Article

Identifications of *Aloe vera* fractions and application as a cure for diseases

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Abstract

Many of the health benefits associated with *Aloe vera* have been attributed to the polysaccharides contained in the gel of the leaves. These biological activities include promotion of wound healing, antifungal activity, hypoglycemic or antidiabetic effects anti-inflammatory, anticancer, immunomodulatory and gastroprotective properties. There are several mechanisms which contribute to the immunological protection enjoyed by normal persons. Among these mechanisms the ingestion of bacteria and other potentially harmful agents by certain white blood cells and the formation of antibodies. Aloe gel contains substances which are active both in stimulating phagocytosis as well as stimulating the formation of antibodies. These effects include the potential of whole leaf or inner fillet gel liquid preparations of *A. vera* to enhance the intestinal absorption and bioavailability of co-administered compounds as well as enhancement of skin permeation. In addition, important pharmaceutical applications such as the use of the dried *A. vera* gel powder as an excipient in sustained release pharmaceutical dosage forms were outlined.

Keywords *Aloe vera*; medicine plant; phenolics; flavonoids; LC-MS; psoriasis.

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1 Introduction

For centuries, the gel of *Aloe vera* has been used for healing and therapeutic purposes, and more than 75 biologically active constituents have been discovered in the inner gel. Most of the therapeutic actions of Aloe leaf have been ascribed to the polysaccharide components (Ni et al., 2004). The biological activities of *Aloe vera* gel are believed to be due to synergistic effect of the chemical components present therein instead of a single chemical entity (Avigan et al., 2014). Anthraquinones are the most important secondary metabolites present in *A. vera* gel which aremainly responsible for various biological activities, such as astringent, hemostatic, antidiabetic, antiulcer, antibacterial, anti-inflammatory, antioxidant and anticancer properties, and also effective in treating gastrointestinal disorders (e.g., constipation, dysentery and diarrhea), radiation injury,

wounds, and burns (Rabe and Staden, 1997; Tan et al., 2011). Currently, *A. vera* is widely used to produce skin care products, cosmetics and nutraceuticals (Gordon and David, 2011).

The *A. vera* is known as a traditional folklore medicine for the treatment of many diseases. It is considered to be the herbal answer to support the health and healing mechanisms of the body. Pharmacologically, it is an immunity booster and detoxifies the mammalian system. It is recommended for adjuvant therapy with antibiotics to eliminate drug-induced gastritis and other adverse effects. It has been claimed that the polysaccharides present in *A. vera* gel havetherapeutic properties, such as immunostimulatory, anti-inflammatory, antioxidant, wound healing, radioprotective, antibacterial, antiviral, antifungal, antidiabetic and antineoplastic activities (Yagi et al., 1999).

A dermatological condition, known as dry skin, is observed mainly in patients with atopic dermatitis (Sator et al., 2003). In these cases, useful cosmetic products must be applied to improve skin hydration. These do not serve only the aesthetic purposes, but also maintain the normal conditions of skin. Studies have shown that *A. vera* extract improves skin moisture by humectants mechanism. Mucopolysaccharides of *A. vera* help in retention of moisture into the skin. Hence for improving skin hydration, *A. vera* freeze-dried extract is a valuable natural product. It is being used widely in commercial moisturizing formulations in cosmetic industries as well as for the treatment of dry skin due to presence of amino acid histidine, arginine, threonine, serine, glycine and alanine, which may improve water retention in the stratum corneum (Dal'Belo et al., 2006). Most in vitro studies on skin protection study the ability of *Aloe vera* and active compounds inwound healing. The immortalized human keratinocyte HaCaT cell line, the primary normal humanepidermal keratinocytes HEKa cell line, and fibroblasts cell lines are the most used. These studies haverevealed that *Aloe vera* and its major compounds (aloesin, aloin, and emodin) exert their protectiveaction mainly through antioxidant and anti-inflammatory mechanisms. Hence, *Aloe vera* up-regulatedTFGβ1, bFGF, and Vegf-A expression in fibroblasts and increased keratinocyte proliferation anddifferentiation by lysosomal membrane stability. Moreover, Aloe vera solution could acceleratecorneal wound closure at low concentrations (≤175 μg/mL) by increasing type IV collagen-degradingactivity in a cellular model of primary cultures of corneal epithelial cells (Hormozi et al., 2017; Negahdari et al., 2017; De Oliveira et al., 2018; Teplicki et al., 2018; Sánchez et al., 2020).

This study aims to identify the fractions of phenolics and flavonoids of Aloe vera using LC-MS. These compounds are known to have several activates against numerous diseases.

2 Materials and Methods

2.1 Plant materials

Aloe vera, often called "cactus-like plant", was purchased from "Special Garden".

