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Research Article

Metabolite fingerprinting and profiling of two locally cultivated edible plants by using nuclear magnetic resonance

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Abstract: Nuclear magnetic resonance (NMR) spectroscopy is a sensitive technique used to analyse the structure, dynamics, reaction state, and chemical environment of molecules. *Abelmoschus esculentus* and *Lagenaria siceraria* are edible plants used traditionally to treat jaundice, diabetes, weight loss, ulcer, hypertension, heart failure, skin diseases and reduced cholesterol. Therefore, based on the medicinal uses the study was designed to analyze fingerprinting of metabolites of the seeds of the selected plants. The dry seeds were powdered and the metabolites were extracted by socking method with a mixture of methanol/chloroform. The extracted metabolites from seeds were subjected to proton NMR using the noesygpprld pulse sequence. A total 18 peaks were obtained from each spectrum. Among the peaks, three peaks with the highest intensities were analyzed by utilizing NMR. The peak metabolites were determined with the correlation with the correct peak using in built Biological Magnetic Resonance Data Bank (BMRB). The results showed that the obtained data varied from known plant metabolites due to the contamination and interaction between the metabolites. In addition, variants in the metabolites from sample to sample may have been the result of errors or limitations in the study. The data generated from this experiment will be used to help to conduct the advanced research in the near future on the selected edible plant species which will be valuable for many different areas of the scientific community. Plant metabolomics has the potential benefit in the medical field, agricultural industry, and many other areas of our economy.

1. INTRODUCTION

Metabolism is an active set of lifesaving chemicals or chemical reactions in body organisms. The focus of metabolism is the energy conversion from food, to run the circulation systems, to block various secondary metabolic such as proteins, fats, several acids, and polyphenolic ingredients, and eliminate body wastes. All these enzymatic reactions permit an organism to develop and preserve structures, to reproduce and retort against the environment. Metabolism is defined as the total chemical reactions happen in an active organism such as digestion and transportation of substances. Nuclear magnetic resonance (NMR) spectroscopy is a sensitive

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analytical tool that is used for determining the structure of organic materials, physical, chemical and biological properties of matter. Nowadays, several analytical techniques are widely used for drug development, drug discovery, as well as to screen metabolites in various organisms such as bacteria, animals, plants, algae, and cancer cell lines.

Among the analytical techniques, NMR (^{1}H- NMR and ^{13}C-NMR) is one of the sensitive analytical techniques to understand the composition of organisms, drugs-metabolic interaction, drugs-drugs interaction etc. (Sarkar, 2008). Therefore, researchers are using the most advanced NMR analytical techniques to solve the complex problems related to plant and animal metabolism. Recently, the researchers are using overlap NMR data technique with built-in library data to identify various aspects such as 3D structure of the molecule, movement of molecules, and interaction with its environment (Berg *et al*., 2002).

One of the best ways to identify and/or quantify metabolites present in organisms is by using NMR spectroscopy. Metabolites are considered as an intermediate product of metabolic reactions catalyzed by various enzymes that naturally occur within cells (Renneber & Loroch, 2016; Dhanjai *et al*., 2018). Generally, there are two types of metabolites: primary and secondary. Primary metabolites are synthesized by the cell because they are vital including amino acids, alcohols, vitamins, and more. Secondary metabolites are made by the organism, but they are not necessary for primary metabolic activities, although they play important roles in several areas including cell survival, signaling, and defense against predators or the environment. In addition, secondary metabolites also serve as a better tool to distinguish between cellular responses of different species which vary from organism to organism (Roessner & Bowne, 2009). The presence or the concentration of some metabolites may be increased or decreased based on stressors in the environment. The collective study of metabolites, primary and secondary is known as metabolomics. Specifically, metabolomics is defined as the comprehensive analysis of metabolites in a biological specimen (Clish, 2015). This approach has helped us understand what drives significant biological activities in the organism being observed.

Both selected plants are edible. *A. esculentus* belongs to Malvaceae family while *L. siceraria* belongs to the Cucurbitaceae family. They are locally known as okra and bottle gourd and their English names are lady`s finger and calabash long squash. Both plants grow throughout the Asian countries and in some of the EU countries, Australia and the USA. The literature review showed that both selected plants are originated in the South Asian, Ethiopian and West African region. However, they spread throughout the world including Greenland. Both fruits are rich sources of active constituents, nutritional values, minerals, fibers, etc. Based on the above nutritive and bioactive constituents these plants are commercially cultivated dible fruit/seed pods throughout the world. Okra is an annual plant that typically grows 3-5' tall (Figure 1). Bottle gourd is also an annual plant that grows up to 12-16 feet long with large, hairy, broad-ovate, entire to shallowly-lobed, dark green leaves (Figure 1). The okra strains from each country have developed characteristics determined by the climates of the location. The fruits of the plant are rich in vitamins, calcium, potassium and other mineral matters. The mature okra seed is a good source of oil and protein (Kumar, *et al*., 2013). Specifically, it contains unsaturated fatty acids such as linoleic acid (András *et al*., 2005). This fatty acid, which comprises almost fifty percent of okra oils, is vital for human nutrition. The fiber that comes from the mature fruit and stem can also be utilized in the paper industry (Kochhar, 1986). The chemical composition of okra has been thoroughly researched. A multicellular fiber found in okra known as okra bast is the target of many studies. The composition of this fiber is identified as 67.5% a-cellulose, 15.4% hemicelluloses, 7.1% lignin, 3.4% pectic matter, 3.9% fatty and waxy matter and 2.7% aqueous extract (Kumar *et al*., 2013).

