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

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Abstract

The phytochemical composition of Aloe vera gel and rind was evaluated using GC-MS to determine their bioactive properties and chemical constituents. Results indicated that mature Aloe vera leaves consist of approximately 55–70% inner gel and 30–45% rind by weight. The gel portion is composed of 98.5–99.5% water and 0.5–1.5% solids, whereas the rind contains 88–91% water and 9–12% solids. Overall, the whole leaf has about 3.5–4.5% total solids, with extractable solids accounting for nearly 1%. The principal components identified across samples included ash, free sugars, organic acids, polysaccharides, and proteins, with protein levels ranging from 3.8–8.3% and typically higher in rind than gel. In contrast, protein content in juice powders was notably lower, reflecting losses during filtration and decolorization processes. GC analysis of ethanolic extracts of Aloe vera (EEAV) revealed 31 significant retention time peaks representing over 25 phytochemical compounds. GC-MS further identified 21 bioactive compounds based on retention time, molecular weight, molecular formula, and peak area, including 2-methyl-1-butanamine, N-hexadecylacetamide, 2-methylisobutanamide, and glycyl-di-alanine. Ethanolic extracts from the rind indicated the presence of compounds such as 3-aminopropionitrile, adenosine derivatives, ethyl-2-(diethoxyphosphoryl)-oxy-3,3,3-trifluoropropanoate, and oxazole-based esters. The gel fraction has widespread application in the development of consumer products, including juices, cosmetics, personal hygiene products, and pharmaceuticals. The identification of key compounds such as hexanoic acid, citronellyl butyrate, phytol, myristic acid, palmitic acid, palmitoyl chloride, and octadecanal is significant, given their established roles in flavoring, fragrances, moisturizing creams, shaving foams, shampoos, bathing oils, lipsticks, and perfumed products.

Keywords: Aloe vera, Gel, Rind, Ethanolic extract, GC-MS

Introduction

Aloe vera (Aloe barbadensis Miller) is a perennial succulent belonging to the family Xanthorrhoeaceae, recognized worldwide for its medicinal and therapeutic properties. Native to the Arabian Peninsula, the plant thrives in arid and semi-arid regions and has since been cultivated extensively in Africa, Madagascar, the Caribbean, the Mediterranean, Japan, and the

United States (Alemdar & Agaoglu, 2021). Its thick, dagger-shaped leaves grow in a rosette formation, with each mature plant weighing nearly 2 kg and producing 15 or more fleshy leaves. These leaves contain three distinct layers: an outer green rind, a middle latex layer, and an inner transparent gel that accounts for most of its therapeutic uses (King, Yates, & Greenlee, 2022).

Historically, Aloe vera has been revered as a "plant of immortality." Ancient Egyptian texts dating back to 1500 BC described its healing properties, while Dioscorides, a first-century Greek physician, documented its application in treating wounds, digestive disorders, constipation, and skin ailments. Across cultures, its use has been diverse: in Africa for stomach disorders, in China during the Sung dynasty for eczema, and in India as the "silent healer" believed to have grown in the Garden of Eden (González-Stuart, 2012). Arab traders are credited with spreading Aloe vera across Persia, India, and the Far East.

In modern medicine, Aloe vera has been investigated for a wide range of therapeutic benefits. Its gel is widely recognized for burn treatment, wound healing, and skin repair. Clinical studies have shown that bioactive compounds in the gel accelerate healing, exert antibacterial effects, and stimulate collagen production, thereby reducing scar formation (Davis, Parker, & Samson, 2014; Schinor, Salvador, & Turatti, 2024). The U.S. has even developed topical creams containing 70% Aloe vera extract, designed to preserve damaged tissue and promote regeneration (Yagi et al., 2023).

Emerging evidence also highlights potential systemic applications. For example, drinking Aloe vera juice has been linked to a reduced risk of lung cancer in smokers in Japan, while other studies suggest it induces tumor necrosis factor alpha, which can suppress tumor vascularization (Yagi et al., 2023). Additional therapeutic uses include alleviating gynecological inflammations, respiratory conditions such as asthma, bone tuberculosis, and even slowing aspects of aging.

Phytochemically, Aloe vera contains diverse bioactive compounds. These include anthraquinones such as aloin and emodin with laxative properties (Yoon, Ahn, Lee, Kim, & Yeom, 2020), saponins with cleansing and antiseptic activity (González-Stuart, 2012), salicylic acid for anti-inflammatory action (Paulsen, Korsholm, & Brandrup, 2016), and glycoproteins that promote wound healing (Reuter et al., 2021). Its rich composition of vitamins (C, E), minerals (calcium, magnesium, zinc), polysaccharides such as acemannan, phenolic compounds, and enzymes contribute to its antioxidant, antimicrobial, and immunomodulatory effects (Rodriguez, Alvarado, & Lopez, 2020; Wang et al., 2022).

