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Antioxidant Potentialof *Borassus flabellifer* Linn.Tuber Sprouts

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Abstract

Borassus flabellifer Linn. belonging to Arecaceae is the state tree of Tamil Nadu. It is a source of livelihood for many people in the state. The tree has not only fruit as a useful part,its leaves,wood and tubers are widely used. During summer the fruitsare a haven to dehydrate the body and replace lost minerals. The sprouts are a good source of minerals and medicinally active compounds. The investigation was undertaken to prove the free radical scavenging property of the sprouts.When samples from trees growing in various regions of Tamil Nadu was compared for antioxidant potential, Madurai samples showed high free radical scavenging activity in both DPPH and ABTS assay, by exhibiting IC_{50} value of 8.34 µg/mL and 3.09 µg/ mL respectively. The antioxidant activity is also correlated to the Total Phenol and Total Flavanoid content of the sprouts.

Introduction:

Medicinal plants have a very important role in the health of human beings. *Borassus flabellifer* Linn. which is distributed widely in the tropical regions of Asian and African countries, slow-growing perennial capable of living for more than 150 years (Artnarong*etal.*, 2016).A review on the pharmacological importance of this planthas been put forward by Shanmugalingam *et al*., 2021, in which the authors have considered the numerous bioactive principles of this plant. The various parts of the palmyra palm have been widely used in traditional medicine for the treatment of several ailments (Paschapur*etal.*, 2009). Traditionally the different parts of the plant such as root, leaves, fruit, and seeds are used for various human disorders. Leaves are used for thatching, mats, baskets, and fans. Flowers of *B. flabellifer* were investigated by Paschapur*et al*., 2009for analgesic and antipyretic effects, anti-inflammatory activity, haematological, and biochemical parameters, and immunosuppressant properties (Révész*et al*., 1999). The different parts of the plant are being used for medicinal properties like anthelminthic and diuretic. The fruit pulp of *B. flabellifer* has been used in traditional dishes and the sap has been used as a sweetener for diabetic

patients. Phytochemical studies of the plant revealed the presence of spirostane-type steroid saponins; steroidal glycoside also contains a bitter compound called flabelliferrin(Yoshikawa *et al*., 2007)Knowledge ofMedicinal plants provides a new way for modern drug development (Brahman. *et al*., 2000) Herbal medicines are in great demand in the developed world of primary health care because of their safety, efficacy, and lesser side effects. Out of these, the real medicinal value of over 4,000 plants is either little known or unknown to the mainstream population(Pushapangadan*et al*., 1995). The healing properties of many herbal medicines have been recognized in many ancient cultures (Rajeshwari*et al*., 2011).In 2022, Bhanu *et al*.,2022have screened the freeze-dried seed coat powderof *B. flabellifer* for various medicinal properties like anticancerous, antidiabetic and antibacterial potential. The present study aims to quantitatively screen the sprout extract of *B. flabellifer* for important phytochemicals and to evaluate its antioxidant properties by *in vitro* free radicals scavenging assays so as to prove its nutraceutical and pharmaceutical importance. The phytochemicals of plants obtained in the form of extract or fractionhave gained considerable interest from researchers working in the field of pharmaceuticals and health sciences. Some previous studies have also reported that the reducing power may serve as a significant indicator of potential antioxidant activity. Antioxidative activity has been proposed to be released to reduce power (Pellegrini *et al.*, 2003). Halvorsen *et al.*, 2006suggested that secondary metabolites are redox-active compounds that will be picked up by the reducing power assay.

MATERIALS AND METHODS

Plant material and preparation of extracts:

The palmyra palmsprouts that weighed between 30 to 50 gm were collected from four Districtsof Tamil Nadunamely Coimbatore, Madurai, Tirunelveli, and Thoothukudi. The plant material waswashed, dried, and made into a fine powder and subjected to successive solvent extraction procedures using methanol as solvent. Phytochemicals like total phenols and flavonoids were quantified in the methanol extract of sprouts. This extract was also subjected to an antioxidant assay to prove the free radical scavenging activity of the tubers using DPPH and ABTS assays.

Determination of Total phenols

Total flavonoid content was determined by the colorimetric method (Jia, Tangand Wu, 1999). 0.25 ml (100 mg ml -1) of each extract was diluted with 4.5 ml of distilled water and

0.3 ml of 5% $NaNO_2$ solution. After 5 min, 0.3 ml of 10 % $AlCl_3$ was added and incubated for 5 min. Then, 2 ml of 1M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured immediately at 510 nm. The results were expressed as catechin equivalents (µg CE/ 100 mg).