Figure 1. Typical picture of Okra and Bottle guard.

Other nutritional values present in okra are carbohydrates, sugars, dietary fibers, fat, protein, water, vitamin A, thiamine (B1), riboflavin (B2), niacin (B3), vitamin, vitamin E, vitamin K, calcium, iron, magnesium, potassium, and zinc. The main constituents of the fiber are a-cellulose, hemicelluloses, and lignin. However, detailed metabolite profiling has not been conducted. Okra seeds are known to contain high amounts of oil (20-40%), which makes it an excellent candidate for essential oilseed (Benchasri, 2012). There are important amino acids in the seeds such as lysine and tryptophan (Kumar *et al*., 2016). Lysine is an essential amino acid whereas tryptophan is important for normal functions of proteins, muscles, enzymes, and also for neurotransmitters production and maintenance (Jenkins *et al*., 2016). The okra seeds also contain oligomeric catechins and flavonol derivatives (Persson *et al*., 2001). Other constituents include hydroxycinnamic and quercetin derivatives, phenolic compounds with important biological properties similar to quartering derivatives, and catechin oligomers, glycol-proteins, procyanidin B2 and B1 (both phenolic compounds), pectin, and rutin (Arapitsas, 2008). Green vegetables are known to contain valuable chlorophyll. Chlorophyllin, a phenolic compound and important component of chlorophyll was studied for its health benefits. These include being an antioxidant, anti-inflammatory and anti-microbial (Mendoza-Núñez *et al*., 2019). Each of these constituents are not only nutritious to humans, but they also play a vital role in the plant's life. There are many properties of okra that are the targets for medicinal uses. These uses include gastro-intestinal aid, endocrine activity, immunoprotective properties, psychological alleviators, and even properties regarding liver detox (Gemede *et al*., 2015). The most notable medicinal properties that have been targeted by researchers are its cardiovascular and endocrine effects. In regard to heart health, consuming okra has shown to assist with reducing cholesterol due to its fiber and the metabolite pectin content (Ngoc *et al*., 2008).

Similarly, phytoconstituents of *Lagenaria siceraria* include carbohydrates such as glucose and fructose, starch, curd fiber, hemi cellulose, cellulose and lagenin. Usually, bitter fruits yield solid foam containing cucurbitacins B, D, G and H, mainly cucurbitacin B. This component is

seen in members of the pumpkin and gourd families. These bitter principles are present in the fruit as aglycones. *Lagenaria siceraria* shows presence of flavone-C glycoside, a well-known antioxidant and anti-tumor agent (Rahman, 2003). *Lagenaria siceraria* seeds contain a wide array of vitamins, minerals, lipids, amino acids, and many other components. These include calcium, magnesium, phosphorous, potassium, vitamin C, niacin and other B vitamins, folate, vitamin A, fatty acids, glutamic acid, aspartic acid, arginine, leucine as well as other amino acids (Roopan *et al*., 2016). Overall, this vegetable provides many important nutrients for human health.

Due to the plethora of nutrients available in bottle gourd, it is used all over the world in traditional and natural medicines. Medicinal properties that have been the targets of research include enzymatic activities of lipase, amylase, and pectinase in the digestive system. Another area is the antimicrobial activity. Antidiabetic activity was also observed. Patients who consumed the juice of bottle gourds showed a marked reduction in cholesterol and blood glucose (Katare *et al*., 2013). Cardioprotective (Adedapo *et al*., 2013), antioxidant (Satvir *et al*., 2012), and anti-inflammatory activities have been observed by monitoring various heart measurements while using bottle gourd powder (Shirsat & Kadam, 2015). There are many more medicinal and pharmaceutical properties of bottle gourd such as anticancer (Thakkar, 2013), analgesic and genetic properties (Roopan *et al*., 2015). Both plants we chose have excellent nutritional values as well as helpful medicinal properties. Understanding their active constituents and important metabolites of the chosen plants will give us new insights into their unique composition. The literature search showed that there is not a single report available on the metabolic identification and profiling of fruits of the selected plants by NMR. Therefore, the objectives of this present study are the isolate the metabolics from the seeds of the selected plants and identify them by using NMR spectroscopy.

2. MATERIAL and METHODS

2.1. Chemicals and Glassware

The solvents were used in this experiment such as methanol, chloroform, and deionized water obtained from E. Merck, Germany. Sodium phosphate buffer and deuterated water were collected from BDH, UK. Other chemicals and solvents were analytical grade. The purities of the chemicals and solvents were above 95%. In addition, all glassware used in this present experiment was purchased from Boroshil, India.

2.2. Instruments

In this experiment, the prepared samples were analyzed by using NMR spectroscopy (Brucker) and centrifuge machine (Model, brand, Country) was used to separate the metabolic from the leaves and seeds of the selected plant species. NMR spectra were noted on a Bruker (400 MHz) instrument in deuterated chloroform with tetramethylsilane as an internal standard (chemical shifts δ, ppm).

