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PHARMACOGNOSTICAL AND PHYTOCHEMICAL ANALYSIS OF *BORASSUS FLABELLFER* LEAVES

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ABSTRACT
Borassus flabellifer L. belongs to the family Arecaceae, commonly known as Palmyra palm is a native of tropical Africa but cultivated and naturalized throughout India. In the previous study, several steroidal saponins, poly saccharides and triterpenes were isolated from the fruit pulp and seeds, young shoots of *Borassus flabellifer* contains gum, albuminoids, fats and the fresh pulp contains vitamin A, B and C, the inflorescence contains borassoside, dioscin and also contains sucrose, bitter compound flabelliferins. Plant yields a black gum, a good source of Vitamin B complex, extract in stroke, head ache, earache, epilepsy, scabies, syphilis, ulcers, vomiting. Bark is used as dentifrices, flowers in uterus tumors. Fruits as tonic for asthmatic patients, gonorrhea and given in gas troubles. Roots used as cooling medicine and restorative, diuretic and anthelmintic, in gastritis. In the present study, leaf powder of Borassus flabellifer has been investigated for isolation and characterization of bioactive compounds. The isolation and characterization of gallic acid was confirmed by spectroscopic methods.

KEYWORDS: *Borassus flabellifer*, Gallic acid, Palmyra tree, Palm tree.

1. INTRODUCTION
Borassus flabellifer L. belongs to the family Arecaceae, commonly known as Palmyra palm is a native of tropical Africa but cultivated and naturalized throughout India. The different parts of *Borassus flabellfer* are being used for medicinal properties. Borassus referred to growing spadix of the palm tree; flabellifer means 'fan bearing or 'fan shaped leaves'. *Borassus flabellifer* trees are the native of tropical Asia, perhaps Indonesian region in specific. It occurs in India, Bangaladesh, Srilanka, China, Cambodia, Laos, Myanmar, Thailand, Vietnam, Indonesia (Java), lesser Suna Island and Sulawesi. In the previous study, several steroidal saponins, poly saccharides and triterpenes were isolated from the fruit pulp and seeds, young shoots of *Borassus flabellifer* contains gum, albuminoids, fats and the fresh pulp contains vitamin A, B and C, the inflorescence contains borassoside, dioscin and also contains sucrose, bitter compound flabelliferins. Plant yields a black gum, a good source of Vitamin B complex, extract in stroke, head ache, earache, epilepsy, scabies, syphilis, ulcers, vomiting. Bark is used as dentifrices, flowers in uterus tumors. Fruits as tonic for asthmatic patients, gonorrhea and given in gas troubles. Roots used as cooling medicine and restorative, diuretic and anthelmintic, in gastritis.

2. MATERIALS METHODS
2.1 Plant Materials
Borassus flabellifer leaves were collected from West Bengal and authentication of the above species was carried out Thirumala college of Pharmacy, Nizamabad, AP.

2.2 Standardization of *Borassus flafellber* leaves
The leaf drugs of *Borassus flabellifer* was dried at room temperature, until they were free from the moisture and subjected to physical evaluation with different parameters. The parameters which were used for evaluation are nature, odour, colour, taste, size, shape, width and length.

Finally leaves were subjected to size reduction by electric mixer grinder to get coarse powder and passed through sieve No. 40 to get uniform powder. Then the uniform powders were subjected to standardization with different parameters as per pharmacopoeias/literatures.

2.2.1 Quantitative Standards[4]
2.2.1.1 Foreign Matter
Foreign organic matter is the material consisting of any or all of the following.

(1) Parts of the organ or organs from which the drug is derived other than the parts named in the definition

and description or for which the limit is prescribed in the individual monograph.
(2) Any organs other than those named in the definition and description.
(3) Matter not coming from the source plant and
(4) Moulds, insects or other animal contamination.

2.2.1.2 METHOD
Weigh 100 to 500 g or the quantity specified in the individual monograph, of the original sample and spread it out in to a thin layer. Inspect the sample with unaided eye or with the use of a 6x lens and separate the foreign matter manually as completely as possible. Weigh and determine the percentage of foreign matter from the weight of the drug taken. Use the maximum quantity of sample for coarse or bulky drugs.

