

Analysis and profiling of naturally occurring and processed *Aloe vera* using NMR spectroscopy

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Abstract: Nuclear magnetic resonance (NMR) spectroscopy uses magnetic fields and radio waves to excite the nuclei within a sample; in turn, this causes the nuclei to re-emit electromagnetic energy that is computed on a spectrum that documents the intensity and absorbance. Overall, this analyzes the structure and dynamics of molecules. *Aloe vera* is renowned for its antioxidant properties and therapeutic topical or oral applications, used to treat sunburn, soothe minor skin burns, acne, and even help with indigestion. This experiment aims to quantify the metabolites present in the *A. vera* plant and in commercially processed *A. vera* gel, and to compare them in order to identify their similarities and differences. In doing so, the study seeks to determine the extent to which products derived from cultivated plants retain or lose specific substances and organic compounds during processing and refinement. Both samples were evaluated through metabolite extraction and NMR. The Biological Magnetic Resonance Data Bank (BMRB) was the database that each peak chosen from the NMR analysis was run through. Through comparison and research of the makeup of both the gel and *A. vera* plant, several options were obtained to determine what molecules were present in each sample. In total, the gel produced 81 options for specific metabolites, and the plant had a total of 50. Similarities between the two samples that were analyzed were D-Fructose-6-Phosphate, 2'-Deoxyguanosine 5'-monophosphate, Mannose compounds, and N-Acetyl-D-glucosamine diastereomers. The major difference between the two samples was the large query pool, as stated previously, and the appearance of preservatives in the gel sample.

INTRODUCTION

Aloe vera (L.) Burm.f. plant, scientifically named *A. vera*, belongs to the family Xanthorrhoeaceae and subfamily Magnoliaceae. Approximately 420 different species of *A. vera* around the world have been discovered and documented, but *A. vera* has been extensively experimented with and used for its variety of beneficial properties (Figure 1a). It is a ceaseless succulent green herb that is represented by thick fleshy leaves. This plant blooms with bright yellow tubular flowers. It is native to hot and dry regions of North Africa, the Southern

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Mediterranean, the Canary Islands, and parts of the Middle East and Asia, demonstrating its striking adaptability to harsh and inhospitable environments (Sánchez *et al.*, 2020).



Figure 1. *A. vera* plant sample extracted from a plant situated at a student's home (A). Generic photo of processed *A. vera* gel (B).

A. vera gel, the colorless glutinous substance extracted from the inner part of its parenchymatous leaf, holds promising, extensive therapeutic properties for which it has earned a reputation over the years in the field of medicine (Figure 1b). It is a rich source of bioactive compounds that include vitamins, amino acids, enzymes, and minerals, which greatly repair and enhance the body's physiological functions, overall promoting the well-being of the individual (Ahluwalia *et al.*, 2022). *A. vera* consists of more than 75 different vitally important compounds that include sugars (monosaccharides such as mannose-6-phosphate and polysaccharides such as glucomannans), vitamins (vitamin A, C, E, and B12), enzymes (peroxidase and amylase), and hormones (auxins and gibberellins) (Maan *et al.*, 2018).

The application of *A. vera* has been traditionally documented in treating dermatological issues, including sunburn, minor epidermal burns, acne, wound healing, and anti-inflammatory processes. Additionally, *A. vera* has exhibited great potential as therapeutic agents, such as anti-cancer, antidiabetic, antioxidant, and antihyperlipidemic. Studies have revealed certain compounds present in *A. vera*, like aloin and aloe-emodin, that have demonstrated remarkable results in halting cancer and tumor growth as well as its progression (Majumder *et al.*, 2019). Certain bioactive compounds, including vitamins, flavonoids, and phenolic compounds, have been discovered in *A. vera*. These are major elements to neutralize free radicals, lowering the levels of oxidative stress, leading to the prevention of cellular damage (Hęś *et al.*, 2019). Several studies have contributed to the mounting evidence of how *A. vera* gel constitutes pharmacophores that greatly promote insulin sensitivity while improving the blood glucose levels, producing a normal HbA1c test in diabetes mellitus (Sharma *et al.*, 2021). *A. vera* has been subjected to investigation to test its potential to manage and reduce hyperlipidemia by promoting the reduction of cholesterol and triglycerides, moving forward towards good cardiovascular health (Alamholo, 2019).