2.3. Sample Collection

The seeds of *A. esculentus* and *L. siceraria* were collected locally on (date) during the day time. Both the samples were collected at three different time and places. The collected samples were stored in a plastic bag at room temperature to protect the metabolic change. After collection of the samples the morphological identification was completed based on wikipedia websites (https://en.wikipedia.org/wiki/Okra and https://en.wikipedia.org/wiki/Calabash) and confirmed by local people. Both collected samples were washed with distilled or deionized water. Then, the samples were ground into paste by mortar and pestle. The paste samples were kept in the aluminum foil at ambient temperature for extraction.

2.4. Extraction of Metabolites

Each sample (0.5 g) was taken in a 100 ml conical flask and added a mixture of aqueous solvent (methanol-water: 4:1.25). The samples mixture was vigorously shaken by using a shaker for few minutes. Then chloroform (4 ml) and water (2 ml) were added to the conical flask and shaken by vortexed for 1 minute. The whole mixture was kept in ice bath for 10 minutes. After incubation, the sample was centrifuged at 5000 rpm for 5 minutes. The sample had two layers and the upper layer was transferred to another tube and dried the mixture solvent completely by using a vacuum centrifuge. Finally, the dried extract sample was dissolved with 250 µL 0.1 M sodium phosphate buffer (pH 7.0) in 90% H_2O and 10% D_2O (Wu *et al*., 2008) (Figure 2).

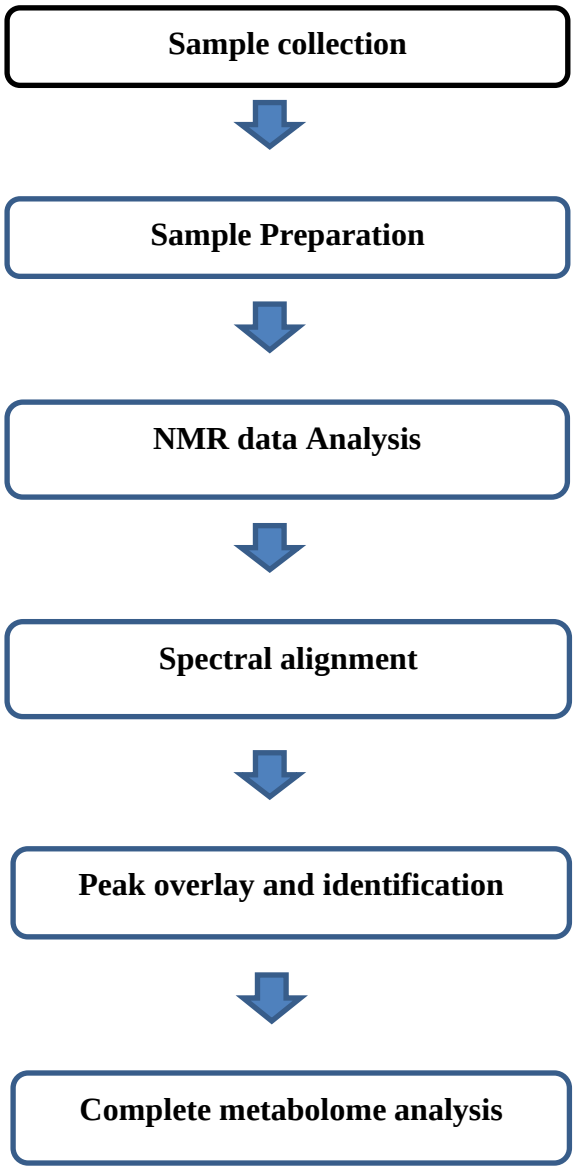


Figure 2. Essential steps involved in NMR based metabolomics studies.

2.5. Analysis of The samples by NMR

The extracted metabolites from okra and bottle guard were analyzed by using NMR with the noesygppr1d pulse sequence. The signals are elucidated by pulses of radiofrequency irradiation and can be related to the contents within the sample (Bligny & Douce, 2001). Utilizing the NMR pulse sequence ensures that all non-exchangeable protons can be detected in a deuterated

buffer solution (Stryeck *et al*., 2018). Deuterated buffers allow for the solvent not to interfere with the sample because the resonance frequency of a deuteron (^{2}H) is vastly different from that of proton (^{1}H). This difference in frequency means there would be no peaks from the solvent in the NMR spectrum. Locking and shimming are also terms used to describe the importance of buffers. Locking refers to keeping the magnetic field stable eliminating the effect of drifting and shimming is to ensure that there is a homogeneous magnetic field over the whole of the sample volume located within the probe's detection coil. If the shimming fails, there will be a distorted line shape leading to poor resolution and decreased sensitivity. Shimming utilizes the coils (shims) around the sample. Applying electrical currents to the coils can make up for any discrepancies in the field homogeneity (Minocha, 2015).

The raw NMR data underwent spectral processing. This process refers to programs in the spectrometer than can be pre-programmed to manipulate certain variables depending on what information we wanted to gather. Along with spectral processing, data processing is also taking place. It is also a part of the pre-processing step. It involves the use of parameters and variables to ensure the data collected is what is desired in the research experiment (Emwas *et al*., 2018).