2.2.2. Ethanol soluble extractive
Macerate 5 g of the air-dried drug, coarsely powdered, with 100 ml of ethanol of the specified strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow to stand for 18 hours. Thereafter, filter rapidly taking precautions against loss of ethanol evaporate 25 ml of the filtrate to dryness in tarred flat-bottomed shallow dish, dry at 105^0C and weigh. Calculate the percentage of ethanol-soluble extractive with reference to the air-dried drug.

2.2.3. Water soluble extractive
Add 5 g to 50 ml of water at 80^0C in a stoppered flask. Shake well and allow to stand for 10 minutes, cool, add 2 g of kieselguhr and filter. Transfer 5 ml of the filtrate to a tarred evaporating dish, 7.5 cm in diameter, evaporate the solvent on a water bath, continue drying for 30 minutes, finally dry in a steam oven for 2 hours and weigh the residue. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

2.2.4. Total ash
Unless otherwise stated in the individual monograph, weigh accurately 2 to 3 g of the air-dried crude drug in a tarred platinum or silica dish and incinerate at a temperature not exceeding 450^0c until free from carbon, cool and weigh. If a carbon –free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper until the ash is white or nearly so, add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450^0C Calculate the percentage of ash with reference to the air-dried drug.

2.2.5. Acid insoluble ash
Boil the ash obtained from the procedure mentioned in the total ash with 25 ml of 2M hydrochloric acid for 5 minutes, collect the insoluble matter in a Gooch crucible or on an ashless filter paper, wash with hot water, ignite, cool in a desiccators and weigh. Calculate the percentage of acid insoluble ash with reference to the air-dried drug.

2.2.6. Moisture content (by Loss on drying)
Weigh a glass-stoppered, shallow weighing bottle that has been dried under the same condition to be employed in the determination. Transfer to the bottle the quantity of the sample specified in the individual monograph, cover it and accurately weigh the bottle and the contents. Distribute the sample as evenly as practicable by gentle sidewise shaking to depth not exceeding 10mn. Place the loaded bottle in the drying chamber (oven or desiccators) as directed in the monograph, remove the stopper and leave it also in the chamber. Dry the sample to constant weight or for the specified time and at the temperature indicated in the monograph. After drying is completed, open the drying chamber, close the bottle promptly and allow it to cool to room temperature (where applicable) in a desiccator before weighing. Weigh the bottle and the contents.

2.3 Extraction of *Borassus flabellifer linn.*leaves
The shade-dried leaves of *Borassus flabellifer Linn*, family Arecaceae was reduced to fine powder (# 40 size mesh) and around 300 gms of powder was subjected to successive hot continuous extraction (Soxhlet) with petroleum ether, and alcohol. Finally the drug was macerated with chloroform-water. Each time before extracting with the next solvent the powdered material was air dried in hot air oven below 50^0C. After the effective extraction, the solvent were distilled off, the extract was then concentrated on water bath and the extract obtained with each solvents was weighed. Its percentage was calculated in terms of air-dried weight of plant material. The colour and consistency of the extracts was noted.

The obtained alcoholic and aqueous extracts were subjected to chemical investigation.

The shade dried powder around 500gm of *Borassus flabellifer* was subjected to continuous hot extraction with methanol. After the effective extraction, the extract was then concentrated on water bath and the extract obtained was weighed. About 100 gm of extract was taken for isolation.

2.4 Qualitative Chemical Investigation of Extract[5,6]
Qualitative chemical tests were conducted for all the extracts of *Borassus flabellifer* to identify the various phytoconstituents. The various tests and reagents used are given below and observations are recorded in the table no.1 and table no.2

Tests for Carbohydrates
Molisch's test (General test): To 2-3 ml aqueous extract, added few drops of α-naphthol solution in alcohol, shaken and added concentrated H_2SO_4 from sides of the test tube was observed for violet ring at the junction of two liquids.

For Reducing Sugars

a) Fehling's test: 1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 min was observed for a yellow, then brick red precipitate.
b) Benedict's test: Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated in boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

Tests for Monosaccharides: *Barfoed's test*: Equal volume of Barfoed's reagent and test solution were added. Heated for 1-2 min, in boiling water bath and cooled. Observed for red precipitate.

Tests for Hexose Sugars: *Cobalt-chloride test:* 3 ml of test solution was mixed with 2ml cobalt chloride, boiled and cooled. Added $FeCl_3$ drops on NaOH solution. Solution observed for greenish blue (glucose), purplish (Fructose) or upper layer greenish blue and lower layer purplish (Mixture of glucose and fructose).

