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Antioxidant Property of Different Parts of Palmyra Palm (*Borassus flabellifer*): A Potential Nutraceutical Food

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NR, KMK, SV, SR planned, conducted the experiment and drafted the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Worldwide, palmyra palm (*Borassus flabellifer*) is utilized in cuisine and is renowned for its many nutraceutical qualities, which include anticancer and antioxidant capabilities. There is a connection between cellular free radicals and diseases like atherosclerosis, cancer, and visual impairment. Antioxidants can support cell renewal and potentially lower these hazards. Using the DPPH method, the antioxidant capacity of methanolic extracts from different sections of the Palmyra palm was evaluated. Several nutraceutical substances, including 5-hydroxy methyl furfural, beta-D-glucopyranose 1,6-anhydride, linoleic acid ethyl ester, 9-octadeceneic acid ethyl ester, butylated

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hydroxytoluene, and stigmasterol, were identified by GC/MS analysis of the crunchy kernel's methanol and hexane extracts. The results show that the crunchy kernel is a valuable addition to food products and has strong antioxidant potential.

Keywords: Borassus flabellifer; DPPH method; GC/MS analysis; antioxidant.

1. INTRODUCTION

Global population depends majorly on six staple crops (wheat, potatoes, rice, oats, barley, and corn) for most of their calorie requirements. Relying on few crops will cause reduction in plant biodiversity and final leads to nutrient shortages in humans. Fruits, vegetables, and tubers have been consumed by humans since prehistoric times. According to studies, eating more of these foods can help avoid diseases and give a number of health benefits since they contain phytochemicals including polyphenols, vitamins, minerals, and proteins. These foods' polyphenol content is impacted by both environmental and genetic factors. It is commonly known that dietary Phyto phenolics, such as flavonoids and phenolic acids, are advantageous antioxidants that have the ability to neutralize dangerous active oxygen species.

In order to prevent chronic diseases such as atherosclerosis and cancer, health agencies have created dietary guidelines. Oxidation is essential for the creation of energy in living things, which drives biological activities. On the other hand, tissue damage and cell death are caused by oxygen-cantered free radicals and reactive oxygen species (ROS), which are constantly generated in vivo. Numerous illnesses, such as diabetes, cancer, heart disease, and aging, have been related to oxygen radicals [1].

Oxidation naturally occurs in the body, so maintaining a balance with antioxidants is crucial for good health. Antioxidants that neutralize reactive oxygen species and free radicals play a vital role in preventing the onset and progression of many diseases caused by oxidative stress. Now a days synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are used widely, but which may results in side effects to the consumers [2].

The search for naturally occurring sources of antioxidants has attracted a lot of attention, with the main goal being to find appropriate chemicals and measure them in order to assess their potential as nutraceuticals and health benefits. In order to comprehend the potential health advantages of numerous nutrients, phytochemicals, and other activities found in diverse portions of Borassus flabellifer, this study intends to explore and characterize them. The towering Borassus flabellifer tree has a black trunk and a crown of leaves at the top. It can reach a height of almost thirty meters. Its leaves are fan-shaped and have stiff, horny, spinescent serrated edges. They range in diameter from 0.9 to 1.5 meters. The tree grows at an estimated rate of 3 cm per year and can live for over 100 years [3]. It bears huge fruits and unisexual flowers. Many tropical Asian nations, including Thailand, Bangladesh, India, Myanmar, Sri Lanka, and Malaysia, are home to this plant's cultivation [4].

The plant's fleshy roots and seedlings are both eaten as staple foods, especially by the underprivileged. In 0.8 square meters of loose, sandy soil, 100–150 drupes are planted in three–four layers with the possibility of producing at least 100–150 seedlings or more. The elongated, club-shaped seedlings are picked when they are between two and four months old, and the starchy, soft substance is then baked, roasted, fried, boiled, or turned into flour. When the roots are about four months grown, they are eaten as a fibrous and nutritious food that is high in starch and low in fats and proteins. The crisp kernel, which tastes like water chestnut but sweeter, is extracted by cracking open the hard shell of the germinated seed.

Numerous chemicals have been found by research on this plant, including a triterpene [5], a polysaccharide [6], and numerous steroidal saponins. Reports suggest that the pulp is high in vitamins A, vitamin B-complex and vitamin C [7]. On the other hand, no information about the crispy kernel's antioxidant activity is currently accessible. Thus, the purpose of this research is to determine the constituents and assess the antioxidant qualities of the crispy kernel's hexane and methanol extracts.

