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PHYTOCHEMICAL CHARACTERIZATION OF *ALOE BARBANDENSIS* MILLER AND ITS ANTIOXIDANT ACTIVITY

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ABSTRACT
The aim of the study was to analyse phytochemical constituents of *Aloe barbadensis* Miller and its antioxidant activity. The qualitative study was done by using 4 different extracts of *Aloe barbadensis* which were petroleum ether, chloroform, ethyl acetate and 70% methanolic extract. Of these extracts 70% methanolic extract have shown the best results as it showed the presence of carbohydrates, anthraquinone glycosides, alkaloids, saponins, flavonoids, triterpenes and fats and oils. On the basis of these tests it was decided to take 70% methanolic extract for quantitative analysis and antioxidant assay. For quantitative analysis total phenolic content and total flavonoid content estimation were done and for antioxidant activity DPPH 2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay and reducing power assay were done. The antioxidant activity was determined at the concentration of 20,40,60 80 and 100µg/ml and IC 50 was calculated.

KEYWORD: *Aloe barbadensis,* carbohydrates, anthraquinone glycosides.

INTRODUCTION
Since time immemorial, plants and plant derived products have been part of the health care system. Various plants are investigated for their phytochemical identification and their antioxidant activity, as certain components have the potential to inhibit free radicals, which are responsible for many diseases.[1] Aloe vera is a monocotyledonous plant and it belongs to the family Asphodelaceae. It is indigenous to the Estern and Southern Africa. The aloe vera gel was used to treat no. of diseases like gastrointestinal problems, skin disease, constipation healing wound and burns etc. Cosmetic, pharmaceutical and food industries also use aloe vera gel as ingredient of their products.[2] The present study was designed for comparative study of phytochemical characterization of different extacts (pet ether, chloroform, ethyl acetate and 70% methanol) extract of aloe vera, quantitative determination of phenolic and flavonoid content and its antioxidant property.

MATERIALS AND METHODS
Collection of plant material: Aloe vera leaves were collected from different sites of Bhopal and plant was identified by Dr. Zia Ul Hasan, professor and head, department of botany, safia college of science, Bhopal.

Extraction: Fresh and healthy leaves were washed in the running tap water, cut longitudinally, air dried and dried material was coarsely powdered and stored for further use. The coarsely powdered material was used for extraction by soxhlation using solvents of different polarity. The solvent used were petroleum ether, Chloroform, ethyl acetate and 70% alcohol.
Phytochemical analysis:-To identify presence or absence of different phytoconstituents the following tests were performed.

Tests for Carbohydrates
- **Fehling's Test**

1 ml of each extract were taken in different test tube and 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in each of the test tube and kept in the water bath for heating, for 10 minutes. Red precipitate was formed which indicated the presence of reducing sugar.

- **Benedict's Test**

Benedict's reagent and aloe vera extract were mixed in a test tube in equal volume and kept in the water bath for heating for 5-10 minutes. Solution appeared green which indicated the presence of reducing sugar.

Tests for Protein and Amino acids
- **Millon's Test**

In 3 ml of extract, 5 ml of Millon's reagent was added. White precipitate formed which on heating not turned to brick red and indicated the absence of proteins.

Tests for Glycosides

➢ **Borntrager's Test**

3 ml of test solution was taken and dilute sulphuric acid was added. The mixture was boiled for 5 minutes and filtered and allowed to cool. In the cold filtrate, equal volume of chloroform was added and shaken. The organic solvent layer was separated, to which ammonia was added. Formation of pink color in ammonical layer indicated the presence of anthraquinone glycosides.

Tests for Alkaloids

In the extract, dilute hydrochloric acid was added, shaken for mixing and filtered. With the filtrate, the following tests were performed.

➢ **Mayer's Test**

In 2ml of filtrate, few drops of Mayer's reagent were added along the sides of the test tube. White precipitate was formed which indicated the presence of alkaloids.

Tests for Saponins

➢ **Froth Test**

In the extract distilled water was added and shaken for 15 minutes. The layer of foam was formed which indicated the presence of saponins.

Tests for Flavonoids

➢ **Lead Acetate Test**

To the extract, few drops of lead acetate solution was added. Yellow precipitate indicated the presence of flavonoids.

