

PHYTO-CHEMICAL ANALYSIS, IN-VITRO ANTIOXIDANT POTENTIAL OF ALOE BARBADENSIS

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Abstract - Aloe barbadensis known for centuries for its herbal remedy belongs to the family of Liliaceae. The aqueous and organic leaf extracts were prepared by using soxhlet apparatus. The evaluated extracts were for qualitative phytochemical analysis and its antioxidant property using DPPH radical scavenging assay, alkaline DMSO method, nitric oxide method, Total antioxidant Assay and Deoxyribose method. The present study showed the presence of alkaloid, flavonoid, reducing sugar, terpenoids and steroids by qualitative phytochemical analysis. The aqueous and organic extracts were prepared by using soxhlet apparatus and screened for anti-oxidant property by different methods. The acetone and methanolic extracts A. barbadensis showed anti-oxidant activity by DPPH radical scavenging assay with IC50 values of 705.2 \pm 8.08 and 182.8 \pm 1.44 µg/ml respectively. Whereas aqueous and methanolic extracts showed anti-oxidant activity by alkaline DMSO method with IC₅₀ values of 237.5 \pm 11.4 and 553.4 \pm 3.28 μ g/ml respectively. While methanolic extract of A. barbadensis showed activity by nitric oxide method with IC₅₀ value of $43.17 \pm 6.29 \mu \text{g/ml}$. The methanolic extract of A. barbadensis gave IC50 value of 589.6 \pm 5.54 by total anti-oxidant activity and aqueous and methanolic extracts gave IC₅₀ value of 579.8 \pm 3.95 and 714.8 \pm 7.21 $\mu g/ml$ respectively by deoxyribose method. The presence of these polyphenols may be responsible for its potent anti-oxidant property. The leaf extracts of Aloe barbadensis showed potent anti-oxidant activity and can be used as a therapeutic agent.

Keywords - *Aloe barbadensis*, DPPH, flavanoids and alkaloids

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I. INTRODUCTION

Aloe barbadensis belong to the family of Liliaceae and species of succulent plant and the plant is cultivated worldwide. The plant extract of A. barbadensis are used widely in herbal products, cosmetics and in medicinal industries. The plant of Aloe barbadensis is succulent short stem plant and grows to a height of about 60-100 cm (24-39 in) in length and the plant spreads by its offsets. The leaves of A. barbadensis are thick and fleshy and color of leaves is green or greyish green. The leaf has a margin which is serrated and has white small teeth⁽¹⁵⁾. The plant is aphrodisiac, hepatic stimulant. stomachic, anti-helmintic, emmenagogue, purgative, hemophilia, wound healing, anti-diabetic and acts particularly on the lower bowels ⁽²⁶⁾. It is also good for skin and skin infections and uterine disorders. The leaves of the plant contains many physiologically and pharmacologically substances which are used as a medicine in spleen enlargement and chronic ulcers⁽⁸⁾ and the hot poultice of leaves are used to relieve swelling⁽³⁾. Many scientists have reported hepatoprotective and anti-oxidant activity in A. barbadensis (33, 32). Mohamed (2011) showed hypoglycemic effect in A. barbadensis gel extract. It is reported the effect may be due to presence of trace elements, Zn, Cr and Mn in plant, enhances the action of insulin and also attenuates oxidative damage in the serum of alloxan induced diabetic rats which leads to low glucose level (25). Kammoun and his co-workers (2011) believe that there is a significant correlation between the total phenolic content and the anti-oxidant capacity (17). The alcoholic extract showed significantly higher antimicrobial activity (7) also showed anti-oxidant activity by DPPH scavenging assay in crude extract (22) and aloin, active compound of A. barbadensis⁽¹⁸⁾.

II. METHODOLOGY

A. Collection and Preparation of plant extract-

The plant leaves were collected from the campus of Integral University, Lucknow. The leaves of the plant were air dried at room temperature and vouchers were sent for identification and stored in National Botanical Research Institute, Lucknow. The dried plant leaves powder was prepared with the help of mortar and pestle and used for extraction in different solvent system using Soxhlet apparatus. The solvents used for extraction were water, benzene, acetone, ethyl acetate and methanol.