Such high interest correlated with the mass production and processing of *A. vera* that quickly raised serious concerns about preserving its key chemical composition, most importantly about its therapeutic properties. Research has revealed how the bioactive molecules in the gel are affected by the manufacturing process, which uses chemicals and certain techniques. One study reported that the compound polysaccharide acemannan, which has a variety of biomedical applications like tissue regeneration, wound healing, and antiviral properties, has a greater chance of degradation, leading to loss of its therapeutic efficacy when subjected to chemical processing to increase its shelf life. Additionally, there stands the possibility of the introduction of certain contaminants into the gel composition, creating a harmful product. Further studies have reported the detection of carcinogenic compounds called anthraquinones in the gel composition after it has been processed. Hence, it is utmost important to emphasize the evaluation of the processing methods of the *A. vera* gel production, to be able to identify which key chemical components are maintained through the process of manufacturing, what type of

substances have been added in the production, and which form of the *A. vera* is suitable for therapeutic utilization (Ramachandra & Rao, 2008).

Several conducted experiments have emphasized the importance of raw *A. vera* in therapeutic applications; however, research into the effects of mass manufacturing of *A. vera* is limited, as the breakdown of polysaccharides reduces the medical functionality. Other studies argue that processing allows for the stabilization of the bioactive compounds (Sánchez *et al.*, 2020).

This study quantifies the possible metabolites in the *A. vera* plant and commercially processed *A. vera* gel and compares results to identify similar compounds and different substances to understand how products that are derived from cultivated plants either maintain or separate substances and organic compounds in their refinement. This research aims to test the hypothesis that specific compounds that are potent and beneficial in the *A. vera* degrade and lose their original therapeutic value. Through the analysis of differences between the two subjects, this paper aims to offer insight into how mass manufacturing affects both effectiveness and quality of *A. vera* products.

METHOD

Analytical Instruments

The analytical instruments utilized in the procedure were an NMR spectroscopy (Bruker Ascend Aeon 400 MHz USA NMR Model), a vortex (Thermo Scientific Sorvall Legend XT, Labnet VX-200 S0200 Low Profile Lab Vortex Mixer 115V), a centrifugal concentrator (Eppendorf 5305 Vacufuge Plus Concentrator Centrifuge), and a digital analytical scale (Mettler Balance ME103E).

Chemicals and Glassware

Solvents like chloroform, methanol, and deionized water were used in the current experiment, which were purchased from E. Merck, Germany. Deionized water and sodium phosphate buffer were obtained from BDH, UK. Also, all glassware was obtained from Boroshil, India.

Consumables

The consumable equipment utilized in the experiment was 4 50 mL centrifuge tubes, 2 NMR tubes, and pipette tips for a P1000 pipette. The items were sterilized before use.

Reusable Equipment

The reusable equipment used in the procedure was a blade and a P1000 pipette. Both objects were sanitized before use.

Analytical Database

The analytical database utilized to interpret the peak levels from the NMR spectra is the Biological Magnetic Resonance Data Bank (Hoch *et al.*, 2023).

Sample Collection

The *A. vera* plant sample was collected locally on September 18th, 2023. The commercially processed *A. vera* gel was bought on September 16th, 2023. Both samples were stored at -20 °C to protect from metabolic change.

Sample Preparation and Extraction

Before beginning, samples were kept at -18 °C for 24 hours. For the *A. vera*, the sample was cut using a sterile blade; roughly half an inch of the sample was placed in a sterile bag and was defrosted, then crushed till there was little to no plant tissue left. This sample includes the green rind and inner gel. 200mg of the sample was weighed and placed into a 50 mL centrifuge tube. For the second sample, the gel was defrosted by setting the bottle in a room-temperature water bath. Once defrosted, 200 mg of the gel was weighed on a scale. The sample was transferred into a 50 mL centrifuge tube as well. To both samples, 1,600 µL of cold methanol and 400 µL of cold water were added to each. Each sample was then vortexed for 10 seconds. Another

