



## EXTRACTION, STABILIZATION AND GC-MS CHARACTERIZATION OF ETHANOLIC LEAF EXTRACTS OF ALOE VERA GEL AND RIND

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**ABSTRACT:** *Aloe vera gel and rind have been examined for their phytochemical properties as well as their chemical composition using GC-MS and the results in general showed that the matured aloe leaf plant is composed of approximately 55–70%, inner leaf and 30 – 45% rind by weight. The inner leaf contains about 98.5–99.5% water and 0.5 – 1.5% solids; the rind contains 88–91% water and 9–12% solids. The whole leaf contains total solids of about 3.5 - 4.5 %, while the extractable solid of the whole leaf was approximately 1%. The main components in these analyzed samples were found to be the ash, free sugars, organic acids, and polysaccharides. The protein contents are relatively high in fresh plants, in the range of 3.8–8.3%, and higher in rind than in gel. Protein content is low in the juice powders, assuming proteins were removed in the filtering and decolorization process. The GC analysis of ethanolic extract of aloe vera (EEAV) resulted in 31 significant retention time (RT) peaks revealing more than 25 phytochemical compounds (approximately 25 compounds/peak) present in the EEAV. In the GC-MS analysis, 21 bioactive compounds, based on their peak area percentage, Retention Time (RT), molecular weight and molecular formula were identified including 2-methyl 1-butanamine, n-hexadecyl acetamide, 2-methyl isobutylamide, glycyl-dialanine. The ethanolic extract of aloe vera Rind indicated the presence of 3-aminopropionitrile, Adenosine 2-methyl acetic acid and 4-(3-acetylamino-2-oxopropyl) phenyl ester), 1,3-dichloro-4,6-dinitrobenzene, Adenosine, 2-methyl alanine, Ethyl-2-(diethoxyphosphoryl)-oxy-3,3,3-trifluoropropanoic, 2-methyl-ethyl,5-(furan-2 yl)-1,2-oxazole-3-carboxylate, 1-butanamine and 2-methyl, 1-butanamide, 2-methyl acetic acid, 2-butyn-1-ol, N-hexadecyl acatanamide, 2-methylisobutanamide and isobutanamide. The gel is mostly used for the preparation of various products like juice, makeup items, tissue papers, moisturizers, soaps, sunscreens, incense, shaving cream, and shampoos. The identification of compounds like Hexanoic Acid, Citronellyl Butyrate, Phytol, Myristic Acid, Palmitic acid, Palmitoyl Chloride and Octadecanal in the present study is important since they are known to be used in flavoring and fragrance, moisturizing creams, shaving creams, shampoos, bathing oils lipsticks and perfumed products.*

**KEYWORDS:** Aloe vera, Gel, Rind, Ethanolic, Extraction.



## INTRODUCTION

Aloe vera is a succulent plant of the Lily family, native to the Cape of Good Hope and growing wild in most parts of Africa and Madagascar. Commercial growers cultivate it in the Caribbean, the Mediterranean, Japan and the USA. It produces a ring of dagger-shaped fleshy leaves that grow up from the base of the plant. Each aloe vera plant grows up to nearly 2kg in weight. It is from the leaf that the soothing aloe vera gel is extracted. The aloe vera plant is drought resistant and grows mainly in subtropical desert like savanna. When the leaves are cut the plant can close off its cell to retain fluid (Alemdar & Agaoglu, 2021). Aloe vera can grow to 20 meters in height but usually grows only to about 1.5 meters in many cases. Each plant has about 15 leaves and blooms intermittently. It produces erect spikes of drooping yellow, orange or red tubular flowers on the woody stem. Research has shown benefits to conditions other than those for which aloe vera is most well-known. These include improvements in bone tuberculosis and broken bones, inflammatory gynecological conditions, paralysis caused by polio, ear, nose and throat conditions and bronchial asthma. It has also been found that aloe vera can help slow the aging process. Researchers have carried out extensive work into the use of aloe vera for all types of burns. They found that compounds within aloe vera can help the burn heal and can also have cleansing and antibacterial effects. The United States has developed a cream containing 70% aloe vera juice extract that prevents partially damaged tissues from dying and allows new skin cells to close off the area, thereby promoting healthy new skin beneath the scalp rather than scar tissue. Research has also been carried out into whether aloe vera can play a role in the treatment of cancer. Aloe vera appears to cause the release of tumor necrosis factor alpha that blocks the blood supply to cancerous growths (Yagi, Kabash, Mizuno, Moustafa, Khalifa & Tsuji, 2023). A study in Japan showed that drinking aloe vera juice regularly may be effective in preventing the onset of lung cancer in smokers. The first recorded evidence of the healing properties of the aloe vera is found in ancient Egyptian texts dating from around 1500 BC. The Egyptians referred to aloe vera as the plant of immortality. Arab traders were probably responsible for the spread of aloe vera into Persia, India and the Far East.

In the first century AD, the Greek physician Dioscorides wrote in his *Material Medical* that aloe vera extract could be used to treat wounds, stomach complaints, constipation, hemorrhoids, headaches, all mouth problems, hair loss, insect bites, kidney ailments and skin irritation. In Africa, aloe vera was used for stomach aches and to prevent infection from insect bites. The Chinese used aloe vera for treating eczema during the Sung dynasty. In India during the fourth century BC, people believed that aloe vera grew in the garden of Eden, they called it “the silent healer” and used it to heal skin conditions and inflammation. In the early Christian era, aloe vera could be found in all advanced medicinal texts (González-Stuart, 2012). By the late 19th century, synthetic laboratory drugs were taking precedence over botanical compounds. There was a naïve optimism that scientific advance would lead to even more effective drugs that would eventually “conquer” all diseases and herbal remedies such as aloe vera fell from favor.



