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Phytochemical Screening and DPPH Free Radical Scavenging Activity of *Aloe vera (Aloe barbadensis miller)* Powder

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Abstract

Natural products are obtained from different plants and are considered a potential source of biologically active compounds. The pharmacological and therapeutic properties of these bioactive compounds remain the subject of research. *Aloe vera* is a cosmetic, medicinal and ornamental plant and it is a perennial succulent herb that grows in tropical and subtropical regions of the world. It is considered as a magical plant which has vast benefits in medicinal history. Its medicinal properties make it worthy for so many health aspects. In this study, phytochemical screening and in-vitro antioxidant activity of methanol and water extracts of *Aloe vera* powder was investigated using the DPPH assay. The results of the present study indicated that the dry matter was 2.65% and the water content was 97.35%. The results of antioxidant activity revealed that water extracts exhibited % inhibition (DPPH) 10.23-35.80, while methanol extracts exhibited 12.84-44.96 at concentrations of 20-100 µg/ml and was compared with standard synthetic antioxidant BHT at same concentration. IC50 (µg/ml) for the water extract was 175, and 134 for the methanol extract at 100 µg/mL. Preliminary phytochemical analysis indicated that the extract contained phenols, flavonoids, alkaloids, tannins, quinone, glycosides, carbohydrates, saponins, terpenoids, chlorophyll and proteins. The present study publicized that *Aloe vera* is a promising plant for phytochemicals and natural antioxidants.

Keywords

Aloe vera, DPPH assay, Antioxidant activity, Phytochemical screening

1. Introduction

Medicinal plants are receiving more attention now than ever because they have the potential to have countless benefits for society and humanity in general, especially in the fields of medicine and pharmacology. In recent times, a substantial body of literature has provided sturdy facts to maintain the pharmacological prospective of plants and their extracts in a variety of pathological circumstances [1]. It provide an important source for the searching of new therapeutics or original active drug and promise to address the need to cure diseases that have plagued humanity for centuries.

Efforts have been made over the past few decades to develop effective treatments for different diseases such as cancer, arthritis, diabetes, cardiovascular diseases and many more. Allopathic medicines are very expensive and have crucial side effects on human health. So, it was necessary to go for herbal treatments from healthcare point-of-view. There are so many traditional medicines used all around the world which are based on plant sources including *Aloe vera* as essential ingredient [2]. Different natural products are being prepared and their active ingredients were tested in clinical trials. Over the past decades the reliance on natural sources has been increased due to health benefits.

Aloe vera from family Liliaceae is a shrubby, xerophytic, succulent, perennial, pea- green colored plant. It is cactus like plant which grows mostly in the dry regions of Asia, Africa, America and Europe [3]. *Aloe vera* has tendency to survive in dry environments and 500 species have been characterized uptil now [4]. It likely evolved in the Arabian Peninsula and likely spread to the Mediterranean region as caravans passed through its native habitat [5].

Aloe vera has been extensively used in world for its beauty, health, skincare and medicinal and properties [6]. It is a natural warrior against various creatures' infection and it is a potent antioxidant. *Aloe vera* is also helpful in heartburn, digestion-related issues, asthma, arthritis, diabetes, stress, rheumatic pain, cancers and AIDS. The gel of *Aloe vera* contains glucomannan polysaccharide which is used in cosmetics. Bradykininase which is anti-inflammatory agent is also present in gel [7].

Aloe vera is also best for diabetic patients [8]. It is antiseptic [9], antitumor [10-11], wound and burn healer [12]. It contains antioxidants which increase cell integrity.

Aloe vera also contains anthraquinones which have laxative properties and help in mucous secretion and protect intestinal linings [13]. *Aloe vera* is best moisturizer and helps in soothing dry skin. It is also helpful to avoid skin wrinkling and has anti-acne aspects [14]. *Aloe vera* plant is given in Figure 1.

Oxidation in living organisms is the biological way of energy production and as a result reactive oxygen species are produced which may contribute in aging and so many diseases like cancer, inflammation and degeneration [15-16].

