

**COMPARISON OF CONSTITUENTS IN ALOE VERA GEL
COLLECTED IN DIFFERENT SEASONS BY CHROMATOGRAPHY
AND SPECTROSCOPY TECHNIQUES.**

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ABSTRACT:

FTIR spectroscopy, NMR and GC-MS mediated predominant functional group detection have been carried out for stabilized Aloe vera juice- "A" collected in monsoon season and stabilized Aloe vera juice – "B" Collected in winter season. The study has been carried out to explore the seasonal influence of aloe vera juice in selection. Maximum transmittance in IR spectrum has been assigned to phenolic OH stretching followed by -CO stretch. CH₃ resonances of acemannan acetylation at 2.1 ppm for Aloe vera juice "A" and 2.11 ppm "B" was observed in NMR spectra which confirms the presence of bioactive polysaccharide- acemannan required for pharmacological activity.

Components vary in both Juices in GC-MS spectra and possess certain activity. On analysis, it was observed that overall contents were more pronounced in the juice "B" than "A". The present work shows that seasonal conditions of Aloe vera plays a decisive role in collection of Aloe vera leaves for making juice.

KEYWORDS: Stabilized Aloe vera juice, Seasonal variation, GC-MS, FTIR, NMR.

INTRODUCTION

Aloe vera L.(syn.Aloebarbadensis Miller) is a plant from Family: Liliaceae. A. vera. gel is widely used in health drink supplements and skin care products. The major components of A.vera gel(AG) can be classified into five different groups namely phenolics, saccharides (mannose, glucomannan, acemannan,etc.),vitamins, enzymes and low molecular weight substances ² Mucilaginous Aloe vera Gel has a diverse range of pharmacological properties which includes anti-viral, anti-bacterial, laxative, protection against radiation, anti-oxidant,

anti-inflammation, anti-cancer, anti-diabetic, anti-allergic, immuno-stimulation, etc^{12,13}. A large part of the pharmacological properties are due to the presence of various polysaccharides, phenolic compounds etc. The diverse array of physical, biochemical and biological activities of Aloe vera gel is the result of synergism among the inherent component compounds of gel, rather than a single ingredient⁵. So, quantitative and qualitative variations in the structural components of Aloe vera gel may alter the physical and biochemical significance of Aloe vera. The structure–function relations of different properties of Aloe vera Gel with reference to various physical and biochemical parameters have been elucidated extensively by different authors^[2,3,7,12,13,14,15,16]

¹H-NMR study has been described as a potential method to analyse the constituents of Aloe vera stabilized juice from the polysaccharides.^[9]

Taking into consideration of the medicinal importance, Aloe vera stabilized juice samples were analysed for using GC-MS, FT-IR, and NMR. This work will help to identify the compounds of therapeutic value. A consolidated effort has been made to analyse the seasonal variations in constituents of Aloe vera stabilized juice by H-NMR, FTIR and GC-MS.

1.1 Objectives:

Phytochemical analysis of stabilized aloe vera juice collected in different seasons by H-NMR, FTIR and GC-MS.

1.2 EXPERIMENTAL

Materials:

1. The *aloe vera* leaves (*Aloe barbadensis*, Family: Liliaceae) were procured from Nisargamitra Aloe Vera Farm-Daheri, Umbergaon, Gujarat.
2. Analysis work done in DIYA LABS, Airoli, Navi Mumbai

MATERIALS AND METHODS

a. Extraction Procedure: Juice Collected after Roller Pressing

Plant material was collected and washed with distilled water. The leaf was cut into cubical pieces (chunks). The upper and lower skin of each leaf was then carefully removed with the help of knife and the mucilaginous mass made up of parenchymatous tissue (filets) was cut into small pieces. Care was taken to avoid contamination of yellow sap with the filet.

Filets were inspected visually for any contamination or adhering yellow sap or any outer green skin and discarded if necessary. The total mass of the uncontaminated fillet was

approximately about 40% of the leaf mass.

The uncontaminated filets were homogenized twice in a blender at a high speed for 5 minutes. The foam was developed which was settled after 10 minutes. The extracted gel was subjected to straining through muslin cloth to remove traces of cellular matter. The clear gel was then subjected to further processing; Preservative 0.1% sodium benzoate was added.

- b. Aloe vera stabilized juice for both A and B Samples analysis was done by H-NMR, FTIR and GC-MS. The work was carried out in DIYA LABS, Airoli, Navi Mumbai. Results were than interpreted.

1.3. RESULTS AND DISCUSSION

Functional group distribution by FTIR spectroscopy

Characterization of Stabilized gel with reference to the pertained functional group distribution was carried out by FTIR spectroscopy. The assignments were compared with pre-existing data for consequent interpretation. Different predominant functional groups namely O-H, C=O, were detected by analysing the FTIR spectrum. Strong and broad intensity of the band at 3400 cm^{-1} in Aloe vera stabilized juice “A” and “B” both assigned to OH stretching was observed because of sugar units. Absorption band at 1640 cm^{-1} confirms the presence of carboxy group in Aloe vera stabilized juice “A” and “B” because of the polysaccharides.

