

# A COMPARATIVE ANALYSIS OF THE ANTIOXIDANT PROPERTIES OF *BORASSUS FLABELLIFER* SAP COLLECTED FROM KANYAKUMARI AND TIRUNELVELI DISTRICTS

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## ABSTRACT

*B. flabellifer* sap was used in folk medicine for multiple purposes, such as a stimulant, anti-laprotic, diuretic and antiphlogistic. The fruits are stomachic, sedative, laxative and aphrodisiac in nature useful in hyperdipsia, dyspepsia, flatulence, skin diseases, haemorrhages, fever and general debility. The fresh pulp is reportedly rich in vitamins A and C while the fresh sap is a good source of vitamin B-complex. Antioxidants that scavenge these reactive oxygen species and free radicals are of major importance in preventing the onset and progression of many diseases caused by oxidative stress. The aim of this study is to examine the antioxidant property of *Borassus flabellifer* L.sap collected from two different localities of South India viz., Kanyakumari District and Tirunelveli District. The dietary phytochemicals like flavonoids and phenolic acids have been recognized largely as beneficial antioxidants that can scavenge harmful active oxygen species, including O<sub>2</sub><sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, -OH, and O<sub>2</sub>. The results reported that while analysing antioxidant properties, both ABTS and DPPH radical scavenging assays demonstrated the antioxidant potential of the sap. With this, the samples collected from Kanyakumari showed higher activity compared to the Palmyra sap sample from Tirunelveli, suggesting the saps pharmacological nature.

## Introduction

Palmyra palm is extensively grown in various geographic, soil and climatic conditions (coastal belt, agricultural margins, and wastelands) of Tamil Nadu (Sakulsathaporn, *et al.* 2017). The Palmyra palm tree is a dioecious plant; India stands

first in the world in terms of its wealth of Palmyra (*Borassus flabellifer* L) palms, with a population of nearly 122 million palms (Vengaiah *et al.* 2012). *Borassus flabellifer* L.sap was collected from the flower section as a sweetener for diabetes patients. Proteins, lipids, carotenoid, vitamin B complex,

ascorbic acid and other minerals are found in the Palmyra, apart from sugars. Besides, all palmyra fruits are also rich in immunosuppressive effects (Kapoor, 2000).

Palm sap is a non-alcoholic, refreshing natural beverage that has a plethora of health benefits of various natures. Palm sap, also called neera (phloem sap extracted with zero percent alcohol), and was extracted from the inflorescence of toddy palms, which is used as a nutritious health drink. Neera is susceptible to natural fermentation at ambient temperature within a few hours of extraction due to enzymatic and microbial fermentation since it is rich in sugars, vitamins, proteins and minerals (Naveen *et al.*, 2018). Neera is obtained by tapping the top shoots and collecting the dripping juice in hanging earthen pots tied to the trees. The juice early in the morning is refreshing and light. The drink has a high nutrient value and is good for health (Bhaskar *et al.*, 2017). Palm sap is tapped from the matured unopened inflorescence of the palm. The palm sap is collected by cutting the head of the inflorescence (Nathanael 1966). Spontaneous fermentation of sap is caused by the accumulation and growth of yeast from the air (Jeyaratnam *et al.*, 1984); at present, arbitrary quantities of lime (calcium hydroxide) are used to arrest fermentation (Mary *et al.*, 2014).