Aloe vera also contains various phytochemicals included flavonoids and polyphenols which help in preventing oxidative stress [17-18]. The oxidation properties of phytochemicals scavenge free radicals by inhibition.

The plan of this research study was to determine antioxidant activity of *Aloe vera* powder in methanol and water extracts and also phytochemicals screening.

Consequently it contain number of biologically active compounds the summary of the class, compounds and their properties of *Aloe vera* are given in Table 1.

2. Materials and Methods

2.1. Plant material collection and cleaning

The fresh plant of *Aloe vera* was collected from PCSIR Laboratories Complex Lahore area and washed with running tap water to remove dirt and dried in an electric oven at 40ºC-50ºC until all plant parts are completely dry. The dried plant material was crushed to fine powder in an electric blender stored in polythene bags and was labeled. Different extracts were then prepared using standard methods described [19].

2.2. Plant materials and preparation of extract

10g of crushed root powder was well mixed with 400 ml of distilled water and methanol in a beaker and was heated at 30°C-40°C on a hot plate. The mixture was mixed by stirring continuously for 20 minutes. The Whatmann filter paper was used to filter the filtrate which was used for further study [20].

2.3. Contents of water and dry matter

The water contents of *Aloe vera* were determined by the method described [21].

Dry matter content (%) = 100 - Water content (%)

Water content (%) = {(w1 – w2)/ w1} × 100

Where; w1= Sample weight before drying

Whereas; w2 = Sample weight after drying

Figure 1. *Aloe vera* plant & its Slices.

Table 1. Review of the Class, Compounds and Properties of *Alove vera*

Class	Compounds	Properties
Anthrones/Anthraquinones	Aloe-emodin, ester of cinnamic acid, emodin, aloetic acid, anthranol, isobarbaloin and barbaloin	Aloin and emodin act as analgesics, antibacterials and antivirals
Carbohydrates	Acetylated mannan, pure mannan, Glucogalactomannan, acetylated glucomannan, galactogalacturan, galactan, pectin substance, cellulose, xylan, arabinogalactan, galactoglucoarabinomannan	Novel anti-inflammatory and anti-allergic properties
Chromones	Neoalosin A, 8-C-glusoly-(2'-O-cinnamoly)-7-O-methlyaloediol A, isoaloeresin D,8-C-glucosyl-(S)-aloesol, isorabaichromone, 8-C-glucosyl-7-O-methylaloediol A, 8-C-glucosyl-noreugenin, 8-C-glucosyl-7-0-methylaloediol	Anti-inflammatory compounds
Enzymes	Amylase, alkaline phosphatese, bradykinase, catalase, superoxide dismutase, oxidase, carboxylase, carboxypeptidase, cyclooxidase, cyclooxygenase, lipase, phosphoenolpyruvate	The related compounds help to breakdown fats and sugars and reduces inflammation of skin
Inorganic compounds	Chlorine, copper, chromium, sodium, calcium, potassium, phosphorous, manganese, iron, magnesium, zinc	They have antioxidant properties and handle various enzymes functions They are essential for the proper
Hormones	Auxins and gibberellins	These help in sore healing process and anti-inflammatory properties
Proteins	Lectins and similar substances	These have anti-inflammatory, anti bacterial and antiseptic properties
Vitamins	Vitamin B12, A, C, folic acid and E. choline	These have antioxidant characteristics

2.4. Procedures for Phytochemical Tests

Freshly prepared *Aloe vera* extracts were subjected to phytochemical analyses of various components, such as phenols, flavonoids, alkaloids, tannins, steroids, quinone, glycosides, carbohydrates, saponins, terpenoids, antiquinones, chlorophyll and proteins [22-27].

I. Tannin Test

2ml of methanolic extract and 2ml of distilled water were mixed and few drops of ferric chloride solution (5% w/v) were added. The green precipitates were formed which showed the presence of tannins.

II. Saponins Test

5ml of methanol extract and 5ml of distilled water were shaken robustly and heated in a test tube. Stable foam was formed indicating saponins.

