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Screening of Nutritional, Phytochemical, Antioxidant and Antibacterial activity of the roots of *Borassus flabellifer* (Asian Palmyra Palm).

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Abstract
Borassus flabellifer Linn., of the Arecaceae family is locally called as Tal, English Name: Palmyra palm. The roots were investigated for it's nutritional, phytochemical and nutraceutical properties. The nutritional analysis of the roots has shown 8.54% protein content, 23.53% carbohydrates, 7.29% crude fibre and negligible fat content. These roots are found to be high in calories. MP-AES analysis has shown these edible roots contain some amount of iron (1.38 ppm) and traces of aluminium, arsenic, strontium, lead, manganese, copper and zinc.
All assays were carried out in hexane, chloroform and methanol extracts of the dried roots. Highest yield was obtained in methanol extract. Total phenolic content of hexane, chloroform and methanol extracts were obtained as 36.3, 89.24 and 99.34 µg GAE/100 mg of extract respectively. Flavonoid content for hexane, chloroform and methanol extracts was obtained as 222.7, 275.25 and 98.48 µg catechin equivalents per 100 mg of extract respectively. The methanol extract of the dried roots obtained an antioxidant potential of ABTS (IC_{50} = 2 mg/ml) and according to FRAP assay, the highest antioxidant activity was obtained in chloroform extract (129.6 µg BHT/100 mg extract).
Moreover, antibacterial assays did not show any activity for the five test organisms taken at different concentrations. The phytochemical analysis showed the presence of 1.61% alkaloids and 0.63% saponins and GC/MS screening of the extracts revealed the presence of fatty acids, alkanes, alkenes, ketones, aldehydes, diterpenes, phytols, and sterols. Thermal Desorption System was used for profiling of volatile and semi-volatile compounds which are responsible for flavor and fragrance in the sample. This also showed that the roots are good source of Vitamin E. Thus, this study depicts that these fruits, with rich nutrients, can be used in health promoting benefits.

Keywords: *B. flabellifer,* Palm tree roots, Nutritional, Phytochemical, Antioxidant, Antibacterial, GC/MS, TDS, MP-AES.

1. Introduction
Many fruits and vegetables have been consumed by humans since ancient times. Scientific investigations have proved that an increased consumption of these, have several health promoting as well as disease preventing benefits because of certain substances known as phytochemicals which include polyphenols, vitamins, minerals, proteins, etc. The presence of polyphenols in fruits and vegetables is largely influenced by genetic factors and environmental conditions. The dietary phytophenolics like flavonoids and phenolic acids have been recognized largely as beneficial antioxidants that can scavenge harmful active oxygen species, including $O_2^{\cdot-}$, H_2O_2, $^{\cdot}OH$, and $^{\cdot}O_2$ [1]. Dietary recommendations for the prevention of cancer, atherosclerosis and other chronic diseases have been established by various health agencies. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centered free radicals and other reactive oxygen species (ROS), which are continuously, produced *in vivo*, result in cell death and tissue damage. The role of oxygen radicals have been implicated in several diseases, including cancer, diabetes, cardiovascular disease and aging [2]. Because oxidation is a naturally occurring process within the body, a balance with antioxidants must exist to maintain good health. 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. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are very effective and are used for industrial processing, but they may possess side effects and toxic

properties that affect human health 1983 [3, 4, 5]. The search for antioxidants from natural sources has received much attention and efforts have been put into the identification of compounds that can act as suitable antioxidants.

In view of the growing interest in these compounds, there is a need to identify and quantify these important compounds in fruits and vegetables so as to evaluate their nutraceutical potential and health benefits. Thus, investigation and characterization of various nutrients, phytochemicals and other activities present in these roots of *Borassus flabellifer* was the aim of the present study in order to understand their health benefits. Analysing the composition and bioactive compounds present in these roots can lead to a better understanding and appreciation of the pharmaceutical, nutraceutical and medicinal value that these roots might offer and an increased consumption by the general public.

Borassus flabellifer is a tall tree attaining a height of about 30 m, with a black stem and crown of leaves at the top; leaves are 0.9-1.5 m in diameter, palmately fan shaped, petiole edges with hard horny spinescent serratures; flowers are unisexual; fruits are large. Trees can live upto more than 100 years. The rate of growth has been estimated at about 3cm per year. This plant is widely distributed and cultivated in tropical Asian countries such as Thailand, Bangladesh, India, Myanmar, Sri Lanka, Malaysia, etc. [6, 7, 8]. In India, it has been cultivated chiefly in the dry or sandy localities of Andhra Pradesh, Bihar, Karnataka, Kerala, Madhya Pradesh, Orissa, Tamil Nadu and West Bengal [9].

Fig 1: Photograph of the roots of *Borassus flabellifer.*

The seedlings as well as the fleshy roots are eaten. These form an important item of food for the poor. About 100 to 150 drupes are sown in 3-4 layers per 0.8 sq m under loose and sandy soils, which may produce at least 100-150 seedlings, sometimes more. They are removed when 2-4 months old and the elongated, club-shaped, starchy, tender material is eaten either baked, roasted, fried or boiled, or made into flour. To preserve the seedlings for future use, they are boiled and dried in the sun. The fleshy roots are eaten when about four months old. They are rich in starch, but poor in fats and proteins. The root is considered cooling, restorative, diuretic and anthelmintic. It is applied as a cure for gonorrhoea. The decoction of the young root and expressed juice from the young terminal buds and leaf-stalks have been used in gastritis and hiccups. It is useful as an antacid in heart-burn and as an anti-periodic.