2. MATERIALS AND METHODS

2.1 Plant Material Collection

The Agricultural College and Research Institute in Madurai served as the experiment's location. The fruit, calyx, pericarp, crisp kernel, leaf, root, root cover, and other parts of the black-skinned fruit-bearing B. flabellifer were obtained from the Madurai district in Tamil Nadu. After gathering, the materials were shade dried and stored for further analysis. To lower the moisture level, they were further dried at 55°C in a hot air oven. After drying, the materials were crushed into a coarse powder and kept in sealed plastic bags at room temperature (28±2°C) until they were extracted using a solvent.

2.2 Preparation of Plant Extract

The powdered samples were sonicated with the solvents for half an hour in order to extract the compounds into methanol and hexane separately. The extracts were filtered and centrifuged at 10,000 rpm. With the help of rotary evaporator set to lower pressure and operate at 35–40°C, the sample quantities were concentrated.

2.3 DPPH Radical Scavenging Property

Samples (root, root cover, kernel, leaf, calyx, pericarp, and fruit) that had been powdered and weighed one gram each were extracted with 100 mm of methanol. Whatman No. 1 filter paper was used to filter the extracts. 0.2, 0.4, 0.6, 0.8, and 1.0 ml of each extract were pipetted into separate vials for the assessment of antioxidant activity, and the volume was corrected to 1 ml using methanol. A blank was made with one mm of methanol. After adding 2 mm of DPPH solution to each vial, the vials were allowed to sit at room temperature for half an hour. Using a spectrophotometer set to 517 nm, the samples' absorbance was determined using Blios's method [8]. The following formula can be used to determine antioxidant activity

%AAT = [(control absorbance - Sample absorbance)/Control absorbance]* 100

2.4 GC-MS Analysis

A Shimadzu QP 2020 instrument with a RxSil 5MS column (0.25 mm × 30 m × 0.25 µm) was used for the GC-MS analysis. The injection port was kept at 280°C and the helium flow rate at 1 ml/min while the oven temperature was progressively raised from 80°C to 280°C. At a ratio of 1:10, samples were injected in split mode with the ionization voltage set to 70 eV. With transfer line and source temperatures of 260ºC and 270ºC, respectively, the mass spectral scan range was 50-500 m/z. For the analysis, NIST 2005 Library was used. Hexane and methanol sample extracts were each diluted with 100 mg ml-1 of the corresponding solvent before analysis. On a fused silica RxSil-5ms capillary column (0.25 mm × 30 m × 0.25 µm), chromatographic separation was accomplished. Each extract was injected in split mode (1:10), resulting in one microliter of each. Eluents were detected in EI mode at an ionization energy of 70 eV. Using deconvolution reporting software (DRS), the mass spectra of the identified peaks were compared with those in the NIST 2014, WILEY spectral library, and F.A.M.E mix (C8:C24). As a proportion of the entire ion chromatogram's peak area, only compounds with quality matches greater than 90% were reported [9].

3. RESULTS AND DISCUSSION

3.1 DPPH Radical Scavenging Property

Antioxidative activity can be quickly assessed thanks to the stable DPPH free radical model. The results of the DPPH radical scavenging activity for different sections of the Palmyra palm in methanolic extract are shown in Table 1, together with reference standards ascorbic acid and butylated hydroxytoluene (BHT). The color changes from purple to yellow when antioxidants give the radical hydrogen, forming a stable DPPH molecule. As a result, absorbance decreases, suggesting that stronger antioxidant activity is correlated with lower absorbance levels.

Of the solvent extracts that were examined, the crispy kernel's methanolic extract showed the greatest levels of free radical scavenging activity. The calyx and leaves, on the other hand, showed the least amount of DPPH radical scavenging activity. The extracts' scavenging efficacy with the DPPH radical is arranged as follows: Crisp kernel, root > Fruit pulp, root sheath > leaf, calyx, and pericarp.

Table 1. Radical scavenging capacity of Crunchy kernel of *Borasus flabellifer* L.

S.No.	Sample	Absorbance	Antioxidant activity	DMRT Ranking
1	Control	0.7636	0	
2	Root	0.6123	19.81404	c
3	Root cover	0.3865	49.38449	b
4	Crunchy kernel	0.6740	11.73389	c
5	Leaf	0.1128	85.22787	a
6	Calyx	0.0623	91.84128	a
7	Pericarp	0.0733	81.03493	a
8	Fruit	0.5055	33.80042	b

Table 2a. List of compounds in Hexane fraction of Crunchy kernel detected by GC/MS

