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Phytochemical Characterization of Bioactive Compound from the *Ensete superbum* Seed Powder

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ABSTRACT

The bioactive components Ensete superbum Seed Powder has been evaluated using GCMS, HPLC, FTIR and HNMR. The chemical compositions of the extract of Ensete superbum Seed Powder was investigated by Gas Chromatography–Mass Spectrometry and GC/MS techniques. The analysis of extract of Ensete superbum Seed Powder revealed that the existence of Eugenol (39.51) n-Hexadecanoic acid (21.97), 9-Eicosyne (5.18), 3-Decanynoic acid(1.87), 1-Tetradecyne(5.30), 7-Methyl-Z-tetradecen-1-ol acetate(1.88), 1-Hexadecyne (9.71), Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate(2.61), Octadecanoic acid (6.01), Tridecanedial (4.78) and cis-13-Eicosenoic acid (1.17). HPLC analysis of Ensete superbum Seed Powder reported that it has mainly contains five flavonoids compounds, namely Gallic acid (5.550 min), Caffeic acid (9.233min), Rutin (10.317min), Quercetin (12.125min) and Ferulic acid (23.200min). The results of FTIR analysis confirmed the presence of Alkynes, Alkanes , Amines , aromatic amines, alkyl halides, alkenes, carboxylic acids and Aromatic Compounds. Further, proton nuclear magnetic resonance spectrum of Ensete superbum Seed Powder was recorded and the chemical shift values of the various signals are identified. The detection of these phytochemical compounds present in the medicinal plants will endow with some information on Ensete superbum Seed Powder as herbal alternative for cure vast array of diseases.

Key words: *GCMS, HPLC, FTIR, HNMR and Ensete superbum*

INTRODUCTION

Medicinal plants are assumed to be much safer and proved elixir in the treatment of several ailments[1]. The medicinal assessment of these plants lies in some chemical constituents that produce a definite physiological accomplishment on the human body.

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The most momentous of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Bestowing to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their major healthcare needs.

Ensete superbum (Roxb.) belonging to the family Musaceae is a native to the Western Ghats, northeastern hills of India. E. superbum does not produce suckers like the other members of banana family; Seeds are the only mode of natural multiplication[2]. Traditionally, the powdered seeds are used for treating kidney stones and painful urination[3] given with milk for diabetes[4], and also for stomach ache[5].

The aim of this study is to determine the bioactive compounds present in the *Ensete superbum* Seed extract with the support of GC MS, HPLC, FTIR and NMR Techniques, which may give an insight in its use of traditional medicine.

MATERIAL AND METHODS

Plant materials

The fresh seeds of *Ensete superbum* were collected. The seeds were shade-dried, made coarse powder and stored in an air-tight container for extraction.

Preparation of Extracts for GC –MS

20 g of the powdered seeds of *Ensete superbum* were soaked in 100ml of 95% methanol for 12 h and filtered through Whatmann filter paper No. 41 along with 2 g sodium sulfate to remove the deposits and traces of water in the remainder. The filtrate was then concentrated and the extract contained both polar and nonpolar phytocomponents of the plant material used. 2 µl of this solution was used for GC/ MS analysis[6].

GC Condition and Identification of Compounds

The sample was examined through Gas Chromatography Mass Spectrometry/Mass Spectrometry Electron Ionization (GC-MS/EI) mode. The GC-MS/MS is a Scion 436- GC Bruker model coupled with a Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl/95% Dimethyl polysiloxane) and Length: 30m; Internal diameter: 0.25 mm; Thickness: 0.25 µm. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was working (split ratio of 10:1). The injector temperature 250°C; ion-source temperature 280°C. The oven temperature was automated from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then5°C/min to 280°C, windup with a 9 min isothermal at 280°C and total GC running time was 41 min. This last escalation was to clean the column from any residues. The mass spectrometer was activated in the positive electron ionization (EI) mode with ionization energy of 70eV. The solvent delay was 0-3.0 min. A scan intermission of 0.5 seconds and fragments from m/z 50 to 500 Da was programmed. The inlet hotness was set at 280 °C, source temperature 250 °C. The relative fraction amount of each component was calculated by comparing its average peak area to the total areas. Software approved to handle mass spectra and chromatograms was MS Work station 8. The NIST Version 2.0 library database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for identifying the chemical components. The GC-MS/MS was performed by Food Safety and Quality Testing Laboratory, Indian Institute of Food Processing Technology, Thanjavur