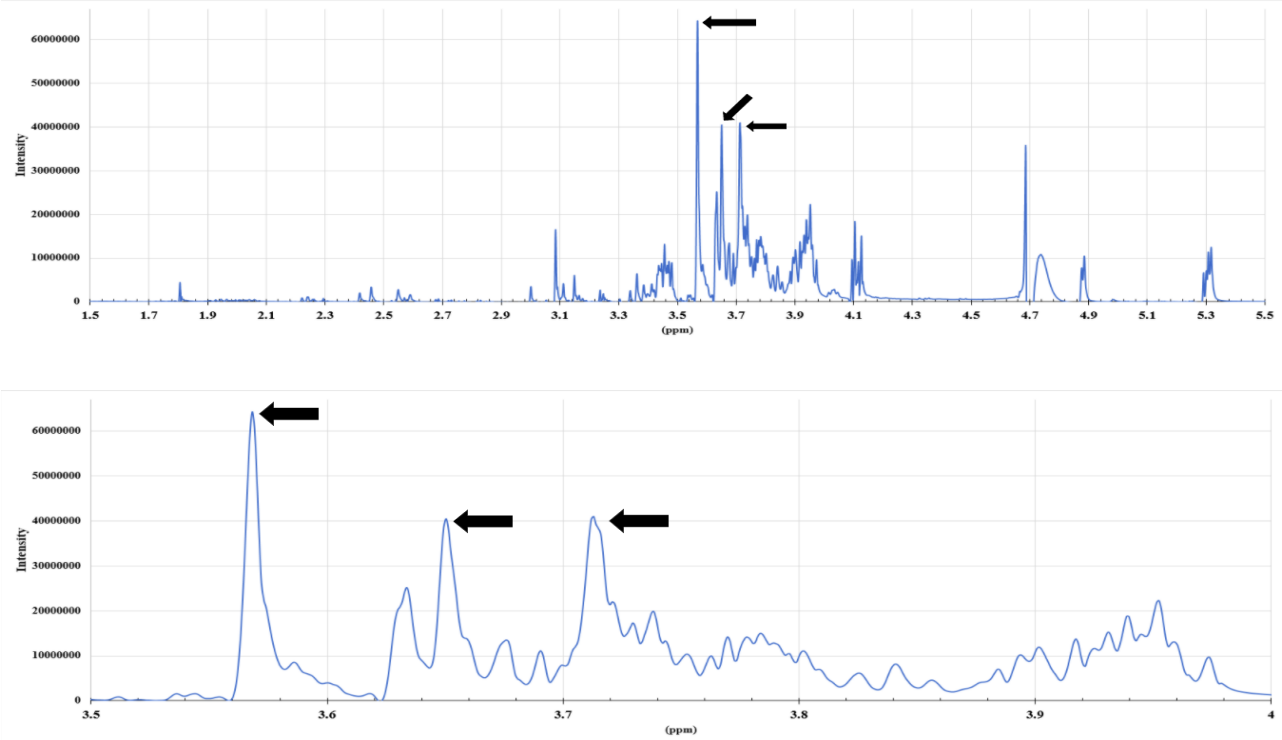
Following the collection of this data from the NMR spectrometer, we then began to analyze the data. The NMR spectroscopy data analysis of the selected plant seeds can be viewed in two different formats that are presented in the result section. The first three sets of samples derived from the *A. esculentus* (okra) and the following three sets of samples are from the *L. siceraria* (bottle gourd). For each of the six samples there are graphs containing the NMR spectroscopy results for the specific sample. The peaks in the spectrums are analyzing and interpreting results of NMR in detail by using the Biological Magnetic Resonance Data Bank (BMRB) and presented in results and discussion section. Based on the BMRM databank as well as previously reported data, it can conclude that which metabolite as a peak is represented in the sample. In addition, based on the BMRB databank and plant metabolomic research, we were able to identify the peaks and discuss their contributions to the plants` inner workings and in near future other areas of the scientific community.

3. RESULTS and DISCUSSION

The people use the fruits of okra and bottle gourd as vegetables as they contain rich sources of bioactive constituents, nutritional values, minerals, fibers etc that can fulfill our body requirements for survival. The previous reports showed that both fruits of the selected plants have significant biological and pharmacological activities due to their active metabolites. All these bioactive compounds play a significant role to protect and treat different ailments. Based on the medicinal benefits, the present work was undertaken to isolate and identify metabolites from the seeds of the selected by using NMR spectroscopy.

3.1. ^{1}H-NMR Spectra of Okra Sample 1

From the spectrum, it showed that several peaks appeared within the range of δ 1.5 to 5.5 ppm. Among the peaks, three peaks at δ 3.5684, 3.6504 and 3.7127 were the major peaks with the highest intensities in the Okra sample 1 as it is presented in Figures 3 and 4.



Figures 3. Proton NMR spectra from okra, *Abelmoschus esculentus* sample one. Figure 4 is an enlarged view of Figure 3. Analyzed peaks are indicated by arrows. X-axis represents the chemical shift (parts per million). Y-axis shows the signal intensity, represented in arbitrary units. The intensity of the signal is proportional to the number of hydrogens that make the signal.