B. Phytochemical Analysis (Qualitative) of Plant Extract-

The phytochemical analysis was carried out to determine the presence of active ingredient of leaf extract. Qualitative estimation of phytochemical analysis has been estimated on the basis of color formation ^(12, 19, 24, 9, 14). The phytochemicals to be determined include alkaloids, anthraquinones, flavonoids, phlobatannins, glycosides, saponins, sterols, tannins, terpenoids and reducing sugar.

Alkaloids

The determination of alkaloids was done by using Wagner's reagent. The preparation of Wagner's reagent was done by dissolving 2 g of iodine and 6 gm of potassium iodide in 100 ml of distilled water. The alkaloids were estimated by preparing extract. The extract was prepared by adding 500 mg of dry plant material in 500 ml methanol and it is kept at 37°C for 20 min in water bath. After cooling the extract was filtered and treated with few drops of Wagner's reagent. The appearance of reddish brown precipitate indicated the presence of alkaloids.

Anthraquinones (Borntrager's test)

The extract of the dried leaves of plant were prepared by weighing 0.5 gm and boiled with 10% hydrochloric acid (HCL) for few minutes in a water bath. The extract was cooled and filtered and to it equal volume of chloroform (CHCl3) and few drops of 10% ammonia was added and heated. The formation of rose pink color indicated the presence of anthraquinones.

Flavonoids

The presence of flavanoids were determined by preparing methanolic extract, mixed with 10 ml of ethyl acetate and boiled for 3 min over steam bath, cooled and filtered. To 4 ml of filtrate 1 ml of dilute

ammonia solution was added. The appearance of yellow color indicated the presence of flavanoids.

Phlobatannins

The aqueous plant extract was prepared and boiled with 1% hydrochloric acid (HCl). The deposition of red precipitate confirmed the presence of phlobatannins.

Glycosides (Fehling's Test)

The extract was prepared by weighing 0.5 gm plant and soaked in 5 ml methanol. The extract was taken 2 ml and to it 10 ml of 50% hydrochloric acid (HCl) was added and kept at water bath for 30 min till boiling. The filtrate was allowed to cool and to it 5 ml of Fehling's solution was added and boiled for 5 min until brick red precipitate appear. The appearance of brick red color indicated the presence of glycosides.

Saponins (Frothing Test)

The methanolic leaves extract was prepared and dried at room temperature. The extract of 0.2 gm was shaken with 5 ml of distilled water and boiled. The appearance of creamy mist of small bubbles appearance confirms the presence of saponins.

Steroids (Salkowski Test)

The methanolic extract was dissolved in methanol and to it 5 drops of concentrated hydrogen sulfoxide (H_2SO_4) was added. The formation of red color indicated the presence of steroids.

Tannins (Ferric Chloride Test)

To estimate the presence of tannins methanolic extract of 0.5 g was weighed and dissolved in 10 ml distilled water, filtered and to it ferric chloride reagent was added. The appearance of blue black precipitate confirms the presence of tannins.

Terpenoids (Salkowski Test)

The dried leaves of the plant was weighed 0.2 gm and mixed with 2 ml of chloroform and 3 ml of concentrated hydrochloric acid (HCl). The reagents were added carefully to form a layer and appearance of reddish brown coloration of the interface indicated the presence of terpenoids.

C. Anti-oxidant Assay

The herbs, spices and medicinal plants have antioxidant property and prevent oxidative stress and increase its protective property. There are number of researchers reported antioxidant property in number of plants. The aqueous and organic extracts were





used for screening anti-oxidant assay by different methods.

DPPH (2, 2 – Diphenyl – 1- Picryl Hydrazyl) Radical Scavenging Assay

The antioxidant property by DPPH radical scavenging assay were determined by the method of Blois (1958) with some modification ⁽⁶⁾. The dried crude extract prepared in different solvet were weighed at different concentrations varying from 1000 µg/ml to 0.46 µg/ml and dissolved in methanol and used for the experiment. The working solution of DPPH prepared in methanol 100 mM. The prepared extract was taken 10 µl and mixed with 200 µl DPPH and incubated at 37° C for 30 min under dark conditions. Control reaction was prepared by using L-ascorbic acid in place of plant extract. The DPPH radical scavenging activity was determined by measuring the absorbance at 490 nm spectrophotometrically⁽⁴⁾. The percentage inhibitions of samples were calculated by the formula given below.