Table 1. FTIR Data of Aloe vera juice “A” and “B”

Sr. No	IR Values		Functional group detection	Probable constituents
	A	B		
1	3401.166 cm^{-1}	3400.166 cm^{-1}	OH stretching	Sugar units
2	1637 cm^{-1}	1637 cm^{-1}	C=O stretching	anthraquinones, saponins, and polysaccharides.

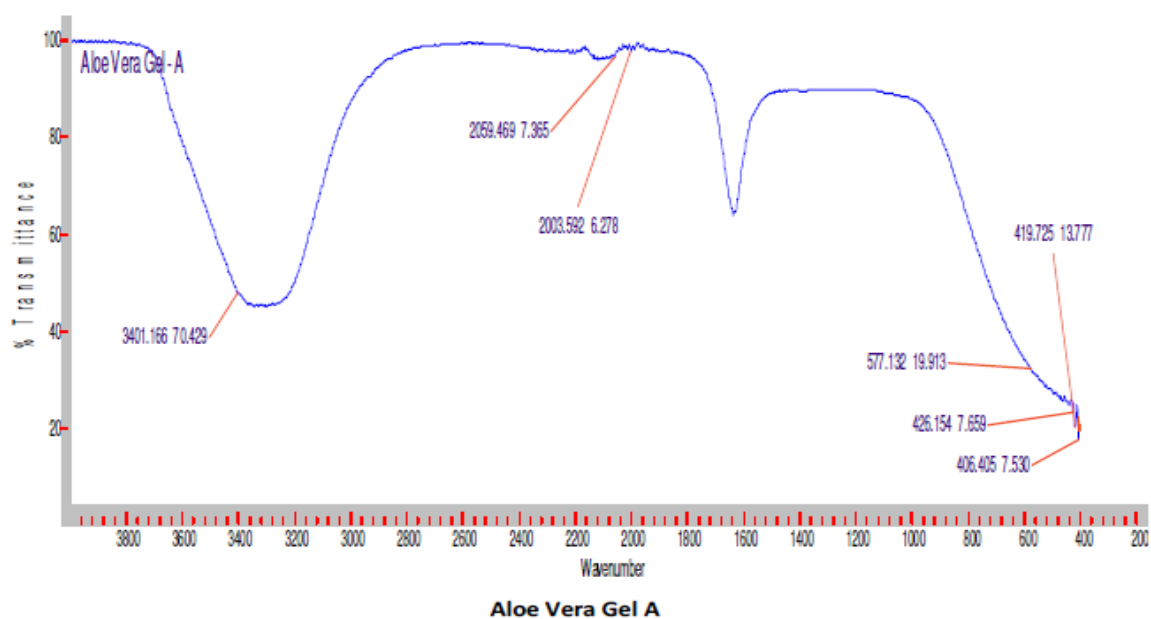


Fig 1: FTIR Spectrum of stabilized Aloe vera juice “A”

