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Evaluation of physiochemical, antioxidant and antimicrobial potential of palmyra palm haustorium

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Abstract
The current study was conducted with germinated seeds of a Palmyra tree called Palmyra haustorium (PH). The nutritional, phytochemical, and antimicrobial assessment was performed to evaluate the pharmacological significance of Palmyra haustorium. Proximate analysis were performed and showed that it is a good source of carbohydrates, protein, and fiber but negligible in fat content. It is a potential source of flavonoids, phenolics, and also ascorbic acid content which contributes to enhanced antioxidant activity. In the antimicrobial assay, the tested sample against different antibiotic disks showed a good inhibitory zone against *Pseudomonas* spp. Indicating that the sample can potentially inhibit *Pseudomonas* organism. The haemolytic assay performed with goat blood showed a lower haemolysis percentage which indicates that PH is suitable for consumption. The results depict that the germinated seed embryo of Palmyra palm possesses adequate macronutrients, phytochemicals, and antimicrobial activity that potentially act as nutraceuticals in enhancing the health condition of humans.

Keywords: palmyra haustorium, haemolytic activity, antimicrobial activity, antioxidants, macronutrients

1. Introduction
Borassus flabellifer commonly called by name Palmyra palm, the name is derived from Greek words "*Borassus*" means the leathery covering of fruit and "*Flabellifer*" -fan-shaped leaves, respectively. The name palmyra was based on the Portuguese word "Palmeira" named because of the resemblance of leaves to the palm of hand [1]. The entire parts of the tree possess economic usages and are also utilized to perform the traditional functions in Tamil customs. It is treated as nature's enduring gift and is called "Karpakatharu" in Tamil language meaning the tree which fulfills all the wishes of man [2]. The palmyra tree is in touch with Tamil culture since ancient times. The old sages, rishis, and researchers utilized leaves of palmyra as composting materials for writings to pass on their gained information and wisdom over more than 2500 years. Around the world, there were 140 million palm trees that were consumed as a source of food, medicine and help to sustain people's livelihood [3]. The tree is immensely grown in India, Sri Lanka, Bangladesh, Burma, Malaysia, the Philippines, and different east Africa regions. It is widely cultivated Indian in states, namely Tamil Nadu, Kerala, Andra Pradesh, Bihar, Bengal, Orissa, and is found along the west coast of India. The palmyra belongs to the Arecaceae family, it is a slow-growing, monocot dioicous tree that grows up to a height of 30 m and life span of 150 years [4]. It is a perennial tree that yields fruit after 15 years of maturity [2]. The trunk of the tree is black, cylindrical with a circumference of 1.5 meters and it can withstand adverse climatic condition [5]. The flowers of a tree are small, appear densely clustered spikes, and later develop into large, brownish, round fruits. The male flowers are usually smaller than female flowers[6]. The fruit of the tree *Borassus flabellifer* is large and fibrous which consists of nut-like portions which consist of seed in it [7]. The fruits are three-sided when young and become semi-spherical to spherical shape and are covered with sepals at the base. When the fruit ripens, it is a deep brown to black [8, 9]. Each part of the palmyra tree possesses its unique pharmaceutical and health-beneficial properties. Palmyra roots are high in calories which is a rich source of Vitamin E and contains Phyto phenolics like flavonoids and phenolic acids that exhibit antioxidant activity[10]. Young roots of palmyra are diuretic in nature, give a cooling effect to the body, and are used as a drug to destroy the parasitic worms [11]. The decoction of roots is used to treat respiratory diseases [12]. The fruits are rich in crude flavonoids, saponins, and phenolic compounds and exhibit anti-inflammatory effects.

Consumption of palmyra fruit during hot summer days keeps the body hydrated by refilling the lost minerals [13]. The consumption of spadix ash from the palmyra tree is used to treat enlarged spleens and livers, as well as heartburn [14]. Further work on palmyra haustorium is needed to exploit nutritional aspects and influence increase consumption of it. As there is no current scientific evidence regarding the nutritional profile of palmyra haustorium. Hence the present study is focused on exploring the nutritional attributes of palmyra haustorium which can enhance the nutritional quality and adds variety to diet.

2. Materials and Methods

2.1 Sample collection

The germinated palmyra seeds were collected from the regions of Thanjavur. The seeds are washed and the outer tough layer was dehusked to obtain the white spongy palmyra haustorium. The haustorium was collected in a polythene bag and stored in a deep freezer maintained at -18 ^{o}C.

