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Chemical composition and biochemical activity of *Aloe vera* (*Aloe barbadensis* Miller) leaves

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Abstract

Aloe.vera has a long history as a medicinal plant with diverse therapeutic applications. This study was conducted to determine chemical composition and biochemical activity of *A. vera* leaves. Proximate composition (moisture, ash, crude protein, crude lipid and crude fibre), ascorbic acid, superoxide dismutase, catalase, peroxidase, amylase, reducing sugars and total soluble sugars were determined. Moisture content of 97.42 ± 0.13% was observed, while average percent ash, fiber, protein and fat contents were 16.88 ± 0.04%, 73.35 ± 0.30%, 6.86 ± 0.06% and 2.91 ± 0.09% respectively along with traces of ascorbic acid (0.004 ± 0.05%). Variable levels (IU/mg) of superoxide dismutase (802.14 ± 55.6-2830.19 ± 37.09), peroxidase (1.46 ± 0.06-3.72 ± 0.19), catalase (1.56 ± 0.14-2.8 ± 0.19) and amylase (0.97 ± 0.82-24.02 ± 1.5) were observed in the extracts. Total soluble and reducing sugars accounted for 120.68 ± 7.24-363.03 ± 9.25 mg/mL and 97.23 ± 0.05-123.33 ± 0.74 mg/mL. Overall, this investigation has provided a succinct resume of information regarding the chemical composition and biochemical activity of *A. vera* leaves. It would be worthwhile embarking on an intensive scientific experimentation and investigation on this valuable medicinal plant and to promote its large-scale utilization.

Key words: *Aloe vera*, proximate composition, ascorbic acid, superoxide dismutase, catalase, peroxidase

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1. Introduction

There has been a revival of interest in herbal medicines. Plants are the basic source of knowledge of modern medicine. The burgeoning worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consumers and national health care institutions to consider plant medicines as alternatives to synthetic drugs [1, 2]. There are approximately 500 species of the genus Aloe (Lilliaceae). *Aloe vera* is the most widely used specie both commercially and for their therapeutic properties [3-5]. Enzymes carboxypeptidase and bradykinase known to relief pain, an anti-inflammatory compound aloeresin I and dihydrocoumarins with immune-modulatory and antioxidative properties are found in *A. vera* [6, 7]. Some polysaccharides in *A. vera* have therapeutic properties such as immune-stimulation, anti-inflammatory, wound healing, promotion of radiation damage repair, anti-bacterial, anti-viral, anti-fungal and anti-oxidant [8-12]. Several pre-clinical and clinical trials showed a blood glucose and lipid lowering effect for *A. vera* gel preparations [13-16].

The hepatoprotective action of *A. vera* was attributed to antioxidant activity [17]. Aloe gels have the ability to cure gastric ulcers or protect ulcer formation. These anti-ulcer activities of *A. vera* are due to several possible mechanisms including its anti-inflammatory properties, healing effects, mucus stimulatory effects and regulation of gastric secretions [18]. *A. vera* gel has also shown chemo-preventative and anti-genotoxic effects on benzo[α]pyrene- DNA adducts [19-23]. The present study was conducted to determine chemical composition and biochemical activity of *Aloe vera* leaves.

2. Materials and methods

2.1. Collection and Preparation of Test Samples

Aloe vera leaves were obtained from the Botanical garden of the Department of Botany, University of Agriculture, Faisalabad, Pakistan. Samples were identified and authenticated at the Department of Botany, University of Agriculture, Faisalabad, Pakistan. Sample collection was conducted during the months of March and April 2011.The leaves were thoroughly washed with tap water and allowed to dry in an air-circulating oven at 50ºC followed by 105ºC until there were no further changes in weight at these two

temperatures. The powder of each sample was sieved through 300µm mesh and stored in an air - tight cellophane bag as stock sample in a refrigerator till further analysis.

2.2. Chemical Analysis

2.2.1. Proximate Analyses: Proximate composition (moisture, ash, crude protein, crude lipid, crude fibre) of the samples was determined following procedures as described by AOAC [24]. Thermal drying method was used in the determination of moisture contents of the samples. The ash contents were determined using the ignition method. Determination of crude protein was done by first determining the total organic nitrogen using the micro-Kjeldahl method and then multiplied by 6.25 to calculate crude proteins. Crude lipid estimation was performed using Soxhlet extraction technique with n-Hexane solvent**.** Moisture free sample was subjected to acid and alkaline hydrolysis to measure crude fibre contents.

2.2.2. Ascorbic acid: Ascorbic acid was estimated by oxidation reduction reaction using 2, 6 dichlorophenol dye. L-ascorbic acid is oxidized to L-dehydroascorbic acid by the oxidation reduction indicator dye, 2,6-dichloroindophenol. At the endpoint, excess unreduced dye appears rose pink in acid solution [25].

