3	Glycosides	Keller Kiliani Test	+	+	+
4	Phenol	Folin's Test	+	+	+
5	Saponin	Distilled water	+	+	+
6	Steroids	Chloroform+H_2SO_4	+	+	+
7	Tannins	Braemer's Test	+	+	+
8	Alkaloids	Dragendorff's Test	+	+	+

Table 2: Quantification of Phenol and Flavonoid in Petroleum Ether, Ethanol and Aqueous Extracts of *B. flabellifer* Sprouts.

Sl. No.	Extract (100 µg/µL)	Phenol		Flavonoid	
		Optical Density	Equivalents of Gallic acid (g/Equivalent)	Optical Density	Equivalents of Rutin (g/Equivalent)
1	Petroleum Ether	0.103	190	0.090	250
2	Ethanol	0.780	610	0.231	270
3	Aqueous	0.804	630	0.443	880

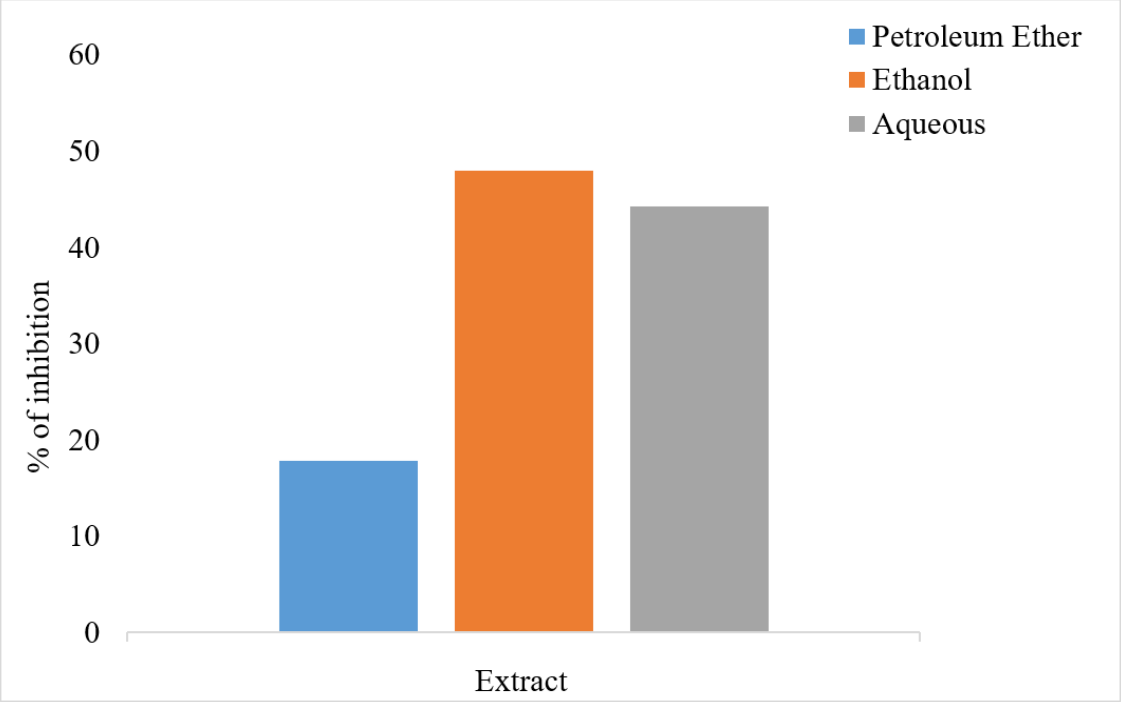

Figure 1: Percentage of inhibition of *in vitro* Antioxidant Activity in Petroleum Ether, Ethanol and Aqueous extracts of *B. flabellifer* Sprouts.

presented by numerous antioxidant studies on plant extracts. [14,15] Numerous biological processes, including metal chelating activity, free radical scavenging, cardio-protective, anti-inflammatory, hepatoprotective and anticancer properties, are linked to flavonoids. Additionally, our study emphasized variation in total yields of flavonoids and phenols in different extracting solvents, which can be explained by variation in the availability of extractable compounds resulting from the formation of different kinds of complexes between these compounds along with other phytochemicals in different plant material samples.

The current study on *B. flabellifer* sprout was to show the presence of phytochemical constituents as well as to depict the antioxidant activity in various extracts. The study reveals the presence of therapeutic properties in *B. flabellifer* sprout. Additional investigation on other individual chemicals found in different medicinal plants and their potential as antioxidants *in vivo* utilizing different antioxidant assays may result from this study.

CONCLUSION

The results of the present study conclude that *B. flabellifer* L. sprouts contain various phytochemical constituents such as alkaloid, tannin, phenol, flavonoid, glycosides, coumarins, steroids as well as saponins so that it has high nutritional value. Antioxidant properties shown in petroleum ether, ethanol, as well as aqueous extracts may characterize the therapeutic properties of sprouts of *B. flabellifer*. This study will contribute to future advancement in the field of research work for the development of therapeutic compounds helpful for mankind.

ACKNOWLEDGEMENT

We are grateful to DBT-STAR, DST-FIST, DST-CURIE and the Department of Botany, Vimala College (Autonomous), Thrissur for providing the necessary facilities for doing this work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DPPH: 1, 1-Diphenyl-2-Picrylhydrazyl; **B. flabellifer:** *Borassus flabellifer*; **Hr:** Hour; **g:** Gram; **µL:** Microliter; **µg:** Microgram; **mL:** Milliliter; **Sec:** Seconds; **Min:** Minutes; **nm:** Nanometer; **°C:** degree Celsius; **UV-Vis:** Ultraviolet-Visible spectroscopy; **Ac:** Absorbance of Control; **As:** Absorbance of testing solution; **(F-C):** Folin-Ciocalteu.

SUMMARY

Borassus flabellifer is an important plant with many traditional medicinal uses. The powder of *B. flabellifer* was extracted using various organic solvents and determined for *in vitro* antioxidant activity and secondary metabolite profile. The preliminary phytochemical analysis revealed that secondary metabolites such as alkaloids, tannin, phenol, flavonoids, glycosides, coumarins and steroids. In the quantitative analysis of the various extracts, the aqueous extract showed significant levels of both phenol and flavonoid. The *in vitro* antioxidant activity was determined using

DPPH radical scavenging assay. The ethanol extract showed the highest percentage of inhibition than other extracts.

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Cite this article: Shaju AC, Dasan A, Vineethan NA, Nargees S, Francis S, Benny AC , Tharakan ST. Phytochemical Screening and Antioxidant Activity of *Borassus flabellifer* L. Sprouts. Pharmacog Res. 2025;17(1):272-6.