International Food Research Journal 25(4): 1450-1456 (August 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Total phenolic and flavonoid content of membrane processed *Aloe vera* extract: a comparative study

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Article history

Received: 21 April 2017
Received in revised form: 25 June 2017
Accepted: 27 June 2017

Keywords

A. vera
Ultrafiltration
Phytochemical
Total phenolic
Flavonoid

Abstract

The aim of the study was to compare the total phenolic and flavonoid contents of membrane processed *Aloe vera* extract. In ultrafiltration studies, both transmembrane pressure (tmp) of 0.2,0.4, 0.6 and 0.8 bar and membrane molecular weight cut-off (MWCO) of 10 and 30 kDa membranes were employed in this research work. In preliminary phytochemical screening, phenols, tannins, saponin, flavonoids, steroids, terpenoids were qualitatively analyzed. Based on presence of flavonoid and phenolic content in aqueous – methanolic extract were used for further study. Results indicate that, In phytochemical evaluation, 10 kDa MWCO, total phenolic content range from 0.07 and 0.03 mg Gallic acid equivalent (GAE)/g. similarly, 30 kDa MWCO total phenolic content range from 0.05 – 0.027 mg GAE/g at 0.2 – 0.8 tmp. In case total flavonoid content, 0.05 – 0.01 mg quercetin/g in 10 kDa MWCO, 0.033 - 0.013 mg quercetin/g in 30 kDa MWCO respectively. Moreover, uniqueness of these study is, In the field of phytochemical quantification, there is no reports available in this membrane processed *A. vera* extract.

Introduction

A. vera (L.) Burm.f. (*A. barbadensis* Miller) is a perennial xerophyte, which can store water in the leaves to survive in dry land or erratic rainfall areas. The native of *A.vera* was originated in Africa and spread over various areas of Asia, Europe and America (Harding, 1979). The genus *Aloe* contains more than 400 species with *A. barbadensis* Miller *(A. vera), A. aborescens* and, *A. chinensis* being the most popular among all other species. By comparing biological properties of *Aloe s*pecies, *A. barbadensis* Miller is considered to be the most biologically active (Bozzi *et al.*, 2007). *A. vera* has been used throughout history in folk medicine as valuable ingredient for the food, pharmaceutical and cosmetic industries (Lanjhiyana *et al.,* 2011). *A. vera* is most widely accepted and used for various medical and cosmetic purposes (Miladi and Damak, 2008). Apart from *A.vera* being used extensively in the pharma industry, it has been described for centuries for its laxative, anti-inflammatory, immunostimulant, antiseptic (Okyar *et al*., 2001), wound and burn healing (Chithra *et al.*, 1998), antiulcer (Koo, 1994), antitumor (Saito, 1993), and antidiabetic activities (Bunyapraphatsara *et al.*, 1996).

In leaves, innermost part is a clear, moist, and slippery tissue that consists of large thin-walled parenchyma cells (Dabai *et al*., 2007). Leaf parenchymatous tissue of *A. vera* has been used for its medicinal properties, which enriched with polysaccharides (Boutagy and Harvey,1978; Rice-Evans, 2004). In the field of medicine, for both the *A. vera* gel and whole leaf extract shows good pharmaceutical properties and recently used to improve the bioavailability of co-administered vitamins in human subjects (Azam *et al*., 2003). Fresh aloe juice from the inner leaf parenchyma contains 96% water, polysaccharides consisting mainly of D-glucose and D-mannose, tannins, steroid, enzymes, plant hormones, amino acids, vitamins and minerals (Mohamed, 2011). Presence of different phytoconstituents such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols is main curative properties of most of medicinal plants etc (Lalnundanga and Lalrinkima, 2012). Apart from phytochemicals, more than 200 different molecules were present in *A. vera* (Davis, 1997). The total solid content of *A. vera* gel is 0.66% and soluble solids are 0.56%. *Aloe* gel contain 55% of polysaccharides, 17% of sugars, 16% of minerals, 7% of proteins, 4% of lipids and 1% of phenolic compounds. The *A. vera* gel contains many vitamins including the important antioxidant vitamins A, C and E. Vitamin B_1, niacin, Vitamin B_2, choline and folic acid (Lawless and Allen, 2000).