1,600 μL of cold methanol and 400 μL of cold water were added to each 50 mL centrifuge tube. Each sample is then vortexed for 1 minute. The samples are then set in an ice bath for 10 minutes. Once the ice bath is completed, the samples are centrifuged at 5,000 rpm for 5 minutes. After completion, there will be two visible layers in each sample; transfer the top layer utilizing a P1000 pipette, carefully, to a separate tube. The top layer contains water and methanol that hold the dissolved secondary metabolites. Now, having two new 50 mL centrifuge tubes containing the top layer, the samples are loaded into a centrifugal concentrator to dry for 3 hours. Lastly, both samples were dissolved utilizing 500 μL of 0.1 M sodium phosphate buffer in 90% H_2O and 10% of D_2O .

To examine the sample using nuclear magnetic resonance spectroscopy, 600 μL of each sample is loaded into individual NMR tubes. The samples are individually loaded into a nuclear magnetic resonance spectrometer that is properly cleaned and sealed. To collect the raw NMR data, the solvent is selected, the Bruker parameters are set, and the preprogrammed software is utilized. The samples are locked using the Bruker TopSpin program. To ensure minimized frequency impedance, enhanced imaging, and spectra data, the magnetic field strength was increased as needed. Once the scan is completed, the NMR receiver is adjusted to have the highest possible signal strength.

RESULTS

H-NMR Spectra Comparison of *A. vera* Plant and Gel Samples

From the *A. vera* plant sample results, there are thirteen noticeable peaks that appear within the spectral domain of 13,500–19,500.

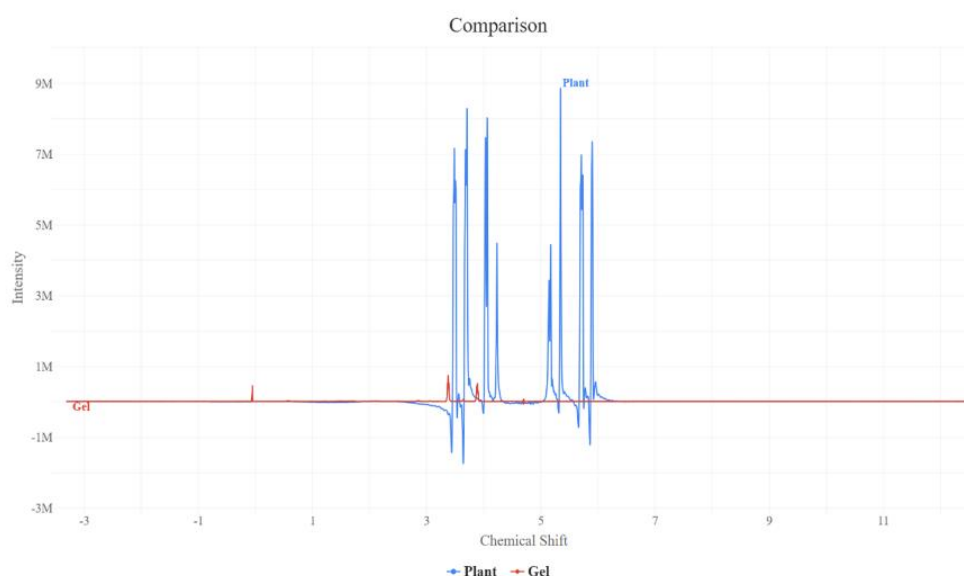


Figure 2. Overlaid H-NMR spectra of *A. vera* plant (blue) and processed *A. vera* gel (red).

H-NMR Spectra of *A. vera* Plant Sample

Within this specific spectral domain, three peaks were selected and evaluated to determine metabolites that had high and noticeable intensities. The peak at δ 5.897975 (ppm) (Peak 1 on Figure 3) when entered in the Biological Magnetic Resonance Data Bank (BMRB) resulted in four possible metabolites: GDP, Cytidine, Selenomethionine, and L(+)-Selenomethionine. The peak at δ 4.228979 (ppm) (Peak 2 on Figure 3) when entered in BMRB resulted in ten possible metabolites: GDP, 2'-Deoxyguanosine 5'-monophosphate, 3-deazauridine, 2'-Fucosyllactose, D-Fructose-6-phosphate, Uridine, 2'-Deoxyguanosine 5'-triphosphate, 5-methyluridine, Cytidine-5'-monophosphate, and Propargyl alcohol.