B. flabellifer is used in folk medicine for multiple purposes, such as a stimulant, anti-laprotic, diuretic, antiphlogistic. The fruits are stomachic, sedative, laxative and aphrodisiac in nature useful in hyperdipsia, dyspepsia, flatulence, skin diseases, haemorrhages, fever and general debility. The roots and juice of the plant are useful in inflammatory reactions. The ash obtained by burning the inflorescence is a good antacid antiperiodic, and is useful in heart burn, splenomegaly and in bilious fever [10, 11]. Studies on this plant have revealed the presence of several steroidal saponins [6, 7, 8], a polysaccharide [12], and a triterpenes [13]. The fresh pulp is reportedly rich in vitamins A and C [14] while the fresh sap is a good source of vitamin B-complex [15]. Male inflorescence constitutes spirostane-type steroid saponins, like borassosides and dioscin. It also contains 20 known steroidal glycosides [16] and carbohydrates like sucrose [17]. *Borassus flabellifer* Linn. has been widely studied for its antidiabetic activity [16, 18].

Flowers of *B. flabellifer* were investigated for analgesic and antipyretic effects [19], anti-inflammatory activity, haematological and biochemical parameters [20, 21], immunosuppressant property [13].Pellets of *B. flabellifer* Linn. showed a significantly reduced capacity to mount a delayed-type hypersensitivity (DTH) [22], and flour from the young shoots of the *B. flabellifer* tested for mutagenicity [23], mitogenic activity [24], neurotoxic effect [25].

2. Material and methods

2.1 Sample collection and preparation

Roots were collected from the coastal region of Darbhanga district of Bihar. Peel was removed from the roots. The fresh root was washed with distilled water, minced into small pieces and dried in the sun for 7 days. The dried roots were ground thoroughly.

2.2 Reagents

All analytical grade chemicals and solvents used in the sample preparation were purchased from local suppliers of SRL, Rankem labs and CDH. The reference standards (rutin, gallic acid, catechin, ascorbic acid) were obtained from Sigma-Aldrich (Sigma- Aldrich, St. Louis, MO, USA**)**. All media were purchased from SRL (Sisco Research Laboratories, Delhi, India) and cultures were obtained from IMTECH (IMTECH, Chandigarh, India). All the other chemicals; TPTZ (2,4,6- tripyridyl-s-triazine), and ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) were procured from Fluka (Fluka, Switzerland).

2.3 Extraction

Extraction from dried *Borassus flabellifer* roots was done using hexane, chloroform and methanol as the extraction solvents. Using direct hot extraction method, 100 gm of the sample with 100 ml of solvent in a round bottomed flask was kept on a heating mantle for two days followed by subsequent filtration using Whatman filter paper # 1. Then, the obtained filtrates were collected and concentrated using a vacuum evaporator until a crude viscous extract was obtained. Then, the combined supernatants were filtered and the filtrates were pooled and concentrated using hot air oven until a crude viscous extract was obtained. After evaporation of organic solvents, these were stored at - 20 ^{0}C till analysis.

2.4 Extract Yield (EY)
The yield of dried extracts based on their dry weights were calculated using the following equation:

Yield (g /100 g of dry plant material) = (W1 × 100) / W2

Where, W1 was the weight of the extract after the evaporation of solvent, and W2 was the weight of the dry plant material.

2.5 Determination of nutritional constituents
Moisture and total ash content were determined by gravimetrical method at 103 ^{0}C to 104 ^{0}C (Ref. 935.29, AOAC, 1995) [26] and at ≤ 525 ^{0}C (Ref. 900.02A, AOAC, 1995) [26] respectively. The total nitrogen content was determined using the Kjeldahl method Ref. 976.05 (AOAC, 1995) [26] and the obtained nitrogen was transformed into protein content by multiplying the total nitrogen by a conversion factor of 6.25. Crude fat content was assessed using the AOAC method, Ref. 2003.06. The amount of total carbohydrates was calculated with the following formula: total carbohydrates (% fresh weight) = 100 - moisture (%) - protein content (% fresh weight) - crude fat (% fresh weight) - ash (% fresh weight) and reported as total carbohydrates in g 100 $^{-1}$ g DM. The calorific value per 100 g of DM was calculated according to the system of Atwater, namely: kcal = (3.36 × % protein fresh weight) + (3.60 × % total carbohydrate fresh weight) + (8.37 × % fat fresh weight).

2.6 Phytochemical analysis
2.6.1 Crude alkaloids and saponins determination
These were determined gravimetrically as per the methods described by Herborne (1973) [27] and Obadoni & Ochuko (2001) [28] and the results were expressed as g 100 g $^{-1}$ DM.

2.6.2 Total Phenolic content
Total phenolic content was determined as per the method described by Singleton and Rossi (1965) [29]. 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 (GAE, µg 100 mg $^{-1}$ EY).

2.6.3 Total Flavonoid content
Total flavonoid content was determined by colorimetric method (Jia, Tang & Wu, 1999) [30]. Briefly 0.25 ml (100 mg ml $^{-1}$) of each extract was diluted with 4.5 ml of distilled water and 0.333 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 (CE, µg 100 mg $^{-1}$ EY).

2.6.4 Flavonol content estimation
For estimation of the flavonol content, 0.25 ml of each extract (100 mg ml $^{-1}$) was added to 1 ml of ethanol followed by 1 ml of 2% aluminium chloride solution with gentle mixing. The solution was then mixed with 3 ml of 5 % sodium acetate solution and incubated at 20 ^{0}C for 2.5 h (Miliauskas, Venskutonis & Beek, 2004) [31]. Absorbance was measured at 440 nm and expressed as rutin equivalents (RE, µg 100 mg $^{-1}$ EY).

2.6.5. Assessment of Antioxidant activities
The antioxidant potential of phenolic compounds was measured by assessing their radical scavenging potential using $ABTS^{\cdot+}$ radical cation scavenging assay or their ability to reduce compounds by donating electrons using FRAP assay.

