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Phytochemicals and Antioxidant Activities of Aloe Vera (*Aloe Barbadensis*)

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Abstract

Fresh Aloe vera leaves were collected from Itahari, Sunsari district Nepal and were well washed with distilled water and subjected to cabinet drying at 50°C until constant weight of sample was obtained. Thus, obtained dry powder was extracted using Soxhlet apparatus in two different solvents i.e. methanol and ethanol and further concentrated using rotatory vacuum evaporator that was used for Total Phenol Content (TPC), Total Flavonoid Content (TFC) and tannin content determination. The mean values of methanolic and ethanolic extract were then statistically analyzed at 5% level of significance by paired t-test. Fresh Aloe vera leaves were extracted in 96% methanol to determine chlorophyll-a, chlorophyll- b and total carotene content. Similarly, 99% methanol was used to determine Total Antioxidant Capacity (TOAC), DPPH radical scavenging activity and reducing power assay of fresh Aloe vera leaves.

The preliminary phytochemical analysis of aqueous extract of Aloe vera showed presence of protein, carbohydrates, phenols, tannin, steroids, terpenoids and glycosides. Total Phenol Content (mg GAE/g), Total Flavonoid Content (mg QE/g) and Tannin Content (mg GAE/g) of methanolic extract of Aloe vera were respectively 30.53±0.30, 14.29±0.44, 73.26±2.4 and that of ethanolic extract were 54.95±2.46, 1.13±0.19, 0.844±0.04 respectively. The methanol and ethanol extract showed significant difference in the phytochemical contents ($p\text{-value}<0.05$). Fresh leaves showed chlorophyll-a, chlorophyll-b and total carotene content to be 0.088±0.007mg/100, 0.017±0.006 mg/100g and 35±0.23μg/100g respectively. Similarly, Total Antioxidant Capacity (TOAC), DPPH radical scavenging assay and reducing power of fresh Aloe vera leaves was found to be 103.49±0.24%, 81.91±0.04%, 67.08±0.85% of dry mass respectively. The study showed Aloe vera is a good source of antioxidants and phytochemicals and can be used as a medicinal herb but the toxicological properties are yet to be studied.

Key Words: Aloe-Vera; Total Phenol Content; Total Flavonoid Content; Total Tannin Content; Antioxidant Activity;

Introduction

Medicinal plants are the richest bio-resource of drugs for traditional systems of medicine. Since evolution, man has been using plant extracts to improve his health and life-style. Prime sources of naturally occurring antioxidants for humans are fruits, vegetables and spices. Search for the novel natural antioxidants from tea, fruits, vegetables, herbs, and spices are continued as efforts have been made by researchers all over the globe. Medicinal plants contain a wide variety of free radical scavenging molecules such as **phenolic compounds** (phenolic acids, flavonoids, catechins, proanthocyanidins, quinons, coumarins, tannins etc.), **nitrogen compounds** (alkaloids, amines, betalains etc.), vitamins, terpenoids, carotenoids and other secondary metabolites [1,2].

Among the reported medicinal plants, Aloe Vera is used as a popular folk medicine throughout the world [3]. It is a succulent plant with triangular fleshy green leaves bearing white teeth at the margins.

It grows 60–100 cm tall. The plant can survive at 40°C temperature and also below freezing temperature depending on the root health [4]. Aloe Vera (L.) Burn.f (Synonym A. Barbadensis Miller) is the legitimate name for Aloe Vera according to International Rules of Botanical Nomenclature [5].

Aloe vera juice has been used traditionally for its purgative effects and fresh leaf gel used in different formulations and cosmetic preparations. Aloe vera contains over hundreds of nutrient and bio-active compounds, including vitamins, enzymes, minerals, sugars, lignin, anthraquinones, saponins, salicylic acid and amino acids, which are responsible for their medicinal properties. Its secondary metabolites have multiple properties such as anti-inflammatory, antibacterial, antioxidant, immune boosting, anticancer, anti-diabetic, anti-ageing and sunburn relief. Several uses of Aloe vera also have been reported such as for burn injury, eczema, cosmetics, inflammation and fever in traditional medicine systems[6].

Interestingly, the genus Aloe comprises 516 individual species; however, the primary focus of most nutritional, pharmacological, and natural product studies is concentrated on Aloe Vera only. This might be because the rationale of pharmacognostic studies is rooted down in the traditional alternative and complementary medicines, where mostly Aloe Vera is used in the treatment of diverse ailments [7]. Among various species of Aloe, Aloe Vera is considered to be the most potent, commercially important and the most popular plant in the research field [8]. Aloe Vera is blessed with all the phytochemicals and marked to be efficient in sustaining in medical field [9]. Aloe Vera gained many reputations, such as "champions among health care medicines," "the best health food in 21st century" and "star in plant" due to its unique effective ingredients and special functions [10]. World Health Organization (WHO) estimates that up to 80% of people still rely on traditional remedies such as herbs for their medicine. One of such herbs is Aloe Vera which has caught the global commercial interest in the world of Science, particularly medical science [11]. It is rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes and coumarins, vitamins, minerals, amino-acids etc. These structures are ubiquitous bioactive compounds and a diverse group of secondary metabolites. Accordingly, bioactive polyphenols have attracted special attention because they can protect the human body from the oxidative stress. The antioxidant properties of phenolic acids and flavonoids are due to their redox properties, ability to chelate metals and quenching of singlet oxygen [12]. Plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants [13]. Phytochemical analysis of plants is commercially important being great interest of pharmaceutical industries for the production of new drugs to cure various diseases [6].