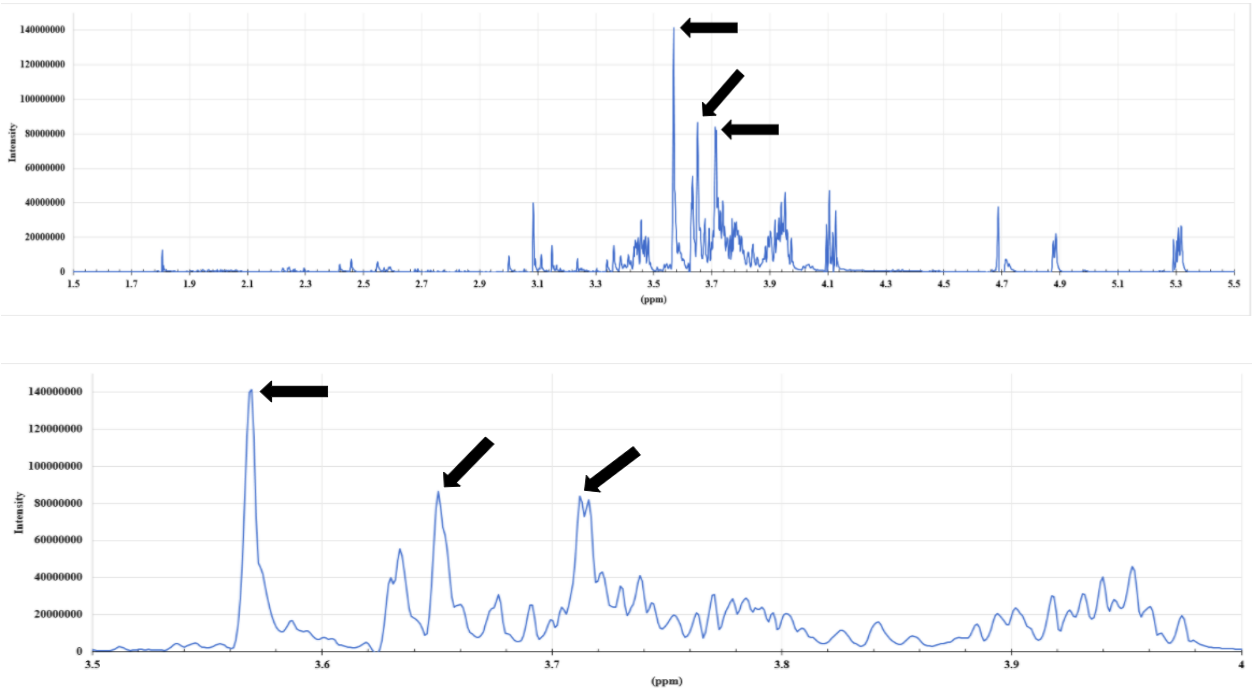


Figure 4. Proton NMR spectra from okra, *Abelmoschus esculentus* sample two. Figure 6 is an enlarged view of Figure 5. Analyzed peaks are indicated by arrows. X-axis represents the chemical shift (parts per million). Y-axis shows the signal intensity, represented in arbitrary units. The intensity of the signal is proportional to the number of hydrogens that make the signal.

The displayed peak at δ 3.5684 ppm had the possibility of being three different substances such as pentoxifylline, aleuritic acid, or H-b-S-OH based on the BMRB databank (Table 1). All of these substances found in Okra sample have been reported earlier by several authors. The bioactive metabolic pentoxifylline is a derivative of theobromine, a methylated xanthine and theobromine is a well-known metabolite that can be found in okra as well as chocolate, tea and cocoa products. Xanthine is a derivative of alkaloids and most closely related to methylated xanthines namely caffeine, theophylline and aminophylline. All of these alkaloid agents are used as a prescription drug to treat relax muscles, particularly bronchial muscles, to stimulate the central nervous system and the kidney to promote diuresis (Zhang, *et al*., 2004). Aleuritic acid is an organic compound derives from the resin. It is a major ingredient of shellac and mainly isolated from plant species. It is used as a starting reagent for manufacturing perfumes. It has also significant health benefits to protect human cell and cure injury (Heredia-Guerrero *et al*., 2017). Lastly, H-b-S-OH according to the BMRB database, falls into the category of b-O-4 dimers and 3-Carbon Sidechains. This compound can be found in the NMR Database of lignin and Cell Wall Model compounds. Usually, it is observed in the plant's cell wall (Biological Magnetic Resonance Data Bank, 2021). Due to the limited resources pertaining to the substance's presence in okra, *A. esculentus*, this peak assigned to compound H-b-S-OH.

Table 1. Summary of peaks and possible matches based on the BMRB databank for sample one of seeds from *A. esculentus*.