2.3. Biochemical analysis

2.3.1. Superoxide dismutase (SOD): The activity of SOD was determined by measuring its ability to inhibit the photo reduction of nitroblue tetra zolium (NBT) following the method [26] of Giannopolitis and Ries (1977). The reaction solution contained 0.222 g methionine in 15 mL of distilled water, 0.015 g of NBT in 17.5 mL of distilled water, 0.0375 mL of Triton-X in 17.5 mL of distilled water, 0.0132 g of Riboflavin in 17.5 mL of distilled water and 0.2 M buffer. Test tubes containing the reaction solution were placed under UV lamp before adding riboflavin for 15 minutes. The absorbance of the solution was determined at 560 nm. One unit of SOD was defined as "the amount of enzyme that inhibited 50 % of NBT photoreduction".

2.3.2. Catalase and Peroxidase: Activities of catalase (CAT) and peroxidase (POD) were measured using the method of Chance and Maehly (1955) and Liu and Huang (2000) with some modifications [27]. The CAT reaction solution contained 50 mL phosphate buffer (pH 7), 5.9 mL H_2O_2, and 0.1 mL enzyme extract. The reaction was initiated by adding the enzyme extract. Change in absorbance of the reaction solution at 240 nm was read every 20 seconds. One unit of CAT activity was defined as "an absorbance change of 0.01 units per minute". The POD reaction solution contained 50 mL phosphate buffer (pH 5), 20 mL guaiacol, 40 mL H_2O_2 and 0.1 mL of enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 20 seconds. One unit of POD activity was defined as "an absorbance change of 0.01 units per minute". The activity of each enzyme was expressed on protein basis. Protein concentration of the plant extracts was measured by the method of Bradford [28].

2.3.3. Amylase: Amylase activity was determined by standard method [29], in which 0.1 mL of the extract was placed in erlenmeyer flask and 1.5 mL, 2 % soluble potato starch solution containing 500 ppm of calcium ion (cofactor) and 1 mL of tri (hydroxymethyl amino methane/HCl) buffer (pH 7.0) was added. The mixture was incubated with constant shaking at 40^o C for 15-30 minutes. The reaction mixture was measured for reducing sugars by DNS (dinitrosalicylic acid) method.

2.3.4. Reducing sugars: Total sugar content was determined by a modified method [30] was used. 100 µL of extract was taken and 900 µL of distilled water and 1.5 mL of DNS reagent were added to it. The mixture was mixed well and boiled for 5 minutes until dark brown colour was developed. Then 1 mL of Rochelle salt solution was added. These tubes were cooled and absorbance was taken at 510 nm. The amount of reducing sugar in the sample was calculated using a standard graph prepared from working standard glucose solution.

2.3.5. Total soluble sugars: Total soluble sugars contents were determined by modified method [30] using 100 µL of extract taken and 900 µL of distilled water was added to it. One mL of anthrone reagent was added and heated for 8 minutes. The reaction mixture was cooled and absorbance was taken at 630 nm. The amount of soluble sugar in the sample was calculated using a standard graph prepared by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.

3. Results and Discussion

3.1. Chemical Compositions

The results on chemical composition and ascorbic acid (vitamin C) of *Aloe vera* leaves are shown in Table 1. Determination of moisture contents is one of the most fundamental and important analytical procedure. The dry matter that remains after moisture removal is commonly referred to as total solids. This analytical value is of great economic importance to a food manufacturer because water is inexpensive filler [31]. The main feature of all the Aloe leaf was their high water content. Approximately 97.42 ± 0.13% moisture contents were observed. Earlier investigations [32-34] indicated 90-98% moisture in *A. vera* leaf. Ash content represents the total mineral content. Determining the ash content may be important for several reasons. It is a part of proximate analysis for nutritional evaluation. Ashing is the first step in preparing a biological sample for specific elemental analysis. Because certain samples are high in particular minerals, ash content becomes important [31]. Ash contents determined in present study (16.88 ± 0.04%) were quite high than previously estimated 7.12-7.30% previously [33]. Carbohydrates are important in foods as a major source of energy, to impart crucial textural properties and as dietary fiber which influences physiological processes. Non-digestible polysaccharides (all those other than starch) comprise the major portion of dietary fiber. Carbohydrates also contribute other attributes, including bulk, body, viscosity, stability to emulsions and foams, water-holding capacity, freeze-thaw stability,

browning, flavors, aromas and a range of desirable textures. They also provide satiety [31]. *Aloe vera* leaves are rich source of fibres, as these represented greater than 70% proportion (73.35 ± 0.30%) in this study. These results were partially in accordance with the findings of [33], as they observed 60.34-72.17% crude fibres in *Aloe vera* tissues.