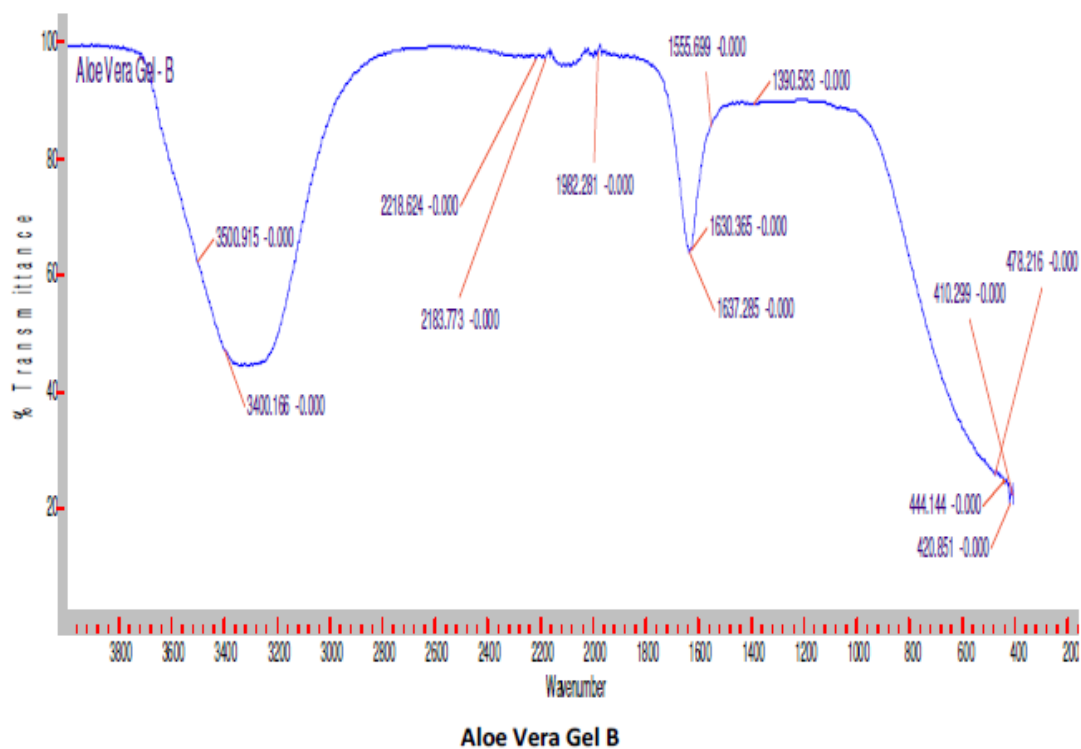


Fig 2: FTIR Spectrum of stabilized Aloe vera juice “B”

¹H NMR spectral analysis

Chemical shift values, peak are used for detection and quantification of aloë vera leaf juice constituents. In the ^1H -NMR spectrum the characteristic peaks located in the range assigned are confirmed.

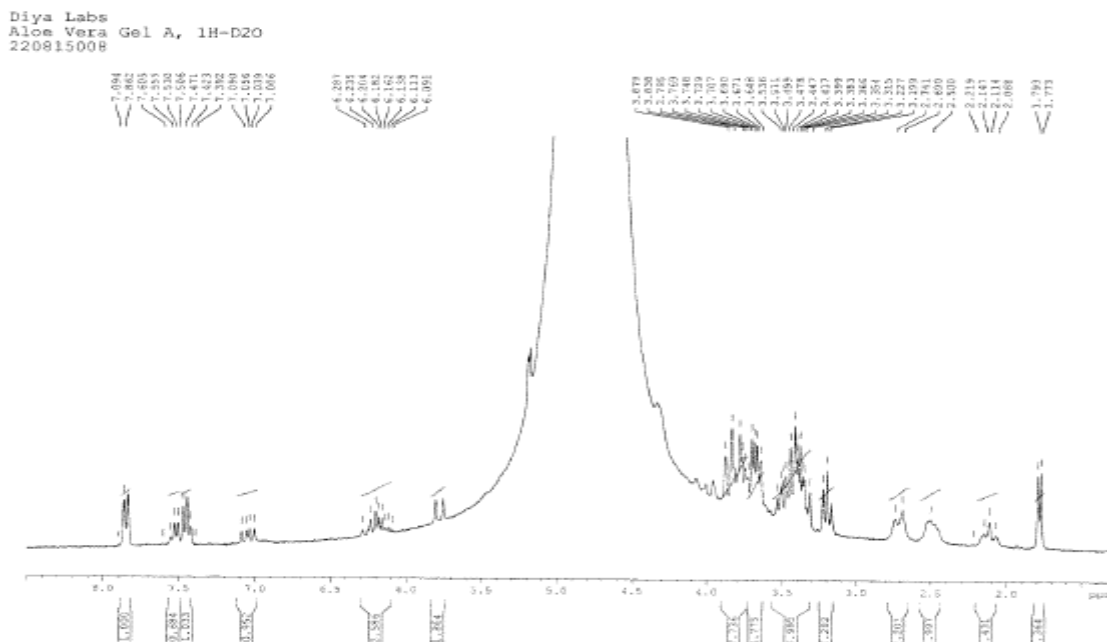


Fig :3 NMR Spectra of Aloe vera juice “A”