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A.vera consists of high content of phenolic compounds, glycosides (aloins), 1,8-dihydroxyanthraquinone derivatives (aloe emodin), beta-1,4 acetylated mannan, mannose-phosphate and alprogen glucoprotein. Phytochemical analysis revealed the presence of alkaloid, carbohydrate, tannin, steroid, triterpenoid in *A. vera* extracts by HPTLC method (Patel *et al*., 2012). Among these phytoconstituents, flavonoid play a major role in antioxidants potential and shows protective potential against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumor (Aiyegoro *et al.*, 2010; Hussain *et al.*, 2011; Patel *et al*., 2011; Eleazu *et al.*, 2012). Similarly phenolic also act as good antioxidant potential. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers, and metal chelators (Scartezzini and Speroni, 2000; Ivanova *et al*., 2005; Kiselova *et al.,* 2006).

In filtration and membrane separation studies, using ultrafiltration method a clear and stable aloe gel juice was obtained by using membrane pore size of 100,000 Da, Aloe powder with moisture content below 4% and prepared freeze dried powder from ultra-filtration and reverse osmosis of concentrated *A. vera* gel (Qian, 2002). By using ultrafiltration membrane, Aloe polysaccharides with desalination (Jian and Fen, 2009). *A. vera* gel processing, both quality and safety assurance were studied at the time of *A. vera* jel juice preparation. In this process, reception of raw materials, filleting operation, grinding or homogenization, pectolytic enzyme addition, filtration, addition of vitamin C and citric acid, dearation, pasteurization, flash cooling, and storage were studied (He *et al*., 2005)

In view of these considerations, the present study was carried out to estimate total phenol, total flavonid contents of membrane processed *A. vera* extract. In this connection, by studying effect of tmp and MWCO. The overall objective has been to develop a novel qualitative and quantitative technique, and concentration, separation of phytochemicals such as total phenolic content and flavonoid content, which can help the quantification of different phytochemicals from *A. vera* herbal extract.

Materials and Methods

Chemicals

Methanol, Aluminium chloride, Sodium hydroxide, Sodium chloride and Sodium carbonate were obtained from SD Fine - Chemicals Limited, India. Folin-Ciocalteau reagent, Quercetin and gallic acid standards were obtained from Hi-Media Chemicals, Mumbai, India.

Sample collection

A. vera were collected from local market, Saravanampatti, Coimbatore – 641 049, Tamil Nadu, and the plant parts washed with tap water and dehydrated (in a chamber below 30- 35°C for 48 h), powdered with a mechanical grinder and stored in an air-tight container for phyto-chemical extraction.

Preparation of A. vera *extract*

A. vera aqueous-methanolic extracts were prepared by adding 1 g of dry powder of *A. vera* in 100 mL aqueous methanol (1:1 ratio), further stirring at 150 rpm (Steelmet incubator shaker, India) at room temperature (28±2°C) for 3 h. similarly, aqueous and methanolic extract were prepared. Insoluble residues and debris from the mixture were removed by centrifugation at 8,000 X g for 10 min and the clear supernatants were collected and concentrated. The extracts were stored at 4°C in plastic vials, till further use. All the estimations and experiments were performed in triplicates.

Phytochemical analysis

The aqueous-methanolic, aqueous and methanolic extracts of *A. vera* subjected to qualitative phyto-chemical analysis. Presence of phenols, steroids, glycosides, saponins, flavonoids, phlobatannins, terpenoids, alkaloids and reducing sugars were determined by various phyto-chemical tests (Trease and Evans, 1989; Sofowora, 1993; Harborne, 1998).

Ultrafiltration unit and procedures

The membrane system used was a polysulfone hollow fiber module (PALL Biosciences, USA) with a fiber diameter and length of 1 mm and 30 cm, respectively. The membrane system consisted of an 8l stainless steel jacket-feed tank and a variable- feed pump (Leeson, USA) and transducers (MBS 3000, Danfoss, Denmark) were used for measuring the pressure of the feed, retentate and permeate.

In studying the effect of Transmembrane pressure (tmp) and molecular weight cut-off (MWCO) of 10 and 30 kDa on the Phyto-chemical constituents of *A. vera* extract, the experiments were carried out and both retentate and permeate were collected separately. Initially, two liters of aqueous-methanolic extract were used for each MWCO (10 and 30 kDa) membrane. The experimental operating conditions included a constant cross-flow velocity of 1.2 ms-1, room temperature (28 ± 2°C) and variable tmp

of (0.2,0.4,0.6,0.8 bar) and 2 different MWCO (10 and 30 kDa) were studied (Figure 1). The cross-flow velocity and tmp were controlled using a needle permeate valve and a variable speed-feed pump. The optimum condition obtained was then employed for estimation of total phenolic, flavonoids in membrane processed *A. vera* extract (Aporn *et al*., 2010).

