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PROSPECTS OF ALOE VERA JUICE IN SUPPLEMENTING NUTRITION AND ANTIOXIDANTAL PROPERTIES

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Abstract

Fresh *Aloe vera* leaves were collected from Lucknow and adjacent areas. The aloe leaves were washed with distilled water and then kept dry at 50°C to obtain the constant weight of leaves. After preparing aloe vera juice, protein, carbohydrates, fat, vitamins, and minerals contents were estimated. For the determination of Total Phenol Content (TPC), Total Flavonoid Content (TFC), saponins and terpenoids, dry powder of aloe vera juice prepared, which were undergoes Soxhlet apparatus in two different solvents i.e. methanol and ethanol and further concentrated using rotatory vacuum evaporator. The mean values of methanolic and ethanolic extract were then statistically analyzed at 5% level of significance by paired t-test. Total Phenol

Content (g /100g), Total Flavonoid Content (g /100g), Saponin Content (g /100g) and Terpenoids content (g /100g) of extract of Aloe vera were respectively 2.39, 3.85, 1.10 and 2.62. Total Antioxidant Capacity (TOAC) was determined using fresh Aloe vera leaves and extracted in 99% methanol of dry mass respectively. The study showed Aloe vera is good source nutrition and antioxidants.

Key Words: *Aloe-Vera; Nutritional quality, Total Phenol Content; Total Flavonoid Content; Antioxidant Activity; saponins, terpenoids.*

1. Introduction

Aloe vera (Aloe barbadensis Miller) is part of the Liliaceae family, which includes more than 360 different species found in arid regions of Africa, Asia, Europe and the Americas. Recently, the family has been named Aloaceae (Eshun and He, 2004). Aloe vera is widely used as a natural treatment and alternative therapy for a variety of ailments, and several studies have suggested the medicinal, aesthetic, and nutritional benefits of this vegetable (Femenia et al., 1999, 2003; Simal et al., 2000; Hu et al., 2003; Chang et al., 2006; Hernández et al., 2006; Vega et al., 2007).

Like other fruit and vegetables, Aloe vera is also rich in vitamins (E, C and A) and has a low fat and high fiber content (Eshun and He, 2004), which is responsible for its healing properties and beneficial effects. Vitamin E (tocopherol) is widely distributed in the plant kingdom, especially in cereals, where tocotrienol predominates, except in maize and soybeans, where tocopherols b and c predominate, and vegetable oils rich in tocopherols a and c (Ubaldi et al., 2005) Vitamin E plays an important role in nutrition due to its function as a cholesterol synthesis inhibitor and antioxidant (Yoshida et al., 2006).

Like fruits and vegetables, Aloe vera are rich in antioxidants, it is because of the presence of carotenoids, ascorbic acid, tocopherols, flavonoids and phenolic acids. These bioactive compounds are known to have the ability to reduce free radicals that induce oxidative stress associated with biological complications such as aging, cardiovascular disease, and carcinogenesis (Lee and Shibamoto, 2002; Hu et al., 2003). These Aloe vera based antioxidants are better alternative to synthetic antioxidants (e.g., butylated hydroxyanisole and butylated hydroxytoluene) as well as other synthetic food additives, which has been criticised, mainly due to their potential toxic effects (Garau et al., 2007, McCarthy et al., Singh et al., 2007).

In the present study, Aloe vera based food product will be developed using fleshy leaves and natural ingredients. Afterwards the developed aloe based food will determine for antioxidants and other important nutritional aspects. This novel approach can be forgotten the problems associated with antioxidant and other nutritional deficiency.

2. Material and methods

2.1 Development of nutraceutical juice using Aloe vera

To harvest the *Aloe vera* plant for juice, remove 3-4 leaves at a time, choosing healthy and thick leaves from the outer sections of the plant. Wash and dry the leaves and later trim the prickly edges with a knife. Now separate the interior gel from the outside of the leaf and cut the aloe gel into slices or cubes. To make aloe juice, use 1 cup of liquid for every 2 tablespoons of aloe gel. Include any other ingredients, like fruit, and use a blender or food processor to mix up your drink.