**Fig. 1: Structure of aloe vera leaf**

The structure of aloe vera leaf reveals three layer structures: the inner transparent layer composed of the gel and the middle lower layer consisting of the yellow latex and the top green layer consisting of the rind. Aloe vera gel is a popular natural remedy known for its numerous health and skincare benefits. Aloe vera, also known as aloe barbadensis miller, belongs to the family Xanthorrhoeaceae. It is a perennial succulent plant with thick, fleshy leaves that grow in a rosette pattern. The leaves are typically green or gray-green in color and can grow up to 60-100 cm in length. The plant produces tubular yellow flowers on a tall spike, which can reach up to 90 cm in height. aloe vera is native to the Arabian Peninsula but is now cultivated in many parts of the world for its medicinal and cosmetic properties (King, Yates & Greenlee, 2022).

Previous methods of extraction concentrate only on the aloe vera gel found in the middle of the leaf. The whole leaf processing was not used because it was difficult to prevent contamination with aloe latex (Reynolds, 2004). Aloe latex is a consistent, colorless and translucent extract from the inner leaf of the plant that acts as a laxative and can cause severe cramps and diarrhea. The very latest processing methods now allow the whole aloe vera to be processed without the latex contamination (Jones & Sacamano, 2000). This is a hugely important step forward as the juice/gel can now contain the healing power of all parts of the plant rather than just the inner gel. The polysaccharide count in whole leaf juice can reach much higher values using these latest processing methods (Shukla, 2008).

Aloe vera contains a wide range of bioactive compounds, including water; aloe vera gel primarily consists of water, making up the majority of its composition (Yagi et al., 2023).

**Anthraquinones:** These are organic compounds with laxative effects found in aloe vera, such as aloin and emodin (Yoon, Ahn, Lee, Kim & Yeom, 2020).

**Saponins:** Aloe vera contains saponins, which have cleansing and antiseptic properties, making them beneficial for skincare (González-Stuart, 2012).



**Salicylic acid:** Aloe vera gel contains salicylic acid, known for its anti-inflammatory and acne-fighting properties (Paulsen, Korsholm & Brandrup, 2016).

**Glycoproteins:** These are proteins with attached carbohydrate molecules that help reduce inflammation and promote wound healing (Reuter, Jocher, Stump, Grossjohann, Franke & Schempp, 2021).

Aloe vera gel has long been recognized for its numerous skincare benefits. Rich in vitamins, minerals, and antioxidants, aloe vera gel possesses properties that contribute to skin health and vitality. Its hydrating properties help to replenish moisture in the skin, making it particularly beneficial for individuals with dry or sensitive skin (Morton, 2022). Aloe vera gel is commonly used in skincare products due to its moisturizing, anti-inflammatory, and wound-healing properties. It is effective in treating sunburns, minor cuts, insect bites, and various skin conditions such as eczema and psoriasis (Lans, 2021). The gel is also utilized in hair care products for its ability to nourish the scalp, promote hair growth, and improve the overall health and appearance of hair. It helps in reducing dandruff, soothing scalp irritation, and adding shine to the hair (Petrovil, Ivanovic, Milovanovic & Zizovi, 2022). Due to its antimicrobial and anti-inflammatory properties, aloe vera gel is used in oral care products such as toothpaste and mouthwash. It helps in reducing plaque buildup, gingivitis, and oral ulcers (Radha et al., 2015).

Furthermore, the gel's ability to stimulate collagen production can contribute to the reduction of fine lines and wrinkles, promoting a more youthful complexion (Radha et al., 2015). Aloe vera gel has long been recognized for its wound healing properties. These compounds exhibit anti-inflammatory, antimicrobial, and antioxidant properties, promoting wound healing by reducing inflammation, preventing infection, and enhancing tissue regeneration (Davis, Parker & Samson, 2014). Additionally, aloe vera gel has been shown to accelerate the healing process by stimulating collagen production and increasing fibroblast activity, leading to faster wound closure (Schinor, Salvador & Turatti, 2024). Furthermore, its moisturizing effect helps maintain a moist wound environment, which is conducive to optimal healing (Suslick, Didenko, Patel, Gadewar, Tahilyani & Patel, 2021).

The effect of aloe vera gel on prevention of pressure ulcers in patients hospitalized in the orthopedic wards (Suslick *et al.*, 2021). Overall, aloe vera gel represents a promising natural remedy for wound management, offering a safe and effective alternative to traditional treatments. Aloe vera gel has been traditionally used for centuries for its medicinal properties. Among its many purported health benefits, aloe vera gel has been reported to possess significant anti-inflammatory properties (Radha et al., 2015). Inflammation is a complex biological response to injury or infection, and chronic inflammation is associated with various diseases, including arthritis, asthma, and inflammatory bowel disease (IBD) (Simeonov, Tsibranska & Minchev, 2021). Therefore, natural anti-inflammatory agents like aloe vera gel hold promise for the management of inflammatory conditions. These bioactive compounds exert their anti-inflammatory effects through various mechanisms, such as inhibition of pro-inflammatory cytokines, modulation of immune responses, and suppression of oxidative stress (Radha et al., 2015).