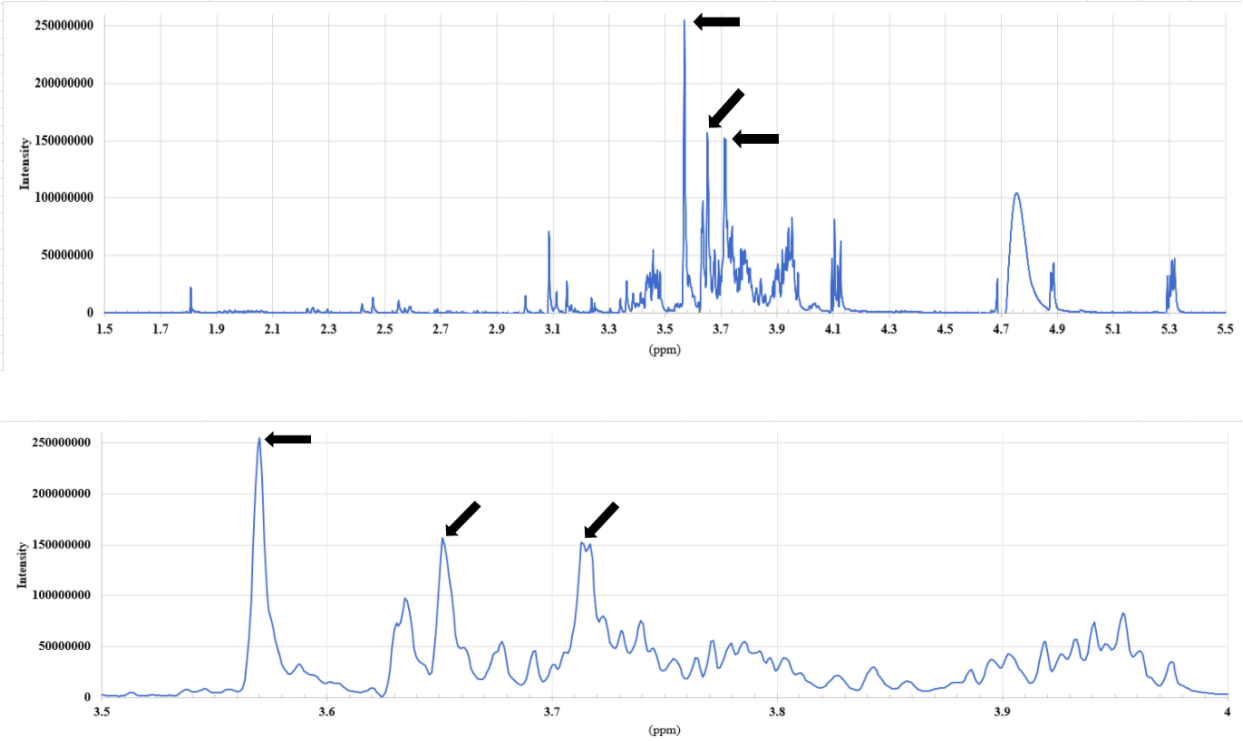
Chemical shift (ppm)	BMRB Databank Results	Predicted results
3.5684	1. Pentoxifylline 2. Aleuritic acid 3. H-b-S-OH	H-b-S-OH
3.6504	1. Lignin_cw_compound_3021 2. Lignin_cw_compound_2028 3. Lignin_cw_compound_2029 4. Lacto-N-fucopentaitolI	Lignin
3.7127	1. Schisandrin 2. L-(-)-Threitol	Lignin

The peak at δ 3.7127 ppm can be attributed to schisandrin, L-(-)-threitol, or lignin_cw_compound_3021 and it is naturally occurring compound found in *Schisandra chinensis*. All those metabolites have significant effects on the human body including hypertension and liver problems (Nasser *et al*., 2020). Recently, L-(-)-threitol has been observed in the edible fungus *Armillaria mellea,* Alaskan beetle, and *Upis ceramboides.* It serves as an antifreeze agent (Huang *et al*., 2015; Wong *et al*., 2019). Lignin_cw_compound_3021 is one of the main components of the cell wall. It acts as a polymer in the cell wall and it is important for many functions including transport, support, and stress resistance. (Liu, *et al*., 2018). Lignin's is a common chemical; therefore, the assigned peak is for lignin (Table 1).

On the other hand, the peak at δ 3.6504 ppm had two candidates, lignin and Lacto-N-fucopentaitolI. The chemical Lacto-N-fucopentaitolI is an oligosaccharide found in human milk. It makes up a very large part of human milk and assist with various functions including immunity (Sotgiu *et al*., 2006). Therefore, once again this peak is also for lignin. Lignin compound is very important for plant structure and cell wall function. Its high prevalence in plants is to be expected.

3.2. ^{1}H-NMR Spectra of Okra Sample 2

The NMR spectrum showed that most of the peaks within the range of δ 1.5 to 5.5 ppm (Figure 5). The peaks at δ 3.5688, 3.6504, and 3.7123 ppm are the major peaks in the okra sample 2 due to the peak intensity. Almost similar results were obtained from the okra sample 2 (Table 2).



Figures 5. Proton NMR spectra from okra, *Abelmoschus esculentus* sample three. Figure 5a is an enlarged view of Figure 5. Analyzed peaks are indicated by arrows. X-axis represents the chemical shift (parts per million). Y-axis shows the signal intensity, represented in arbitrary units. The intensity of the signal is proportional to the number of hydrogens that make the signal

Table 2. Summary of peaks and possible matches based on the BMRB databank for sample one of seeds from *A. esculentus*.

Chemical shift (ppm)	BMRB Databank Results	Predicted results
3.5684	4. Pentoxifylline	H-b-S-OH
	5. Aleuritic acid	
	6. H-b-S-OH	
3.6504	5. Lignin_cw_compound_2028	Lignin
	6. Lignin_cw_compound_2029	
	7. Lacto-N-fucopentaitolI	
3.7127	3. Schisandrin	Lignin
	4. L-(-)-Threitol	
	5. Lignin_cw_compound_3021	

3.3. ^{1}H-NMR Spectra of Okra Sample 3

The NMR spectrum showed most of the peaks within the range of δ 1.5 to 5.5 ppm (Figure 6). The peaks at 3.5699, 3.6516, and 3.7134 ppm are the major peaks in the Okra sample 2 due to the peak intensity. The Okra sample 3 is slightly different from sample 1 and 2 and the obtained results are presented Table 3.