To consider a natural composite as an antioxidant substance, it is important to investigate its antioxidant activities in vitro. Our study adopts this approach and it aims at showing the value of the antioxidants and phytochemicals content of the plant taken under study.

Materials and Methodology

Materials

The plant under the study was Aloe vera (Aloe Barbadensis). The plant specimen was taxonomically identified as Aloe vera from the Department of Biology, Central Campus of Technology, Dharan Nepal.

Collection and Preparation of Sample

The plant specimen under study i.e. Aloe vera leaves were collected in October 2017 from Itahari, Sunsari District of Nepal. The basic flow diagram of methodology is made by modifications from the methodologies described by Jaradat et al.[14] and is shown in Figure 2.2.

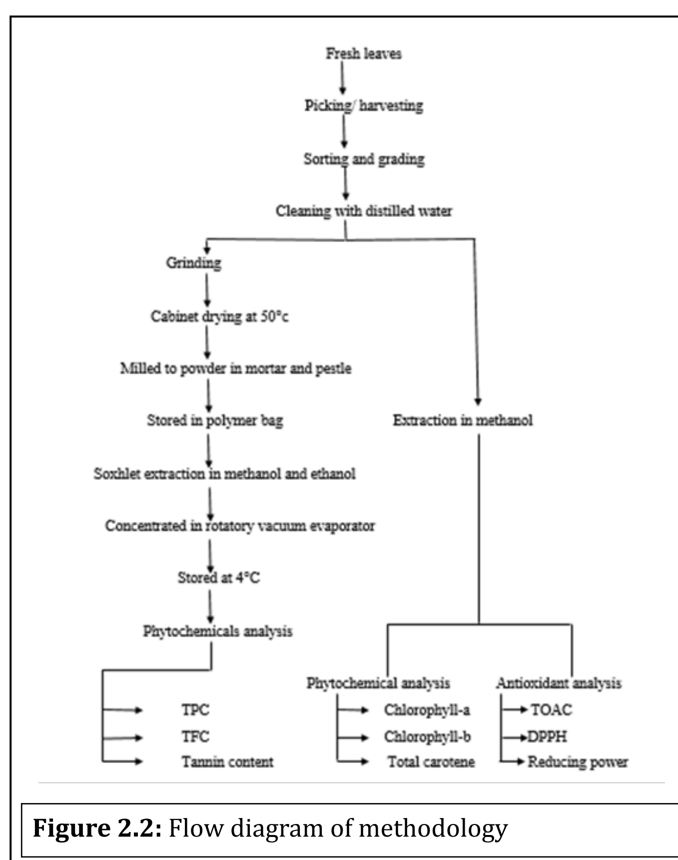

Figure 2.2: Flow diagram of methodology

Drying

The fresh leaves of *Aloe vera* were well washed with distilled water and then dried at 40°C-50°C in cabinet dryer till until all plant parts become well dried. After drying, the plant materials are then powdered by using grinder and placed into a well closed container.

Water and Dry Matter Contents

The water content is determined following the method of Benzidia *et al.* (2018)[15].

Calculation,

Water content (%) = [(W1 – W2)/ W1] × 100

W_1 stands for = Weight of the sample before drying; whereas W_2 = stands for Weight of the sample after drying. Thus, dry matter content was extracted from water content as shown in the formula below:

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

Preliminary Qualitative Phytochemical Screening of Plant Specimen [14]

Preparation of Aqueous Extract

The aqueous extraction is done by taking 5 grams of the plant powder 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 phytochemical analysis.

Qualitative Analysis for Phytochemicals

The plant methanolic extracts were screened for the presence of the phytochemical classes by using the standard following methods [14].

a. Tests for Proteins

- Ninhydrin test: Boil 2 ml of 0.2% Ninhydrin solution with the entire plant crude extract, appeared violet color indicate the presence of proteins and amino acids.

b. Tests for Carbohydrates

- Fehling's solutions test: Boil a mixture of Fehling solutions A and B with equal volumes were added to crude plant extract. A red color precipitate indicated the presence of reducing sugars.

- Benedict's reagent test: Boil 2 ml of Benedict's reagent with a crude extract, a reddish-brown color indicated the presence of the carbohydrates.

- Molisch's solution test: Shake 2 ml of Molisch's solution with crude plant extract then add 2 ml of H_2SO_4 concentrated and poured carefully along the side of the test tube a violet ring appeared at the inter phase of the test tube indicated the presence of carbohydrate.

- Iodine test: 2 ml of iodine solution mixed with crude plant extract. Purple or dark blue colors prove the presence of the carbohydrate.

c. Test for Phenols and Tannins

- Two milliliters of 2% solution of $FeCl_3$ mixed with crude extract. Black or blue-green color indicated the presence of tannins and phenols.

d. Tests for Flavonoids

- Alkaline reagent test: 2 ml of 2% NaOH solution was mixed with plant crude extract, intensive yellow color was formed, which turned into colorless when added 2 drops of diluted acid to solution, this result indicated the presence of flavonoids.

e. Test for Saponins

- Five milliliters of distilled water was added to crude plant extract in a test tube and it was shaken vigorously. The foam formation indicated the presence of saponins.

f. Tests for Glycosides

- Liebermann's test: 2 ml of acetic acid and 2 ml of chloroform mixed with entire plant crude extract. The mixture was then cooled and added H_2SO_4 concentrated, green color indicated the entity of aglycone steroidal part of glycosides.