Additionally, aloe vera gel has been shown to inhibit the activity of enzymes involved in the inflammatory process, such as cyclooxygenase (COX) and lipoxygenase (LOX) (Simeonov et al., 2020). Aloe vera gel has been extensively studied for its various therapeutic properties. Several studies have demonstrated the potential of aloe vera gel in wound healing due to its anti-inflammatory, antimicrobial, and antioxidant properties (Patel, Singh & Patel, 2022; Davis, 2016). Furthermore, aloe vera gel has been found to possess moisturizing and skin-repairing effects, making it a common ingredient in skincare products (Reuter et al., 2008). In addition to its topical applications, aloe vera gel has also been investigated for its potential oral health benefits. Studies suggest that aloe vera gel may aid in the treatment of oral ulcers, gingivitis, and periodontitis due to its anti-inflammatory and antimicrobial properties (Radha *et al.*, 2015; Patel et al., 2022). Aloe vera gel has been explored for its potential benefits in promoting hair health. Studies suggest that aloe vera gel contains enzymes that may stimulate hair growth and prevent hair loss by nourishing the scalp and improving blood circulation (Chung, Cheong, Lee, Roh & Cha, 2021). Additionally, aloe vera gel has been studied for its potential immune-modulatory effects. Research indicates that the polysaccharides present in aloe vera gel may enhance immune function by stimulating the activity of macrophages and promoting the production of cytokines (Treutlein, Smith, van Wyk & Wink, 2023). Furthermore, the antioxidant properties of aloe vera gel may help reduce oxidative stress and support overall immune health (Tian & Hua, 2021). Aloe vera gel is composed of various bioactive like polysaccharides, such as acemannan, are considered one of the major components and are responsible for the gel's viscosity and mucilage properties (Wang, Li, Wang, Li, Li, Yang & Li, 2022). Phenolic compounds, such as aloin and barbaloin, exhibit antioxidant and anti-inflammatory properties (Tanaka, Misawa, Ito & Habara, 2019). Enzymes like catalase and peroxidase contribute to the gel's enzymatic activity, which plays a role in wound healing (Thamlikitkul, Bunyapraphatsara, Riewpaiboon, Theerapong, Chantrakul & Thanaveerasuwan, 2022). The physical properties of aloe vera gel have been extensively studied due to its wide range of applications in the pharmaceutical, cosmetic, and food industries (Vardy, Cohen & Tcheto, 2021). It appears as a consistent, translucent, colorless and odorless gel that stretches continuously with strong adhesive properties (Vardy et al., 2021).

Aloe vera gel contains a plethora of chemical constituents that contribute to its therapeutic properties (Rodriguez, Alvarado & Lopez, 2020). The main chemical components of aloe vera gel include polysaccharides, glycoproteins, vitamins, minerals, and phenolic compounds. Moreover, aloe vera gel contains vitamins C and E, which possess antioxidant properties, along with minerals such as calcium, magnesium, and zinc, contributing to its nutritive value (Vogler & Ernst, 1999). Overall, the existing literature highlights the diverse therapeutic effects of aloe vera gel, making it a promising natural remedy for various health conditions. aloe vera gel has gained significant attention for its diverse applications in various industries, including pharmaceuticals, cosmetics, and food. According to Surjushe et al. (2008), the gel's therapeutic properties make it a valuable ingredient in skincare products, where it is utilized for its moisturizing, anti-inflammatory, and wound-healing effects. In the pharmaceutical industry, aloe vera gel has been studied for its immunomodulatory, antioxidant, and antimicrobial properties (Davis et al., 2014). The gel's bioactive compounds, including polysaccharides, glycoproteins, and phenolic compounds, contribute to its pharmacological activities, making it a promising candidate for the development of novel therapeutic agents (Rodriguez-Rodriguez et al., 2020). Moreover, aloe vera gel has found



applications in the food and beverage industry, where it is used as a natural additive due to its nutritional value and functional properties (Rodriguez-Rodriguez *et al.*, 2020).

Recent studies have highlighted the moisturizing effects of aloe vera gel due to its rich composition of polysaccharides, vitamins, and minerals (Davis, Philpott, Kumar & Mendall, 2021). These components contribute to enhanced hydration of the skin and aid in the repair of damaged epithelial tissue (Surjushe *et al.*, 2018). Furthermore, aloe vera gel has been shown to accelerate wound healing by stimulating fibroblast proliferation and collagen synthesis (Mason, Paniwnyk & Lorimer, 2021). The controversy over the identity of the active substance(s) in aloe vera has not been settled. Also various mechanisms have been proposed for the alleged healing properties of aloe vera. Since no single definite active ingredient has been found, it is commonly suggested that there may be some synergistic action between the polysaccharides base and other components. According to Mackee (2021), vitamin D was the healing agent. However, Knorr (2022) proposed a theory stating the seeming efficacy of aloe vera pulp may be attributed to its high water content (i.e. 96%+), providing a means of making water available for injured tissue without scaling it off from the air. This recovery would explain the instant soothing effect of aloe vera gel on burns but would not account for the long term effect of healing. The action of aloe vera is simply due to its moisturizing and emollient effects, hence its use in cosmetics. Various researchers reported that the effective component for wound healing may be tannic acids and a type of polysaccharide. The anti-inflammatory properties of aloe vera gel have been well-documented in recent literature, with studies demonstrating its ability to inhibit the production of pro-inflammatory cytokines and reduce edema in experimental models (Paul & Crepeau, 2023). Moreover, aloe vera gel exhibits broad-spectrum antimicrobial activity against various pathogens, including bacteria, fungi, and viruses, making it a promising agent for the treatment of infections (Chang, Wang, Feng & Liu, 2021; Bloomfield, 2021).

## Experimental

The collected samples were firstly washed under running tap water and then with distilled water to remove dust particles. The rind was cleanly peeled off with a sharp knife to expose the gel. The separated rind and gel were then kept in two separate beakers before crushing and grinding using an electric blender. The 20 g ground pulp of aloe vera gel and rind was mixed in 200 ml AR grade ethanol solvent (HiMedia) in a ratio of (1:10) and placed in a soxhlet apparatus for extraction for 16-18 hours at 78.6 °C (boiling point of the ethanol). The collected pure ethanolic extract of aloe vera, EEAV was then filtered and the extract concentrated using a rotary evaporator connected with the water bath set at 45.0 – 50.0 °C. The resulting solution was then stored in a refrigerator at 4 °C for further analysis.