Determination of Flavonoids

Total phenolic content was determined as per the method described by Singleton and Rossi (1965). Briefly, appropriate volumes of sample extracts were oxidized with Folin-Ciocalteu reagent and the reaction was neutralized with sodium carbonate. The results were expressed as gallic acid equivalents (µg GAE/ 100 mg).

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay

DPPH (1, 1-diphenyl -2picryl –hydrazyl) free radical scavenging activity of the methanolic fruitextract of *B. flabellifer* was determined by the method of Lamaison *et al*.,2004 which depends on scavenging of coloured free radical (DPPH) in methanol solution by seed extract of *B. flabellifer*. The reaction mixture contains DPPH and extracts in a final concentration of 3ml. Absorption of DPPH at its absorption maximum of 516nm is inversely proportional to the concentration of extract which depends on the scavenging potential of extract. The activity was expressed as inhibitory concentration 50 (IC_{50}) i.e. the concentration of extract required to give a 50% reduction in absorbance of test solution compared to that of blank solution.

ABTS•+ (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay

ABTS•+radical cation scavenging activity of methanolictuber extract was determined according to the method of Re *et al.*, 1999. ABTS radical cation (ABTS•+) in 20 mmol sodium acetate buffer (pH-4.5) was combined with 2.45 mmol potassium persulfate to generate a stable dark blue-green radical following 12-16 h of incubation at 4 °C in the dark. The reaction mixture is suitably diluted to an absorbance of 0.7±0.01 at 734 nm spectrophotometrically to form the test reagent. The reaction mixture of tuber extract and 3.0 ml of test reagent were incubated in a water bath at 30 °C for 30 min. The test reagent was added to the test solution mixture turns colourless and the absorbance is reduced due to the sequestration of unpaired electrons in the test reagent by the antioxidants in the tuber extract. Trolox standards (final concentration 0-15 µM) in ethanol were used as a reference. The unit

of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts on dry matter.

The percentage of inhibition for all assays was calculated as follows:

% inhibition = [(Absorbance of Control – Absorbance of Test)/Absorbance of Control] x100

Results and Discussion

The present investigation was done to screenthe biochemical and antioxidant potential of *Borassus flabellifer*tubers (sprouts). Methanolicextractof sprouts of this plantgavemore number ofphytochemicals and hence biochemical and antioxidant activity was screened in thisextract. The phytochemicals quantifiedwere totalflavonoids and total phenols. The methanolic extract of*B. flabellifer*sproutsfromtrees growing at the Madurai site showed a total flavonoid was 96.±0.01µg/GAE/100mgandphenolic content calculated was 91.17±0.04 µg/CE/100mg(Table 1).

Table :1 Total Phenol and Flavanoid Contentof *B. flabellifer* Sprouts Extracted using Methanol.

No.	Assays	Unit
1	Total Phenols	91.17 ± 0.04 µg/GAE/100mg
2	Total Flavanoids	96.41 ± 0.01 µg/CE/100mg

Phenolic compounds are partially responsible for colour, astringency, bitterness, flavour, and nutritional qualities in fruits and vegetables (Macheix*et al.,* 1990). In-plant cells, phenolic compounds are located in the vacuole whereas polyphenol oxidase is located in plastids. Damaged areas in cells allow contact with phenolic compounds, triggering the reaction known as enzymatic browning (Vaughn *et al*., 1984). Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl group thereby contributing to theantioxidant action (Stankovic, 2011). In the present investigation, the Total Phenolic content of methanolicextract of sprouts from trees growing at Madurai was91.17 ± 0.04 µg/GAE/100mg(Table 1). The present investigation agrees with

Sahini *et al*., 2014, in the level of above said biomolecules. Bagashwar and Desai, 2021 has extracted almost similar amounts of these two biomolecules from waste produced during the processing of fruits of *B.flabellifer*. Sahni *et al*., 2014, observed that chloroform extract gave more of Total Flavanoid content while high Total Phenols werecalculated in chloroform extracts of sprouts, which is contraryto the present work in which both Total Phenol and Total Flavanoidwere present in the methanol extract of sprouts.

In this investigation, the Total Flavanoid content cameto 96.41 ± 0.01 µg/CE/100mg (Table 1).Flavonoids are the low molecular weight polyphenolic secondary metabolic compounds universally distributed in the green plant kingdom, located in cell vacuoles.. In plants, flavonoids have long been known to be synthesized in particular sites and are responsible for the colour, and aroma of flowers and fruits to attract pollinators (Samanta *et al.*, 2009). Flavonoids may act as antioxidant factors by preventing the generation of reactive oxygen species and even scavenging it when formed.