2.2 Extraction procedure

Aloe vera is a versatile plant that has umpteen benefits, and it is highly effective. As it is one of nature's great healers. It helps in soothing irritation like "eczema" and heals all kinds of wounds like sunburns and any sort of inflammation. *Aloe vera* gel can be mixed with other ingredients to help preserve it for more than a few days.

Steps to make *Aloe vera* gel.

1. Wash your hands and wash all the tools (to make sure that tools are clean, and the gel doesn't get contaminated).
2. Slice off an outer leaf of an Aloe plant (use a sharp knife to make a clean cut near base).
3. Drain the resin (dark yellow substance) for 10 minutes (As resin contain latex which causes irritation).
4. Peel the leaves (use the peeler to peel away green portion and cut inner white layer to gel underneath).
5. Scoop the gel out with the spoon or knife.

6. Mixing the gel with a natural preservative (mix in 500mg powdered vitamin (C) or 400 IU vitamin(E) for every ¼ cup of the gel) then put them in blender to blend them well and the gel will like a foamy.
7. Put the gel in sterilized , clean jar.
8. Use the gel.

The gel of *Aloe vera* (5 g) was separately extracted by maceration with 10 ml methanol using a shaker apparatus at room temperature for 24 h. The residual was rinsed with 50 ml of solvent and the recovered fractions were filtered and evaporated completely under vacuum.

2.3 Sample preparation

The gel materials of *Aloe vera*was transferred into a screw-capped extraction tube and extracted with 10 ml of 70% aqueous methanol at 75ºC for 60 min. After centrifuging at 4000 rpm for 5 min, 3 ml of supernatant was transferred to a 5 ml vial. The solution was filtrated through a syringe filter of 0.2 μm membrane for quantification of apigenin and apigenin 7-glucoside in plant material by LC-MS.

2.3.1 Alkaline hydrolysis

Apigenin is normally present both free and as esters with glycoside in this herb. For measurement of total apigenin 7-glucoside, the herb and extracts were separately subjected to alkaline hydrolysis according to the procedure reported in British Pharmacopoeia (British Pharmacopoeia, 2010). In this process, various acetylated derivatives of apigenin 7-glucoside are converted to apigenin 7-glucoside. The hydrolysates were filtrated through a syringe filter of 0.2 μm membrane to assay total apigenin 7-glucoside by UPLC.

2.3.2 Acid hydrolysis

A simple approach to obtain the aglycone is mild acid hydrolysis of the samples which releases the glycoside moiety without promoting decomposition of the remaining aglycone skeleton. Hydrolysis of apigenin glycosides in the extracts and herb were performed in acidic medium following the procedure described in the literature with minor modifications (Haghi and Hatami, 2010). A 100 mg of herb or 20 mg of dry extract were separately hydrolysed with 10 ml of a mixture of methanol: HCl (7:1) at 100°C in a caped tube for 2 h. The solution was cooled, centrifuged for 5 min and filtered through a 0.2 μmfilterfor the measurement of total apigenin by UPLC method.

2.4 Total phenolic content (TPC)

The total phenolic content of were aqueous-methanolic extract of *A. vera* quantified by Folin-Ciocalteau reagent and concentration of phenolic content was expressed as mg of GAE/g. For quantification of total phenol, 0.1 mL of membrane processed samples, added 3.9 mL of distilled water followed by 0.5 mL of freshly prepared Folin's reagent. The reaction mixture was incubated at room temperature (28±2°C) for 3-4 min. To this added 2 mL of 20% Sodium carbonate ($NaCO_3$) and kept at boiling water bath for 1-2 min. The blue color formed was read at 650 nm by using an UV-Vis spectrophotometer (Beckman DU-530). Data were reported as mean ±SD for three replicate measurements (Singleton and Rossi, 1965).

2.5 Total flavonoid content (TFC)

Stock solution of quercetin (0.5 mg/ml) prepared in methanol was diluted with 80% (v/v) ethanol (ranging from 25 to 100 μg/ml) to construct the calibration curve. A 0.5 ml of standard solutions or the crude extract were separately transferred into a 5 ml volumetric flask containing 2 ml of 80% ethanol, 0.1 ml of 10% (w/v) aluminum chloride and 0.1 ml of 1 M potassium acetate, diluted with distilled water to the mark and mixed. After 30 min, the absorbance was measured at 415 nm with a Perkin-Elmer Lambda EZ-210 spectrophotometer. The TFC in the crude extracts (1 mg/ml) were determined as above method. TFC expressed as g of quercetin equivalent per 100 g of dry material (Chang et al., 2002).

3 Results

3.1 Determination of total phenolic and total flavonoid contents

The dry extract yield of dry material was methanol extract. The total phenolic and total flavonoid contents were calculated on the basis of gallic acid and quercetin and expressed as equivalents of gram gallic acid and quercetin per 100 g of dry material, respectively. The amounts of total phenolics and total flavonoid are shown in Fig. 1.