Sample preparation for HPLC

Seeds of *Ensete superbum* (2 g) suspended in 50 ml of 95% Methanol was extracted at 80 KHz using an ultrasonic device for 30 min (twice) at 45 °C. The consequential extract was collected, filtered and dried at 50 °C under reduced pressure. The dry crude extract was dissolved in the 100 ml mobile phase, filtered through 0.45 mm membrane filter (Millipore) and the extract was injected into HPLC.

HPLC conditions

Flavonoids were analyzed employing a RP-HPLC technique[7], Shimadzu firm., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu

SPD- 10ATVp ultraviolet illumination VIS detector and a loop gizmo by a loop size of twenty µl. The height space was thought-about with a CLASSVP software system. Reverse part action analysis was meted out in isocratic conditions employing a C-18 reverse part column (250×4.6 mm i.d., particle size five µm, Luna 5µ C-18; phenomenex, Torrance, CA, USA) at 25°C. The gradient extraction of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a big impact on the resolution of compounds. As a result, solvent gradients were fashioned, victimization twin pumping system, by varied the proportion of solvent A [water-acetic acid (25:1, v/v)] to solvent B (methanol). Solvent B was augmented to five hundredth in four min and later on augmented to eightieth in ten min at a rate of one.0 mL/min. Detection wavelength was 280 nm. Gallic acid (GA), Caffeic acid (CA), Rutin (RU), Ferulic acid (FA) and Quercetin (QU) was used as internal and external standards. Synthetic resin acids gift in every sample were known by scrutiny action peaks with the retention time (RT) of individual standards and more confirmed by co-injection with isolated standards.

FTIR Spectroscopic Analysis

Fourier remodel infrared photometer (FTIR) is probably the foremost powerful tools for distinguishing the kinds of chemical bonds (functional groups) gift in compounds. Dried powders of various solvent extracts of every material were used for FTIR analysis. 10mg of the dried extract powder was encapsulated in a hundred mg of KBr pellet, so as to organize clear sample disc. The fine sample of every plant specimen was loaded in FTIR prism spectroscope (Shimadzu, IR Affinity1, Japan), with a scan vary from four hundred to 4000 cm-1 with a resolution of 4cm-1.

HNMR Spectroscopic Analysis

For 1H-NMR analysis, spectrum was obtained from NMReady-60 bench prime prism spectroscope with the resonance frequency of sixty megahertz in static magnet. The sample was ready in an exceedingly confirmed inside the enclosure of normal five millimetre nuclear magnetic resonance tubes (NORELL) and dissolved in five hundred uL of deuterated Methanol-d4 and solvent peak was detected at four.78 ppm. Collected the information within the vary zero-15 ppm as spectral dimension and variety of scans given for every sample is 128 scans generally 0.06 seconds per scan at temperature. information were exported from the NMReady to be a part, baseline corrected was done exploitation mest Renova software package.

RESULTS AND DISCUSSION

Plants area unit important supply of doubtless helpful bioactive principles for the event of latest chemotherapeutical agents[8] The biological and pharmacologic properties of the many plants area unit still unknown. World over, the scientists area unit exploring the potential of utilizing pharmacologically active compounds from healthful plants[9]. flavorer medicines area unit employed by eightieth of the folks worldwide thanks to its high potency, cheap cost, non narcotic nature and fewer aspect effects[10].

GC/MS analysis

The bioactive phytoconstituents gift within the methanolic extract of genus Ensete superbum (Musaceae) seed powder known by GC-MS analysis. On comparison of the mass spectra of the constituent with the National Institute of Standards and Technology library, the eleven phytoconstituents were characterized and known. The active principles with their retention time (RT), chemical formula, mass and concentration of that eleven phytoconstituents gift in genus Ensete superbum (Musaceae) seed powder are conferred in Table 1. The GC-MS analysis result reveals the presence of eleven phytoconstituents within the seed extract of genus Ensete superbum (Musaceae) were Eugenol (39.51) n-Hexadecanoic acid (21.97), 9-Eicosyne (5.18), 3-Decanynoic acid (1.87), 1-Tetradecyne (5.30),7-Methyl-Z-tetradecen-1-ol acetate (1.88), 1-Hexadecyne (9.71), Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate (2.61),Octadecanoic acid (6.01), Tridecanedial (4.78) and cis-13-Eicosenoic acid (1.17). The

spectrum profile of GC-MS confirmed the presence of eleven major parts with retention time were seven.73, 11.12, 12.88, 14.16, 14.43, 15.44, 17.28, 18.22, 20.40, and 20.92 were shown in Table -1 and Figure 1.