- Salkowski's test: H_2SO_4 concentrated (about 2 ml) was added to the entire plant crude extract. A reddish-brown color produced indicated the entity of steroidal aglycone part of the glycoside.

- Keller-kilani test A mixture of Acetic acid glacial (2 ml) with 2 drops of 2% $FeCl_3$ solution was added to the plant extract and H_2SO_4 concentrated. A brown ring produced between the layers which indicated the entity of cardiac steroidal glycosides.

g. Test for Steroid

- Two milliliter of chloroform and concentrated H_2SO_4 were mixed with the entire plant crude extract. In the lower chloroform layer produced red color that indicated the presence of steroids. Another test was performed by mixing 2 ml of each of acetic acid with H_2SO_4 concentrated and crude extract with 2 ml of chloroform. Green color indicated the entity of steroids.

h. Test for Terpenoids

- Two milliliter of chloroform was mixed with the plant extract and evaporated on the water path then boiled with 2 ml of H_2SO_4 concentrated. A grey color produced indicated the entity of terpenoids.

Phytochemicals Quantitative Analysis

The plant aqueous is screened for the presence of the phytochemical classes by using the standard following methods as per Jaradat et al., 2015.

Preparation of Plant Extract

The phytochemical extraction was performed using organic solvent extraction. The organic extraction was performed by Soxhlet extraction method. This extraction was done by taking 20 gm of dried plant powder and was placed into a glass thimble then extracted with 250 ml of different solvents separately (methanol and ethanol). The extraction processes carry on till the solvent in siphon tube of Soxhlet apparatus become colorless. After that the extract was heated on rotatory vacuum evaporator at 35°C until all the solvent evaporated. The dried plant crude extract was kept in refrigerator at 2-8°C for their future use[14].

Total Phenolic Content of Plant

Total Phenolic Content (TPC) in the plant methanolic and ethanolic extracts was determined using spectrophotometric method with some modifications. 1 mg/ml aqueous solutions for both methanolic and ethanolic extracts were prepared in the analysis [14].

TPC was determined using the Folin–Ciocalteu method[16] with slight modifications. 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 Na_2CO_3 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. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of gallic acid equivalent expressed in terms of (mg of GAE/g of extract).

Total Flavonoid Content of Plant

Flavonoid content in the plant methanolic and ethanolic extracts was determined using spectrophotometric method with some modifications. 1 mg/ml aqueous solutions for both methanolic and ethanolic extracts were prepared in the analysis[14].

Total flavonoid content was determined using a modified aluminium chloride assay method as described by Barek and Hasmadi [17]. 2 ml of solution was pipette out in a test tube in which 0.2 ml of 5% Sodium Nitrate ($NaNO_3$) was mixed and stand for 5 minutes. 0.2 ml of 5% Aluminium Chloride ($AlCl_3$) was pipetted out, mixed in the tube and allowed to stand for 5 minutes. This followed addition of 2 ml of 1N Sodium Hydroxide (NaOH) in the tube and finally volume was made up to 5ml. The absorbance was measured after 15 minutes at 510nm against a reagent blank. The test result was correlated with standard curve of Quercetin (20, 40, 60, 80, 100µg/ml) and the total flavonoid content is expressed as mg QE/g of dry weight.

Tannin Content of Plant Materials

Tannin content in the plant methanolic and ethanolic extracts was determined using spectrophotometric method with some modifications. 1 mg/ml aqueous solutions for both methanolic and ethanolic extracts were prepared in the analysis[14].

The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20,40,60,80 and100µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract[18-20].

Total Chlorophyll and Total Carotene Contents

The chlorophyll and carotene contents were analyzed according to the method described byTurlerinde[21]. The weighed samples were mixed with 96% methanol (50 mL for each gram) for one minute using a vortex. The homogenate was filtered through a filter paper (No: 42 Whatman) and centrifuged using the centrifuge (EBA 20) for 10 min at 245g. The supernatant was separated and the absorbance was read at 470, 653, and 666 nm on UV/VIS spectrometer (SP-3000). The concentration of each pigment was calculated according to the formulas ofKichtenthaler and Wellburn[22] and was reported as µg per g dry weight of sample.

$$\text{Chlorophyll a} = 15.65\,(A_{666}) - 7.34(A_{653}).$$

$$\text{Chlorophyll b} = 27.05(A_{653}) - 11.21(A_{666}).$$

$$\text{Carotene} = (1000\,(A_{470}) - 2.270\,(C_a) - 81.4\,(Cb))/245.$$

Where,

Ca is chlorophyll a

Cb is chlorophyll b

Antioxidant Assays

Preparation of Plant Extracts for Antioxidant Evaluation

About 10 g of the grounded plant were soaked in 1 Liter of methanol (99%) and put in a shaker device at 100 rounds per minute for 72 hours at room temperature and stored in refrigerator for 4 days. The extracts were then filtered using filter papers and concentrated under vacuum on a rotator evaporator. The crude extract was stored at 4°C and the antioxidant test was done directly within five minutes[14].