2.6.5.1 ABTS radical scavenging assay
The ability of the test sample to scavenge $ABTS^+$ radical cation was compared to Ascorbic acid standard. The total antioxidant activity of the three extracts was evaluated according to the decolorization of the ABTS radical cation ($ABTS^+$) as percentage inhibition by Re *et al*, (1999) [32]. The cation was pre-generated by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate and incubating for 12–16 h in the dark at room temperature until the reaction was complete and the absorbance was stable to 0.70 (± 0.02). Next, 1 ml was mixed with 10 µl of the test sample (0.05–10 mg ml $^{-1}$) and the absorbance was measured at 734 nm after 6 min. The percent inhibition was calculated and plotted as a function of the concentration of standard and sample to determine the ascorbic acid equivalent antioxidant concentration.

2.6.5.2 Ferric reducing activity power (FRAP) assay
The ability of the extract to reduce ferric ions was determined using the FRAP assay developed by Benzie and Strain (1996) [33]. Appropriate dilutions of extracts were prepared and 100 µl was mixed to 900 µl of FRAP reagent, vortexed and incubated at 37 ^{0}C for 4 min. The absorbance was measured at 593 nm and reported as BHT equivalents (µg 100 mg $^{-1}$ EY).

2.7 Chromatographic analysis
2.7.1. GCMSD profiling of extracts of the roots of Borassus flabellifer
The three extracts namely hexane, chloroform and methanol were diluted with their respective solvents (100 mg ml^{-1}) and analyzed by Agilent 6890 GC and 5975B MSD. The chromatographic separation was done on a capillary column of fused silica HP-5ms (0.25 mm × 30 m × 0.25 µm). 1 µl of each extract was injected in the split mode (1:50) by empty baffled liner at 280 ^{0}C (Agilent#5183-2037). The oven was programmed under the same condition as described above (Medini, Marzouki, Chemli, Khouja & Marongiu, 2009; NIST, 2005) [34]. Eluents were detected in EI mode with ionization energy of 70 eV. All the mass spectra of the identified peaks were compared with the spectra from the NIST'05a, WILEY spectral library and F.A.M.E mix (C_8:C_{24}) in combination with deconvolution reporting software (DRS). The results for individual compound those quality matches > 90% is only reported (as their percentage of the total area of peaks in the total ion chromatogram).

2.7.2 Flavor and fragrance profiling of the roots of Borassus flabellifer
The dried powdered sample of roots was analysed by the thermal desorption system. The conditions for GC were same as mentioned above, except the oven was programmed from 60 °C (0 min) at 3 °C/min to 240 °C (6 min) at 5 °C/min to 280 °C (15 min). The compounds were eluted from GC in Total Ion Chromatograph (TIC), which were searched against two databases,

- Agilent NIST'05 library developed by the National Institute of Standards and Technology
- Flavor 2 developed by Agilent.

2.8 Antimicrobial activity

Antibacterial activity of the seed extracts was tested against 3 gram positive i.e. *Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus subtilis* and 2 gram negative bacterial strains i.e. *Escherichia coli* and *Proteus mirabilis* on NA plates by disc diffusion method (Igbinosa., Igbinosa., & Aiyegoro, 2009) [35]. Extracts were reconstituted to a final concentration of 100 mg ml $^{-1}$. Nutrient agar was inoculated by spreading 100 µl of the bacterial inoculums. Wells (8 mm diameter) were punched in the agar and 100 µl of extracts were loaded into the wells. The plates were incubated at 37 ^{0}C for 24 h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition and reported on the scale of millimeter.

3. Results and discussion

3.1. Nutritional composition

In the present study, the potential benefits were shown by nutritional attributes of *B. flabellifer* roots (Table1). Moisture content and dry matter analysis, reporting during nutritional analysis is very important because it directly affects the nutritional content of fruit. Palm tree roots were found to be very rich in carbohydrates. There was a moderate amount of protein, making it a good source of protein, while the fiber content obtained was also good. It has a negligible amount of fat, which makes it an ideal diet for overweight people. The energy value of dried roots was calculated and the value obtained was 118.42 K cal.

The dried root extracts were analysed by Microwave Plasma-Atomic Emission Spectroscopy to identify and quantify the mineral content of the samples. The results of the analysis are tabulated below. It was found to contain iron, manganese and zinc, followed by many other beneficial nutrients. Iron is used against anaemia, tuberculosis and disorders of growth [36]. Zinc supplementation in diabetes mellitus proved to have antioxidant effect [37]. Other minerals were present in trace quantities.

Also, low content of Pb, Ni, As show that they are free from toxic metals. Therefore the collected species can be used in well-balanced diets and can be consumed unreservedly without any health risk.

Table 1: Nutritional composition of roots of *Borassus flabellifer*.

Composition	Content
Moisture (g 100 g $^{-1}$ FW)	62.38
Ash (g 100 g $^{-1}$ DM)	4.95
Protein (g 100 g $^{-1}$ DM)	8.54
Fat (g 100 g $^{-1}$ DM): (Fresh, dried)	0.6
Crude fiber (g 100 g $^{-1}$ DM)	7.29
Carbohydrates (g 100 g $^{-1}$ DM)	23.53
Food energy (Kcal)	118.42
Mineral elements in *B. flabellifer* roots (ppm)	
Iron	1.38
Strontium	0.14
Copper	0.09
Manganese	0.11
Zinc	0.08
Aluminum	0.48
Trace element and heavy metals (ppm)	
Arsenic	0.22
Lead	0.14
Nickel	0.03

3.2 Yield obtained

Quantity of yield obtained from hexane, chloroform and methanol was found in terms of (%).

Highest yield was obtained in methanol extracts. Values are listed in Table 2.