The peak at δ 4.059831 (ppm) (Peak 3 on Figure 3) when entered in BMRB resulted in nineteen possible metabolites: D-Ribose 5-phosphate, O-Acetyl L-serine, 3-acetamido-5-(acetyl-

methylamino)-2,4,6-triiodo-N-[2,4,5-trihydroxy-6-(hydroxymethyl)oxan-3yl]benzamide, Tropic acid, D-Saccharate, 2-alpha-Mannobiose, O-Phospho-L-serine, S-(5'-Adenosyl)-L-methionine, apocholic acid, 2-methyl-3,4,5,6-tetrahydropyrimidine-4-carboxylic acid, Amygdalin, NADPH, FMN, FAD, Choline, D-Ribulose 5-phosphate, 2'-Deoxycytidine, 1,4-D-xylobiose, and Cytidine-5'-monophosphate. The candidate metabolites identified for the *A. vera* plant sample based on BMRB matching are summarized in Table 1.

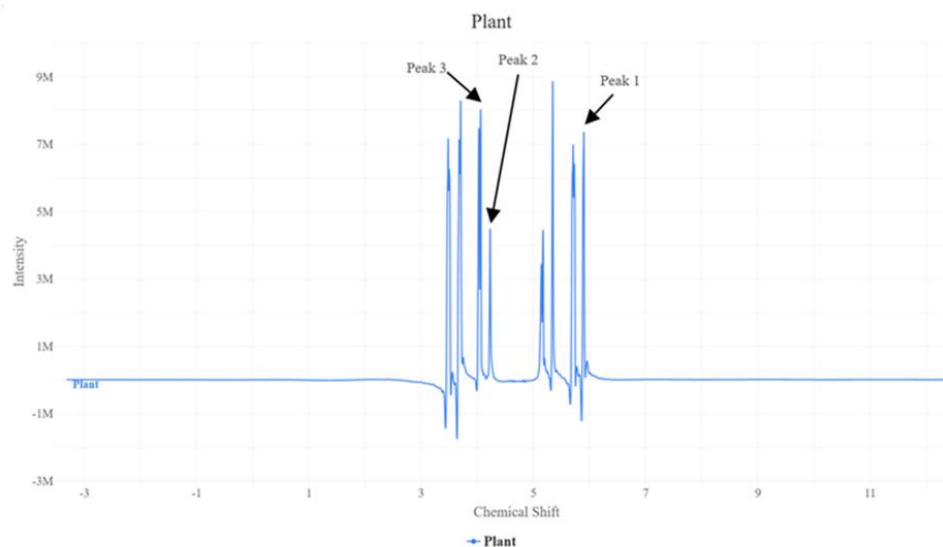


Figure 3. *H*-NMR spectrum of the *A. vera* plant sample showing three selected peaks in the 13,500–19,500 domain.

Table 1. Summary of the results from the *A. vera* plant sample determined by the Biological Magnetic Resonance Data Bank.

Peak Number	Metabolite	Peak Number	Metabolite
Peak 1	GDP	Peak 3	O-Phospho-L-serine
Peak 1	Cytidine	Peak 3	S-(5'-Adenosyl)-L-methionine
Peak 1	Selenomethionine	Peak 3	apocholic acid
Peak 1	L(+)-Selenomethionine	Peak 3	2-methyl-3,4,5,6-tetrahydropyrimidine-4-carboxylic acid
Peak 2	GDP	Peak 3	Amygdalin
Peak 2	2'-Deoxyguanosine 5'-monophosphate	Peak 3	NADPH
Peak 2	5-methyluridine	Peak 3	FMN
Peak 2	Cytidine-5'-monophosphate	Peak 3	FAD
Peak 2	Propargyl alcohol	Peak 3	Choline
Peak 3	D-Ribose 5-phosphate	Peak 3	D-Ribulose 5-phosphate
Peak 3	O-Acetyl L-serine	Peak 3	2'-Deoxycytidine
Peak 3	3-acetamido-5-(acetyl-methylamino)-2,4,6-triiodo-N-[2,4,5-trihydroxy-6-(hydroxymethyl)oxan-3yl]benzamide	Peak 3	N/A
Peak 3	D-Saccharate	Peak 3	1,4-D-xylobiose
Peak 3	2-alpha-Mannobiose	Peak 3	Cytidine-5'-monophosphate

H-NMR Spectra of *A. vera* Gel Sample

From the *A. vera* gel sample results, there are nine noticeable peaks that appear within the spectral domain of 16,000–19,500.