Using Dr. Duke's phytochemical and ethanobotanical info (online), the biological activity of the known phytocomponents was determined. the assorted vital phytochemicals that contributes to the medicative activity of the plant given in Table: 1. Biological activities listed ar supported Dr. Duke's Phytochemical the results indicated eleven phytochemical constituents are known from methanolic extract of the seed of genus Ensete superbum (Musaceae)by Gas Chromatogram-Mass chemical analysis (GC-MS) analysis. In terms of proportion amounts Eugenol (39.51) and n-Hexadecanoic acid (21.97) were predominant within the extract. These 2 major compounds ar shown to possess medicinal drug, antiepileptic drug, Antiestrogenic, Antiinflammatory, inhibitor, Antitumor, Antiandrogenic, 5-Alpha enzyme matter ,Hepatoprotective, Haemolytic, Hypochloesterolemic ,Insecticide, Lubricant ,Pesticide, Nematicide, dilator activity. By decoding these compounds, it had been found that genus Ensete superbum (Musaceae) seed possess varied valuable phytoconstituents which is able to definitely finds application in drug discovery and can function pharmacologic tool to treat varied chronic diseases.

Determination of HPLC retention times

A selective and sensitive high-performance liquid chromatographic method is developed for the quantitative analysis of five naturally occurring flavonoids of methanolic extract of *Ensete superbum (Musaceae)* seed powder , namely Gallic acid (GA), caffeic acid (CA), rutin (RU), Ferulic acid (FA) and quercetin (QU). The HPLC result shows based on the Retention time (Rt), Gallic acid (5.550 min), Caffeic acid (9.233min), Rutin (10.317min), Quercetin (12.125min) and Ferulic acid (23.200min) content in *Ensete superbum (Musaceae)* was found to be 17.379 % , 1.433 % , 0.571% , 0.302 %, and 80.196 % were shown in Table 2 and Figure 2. Simultaneous analysis of Gallic acid, Caffeic acid, Rutin, Quercetin and Ferulic acid by HPLC method has been developed. This HPLC procedure provides a wonderful identification and quantification tool for these 5 Flavonoids compounds are gift within the methanolic extract of the seed of Ensete superbum (Musaceae) with a brief analysis time of half-hour. The experimental results indicated that methanolic extract of seed of Ensete superbum (Musaceae) contained high proportion of Ferulic acid followed by acid, Caffeic acid, Rutin and Quercetin so as .

The polyphenolic compounds most ordinarily found in plant extracts are the phenolic acids, flavonoids and tannins[11].These compounds beside alternative phenolic structures of plant origin are according as scavengers of Reactive atomic number 8 Species (ROS) and are seen as promising therapeutic medication at no cost radical mediate pathologies together with diabetic, vascular diseases[12].Most flavonoidic compounds exhibit antipyretic, analgesic, medicament, anti-arthritic, inhibitor and immuno-modulatory properties[13].

The results higher than showed, therefore, genus Ensete superbum could be a wealthy supply of the necessary biologically active flavanoids delineate here for the primary time, within the plant. The delineate HPLC procedure may well be helpful for the qualitative and quantitative chemical analysis of flavonoids in plant materials, particularly those of the (Musaceae) family. It may also be employed in the standard management of phytopreparations containing Gallic acid, Caffeic acid, Rutin, Ferulic acid and Quercetin likewise as in chemosystematics. based mostly upon the HPLC fingerprints, it will be over that this analytical technique could be a convenient methodology to spot the presence of various constituents gift within the methanolic extract. Thence the plant will be

thought of as a possible supply notably for flavonoids compounds. The findings from this work could raise the price of the medicative potential of plants.