III. Detection of Chlorophyll

2ml of methanolic extract to 2ml hydrochloric acid (1%) was added and the mixture was boiled. The red precipitates deposition showed chlorophyll presence.

IV. Test for Flavonoids

1ml methanol extract was added to 1ml lead acetate (10%) solution. The yellow precipitates were formed which indicated flavonoids.

V. Anthraquinone Detection

Borntrager test: 3ml of methanolic extract and 3 ml of benzene were well shaken and then filtered. 5ml of ammonia (10%) solution was added to the filtrate. The mixture was shaken well and the ammonia phase (lower part) appears pink, red or violet which showed the presence of anthraquinone.

VI. Test for terpenoids

2ml of chloroform was mixed with 2ml of extract and evaporated to dryness. 2ml of concentrated sulphuric acid) was added and heated for 2minutes. A grey coloration confirmed the incidence of terpenoids.

VII. Steroid Testing

2ml of the extract was mixed with 2ml of chloroform. 2ml of concentrated sulfuric acid was then added to the mix-

ture. The development of red color in the lower layer of chloroform indicated steroids presence.

VIII. Alkaloids Test

3ml of 1% hydrochloric acid was added to 3ml of methanolic extract on a steam bath. Wagner's and Mayer's reagents were added. The turbidity due to precipitates formation indicated alkaloids presence.

IX. Carbohydrates Test

Molisch test: 2ml of Molisch reagent was added to 3 ml of methanolic extract. The mixture was well shaken. 2ml concentrated sulphuric acid was added carefully to the test tube. A purple ring at the junction indicated carbohydrates presence.

X. Glycosides Test

Liebermann test: 2ml of chloroform and 2ml of acetic acid were added to 2ml of extract carefully. The change in color from purple to blue to green indicated the incidence of a steroidal core.

IX. Phenols Detection

2mL of 2% ferric chloride solution was mixed with the extract. The black or blue-green color indicated phenols.

IIX. Proteins Test

Ninhydrin test: 2ml of 0.2% ninhydrin solution was boiled with the methanolic extract of Aloe vera powder. The emergence of violet color indicated the occurrence of amino acids and proteins.

2.5. Antioxidant study by DPPH (diphenyl-2-picrylhydrazyl) radical scavenging assay

DPPH was used to find out the antioxidant activity of the extracts by using method described by Brand-Williams, 1995 [28] with certain alterations [29]. 2.9 mL of DPPH (0.004 % in methanol) was added to 0.1 mL aliquot of the extract solution (100-500 µg/mL) and were mixed well by shaking briskly. The mixture was left to stand for 30 minutes. The absorbance of the resulting solution mix was measured by using UV-visible spectrophotometer (1700, Shimazdu, Japan) at 517nm. The results were articulated as percentage inhibition of the radical DPPH. The antioxidant activity can be calculated by following equation:

Antioxidant activity % = $1 - [A_{sample}/A_{control}] \times 100$

2.6. Statistical analysis:

The descriptive statistics was used to analyze all generated data as [30]. The statistical values including mean and standard deviations were calculated.

3. Results and Discussion

3.1. Water and Dry Matter Content

The moisture and dry matter contents of fresh *Aloe vera* leaves were 97.35% and 2.65%, respectively as shown in Figure 2. The results showed that *Aloe vera* contains mucus so it is rich in water, which keeps it hydrated. It was confirmed from present findings that *Aloe vera* plant leaves are mainly composed of 97.40-99.50% water. This result was similar to the study done by [31], where the water and dry matter contents were 97% and 3%, respectively. The results of current study were also same in which dry matter 2.47% & water content 97.53%. A good determination of water content remains a fundamental and important factor in analytical procedures for various reasons. Since water is an inexpensive load and determining the total amount of water is very beneficial for the manufacture of *Aloe vera* products. The total solids are same as dry matter remaining after moisture analysis [32].