## Preliminary Phytochemical Screening

The preliminary phytochemical screening of EEAV will be carried out for the detection of phytochemicals such as alkaloids, steroids, triterpenoids, glycosides, carbohydrates, flavonoids, tannins, phlobatannins, anthraquinones and saponins in leaf samples' extract by standard methods described by Harborne (1998).



### **Test for Tannins**

About 2.0 ml EEAV was mixed in 2 ml distilled water and then a few drops of FeCl<sub>3</sub> solution (5% w/v) were added. The formation of a green precipitate (an indicator of the presence of tannin in the solution) in a few minutes will be observed.

### **Test of Saponins**

About 5.0 ml MEAV was mixed with 5 ml distilled water, poured into a test tube and thoroughly shaken. The tube was then observed for the formation of stable foam (an indicator of the presence of saponins after a few minutes).

### **Test for Flavonoids**

About 1.0 ml EEAV was mixed in 1 ml of 10% lead acetate solution and then observed for the formation of a yellow precipitate (an indicator of the presence of flavonoids).

### **Tests for Anthraquinones Bontrager's Test**

About 3.0 ml EEAV was added in 3 ml benzene, shaken well, filtered and then added 5 ml of 10% ammonia solution to the filtrate. The mixture was then observed for the presence of a pink, red or violet color in the ammoniacal (lower) phase (an indicator of the presence of free anthraquinones). About 3 ml MEAV will be boiled with 3 ml aqueous sulphuric acid and filtered in hot conditions. Further, 3 ml benzene was added to the filtrate and shaken. A benzene layer was separated and 3 ml of 10% NH<sub>3</sub> was added to it and observed for the pink, red or violet color in the ammoniacal (lower) phase (an indicator of the presence of anthraquinone derivatives).

### **Test for Terpenoids**

About 2.0 ml EEAV was dissolved in 2.0 ml chloroform and evaporated to the state of dryness. Then 2.0 ml concentrated sulphuric acid was added to it and allowed to heat for 2 minutes, then observed for the presence of a grayish color (an indicator of the presence of terpenoids).

### **Test for Alkaloids**

About 3 ml MEAV was stirred with 3 ml of 1% HCl in a steam bath and then Mayer's and Wagner's reagents were added to the mixture. Further, turbidity of the resulting precipitate was taken as evidence of the presence of alkaloids.

### **Test for Carbohydrates**

About 3 ml EEAV will be added to 2 ml Molisch's reagent and the resulting mixture will be shaken properly, then 2 ml concentrated H<sub>2</sub>SO<sub>4</sub> will be added carefully to the test tube, and then observed for the formation of a violet ring (an indicator of the presence of carbohydrate).



## Gas Chromatography-Mass Spectrometer (GC-MS) Analysis

The GC-MS analysis of EEAV was carried out on GC Clarus 680 TurboMass Perkin Elmer gas chromatography coupled to a mass identifier system comprising an AOC-20i auto-sampler and gas chromatograph, interfaced to a mass spectrophotometer. The sample was analyzed on the acquisition parameters where the oven was set to an underlying temperature of 40 ° C for 5 min, ramp at 120 ° C/min to 260 ° C and hold up at this temperature for 10 min. Injection temperature was kept up at 250 ° C, helium (99.999 %) stream rate as 1.5 ml/min, transfer temperature at 180 ° C and particle source temperature at 200 ° C. Infusion was performed in the split mode at 50:1 and the volume was 0 µL. Solvent delay was for 2 min. The mass spectra were obtained by electron ionization (EI) and scanned between 50 to 500 Da, Kushwaha et al., (2021). The GC chromatogram was interpreted by MS by comparing the obtained spectrum with the library of compounds spectrum stored in the associated National Institute of Standard and Technology (NIST) database.

## RESULTS AND DISCUSSION

Aloe vera gel and rind have been examined for their phytochemical properties as well as their chemical composition. Using GC-MS, the results are presented below.

In general, the matured aloe leaf plant is composed of approximately 55–70% inner leaf and 30 – 45% rind by weight. The inner leaf contains about 98.5–99.5% water and 0.5 – 1.5% solids; the rind contains 88–91% water and 9–12% solids. The whole leaf contains total solids of about 3.5 - 4.5%, while the extractable solid of the whole leaf was approximately 1%. The main components in these analyzed samples were found to be the ash, free sugars, organic acids, and polysaccharides. The protein contents are relatively high in fresh plants, in the range of 3.8–8.3%, and higher in rind than in gel. Protein content is low in the juice powders, assuming proteins were removed in the filtering and decolourization process.

**Table 1: Results of Phytochemical Analysis**

Phytochemical	Observation	Gel	Rind
Alkaloids	Cream color	+	+
Mayer's test	Reddish brown	+	+
Flavonoids	Yellow orange	+	+
Lead acetate test	Reddish brown	+	+
Steroids	Violet to blue	+	+
Terpenoids	Reddish brown precipitate	+	+
Antraquinone	Pink color	+	+
Saponins	Stable persistent	-	+
Tannins	Brownish green	+	+
Carbohydrates	Blue green color	+	+