Major band assignments for the IR Spectra

The FTIR spectrum was wont to determine the practical cluster of the active elements supported the height worth within the region of infrared. The seed powder of genus Ensete superbum (Musaceae) was passed into the FTIR and also the practical teams of the elements were separated supported its peak quantitative relation. The results of genus Ensete superbum (Musaceae) seed powder FTIR analysis confirmed the presence of Alkynes, Alkanes, Amines , aromatic amines, chemical group halides , acyclic amines , alkenes and radical acids compounds that shows major peaks at 3291.23,2927.59, 1635.96, 1336.45, 1149.25, 1076.94, 999.78, 927.94 and 859.96 severally teams (Table 3 and Figure 3).Therefore, the FT-IR analysis on genus Ensete superbum (Musaceae) seed displayed new phytochemical markers as helpful diagnostic tool to visualize out not solely the standard of the powder however conjointly to spot the medicinally vital and bio molecular composition of the plant.

^{1}H-NMR structural analysis

In nuclear magnetic resonance study, Ensete superbum seed powder were subjected to analyze the structural assignment and further as confirmation of purity. Table 4and Figure 4 shows the nucleon nuclear magnetic resonance spectra of seed recorded in deuterated solvent at temperature.

Nuclear magnetic resonance spectrographic analysis is far and away the foremost powerful spectroscopical techniques for getting elaborate structural info concerning organic compounds in plants[14]. A nuclear resonance spectrum provides the most important quantity of knowledge concerning the structure of a compound. In nuclear magnetic resonance spectroscopical methodology, a substance is placed during a sturdy magnetic flux that affects the spin of the atomic nuclei. A radio emission passes through the substance, and reorients these nuclei. Once the wave is turned off, the nuclei unharness a pulse of energy that gives information on the molecular structure of the substance which will be remodeled into a picture by laptop techniques[15].

Plants turn out a various vary of bioactive molecules creating them made supply of various kinds of medicines. Varied techniques square measure utilized for his or her investigation which incorporates bioassays for chemical screening and their analysis for presence of biological activities. GC/MS results signification the presence of 11 phytochemical constituents. The prevailing compounds were Eugenol (39.51) and n-Hexadecanoic acid (21.97). HPLC analysis provided an honest platform for identification and quantification of 5 phenolic resin compounds as acid (GA), Caffeic acid (CA), Rutin (RU), Ferulic acid (FA) and Quercetin (QU) gift in monocot genus superbum (Musaceae) seed powder. The results of FTIR analysis confirmed the presence of Alkynes, Alkanes, Amines, aromatic amines, alkyl radical halides, open-chain amines, alkenes and group acids compounds. H proton magnetic resonance analysis has given sensible data to figure more for its purification and detection of the compound to use its healthful functions. The presence of varied bioactive compounds justifies the employment of the Ensete superbum (Musaceae) seed powder for various ailments by ancient practitioners. The results of this study supply a platform of mistreatment Ensete superbum (Musaceae) seed powder indulge effective potential bioactive compounds. Moreover, isolation of individual phytochemical constituents might cause the event of novel medication to treat the varied chronic diseases.

Table 1: Compounds Identified in the *Ensete Superbum (Musaceae)* Seed Powder

S.No.	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %	Activity
1	7.73	Eugenol	$C_{10}H_{12}O_2$	164	39.51	Antibacterial, Anticonvulsant, Antiestrogenic, Antiinflammatory, Antioxidant, Antitumor, Hepatoprotective, Insecticide, Pesticide, Vasodilator
2	8.39	3-Decanynoic acid	$C_{10}H_{16}O_2$	168	1.87	Acidifier, Arachidonic acid-Inhibitor, Inhibit Production of Uric Acid, Urinary-Acidulant
3	11.12	1-Tetradecyne	$C_{14}H_{26}$	194	5.30	Antineoplastic, Membrane integrity agonist, Ubiquinol-cytochrome-c reductase inhibitor, Testosterone 17beta-dehydrogenase (NADP+) inhibitor
4	12.88	9-Eicosyne	$C_{20}H_{38}$	278	5.18	Anti-microbial and cytotoxic properties
5	14.16	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	1.88	Anti cancer, antiinflammatory, hepatoprotective
6	14.43	1-Hexadecyne	$C_{16}H_{30}$	222	9.71	Antibacterial
7	15.44	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	21.97	Antioxidant, Hypochloesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Haemolytic, 5-Alpha reductase inhibitor.
8	17.28	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268	2.61	Antioxidant, Haemolytic.
9	18.22	Octadecanoic acid	$C_{18}H_{36}O_2$	284	6.01	Anti-inflammatory Hypocholesterolemic Cancer preventive Hepatoprotective Nematicide Insectifuge, Antihistaminic,Antieczemic Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic ,Antiarthritic, Anticoronary ,Insectifuge
10	20.40	Tridecanedial	$C_{13}H_{24}O_2$	212	4.78	Not found
11	20.92	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310	1.17	A rare omega 7 fatty acid.Reported only in Sapindaceae Family Paullinia elegans (Sapindaceae).