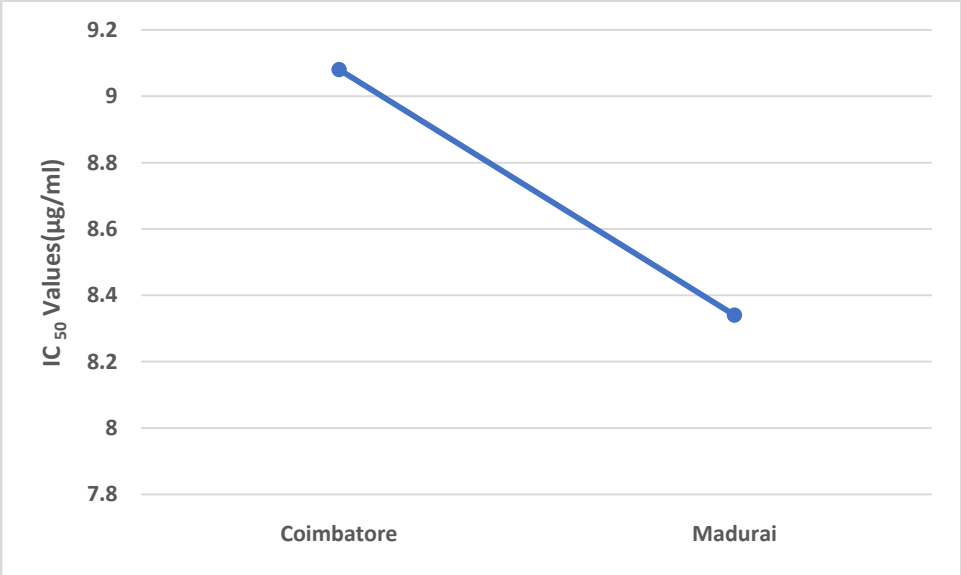
Among the several *in vitro* antioxidant assays, DPPH* and ABTS^{*+}assays have been widely used as more reliable methods in determining the free radical scavenging efficacy of unknown compounds. DPPH is a stable radical with maximum absorption at 517nm that can readily undergo scavenging by antioxidants. It has been widely used to test the ability of compounds as free radicals scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods. Antioxidants with DPPH radical scavenging activity could donate hydrogen to free radicals, particularly to the lipid peroxides or hydroperoxide radicals that are the major propagators of the chain auto-oxidation of lipids and form non-radicals species, resulting in the inhibition of propagating phase of lipid peroxidation (Kandhasamy and Sun chul, 2013). The concentration of the sample necessary to decrease the initial concentration of DPPH· by 50% (IC_{50}) under the experimental condition was determined. Therefore, a lower value of IC_{50} indicates a higher antioxidant activity(Kang*et al*., 2008).

In the present work,DPPH radical scavenging activity was compared with the standard ascorbic acid (IC_{50} 2.952µg /mL).The highest percentage of inhibition of free radicals was shown by the methanolic extractof sprouts collectedfrom Madurai (65.01%) when 10µg/mL of extract was used. The second highest percentage of free radical inhibition was shown by samples from Coimbatore (54.71%) (Table 2). The samples from Madurai District showed an IC_{50} value of 8.34 µg/mLwhich was the leastwhen compared to other Districts samples(Fig 1). Sastry *et al*., 2015agree with the present investigation that the *B.flabellifer* seed coat has high DPPH scavenging activity.

Table 2: DPPH Assay of*B. flabelliferdifferent* concentrations of Methanolic Sprout Extract expressed as Percentage of Inhibition

Sl.N	Sampling sites	Percentage of Inhibition (%)			
		2.5	5	10	1
1	Coimbatore	20.75	37.97	54.71	18.32
2	Madurai	26.98	41.05	65.10	21.24
3	Tirunelveli	18.45	34.73	57.04	16.37
4	Thoothukudi	16.21	32.16	49.12	14.82
5	Ascorbic Acid (Control)	35.36	57.21	24.15	16.34

Fig : 1 IC $_{50}$ Values of DPPH radical scavenging activity of *B. flabellifer*Methanolic Fruit Extract.