Total Antioxidant Capacity

The total antioxidant capacity of leaf extracts was analyzed according to the method described by Prieto [23]. The tubes containing leaf extract (0.3 mL) and 3 mL reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95°C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm spectrophotometrically against a blank. The antioxidant capacity was expressed as Ascorbic Acid Equivalents (AAE).

DPPH Radical Scavenging Assay

The capacity of prepared extracts to scavenge the 'stable 'free radical DPPH was monitored according to the method ofHatano[24] with slight modifications. Extracts (100μL) were dissolved in 3.9 mL freshly prepared methanolic solution of DPPH (1 mM, 0.5 mL). The mixture was vortexed for 15 seconds and then left to stand at room temperature for 30 min in the dark. The absorbance of the resulting solution was read spectrophotometrically (UV/VIS spectrometer) at 517 nm. The percentage inhibition of the radicals due to the antioxidant activity of leaf extracts was calculated using the following formula.

$$\% \text{ inhibition} = (A_{control} - A_{sample})/A_{control} \times 100$$

$A_{control}$ is the absorbance of the DPPH solution with nothing added (control).

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity[25].

Reducing Power Assay

The reducing power of the prepared extracts was determined according to the method of Oyaizu [26]. Briefly, each extract (1mL) was mixed with 2.5 mL of a 0.2M phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 min and then 2.5 mL of 10% (w/v) trichloroacetic acid solution was added and the mixture was then centrifuged for 10 min at 3000rpm. A 2.5 mL aliquot of the upper layer was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride solution. Absorbance of the reaction mixture was read using UV/VIS spectrometer (SP-3000) at 700 nm. Mean values from three independent samples were calculated for each extract. Here, ascorbic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard.

Statistical Analysis

Analysis was carried out in triplicate. Statistical calculations were performed in Microsoft office Excel 2010. All the data obtained in this experiment were analyzed for significance by paired t- test by using Microsoft office Excel 2010. Significant difference of means was analyzed with paired t-test at 5% level of significance.

Results and Discussion

A common variety of Aloe (*Aloe vera*) was collected from Sunsari district which was used to test the presence of phytochemicals. The *Aloe vera* leaves were collected fresh in November 2017. Fresh Aloe vera leaves were subjected to drying at 50°C and their phytochemical composition, B-carotene and chlorophyll content were analyzed.

Water and Dry Matter Content

As shown above in Figure 3.1, the content of water and dry matter of fresh Aloe vera leaves were 97.5% and 2.47% respectively. The results show that Aloe vera is rich in water because of its richness in mucilage which allows the retention of water. Our results confirm that the *Aloe vera* plant leaf is composed primarily of water 97.4–99.5%. This result is similar to the studies done byAhmed and Hussain, Boudreau[27,28]where water and dry matter content were respectively 97% and 3% respectively.

For various reasons, a good determination of the moisture content is still an essential and important element for the analytical procedures. This determination of the total amount of water is of great benefit to product manufacturers since water is an inexpensive load. The dry matter that remains after the moisture analysis is called total solids[29].

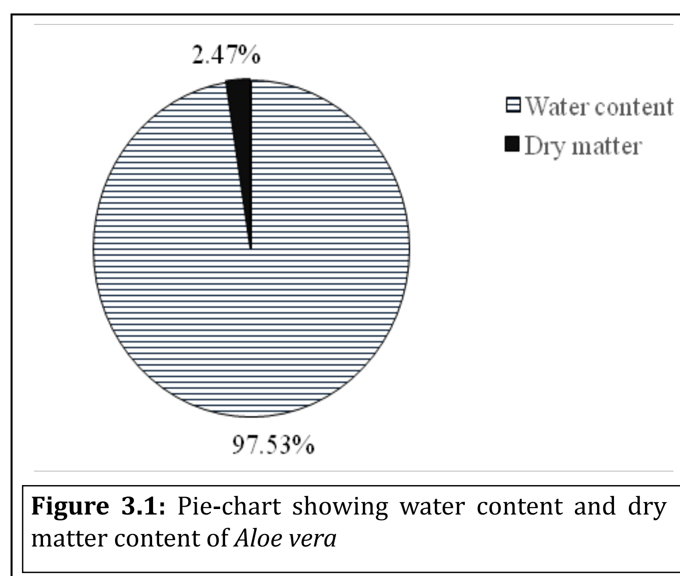

Figure 3.1: Pie-chart showing water content and dry matter content of *Aloe vera*

Preliminary Phytochemical Qualitative Analysis of Preliminary Phytochemical Qualitative Analysis of *Aloe Vera*

The aqueous extract of *Aloe vera* was prepared to conduct preliminary phytochemical analysis. Table 3.2 shows the phytochemicals present in *Aloe vera.*

Table 3.2 Phytochemical screening of *Aloe vera* aqueous extract

S.N.	Test	Aqueous extract
i	Protein	+
ii	Carbohydrate	+
iii	Reducing sugar	+
iv	Starch	+
v	Phenols and Tannins	+
vi	Flavonoids	+
vii	Saponins	-
viii	Glycosides	+
ix	Steroids	+
x	Terpenoids	+

Where, Plus (+) = positive test; Minus (-) = negative test

The phytochemicals screening of aqueous extract of Aloe Vera showed that bioactive compounds such as 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 negative as shown in Table 3.2. These findings are in total agreement with those existing in the literature [30,31]. Other studies have shown that the presence of saponins depends on extraction solvents. They are positive with ethanol, methanol, ethyl acetate, petroleum ether, acetone and hexane extracts and negative with the aqueous extract [30,32].