RT-Retention Time

Table 2: Hplc Analysis *of Ensete superbum (Musaceae)* Seed Powder Extract

Peak	Area	Area%	Height	Height %	Retention Time	Name of the compound
1	72273	17.379	40	0.313	5.550	Gallic acid
2	5960	1.433	17	0.133	9.233	Caffeic acid
3	2375	0.571	10	0.078	10.317	Rutin
4	1254	0.302	1	0.008	12.125	Quercetin
5	333507	80.196	12716	99.468	23.200	Ferulic acid

Table 3. Ftir Peak Values of *Ensete superbum (Musaceae)* Seed Powder

S. No.	Peak Values	Functional groups
1.	3291.23	Alkynes
2.	2927.59	Alkanes
3.	1635.96	Amines
4.	1336.45	Aromatic Amines
5.	1149.25	Alkyl Halides
6.	1076.94	Aliphatic Amines
7.	999.78	Alkenes
8.	927.94	Carboxylic Acids
9.	859.96	Aromatics

Table 4. H NMR Data and Their Assignment from the *Ensete superbum (Musaceae)* Seed Powder

S.NO	Chemical shift range, ppm	Type of proton
1.	7.35-7.46	Ar OH
2.	6.01-6.90	A r–H
3.	4.00-4.10	HC–F
4.	3.31-3.63	HC–OR
5.	2.08-2.86	C≡C–H
6.	0.89	RCH3