Scavenging activity (%) = <u>Absorbance of Control - Absorbance of test x 100</u> Absorbance of Control

Scavenging of Superoxide Radical with the Alkaline DMSO (dimethyl sulfoxide) Assay

The scavenging of superoxide radical assay with alkaline DMSO were determined by the method of Kunchandy and Rao (1990) with slight modification (20, 31, 23). The dried plant extract was weighed at different concentrations varies from 1000 µg/ml to 0.46 µg/ml and dissolved in dimethyl sulfoxide. The prepared extract at different concentration were mixed with 0.1 ml of nitro blue tetrazolium (1 mg/ml in DMSO) and 1 ml of alkaline DMSO. The alkaline DMSO was prepared by adding 1 ml of DMSO with 5 mM sodium hydroxide and 0.1 ml of water. The final volume was make up to 1.4 ml by adding 0.3 ml of crude extract prepared in DMSO. The absorbance was measured at 560 nm spectrophotometrically. For Control L-ascorbic acid was used in place of extract. The percentage inhibition was calculated by the formula given below.

Percentage super oxide scavenging activity = <u>Test absorbance - Control absorbance x</u> 100 Test absorbance

Nitric Oxide Radial Inhibition Assay

The dried plant extract was weighed at varying concentrations from $1000\mu g/ml - 0.45\mu g/ml$ and

dissolved in methanol. The prepared extract of 1 ml was mixed with 1 ml phosphate buffer saline and 4 ml of 10 mM sodium nitroprusside. The reaction was incubated at 25° C for 150 min and after incubation 1 ml sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was added and again incubated for 5 min at room temperature and the process called diazotization reaction. Finally 1 ml of N-(1-naphthyl) ethylenediamine dihydrochloride was added to the reaction mixture and kept for 30 min in diffused light, after 30 min the absorbance was measured at 540 nm ⁽⁵⁾. The control used was L-ascorbic acid. Scavenging activity (%) = <u>Absorbance of control</u> – Absorbance of extract x 100 Absorbance of control

TotalAnti-oxidantCapacitybyPhosphomolybdenum Method

The antioxidant assay by phosphomolybdenum method were determined in crude extrct and L-ascorbic acid ⁽²⁸⁾. Different concentrations of plant extracts were taken like other assays. The reagent mixture was prepared by taking 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The plant extract 0.1 ml were mixed with 1 ml of reagent mixture and incubated at 95° C for 90 min, after incubation the samples were cooled to 25° C and absorbance measured at 695 nm against blank. The blank contained 1 ml of reagent solution without the sample. The results were expressed as absorbance value at 695 nm ⁽¹⁶⁾.

Hydroxyl Radical in the Deoxyribose Method

The method of Halliwell and co-workers (1987) with slight modifications was used for scavenging of hydroxyl free radical ⁽¹¹⁾. The crude extract of different concentration was prepared in DMSO. The reaction mixture was prepared by adding 0.2 ml (3mM) deoxyribose, 0.2 ml (0.1mM) ferric chloride, 0.2 ml (0.1 mM) ethylenediaminetetraacetic acid disodium salt, 0.2 ml (0.1 mM) ascorbic acid and 0.2 ml (2 mM) hydrogen peroxide in 20 mM phosphate buffer. The extract was added in 0.2 ml reaction mixture to make up volume to 1.2 ml and the sample was incubated at 37° C for 30 min. After incubation to the reaction mixture 0.2 ml (15% w/v) chilled trichloroacetic acid, 0.2 ml (1% w/v) thiobarbituric acid dissolved in 0.25 N hydrochloric acid was added. The sample was kept in boiling water bath for 30 min, cooled and absorbance was measured at 532 nm spectrophotometrically (13, 23). The percentage scavenging were calculated by the formulae given below.

Scavenging activity (%) =



Absorbance of Control - Absorbance of test x 100 Absorbance of Control

D. Statistical Analysis

All results are expressed as mean \pm standard deviation (SD). The mean, standard deviation and percentage inhibition values were calculated in MS excel. Linear regression analysis was used to calculate the efficient concentration (IC₅₀) values.