Estimation of total phenolic content

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).

Estimation of total flavonoid content

Total flavonoid content was estimated and expressed as mg quercetin / g of plant tissue. For flavonoid estimation, 0.1 mL of membrane processed samples were taken and made up to 5 mL with distilled water. 0.3 mL of 5% Sodium Nitrite ($NaNO_2$) was added. 3 ml of 10% Aluminum Chloride $AlCl_3$) was added after 5 minutes and were shaken well. 2 mL of 1M Sodium Hydroxide (NaOH) was added after 6 minutes and the absorbance was read at 510 nm (Sathishkumar *et al*., 2013).

Statistical analysis

Each assay was performed three times. All data obtained were subjected to analysis and means were compared and presented as mean ± SE.

Results and Discussion

Phytochemical analysis

In addition to the estimation of total phenolic and flavonoid content aqueous-methanolic extract of *A. vera*, phytochemical analysis were carried out. The results of phytochemical analysis showed in Table 1. In phytochemical analysis, tests for phenols, tannins, quinones, flavonoids, steroids, terpenoids, glycosides, saponins were carried out after extraction with aqueous-methanolic extract. These phytochemicals and other aromatic aromatic

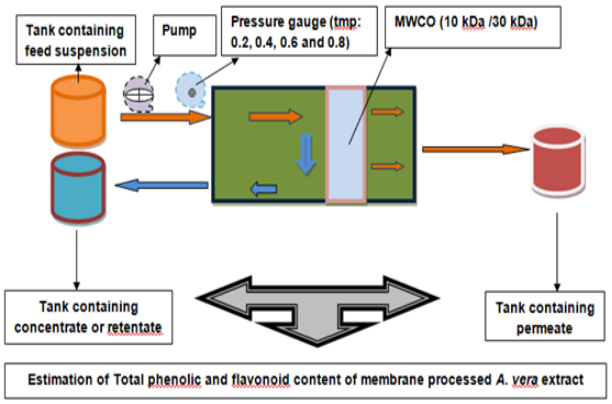

Figure 1. Ultra filtration process: Overall flow operation

compounds directly involved in defense mechanism against insect, pathogens and other herbivore (Shihabudeen *et al.*, 2010). Apart from defense properties of most of the medicinal plants, medicinal properties also determined based on presence of those phytochemicals (Lalnundanga and Lalrinkima, 2012).

Total phenolic content

Total phenolic content of the membrane processed samples was measured using the Folin-Ciocalteu method and the results are presented in Figure 2 and 3. There was a wide range of phenolic content in both permeate and retentate of both MWCO 10 and 30 kDa. In 10 kDa membrane processed permeate phenolic content value range from 0.07–0.03 mg GAE/g at 0.2 – 0.8 tmp (bar). Similarly, retentate shows 0.05- 0.03 mg GAE/g. In case of MWCO 30 kDa, When comparing total phenolic content of both permeate and retentate, permeate shows higher phenolic content in two different MWCO membrane processed *A. vera* extracts. In case of retentate, phenolic content range from 0.043– 0.03 mg GAE/g in 30 kDa membrane processed samples. Similarly, in permeate, total phenolic content range from 0.05 – 0.027 mg GAE/g at 0.2 – 0.8 tmp (bar).

Total flavonoid content

Estimation of total flavonoids is directly related to the formation of complex between $AlCl_3$ and flavonoid that produces a yellow colored complex. This complex absorbance is measured spectrophotometrically at maximum wavelength of 415 nm. Total flavonoid content of the membrane processed samples was estimated and results are presented in Figure 4 and 5. In comparison with *A.vera* extracts, there was a wide range of total flavonoid content in both 10 kDa and 30 kDa membrane processed samples. In 10 kDa membrane processed samples, range of total flavonoid content is 0.05 – 0.01mg quercetin/g. In increasing transmembrane pressure from 0.2 to 0.8

Table 1. Phytochemical screening of various extracts of *A. vera*

Phyto-chemical constituent	Aqueous extract	Methanolic Extract	Aqueous methanolic extract
Phenols	+	++	+++
Tannins	+	++	++
Saponins	+	–	+
Flavonoids	+	++	+++
Steroids	+	+	+
Terpenoids	+	+	+
Cardiac glycosides	-	-	-
Anthroquinones	-	-	-
Phlobatannins	-	-	-