S.No	Peak Name	Retention Time(min)	Area	Area%
1	Cyclohexane,1,3-dimethyl-,trans-	3.143	150335	0.50
2	Oxirane,2,2-dimethyl-3-propyl-	3.771	58648	0.19
3	1-Propene,2-methyl-3-propoxy-	3.929	70405	0.23
4	Phosphonousdibromide,cyclohexyl-	4.305	332725	1.10
5	D-Limonene	4.886	173653	0.58
6	Undecane,5,7-dimethyl-	5.025	64283	0.21
7	Benzene,1,3-bis(1,1-dimethylethyl)-	6.815	225152	0.75
8	Dodecane,2,6,11-trimethyl-	7.006	157050	0.52
9	Dodecane,4,6-dimethyl-	7.515	93562	0.31
10	Hexadecane	8.506	150154	0.50
11	D-Alanine,N-neopentyloxycarbonyl-,octadec	9.393	194669	0.64
12	2,5-cyclohexadien-1-one,2,6-bis(1,1-dimethyl	9.488	363067	1.20
13	2-Tridecenal,(E)-	9.634	132559	0.44
14	Dodecane,4,6-dimethyl-	9.835	66391	0.22
15	Heptadecane	9.883	316030	1.05
16	Pentadecane	10.047	378891	1.25
17	Acetone	10.110	115493	0.38
18	ButylatedHydroxytoluene	10.255	3403009	11.27
19	Hexadecane	10.659	130710	0.43
20	Dodecanoic acid	11.033	139911	0.46
21	Hexadecane	11.887	462736	1.53
22	Heneicosane	14.000	569822	1.89
23	Phosphorin,2,4,6-tris(1,1-dimethylethyl)-	14.639	167283	0.55
24	Eicosane	14.965	226832	0.75
25	Tetradecanoicacid	15.205	245654	0.81
26	E-15-Heptadecenal	16.051	213853	0.71
27	Octadecane	16.209	231935	0.77
28	Nonadecane	18.529	151825	0.50
29	Hexadecane,1-iodo-	18.817	349997	1.16
30	2-Bromotetradecane	19.805	314972	1.04
31	n-Hexadecanoicacid	19.920	2108720	6.98
32	Hexadecanoicacid,ethylester	20.657	1818073	6.02
33	Octadecane	20.873	175967	0.58
34	2-Hydroxy-(Z)9-pentadecenylpropanoate	22.976	141881	0.47
35	Heneicosane	23.182	477794	1.58
36	Eicosane	23.695	355622	1.18
37	Linoelaidicacid	23.892	803867	2.66
38	9-Eicosenoicacid,(Z)-	24.033	1667334	5.52
39	11-Hydroxy-11-methyl-tricyclo[4.3.1.1(2,5)]u	24.130	282612	0.94
40	Linoleicacidethyl ester	24.513	5042943	16.70

S.No	Peak Name	Retention Time(min)	Area	Area%
41	(E)-9-Octadecenoicacidethylester	24.663	4533951	15.01
42	Hexadecanamide	24.909	97899	0.32
43	Octadecanoicacid,17-methyl-,methylester	25.255	595043	1.97
44	1-Tricosene	25.325	266936	0.88
45	Nonadecane	25.445	167625	0.56
46	Heptacosylacetate	25.591	166057	0.55
47	Hexadecanoicacid,2-hydroxy-1-(hydroxymet	31.998	248478	0.82
48	Di-n-octylphthalate	32.413	290173	0.96
49	Squalene	37.790	1026651	3.40
50	Stigmasterol	38.888	279326	0.92
			30198558	100.00

Table 2b. List of compounds in Methanol fraction of Crunchy kernel detected by GC/MS