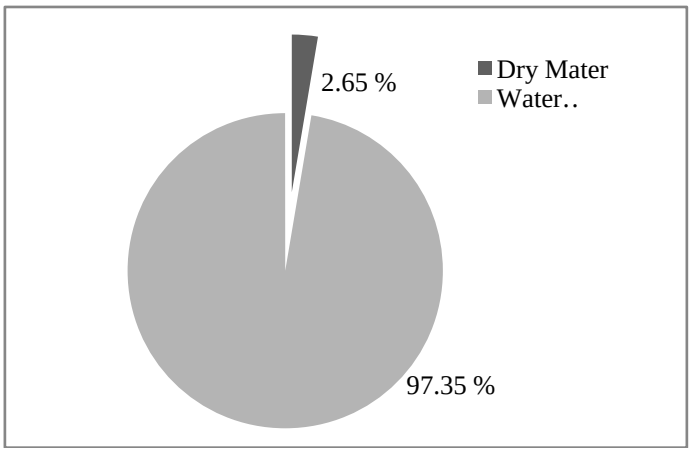

Figure 2. % Recovery of *Aloe vera* powder (Dry Matter).

3.2. Phytochemical Screening of *Aloe vera* Powder

Aloe vera plants, a miracle of nature and are able to synthesize hundreds of compounds with various metabolic functions. A number of potentially biologically active phytochemicals (secondary metabolites) have been identified in most plant species. In this study, Table 1 showed the results of groundwork phytochemical screening of *Aloe vera* extracts. The results showed that the extract contained phenols, flavonoids, alkaloids, saponins, tannins, terpenoids, glycosides, quinones, chlorophyll, carbohydrates and reducing sugars. The results of current study are in accordance with Ashour [33]. These metabolites are known to be biologically active [34-36].

Table 2. Phytochemical constituents of *Aloe vera* powder

Sr. No.	Phytochemical constituents	Qualitative Results
1	Flavonoids	++
2	Phenols	++
3	Terpenoids	++
4	Alkaloid	+
5	Glycosides	+
6	Tannins	+
7	Saponin	+
8	Anthraquinones	++
9	Carbohydrate	+++
10	Protein	+++
11	Chlorophyll	+++
12	Steroid	--
13	Volatile Oils	--

Small amount +; Moderate amount ++; High amount +++; Not detected --

3.3. Antioxidant activity using DPPH assay

The antioxidant activity of *Aloe vera* extract was measured by free radical scavenging assay using the DPPH method as described previously. The method relies on a decrease in DPPH in the presence of antioxidants and a gradual change in DPPH color from purple to yellow depending on the concentration of antioxidants, which is indicated by a decrease in absorbance [37]. % Inhibition DPPH methanol and water extracts of *Aloe vera* are shown in the Figs. 3 & 4. The percentage inhibition (DPPH) of the water extract was 10.23-35.80, while the methanol extract was 12.84-44.96 at the concentration of 20-100 µg/ml which is slightly lower than standard synthetic antioxidant BHT. These results suggest that DPPH scavenging is correlated with the concentration of *Aloe vera* powder extract. These % inhibitions are higher than to those of Waris et al., 2018 [38], who describe % inhibition (DPPH) of 24±0.7 at a same concentration of 100µg/ml.

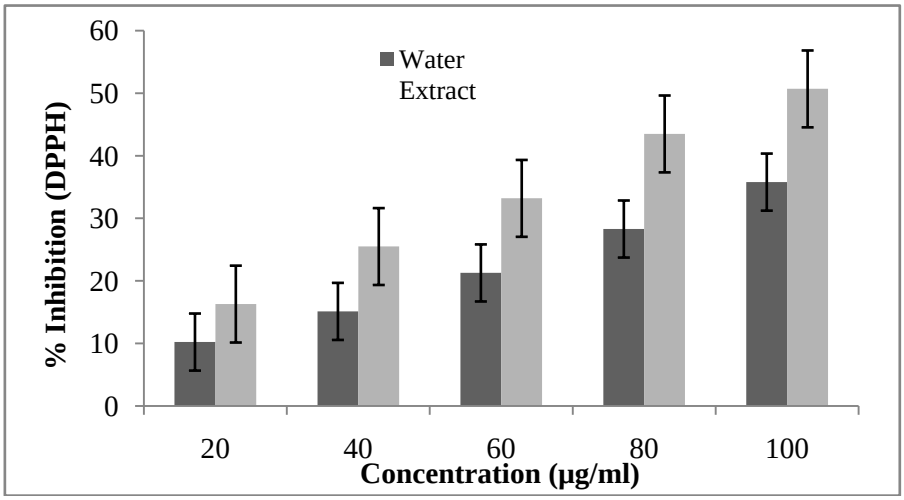

Figure 3. % inhibition (DPPH) of Water extract of *Aloe vera* powder.