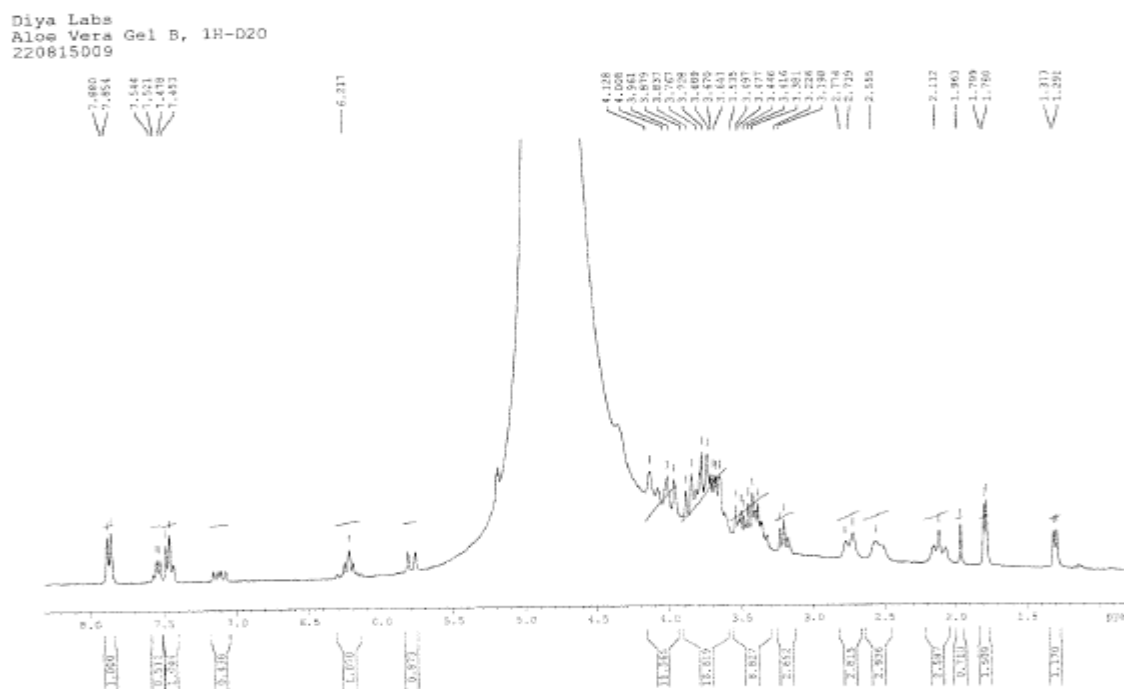


Fig :4 NMR Spectra of Aloe vera juice “B”

The characteristic peaks located in the range of 1.77–1.79 ppm assigned to the proton signals to CH₃ confirms the presence of Sorbate observed at 1.77 ppm (doublet) in Aloe vera juice “A” and 1.78 ppm (doublet) in Aloe vera juice “B”. Succinic Acid – 2 x CH₂ peak at 2.5 ppm (singlet) was observed in the juice “A” and 2.55 ppm in “B”. CH₃ resonances of acemannan acetylation at 2.1 ppm for Aloe vera juice “A” and 2.11 ppm “B” was observed. Benzoate – ortho-protons (2H) give peaks at 7.8 ppm was observed for both the juice “A” and “B”. The peak in the area 3.2-4.3 ppm represents polysaccharide and glucose sugar signals which was observed in both the juice “A” and “B”. The data is summarised in following table.

Table 2. NMR Data of Aloe vera juice “A” and “B”

Sr. No	Signal type	Chemical Shift values	Probable Components present in Aloe vera juice “A”	Chemical Shift values	Probable Components present in Aloe vera juice “B”
1	CH ₃ peak, doublet	1.77 ppm	Sorbate	1.78 ppm	Sorbate
2	CH ₂ peak, Singlet	2.5 ppm	Succinic Acid	2.55 ppm	Succinic Acid
3	CH ₃ resonances of acemannan acetylation	2.1 ppm	acemannan	2.11 ppm	acemannan
4	2 x CH, doublet	7.8 ppm	Benzoate	7.8 ppm	Benzoate

GC-MS Analysis: GC-MS is one of the best techniques to identify the volatile phytochemicals present in the Aloe vera stabilized juice. The chromatogram of the GC-MS analysis is given below,