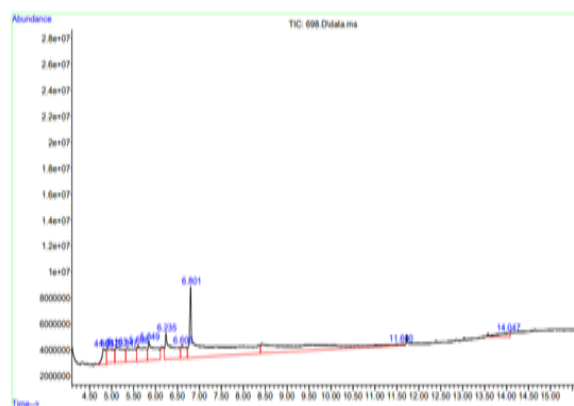
### GC-MS Results



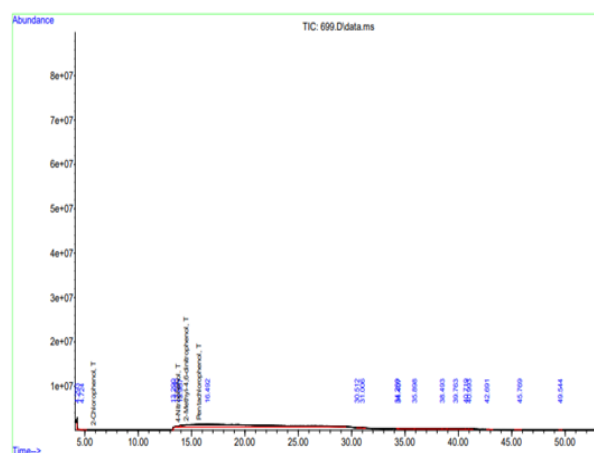
**Table 2: Result of Chromatographic Analysis**

SN	Name of compound	Formula	Molecular weight	Structure
1	3-Butyn-1-ol	C <sub>4</sub> H <sub>6</sub> O	70.0	
2	Glycol-di-alanine	C <sub>5</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub>	146.06	
3	2-Bromoethanol	C <sub>2</sub> H <sub>5</sub> BrO	123.15	
4	1- Heptanamine	C <sub>7</sub> H <sub>17</sub> N	115.13	
5	1-Hexanamine	C <sub>6</sub> H <sub>15</sub> N	99.13	
6	Propane	C <sub>3</sub> H <sub>8</sub>	44.0	
7	1-H-Pyrrole-2,5-dione	C <sub>4</sub> H <sub>3</sub> NO <sub>2</sub>	97.016	
8	2-Methyl-adenosine	C <sub>11</sub> H <sub>15</sub> N <sub>5</sub> O <sub>4</sub>	281.112	
9	4(-3-acetylamino-2 oxopropyl)phenyl ester	C <sub>13</sub> H <sub>15</sub> NO <sub>4</sub>	249.0	
10	Oleylamine	C <sub>18</sub> H <sub>37</sub> N	267.29	
11	1-Butanamine	C <sub>4</sub> H <sub>11</sub> N	73.0	

File : D:\MassHunter\GCMS\1\data\2024\JULY\MS-SCAN\01072024\699.D  
 Operator : OPERATOR 1  
 Acquired : 01 Jul 2024 12:11 using AcqMethod MS-SCAN 01072024.M  
 Instrument : GCMSD  
 Sample Name: A  
 Misc Info : SAMPLE  
 Vial Number: 41

**Fig. 2: GC-MS of EEA V Gel**

File : D:\MassHunter\GCMS\1\data\2024\JULY\MS-SCAN\01072024\699.D  
 Operator : OPERATOR 1  
 Acquired : 01 Jul 2024 13:10 using AcqMethod MS-SCAN 01072024.M  
 Instrument : GCMSD  
 Sample Name: B  
 Misc Info : QC  
 Vial Number: 42

**Fig. 3: GC-MS of EEA V Rind**



The GC analysis of MEAV resulted in 31 significant retention time (RT) peaks (shown in figure 2) which on the interpretation by a Mass Spectrometer (MS) revealed more than 25 phytochemical compounds (approximately 25 compounds/peak) present in the EEAV. In the GC-MS analysis, 21 bioactive compounds, based on their peak area percentage, Retention Time (RT), molecular weight and molecular formula were identified in the ethanolic extract of aloe vera gel (Table.2) out of which include, 2-methyl 1-butanamine (RT:35.89 ), n-haxadecylacetamide, (RT:39.76), 2-methyl isobutanamide, (RT: 5.19), glycyl-di-alanine, (RT: 5.103). Also present were 1-heptane,1-tridecanamine and 1-octadecanamine at RT of 5.598, 2-bromopropane and propane (RT: 5.849), N-(n-propyl)acatanamide (RT: 6.235), Ethanamide, (RT: 35.662), 1-H-pyrrole-2,5-dione, (RT: 6.606), Oleylamide and 3-amino1, 2-propanediol, (RT:14.047) and Diethyl, 3-chloro—2-hydroxypropylmalonate (RT: 18.730).

The ethanolic extract of aloe vera Rind (Table 3) indicated the presence of 3-aminopropionitrile, (RT: 4.724), Adenosine 2-methyl acetic acid and 4-(3-acetylamino-2-oxopropyl) phenyl ester), (RT:13.29), 1,3-dichloro-4,6-dinitrobenzene, (RT:13.29), Adenosine,2-methyl alanine, (RT:13.957), Ethyl-2-(diethoxyphosphoryl)-oxy-3,3,3-trifluoropropanoate, (RT:13.590), 2-methyl-ethyl,5-(furan-2yl)-1,2-oxazole-3-carboxylate, (RT:30.512), 1-butanamine and 2-methyl,1-butanamide, (RT:34.26), 2-methyl acetic acid, (RT: 13.290), 2-butyn-1-ol, (RT: 4.724), N-hexadecyl acatanamide, (RT: 39.763), 2-methylisobutanamide, (RT: 45.769) and isobutanamide, (RT:49.544).