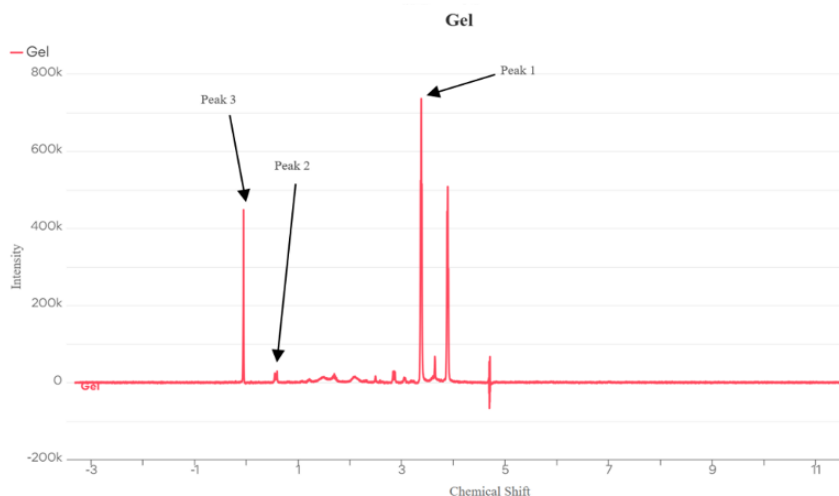


Figure 4. H-NMR spectrum of the processed *A. vera* gel sample showing three selected peaks in the 16,000–19,500 domain.

The peak at δ 3.90046 (ppm) (Peak 1 on Figure 4) when entered in BMRB resulted in sixteen possible metabolites: 3-Fucosyllactose, 3-Iodo_L-tyrosine, Adenosine-3'-monophosphate, Betaine, D-Fructose-1,6-bisphosphate, D-Galacturonic acid, D-Xylulose, FAD, Kanamycin, Lacto-N-tetraose, Methyl N-acetyl-alpha-D-glucosaminide, N-Acetylneuraminic acid, Thiamin, Uridine, Alpha-D-Galactose 1-phosphate, and Beta-gentiobiose.

The peak at δ 3.638427 (ppm) (Peak 2 on Figure 4) when entered in BMRB resulted in twenty possible metabolites: D-(+)-Galactose, D-Trehalose, D-pinitol, Meso-Erythritol, Xylitol, DL-alpha-Glycerol phosphate, Diethyl oxalacetate, N-Acetyl-D-glycosamine 6-phosphate, D-Psicose, D-Panthenol, D-(+)-Mannose, Ethanol, LNnT, Argininosuccinic acid, Acetonitrile, D-Fructose-6-phosphate, 3'-Sialyllactose Sodium Salt, Lacto-N-tetraose, Lacto-N-fucopentaose, L-Histidinol, and 6'-Sialyllactose sodium salt.

The peak at δ 3.374927 (ppm) (Peak 3 on Figure 4) when entered in BMRB resulted in seven metabolites: D-(+)-Mannose, Methyl N-acetyl-alpha-D-glucosaminide, Beta-gentiobiose, 2-ethylpiperidine, 2-phenylbutyric acid, Alpha-D-Glucose-1-phosphate, and Kanamycin. The candidate metabolites identified for the processed *A. vera* gel sample are summarized in Table 2.

Table 2. Summary of the results from the *A. vera* gel sample determined by the Biological Magnetic Resonance Data Bank.