Tests for Non-Reducing Sugars

a***)*** Test solution does not give response to Fehling's and Benedict's tests.
b) Tannic acid test for starch: With 20% tannic acid, test solution was observed for precipitate.

Tests for Proteins

a) Biuret test (General test): To 3 ml T.S added 4% NaOH and few drops of 1% C_4SO_4 solution observed for violet or pink colour.
b) Million's test (for proteins): Mixed 3 ml T.S. with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red colour was observed.
c) Xanthoprotein test (For protein containing tyrosine or tryptophan): Mixed 3ml T.S. with 1 ml concentrated H_2SO_4 observed for white precipitate.
d) Test for protein containing sulphur: Mixed 5 ml T.S. with 2 ml 40% NaOH and 2 drops 10% lead acetate solution. Solution was boiled it turned black or brownish due to PbS formation was observed.
e) Precipitation test: The test solution gave white colloidal precipitate with following reagents.
i) Absolute alcohol
ii) 5% $HgCl_2$ solution
iii) 5% C_4SO_4 solution
iv) 5% lead acetate
v) 5% ammonium sulphate

Tests for Steroid

a) Salkowski Reaction: to 2 ml of extract, 2 ml chloroform and 2 ml concentrated H_2SO_4 was added. Shaked well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.
b) Liebermann-Burchard Reaction: Mixed 2ml extract with chloroform. Added 1-2 ml acetic anhydride and 2 drops concentration H_2SO_4 from the side of test tube observed for first red, the blue and finally green colour.
c) Liebermann's reaction: Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H_2SO_4 observed for blue colour.

Tests for Amino Acids

a) Ninhydrin test (General test):- 3 ml T.S. and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min. observed for purple or bluish colour.
b) Test for Tyrosine: Heated 3 ml T.S. and 3 drops Million's reagent. Solution observed for dark red colour.
c) Test for tryptophan: To 3 ml T.S. added few drops glyoxalic acid and concentrated H_2SO_4 observed for reddish violet ring at junction of the two layers.

d) Tests for Glycosides

Tests for Cardiac Glycosides

a) Baljet's test:- A test solution observed for yellow to orange colour with sodium picrate.
b) Legal's test (For cardenolides):- To aqueous or alcoholic test solution, added 1 ml pyridine and 1 ml sodium nitroprusside observed for pink to red colour.
c) Test for deoxysugars (Kellar Killani test):- To 2 ml extract added glacial acetic acid, one drop of 5% $FeCl_3$ and concentrated H_2SO_4 observed for reddish brown colour at junction of the two liquid and upper layers bluish green.
d) Liebermann's test (For bufadenolides):- Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H_2SO_4 observed for blue colour.

Tests for Saponin Glycosides

a) Foam test: The drug extract or dry powder was shaked vigorously with water. Persistent foam was observed.
b) Haemolytic test: Added test solution to one drop of blood placed on glass slide. Haemolytic zone whether appeared was observed.

Tests for Coumarin Glycosides

Test solution when made alkaline, observed for blue or green fluorescence.

Tests for Flavonoids

a) Shinoda test: - To dried powder or extract, added 5 ml 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink colour was observed.
b) To small quantity of residue, added lead acetate solution observed for Yellow coloured precipitate.

c) Addition of increasing amount of sodium hydroxide to the residue whether showed yellow colouration, which was decolorized after addition of acid was observed.
d) Ferric chloride test: - Test solution, added few drops of ferric chloride solution observed for intense green colour.

e) **Tests for Alkaloids**
a) Dragendroff's test: To 2-3 ml filtrate added few drops Dragendroff's reagent observed for orange brown precipitate.
b) Mayer's test: - 2-3 ml filtrate with few drops Mayer's reagent observed for precipitate.
c) Hager's test: - 2-3 ml filtrate with Hager's reagent observed for yellow precipitate.
d) Wagner's test: - 2-3 ml filtrate with few drops of Wagner's reagent observed reddish brown precipitate.

Tests for Tannins and Phenolic Compounds
To 2-3 ml test solution, added few drops of whether showed following was observed.
a) 5% $FeCl_3$ solution: - Deep blue-black coloured.
b) Lead acetate solution: - White precipitate.
c) Gelatin solution: - White precipitate.
d) Bromine water: - Decoloration of bromine water.
e) Acetic acid solution: - Red colour solution.
f) Potassium dichromate: - Red precipitate.
g) Dilute iodine solution: - Transient red colour.
h) Dilute HNO_3:- Reddish to yellow colour.