The dietary phytophenolics like

phenolic acids and flavonoids have been greatly recognized as beneficial antioxidants that can delete harmful active oxygen species such as  $O^2$ ,  $H^2O^2$ , and  $OH$  (Sahni *et al.* 2014). It contains 20 known steroidal glycosides and carbohydrates, like sucrose. It also contains a bitter compound called flabelliferins; these are steroidal saponins (Masayuki and Fengming, 2007). Palm fruit has anti-inflammatory and antioxidant properties. The antioxidant activity could be attributed due to the presence of higher content of crude flavonoids, saponins and phenolic compounds (Pramod *et al.* 2013). Sap from the flower of the matured tree stalk is prized as a tonic, diuretic agent, stimulant, laxative, anti-phlegmatic, and amebicide that are considered to be the best for day-to-day life. Sugar made from this sap is said to counteract poisoning, and it is fairly prescribed for liver disorders. The ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* L. (Arecaceae) has analgesic activity (Jamkhande *et al.* 2016). In the present research, the antioxidant property of the palmyra sap collected from two different localities viz., Kanyakumari and Tirunelveli District of South India were analysed.

## Materials and Methods

### Collection of Palmyra palm sap samples

Fresh Palmyra palm sap samples were collected from two geographically distributed areas, namely Kanyakumari and

Tirunelveli districts of Tamil Nadu. *Borassus flabellifer* is extensively found in the Kanyakumari region, particularly in Karungal, where the extraction of sap reaches its peak from August to November. In the Tirunelveli region, it is widely distributed in the area of Aral, and the extraction of sap is high from April to June. Palm sap is tapped from the mature, unopened inflorescence of the palm. The palm sap was collected by cutting the head of the inflorescence. In rural areas, palm sap was traditionally collected from

Palmyra trees by organized practice for its local consumers. The fresh samples were collected in the early morning by professional tappers. As soon as the collected fresh Palmyra sap samples were brought down from the Palmyra tree, they were transferred to sterilized bottle, packed in a sterile ice box to avoid fermentation during transportation, and transported to the laboratory for analysis. On reaching the laboratory, the Palmyra sap samples were stored in the refrigerator at about 4°C for further analysis.



**Figure 1: Collection of Palmyra sap sample**

### **Antioxidant Assay**

#### **a. ABTS radical scavenging Assay (*Re et. al 1999*)**

The reaction was initiated by the addition of 200µl of diluted ABTS to 1.56–1000µg/ml of different concentrations of Palmyra sap sample. 50 µl of methanol was used in the control tube instead of the

sample. Methanol was used as a blank. The absorbance was measured at 734 nm, and the percentage of inhibition was calculated according to the following equation:

$$\% \text{ of inhibition} = A_0 - A_1 / A_0 \times 100$$

Where,  $A_0$  is the absorbance of control,  $A_1$  is the absorbance of test compound.

## b. DPPH radical scavenging Assay (Kevin *et al.*2013)

The radial scavenging activity of the test sample against stable 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) was determined according to the method of Brand-William *et al.* (1995), with a slight modification. For the DPPH assay, ascorbic acid was used as a reference standard. The ascorbic acid stock solution was prepared in distilled water (1 mg/ml, w/v). A 60 µm solution of DPPH in methanol was freshly prepared and 200µl of this solution was mixed with 50µl of test sample at various concentrations (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, and 800 µg/ml). The plates were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured at 515 nm. The control was prepared with DPPH solution only, without any extract or ascorbic acid. 95% methanol was used as a blank.

Radical scavenging activity was calculated by the following formula;