2.2 Nutritional analysis

Preparation of Aqueous Extract

The aqueous extraction is done by taking 5 grams of the aloe vera pulp and mixed with 200 ml of distilled water in a beaker. The mixture is heated on a hot plate at 30°C-40°C and mixed with continuous stirring for 20 minutes. The mixture is filtered using Whatmann filter paper and the filtrate is used for further preliminary nutritional analysis.

2.2.1Total Energy

The caloric value of a foodstuff can be determined by measuring the heat produced when a given amount is completely burnt in oxygen. It is done in a ‘bomb calorimeter’ where the oxygen is put in under considerable pressure. Since it requires a calorimeter of robust construction, it has been called a bomb calorimeter.

2.2.2Total carbohydrate

Carbohydrates are dehydrated by conc.H_2SO_4 to form furfural. Active form of the reagent is anthranol, the enol tautomer of anthrone, which reacts by condensing with the carbohydrate furfural derivative to give a green color in dilute and a blue color in concentrated solutions, which is determined colorimetrically. The blue - green solution shows absorption maximum at 620 nm.

2.2.3 Total Sugars

A 1 mL aliquot of carbohydrate solution was rapidly mixed with 3 mL of concentrated sulfuric acid in a test tube and vortexed for 30 s. The temperature of the mixture was raised rapidly within 10–15 s after addition of sulfuric acid. The solution was cooled in ice for 2 min to bring it to room temperature. Finally, UV light absorption at 315 nm was measured using a UV spectrophotometer. Reference (reagent blank) solutions were prepared following the same procedure as above, except that the carbohydrate aliquot was replaced with distilled deionized water.

2.2.4 Total fat

Fat content is measured by the weight of fat loss of the sample. Soxhlet method is a semicontinuous solvent extraction approach. In this procedure, the sample is soaked completely for 5–10 min in a solvent and then siphoned back into the boiling flask. The Mojonnier test is an example of the discontinuous solvent extraction method.

2.2.5Total Dietary fiber

Total Dietary Fiber measures total dietary fiber using phosphate buffer systems. Duplicate portions of sample of dried (defatted if necessary) foods are gelatinized and partially digested with alpha-amylase and then enzymatically digested with protease and amyloglucosidase to remove the protein and starch present in the sample, simulating human digestion. One portion of the sample is analyzed for protein and the other is ashed. Total dietary fiber is calculated as the weight of the residue minus the weight of the protein and ash, reported as a percentage of the original sample weight.

2.2.6 Total protein

For the determination of crude protein, 2 gm fine powder of aloe vera leaves was mixed with 10 mL of extraction buffer (100 mM monobasic potassium phosphate, 1% polyvinylpyrrolidone-40 (PVP-40) 2mM EDTA, pH 7.0) and vortex shake till the tissue was homogenized which may takes about 20-25 seconds, then centrifuged for12 min at 4ºC. The supernatant was collected in Eppendorf tubes and stored at -20ºC. Further, the quantification was carried out by the Bradford method (1976).

2.2.7 Vitamin analysis

L-ascorbic acid was determined by the 2,6 dichlorophenol–indophenol (Merck KGaA, Darmstadt, Germany) tritimetric method according to AOAC method No. 967.21 (AOAC, 2000). The vitamin C content in fresh and rehydrated A. vera gel samples was similarly evaluated. A total of 10 ± 0.1 g of triturated sample was weighed, filtered, and diluted to a volume of 50 mL. All measures were done in triplicate; the vitamin C content is expressed as mg AA/100 g d.m.