2.5 Isolation of Phytoconstituents by using column chromatography
The methanolic extract of coarsely powdered (500gm) was prepared by using soxhlet apparatus. The crude extract was evaporated to dryness in a water bath to give dark brown mass. The methanolic extract around 100 gm was subjected to column chromatography on silica gel (60-120 mesh) using varying polarities, starting from Chloroform and Methanol to yield several fractions. The column was eluted firstly with pure chloroform and the methanolic extract was subjected to the column for isolation. The column eluted with chloroform and methanol (95:5, 90:10, 80:20 and 50:50). The elute from different solvents were concentrated to give dry residues The obtained residues were further purified by rechromatogram by using pure solvent chloroform. The samples were confirmed by using TLC, by their melting point, IR NMR and MASS spectroscopy.

2.6 Spectroscopic Characterization
Different spectroscopic methods were used to elucidate the structure elucidation of isolated compounds. Among the spectroscopic techniques IR, ^{1}H NMR, ^{13}C-NMR, MASS and GC-MS were carried out.

2.6.1 IR
The infrared spectra were recorded on PPERKIN ELMER SYSTEM ONE FTIR/ATR (Model: Spectrun one: FT-IR Spectrometer) Scan range 1-o cm^{-1} with globar and mercury vapour lamp.

2.6.2 Mass
The mass spectra were recorded by using the GEOL GCMATE II GC-MS double focusing instrument, the maximum resolution 6000, and maximum calibrated mass: 1500 Daltons and source options are electron impact and chemical ionization.

2.6.3 NMR
The 1HNMR and 13CNMR spectra were recorded by using Bruker AVANCE III 500 MHz (AV500 MHz) multinuclei solution NMR Spectrometer with 11.7 Tesla super conducting long hold magnet, activity shielded with standard bore (narrow bore: 5-5.4 cm) built in cryoshims, 34 channel room temperature shims, amplifier power 300W, HP Work station with LCD TFT monitor and Windows XP based TOPSPIN-2 software with an additional software for processing. The IR, MASS and NMR spectral studies were carried out in Sophisticated Analytical Instrumental Facility (SAIF), Indian Institute of Technology Madras**.**

2.6.4 Melting points were measured by SRS Optimelt MPA 100 instrument.

3. RESULT AND DISCUSSION
In the present study, *Borassus flabellifer* leaves were collected and authentication of the drugs was carried out at Thirumala college of Pharmacy, Nizamabad, Telungana which was subjected to physical evaluation with different parameters which were prescribed in the pharmacopoeias/literatures, such as Foreign matters, Ethanol soluble extractive, Water soluble extractive, Total ash, Acid insoluble ash, Moisture content etc. the results of the standardized *Borassus flabellifer* leaves are given in the Table No. 1

The standardized powdered leaf drugs of *Borassus flabellifer* was subjected to extraction by continuous or successive hot Soxhlet method according to increasing polarity order of the solvent, extracts were filtered, concentrated to dryness and weighed. The percentage was calculated in terms of air dried weight of the plant materials subjected to extraction by successive hot Soxhlet method. The resultant extracts were subjected to phytochemical investigation by the standard procedure prescribed in the text books. Methanolic extract was prepared and subjected to isolation of constituents.

The results of qualitative chemical investigation of various extracts of *Borassus flabellifer* have indicated the presence of the following compounds.

Pet ether extracts - Steroids, triterpenes, Glycoside flavanoids.
Chloroform extract - Steroids, glycoside, flavanoids.
Alcohol extract - Carbohydrates, tannins and phenolic compounds.,

Aqueous extract - Carbohydrates, protein, amino acid and glycoside.

The methanolic extract (of coarsely powdered500gm) was prepared by using soxhlet apparatus. The crude extract was evaporated to dryness in a water bath to give dark brown mass. The methanolic extract around 80 gm was subjected to column chromatography on Silica Gel (230-400 Mesh) using varying polarities, starting from chloroform and methanol to yield several fractions. The column was first eluted with pure chloroform and methanolic extract was subjected to column for isolation.

The column eluted with Chloroform: Methanol (95:5.90:10, 80:20 and 50:50). The elute from different solvents were concentrated to get residue, further purified by rechromatogram with pure chloroform or methanol.