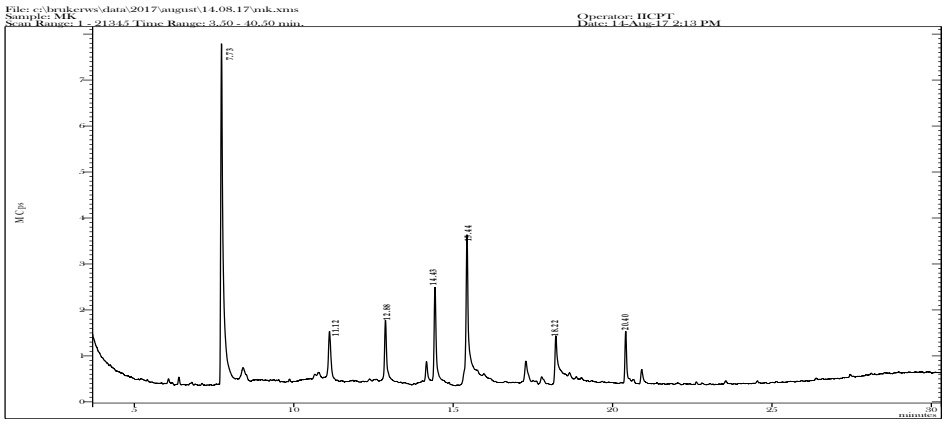

Fig. 1: GC- MS/MS Chromatogram

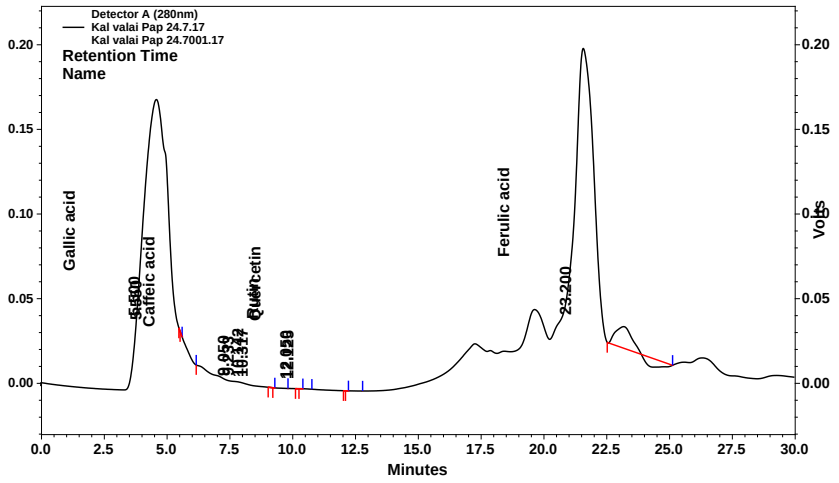

Fig. 2: HPLC analysis of *Ensete superbum (Musaceae)* seed powder extract

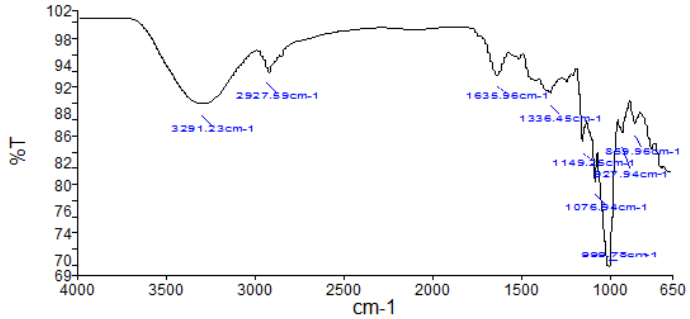

Fig. 3: FTIR analysis of *Ensete superbum (Musaceae)* seed powder

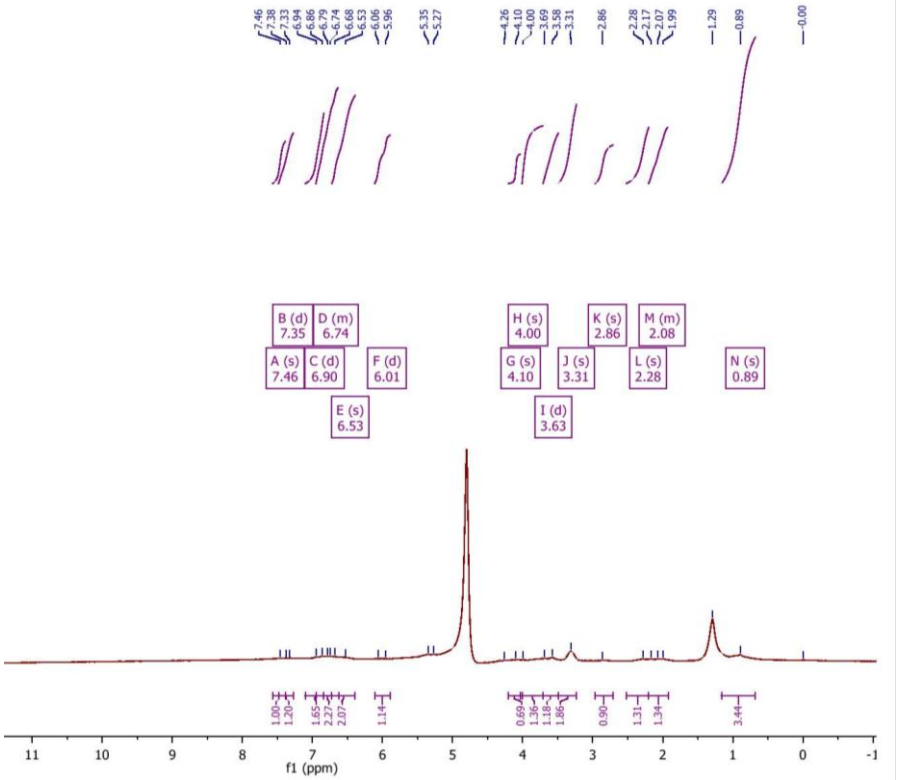

Fig. 4: 1H NMR spectrum of the *Ensete superbum (Musaceae)* seed powder

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