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of Control}} \times 100$$

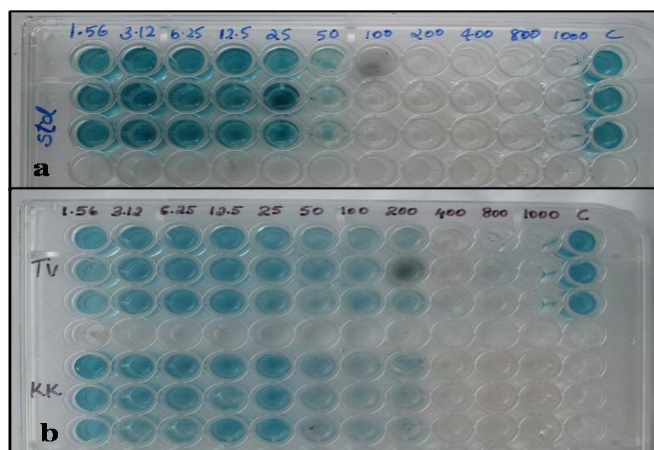
## Result and Discussion

### Results

#### a. ABTS radical scavenging Assay

In the present study, an attempt was made to investigate the antioxidant potentials of Palmyra sap collected from two different geographical (Kanyakumari and Tirunelveli) regions of Tamil Nadu through ABTS radical scavenging assay. Amongst the two Palmyra sap samples, Kanyakumari showed considerably higher ABTS radical scavenging activity than Tirunelveli. For instance, 1.56 µg/ml for Kanyakumari and Tirunelveli recorded minimal antioxidant activity of 5.26% and 7.72%, respectively. The results of the ABTS radical scavenging activity of Palmyra sap samples collected from two different regions of Tamil Nadu are displayed in Table 1 and Figure 2 respectively. So the overall results of ABTS radical scavenging activity were higher in the Palmyra sap samples of Kanyakumari District, with the lowest IC<sub>50</sub> value of 61.50 µg/ml compared to the Palmyra sap samples of Tirunelveli District.

**Fig.2. ABTS radical scavenging assay for a) standard b) samples**



**Table 1. ABTS radical scavenging assay (% of inhibition) in *Borassus flabellifer* sap extracted from two different regions of Tamil Nadu**

Concentration ( $\mu\text{g/ml}$ )	ABTS radical scavenging assay(% of inhibition)		
	Control (Ascorbic acid)	Kanyakumari	Tirunelveli
1.56	8.20	5.26	7.72
3.12	14.80	13.97	11.86
6.25	27.58	17.77	20.52
12.5	43.04	32.62	24.31
25	56.42	40.81	32.21
50	76.73	49.27	42.98
100	90.48	57.84	47.03
200	94.72	69.87	55.57
400	96.10	72.61	65.26
800	96.40	79.85	72.08
1000	97.24	83.52	75.56
IC50	19.69	61.50	134.12

#### **b. DPPH radical scavenging Assay**

The DPPH radical scavenging activity of both experimental samples and ascorbic acid (the positive control) showed a dose-dependent radical scavenging property. Among the experimental samples, Palmyra sap obtained from Kanyakumari region showed consistently

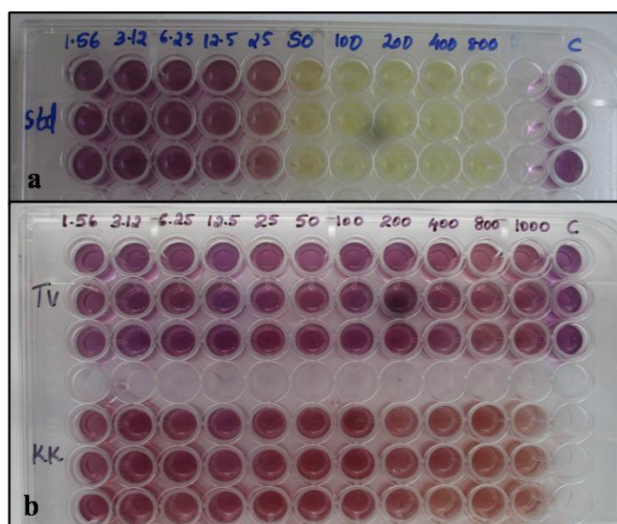


higher DPPH radical scavenging activity of 4.51% to 52.25% than the Tirunelveli region sap (4.27% to 38.29%) across the tested concentrations (1.56 to 1000 µg/ml). The results on the DPPH radical scavenging activity of Palmyra sap of two different geographical regions (Kanyakumari and Tirunelveli) are shown in Table 2 and Figure 3. The overall results of DPPH radical scavenging activity showed that Palmyra sap samples collected from Kanyakumari District recorded higher antioxidant activity with an IC<sub>50</sub> value of 950.74 µg/ml compared to Tirunelveli District.