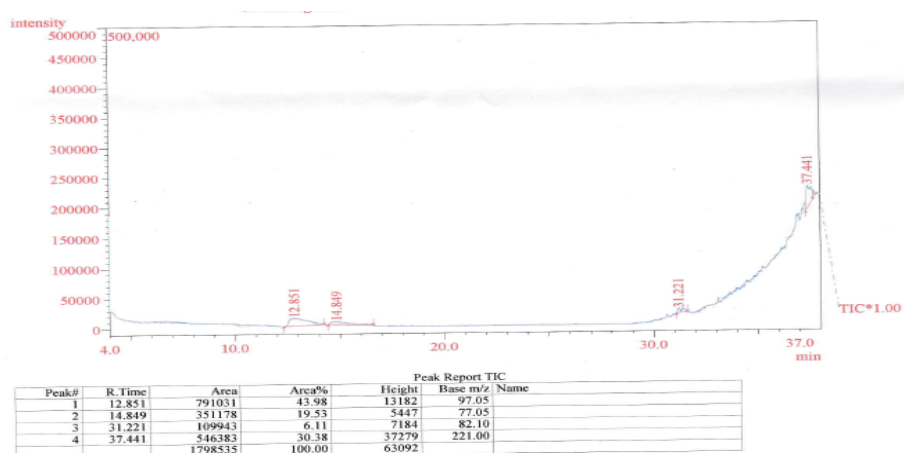


Fig 5. GC-MS Spectra of Aloe vera gel A

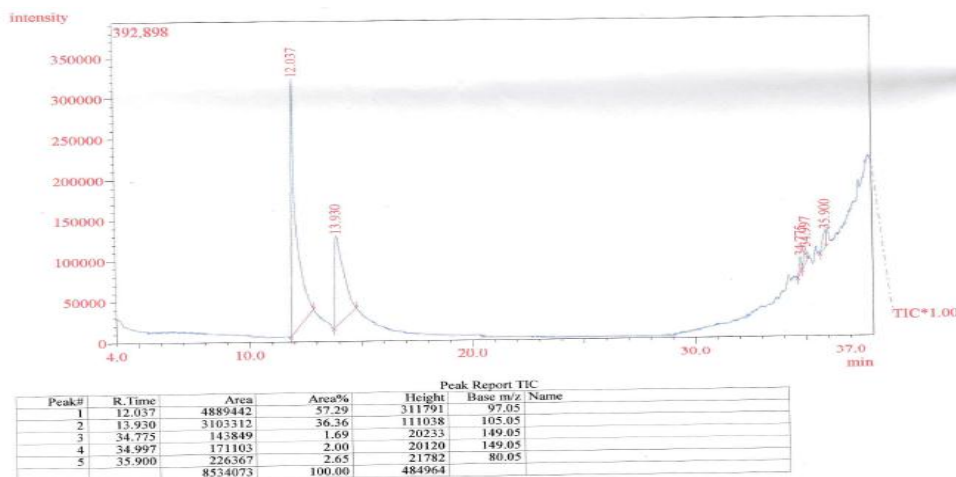


Fig 6. GC-MS Spectra of Aloe vera gel A

The GC-MS analysis of Aloe barbadensis leaf revealed the presence of compounds. The following are the volatile components of Aloe vera juice "A" Sorbic acid; 2,4-hexadienoic acid; Methanol, oxo, Benzoate; Acetylene dicarboxylic acid, di-(-)menthyl Hexasiloxane, tetradecamethyl-

Following spectrum are of Aloe vera juice "A".