Table 2: Total yield obtained from different extracts of *Borassus flabellifer*

Solvent	Yield (%)
Hexane	0.56
Chloroform	1.29
Methanol	4.12

3.3 Phytochemical analysis

Phytochemicals, mainly phenolics are considered to be the important bioactive compounds for health benefits. The extracts which contain different classes of polyphenols are not only attractive in phytotherapy but also in the food industry. Hence, the total phenolics, total flavanoids, total flavanols, alkaloids and saponins were investigated.

The quantitative estimates of the crude phytochemicals of *B. flabellifer* DM were obtained as: alkaloids, 1.61 g 100 g $^{-1}$ and Saponins, 0.63 g 100 g^{-1} (Table 3). Alkaloids are good spasmolytic and anesthetic agents while saponins help in boosting the immune system, in lowering cholesterol levels in the blood and reducing the risk of getting intestinal cancer. Also, alkaloids are the most efficient therapeutically significant plant substance and saponins are known as anti-nutritional factors that can reduce the uptake of certain nutrients, including cholesterol and glucose at the gut through intra lumenal physicochemical interaction or other yet unidentified activity (Okwu & Okwu, 2004) [38].

Phenolic content (TPC) contributes directly to the anti-oxidative action. The highest phenolic content was found to be in methanolic fraction followed by chloroform and hexane fractions. Highest flavonoid content (TFC) and flavanol content were found to be present in chloroform fraction of roots. (Table 4).

Table 3: Total alkaloids and saponins contents

Parameter	Amount (g 100^{-1})
Alkaloids	1.61
Saponins	0.63

Table 4: Antioxidant activity and flavanol content

Solvent	Hexane	Chloroform	Methanol
TPC (µg GAE) 100 mg $^{-1}$ EY	36.3	89.24	99.34
TFC (µg CE) 100 mg $^{-1}$ EY	222.7	275.25	98.48
Flavanol (µg BHTE) 100 mg $^{-1}$ EY	3.34	3.89	0.43

3.4 Antioxidant activities

Polyphenolics are considered to function as antioxidants by various mechanisms like radical scavenging by H-donation, prevention of chain initiation by donating electrons. Hence, ABTS and FRAP assays were performed to find out the potential of *Borassus flabellifer* roots as a candidate for nutraceutical and determine its pharmacological significance.

3.4.1 ABTS radical scavenging assay

ABTS scavenging activity is applicable for screening both lipophilic and hydrophobic antioxidants. The total antioxidant activity of the *Borassus flabellifer* extracts was evaluated according to the decolorization of the ABTS radical cation ($ABTS^{+}$) as percentage inhibition by Re *et al* method, (1999) [32]. The highest antioxidant potential was found in the methanolic extract of the roots of *B. flabellifer* (IC_{50} = 2 mg/ml). This suggests that they have good antioxidant and free radical scavenging activity.

3.4.2 FRAP assay

The ability of the plant extracts to reduce ferric ions into ferrous ions under low pH was determined using the FRAP assay developed by Benzie and Strain (1996) [33]. The highest antioxidant activity was found to be 129.6 µg BHTE 100 mg^{-1} in the chloroform fraction of the roots.

3.5 GC/MS analysis

The details of all identified compounds in hexane, chloroform and methanolic extracts are grouped by their chemical nature (Table 4a, 4b and 4c). The reported saturated fatty acids such as oleic acid is found in animals and plants and are primarily used to produce hormone-like substances that regulate a wide range of functions, including blood pressure, blood clotting, blood lipid levels, the immune response, and the inflammation response to injury infection (Meechaona, Sengpracha, Banditpuritat, Kawaree & Phutdhawong, 2007) [39]. The presence of phytosterols in seed extract may be contributing towards antimicrobial and antioxidant activity. They are well known towards their medical, cosmetic, functional food applications and also known for their saturated fat reducing and cholesterol lowering activity; thus they may reduce risk of heart disease (Gabay *et al*., 2010) [40]. Hexadecanoic acid (used in cosmetics, soaps), Tetradecanoic acid (cosmetics and medicinal preparations) and Pentadecanoic acid were also found.

Table 4a: List of compounds in Hexane fraction of dried roots detected by GC/MS

S. No.	Compounds Detected	CAS#	Area (%)	R.T.
1.	Decamethylcyclopentasiloxane	000541-02-6	0.94	9.895
2.	DodecamethylCyclohexasiloxane	000540-97-6	0.73	12.721
3.	TetradecamethylCycloheptasiloxane	000107-50-6	0.45	15.099
4.	Dodecanoic acid	000143-07-7	0.19	15.895
5.	HexadecamethylCyclooctasiloxane	000556-68-3	0.29	17.140
6.	Tetradecanoic acid	000544-63-8	1.76	18.194
7.	Octadecane	000593-45-3	0.18	18.598
8.	Nonadecane	000629-92-5	0.18	18.598
9.	Octadecamethyl-Cyclononasiloxane	000556-71-8	0.37	18.889
10.	Pentadecanoic acid	001002-84-2	0.52	19.226
11.	Tetradecanoic acid	000544-63-8	0.52	19.226
12.	Tetradecane	000629-59-4	0.22	19.630
14.	Methyl ester Hexadecanoic acid	000112-39-0	1.16	19.889
15.	n-Hexadecanoic acid	000057-10-3	18.07	20.325
16.	Eicosamethyl-Cyclodecasiloxane	018772-36-6	0.79	20.448
17.	Ethyl ester Hexadecanoic acid	000628-97-7	0.88	20.572
18.	Pentadecane	000629-62-9	0.58	20.617
19.	Eicosane	000112-95-8	0.58	20.617
20.	Heptadecanoic acid	000506-12-7	0.13	21.211
21.	Methyl ester 9,12-Octadecadienoic acid	002462-85-3	1.54	21.547
22.	Methyl ester 8,11-Octadecadienoic acid	056599-58-7	1.54	21.547
23.	9-Eicosyne	071899-38-2	19.61	21.985
24.	(Z)-6-Octadecenoic acid	000593-39-5	13.14	22.018
25.	Octadecanoic acid	000057-11-4	4.45	22.164
26.	Heptadecane	000629-78-7	0.84	22.456
27.	Tritetracontane	007098-21-7	0.84	22.456
28.	Hexadecamethyl-Cyclooctasiloxane	000556-68-3	0.51	23.151
29.	Heptadecane	000629-78-7	0.87	23.319
30.	Nonadecane	000629-92-5	0.87	23.319
31.	Eicosane	000112-95-8	0.87	23.319
32.	Oleic Acid	000112-80-1	0.19	23.858
33.	Tetracosane	000646-31-1	0.87	24.149
34.	Heneicosane	000629-94-7	1.26	25.719
35.	1-Bromo-octadecane	000112-89-0	2.88	26.471
36.	Squalene	007683-64-9	2.18	27.682
37.	Vitamin E	000059-02-9	1.28	31.731
38.	gamma-Sitosterol	000083-47-6	1.51	35.510
39.	beta-Sitosterol	000083-46-5	1.51	35.510