III. RESULTS AND DISCUSSIONS

The crude extract of Aloe barbadensis showed the presence of alkaloid, flavonoid, terpenoids and steroids by qualitative phytochemical analysis. The presence of these phytochemicals like polyphenols and flavonoids in plant plays an important role in reducing risk for chronic diseases and ability to trap highly reactive free radicals species present in biological systems. The presences of these polyphenols are found to be responsible for its antioxidant property ⁽²¹⁾. In the present study, different aqueous and organic plant extracts were used to evaluate anti-oxidant activity and all showed significant anti-oxidant activity. The number of scientists reported that polyphenols are anti-oxidants with redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. Some show metal chelation properties (29). The anti-oxidant properties were evaluated by DPPH, Alkaline DMSO, Nitric oxide, Total anti-oxidant and Hydroxyl Radical in the Deoxyribose method.

A. DPPH (2, 2 – Diphenyl – 1- Picryl Hydrazyl) Radical Scavenging Assay

The assay showed the property of crude extracts and standard to scavenge DPPH free radicals. In the DPPH radical scavenging assay, anti-oxidants react with the DPPH radicals exists in stable free radical naturally in deep violet color turns in yellow colored diphenvl picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of the anti-oxidant (10). The reducing capability of the DPPH is determined by the reduction in its absorbance and was measured at 490 nm induced by anti-oxidant ⁽⁶⁾. The A. barbadensis methanolic extract showed strongest scavenging activity of 62.9 ± 0.12 percent. The other extracts gave their activity in the order of acetone > aqueous extracts and the percentage inhibition was found to be 52.5 \pm 0.25 and 45.3 \pm 0.22 percent respectively at 1000 µg/ml concentration of plant extract. The benzene and ethyl acetate extract gave negative result by DPPH assay. The percentage inhibition value of standard, L-ascorbic acid by DPPH method was found to be 78.2 ± 1.79 percent at concentration of 1000 µg/ml. The values of *A. barbadensis* extracts at different concentrations are shown in graphical representation (Figure 1).

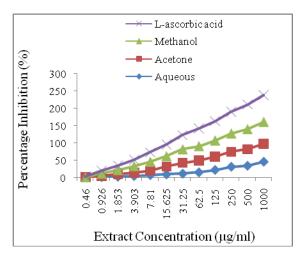


Fig. 1.The scavenging effect of aqueous and organic extracts of *Aloe barbadensis* and L-ascorbic acid (standard) by DPPH method. The different concentrations of extracts used were 1000 to 0.46 μ g/mL. The data represent the percentage DPPH inhibition and values are expressed as mean \pm SD (n=3).

B. Scavenging of Superoxide Radical with the Alkaline DMSO (dimethyl sulfoxide) Assay

The superoxide radicals are known to be very harmful to the cellular component. The superoxide free radical formed by alkaline DMSO in the reaction reacts with Nitro blue tetrazolium (NBT) and produce coloured di formazan which are absorbed at 560 nm ⁽³¹⁾. Superoxide anion widely occurs in nature is an important product of one electron reduction of dioxygen O_2^- . The superoxide ion is a free radical with one unpaired electron and like dioxygen is paramagnetic. The superoxide was a byproduct of mitochondrial respiration mostly by Complex I and Complex III, as well as several other enzymes for example xanthine oxidase. The superoxide radicals are involved to kill and invade micro-organisms by immune system and produced by enzyme NADPH oxidase (23). The methanolic extract of A. barbadensis showed strongest anti-oxidant activity, followed by aqueous > acetone > benzene > ethyl acetate extract and the percentage inhibition values were found to be $60.3 \pm 0, 57.3 \pm 6.37, 45.6 \pm 0.56, 44.5 \pm 1.75$ and 34.8 ± 0.49 respectively, at the concentration of 1000 µg/ml of plant extracts. The percentage inhibition



value of standard, L-ascorbic acid was found to be 54.0 ± 0.12 percent at concentration of $1000 \ \mu g/ml$. The percentage inhibition values of *A. barbadensis* extracts by Alkaline DMSO method at different concentrations are shown in graphical representations Figure 2.

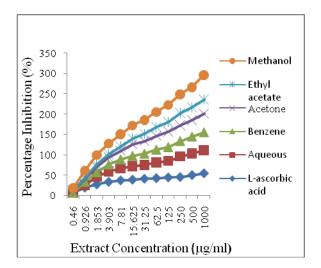


Fig. 2. The scavenging effect of aqueous and organic extracts of *Aloe barbadensis* and L-ascorbic acid (standard) by Alkaline DMSO method. The different concentrations of extracts used were 1000 to 0.46 μ g/mL. The data represent the percentage alkaline DMSO inhibition and values are expressed as mean \pm SD (n=3).