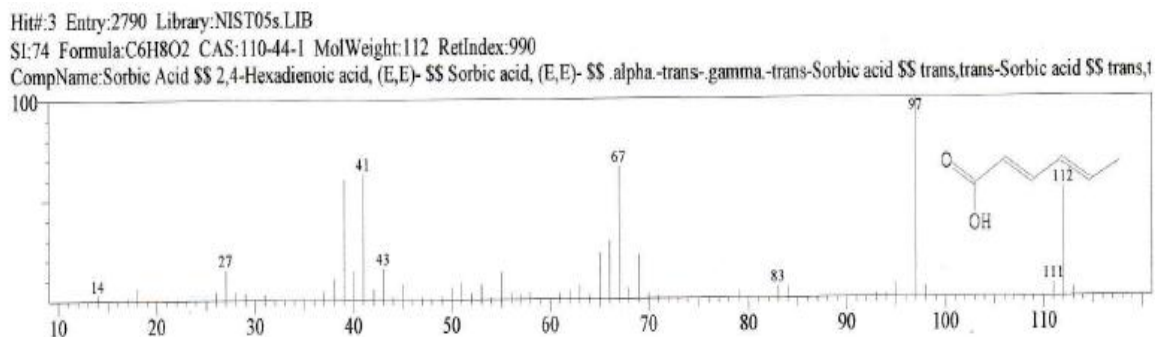


Fig 7. Sorbic acid; 2,4-hexadienoic acid

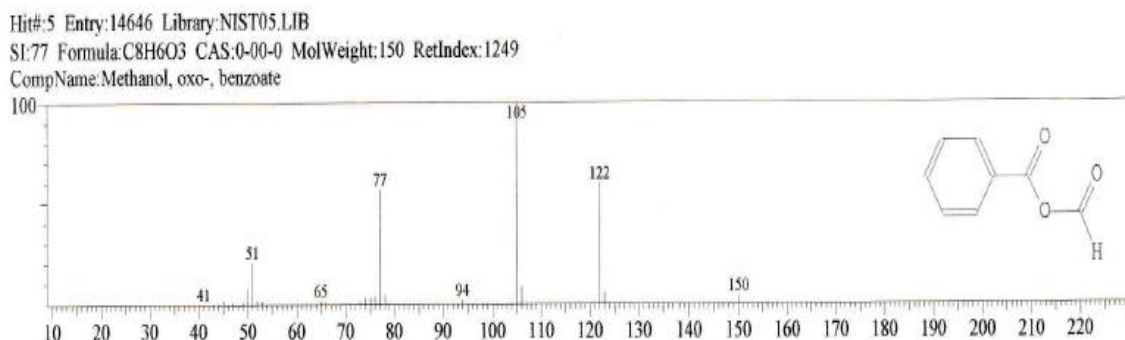


Fig 8. Methanol, oxo, Benzoate

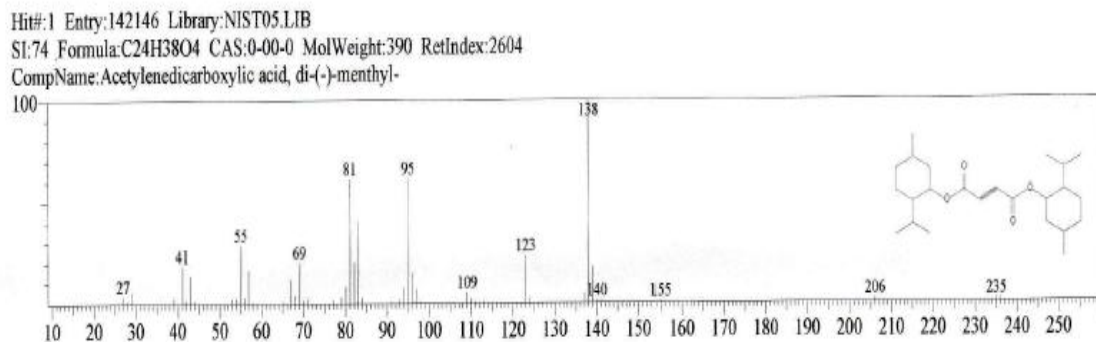


Fig 9. Acetylene dicarboxylic acid, di-(-)-menthyl-

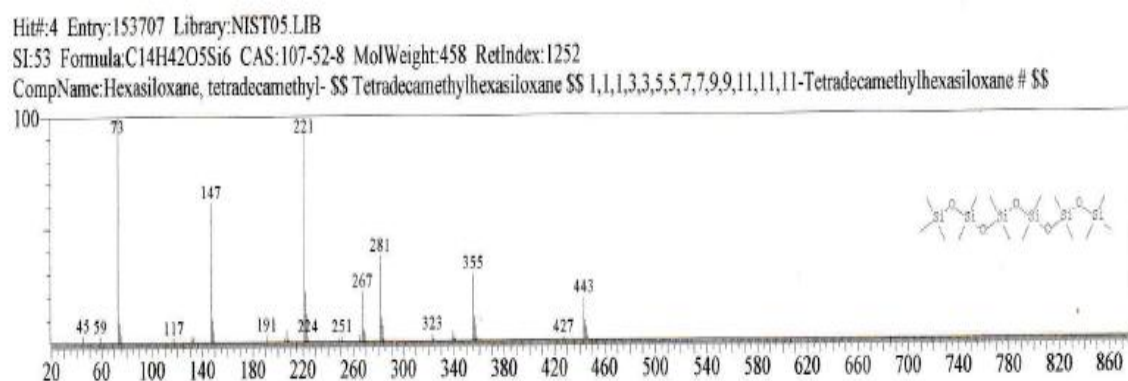


Fig 10. Hexasiloxane, tetradecamethyl-

The following are the volatile components of Aloe vera juice “B” Sorbic acid; 2,4-hexadienoic acid, Benzene carboxylic acid, 1,2-Benzenedicarboxylic acid, bis (1-methylethyl) ester, 1,2-Benzenedicarboxylic acid, 2 butoxy ethyl butyl ester, 2-Butenedioic acid (E)-, bis [5-methy-2-91-methy ethyl) cyclo hexyl] ester

Following spectrum are of Aloe vera juice “B”.