➢ **Alkaline Reagent Test**

To the extract, few drops of sodium hydroxide was added in a test tube. Intense yellow color was formed, which become colorless on addition of few drops of dilute acid, indicated the presence of flavonoids.

➢ **Shinoda test**

5 ml (95%) of ethanol was added in the extract. Few fragments of magnesium turning was added in the mixture. After that dropwise concentrated hydrochloric acid was added. Pink color indicated the presence of flavonoids.

Tests for Triterpenoids and Steroids

➢ **Salkowski's Test**

Chloroform was added in the extract and filtered. Few drops of concentrated sulphuric acid were added in the filtrate, shaken and allowed to stand. Lower layers turned red in chloroform and ethyl acetate extract, which showed the presence of sterols in that. In the 70% methanolic extract golden yellow layer formed at bottom, which showed the presence of triterpenes.

Tests for Tannin and Phenolic compounds

➢ **Ferric Chloride Test**

Extract was dissolved in distilled water and 2 ml of 5% ferric chloride solution was added to it. Formation of green color showed the presence of phenolic compounds.

Tests for Fats and Oils

➢ **Solubility test**

2-3 ml alcoholic solution of extract was taken and few ml of chloroform were added to it and observed for solubility.

2-3 ml alcoholic solution of extract was taken and few ml of 90% ethanol were added to it and observed for solubility.[3]

Total Phenolic Content Estimation

- Different concentrations of Gallic acid (20,40,60,80,100µg/ml) were prepared in methanol.
- Test sample100µg/ml prepared in methanol
- In 0.5 ml of different concentrations of Gallic acid/ test sample, 2 ml Folin-Ciocalteu Reagent (1:10 in deionized water) was added.
- 4 ml of 7.5% sodium carbonate solution was added.
- Incubated at room temperature for 30 min with intermittent shaking.
- Absorbance was taken at 765 nm (due to developed blue colour) using methanol as blank.

Total Flavonoid Content Estimation

- Different concentrations of Rutin/ Quercetin (20 to 100 µg/ml)were prepared in methanol.
- Test sample prepared in methanol (100µg/ml).
- 0.5ml diluted sample solutions were mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution.
- After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, then 2 ml of 4% NaOH solution was added to the mixture.
- Immediately, water was added to bring the final volume to 5 ml, and then mixture was mixed thoroughly and allowed to stand for another 15 min.
- Absorbance was taken of at 510 nm versus a prepared water blank.

DPPH radical scavenging activity

- DPPH solution (4mg/100ml) was prepared in methanol.
- Different concentration of test sample (20,40,60,80 and 100µg/ml)were prepared with methanol.
- In 2ml of test sample, 1ml of DPPH solution was added.
- Incubated at room temperature for 30 min.
- Absorbance was taken at 515 nm against blank (methanol).
- %Inhibition was calculated using the formula:

  % Inhibition = [(AC 515 nm- AS 515 nm/ AC 515 nm) x 100].
- A curve for % Inhibition and concentration was ploted and using line of regression IC50 was estimated.

Reducing Power Assay

- Different concentrations (20,40,60,80 and100µg/ml) of test sample were prepared.

- In 0.5 ml of different concentrations of sample, 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (0.5 ml, 1%W/V) were added.
- Reaction mixture was incubated at 50° C for 20 min.
- After cooling, 1.5 ml of trichloroacetic acid solution (10% W/V) was added.
- 0.5 ml ferric chloride (0.1% W/V) was added and absorbance was measured at 770 nm.

RESULTS and DISCUSSION

The different extracts were analysed qualitatively for the carbohydrates, protein and amino acids, anthraquinone glycosides, alkaloids, saponins, flavonoids, triterpenoids and steroids, tannins and phenolic compounds and fats and oils of which different extracts showed different results which is tabulated in table 1.Of the individual extracts investigated best results were shown by 70% methanolic extract, this was the reason, for the selection of 70% methanolic extract, for the quantitative analysis. In quantitative analysis total estimation of phenolic content, total estimation of flavonoids contents was done and antioxidant activity were determined by using DPPH assay and reducing power assay.[4,5,6,7]

Table 1: Phytochemical analysis of Aloe Vera extracts.