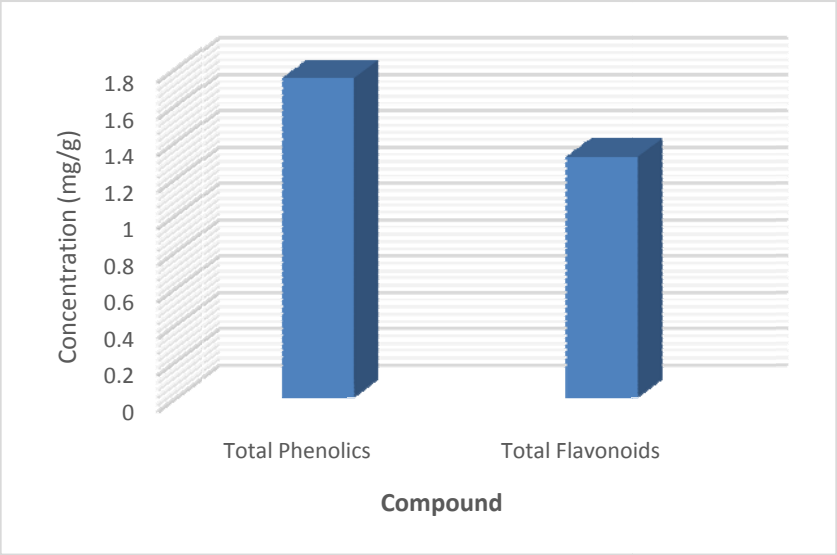

Fig. 1 The total phenolics and flavonoids of *Aloe vera* plant.

3.2 Estimation of the phenolics and flavonoids fractions

The identification of the total fractions (phenolic and flavonoid) in chamomile plant were identified using LC-MS analysis. The identified fractions were tabulated in Table 1. Some of the fractions were illustrated in Fig. 2.

Table 1 The identification of the different compounds in *Aloe vera* plant.

Fraction No.	Fraction/Compound	Fragment size m/z
1	Isoaloeresin D	237
2	7-O-methyl aloeresin A	375
3	Aloenin	293
4	Aloesin	245
5	Aloin B	268
6	Aloin A	297
7	8-C-glucosyl-R-aloesol	229
8	7-O-methyl aloesin	240
9	8-O-methyl-7-hydroxyaloin	336
10	Aloeresin A	299
11	Dihydroisocoumarin glucoside	458

12	Homonataloin B	271
13	Aloeresin E	523
14	Homonataloin A	271
15	Aloinoside B	419
16	Aloinoside A	211
17	Microdontin A	299
18	Microdontin B	228
19	2'-O-Feruloylaloesin	510
20	8-C-(20-O-coumaroylglucosyl)-7-hydroxy-5-methyl-chromone-2-carboxylic acid	485