Since this plant had been used in the treatment of different ailment such as malaria, dysentery, diarrhea, skin burn etc., the medicinal roles of these plants could be related to such identified bioactive compounds. The presence of these biologically active compounds in the extracts has made the plant to be known for its medicinal use especially for antimicrobial activity against pathogenic organisms[33].

Quantitative Analysis of Phytochemicals in *Aloe Vera* Leaves

Table 3.3: Phytochemical content of *Aloe vera*showing p-value

Assays	Methanolic extract	Ethanolic extract	P-value
Total Phenol Content (TPC)	30.53±0.30[a]	14.29±0.44 [a]	0.00*
Total Flavonoid Content (TFC)	73.26±2.46[b]	54.95±4.15[b]	0.0005*
Tannin Content	1.13±0.19[c]	0.844±0.0.04[c]	0.01*

Data expressed as mean ± SD; n =3; [a] mg GAE/g dw; [b] mg RE/g dw; [c] mg GAE/g dw;* indicates significant difference

The Table 3.3 shows the phytochemical content of Aloe Vera

Total Phenolic Content (TPC) of *Aloe Vera*

TPC of methanolic extract of whole Aloe Vera leaves was found to be 30.53±0.30 mg GAE/g of dry weight which is shown in Figure 3.3.1(1). This finding is in agreement with the study done by Kumar et al.[6] 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.[6]. Different agro-climatic conditions have effects on phytochemical diversity and antioxidant potential of Aloe Vera plant [34].

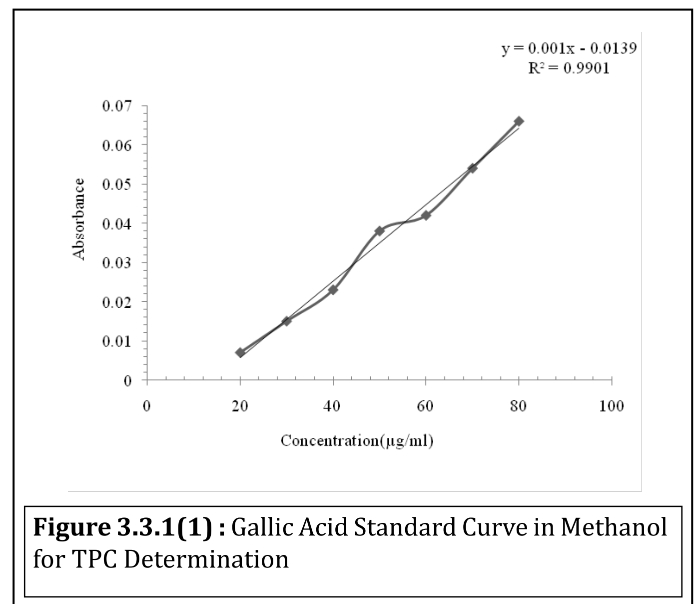

Figure 3.3.1(1) : Gallic Acid Standard Curve in Methanol for TPC Determination

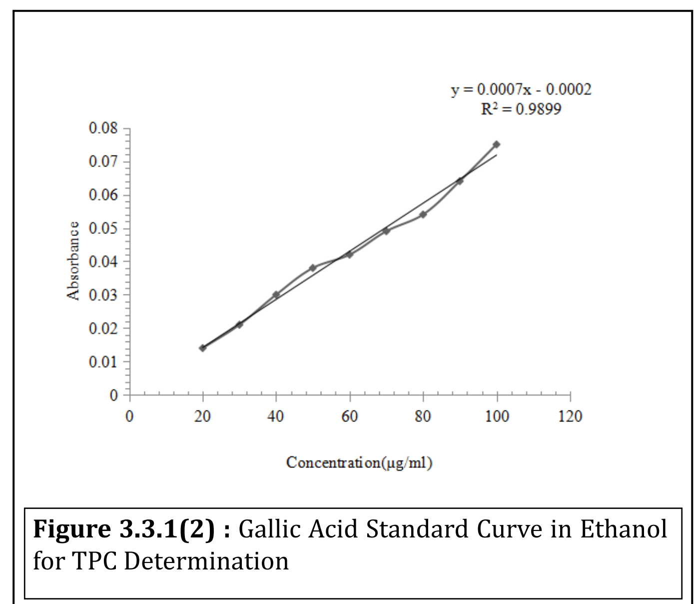

Figure 3.3.1(2) : Gallic Acid Standard Curve in Ethanol for TPC Determination

In this study, ethanolic extract of Aloe Vera showed TPC of 14.29±0.44 mg GAE/g of dry weight. Whereas study done by Nejatzadeh-Barandozi [35] shows that 95% Ethanol Leaf Gel Extracts (ELGE) gives TPC of 413 ± 9.88 mg GAE/100g dw of extract which is less, it may be due to the difference in the degree of solvent used for extraction of polyphenols[6,36,37] and difference in the part of the plant used [38] where ethanolic extract of gel and peel were 2.06±0.25 mg GAE/g and 7.99±0.26 mg GAE/g respectively. The findings of Aloe Vera is also comcomittant to the study of Ficus racemosa where TPC of ethanolic extract was found to be 12.36 mg GAE/g [39].