S.No	Peak Name	Retention Time(min)	Area	Area%
1	N-Ethyl-N'-nitroguanidine	3.079	2981654	0.27
2	2-Propanone,1-hydroxy-	3.154	14043961	1.29
3	p-Dioxane-2,3-diol	3.229	9187575	0.84
4	2,2'-Bioxirane	3.566	3425784	0.31
5	Aceticacid,(acetyloxy)-	3.661	1967182	0.18
6	Acetic acid,methylester	3.700	7228833	0.66
7	Diethoxymethylacetate	3.761	12773483	1.17
8	Propanoic acid,2-oxo-,methylester	3.821	6706133	0.62
9	2,3-Butanediol,[R-(R*,R*)]-	3.924	8090005	0.74
10	(S)-5-Hydroxymethyl-2[5H]-furanone	3.963	3475382	0.32
11	Furfural	4.228	39313567	3.61
12	2-Furanmethanol	4.467	12591687	1.16
13	4-Penten-2-one,3-methyl-	4.531	4219125	0.39
14	(+-)-4-Amino-4,5-dihydro-2(3H)-furanone	4.740	14716308	1.35
15	Ethanone,1-(2-furanyl)-	4.945	1303285	0.12
16	2(5H)-Furanone	5.000	4946350	0.45
17	2-Cyclopenten-1-one,2-hydroxy-	5.148	8933974	0.82
18	3-Methyl-3-buten-1-ol,acetate	5.239	2820096	0.26
19	1H-Imidazole-4-carboxylicacid	5.321	804897	0.07
20	2-Furancarboxaldehyde,5-methyl-	5.427	11273237	1.04
21	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-on	5.610	9475780	0.87
22	Triethylenediamine	5.795	14377683	1.32
23	1,4-Butanediol	5.950	4606368	0.42
24	2-Methyl-3-oxobutyronitrile	6.040	4058631	0.37
25	1,5-Hexadien-3-ol,acetate	6.085	1530681	0.14
26	Benzeneacetaldehyde	6.130	2728539	0.25
27	2-Nonene	6.167	4644637	0.43
28	Furaneol	6.374	23949730	2.20
29	6,7-Dioxabicyclo[3.2.2]nonane	6.481	18018873	1.66
30	2(3H)-Furanone,5-methyl-	6.625	1666430	0.15
31	Undecane,6-methyl-	6.757	2353315	0.22
32	4H-Pyran-4-one,,3-dihydro-3,5-dihydroxy-6	7.111	64842116	5.96
33	4H-Pyran-4-one,3,5-dihydroxy-2-methyl-	7.362	3129726	0.29
34	5-Acetoxymethyl-2-furaldehyde	7.474	5866822	0.54
35	5-Hydroxymethylfurfural	7.994	605183228	55.62
36	5-Acetoxytridecane	8.400	11419522	1.05
37	1,3,2-Dioxaborolane,4,4-dimethyl-5-oxo-,2-e	8.638	18715273	1.72
38	Sulfoxide,butylpropyl	8.820	7196341	0.66
39	9-Imino-12-phenyl-10,11-dioxa-tricyclo[6.2.2.	8.930	3708196	0.34

S.No	Peak Name	Retention Time(min)	Area	Area%
40	Succinicacid,3-hex-4-ynyl3-methylbutylste	9.192	8528993	0.78
41	6-Oxa-bicyclo[3.1.0]hexan-3-ol	9.320	2073607	0.19
42	Isopropylphosphonicacid,dicylcopentylester	9.453	6496619	0.60
43	3-Bromo-5,5-dimethyl-cyclohex-2-enol	10.090	7894453	0.73
44	.beta.-D-Glucopyranose,1,6-anhydro-	10.663	43546394	4.00
45	n-Hexadecanoicacid	17.640	8802096	0.81
46	9-Oxabicyclo[6.1.0]non-6-en-2-one	18.375	16915165	1.55
47	Linoelaidicacid	21.296	3508597	0.32
48	cis-Vaccenicacid	21.426	5582435	0.51
49	Octadecanoicacid	21.919	3011270	0.28
50	OleicAcid	26.182	3519928	0.32
			1088153966	100.00

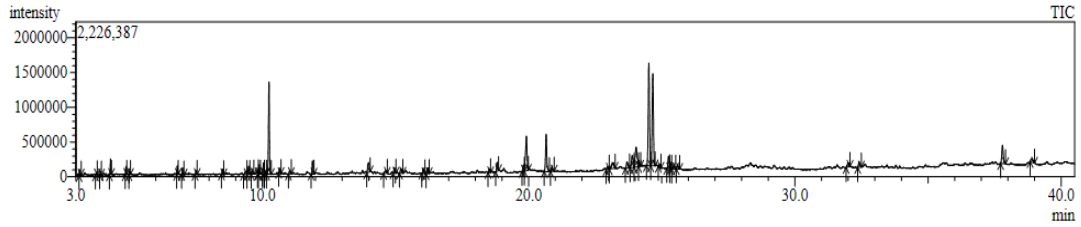

Fig. 1a. GC/MS chromatogram of hexane extract of Crunchy kernel of *Borassus flabellifer* L.

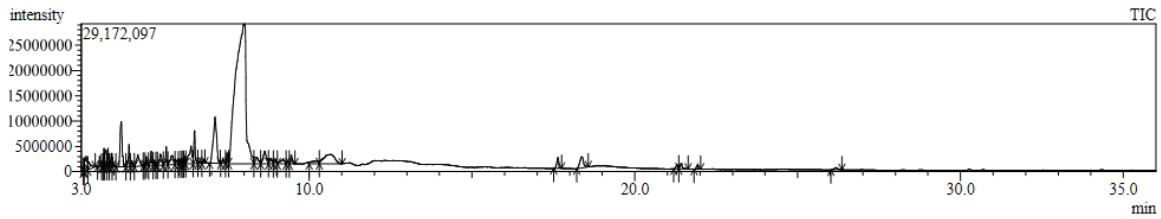

Fig. 1b. GC/MS chromatogram of methanol extract of Crunchy kernel of *Borassus flabellifer* L.