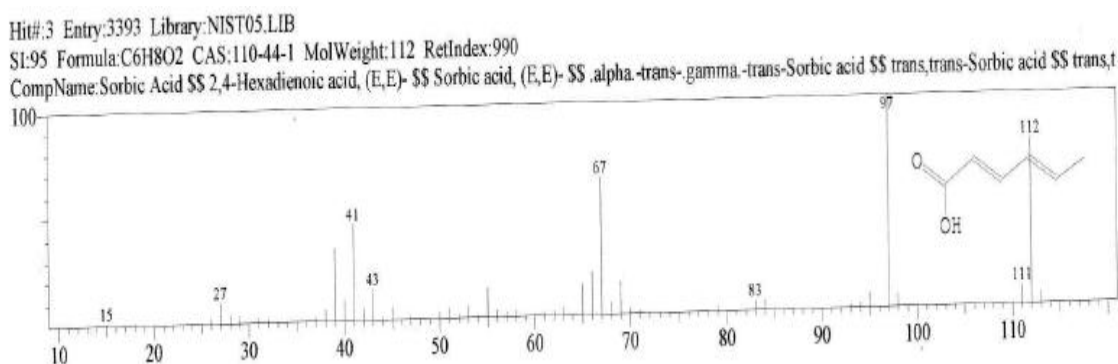


Fig 11. Sorbic acid; 2,4-hexadienoic acid

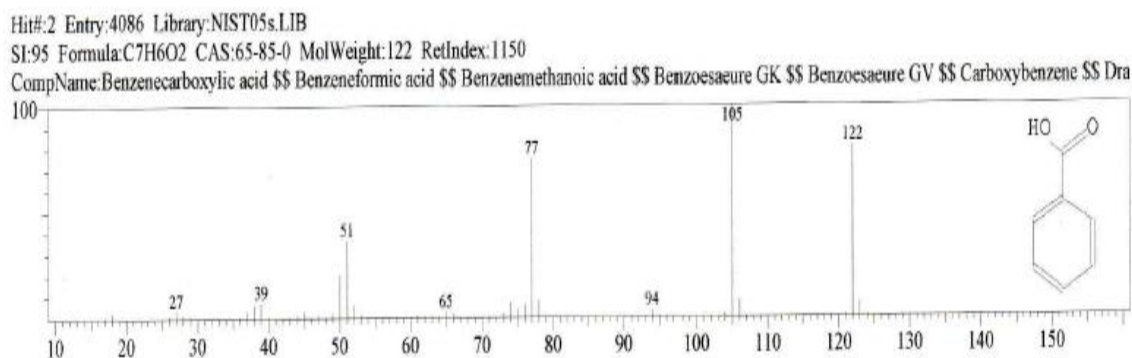


Fig 12. Benzene carboxylic acid

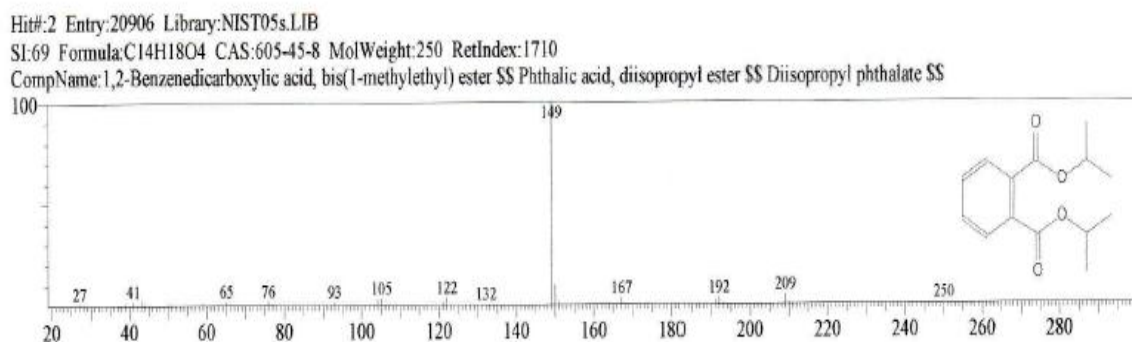


Fig 13. 1,2-Benzenedicarboxylic acid, bis (1-methylethyl) ester

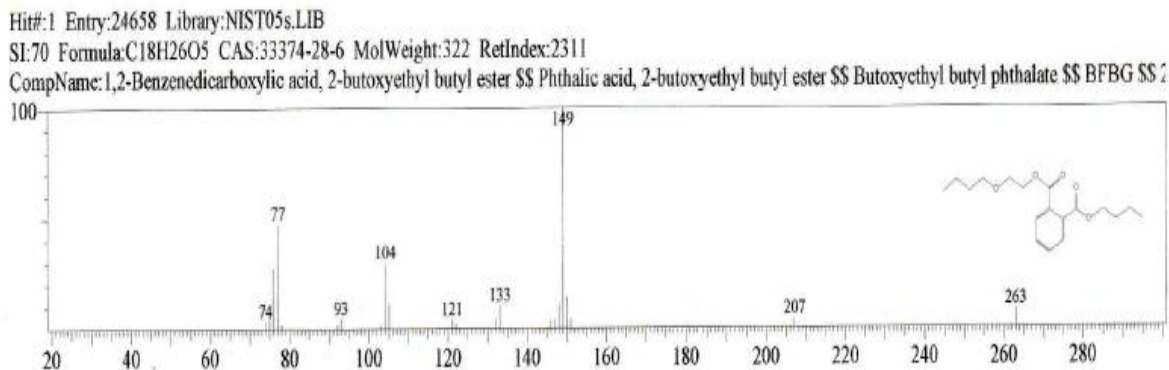


Fig 14. 1,2-Benzenedicarboxylic acid,2-butoxyethyl butyl ester

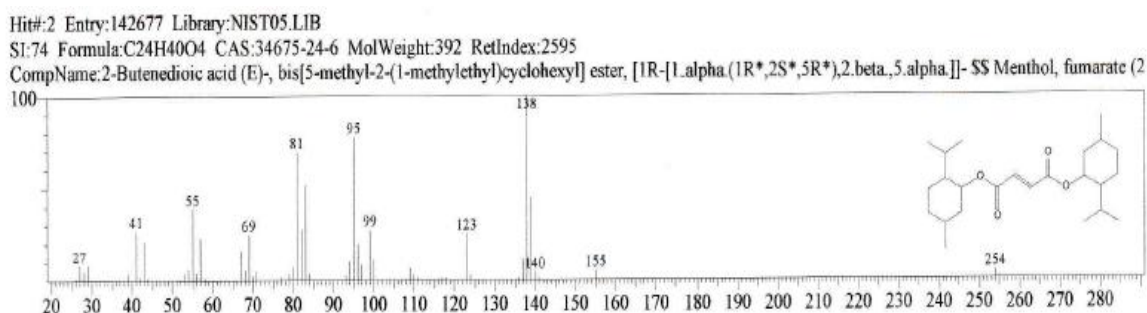


Fig 15. 2-Butenedioic acid (E)-, bis [5-methyl-2-(1-methylethyl) cyclo hexyl] ester

Following observation table gives retention time and Percent peak area of **Aloe vera juice** “A”, and “B”.

Table 3. GC-MS data of Aloe vera Juice “A”, “B”

Sr. No	Name of compound	Molecular formula	Molecular weight	Retention time		Peak area percent	
				Juice A	Juice B	Juice A	Juice B
1	Sorbic acid;2,4-hexadienoic acid	C ₆ H ₈ O ₂	112	12.85	12.03	43.98	57.29
2	Benzene carboxylic acid	C ₇ H ₆ O ₂	122	-	13.93	-	36.36
3	1,2-Benzenedicarboxylic acid, bis (1-methylethyl) ester	C ₁₄ H ₁₈ O ₄	250	-	34.77	-	1.69
4	1,2-Benzenedicarboxylic acid, 2 butoxy ethyl butyl ester	C ₁₈ H ₂₆ O ₅	322	-	34.99	-	2
5	2-Butenedioic acid (E)-, bis [5-methy-2-91-methy ethyl) cyclo hexyl] ester	C ₂₄ H ₄₀ O ₄	392	-	35.90	-	2.65
6	Methanol,oxo,Benzoate	C ₈ H ₆ O ₃	150	14.84	-	19.53	
7	Acetylene dicarboxylic acid,di-(-)menthyl-	C ₂₄ H ₃₈ O ₄	390	31.22	-	6.11	
8	Hexasiloxane,tetra decamethyl-	C ₁₄ H ₄₂ O ₅ Si ₆	458	37.44	-	30.38	

GC-MS is the best technique to identify the bioactive constituents.

Among the identified phytochemicals, in Aloe vera juice “A” and “B”,

It was found that, Sorbic acid;2,4-hexadienoic acid has the properties of antioxidant and nematocidal activity in both juice.¹⁸

Among the identified phytochemicals, the activity found in Aloe vera juice “A” and “B” are

Table 4. Phytochemicals possessing activity in Aloe vera juice “A”