ABTS radical scavenging assay

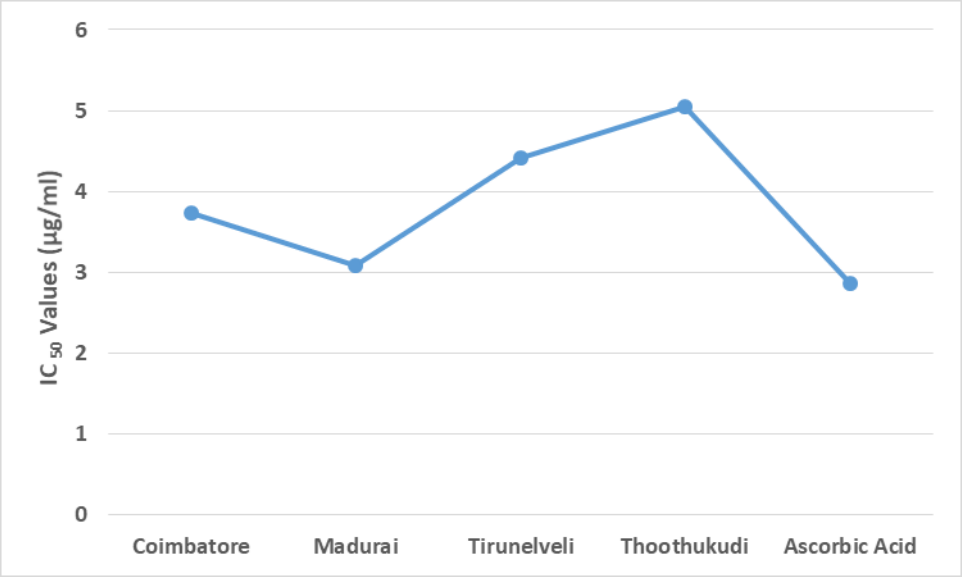
ABTS*+produce more powerful free radicals than DPPH* radicals and the reactions with ABTS^{*+}radicals involve a single electron transfer process (Luo*et al*., 1998). The principle of ABTS^{*+}assay is that the preformed radical monocation of ABTS*+ is generated by the oxidation of ABTS*+ with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants.ABTS·+, a protonated radical, has a characteristic absorbance maximum at 734 nm which decreases with the scavenging of the proton

radicals.ABTS·+ was generated by incubating ABTS with potassium persulfate. The efficacy of ABTS cation radical scavenging activityof tuberextracts of *B. flabellifer* is shown in Table 3 and the comparative chart is shown in Fig 2. In the present investigation, the highest percentage of free radical inhibition was shown byextract of sprouts from trees growing at the Madurai site and the least free radical inhibition was shown by methanolic extract of fruits from trees grown in the Thoothukudi area (Table 3). Theradical scavenging activity was found to be the leastin the methanolic extract of the Thoothukudi sample with an IC_{50}value of (5.05 µg/ mL of theextract) and highest in the methanolic sproutextract of the Madurai samples IC_{50}which came to (3.09 µg/ mL of theextract). The scavenging effect of various sproutextracts with the ABTS radical is in the following order:Madurai>Coimbatore>Tirunelveli>Thoothukudi.Earlier reports by Sahni *et al.*, 2014 agree that the methanol extract of tubers expressed more antioxidant activity in the ABTS assay.

Table 3: ABTS Assay of *B. flabellifer* different concentration of Methanolic Sprout Extract expressed as Percentage of Inhibition

Sl.No	Sampling sites	Percentage of Inhibition (%)			
		2.5	5	10	1
1	Coimbatore	37.09	46.01	58.07	28.31
2	Madurai	46.78	59.29	73.81	37.94
3	Tirunelveli	31.79	40.76	61.04	25.56
4	Thoothukudi	24.01	35.16	53.12	19.29
5	Ascorbic Acid (Control)	38.06	59.51	29.45	19.64

Fig 2 IC_{50} Values of ABTS radical scavenging activity



Conclusions

The reports involving the pharmacological properties of palmyra tubers are sparse. The results of the present study evidenced the presence of pharmacologically active phytochemicals in the methanolic extract of palmyra tubers. The total phenolic and flavonoid contents readily account for the *in vitro* free radical scavenging activity of the tuber extract. Thus, it can be concluded that the Palmyrasprouts may be considered a rich source of diversified medicinal compounds. However, to be considered effective in the field of medicine, clinical tests involving animal and human trials should be conducted. If the *in vivo* findings match the *in vitro* results, this *B. flabellifer* seed powder can be considered a natural ingredient for therapeutic purposesand may serve as a candidate for nutraceutical.

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