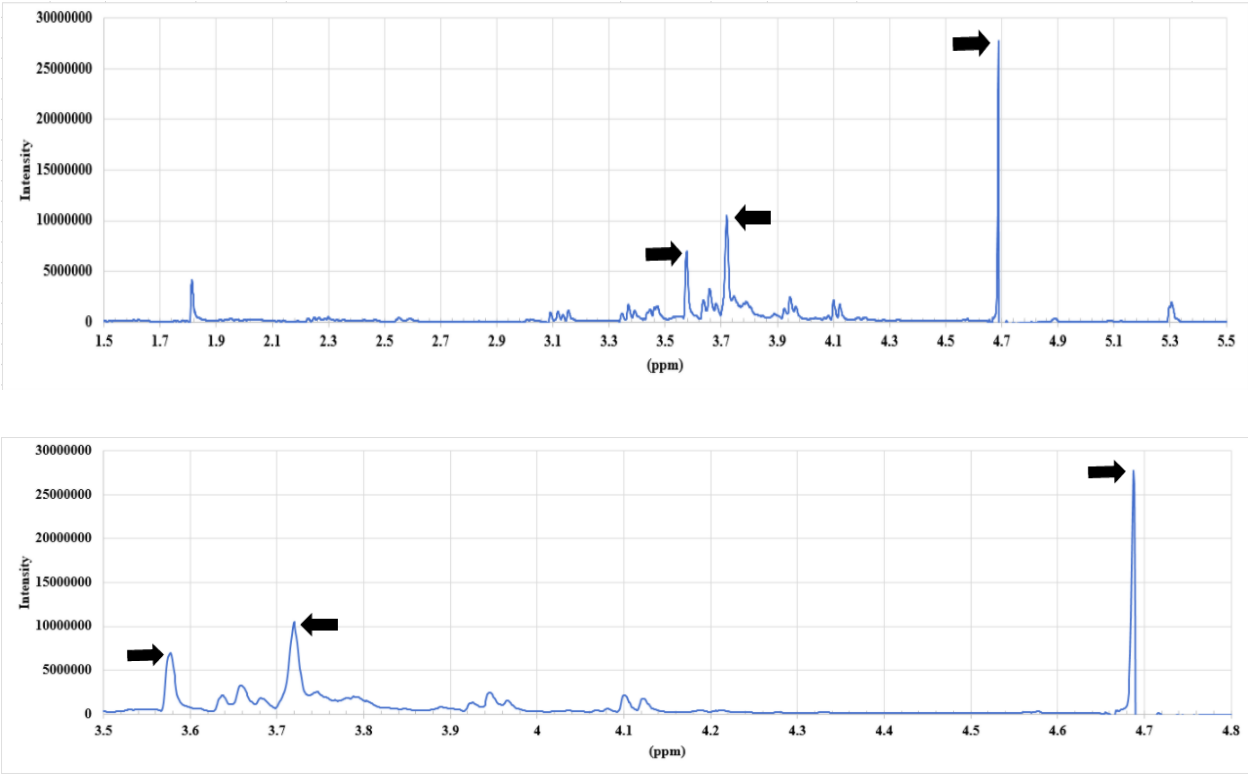


Figure 6. Proton NMR spectra from bottle gourd, *Lagenaria siceraria* sample one. Figure 6a is an enlarged view of Figure 6. Analyzed peaks are indicated by arrows. X-axis represents the chemical shift (parts per million). Y-axis shows signal intensity, represented in arbitrary units. The intensity of the signal is proportional to the number of hydrogens that make the signal.

Table 3. Summary of peaks and possible matches based on the BMRB databank for sample one of seeds from *A. esculentus*.

Chemical shift (ppm)	BMRB Databank Results	Predicted results
3.5684	7. Pentoxifylline 8. Aleuritic acid 9. H-b-S-OH	H-b-S-OH
3.6504	8. L-(-)-Threitol 9. Indole-3-acetic acid 10. lignin_cw_compound_2028	Indole-3-acetic acid
3.7127	6. Bis (2-butoxyethyl) phthalate 7. Rutin trihydrate 8. Schisandrin	Rutin trihydrate

Based on the BMRB databank, the peak at δ 3.6516 ppm indicates the presence of L-(-)-Threitol and lignin _cw_compound_2028 where two possibilities have already been discussed. Another new metabolite Indole-3-acetic acid observed is common naturally occurring plant hormone of the auxin class. Auxins are a class of plant hormones with some morphogen-like characteristics. They are important signaling molecules in the plant. Auxins play an important

role in coordination of growths and behavioral processes in plant life cycles and they are also important for plant body development. (Dhande *et al*., 2012; Woodward & Bartel, 2005). The most likely match for this peak is indole-3-acetic acid. This hormone is vital for plant growth and development.

The third peak in the sample 3 is at δ 3.7134 that indicates the presence of schisandrin that was already discussed. The other two metabolites are biologically important substances. First is bis(2-butoxyethyl) phthalate which is an organic compound derived from 2-butoxyethanol. It is a well-known plasticizer in PVC and PVA and works as an adhesive. It has rarely been found in plants (Cheriti, *et al*., 2006; Thiemann, 2021). The most well-known secondary metabolite is rutin trihydrate. Rutin trihydrate is an organic polyphenolic compound and it has several pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities. It is also natural product isolated from several plant species (Ganeshpurkar & Saluja, 2017; Tongjaroenbuangam et. al, 2011). Therefore, the third peak in the NMR spectrum is rutin trihydrate.

3.4. ^{1}H-NMR Spectra of Bottle Gourd Sample 1

In the NMR spectrum of bottle gourd sample 1 showed that many peaks that ranged from δ 1.5 to 5.5 ppm. Among the peaks, three peaks at δ 4.688, 3.7195, and 3.5768 ppm showed the major peaks with the highest intensities in the bottle guard sample 1 that are presented in Figures 9 and 10.