Proteins are composed of elements including hydrogen, carbon, nitrogen, oxygen, and sulfur. Nitrogen is the most distinguishing element present in proteins. However, nitrogen content in various proteins ranges from 13.4 to 19.1% due to the variation in the specific amino acid composition of proteins. Generally, proteins rich in basic amino acids contain more nitrogen [31]. The protein content of a lyophilized product indicated a level that corresponds to about 0.013% in commercial aloe gel products [34]. However, a study on compositional features of *Aloe vera* tissues reported 7.56-15.4% crude proteins [33]. This data was similar to our results (6.86 ± 0.06%). Lipids, proteins and carbohydrates constitute the principal structural components of foods. Analysis of lipids in foods is important for accurate nutritional knowledge [31]. On dry matter basis, lipids represented a minor fraction in present analysis (2.91 ± 0.09%). Vitamins are defined as relatively low-molecular weight compounds required in small quantities for normal metabolism. With few exceptions, humans cannot synthesize most of the vitamins. Vitamin analysis of biological samples has played a critical role in determining human nutritional requirements [31]. Presence of important antioxidant vitamin C in *Aloe vera* leaves in present study (0.004 ± 0.05%) was in compliance with previous report [35].

3.2. Biochemical analysis

The data on biochemical activity of *Aloe vera* leaf is summarized in Table 2. Determination of enzymes represents one of several alternatives for exploiting natural substances as antioxidant agents. Oxidase and catalase enzymes have been shown to exert significant antioxidative effects in food systems [36]. Although, catalase (1.56 ± 0.14-2.8 ± 0.19 IU/mg), peroxidase (1.46 ± 0.06-3.72 ± 0.19 IU/mg) and amylase (0.97 ± 0.82-24.02 ± 1.5 IU/mg) represented minor levels, the most prominent feature of biochemical assay was higher SOD measurements (802.14 ± 55.6-2830.19 ± 37.09 IU/mg) in *A. vera* Leaf. [32] and [37] studied same enzymes in Aloe tissues.

Carbohydrates are widely prevalent in the plant kingdom, comprising the mono-, di-, oligo-, and polysaccharides. Aloe polysaccharides pretreatment can attenuate the cerebral ischemia and reperfusion injury in severe traumatic-hemorrhagic rats through inhibiting systemic inflammatory response and leukocyte aggregation and lipid peroxidation in the brain [38]. The amount of soluble sugars detected differed depending on the *Aloe vera* portion. [33] determined 11.22% in skin, 16.48% in the filet and 27.81% of soluble sugars in the gel. Waller et al. [39] identified two monosaccharides, D-glucose and D-mannose together with trace amounts of xylose, rhamnose, galactose and either arabinose or fucose in *A. vera*. Whereas, present study measured 97.23 ± 0.05-123.33 ± 0.74 mg/mL reducing sugars and 120.68 ± 7.24-363.03 ± 9.25 mg/mL total soluble sugars.

Table 1: Chemical Composition of *Aloe vera* leaves

Constituents	
Ash	16.88 ± 0.04
Crude fiber	73.35 ± 0.30
Crude protein	6.86 ± 0.06
Crude fat	2.91 ± 0.09
Ascorbic acid	0.004 ± 0.05

Results are expressed as percentages ± standard deviation on dry matter basis

Table 2: Biochemical activity of *Aloe vera* leaves

Constituents	**Extract concentration**		
	1/10	**1/20**	**1/30**
Superoxide dismutase (IU/mg)	2830.19 ± 37.09	1244.81 ± 70.4	802.14 ± 55.6
Peroxidase (IU/mg)	3.72 ± 0.19	2.32 ± 0.11	1.46 ± 0.06
Catalase (IU/mg)	2.8 ± 0.19	1.81 ± 0.02	1.56 ± 0.14
Amylase (IU/mg)	24.02 ± 1.5	12.33 ± 1.25	0.97 ± 0.82
Reducing sugars (mg/mL)	123.33 ± 0.74	106.28 ± 2.16	97.23 ± 0.05
Total soluble sugars (mg/mL)	363.03 ± 9.25	216.17 ± 6.03	120.68 ± 7.24

Data is represented as mean ± standard deviation of triplicate measurements, 1/10: 1 mL extract/10 mL distilled water, 1/20: 1 mL extract/20 mLdistilled water, 1/30: 1 mL extract/30 ml distilled water

4. Conclusions

A. vera has a long history as a medicinal plant with diverse therapeutic applications. Overall, this investigation has provided a succinct resume of information regarding proximate chemical composition and biochemical activity of *A. vera* leaves. It would be worthwhile embarking on an intensive scientific experimentation and investigation on this apparently valuable medicinal plant and to promote its large-scale utilization.

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