TPC of methanolic and ethanolic extract of the plant was significantly different from each other (p-value <0.05) as shown by Figure 3.3.1(1) and Figure 3.3.1.(2). Methanolic extract yielded high TPC content than ethanolic extract. Similar result was found in the study by Anwar et al., Kumar et al., Sonam and Tiwari [9,34,40].

Plant polyphenols have been implicated in diverse functional roles, including plant resistance against microbial pathogens and animal herbivores such as insects (antibiotic and anti-feeding actions), protection against solar radiation, besides reproduction, nutrition, and growth. Phenolic compounds have also been reported to prevent diseases resulting from oxidative stress [41, 42].

Total Flavonoid Content (TFC) of *Aloe Vera*

This study showed Total Flavonoid Content (TFC) of methanolic extract of Aloe Vera to be 73.26 ± 2.46 mg QE/g of dry weight. The findings of this study is more than the study done byTaukoorah and Mahomoodally [43] 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 [44] and also due to difference in the chemical used to prepare standard curve [41].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 [45].The findings of 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 [46].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 Figure 3.3.2.(2). This result is higher than the study done by Botes et al.[47]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 [48]. The difference is probably due to difference in the part of plant used for analysis and also standard curves were prepared from two different chemicals and maximum value was obtained at quercetin equivalent than catechin and rutin equivalents. This decrease is due to the unstable nature of rutin and catechins. The flavonoid content of Cistus incanus expressed as quercetin, rutin and catechins equivalent were respectively 40.80 ± 4.52mg/g, 6.00 ± 0.75mg/g and 20.40 ± 2.26mg/g [41].

TFC of methanolic and ethanolic extract of the plant was significantly different from each (p-value <0.05) other as shown by Figure 3.3.2.(1) and Figure 3.3.2.(2) below. Methanolic extract yielded high TFC content than ethanolic extract. Similar result was found in the study byAnwar et al., Asuk et al., Kumar et al., Sonam and Tiwari[9,34,40,44].

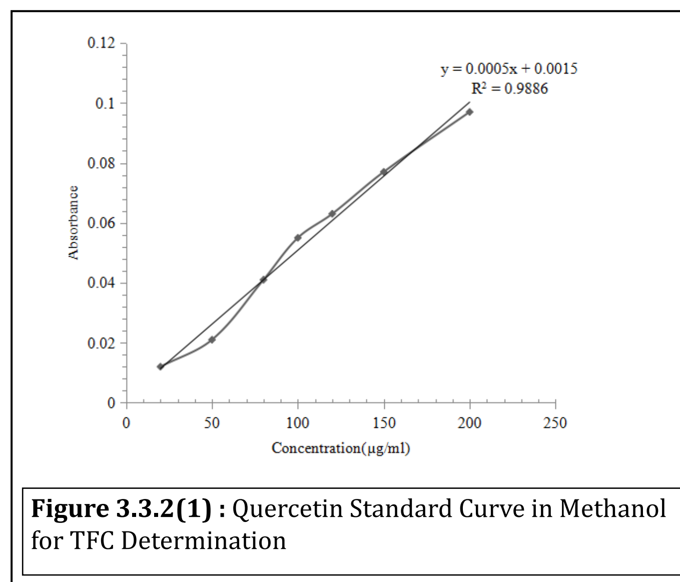

Figure 3.3.2(1) : Quercetin Standard Curve in Methanol for TFC Determination

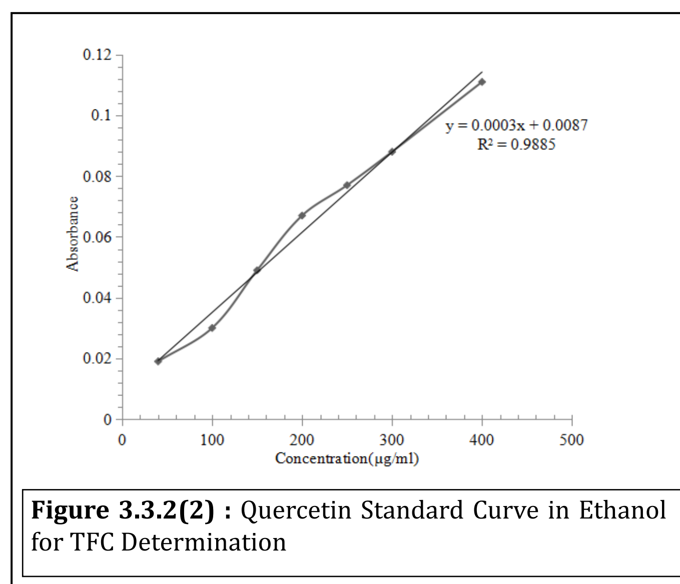

Figure 3.3.2(2) : Quercetin Standard Curve in Ethanol for TFC Determination

Some flavonoids are antioxidants and has been proved to exhibit a wide range of biological activities like antimicrobial, anti-inflammatory, antiangionic, analgesic, anti-allergic, cytostatic and antioxidant properties[11].

Tannin Content of *Aloe Vera*

Methanolic extract of Aloe vera showed total tannin content of 1.13±0.19 mg GAE/g of dry weight of extract. This finding is concomitant with the study of Bael (*Aeglemarmelos*) powder from

cold treated pulp where tannin content was 2.90±1.13 mg GAE/g dw[49].