Table 4b: List of compounds in Chloroform fraction of dried roots detected by GC/MS

S. No.	Compounds detected	CAS#	% Area	R.T.
1.	Trichloromethane	000067-66-3	6.05	3.412
2.	Dimethyl Sulfoxide	000067-68-5	12.22	5.285
3.	Chloroacetyl chloride	000079-04-9	0.09	5.745
4.	1,1,2,2-tetrachloro- Ethane	000079-34-5	0.52	6.093
5.	Dimethyl sulfone	000067-71-0	1.53	6.272
6.	1,1,3-trichloro- 2-Propanone	000921-03-9	0.63	6.788
7.	Pentaborane(9)	019624-22-7	0.21	6.934
8.	Benzyl Alcohol	000100-51-6	0.13	7.910
9.	3-chloro-2-methyl-1-propene	000563-47-3	0.20	9.581
10.	3-chloro- 1-Butene	000563-52-0	0.20	9.581
11.	1,1,2,3,3-pentachloro-propane	015104-61-7	0.76	9.670
12.	1-chloro-2-methyl- 1-Propene	000513-37-1	0.22	9.805
13.	3-chloro-2-methyl- 1-Propene	000563-47-3	0.22	9.805
14.	1,3-dichloro-propane	000142-28-9	0.15	11.151
15.	Dodecamethylcyclohexasiloxane	000540-97-6	0.05	12.732
16.	2,5-bis(1,1-dimethylethyl) Phenol	005875-45-6	0.05	15.334
17.	Dodecanoic acid	000143-07-7	0.06	15.906
18.	Hexadecane	000544-76-3	0.04	16.355
19.	Heptadecane	000629-78-7	0.05	17.510
20.	Tetradecanoic acid	000544-63-8	0.66	18.228
21.	Hexadecyl ester Trichloroacetic acid	074339-54-1	0.05	18.530
22.	(E)- 5-Octadecene	007206-21-5	0.05	18.530
23.	Z-8-Hexadecene	1000130-87-5	0.05	18.530
24.	Octadecane	000593-45-3	0.05	18.598
25.	Tetratetracontane	007098-22-8	0.05	18.598
26.	n-Hexadecanoic acid	000057-10-3	3.32	20.325
27.	Eicosane	000112-95-8	0.10	20.617
28.	Pentadecane	000629-62-9	0.10	20.617
29.	2-propyl tridecyl ester Sulfurous acid	1000309-12-4	0.10	20.617
30.	Methyl ester 9,12-Octadecadienoic acid	002462-85-3	0.21	21.559
31.	Methyl ester Octadecanoic acid	000112-61-8	0.03	21.828
32.	Octadecanoic acid	000057-11-4	1.71	22.187
33.	Eicosane	000112-95-8	0.18	22.467
34.	Nonadecane	000629-92-5	0.18	22.467
35.	Tritetracontane	007098-21-7	0.18	22.467
36.	Tetracosane	000646-31-1	3.68	26.505
37.	Heptacosane	000593-49-7	3.68	26.505
38.	Squalene	007683-64-9	0.20	27.693
39.	(3.beta.)- Cholest-5-en-3-ol	000057-88-5	0.17	31.619
40.	Campesterol	000474-62-4	0.48	3.593
41.	Stigmasterol	000083-48-7	0.53	34.277
42.	22,23-dihydro- Stigmasterol	1000214-20-7	1.55	35.600
43.	.gamma.-Sitosterol	000083-47-6	1.55	35.600
44.	.beta.-Sitosterol	000083-46-5	1.55	35.600

Table 4c: List of compounds in methanol fraction of dried roots detected by GC/MS

S. No	Compounds detected	CAS#	% Area	R.T.
1.	n-Hexadecanoic acid	000057-10-3	3.83	20.213
2.	Octadecanoic acid	000057-11-4	0.95	22.19
3.	.alpha.-Amyrin	000638-95-9	1.97	27.559
4.	.beta.-Amyrin	000559-70-6	1.97	27.559
5.	Olean-12-ene	000471-68-1	15.67	29.521
6.	.beta.-Sitosterol	000083-46-5	2.19	35.510
7.	22,23-dihydro- Stigmasterol	1000214-20-7	2.19	35.510
8.	Olean-12-ene	000471-68-1	8.26	52.490