3.2 GC-MS Analysis

Plants are a great source for new drug discovery; several compounds produced from plants are currently widely used in pharmaceuticals [10]. Medicinal plants are vital therapeutic tools that are important to the health systems of both humans and animals. Because they are less expensive, more readily available, and non-toxic than contemporary medications, plant-based medications continue to be significant [11]. Bioactive principles can be found using the DPPH technique for preliminary antioxidant screening (Table 1). Alcohols, acids, esters, long- and branched-chain hydrocarbons, volatile matter components, etc. can all be identified with great accuracy using GC-MS [12]. In this study, 50 main peaks were identified by GC-MS analysis of hexane and methanolic extracts from the crunchy kernel of Borassus flabellifer (Tables 2a and 2b). The NIST library was used in order to identify different phytocomponents from the mass spectra.

The major compounds found in the hexane extract were found to be linoleic acid ethyl ester, (E)-9-Octadecenoic acid ethyl ester, and butylated hydroxytoluene; the major compounds found in the methanolic extract were found to be beta-D-Glucopyranose, 1,6-anhydro-, 4H-Pyran-4-one, 3-dihydro-3,5-dihydroxy-6, 5-Hydroxymethylfurfural, and furfural. Food additives and flavoring agents can be made from 9-octadecenoic acid ethyl ester ($C_{20}H_{38}O_2$), a fatty acid ester generated during ethanol intoxication [13].

As a synthetic counterpart of vitamin E and a phenol derivative, butylated hydroxytoluene (BHT) ($C_{15}H_{24}O$) is a lipophilic chemical molecule that is prized for its antioxidant qualities. BHT is included in a number of databases and catalogues as a food additive, industrial additive, pesticide ingredient, plastic/rubber ingredient, personal care product/cosmetic ingredient, and medical/veterinary/research agent. BHT is generally utilized as an antioxidant food additive

that keeps food fresh, keeps it from spoiling, and slows down food texture, color, and flavor changes. BHT has additionally shown antiviral action [14].

When applied topically, linoleic acid ethyl ester ($C_{20}H_{36}O_2$), a lipid-soluble version of linoleic acid, has anti-inflammatory, acne-reducing, skin-lightening, and moisture-retentive qualities. It is an important polyunsaturated omega-6 fatty acid. Deficiencies can cause dermatitis, growth retardation, and poor wound healing. The chemical molecule known as beta-D-glucopyranose 1,6-anhydro (Levoglucosan) ($C_6H_{10}O_5$) has a six-carbon ring structure and is produced when carbohydrates such as cellulose and starch are pyrolyzed. Because of its substantial presence in the gas released by pyrolyzed wood (biomass), it is utilized as a chemical tracer for biomass burning in atmospheric chemistry research. Levoglucosan is regarded as a clear-cut tracer for biomass combustion in brush and forest fires. Fermentable glucose, which is created by hydrolyzing levoglucosan, can be utilized to create chiral polymers similar to unhydrolyzable glucose polymers.

Dehydration of certain sugars produces hydroxymethylfurfural (HMF) ($C_6H_6O_3$), which is then broken down into 5-hydroxymethyl-2-furoic acid (HMFA) and eliminated in the urine. HMF binds intracellular sickle hemoglobin (HbS). HMF has been investigated as a potential treatment for sickle cell disease under the development code Aes-103 [15]. Through catalytic reductions, furfural ($C_5H_4O_2$), a renewable, non-petroleum-based chemical feedstock, can be transformed into a variety of solvents, polymers, fuels, and other valuable compounds. Furfuran resins are derived from furfuryl alcohol (FA) and found in coatings, cements, adhesives, casting resins, and thermoset polymer matrix composites.

4. CONCLUSION

Two primary areas of attention were this study. First, it demonstrated the crunchy kernel of Borassus flabellifer's great potential for antioxidants. Second, the metabolite characterization using GC/MS revealed significant compounds like butylated hydroxytoluene (BHT) ($C_{15}H_{24}O$), which is recognized for its health-promoting qualities and antioxidant qualities, and 9-octadeceneic acid ethyl ester ($C_{20}H_{38}O_2$), which can be used as a food additive or flavoring agent. The substantial antioxidant capacity of the crispy kernel indicates that it can be successfully used as a food product with added value.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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