Ethanolic extract of Aloe Vera showed total tannin content of 0.844±0.04 mg GAE/g of dry weight. A study of Wintola and Afolayan [50] showed tannin content of ethanolic extract of Aloe ferox is 0.026 ± 0.01 mg CE/g of dry plant materials which is less than the value of Aloe Vera. The tannin content of Aloe Vera is concomitant to the study byMohammed and Manan [51]where tannin content of Moringa olifera seed extract was 0.890 ± 0.020 mg GAE/g of dry weight.

Tannin content of methanolic and ethanolic extract of the plant was significantly different from each other (p-value<0.05) as shown by Figure 3.3.3(1) and Figure 3.3.3(2)below. Methanolic extract yielded higher tannin content than ethanolic extract. Similar result was found in the study byAnwar et al., Kumar et al., Sonam and Tiwari,[9,34,40].

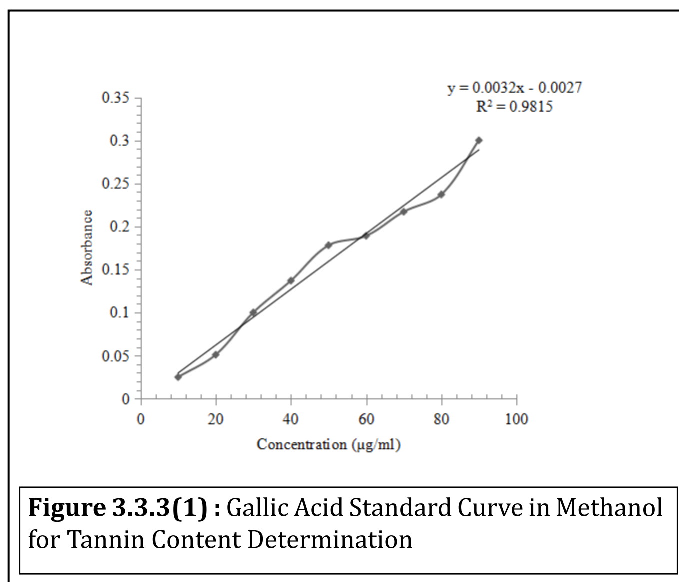

Figure 3.3.3(1) : Gallic Acid Standard Curve in Methanol for Tannin Content Determination

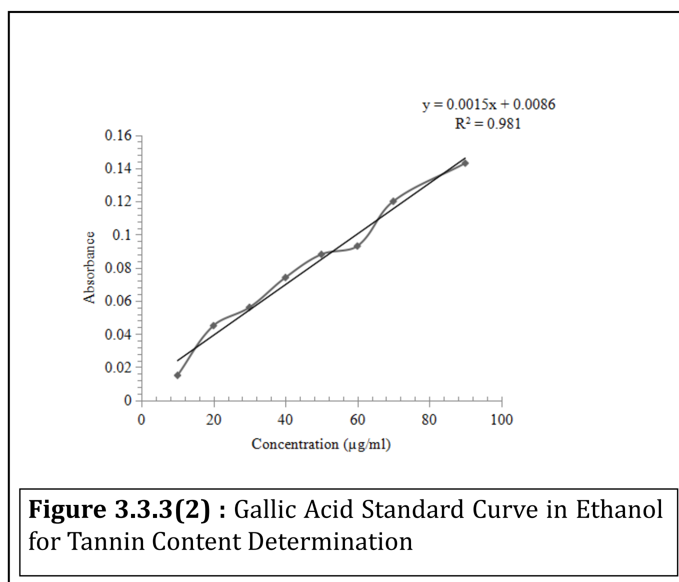

Figure 3.3.3(2) : Gallic Acid Standard Curve in Ethanol for Tannin Content Determination

Tannin has been reported to interfere with bacterial cell protein synthesis and is important in the treatment of ulcerated or inflamed tissues and also in the treatment of intestinal disorders[52]. Thus, these observations therefore support the use of *Aloe vera* in herbal cure remedies.

Total Chlorophyll and Total Carotene Content

The study showed chlorophyll-a and chlorophyll-b content of Aloe Vera were 0.088±0.007mg/100 g and 0.017±0.006mg/100g fresh weight of leaves respectively. Summarizing chlorophyll-a and chlorophyll-b, the total chlorophyll content of Aloe Vera was 0.105mg/100g which is similar to the study of germ plasm of Aloe Vera, where total chlorophyll content varied from 0.19-0.20 (mg/100 gm) [53]. Plants under diffused light had greatest chlorophyll content than plants grown under direct sunlight. Significant increases in biomass are noticed for plants grown under shade than plants grown under direct sunlight [54].

In the study, total carotene content of fresh Aloe Vera leaves was found to be 35±0.23µg/100g. Whereas study done by Kaur et al.[55] showed that Aloe Vera leaves contain B-carotene of 19.08 µg/100g. Gautam and Awasthi [56]analyzed the β-carotene in Aloe Vera leaf powder and fresh leaf. In the powder, β-carotene levels reported was 335.80µg/100 g where as in the fresh leaf was 10.80µg/100g. Since total carotene consists not only of B-carotene [57] and the findings of this study is in equivalence with these studies mentioned aboveGautam and Awasthi, Kaur et al. [55,56].