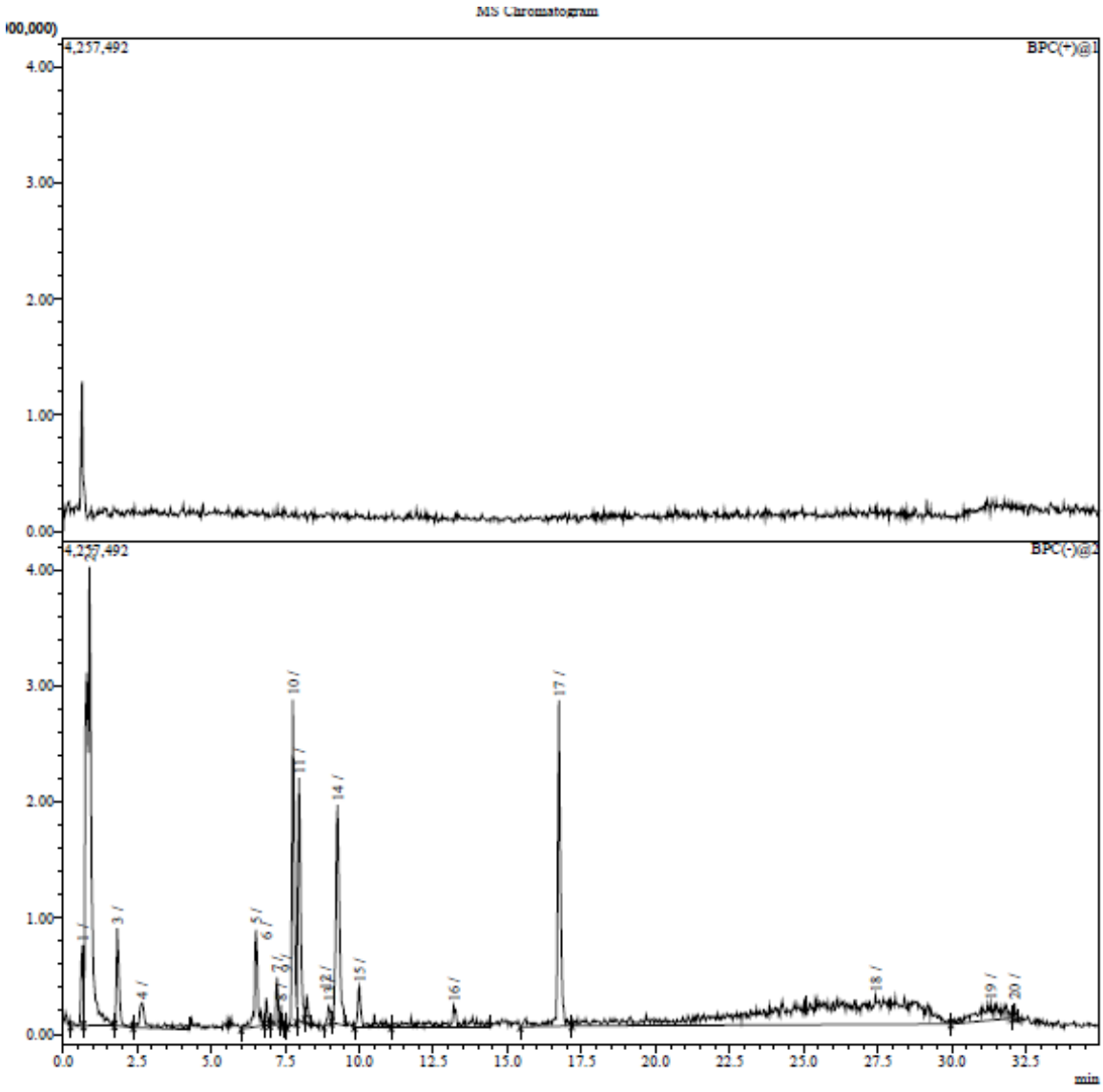

Fig. 2 The MS chromatogram of the fractions of *Aloe vera* plant.

3.3 The total extracted gel

The extracted gel was used in the production of gel supplemented with vitamins for treatment of eczema disease. The general steps for gel preparation were shown in figure 5 and the final product was shown in Fig. 3.

Fig. 3 The general steps for gel preparation.

4 Discussion

There are a number of clinical reports that have found that *A. vera* gel is devoid of therapeutic activities in some of the aforementioned disorders or even to cause undesirable effects, such as retardation of wound healing. As mentioned before, these conflicting results could be due to the use of plants from different locations with variations in their chemical compositions and also because of different isolation techniques that were used to extract compounds from the *A. vera* leaf pulp(Yagi et al., 1999).

Malaria has been reported to cause 2-3 million deaths every year. Most of the deaths occur in children below five years of age and pregnant women. Gradually increasing resistant has been reported in Plasmodium falciparum to standard antimalarials drugs. Some Aloe species have potential for treatment of malaria-related symptoms. Tritriated [3H]-hypoxanthine incorporation assay was used for antiplasmodial activity evaluation of Aloe species. The results indicated that methanol extracts obtained from *A. viridiflora*, *A. wickensii* and *A. speciosa* inhibited *P. falciparum* growth by inhibiting intra-erythrocytic growth of the malaria parasite (Van

Zyl and Viljeon, 2002). Several other investigators have also reported antimalarial activity of *A. vera* (Clarkson et al., 2004).

Many natural products have shown antiviral activity (Kumar et al., 2014). Antiviral activity of *A. vera* is mediated by acemannan. It has direct effect on the cells of the immune system, activating and stimulating macrophages, monocytes, antibodies, and T-cells. Preliminary reports have suggested that consumption of *A. vera* might be helpful to human immunovirus-infected individuals since it enhances the CD4 count and thereby improves the functioning of the immune system (Olatunya et al., 2012).

5 Conclusion

A. vera is widely used in food, healthcare, skincare, cosmetic and pharmaceutical industries as a source of active ingredients withextratherapeutic, hygienic, rejuvenating and health enhancing properties. *A. vera* has high value functional constituents that exhibit remarkable biological and pharmacological functions. It is quite promising natural source for developing multifunctional medicinal agents. *A. vera* has a long history as a medicinal plant with diverse therapeutic applications. Although it has been claimed that some of the biological and pharmacological activities of this plant can be attributed to the polysaccharides found in the leaf gel, it is a daunting task to link individual polysaccharides to specific therapeutic properties. Differences in plant composition due to geographic location as well as differences in gel extraction methods and sample preparation techniques have contributed to discrepancies in the results obtained from many studies in terms of the chemical composition and biological activities of *A. vera* leaf gel.

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