(+++) appreciable amount; (++) moderate; (+) trace amount; (-) completely absent

tmp, both permeate and retentate total flavonoid content increased from 0.01 to 0.05 mg quercetin/g. In case of 30 kDa MWCO, total flavonoid content of retentate is drastically reduced from 0.051 to 0.016 mg quercetin/g, but in permeate it was high in 0.4 tmp (0.033 mg quercetin/g) and low in 0.8 tmp (0.013 mg quercetin/g). When compared with 10 kDa MWCO, concentration of total phenolic and flevonoid content increased. It might be, increasing tmp (bar) from 0.2 – 0.8 and moreover, membrane pore size (30 kDa MWCO), directly influence the concentration of total phenolic and flavonoid content in permeate and retentate.

In these studies, we estimate total flavonoid and phenolic content of membrane processed *A. vera* extracts. In estimation of phenolic content, by comparing total phenolic content of both permeate and retentate, permeate shows higher phenolic content in two different MWCO membrane processed *A.vera* extracts. In general, by comparing other medicinal plants, *A. vera* had significantly lower concentration of total phenolic in ethanolic extracts ((0.11±0.01 GAE)/g) Vidic *et al.*, 2014). Yield of total phenolic content is determented by samples as well as solvent system. Previous findings (Miladi and Damak, 2008; Kammoun *et al*., 2011) showed that content of phenolics is low in aqueous extract (2 mg GAE/g), while in chloroform-ethanol extract, total phenolic content was about 40 mg GAE/g. Similarly, reports from Zheng and Wang (2001) also observed lowest phenolic content (0.23 mg of GAE/g of fresh weight) among 39 plants used for their study. Abdulbasit, (2014) reported that, Aqueous extracts of *A. vera* showed the highest phenolic content (5477.53 mg GAE /100g). Similarly, Methanolic plant extract of A. barbadensis shows only 2.34 ±0.370.13 mg GAE/g of phenolic content when compared with 19 dietary

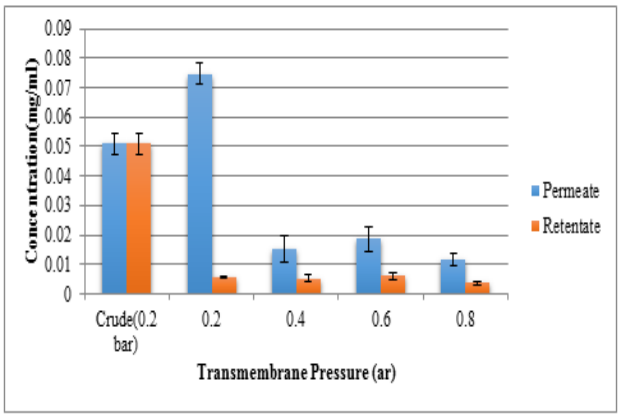

Figure 2. Total phenolic content of membrane processed *A. vera* extract using molecular weight cut-off of 10 kDa

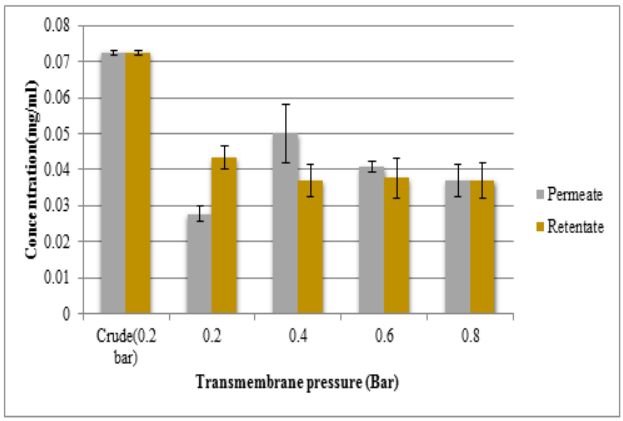

Figure 3. Total phenolic content of membrane processed *A. vera* extract using molecular weight cut-off of 30 kDa

plants (Tupe *et al.*, 2013). Another report shows that, total phenolic content of the methanolic extract of *A. vera* found to be 38.94 ± 7.64 mg GAE/g (Shashank and Vidhya, 2011).