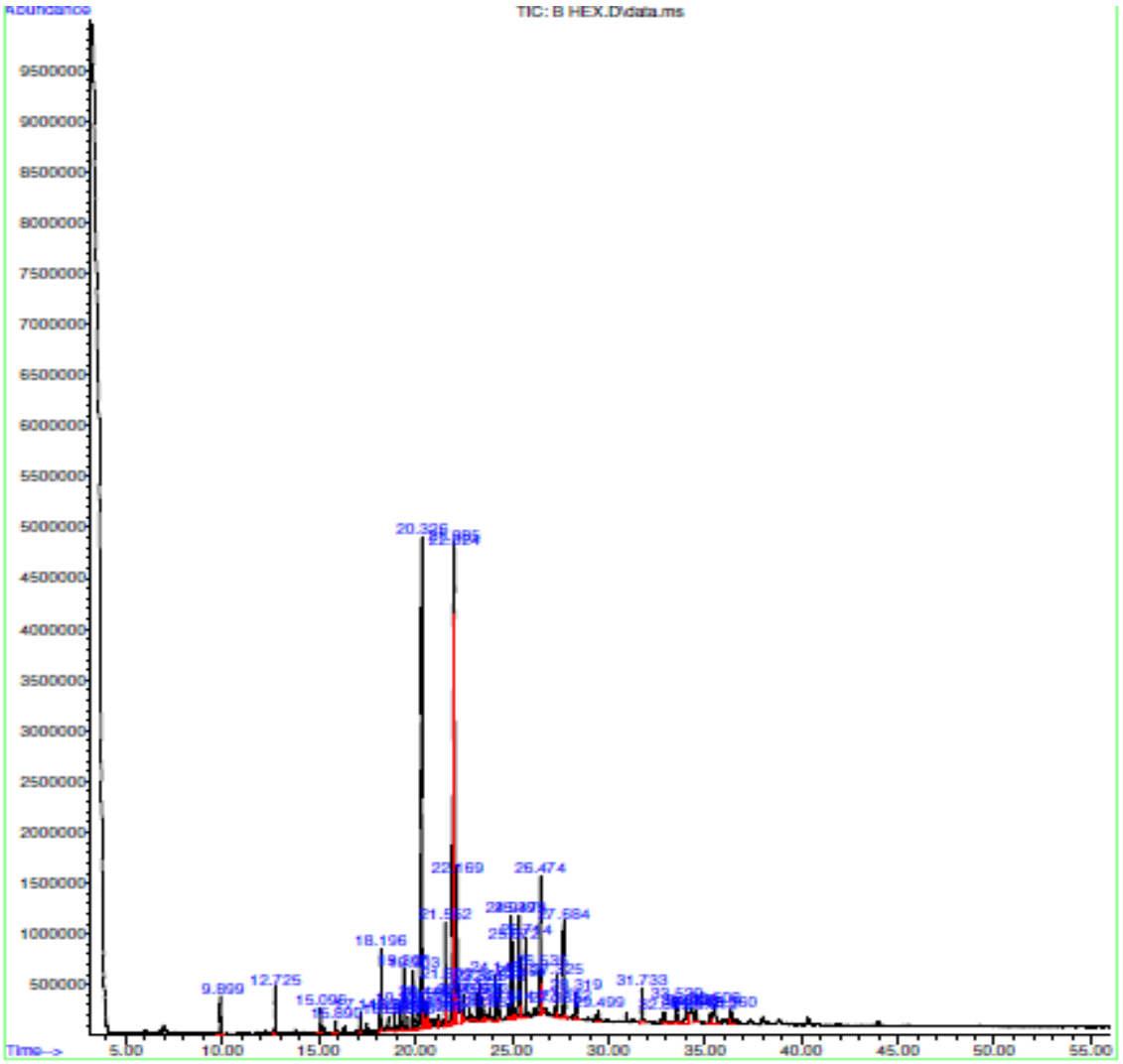

Fig 2(A): GCMS chromatogram of hexane extract of the roots of *Borassus flabellifer*.

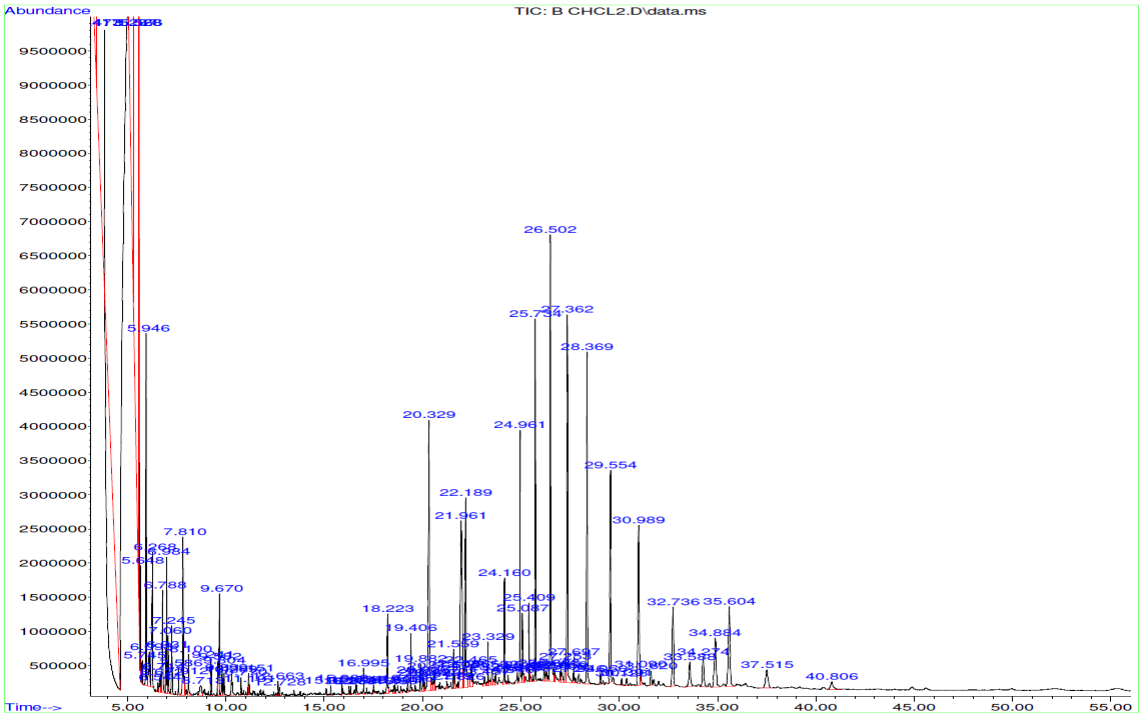

Fig 2(B): GCMS chromatogram of chloroform extract of the roots of *Borassus flabellifer*.