The compound was shown from polar fraction of methanolic extract of Borassus flabellifer. The compound was separated from Chloroform: Methanol (8:2). Further separated compounds were confirmed by using TLC method. The solvent system used for the separation is Ethyl acetate: Benzene (9:11), the R_f values were found to be 0. 40 for gallic acid shown pink colour spots when sprayed Vanillin sulphuric acid reagent. The spectral details are as follows. The isolated compound gave blue colour with Ferric chloride reagent.

IR Spectroscopy

The absorption band (Fig.1) shows the following frequencies: 3383-(OH) broad band, 2923 along with broad band, 1652 (C=O) corboxylate.1403&1260(C=O), (O=C-H). Other peaks are 1543, 1058, 679 and 568.

MASS

The M+ ion peak is 170.12 and other m/z peaks are 162, 154, 144, 137, 125, 119, 113, 109, 97 and 91. The results are shown in Fig.2.

^{1}H-NMR:

The proton NMR peaks are as follows.
9.44(OH, 2H), 8.885(OH, H), 5.007, 8.003, 7.981, 7.945, 7.941, 7.439, 7.199, 7.196, 6.894, 6.890, (aromaticity, 2H), 5.764, 3.73 and 3.693. The results are shown in Fig 3.

^{13}C-NMR

The ^{13}C- NMR spectrum Fig.4.shows the following peaks.
The ^{13} CNMR peaks are follows.
188.60 (C-6), 165.47 (C-7), 156.13 (C-4), 148.16 (C-3), 135.31 (C-1) 129.54, 116.63 (C-2), 104.98. The compound is confirmed from the above spectral data as 3, 4, 5 Tri hydroxy benzoic acid (Gallic acid).

Table 1: Certificate of Analysis (*Borassus flabellifer*).

Sl.No.	Parameter	IP/BP as per literature	Observation
I.	Physical Test Colour Odour Taste	 -Pale Green colour - Slight -Astringent	 Pale Green colour Slight Astringent
II.	Extractive value Aqueous Alcohol soluble	 - Not less than 37.0% - Not less than 21%	 40.53% 25.0%
III.	Moisture content (By Loss on Drying)	Not more than 8.0%	5.20%
IV.	Ash values Total Ash Acid Insoluble Ash -	 Not more than 13.0% Not more than 4.0%	 10.6% 5.6%

Table 2: Results of Qualitative Chemical Investigation of *Borassus flabellifer* leaves.

Sl. No.	Name of Test	Extracts			
		Pet. Ether	Chloroform	Alcohol	Aqueous
I.	**Test for Carbohydrates**				
	Molisch's test (General test)	-	+	+	+
	C) Test for Hexose Sugar a) Cobalt chloride test	 -	 -	 -	 +
II	**Test for Amino acids** a) Ninhydrin test (General test) b) Test for Tyrosine c) Test for Tryptophan d) Test for Cystein	 - - - -	 - - - -	 + + - -	 + - - -
III	**Test for Proteins**	-	-	-	+
IV	**Test for Steroids**				

	a) Salkowski reaction	+	-	-	-
	b) Liebermann-Burchard reaction	+	+	-	-
		+	-	-	-
V	**Test for Triterpenoids**				
	a) Salkowski reaction	+	+	-	-
	b) Liebermann-Burchard reaction	+	+	-	-
VI	**Test for Glycosides**				
	A) Test for cardiac glycosides	+	+	-	-
	a) Baljet test	+	+	-	-
	b) Legals test (Test for Cardenolides)	+	+	-	-
	c) Test for deoxy sugars	+	+	-	-
	B) Test for Anthraquinone glycosides	-	-	+	-
	a) Borntragger's test	+	+	+	+
	C) Test for Saponin glycosides				
	a) Foam test	-	-	-	-
	b) Haemolysis test	-	+	-	-
	D) Test for Coumarin glycosides				
	a) Alkaline reagent test	+	-	-	-
	b) NaOH soaked paper test	+	-	-	-
VII	**Test for Flavonoids**				
	a) Ferric chloride test	-	+	-	-
	b) Shinoda test	-	+	-	-
	c) Alkaline reagent test	-	+	-	-
	d) Lead acetate test	-	+	-	-
VIII	**Test for Alkaloids**				
	a) Dagendroff's test	-	+	+	-
	b) Mayers test	-	-	-	-
	c) Hagers test	-	-	-	-
	d) Wagners test	-	-	-	-
	e) Murexide test	-	-	-	-
IX.	**Test for tannins and phenolic compounds**				
	a) 5% $FeCl_3$ solution	-	-	+	-
	b) Lead acetate solution	-	-	-	-
	c) Gelatin solution	-	-	+	-
	d) Bromine water	-	-	-	-
	e) Acetic acid solution	-	-	+	-
	f) Dilute iodine solution	+	-	-	-
	g) Dilute HNO_3	-	-	-	-
	h) Dilute $KMNO_4$	-	-	+	+
X.	**Test for Lipids**	+	-	+	-