## DISCUSSION

The phytochemical analysis of the ethanolic extracts of aloe vera gel and rind reveals the presence of alkaloids, flavonoids, steroids and anthraquinone. However, saponin was present in the EEAV rind but absent in the gel. Carbohydrate and tannins were also present in both the gel and the rind, as seen in Table 1. This is indicative of high phytochemical properties. The GC-MS analysis reveals the presence of unsaturated aliphatic alcohols like 3-butyn-1-ol, haloalkanes like bromoethane and amino acids like glycol-di-alanine, 1-heptamine, 1-hexamine, 1-butanamine and oleylamine, as seen in Table 2. Simple hydrocarbons like propane were also present as well as large molecules like the aromatic 1-H-Pyrrole -2, 5-dione, 2-methyladenosine and 4(-3-acetylamino-2-oxopropyl) ester.

The traditional medicines have been an integral part of human civilization since the beginning of civilization. In the whole world, about 80% people depend on herbs as a conventional method of treating various ailments, among which aloe vera has been considered and used as a popular folk medicine all across the world (Arunkumar & Muthuselvam, 2009). Considering its use, the aloe vera is well studied by various scientists in the last 4 decades for its countless properties. Being rich in several types of phytochemicals such as tannins, carbohydrates, saponins, flavonoids, alkaloids, anthraquinones, and terpenoids as detected in the present study, it is used in the formation of various pharmaceutical and industrial products. Other workers reported its role in treating diabetes (Yongchaiyudha et al., 1996; Choo, 2003). Rosi et al. (2021) reported the antibacterial effects of aloe vera but it also possesses antifungal compounds like undecanoic acid,



nonanoic acid. Faustino et al. (2016) emphasized the need of its exploration towards the use of this plant in the treatment of deadly disorders like cancer and HIV. Buchhaupt et al. (2014) tapped to the further uses in cosmetic and personal care products industry. Shukla et al. (2008) gave the hint of the use of aloe vera seeds in the production of biodiesel which is confirmed here by the detection of compound named 12-Hydroxy-8-(1-Hydroxyethyl) reported to be used to produce detergents and biodiesel. Considering its huge industrial applications, the aloe contents are used in the formation of different market products such as sunburn lotions, creams (about 20%), juices (about 95%), beverages (about 50%), drinks (about 10%) and capsules (about 5-10%) (Perez, 2020). One of the identified compounds named 2-hexadecanone and 4, 5-dimethyl-4-hexene -3-one has been reported to be used in the production of gasoline, electricity, transportation fuel. It is suggested that aloe vera is a medicinal and cosmetically important plant species. There are various compounds that we identified through GC-MS screening of EEAV that suggest that aloe vera has a great commercial importance also other than its medicinal and pharmaceutical uses. It has a wide industrial application in food and cosmetic industries along with its already proven nutritional and pharmacological properties. So, it is assured that the phytochemical constituents of aloe vera are the profitable source that meet the needs of the hour medicinally as well as industrially both according to the requirement of public domain.

## CONCLUSIONS

In this study, comprehensive investigations on the aloe chemical constituents carried on the ethanolic extracts of the gel and the rind show that fresh aloe vera leaf is mainly composed of organic acids, minerals, proteins, free sugars, polysaccharides, and insoluble fibers. In the analyzed commercial juice products, the major constituents in non-ethanol-precipitated products are organic acids, minerals, free sugars, and polysaccharides, which account for greater than 90% of the entire composition. As fresh aloe leaf juice contains over 98% water, the juice preparation process is similar to a water extraction and all the water-soluble components mentioned above are extracted, while the water-insoluble substances, such as lipids, are not favorable for extraction. The majority of phenolic compounds existing in latex are removed through activated charcoal absorption and filtration during the manufacturing process because they are considered to be unwanted compounds (e.g., anthraquinone aloins). Ethanol precipitation increases the concentrations of polysaccharides and reduces the presence of small molecule compounds. The main components of the ethanol-precipitated products comprise only organic acids, polysaccharides, minerals, and proteins. The present work has provided the general composition of aloe vera products. Although the chemistry varies with different manufacturing processes, the variations of ratios of the individual components remain consistent for each type of process. It is always a challenge to obtain accurate MW and quantity of the polysaccharides. aloe polysaccharides are generally believed to be the main bioactive constituents of the plant and responsible for a number of reported biological activities and clinical effects. The gel is mostly used as part of the plant for the preparation of various products like juice, makeup items, tissue papers, moisturizers, soaps, sunscreens, incense, shaving cream, and shampoos, among others. The identification of compounds like Hexanoic Acid, Citronellyl Butyrate, Phytol, Myristic Acid, Palmitic acid, Palmitoyl Chloride and Octadecanal in the present study is germane since they are known to be used in flavoring and



fragrance, moisturizing creams, shaving creams, shampoos, bathing oils lipsticks and perfumed products (Babu *et al.*, 2015), toilet soaps, household cleaners and detergent production (Ifeanacho *et al.*, 2019; Liu *et al.*, 2013), as surfactant, opacifying agent, texture enhancer, emollient, cleansing agent emulsifier in cosmetics and other personal products (Cerone & Smith, 2021), producing fragrance in perfume production and as flavor compounds. Buchhaupt *et al.* (2014) has tapped their further uses in the cosmetic and personal care products industry.