The BMRB databank, displayed that 4.688 ppm peak had the possibility of being three different substances. Betulin, quinidine, or 1-(4-acetoxy-3,5-dimethoxyphenyl)-1,3-diacetoxy-2-[4-(1-acetoxyethyl)phenoxy] propane. Betulin is a naturally occurring triterpene (Boparai, *et al*., 2017). It has a role as a metabolite, an antiviral agent, an analgesic, an anti-inflammatory agent and an antineoplastic agent (Hordyjewska, 2019). The activity of betulin acid has been linked to the induction of the intrinsic pathway of apoptosis and it is being explored for its anti-tumor properties (Fulda, 2008; Zdzisińska *et al*., 2003). The final candidate for this peak is 1-(4-acetoxy-3,5-dimethoxyphenyl)-1,3-diacetoxy-2-[4-(1-acetoxyethyl)phenoxy] propane. The BMRB states that this compound is a lignin. As a lignin, its main functions in the cell are to provide structure and adhesiveness in the wall (Ralph *et al*., 2004). Out of these three options, the best option for our sample is betulin.

Peak two of this sample is 3.7195 ppm. Possible matches for his sample include 2'-fucosyllactose, papaverine hydrochloride, or 6'-sialyllactose sodium salt. 2′-Fucosyllactose (2-FL) is the most abundant fucosylated oligosaccharide in human milk. It has pharmaceutical properties. Human milk oligosaccharides are important components of human milk that prevent infant health (Yu *et al*., 2018). Papaverine is an natural opiate alkaloid isolated from the plant and it is a vasodilator that relaxes smooth muscles in blood vessels to help them dilate. This effect lowers blood pressure and allows blood to flow more easily through your veins and arteries (National Center for Biotechnology Information, 2021; Prabhakara, *et al*., 2010). Lastly, 6'-sialyllactose sodium salt, also known as Neu5Ac-a-2-6-Gal-b1-4-Glc;6'-SL;6'-N-Acetylneuraminyl-D-lactose, is one of the most abundant sialylated (acidic) oligosaccharides found in milk observed in humans and other mammals. It has many immunoprotective properties and aims to protect newborns against pathogens. Based on the data collected, this peak is papaverine hydrochloride.

Another candidate for this peak is dihydroisorescinnamine. It is a naturally occurring Reserpine type alkaloid. A Reserpine is a compound of the alkaloid class used for treating hypertension. The reserpine type alkaloids are common secondary metabolites in gourd species. It is believed that alkaloid toxicity is what helps protect the diseases. (Kumar *et al*., 2012). The final possibility for this peak is lignin_cw_compound_3027. As stated in the above samples,

lignin is a prevalent metabolite thoughout the plant kingdom. However, based on the information the peak is for dihydroisorescinnamine.

3.5. ^{1}H-NMR Spectra of Bottle Gourd Sample 2 & 3

Proton NMR spectra of bottle gourd sample 2 & 3 are shown in Figures 11, and 12. Many peaks that ranged from chemical shift values 1.5 to 5.5 ppm were observed and focused on peaks of 4.695, 3.7213, and 3.5789 ppm because they have the highest intensities in sample two (Table 4). Almost similar metabolites were obtained from the samples 2 & 3.

Table 4. Summary of peaks and possible matches based on the BMRB databank for sample one of seeds from *L. siceraria*.

Chemical shift (ppm)	BMRB Databank Results	Predicted results
4.688	10. Betulin 11. Quinidine 12. 1-(4-acetoxy-3,5-dimethoxyphenyl)-1,3-diacetoxy-2-[4-(1-acetoxyethyl)phenoxy] propane	Betulin
3.7195	11. 2'-Fucosyllactose 12. Papaverine hydrochloride 13. 6'-Sialyllactose sodium salt	Papaverine hydrochloride
3.5768	1. Dihydroisorescinnamine 2. Lignin_cw_compound_3027 3. 3'-Sialyllactose Sodium Salt	Dihydroisorescinnamine

4. CONCLUSION

The benefits of understanding plant metabolomics and the inner workings of plants are vital for many industries. The metabolites that are identified often contribute to other areas of science including agriculture and nutrition, health and medicine, as well as production of biofuels and utilizing alternative energy sources. NMR spectroscopy may be used to assist the development of these areas by discovering new ways to identify and analyze plant metabolites. Our experimental results showed exciting results. In addition, the results are also identified areas of strengths and weaknesses in our study and plan on eliminating as many errors and limitations as possible. The future of plant metabolomics is the ability to identify and quantify the major metabolites of plants effectively and efficiently. The use of NMR spectroscopy is vital in this quest to further explore the world of plant metabolomics.

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Declaration of Conflicting Interests and Ethics

The authors declare that in this review there is no conflict of interest. This present study complies with publishing ethics. Each author is responsible for scientific and legal responsibility for manuscripts published in IJSM.

Authorship Contribution Statement

Megan Huerta: Data curation; Data analysis; **Jyoti Tamang**: Edit manuscript. **Mohammad Amzad Hossain**: Literature survey; Reviewing and Editing. **Gen Kaneko**: Sample collection and data curation **Hashimul Ehsan:** Supervision, Planning, draft writing, interpretation.

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