Note: '+' = Present '-' = Absent

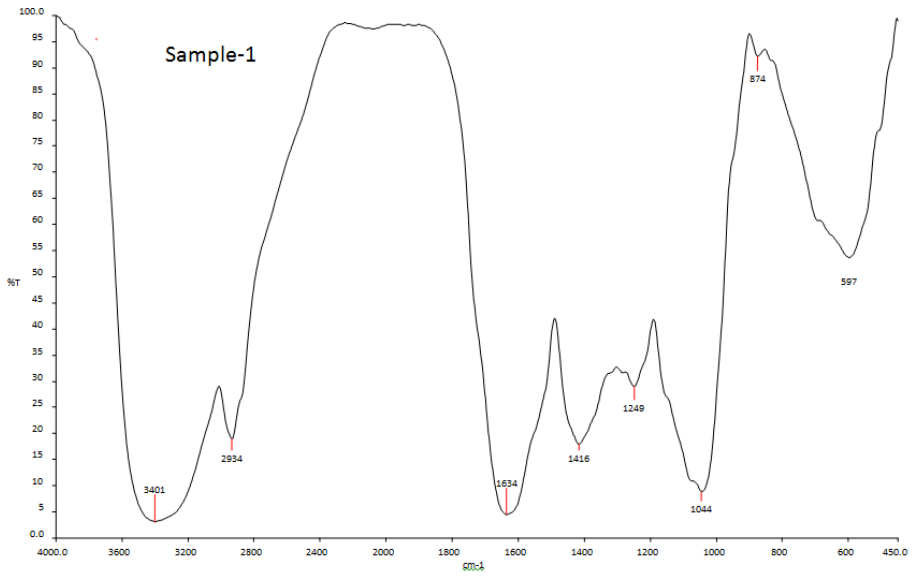

Figure1: IR Spectra of compound1.