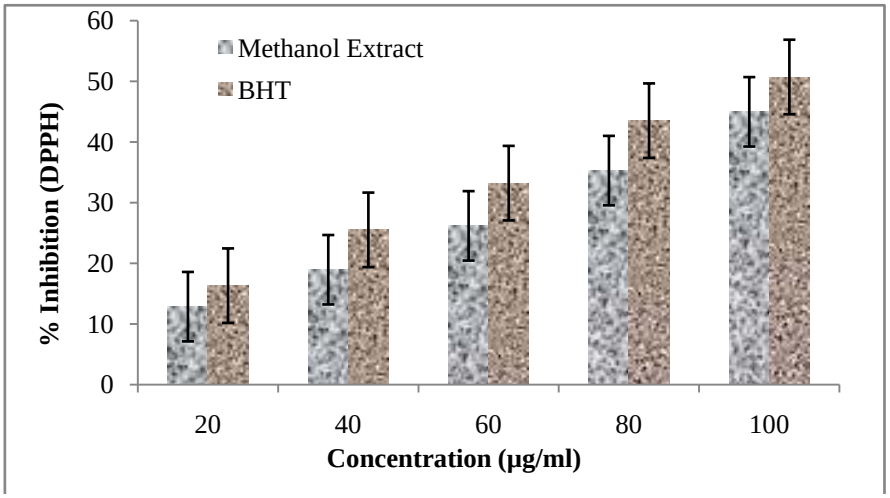

Figure 4. % inhibition (DPPH) of Methanol extract of *Aloe vera* powder.

3.4. Inhibition concentration (IC50)

The inhibitory concentration (IC_{50}) parameter was also used to elucidate the outcome of the DPPH process. IC_{50} is actually the concentration of extract that achieves 50% DPPH inhibition. It is inversely related to scavenging power and represents the amount of antioxidants required to decrease free radicals by 50%. The smaller the IC_{50} value of the extract, the greater its antioxidant activity. IC50 (µg/ml) was 175 at 100 µg/mL, for the water extract and 134 for the methanol extract. These results are consistent with those obtained where, IC_{50} µg/ml = 142.34 and % Inhibition 38±0.8 at 100µg/mL. The antioxidant properties of *Aloe vera* powder may be due to antioxidant protein called "metallothionein". This protein scavenges hydroxyl radicals and thwarts superoxide dismutase and glutathione peroxidase inhibition [39]. It has been pointed out that the antioxidant properties of plants are due to phenolic compounds like terpenes, flavonoids, saponins and polyphenols present in *Aloe vera* powder. The presence of polyphenols and flavonoids in most plants were responsible for the observed free radical scavenging effects [40-41].

4. Conclusion

The results confirmed the existence of alkaloids, flavonoids, steroids, carbohydrates, saponins and other phytochemical components in *Aloe vera* extract. *Aloe vera* plant has been used to treat various diseases like cardiomyopathy, diabetes, skin burns, etc. The medicinal effects of this plant may be related to these detected bioactive compounds. As a result, this plant should be used for diverse medicinal aspects on the basis of its biologically active compounds. These extracts also have excellent DPPH scavenging activity. These data suggest that these extracts are good sources of natural antioxidants.

5. Author Contribution

Dr. M. Khalid Saeed and Dr. Naseem Zahra conducted research and drafted the article. Dr. Syed Hussain Abidi and Dr. Qurat-ul-Ain Syed supervised and finalized the research.

6. Declaration of Competing Interest

The authors have no conflict of interest about the work illustrated in this current manuscript.

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