Peak Number	Metabolite	Peak Number	Metabolite
Peak 1	3-Fucosyllactose	Peak 2	N-Acetyl-D-glycosamine 6-phosphate
Peak 1	3-Iodo_L-tyrosine	Peak 2	D-Psicose
Peak 1	Adenosine-3'-monophosphate	Peak 2	D-Panthenol
Peak 1	Betaine	Peak 2	D-(+)-Mannose
Peak 1	D-Fructose-1,6-bisphosphate	Peak 2	Ethanol
Peak 1	D-Galacturonic acid	Peak 2	LNnT
Peak 1	D-Xylulose	Peak 2	Argininosuccinic acid
Peak 1	FAD	Peak 2	Acetonitrile
Peak 1	Kanamycin	Peak 2	D-Fructose-6-phosphate

Peak 1	Lacto-N-tetraose	Peak 2	3'-Sialyllactose Sodium Salt
Peak 1	Methyl N-acetyl-alpha-D-glucosaminide	Peak 2	Lacto-N-tetraose
Peak 1	N-Acetylneuraminic acid	Peak 2	Lacto-N-fucopentaose
Peak 1	Thiamin	Peak 2	L-Histidinol
Peak 1	Uridine	Peak 2	6'-Sialyllactose sodium salt
Peak 1	Alpha-D-Galactose 1-phosphate	Peak 3	D-(+)-Mannose
Peak 1	Beta-gentiobiose	Peak 3	Methyl N-acetyl-alpha-D-glucosaminide
Peak 2	D-(+)-Galactose	Peak 3	Beta-gentiobiose
Peak 2	D-Trehalose	Peak 3	2-ethylpiperidine
Peak 2	D-pinitol	Peak 3	2-phenylbutyric acid
Peak 2	Meso-Erythritol	Peak 3	L-Proline
Peak 2	Xylitol	Peak 3	Alpha-D-Glucose-1-phosphate
Peak 2	DL-alpha-Glycerol phosphate	Peak 3	Kanamycin
Peak 2	Diethyl oxalacetate	Peak 3	L-Histidinol

DISCUSSION and CONCLUSION

GDP, commonly known as guanosine diphosphate, is a purine ribonucleotide that is found in all living organisms; however, GDP would not be a candidate for this specific peak due to the intensity level (Wishart *et al.*, 2022). While GDP would be found in the *A. vera* plant sample, it would have a lower intensity level than this chemical shift does. This was the only metabolite that could be narrowed out.

The three metabolites that are still within the possibility pool are Cytidine, selenomethionine, and L(+)-Selenomethionine. In a recent study that was observing metabolites from *A. vera* and DNA structures to inhibit cell proliferation in tumor cells, cytidine was identified via Total Correlation Spectroscopy (TOCSY) in the *A. vera* sample (Dallavalle *et al.*, 2022). For both the selenomethionine and L (+)-selenomethionine, a research paper published this year biosynthesized selenium nanoparticles to increase disease resistance in lettuce; the selenium nanoparticles were biosynthesized from *A. vera* gel (Shang *et al.*, 2023).

As discussed for Peak 1, GDP would be found in the *A. vera* plant sample; it could not be this specific chemical shift due to the high intensity (Ramirez-Gaona *et al.*, 2017). 2'-Deoxyguanosine 5'-monophosphate is a purine nucleotide that has a monophosphate group attached to a ribose moiety and has a nucleobase of guanine; while it is found in all living organisms, the intensity of this chemical shift is too great for this specific metabolite to be identified.

2'-Fucosyllactose is an oligosaccharide that is mainly found in breast milk; this metabolite would not be found in the *A. vera* plant sample unless through contamination (Hegar *et al.*, 2019). Uridine consists of uracil and D-ribose, making it a nucleoside; while uridine is found in all organisms, it would not appear at this level of intensity in the *A. vera* plant sample. It would still be on the NMR spectrum, but not at this specific chemical shift with this height of intensity.

2'-Deoxyguanosine 5'-triphosphate is a nucleoside triphosphate with a base of guanine; much like the previous metabolites, 2'-Deoxyguanosine 5'-triphosphate is found in all living organisms, but with the high intensity of the chemical shift, the metabolites cannot be identified at this peak, but can still be located within the NMR spectrum for the *A. vera* plant sample.