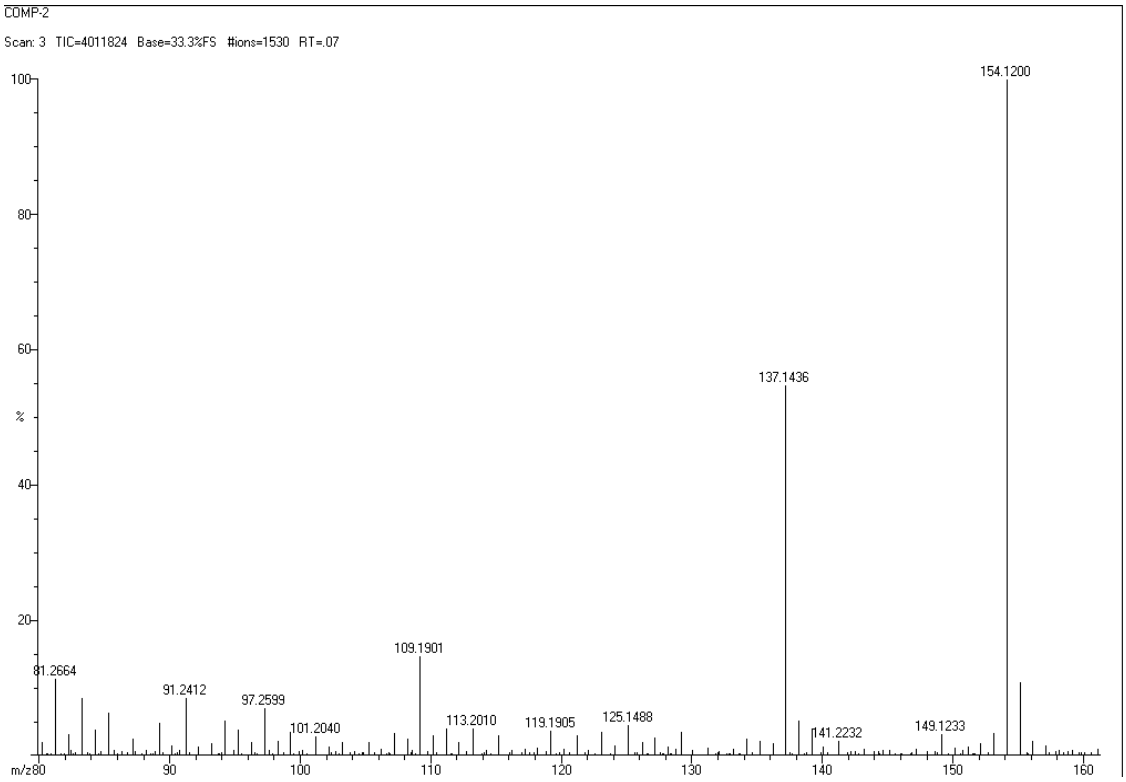

Figure 2: Mass spectra of compound 1.

Fig:3 Compound-1 HNMR

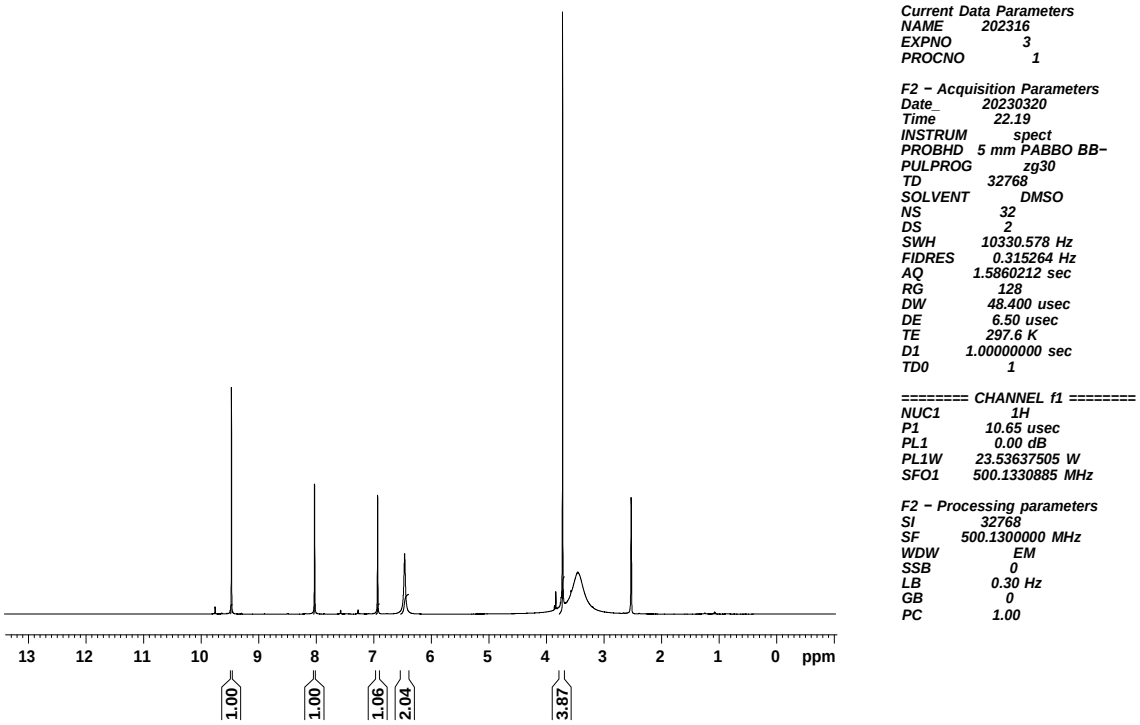

Figure: 3 HNMR Spectra of compound1.