C. Nitric Oxide Free Radical Scavenging Assay

The nitric oxide free radical scavenging activity showed the ability of the extracts and the standard to scavenge nitric oxide free radical. Nitric oxide is unstable under aerobic conditions and reacts with oxygen to reduce stable product nitrates and nitrite through intermediates through NO₂, N₂O₄ and N₃O₄. These compounds alter cellular structure and components by causing harm. The test compound scavenge these and decreases the amount of nitrous In above scavenging assav. acid. sodium nitroprusside in aqeous solution and physiological pH generates nitric oxide which interacts with oxygen to produce nitrite ions and they are scavenged by sample used, this is measured at 540 nm spectrophotometrically ⁽²⁷⁾. The scavenging of nitric oxide by extracts are concentration dependent. The methanolic extract of A. barbadensis showed strongest activity, followed by ethyl acetate > aqueous > acetone > benzene extract and the percentage inhibition values were found to be 70.2 \pm 2.54, 47.7 \pm 0.76, 43.1 \pm 0.76, 41.7 \pm 1.92 and 22.5 \pm 2.5 percent respectively, at the concentration of 1000 μ g/ml of plant extracts. The percentage inhibition value of standard, L-ascorbic acid was found to be 84.4±4.56 percent at concentration of 1000 μ g/ml. The percentage inhibition values of *A. barbadensis* extracts by Nitric oxide scavenging assay at different concentrations are shown in graphical representations Figure 3.

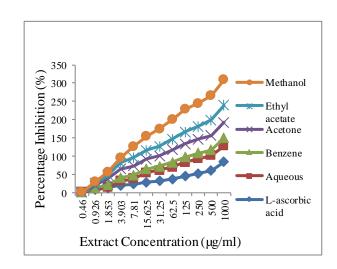


Fig. 3. The nitric oxide radical scavenging activity of aqueous and organic extracts of *Aloe* barbadensis and L-ascorbic acid (standard). The different concentrations of extracts used were 1000 to 0.46 μ g/mL. The data represent the percentage nitric oxide inhibition and values are expressed as mean \pm SD (n=3).

D. Total Anti-oxidant Capacity by Phosphomolybdenum Method

Total anti-oxidant capacities of the plant extracts and L-ascorbic acid were determined by the method of Phosphomolybdenum and measured at 695 nm spectrophotometrically. The higher absorbance value indicates the greater anti-oxidant activity. The method is based on the reduction of Mo (IV) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) compounds ⁽¹⁾. A high absorbance value of the sample indicates its strong anti-oxidant activity. The total anti-oxidant capacity may be attributed to their chemical composition and phenolic acid content. The A. barbadensis methanolic extract showed strongest scavenging activity of 58.7 ± 0.52 and the other extracts gave their activity in the order of aqueous > acetone > ethyl acetate > benzene extracts and the

percentage inhibition was found to be 48.1 ± 2.56 , 34.2 ± 5.55 , 34.2 ± 2.18 and 18.5 ± 1.00 percent respectively at 1000 µg/ml concentration of plant extract. The percentage inhibition value of standard, L-ascorbic acid was found to be 73.3 ± 1.15 percent at concentration of 1000 µg/ml. The percentage inhibition values of *A. barbadensis* extracts by Phosphomolybdenum method at different concentrations were shown in Figure 4.

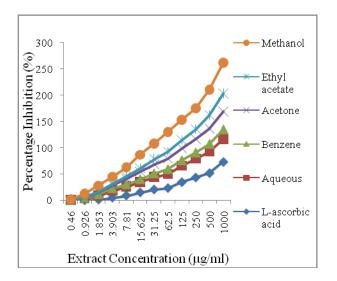


Fig. 4. The scavenging activity of aqueous and organic extracts of *Aloe barbadensis* and L-ascorbic acid (standard) by Total anti-oxidant assay. The different concentrations of extracts used were 1000 to 0.46 μ g/mL. The data represent the percentage inhibition values and values are expressed as mean \pm SD (n=3).