## REFERENCES

- Alemdar S, Agaoglu S (2021). Investigations of in-vitro antimicrobial activity of aloe vera juice. *J Anim Vet Adv* 8(1):99–102.
- Akhtar MA and Hatwar SK (2022). Efficacy of aloe vera extract cream in management of burn wounds. *J ClinEpidemiol.* ;49:24.
- Arunkumar S, and Muthuselvam M (2009) Analysis of phytochemical constituents and antimicrobial activities of aloe vera L. against clinical pathogens. *World Journal of Agricultural sciences* 5(5): 572- 576
- Bloomfield F: (2021). *Miracle Plants: aloe vera*. Century, London
- Chang, L. X., Wang, C., Feng, Y., Liu, Z. (2021). Effects of heat treatments on the stability of polysaccharides substances and barbaloin in gel juice from aloe vera Miller. *J Food Eng* 75:245–251.
- Choo C (2003) *Vital vera*. *Asia Pacific Food Ind* 15:36-37.
- Collins, C.E. and C. Collins, 2022. Roentgen dermatics treated with fresh aloe leaf. *Am. J. Roentgenol.*, 33: 396 397.
- Dal Belo SE, Gaspar LR, Maia Campos PM. (2021). Moisturizing effect of cosmetic formulations containing aloe vera extract in different concentrations assessed by skin bioengineering techniques. *Skin Res Technol.* ;12(4):241-46.
- Grindlay D, Reynolds T. (2023). The aloe vera phenomenon: a review of the properties and modern uses of the leaf parenchyma gel. *J Ethnopharmacol*;16(2-3):117-51.
- King GK, Yates KM, Greenlee PG, 2022. The effect of Acemannan Immunostimulant in combination with surgery and radiation therapy on spontaneous canine and feline fibrosarcomas. *J Am Anim Hosp Assoc.* ;31(5):439-47.
- Lans CA. 2021. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *J Ethnobiol Ethnomed.* ;2:45.
- Morton JF. (2022). Folk uses and commercial exploitation of aloe leaf pulp. *Economic botany.* ;15(4):311-19.
- Reuter J, Jocher A, Stump J, Grossjohann B, Franke G, Schempp CM. (2021). Investigation of the anti-inflammatory potential of aloe vera gel (97.5%) in the ultraviolet erythema test. *Skin Pharmacol Physiol.* ;21(2):106-10.
- Lee, K. H., Kim, J. H., Lim, D. S., and Kim, C. H. Anti-leukaemic and anti-mutagenic results of di(2-ethylhexyl)phthalate removed from aloe vera Linne. *J Pharm Pharmacol* 2000;52(5):593-598.



- Thamlikitkul V, Bunyaphatsara N, Riewpaiboon W, Theerapong S, Chantrakul C, Thanaveerasuwan T. 2022. Clinical trial of aloe vera Linn. for treatment of minor burns. *SirirajHosp Gaz.* ;43(5):313-316.
- Vardy AD, Cohen AD, Tchetov T. 2021. A double-blind, placebo-controlled trial of aloe vera (*A. barbadensis*) emulsion in the treatment of seborrheic dermatitis. *J Derm Treatment*;10(1):7-11
- Davis K, Philpott S, Kumar D, Mendall M, 2021. Randomized double-blind placebo-controlled trial of aloe vera for irritable bowel syndrome. *Int J ClinPract.* 2006;60(9):1080-86.
- Knorr, D., 2022, Impact of non-thermal processing on plant metabolites. *Journal of Food Engineering*, 56, p.131.
- Mason, T.J.,Paniwnyk, L.,Lorimer J.P., 2021,The uses of ultrasound in food technology, *UltrasonicsSonochemistry*, 3,Elsevir, p.253.
- Paul, N., Crepeau, 2023,Mixer design optimization for high solids contents media : methodology and application to the, Pechiney’s high density predesilication process, *Light Metals, The Minerals, Metals & Materials Society*, p.43
- Petrovi1, S., Iivanovi, J.,Mmilovanovi, S.,Zižovi, I., 2022,Comparative analyses of the diffusion coefficients from thyme for different extraction processes; *J. Serb. Chem. Soc.* 77 (6) ,p.799.
- Schinor, E.C., Salvador, M.J., Turatti, I.C.C., 2024, Comparison of classical and ultrasound-assisted extractions of steroids and triterpenoidsfromthreeChrestaspp.,*UltrasonicsSonochemistry*, 11,p. 415.
- Simeonov, E. , Tsibranska, I. , Minchev, A. 2021, Solid – liquid extraction from plants – experimental kinetics and modeling ,*Chem.Eng.Journal*, 73, p.255.
- Suslick, K.S., Didenko, Patel K, Gadewar M, Tahilyani V, Patel DK 2021. A review on pharmacological and analytical aspects of diosgenin: a concise report. *Nat Prod Bioprospect*; 2(2): 46-52
- Patel K, Singh RB, Patel DK 2022. Medicinal significance,pharmacological activities, and analytical aspects of solasodine:A concise report of current scientific literature. *J Acute Dis*2013;2(2): 92-98
- Perez, Garcia, Garcia, (2020), *Anti Ulcer*, (2007)
- Chung JH, Cheong JC, Lee JY, Roh HK, Cha YN 2021. Acceleration Of the alcohol oxidation rate in rats with aloin, a quinine derivative of aloe.*BiochemPharmacol* 2021; 52(9): 1461-1468.
- Tian B, Hua Y. Concentration-dependence of prooxidant and antioxidant effects of aloin and aloemodin on DNA. *FoodChem*2005; 91(3): 413-418.
- Wang H, Li F, Wang T, Li J, Li J, Yang X, Li J (2022). "[Determination of aloin content in callus of aloe vera var. chinensis]". *Zhong Yao Cai (in Chinese)*. 27 (9): 627–8.
- Treutlein J, Smith GF, van Wyk BE, Wink W (2023). "Phylogenetic relationships in Asphodelaceae (Aloaceae) inferred from chloroplast DNA sequences (rbcL, matK) and from genomic finger-printing (ISSR)". *Taxon*. 52 (2): 193–207
- Jones WD, Sacamano C. (2000). *Landscape Plants for Dry Regions: More Than 600 Species from Around the World*. California Bill's Automotive Publishers. USA.
- Yagi, A, A. Kabash, K. Mizuno, S. M. Moustafa, T. I. Khalifa and H. Tsuji, (2023). “Radical Scavenging Glycoprotein Inhibiting Cyclooxygenase-2 and Thromboxane A2 Syn- thase from aloe veraGel,” *PlantaMedica*, Vol. 69, No. 3, pp. 269