2.2.8 Mineral analysis

The mineral content was measured in roots, stems, and leaves dried tissue. At the end of pot experiment the mature plants were carefully removed and surface-sterilized, to remove all kinds of impurities sticking their asparagus, and oven dried then analyzed for mineral updates. Mineral content of leaves were measured in mature leaves approximately 20–30 cm of length. The leaves were separated from the main plant. All the plants were placed in a pre-heated hot air oven to dry at 80°C in which the root, stem and leaves of the main plant were kept separately. Further, Na, Ca, Fe, and K content were determined by atomic absorption spectrophotometer. The AAS samples were prepared via acid digestion with H_2SO_4.

2.3 Antioxidant capacity

For estimation of antioxidant activity, Aloe vera peel sample was dissolved in 6 ml of 0.008% methanol solution containing 1, 1diphenyl-2-picrylhydrazyl (DPPH) as radical scavenging agent (Braca et al. 2001).

After the reaction mixture, sample was analyzed through spectrophotometer at 517 nm for examination of antioxidant activity.

2.4 Flavonoid estimation

Aloe vera sample was crushed and dissolved in solvent (1mg/ml) and further 1 ml of 2% aluminium chloride ($AlCl_3$) solution (methanol) was added and mixed well. Afterwards, mixed sample was incubated at room temperature for 1 h. After incubation, sample was examined through spectrophotometer at 415 nm as per Quettier et al. (2000).

2.5 Phenolic Content

The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of Na2CO3 aqueous solution. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at wave length 765nm.

2.6 Saponins

Saponins were extracted in 20% aqueous ethanol by several repetition of this process. The concentrate was transferred in to diethyl ether in separating funnel to obtain two separate layers; out of which aqueous layer was recovered. It is followed many times, n- butanol was mixed, which washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample obtained were dried in oven to the constant weight and the saponin percentage was calculated (Obadoni and Ochuko, 2001).

2.7 Terpenoids

About 10 gm of Aloe vera powdered was taken and soaked in alcohol for 24 hours. It was filtered and filtrate extracted with petroleum ether; this ether extract was treated as total terpenoids (Ferguson, 1956).

Result and Discussion

Aloe vera was collected from Lucknow and adjacent areas which was used to test for the nutritional analysis and various phytochemicals. Fresh Aloe vera leaves were subjected to drying at 50°C and their energy, carbohydrate, fat, protein, fiber and mineral content were analyzed. These findings are in total agreement with those existing in the literature (Dharajiya et al., 2017; Kumar et al., 2016).

Table-1- Nutritional Analysis

S. No.	Parameter	Test Result as per 100 gm	Protocol
1.	Total Energy	73.96 Kcal	By Calculation
2.	Total Fat	0.10 gm	AQAC
3.	Protein	1.10 gm	IS: 7219
4.	Dietary Fiber	14.28 gm	AQAC
5.	Total carbohydrate	17.29 gm	By Calculation
6.	Sugar	0.85 gm	AQAC
7.	Vitamin C	7.38 gm	AQAC
8.	Calcium	28 mg	AAS
9.	Potassium	47 mg	AAS
10.	Vitamin A	0.0IU	AQAC
11.	Iron	2.17 mg	AAS
12.	Sodium	2.90 mg	Spectrophotometer

Total Phenolic Content (TPC)

TPC of methanolic extract of whole Aloe Vera leaves was found to be 2.39 gm/ 100g of dry weight which is shown in Table-2. This finding is in agreement with the study done by Kumar et al.(2016) where the values ranged from 32.9 to 65.7 mg GAE per g of dry weight. And this finding is concomitant with sample from Kerala whose TPC of methanolic extract of whole Aloe Vera plant was 32.9 ± 0.19 mg GAE/ g of dry weight. Maximum values of TPC were obtained for Punjab, Jammu and Himachal accessions. Kerala, Telangana and West Bengal showed low TPC values as compared with other accessions in the study by Kumar et al. (2016). Different agro-climatic conditions have effects on phytochemical diversity and antioxidant potential of Aloe Vera plant (Kumar et al., 2017).