The gel has broad applications across industries. In cosmetics, it is a key ingredient in moisturizers, sunscreens, and anti-aging creams due to its hydrating and rejuvenating properties (Morton, 2022; Lans, 2021). In hair care, it nourishes the scalp, reduces dandruff, and promotes hair growth (Petrovil, Iivanovi, Mmilovanovi, & Zižovi, 2022). In dentistry, it reduces plaque accumulation, gingivitis, and oral ulcers (Radha et al., 2015). Its immunomodulatory potential is also gaining attention, with polysaccharides shown to enhance macrophage activity and

cytokine production, thereby supporting immune health (Treutlein, Smith, van Wyk, & Wink, 2023; Tian & Hua, 2021).

Processing methods have evolved from isolating only the inner gel to modern whole-leaf processing technologies that prevent contamination from the latex, which contains anthraquinones with strong laxative effects (Reynolds, 2004; Shukla, 2008). Advances in processing ensure higher polysaccharide content and greater therapeutic potential (Jones & Sacamano, 2000).

Overall, Aloe vera remains a plant of remarkable pharmacological and industrial importance. Its bioactive compounds, diverse therapeutic applications, and integration into cosmetics, pharmaceuticals, and food industries underscore its global relevance. However, the precise mechanisms behind its healing effects remain debated, with suggestions ranging from synergistic interactions of polysaccharides to simple moisturizing effects (Knorr, 2022; Paul & Crepeau, 2023). Further research is required to isolate definitive active compounds, clarify their mechanisms, and expand its evidence-based applications.

Experimental

The collected samples was 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 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 0 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 º C. The resulting solution was then stored in a refrigerator at 4 º C for further analysis.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of EEAV will be carried out for the detection of phyto chemicals 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 FeCl3 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 colour 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% NH3 was added to it and observed for the pink, red or violet colour 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 greyish colour (an indicator of the presence of terpenoids).

Test for Alkaloids

About 3 ml MEAV was stirred with 3 ml of 1% HCl on 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.

Tests for carbohydrates

Molisch's test: 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 H2SO4 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 Turbo Mass Perkin Elmer gas chromatogram 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 º C for 5 min, ramp at 120 º C/min to 260 º C and hold up at this temperature for 10 min. Injection temperature was kept up at 250 º C, helium (99.999 %) stream rate as 1.5 ml/min, transfer temperature at 180 º C and particle source temperature at 200 ºC. 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 attained 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.

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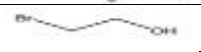
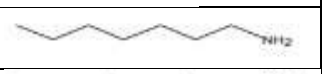
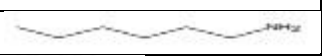
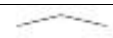
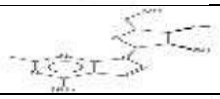
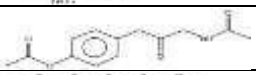
Results and Discussion

Aloe vera gel and rind has been examined for their phytochemical properties as well their chemical composition and using GC-MS and the results are as presented. 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

Table 1. GC-MS Results

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

Table 2. Result of chromatographic analysis

SN	Name of compound	Formula	Molecular weight	Structure
1	3-Butyn-1-ol	C_4H_6O	70.0	
2	Glycil-di-alanine	C5H10N2O3	146.06	
3	2-Bromoethanol	C_2H_5BrO	123.15	
4	1- Heptanamine	C7H17N	115.13	
5	1-Hexanamine	C6H15N	99.13	
6	Propane	C3H8	44.0	
7	1-H-Pyrrole-2,5-dione	C4H3NO2	97.016	
8	2-Methyl-adenosine	C11H15N5O4	281.112	
9	4(-3-acetylamine-2 oxopropyl)phenyl ester	C13H15NO4	249.0	
10	Oleylamine	C18H37N	267.29	
11	1-Butanamine	C4H11N	73.0	

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 1butanamine (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)acatamide (RT: 6.235), Ethanamide, (RT: 35.662), 1-H-pyrrole-2,5-dione, (RT: 6.606), Oleylamide and 3-amino1, 2propanediol, (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-methylethyl,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, 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, haloalkanols like bromoethanol and amino acids like glycil-di-alanine, 1-heptamine, 1-hexamine, 1-butamine and oleylamine, 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-acetylamine-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 conventional method of treating various ailments, among which Aloe vera has been considered & used as a popular folk medicine all across the world, Arun kumar and Muthuselvam, (2009). Considering its use, the Aloe vera is well studied by various scientists in last 4 decades for its countless properties. Being rich in several types of phytochemicals such as Tannins, Carbohydrates, Saponins, Flavonoids, Alkaloids, Anthraquinones, Terpenoids as detected in the present study also it is very well 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 anti-bacterial effects of Aloe vera but it also possess anti-fungal compounds like Undecanoic Acid, Nonanoic acid Rossi et al., (2021). Faustino et al., (2016)

has 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) has tapped to their further uses in cosmetic and personal care products industry. Shukla et al., (2008), had given the hint of use of A. 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, Dahiya, (2020). 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 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 determined. 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 ethanolprecipitated 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 part of the plant for the preparation of various products like juice, makeup items, tissue papers, moisturizers, soaps, sunscreens, incense, shaving cream, and shampoos etc. 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 flavouring 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 and Smith, (2021), producing fragrance in perfume production and as flavour compounds Buchhaupt et al., (2014) has tapped to their further uses in cosmetic and personal care products industry.

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