Fig:4 Compound-1 CNMR

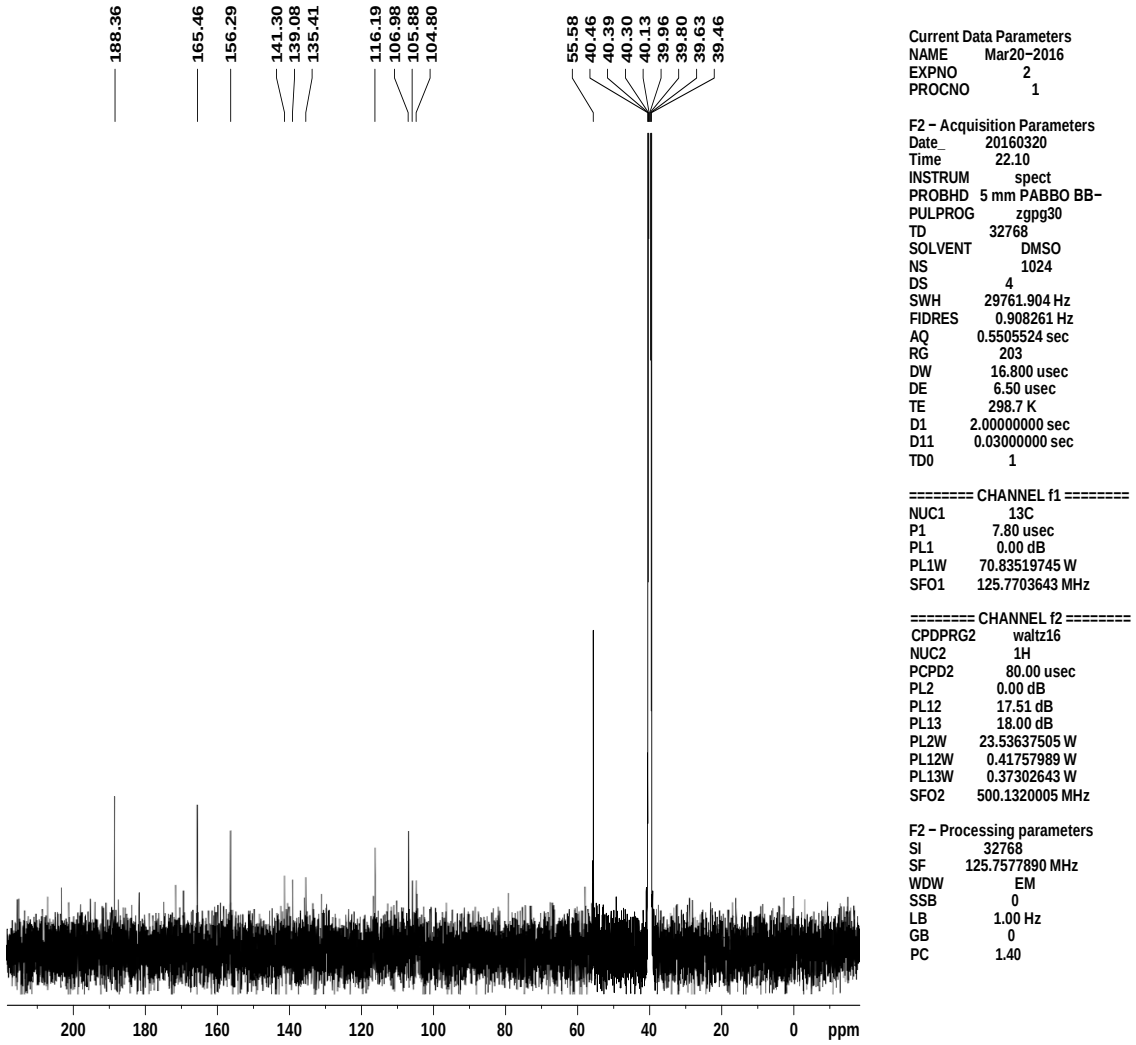

Figure 4: CNMR Spectra of Compound 1.

CONCLUSION

The standardized leaf powder of *Borassus flabellifer* was subjected to extraction with various solvents, the resultant extracts were subjected to phytochemical investigation. As per phytochemical investigation, steroids flavanoids, triterpenes, carbohydrates glycosides amino acids and tannins were found to be present in the various extracts. One chemical constituent was isolated from *Borassus flabellfer* is gallic acid. Further confirmation was carried out by IR, MS, HNMR and CNMR spectroscopy.

The melting points of the compound is 223, The structures of the compounds were established by IR, MASS & NMR spectroscopy and chemical tests. The base peak of the compound was 154, The compound was identified by TLC using ethyl acetate: formic acid (9:1) ,the compounds showed the Rf value is 0.40 matching with 3,4,5 trihydroxy benzoic acid(Gallicacid).

All the data were compared with the available sources.[7,8,9]

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