Total flavonoid content was estimated and expressed as mg quercetin / g of plant tissue. Vidic *et al.* (2014) reported that, in comparison with plant extracts, commercial product of *A. vera* had significantly lower concentration of total flavonoid content (0.005±0.003 GAE)/g). Previous results published by Hu *et al.* (2003), showed that 3 years old *A. vera* plant had significantly higher levels of polysaccharides and total flavonoids than 2 and 4 years old *A. vera* plant and no significant differences in total flavonoid levels were found between 3 and 4 year old *A. vera*. Similarly, methanoic extract of *A. vera* showed the highest content of total flavonoid 1958.27 mg QE/100g when compared with 10 different arabian herbs and spices (Abdulbasit, 2014). Another reports shows, total flavonoid content of methanolic extract of *A. vera* was found to be 14.10 ± 1.60 mg catechin equivalents/g (Shashank and Vidhya, 2011).

In these studies, uniqueness is, no one reported

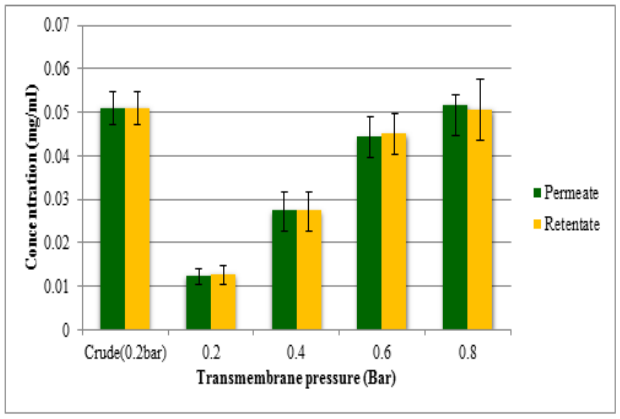

Figure 4. Total flavonoid content of membrane processed *A. vera* extract using molecular weight cut-off of 10 kDa

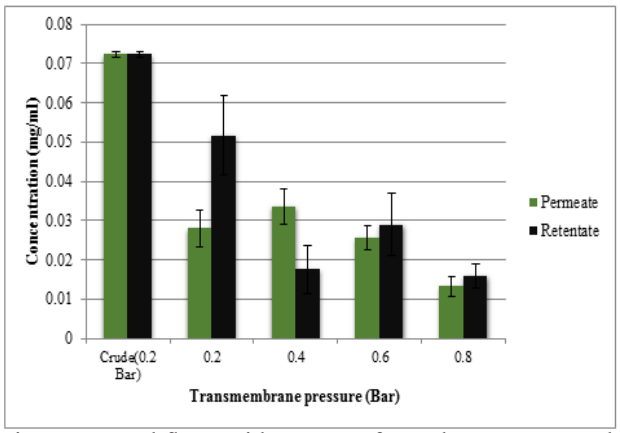

Figure 5. Total flavonoid content of membrane processed *A. vera* extract using molecular weight cut-off of 30 kDa

earlier in the area of estimation of total phenolic and flavonoid content of methanolic extracts of *A. vera* by using two different MWCO (10 and 30 kDa) as well as various tmp (0.2, 0.4, 0.6 and 0.8 bar)

Conclusion

In this study, total phenolic and flavonoid contents of membrane processed *A. vera* extract were carried out. Tmp of 0.2, 0.4, 0.6 and 0.8 bar and MWCO of 10 and 30 kDa membranes were employed in this study. Initially, phytochemical screening were carried out, from this study, phenols, tannins, saponin, flavonoids, steroids, terpenoids were qualitatively analyzed. Further estimation of total phenolic and flavonoid content were estimated based on the concentration. In comparison with tmp and MWCO, total phenolic and flavonoid content of *A. vera* extracts, both permeate and retentate did not shows any significant differences, its may be due to both operating conditions influence the concentration of total phenolic and flavonoid content as well as compound denature. In summary, from these results we can conclude that extraction and separation condition had very important role, because during separation as well as extraction process, there is chance of degradation of compounds could occur. In the final conclusion, both extraction conditions and optimizing operating conditions, we can retain activity of total phenolic and flavonoid content of *A. vera* extracts. However in future, further studies may be focused to isolate specific compounds with improved activity. In the field of medicine, we can go for membrane based separation or concentration of clinically important phytochemicals from *A. vera* extracts.

Acknowledgements

We thank Dr. M. Shanmugaprakash, Assistant Professor (SrG) and Mrs. S. Nithyapriya, Assistant Professor (SrG) for helpful suggestions and critical comments. The authors would like to thank the management of Kumaraguru College of Technology for providing research facilities.

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