5-methyluridine, more commonly known as Ribothymidine, is a nucleoside found in human fluids; this metabolite would not be found in the *A. vera* plant sample unless through contamination (Wishart *et al.*, 2022). Cytidine-5'-monophosphate, commonly abbreviated as

CMP, is a nucleotide that aids in synthesizing nucleotide derivatives; though this metabolite is found in all living organisms, much like previous metabolites, the intensity is too great for it to be this specific peak (Ramirez-Gaona *et al.*, 2017; Li *et al.*, 2020).

With the elimination of seven metabolites for the query, we are left with three metabolites that are possibly the identity of the chemical shift: 3-deazauridine, D-Fructose-6-phosphate, and Propargyl alcohol. 3-deazauridine does not have a lot of research published about the metabolite; however, with the papers that have been published,

3-deazauridine, an analog of uridine, has been determined to inhibit the growth of tumor cells by hindering nucleic acid synthesis (Brockman *et al.*, 1975; Moriconi *et al.*, 1986). From previous research into cytidine, 3-deazauridine, which has yet to be detected or quantified in *A. vera*, could be the metabolite for this chemical shift.

D-Fructose-6-phosphate, a derivative of fructose, has been detected and quantified in a study that used GC-MS analysis to examine multiple *A. vera* grown under various levels of hydration; this metabolite was visible in every plant but at different abundance levels (Salinas *et al.*, 2016).

Propargyl alcohol, C₃H₄O, is a moderately volatile alcohol; while this is not located in the plant *A. vera* sample, it is possible that this organic compound could have been produced by a reaction formed by the chloroform reacting with the sodium phosphate buffer.

3-acetamido-5-(acetyl-methylamino)-2,4,6-triiodo-N-[2,4,5-trihydroxy-6-(hydroxymethyl)oxan-3yl] benzamide, commonly known as metrizamide, is a solute only found within a person who has taken it as a medication; this metabolite would not be found in the *A. vera* plant sample unless through contamination (Wishart *et al.*, 2022).

O-Phospho-L-serine, commonly recognized as DL-O-Phosphoserine, is known as an α -amino acid and is found in all living organisms; O-Phospho-L-serine would be found in the *A. vera* plant sample; however, it could not be for this specific peak due to the intensity of the chemical shift.

Apocholeic acid is a bile acid formed through mild hydration of cholic acid; apocholeic acid is primarily found in vertebrates. With this intensity, the metabolite being the identity of this peak would be through contamination.

2-methyl-3,4,5,6-tetrahydropyrimidine-4-carboxylic acid, commonly known as ectoine, is a primary metabolite that is found mainly in bacteria. While ectoine is not found in *A. vera*, it is often paired with *A. vera* in cosmetics due to the ability to protect biological membranes from environmental conditions (Bilstein *et al.*, 2021).

Amygdalin is a glycoside that is found in almonds and the kernels and pits of fruits (Wishart *et al.*, 2022). NADPH, nicotinamide adenine dinucleotide phosphate, is a coenzyme that is the reduced form of NADP⁺; while this metabolite is found in all living organisms, the intensity level of this chemical shift would not accurately represent NADPH. FMN, commonly known as flavin mononucleotide, is a biomolecule that is produced by vitamin B₂; vitamin B₂ has been identified in *A. vera* amongst other vitamins; however, the form of FMN would not be at this high of an intensity (Hamman, 2008). FAD, more recognized as flavin adenine dinucleotide, is a drug used to treat illnesses caused by vitamin B₂ deficiency; this would not be found in the *A. vera* plant sample unless through contamination (Wishart *et al.*, 2022).

D-Ribulose 5-phosphate, a carbohydrate derivative, is found in all living organisms and has been detected in a multitude of foods, but it would not have the level of intensity that the identity of this peak has. 2'-Deoxycytidine, primarily known as deoxycytidine, is a component of DNA and exists in all living organisms; like many of the metabolites that are a part of nucleic acids, this metabolite does not have the level of intensity that would be needed to be the identity of the peak (Wishart *et al.*, 2022). After narrowing down eleven metabolites, the remaining eight are what are left as possible identities of Peak 3: D-Ribose 5-phosphate, O-Acetyl-L-serine, tropic acid, D-Saccharate, 2- α -Mannobiose, S-(5'-Adenosyl)-L-methionine, choline, and 1,4-D-xylobiose. D-Ribose 5-phosphate, a metabolite found in all living organisms, is a

carbohydrate derivative that has recently been researched for it being a biocatalyst for the production of D-allose and other rare sugars; D-allose is a medicinal bioactive compound that was identified via GC-MS analysis in a paper that examined bioactive principles found in *A. fleurentiniorum* (Chen *et al.*, 2020; Moni *et al.*, 2021).