**Table 3. DPPH radical scavenging activity (% of inhibition) of *Borassus flabellifer* sap extracted from the different regions**

Concentration (µg/ml)	DPPH radical scavenging assay (% of inhibition)		
	Control (Ascorbic acid)	Kanyakumari	Tirunelveli
1.56	3.36	4.51	4.27
3.12	11.61	13.30	7.49
6.25	17.22	16.33	11.40
12.5	32.98	22.43	14.59
25	44.26	26.13	19.73
50	82.19	33.39	22.78
100	88.75	35.13	24.92
200	89.75	39.86	28.82
400	92.11	43.30	32.17
800	94.95	45.55	35.91
1000	-	52.25	38.29
IC <sub>50</sub>		950.74	-

**Fig.3.DPPH radical scavenging activity for a) standard b) Palmyra sap samples**



## Discussion

Plants are capable of producing a wide range of bioactive chemicals. Fruits and vegetables build high levels of phytochemicals, which may protect against free radical damage. Plants that possess beneficial phytochemicals may supplement human needs by functioning as natural antioxidants. (Altemimi *et al.* 2017). Flavonoids are dominant water-soluble antioxidants as well as free radical scavengers, which can forestall oxidation-instigated cell damage coupled with effective anticancer attributes. They can also defend the body from diabetes-linked oxidative disruptions (Yadav *et al.* 2014). In order to analyse the antioxidant potential of Palmyra palm sap, the DPPH and ABTS radical scavenging assays were performed. The ABTS and DPPH assays are widely used methods for the assessment of the antioxidant potentials of natural products. Both of these assays are spectrophotometric

techniques based on the quenching of stable coloured radicals (ABTS or DPPH) and by this means, they reveal the radical scavenging potential of antioxidants even if they are present in complex biological mixtures (Mustafa *et al.* 2021). Sivajia and Aheeshan (2021) depicted that the DPPH scavenging activity was  $1.36 \pm 0.35$  mg/mL, and the total phenolic content and ascorbic acid content were recorded as  $186 \pm 12.27$  (mg GAE/100g) and  $12.16 \pm 0.31$  (mg/100g), respectively. Aheeshan *et al.* (2020) compared the DPPH's scavenging ability of Palmyra, coconut, and kithul samples with the IC values  $1.69 (\pm 0.13)$ ,  $2.91 (\pm 0.04)$ , and  $2.21 (\pm 0.14)$  mg/ml, respectively. In the present research, both samples manifested concentration-dependent enrichment in free radical scavenging efficiency along with effective antioxidant potential. The better efficacy was elicited by the sample from Kanyakumari district. The overall results of

ABTS and DPPH radical scavenging antioxidant activity showed that Palmyra sap samples collected from Kanyakumari District recorded higher antioxidant activity with the lowest IC<sub>50</sub> value of 61.50 µg/ml in the ABTS assay and 950.74 µg/ml in the DPPH assay compared to Tirunelveli District, which showed a lower percentage of inhibition. Hence, we can advocate that the existence of rich concentrations of secondary metabolites may be the principal reason backing the regulation of oxidation-reduction reactions in our selected Palmyra palm sap samples.

### Conclusion

The present study was focused on the examination of antioxidant property of *B. flabellifer* sap sample collected from two different localities of South India viz., Kanyakumari and Tirunelveli Districts using DPPH and ABTS assay methods. The results indicates that both the sample expertise a good antioxidant property whereas the sample collected from Kanyakumari District portrait a better antioxidant property compared to Tirunelveli district. These may be due to the climatic and soil nature of the two variant localities. Other than this the phytochemicals present in both the Palmyra sap samples directly plays a vital role in the antioxidant property of the fresh Palmyra sap sample which can be acted as remedy for various diseases and it procured many

health benefits naturally.

### Conflicts of Interest

The authors declare that this study does not have any conflict of interest.

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