2.2 Proximate Analysis

The moisture, protein, fat, fiber, and ash content were analyzed using the AOAC method 1995. Moisture and total ash content were determined by gravimetrical method at 103 ^{0}C for 3 hours and ash at 550 ^{0}C for 3-5 hours respectively. The protein content of samples is determined by the Kjeldahl method and obtained nitrogen content was multiplied with a conversion factor of 6.25 to obtain protein value. Soxhlet apparatus is used to estimate the fat content using hexane as a solvent. The crude fiber was estimated using a reference AOAC 978.10 method[15]. The total amount of carbohydrate was estimated by the difference method using the formula: total carbohydrate (%) = 100 - [moisture (%) -protein (%) - fat (%) - ash (%)] and finally expressed in g/100g. The calorific value was calculated according to Atwater method: kcal/100g = (3.36 ×% protein) + (3.60 ×% total carbohydrate) + (8.37 ×% fat) [10].

2.3 Ascorbic acid analysis by indophenol dye method

Ascorbic acid content is estimated using 2,6 dichloro indophenol dye method [16]. About 5 ml of working standard with 4% 10 ml oxalic acid was titrated against 2,6 dichloro indophenol dye (V_1 ml) until pale pink color appears. The 5 ml of sample extract with 4% 10 ml oxalic acid titrated against dye (V_2 ml). The results are expressed as Ascorbic acid (mg/100g).

$$\text{Ascorbic acid (mg/100g)} = \frac{V_1\text{ml} \times 5\text{ ml} \times \text{Weight of sample}}{0.05 \times V_2\text{ ml} \times 100\text{ ml}} \times 100$$

2.4 Physiochemical Parameters

Water activity was estimated by a water activity meter (Aqua lab 4TE water activity meter). TSS was measured by hand refractometer and pH by digital pH meter. The titratable acidity was determined by titrating with 0.1N NaOH. The Colour of samples were measured by hunter lab colorimeter, where 'L' is lightness ranging from 0 (black) to 100 (white), 'a' and 'b' does not possess numerical values and are indicated in positive (+) and negative (-) sign; for redness (+a) to greenness (−a) and yellowness (+b) to blueness (−b) [17]. The bulk density, tap density, True density, Compressibility index, Hausner ratio, Porosity (%) were performed according to a slight modified method of Smita *et al*., 2018 [18].

2.5 Antioxidant activity

The antioxidant activity PH was determined by 2,2-diphenyl picryl-1-picryl-hydrazyl (DPPH) method [19]. 1 gram of sample is dissolved in 10 ml of methanol. For 1 ml of sample extract, 3 ml of DPPH solution was added and incubated for 30 minutes. The absorbance is measured at 517 nm using a spectrophotometer and is expressed in inhibition percentage.
Inhibition (%) = (Absorbance of Control – Absorbance of Sample) / Absorbance of Control

2.6 Total phenolic and flavonoid content

Total phenolic content was estimated by Folin–Ciocalteu method using gallic acid as a reference standard. About 0.1 g of standard gallic acid was diluted to a concentration of 100 µg/ml with distilled water. 0.5 g of sample dissolved in 80% of 10 ml of methanol. 0.5 ml of FC reagent and 2 ml of sodium carbonate were added to the mixture. The absorbance is measured at 650 nm and results are expressed as mg gallic acid equivalents (GAE). Total flavonoid content was determined by the aluminum chloride method by using quercetin as a standard for reference. The absorbance is measured at 510 nm and the results are expressed as quercetin equivalents (QE) [20].