- Mackee, G.M., (2021). X-ray and Radium in the Treatment of Diseases of the Skin. Lea and Febiger (Eds.). Philadelphia, PA, pp: 319-320.
- Faustino C, Serafim C, Rijo P and Reis C P (2016) Bile acids and bile acid derivatives: use in drug delivery systems and as therapeutic agents. *Expert Opinion on Drug Delivery* 13(8):1133–1148 <https://doi.org/10.1080/17425247.2016.1178233>
- Davis RH, Kabbani JM, Maro NP. aloe vera and wound restoration. *J Am Podiatric Med Assoc* (2016); 77:165-9
- Davis, R. H., Donato, J. J., Hartman, G. M., and Haas, R. C. Anti-inflammatory and wound restoration hobby of a boom substance in aloe vera. *J Am Pediatric Med Assoc* (2014); 84(2):77-81.
- Buchhaupt M, Kähne F, Etschmann M M W and Schrader J (2014) Biotechnological Production of Fatty Aldehydes. *Flavour Science*: 195–199 <https://doi.org/10.1016/b978-0-12-398549-1.00037-4>
- Cerone M, & Smith T K (2021) A Brief Journey into the History of and Future Sources and Uses of Fatty Acids. *Frontiers in Nutrition* 8: 570-401 <https://doi.org/10.3389/fnut.2021.570401>
- Harborne A. and Harborne JB (1998) *Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis*: 317
- Ifeanacho M O, Ogunwa S C and Amadi P U. (2019) Phytochemical Composition of Vitex doniana. *Analytical Chemistry Letters* 9(6): 863–875.
- Kushwaha P, Alok S and Dwivedi L. K. (2021) The GC-MS analysis of methanolic extract of Chlorophytum borivilianum and compounds' activities validation at standard databases. *South Asian J Exp Biol* 11(6): 768-774 DOI: 10.38150/sajeb.11(6).p768-774
- Rossi A, Martins M P, Bitencourt T A, Peres N T A, Rocha C H L, Rocha F M G, Neves-da-Rocha J, Lopes M E R, Sanchez P R, Bortolossi J C and Martinez-Rossi N M (2021). Reassessing the Use of Undecanoic Acid as a Therapeutic Strategy for Treating Fungal Infections. *Mycopathologia* 186(3): 327–340 <https://doi.org/10.1007/s11046-021-00550-4>
- Liu P, Chen D and Shi J (2013) .Chemical Constituents, Biological Activity and Agricultural Cultivation of aloe vera. *Asian Journal of Chemistry* 25(12): 6477–6485
- Yongchaiyudh S, Rungpitarangsi V, Bunyapraphatsara N and Chokechaijaroenporn O (1996) Antidiabetic activity of aloe vera L. juice. I. Clinical trial in new cases of diabetes mellitus. *Phytomedicine* 3(3): 241–243
- Babu R J, Chen L, and Kanikkannan N (2015). Penetration enhancers and their mechanism studied on a molecular level. *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*:133–150 <https://doi.org/10.1007/978-3-662-47039-8>
- Dahiya A, (2020) Cutting-edge biofuel conversion technologies to integrate into petroleum-based infrastructure and integrated biorefineries. In *Bioenergy: Biomass to Biofuels and Waste to Energy*. Second Elsevier:649–670). <https://doi.org/10.1016/B978-0-12-815497-7.00031-2>
- Shukla S, (2008). aloe vera has biodiesel potential, reveals MSU study. <http://www.expressindia.com/latestnews/aloe-vera-has-biodiesel-potential-reveals-MSUstudy/324861/> (21.06.08)
- Perez R M, Garcia E and Garcia F E (2020). Antidiabetic activity of aloe vera leaves. *Evidence-Based Complementary and Alternative Medicine*: 1–15. <https://doi.org/10.1155/2020/6371201>



- Reynolds, (2004). *aloes: The Genus aloe*. CRC Press: Boca Raton .
- Rodríguez D Jasso de, Hernández-Castillo D, Rodríguez-García R , Angulo-Sanchez J L. (2020). Antifungal activity in vitro of aloe vera pulp and liquid fraction against plant pathogenic fungi. *Industrial Crops and Products*. 21:81-87
- Paulsen-Sandfeld Birgitte, Kristine Raaby Jakobsen, Rikke Bæk, Birgitte Holst Folkersen, Torben Riis Rasmussen, Peter Meldgaard, Kim Varming, Malene Møller Jørgensen and Boe Sandahl Sorensen, (2016). Exosomal Proteins as Diagnostic Biomarkers in Lung Cancer. *Thorac Oncol*. 2016 Oct; 11(10):1701-10.
- Yoon Jin Gu , Jung Yoon, Joon Young Song, Soo Young Yoon, Chae Seung Lim, Hye Seong , Ji Yun Noh, Hee Jin Cheong and Woo Joo Kim, (2020). Clinical Significance of a High SARS-CoV-2 Viral Load in the Saliva. *J Korean Med Sci*. 2020 May 25;35(20):e195.
- Babu R J, Chen L, & Kanikkannan N (2015) Penetration enhancers and their mechanism studied on a molecular level. *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*:133–150 <https://doi.org/10.1007/978-3-662-47039-8>
- Surjushe A, Vasani R, & Saple D (2008) aloe vera: A short review. *Indian Journal of Dermatology* 53(4): 163–166 <https://doi.org/10.4103/0019-5154.44785>
- Vogler BK, Ernst E. aloe vera: A systematic evaluation of its scientific effectiveness. *Br J Gen Pract* 1999; 49:823-8.