Thus, the study shows that Aloe Vera is a good source of carotenoids and chlorophyll. Chlorophyll exerts therapeutic properties (Inanc, 2011). Carotenoids are pigments that play a role in the protection of plants against photo-oxidative processes and they are much effective antioxidant scavenging singlet molecular oxygen and peroxyl radicals [58].

Total Antioxidant Capacity (TOAC)

In the study, TOAC of 99% methanolic extract of Aloe Vera was found to be 87.13±0.27% [Figure 3.3.5]. This finding is concomitant to the study done in Ethiopia in green tea where TOAC was 80.0 ± 0.63% [59]. 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 [58]. Antioxidants prevent free radical induced tissue damage by preventing the formation of

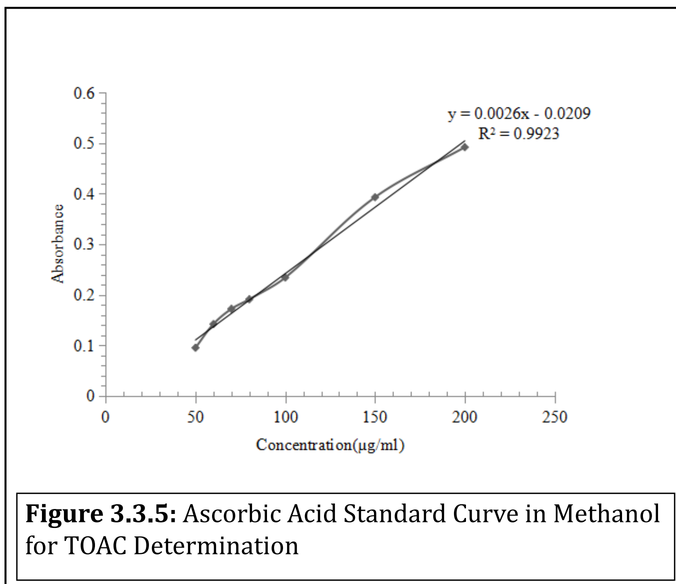

Figure 3.3.5: Ascorbic Acid Standard Curve in Methanol for TOAC Determination

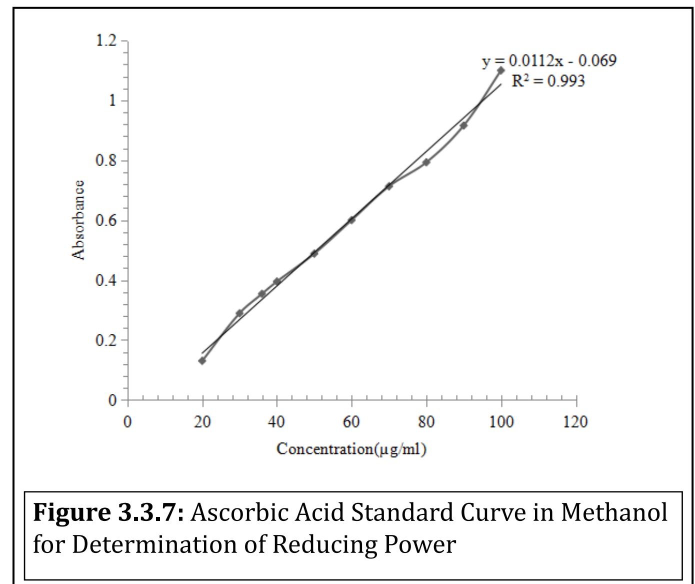

Figure 3.3.7: Ascorbic Acid Standard Curve in Methanol for Determination of Reducing Power

Table 3.3.5 Antioxidant Potential of Methanolic *Aloe Vera* Extract

Parameter	Antioxidant potential
Total antioxidant capacity (TOAC) (%)	87.13±0.27
DPPH radical scavenging assay (%)	81.91±0.04
Reducing power (%)	67.08±0.85

Data expressed as mean ± SD

radicals, scavenging them, or by promoting their decomposition [60]. The Table 3.3.5 shows antioxidant potential of methanolic Aloe Vera extract.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of 99% methanolic extract was found to be 81.91±0.04% which is similar to the study done bySultana et al. [61] where 80% methanolic and ethanolic extract shows DPPH scavenging activity was 80.1±2.3% and 70.7±1.2% respectively. It has been documented that growth periods of A. Vera is critical in the regulation of the DPPH radical scavenging activity of gel [62].

Environmental temperature plays a significant role on antioxidant activity evaluation and it is more pronounced in cold weather [63]. Thus, the higher value of DPPH indicates that majority of free radicals produced can be scavenged by Aloe Vera methanolic extract which protects the cells from being oxidized and thus help prevent many degenerative diseases[64].

Reducing Power

The reducing power of 99% methanolic extract of Aloe Vera was found to be 67.08±0.85%. One study by Akhtar et al.[65]showed that reducing power of Berberis lycum Royale was 80±2% and is nearly equal to the values from our study. The various biological and environmental factors in which the plant grew also contribute to the plant antioxidant power [41].

The reduction of Fe^{3+} has been described as an indicator of electron donating activity which can demonstrate the antioxidant potential of different phenolic compound of phyto origin [58, 66]. The reducing power is generally associated with the presence of reductones [67], which has been shown to exhibit antioxidant potential by splitting the free radical chains by donating hydrogen atoms. Reductones can prevent the peroxide formation by reacting with the precursors of peroxides.

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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