Phytochemical tests	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	70% Methanolic extract
Carbohydrates	-ve	-ve	+ve	+ve
Proteins and amino acids	-ve	-ve	-ve	-ve
Anthroquinone glycosides	-ve	-ve	-ve	+ve
Alkaloids	-ve	-ve	+ve	+ve
Saponins	-ve	-ve	-ve	+ve
Flavonoids	-ve	-ve	+ve	+ve
Triterpenoids and steroids	Both -ve	Sterol +ve	Sterol +ve	Triterpenes +ve
Phenols	-ve	+ve	+ve	+ve
Fats & oils	+ve	+ve	+ve	+ve

Quantitative Phytochemical estimation

Total Phenolic Contents: 70% methanolic aloe vera extract was found to contain 280.50mg/g of gallic acid equivalent (GAE) of total phenolics in dried extract. Based on the calibration curve of gallic acid the total phenolic content were calculated using the following linear equation:-

$y = 0.002x + 0.063$

$R^2 = 0.0995$

Where y is the absorbance and x is the amount of gallic acid in µg.

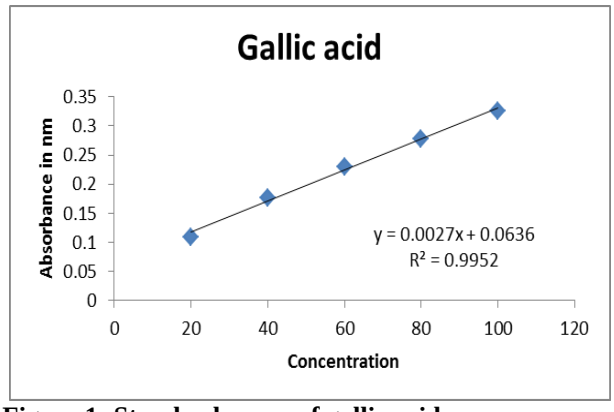

Figure 1: Standard curve of gallic acid.

Table 2: Total phenolic content of 70% methanolic aloe vera extract.

Sample	Test
70 % Methanolic extract	280.50 mg/g equivalent to Gallic acid

Total Flavonoid Content

The total flavonoid content of the 70% methanolic aloe vera extract was 340.00mg/g equivalent to rutin, which was calculated by the calibration curve (R^2=0.979). The linear equation was:

$$y = 0.001x + 0.092$$

Where y is the absorbance and x is the amount of rutin in µg.

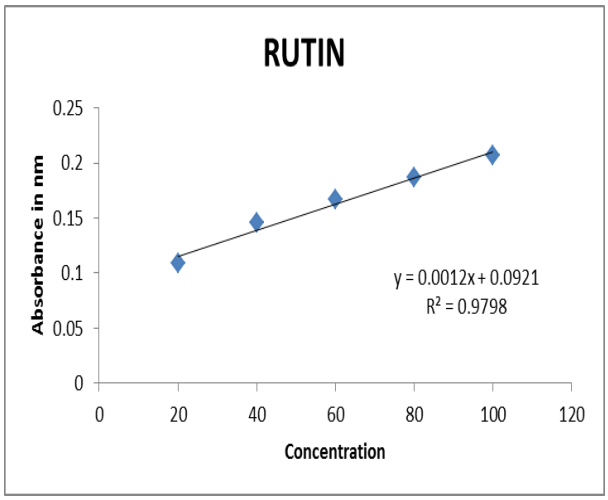

Figure 2: Standard curve of rutin.

Table 3: Total flavonoid content of 70% methanolic aloe vera extract.

Sample	Test
70 % Methanolic extract	340.00 mg/g equivalent to Rutin

DPPH Assay

Free radicals are a major cause of biological damages and DPPH which is stable free radical decolourizes from purple to yellow colour in the presence of antioxidants. The reduction capacity of DPPH (2,2 diphenyl-1-picrylhydrazyl)is determined by decrease in its absorbance at 515nm, which is induced by antioxidants.

Figure-4 shows the DPPH radical scavenging activity of 70% methanolic aloe vera extract which is expressed in terms of IC50 value with respect to ascorbic acid as standard.

Lower the IC50 value shows more antioxidant potential.

Table 4: Different concentrations of ascorbic acid and its absorbance in DPPH assay.