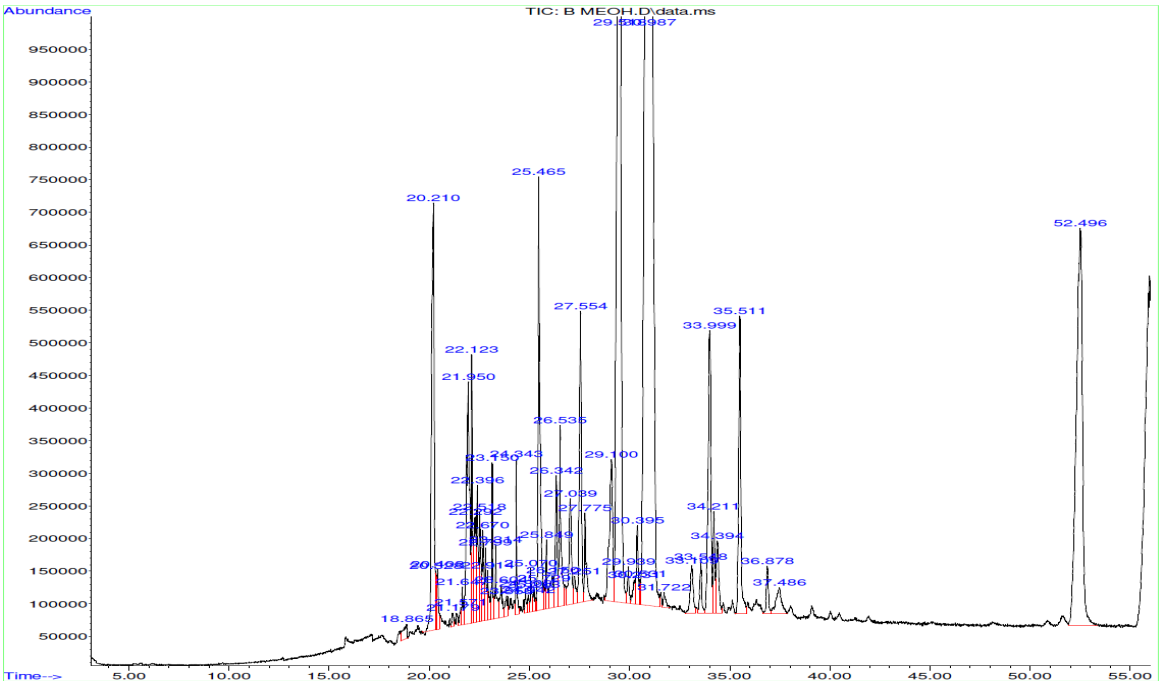

Fig 2(C): GCMS chromatogram of methanol extract of the roots of *Borassus flabellifer*.

3.6 Fragrance and Flavor profile

Thermal Desorption System (TDS) was used for profiling of volatile and semi-volatile compounds which are responsible for flavor and fragrance in the sample.

Table 5: List of compounds in dried roots sample detected by GC/MS

S. No.	Compounds detected	CAS#	% Area	R.T.
1.	Dimethyl Sulfoxide	000067-68-5	57.79	3.542
2.	Dimethyl sulfone	000067-71-0	0.72	4.564
3.	5-methyl- 2-Furancarboxaldehyde	000620-02-0	0.44	5.264
4.	1,3,5-Benzenetriol	000108-73-6	0.48	7.341
5.	6-Methyluracil	000626-48-2	0.48	7.341
6.	5-(hydroxymethyl)- 2-Furancarboxaldehyde	000067-47-0	0.95	10.019
7.	(E)-9-Octadecene	007206-25-9	0.08	12.407
8.	1-Tridecene	002437-56-1	0.08	12.407
9.	(E)- 9-Eicosene	074685-29-3	0.08	12.407
10.	4-Pyridinecarboxamide	001453-82-3	0.13	12.818
11.	D-Allose	002595-97-3	0.58	13.818
12.	3,4-Altrosan	1000129-76-4	0.58	13.818
13.	3-Hydroxy-4-methoxybenzoic acid	000645-08-9	0.23	14.784
14.	Cyclododecane	000294-62-2	0.61	15.018
15.	Methyl.alpha.-d-Ribopyranoside	006207-03-0	2.56	15.606
16.	ethyl octadecyl ester Phthalic acid	1000309-10-9	0.18	19.083
17.	(3.beta.)- Ergost-5-en-3-ol	004651-51-8	1.29	20.105
18.	24-methyl-5-Cholestene-3-ol	1000214-17-4	1.29	20.105
19.	Campesterol	000474-62-4	1.29	20.105
20.	Stigmasterol	000083-48-7	0.33	22.349
21.	.beta.-Sitosterol	000083-46-5	3.20	24.482
22.	.gamma.-Sitosterol	000083-47-6	3.20	24.482
23.	.alpha.-Amyrin	000638-95-9	1.41	26.304
24.	.beta.-Amyrin	000559-70-6	1.41	26.304