O-Acetyl-L-serine is an α -amino acid used in the biosynthesis of cysteine in all living organisms; while O-Acetyl-L-serine has not been specifically detected, acetyl-groups of acemannan polymers have been detected within an unprocessed *A. vera* gel sample (Sonawane *et al.*, 2020). Tropic acid, commonly known as tropate, is determined to be both a β -hydroxy acid and/or a β -hydroxy acid derivative that is mostly found in plants; there has not been much research published on this metabolite, so there is not enough evidence to rule it out. D-Saccharate, more commonly known as Glucaric acid, is a sugar acid derivative of D-glucose that is primarily found in plants and mammals; while D-Saccharate has not been determined within an *A. vera* sample, there is no evidence to consider D-Saccharate as the identity of the Peak 3.

2- α -Mannobiose is a disaccharide that consists of a mannosyl and mannose; mannose-6-phosphate is one of the most notable monosaccharides within *A. vera* (Surjushe *et al.*, 2008). *A. vera* is a mannoprotein, meaning that it is made up of a high amount of mannose in the cell wall (Marangon *et al.*, 2018). Mannoproteins are known for their ability to heal topically, which *A. vera* is typically used for (Patil *et al.*, 2015). S-(5'-Adenosyl)-L-methionine, known as S-Adenosylmethionine, possesses anti-inflammatory activity that is found in all living organisms; s-(5'-Adenosyl)-L-methionine is a part of the biosynthesis of phospholipids, epinephrine, melatonin, creatine, proteins, both DNA and RNA nucleic acids, and other molecules. Choline is considered an essential vitamin and can be found in all living organisms, especially in liver, eggs, milk, and peanuts; choline is one of the vitamins that has been determined in *A. vera* (Hamman, 2008). 1,4-D-xylobiose, more commonly known as xylobiose, is a glycoside that has an O-glycosidic bond; there is not a lot of research written with this metabolite, so there is no evidence to rule it out as a possibility (Wishart *et al.*, 2022).

In summary, through comparison and research into what was in the makeup of both the gel and *A. vera* plant, several options were obtained for what molecules were present in each sample using NMR. In total, the gel produced 81 options for specific metabolites, and the plant had a total of 50 metabolites. Similarities between the two samples that were analyzed were D-Fructose-6-Phosphate, 2'-Deoxyguanosine 5'-monophosphate, Mannose compounds, and N-Acetyl-D-glucosamine diastereomers. The major difference between the two samples was the large query pool, as stated previously, and the appearance of preservatives in the gel sample. *A. vera* has endless uses for medical use for scrapes, cuts, and burns, as well as use in the cosmetic industry. Through understanding what metabolites are within *A. Vera* and processed *A. vera* gel, which offers a comprehensive understanding and insight into the many applications and how their chemical components are being utilized within diverse industries, and if the preservation of key attributes of *A. vera* is maintained in the refinement. Regarding future research, tests need to be rerun so the pool of specific metabolites in both the plant and the gel can be narrowed down to a smaller pool, and specific chemical compounds can be distinguished in both samples. By doing this, the results will be narrowed down enough to provide more detailed results on what chemical components make up the *A. vera* plant and the gel.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors. Ethics Committee approval: not required for this study. The study didn't involve human subjects.

Authorship Contribution Statement

E.M.: Methodology, Supervision, and Validation. **T.I.E.:** Data curation and data analysis. **H.S.:** Data curation and data analysis. **E.D.L.G.:** Data curation and data analysis. **D.O.:** Data curation and data analysis. **S.J.-I.:** Data curation, manuscript editing, and manuscript reviewing. **E.Y.:** Manuscript editing and manuscript reviewing. **G.K.:** Sample collection and data curation. **H.E.:** Supervision, planning, draft writing, and interpretation.

Data Availability Statement

The datasets generated during the current study are available from the corresponding author upon reasonable request.

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