2.7 Antimicrobial activity

The antimicrobial efficacy of the PH sample was scrutinized with certain Antibiotic Resistant (ABR) strains (test organism – which was isolated from different food waste) on Muller Hinton Agar (MHA) medium using (measure the inhibition zone) disk diffusion test. The sample for antimicrobial activity was prepared according to a slight modified method of Astal *et al* 2005 [21]. The prepared MHA agar media solution was sterilized by autoclaving at 121 ^{0}C for 15 min at a pressure of 15 lbs. 20 ml of the medium was poured into each sterilized Petri dish and allow to solidifying. After solidification, the test organisms (*Pseudomonas* spp, *Shigella* spp, *Escherichia* spp, *Salmonella* spp, *Bacillus* spp, *Staphylococcus* spp) were spread throughout the Petri dishes uniformly by using a sterile swab. The antimicrobial activity was performed by the combined effect of antibiotic disks and test sample against test organisms. Seven different antibiotic disks [Norfloxacin (NX10), Vancomycin (VA10), Tetracycline (TE10), Chloramphenicol (C10), Erythromycin (E10), Penicillin (P10), Neomycin (N10)] were used in this study to determine the combined effect. Each standard antibiotic disk was further impregnated with 20µl of the test sample. Then, the antibiotic disc + test sample coated discs were placed onto the test organism swabbed on MHA media by using sterile forceps. The plates were incubated at 37±1 ^{0}C for 24-48 hours. After incubation, the inhibition zones were measured using Hi Antibiotic Zone scale-C [22]. An increase in fold area was calculated using the formula. The increase in fold area of PHMCP sample against different microorganism for antibiotic disk and for test sample + antibiotic disk was calculated by the equation (B2-A2)/A2, where A is the zone of inhibition of Antibiotic disk alone and B is the zone of inhibition of test sample + antibiotic disk.

2.8 Haemolytic activity

The goat blood was collected with anticoagulant sodium citrate – EDTA (Ethylene diamine tetraacetic acid) in a 50 ml centrifuge tube. Centrifuge the blood sample at 6000 rpm for 10 minutes to separate serum proteins. The Blood sample was washed with phosphate buffer saline (PBS) and centrifuged 3-

4 times till the supernatant was clear and colorless. The Final concentration of erythrocytes was maintained approximately at 0.5×10 [9] cells /ml with PBS. The different concentrations of a sample (62.5 µg, 125 µg, 250 µg, 500 µg) were prepared with the help of PBS. Incubate the 0.5 ml of the test sample with 0.5 ml of erythrocytes at 37 ºC for 1 hour with gentle shaking. Centrifuge the test samples at 5000 rpm for 10 minutes and dilute the supernatant 10 times with PBS. Measure the absorbance at 540 nm [23].

$$\text{Haemolysis (\%)} = \frac{\text{As - Am}}{\text{A – Am}} \times 100$$

Where,
As - Absorbance of sample
Am - Absorbance of mechanical haemolysis
A - Absorbance of 100% haemolysis

3. Result and Discussion

3.1 Proximate composition

The results of the proximate analysis of PH is tabulated in table 1. The haustorium yielded an energy of 122.95±4.16 Kcal/100g. The palmyra haustorium was found to be high in carbohydrates. It is potentially a good source of protein and also in fiber content. It is lower in fat content and can be advisable for overweight persons. The moisture content is important parameter as it affects the shelf life and stability of the product was found to be 66.48±2.19 g/100g (on a fresh weight basis). The low moisture content recorded makes the product shelf-stable. It also found to be consists of adequate levels of ascorbic acid that play a major role as antioxidants. The carbohydrate content is found to be similar to the results obtained by Sahni *et al*., 2014 [10]. The protein and ash level is found to be in line with the results of Umar *et al*., 2015 [24]. Thus, the result indicates that the PH possesses adequate nutritional profile for human consumption.

Table 1: Proximate analysis and ascorbic acid content of Palmyra haustorium

Parameters	Average value*
Energy (Kcal/100g)	122.95±4.16
Moisture (g/100g)	66.48±2.19
Carbohydrates (g/100g)	24.76± 1.42
Proteins (g/100g)	6.44±0.81
Fat (g/100g)	1.04±0.14
Fiber (g/100g)	2.05±0.18
Ash (g/100g)	1.28±0.08
Ascorbic acid (g/100g)	1.63±0.33

*- All the data values were analysed with triplicate

3.2 Physiochemical parameters

The water activity of the palmyra sample was found to be 0.96± 0.02 and the pH was nearest to neutral which is shown in table 2. The pH values indicate the acidic or alkalinity nature of the sample. The Total soluble solids of sample was found to be 2.73±0.53 ^{0}Brix. Bulk density is a measure of sample heaviness that is determined by the particle size of the samples. Increased bulk density is desired because it improves packaging efficiency by allowing more products to be packed into the same volume [25]. The bulk density of PH was found to be 0.42±0.02 g/cm^3 and tap density of 0.77±0.05 g/cm^3. The compressibility index defines the flow behavioural of flour. The flow property of the sample was found to with a compressibility index of 45.29±0.93. The Hausner ratio determines particle cohesiveness and the sample showed the values with 1.83±0.03 indicating good flow characteristics of the sample. Colour is one of the most important quality attributes of a food product. The colour of the PH sample is whitish in colour. The higher values of 'L' of 74.27±2.12 indicate the degree of white colour of the sample and 'b' values of 31.71±1.47 indicates the slight yellowish colour of the sample. This result indicates that the incorporation of PH samples into food material does not affect the colour of the end product. The pH values are slightly high compared to the values reported by Vijayakumari *et al*., 2014 [26]. The TSS and colour values are similar to the results obtained by Rodiah *et al*., 2019 [17].