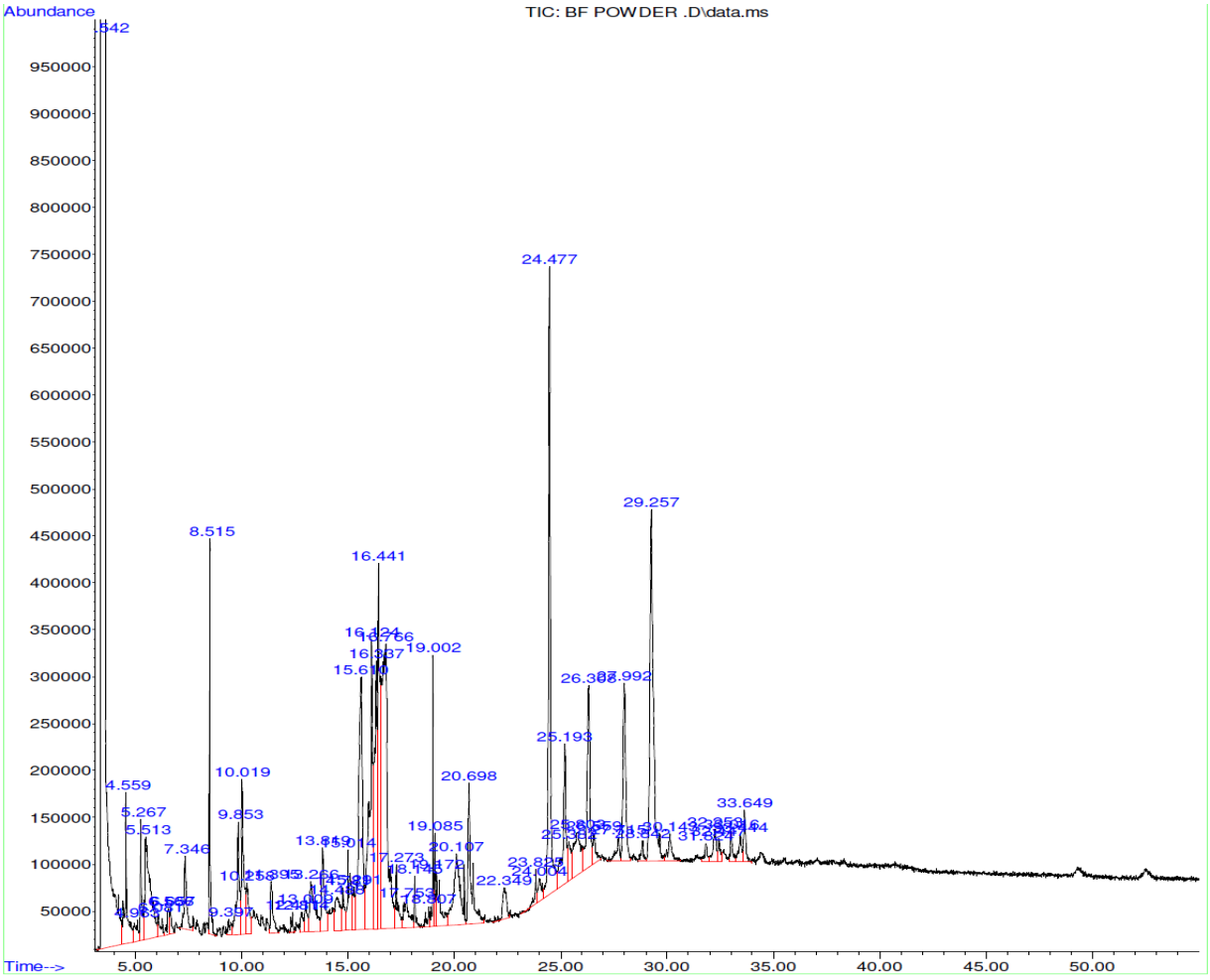

Fig 2(D): Chromatogram of Flavor and Fragrance profile of the roots of *Borassus flabellifer*.

*** % Matching with NIST library; RT** Retention time of the compound, in minute**; "area (%)"** the percentages of the area of the total ion chromatogram represented by the peaks of each of the compounds identified**;; a, b, c** Identified by NIST and WILAY spectral library, mass fragmentation and co-injection with authentic material.

3.7 Antibacterial activity

Screening of the dried root extracts in hexane, chloroform and methanol was done for antibacterial activities by disc diffusion method against gram positive *B. subtilis*, *S. aureus, S. epidermidis* and gram negative *P. mirabilis* and *E. coli*. No zone of inhibition was observed in either of the extracts for all the five test organisms indicating that they do not possess antibacterial activity for them. It may be due to the low concentration of the extract in the sample solution.

4. Conclusion

The present study was focused in three areas of enquiry. The first involved assessment of the nutritional content of the roots. Though the total protein, carbohydrate and crude fibre content was not very high. The fat content was found to be negligible and the fruit was found to be high in calorie content.

The second area involved quantification of phenolics, flavanoids, flavanols, alkaloids and saponins as well as the antioxidant potential using ABTS and FRAP assays. The antioxidant potential was found to be in good correlation showing good antioxidant activity. Thus, eliciting that it could be used as a source of antioxidants in various food preparations.

In the third part of the study, characterization of secondary metabolites was done using GC/MS which revealed the presence of alkanes, alkenes, fatty acids, sterols, and alkaloids indicating that it may be used as a flavouring agent as well as has pharmacological significance. The roots are a good source of Vitamin E. Quantification of the mineral content of roots by MP-AES revealed that some amount of iron, arsenic, aluminium, manganese, zinc and lead are present. Flavor and aroma analysis was also performed using Thermal Desorption System (TDS). The results indicate that the roots of *Borassus flabellifer* possess high phenolic content and antioxidant properties and are a good source of dietary polyphenolics and essential micronutrients in human diet. Therefore, it seems reasonable to consider the roots as a new valuable ingredient for food and nutraceutical applications in the promotion of health.

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