Sr.No	Name of compound	activity
1	Sorbic acid;2,4-hexadienoic acid	antioxidant and nematicidal activity
2	Methanol,oxo,Benzoate	preservative activity
3	Acetylene dicarboxylic acid,di-(-)menthyl-	nematicide, anti-inflammatory, antiarthritic, antibacterial, fungicidal and insectifuge agents
4	Hexasiloxane, tetradecamethyl-	Antifouling compound, immunomodulatory, antimicrobial

Table 5. Phytochemicals possessing activity in Aloe vera juice “B”

Sr.No	Name of compound	activity
1	Sorbic acid;2,4-hexadienoic acid	antioxidant and nematicidal activity
2	Benzene carboxylic acid	preservative activity
3	1,2-Benzenedicarboxylic acid, bis (1-methylethyl) ester	antimicrobial, antioxidant, and anti-inflammatory activity
4	1,2-Benzenedicarboxylic acid, 2 butoxy ethyl butyl ester	Antimicrobial activity
5	2-Butenedioic acid (E)-, bis [5-methy-2-91-methy ethyl) cyclo hexyl] ester	antimicrobial activity

CONCLUSION

The contents were more pronounced in the juice “B” analysed by NMR, GC-MS and FTIR. CH₃ resonances of acemannan acetylation at 2.1 ppm for Aloe vera juice “A” and 2.11 ppm. “B” was observed in NMR spectra which confirms the presence of bioactive polysaccharide-acemannan required for pharmacological activity. In GC-MS analysis the similar component observed in both juices is sorbic acid; 2,4-hexadienoic acid. Percent peak area analysed was more for sorbic acid; 2,4-hexadienoic acid in the juice “B” than “A” and has antioxidant and nematicidal activity. The components vary in both juice and possess certain activity. FTIR analysis gives the probable constituents present in both juices. Therefore, it could be concluded that presence of various bioactive constituents was analysed by GC-MS, NMR, FTIR and thus helps to select Aloe vera juice “B” collected in Winter season.

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