Table 2: Physiochemical properties of Palmyra haustorium

Parameter	Average value*
Water activity	0.96± 0.02
pH	6.24±0.03
TSS (ºBrix)	2.73±0.53
Titratable Acidity (%)	0.53±0.08
Bulk density (g/cm^3)	0.42±0.02
Tap density (g/cm^3)	0.77±0.05
True density (g/ml)	5
Compressibility index	45.29±0.93
Hausner ratio	1.83±0.03
Porosity (%)	84.56±0.97
Colour	
L	74.27±2.12
a	3.51±0.36
b	31.71±1.47

*- All the data values were analysed with triplicate

3.3 Total phenolic content, total flavonoid content, and antioxidant activity

The antioxidant activity of PH was determined by the DPPH method and the results are tabulated in table 3. The antioxidant activity of the food material is due to polyphenolic compounds. It scavenges free radical by donation of a hydrogen atom or preventing the initiation of chain reaction by donating electrons [10]. The Phenolic content of the sample was found to be 0.7±0.02 mg GAE/g and flavonoids of 0.2±0.07 mg QE/g which contributes to the antioxidant activity of 63.73±3.1% of inhibition of DPPH stable free radical. Antioxidants are phytochemicals that decrease the harmful effects of oxidative processes by either suppressing free radical formation or scavenging free radicals [27]. Antimicrobial, antioxidant, antiallergic, anti-inflammatory, and anticancer agents are all found in phenols and flavonoids. They are essential for normal reproduction and growth. They also guard against pathogenic microorganisms that are hazardous to humans [28].

Table 3: Antioxidant activity, Total phenolic and flavonoid content of palmyra haustorium

Parameters	Averge value*
Antioxidant activity (Inhibition%)	63.73±3.1
Total phenolic content (mg GAE/g)	0.7±0.02
Total flavonoid content (mg QE/g)	0.2±0.07

*- All the data values were analysed with triplicate

3.4 Antimicrobial activity

In the combination (test sample + antibiotic) study conducted for the PH sample. In comparation to normal antibiotic disk alone the combination of disk and test sample produces higher inhibition zone. In combined study of Antibiotic disk + test

sample, standard antibiotic disk alone was taken as control. The antimicrobial activity exhibited by antibiotic disks increased effectively with the addition of test sample [22]. The maximum antimicrobial activity with highest increase in fold area was observed in *Pseudomonas* species against the antibiotic, Erythromycin (E10) and highest zone of inhibition was observed in Norfloxacin (NX 10) antibiotic disks as shown in Figure 1 and Table 4. The lowest inhibition zone was showed by antibiotic Norfloxacin (NX10) against *Salmonella* species. Unfortunately, nil record was observed for *Bacillus* species against all antibiotic disks. The antibiotic, Penicillin showed nil record against all the strains of bacteria.