Total Flavonoid Content (TFC)

This study showed Total Flavonoid Content (TFC) of methanolic extract of Aloe Vera to be 3.85 gm QE/ 100 gm of dry weight. The findings of this study is more than the study done by Taukoorah and Mahomoodally (2016) where total flavonoid content of methanolic extract of crude gel was found to be 60.95 ± 0.97µg RE/mg of crude extract. The difference is probably due to the difference in the part of the plant used (Asuk et al., 2015) and also due to difference in the chemical used to prepare standard curve. The flavonoid concentration of methanolic extract of *P. capillacea* was 91.58 ± 3.74QE/mg which is near to the flavonoid content of Aloe Vera (Formagio et al., 2014). Aloe Vera is also concomitant to the study of leaves of *Zapoteca portoricensis* where TFC of methanolic extract was found to 63.67 ± 0.20 mg QE/g (Agbo et al., 2015).This study showed total flavonoids of ethanolic extract of Aloe Vera to be 54.95±2.46 mg QE/g dry weight of extract as shown in Table-2. This result is higher than the study done by Botes et al. (2008) which showed that total flavonoids (mg of CE/100g ± SD) of 95% Aqueous Ethanol Leaf Gel Extracts (ELGE) was 20.2 ± 0.50. Other study showed flavonoid content of ethanol extract of Aloe Barbadensis flower was 13.20 ± 0.09 mg CE/g of dry mass (Debnath et al., 2017). Some flavonoids are antioxidants and have been proved to exhibit a wide range of biological activities like antimicrobial, antiinflammatory, antiangionic, analgesic, anti-allergic, cytostatic and antioxidant properties.

Saponin content

Saponin on the extracted Aloe vera (L.) peel at 800C for 60 minutes was 1.10 gm/ 100 gm. It was decrease compared with saponin from raw material which was 5.43%. Shi et al. (2009) reported that the cooking medium and methods greatly influenced saponin B degradation during cooking. A similar research was carried out by Rickert et al. (2004), the effects of process temperature (250C or 600C) on plant extract which contained saponin lead an increasing number of saponins extracted. There were also similar reported by Alupului et al. (2007), due to process heating at a certain temperature to occur a disorder of mechanical in the cell walls of plant, therefore saponin lead extrication and displacement of the period. The other factors which lead decrease saponin in a material were extraction time. It is also reported that time is influential in the

process of extraction, by increasing the extraction time, quantity and quality of extracted material analyses will lead increased and degradation.

Total Antioxidant Capacity (TOAC)

In the study, TOAC of 99% methanolic extract of Aloe Vera was found to be in good percentage (Table-2). This finding is concomitant to the study done in Ethiopia in green tea where TOAC was 80.0 ± 0.63% (Bizuaychu et al., 2016). The antioxidative potential of plant extracts can be measured using various in vitro assays and each assay is based on at least one feature of antioxidant activity. However, total antioxidant properties of plants cannot be evaluated by any single method because of their complex nature of phytochemicals. Therefore, two or more methods should always be employed in order to evaluate the total antioxidative effects of plant extracts (Gunathilake and Ranaweera, 2016). Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition (Young and Woodside, 2001).

Table- 2- Antioxidant Analysis

S. No.	Parameter	Result as per 100 gm
1.	Total Flavonoid Content	3.85
2.	Saponins	1.10
3.	Tarpenoids	2.62
4.	Total Phenolic Content	2.39

Conclusion

In this work, we have focused on the study of the antioxidant activity of the methanolic and ethanolic extract obtained from Aloe vera. According to the phytochemical tests, flavonoids, steroids, terpenoids, proteins, phenols, carbohydrates, reducing sugar, starch, tannins, glycosides were detected to be present in the leaves of Aloe vera whereas saponin was absent. The result showed Aloe vera is potential plant containing phytochemicals and antioxidant properties of it can utilized in various medicinal preparation and the control of various life-threatening diseases. But the toxicological properties of the plant should be studied further.

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