S. No.	Concentration	Absorbance
1.	Control	0.912
2.	20 µg/ml	0.431
3.	40 µg/ml	0.398
4.	60 µg/ml	0.351
5.	80 µg/ml	0.283
6.	100 µg/ml	0.258

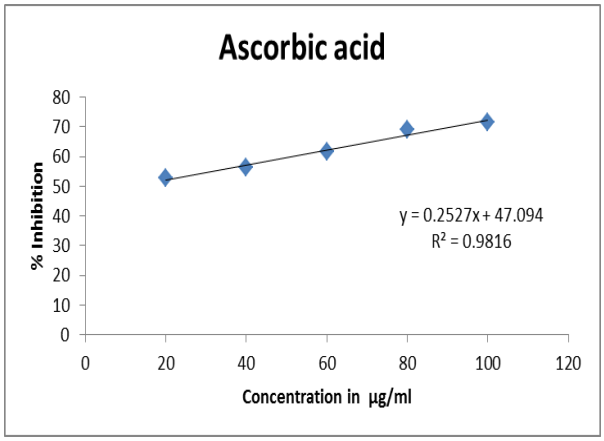

Figure 3: DPPH scavenging activity of standard (Ascorbic Acid).

IC50 value of standard is 11.54µg/ml.

Table 5: Different dilutions of 70% methanolic extract of aloe vera and its absorbance in DPPH assay.

S. No.	Concentration	Absorbance
1.	Control	0.912
2.	20 µg/ml	0.738
3.	40 µg/ml	0.702
4.	60 µg/ml	0.671
5.	80 µg/ml	0.614
6.	100 µg/ml	0.573

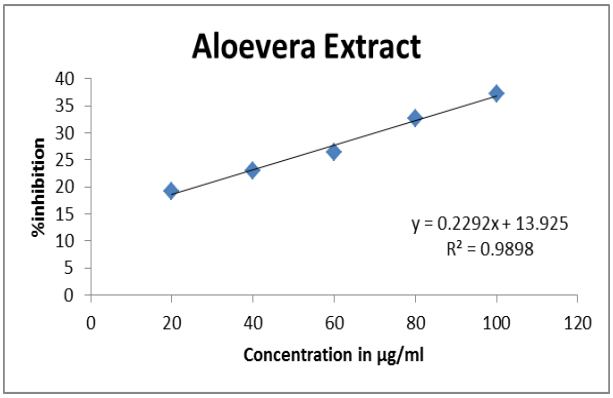

Figure 4: DPPH scavenging activity of extract.

IC50 value of extract is 157.55µg/ml**.**

Reducing Power assay

Table 6: Different concentrations of ascorbic acid and aloe vera and its aborbance.

S. No.	Concentration	Ascorbic acid	Aloevera Extract
1.	20 µg/ml	0.213	0.256
2.	40 µg/ml	0.289	0.267
3.	60 µg/ml	0.382	0.295
4.	80 µg/ml	0.41	0.311
5.	100 µg/ml	0.473	0.349

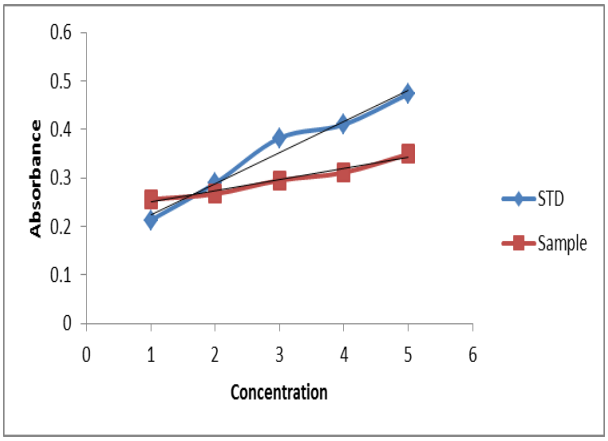

Figure 5: Free radical reducing potential of standard Ascorbic acid and 70% methanolic extract of aloe vera.

Reducing power of extract was found to be less, when compared with the standard. Reducing power reflects the antioxidant capacity, which is due to their hydrogen donating ability. In the present study, extract as well as standard showed that with increase in concentration, absorbance has also increased.

CONCLUSION

On the basis of the study it was concluded that out of the 4 different extracts tested for the presence of phytochemicals, 70% methanolic extract has shown the best results and chosen for quantitative determination. It has shown good value of total phenolic and total flavonoid content which has justified the therapeutic use of this plant. It has also shown good free radical inhibiting capacity and reductive potential. As free radicals are involved in the pathogenesis of many diseases, use of this plant can be fruitful in developing new drug.

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