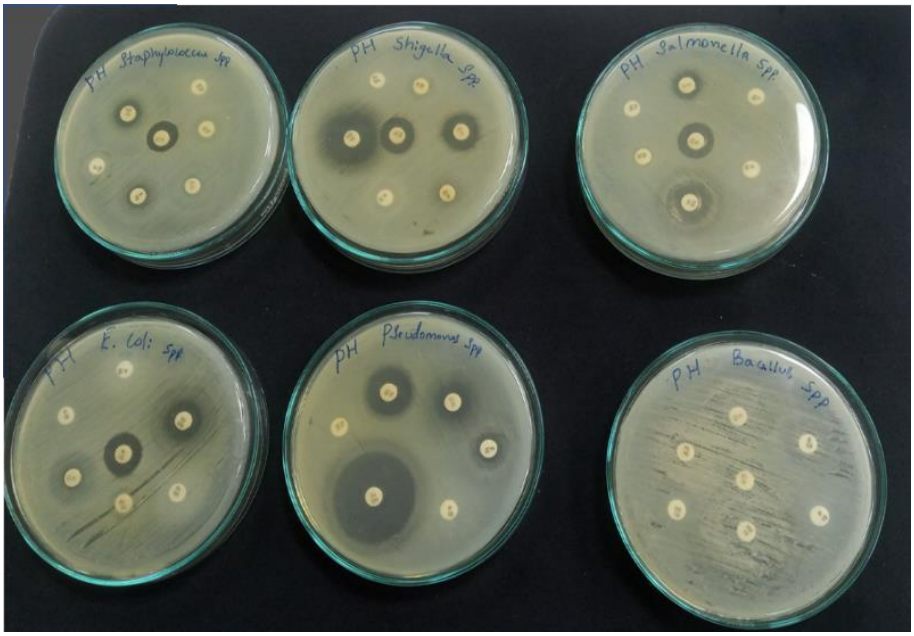

Fig 1: Antimicrobial activity of Palmyra haustorium

Table 4: Antimicrobial activity of Palmyra haustorium with values of zone of inhibition

Microbial Strains	Antibiotic name	Positive control (PC) zone of inhibition (mm)	PC + PH zone of inhibition (mm)	Increase in fold area
Pseudomonas Species	Norfloxacin (NX10)	20	30	1.25
	Vancomycin (VA10)	6	6	-
	Tetracycline (TE10)	13	18	0.91
	Chloramphenicol (C10)	18	18	-
	Erythromycin (E10)	6	12	3
	Penicillin (P10)	11	11	-
	Neomycin (N10)	10	14	0.96
Staphylococcus Species	Norfloxacin (NX10)	10	12	0.44
	Vancomycin (VA10)	6	6	-
	Tetracycline (TE10)	6	6	-
	Chloramphenicol (C10)	6	6	-
	Erythromycin (E10)	6	10	1.77
	Penicillin (P10)	18	18	-
	Neomycin (N10)	11	14	0.61
Shigella Species	Norfloxacin (NX10)	19	23	0.46
	Vancomycin (VA10)	6	6	-
	Tetracycline (TE10)	6	6	-
	Chloramphenicol (C10)	16	16	-
	Erythromycin (E10)	6	6	-
	Penicillin (P10)	11	11	-
	Neomycin (N10)	12	15	0.56
Salmonella Species	Norfloxacin (NX10)	14	16	0.30
	Vancomycin (VA10)	6	6	-
	Tetracycline (TE10)	6	6	-
	Chloramphenicol (C10)	12	12	-
	Erythromycin (E10)	6	6	-
	Penicillin (P10)	6	6	-
	Neomycin (N10)	12	14	0.36
Bacillus Species	Norfloxacin (NX10)	6	6	-
	Vancomycin (VA10)	6	6	-
	Tetracycline (TE10)	6	6	-
	Chloramphenicol (C10)	6	6	-
	Erythromycin (E10)	6	6	-

	Penicillin (P10)	6	6	-
	Neomycin (N10)	6	6	-
Eschirechia Species	Norfloxacin (NX10)	20	20	-
	Vancomycin (VA10)	6	6	-
	Tetracycline (TE10)	16	16	-
	Chloramphenicol (C10)	22	22	-
	Erythromycin (E10)	9	9	-
	Penicillin (P10)	10	10	-
	Neomycin (N10)	12	15	0.56

3.5 Haemolytic activity

Haemolytic activity of the PH sample against goat erythrocytes indicates the percentage of lysis of RBC cells and whether it is suitable for consumption. The haemolytic activity of PH was studied at different concentration of 62.5 to 500 µg and results showed a lower 1.7% of haemolysis at 500 µg of concentration. This may be due to the presence of antinutritional factors like saponin that cause the RBC lysis [29]. The saponin is reported in palmyra seed powder by Vengaiah *et al*., 2019 [30]. Since the haemolysis percentage is low can be recommended for consumption.

4. Conclusion

The palmyra haustorium has a potential source of nutraceuticals and therapeutic values. The macronutrients and phytochemicals in Palmyra haustorium alleviate malnutrition, especially in women and children. The current study concludes that it is a good source of carbohydrate, fiber, and protein and is limited to fat content. The phenolic compounds in PH scavenge free radicals and possess antioxidant activity thereby enhancing the health-promoting potential. The antimicrobial activity showed by sample safeguard body against microbes. Therefore, the results of the study confirmed that germinated seed embryo of palmyra haustorium is a new valuable component of food and possesses nutraceuticals that help in the promotion of health

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