E. Deoxyribose Method for Scavenging of Hydroxyl Radical

The potentially reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. The plant extract scavenge free radicals mediated by deoxyribose damage and was assessed by means of iron (II)-dependent DNA damage assay. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe2+/ EDTA/ H_2O_2 system (Fenton reaction)⁽³⁴⁾. The radicals attack deoxyribose which hydroxyl eventually results in thiobarbituric acid reacting substances (TBARS) formation. Deoxyribose degradation was measured as TBA reactive substances and the percentage of inhibition was calculated from the control where no test compound was added ⁽²⁾. The aqueous extract *A. barbadensis* showed strongest scavenging activity of 65.1 ± 0.08 and the other extracts gave their activity in the order of methanol > acetone > ethyl acetate extracts and the percentage inhibition values were found to be 60.2 ± 0.16 , 48.6 ± 0.81 and 40.4 ± 0.73 percent respectively at the concentration of 1000 µg/ml of plant extract. The percentage inhibition value of standard, L-ascorbic acid was found to be 53.7 ± 0.08 percent at concentration of 1000 µg/ml. The benzene extract gave negative result by Deoxyribose method. The percentage inhibition values of *A. barbadensis* extracts by Deoxyribose method at different concentrations were shown in Figure 5.

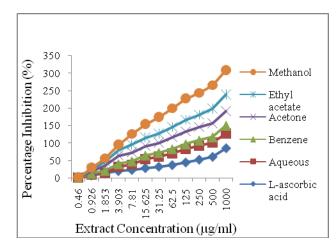


Fig. 5. The hydroxyl radical scavenging activity of aqueous and organic extracts of *Aloe* barbadensis and L-ascorbic acid (standard) by deoxyribose method. The different concentrations of extracts used were 1000 to 0.46 μ g/mL. The data represent the percentage inhibition values and values are expressed as mean \pm SD (n=3).

F. Comparative Chart of IC₅₀ Values of Aqueous and Organic Extracts

The extracts of *Aloe barbadensis* also showed potent anti-oxidant activity by different methods. Comparative values of IC_{50} values of aqueous and organic extracts are given in table 1. The present study shows acetone and methanolic extracts *A. barbadensis* showed anti-oxidant activity by DPPH radical scavenging assay with IC_{50} values of 705.2 \pm 8.08 and 182.8 \pm 1.44 µg/ml respectively. Whereas aqueous and methanolic extracts showed anti-oxidant activity by alkaline DMSO method with IC_{50} values of 237.5 \pm 11.4 and 553.4 \pm 3.28 µg/ml respectively. While methanolic extract of *A. barbadensis* showed



activity by nitric oxide method with IC₅₀ value of 43.17 \pm 6.29 µg/ml. The methanolic extract of *A. barbadensis* gave IC₅₀ value of 589.6 \pm 5.54 by total anti-oxidant activity and aqueous and methanolic extracts gave IC₅₀ value of 579.8 \pm 3.95 and 714.8 \pm 7.21 µg/ml respectively by deoxyribose method. Similarly, Ray and co-workers (2013), reported potent anti-oxidant activity in *A. barbadensis* by DPPH method, superoxide radical scavenging assay, hydroxyl radical scavenging assay ⁽³⁰⁾. In other study, done by researchers on *A. barbadensis* found significant free radical scavenging activity by DPPH and ferric reducing anti-oxidant power (FRAP) assay ⁽²¹⁾.

Extract in	IC ₅₀ values ± SD (µg/ml) of different anti-oxidant		
different solvents	DPPH	Alkaline DMSO	Nitric oxide
Aqueous	-	237.5±11.4	-
Acetone	705.2±8.08*	-	-
Methanol	182.8±1.44*	553.4±3.28	43.17±6.29

Table 1 Comparative chart of IC₅₀ values of aqueous and organic extracts of *Aloe barbadensis*. Unit for IC₅₀ for all the activities are μ g/ml. Data are expressed as mean \pm SD (n=3). *p< 0.0001 vs 0 μ g/ml.

Extract in different	IC ₅₀ values ± SD (µg/ml) of different anti-oxidant		
solvents	Total anti-oxidant capacity	Deoxyribose	
Aqueous	-	579.8±3.95*	
Acetone	-	-	
Methanol	589.6±5.54*	$714.8 \pm 7.21*$	

Table 2 Comparative chart of IC₅₀ values of aqueous and organic extracts of *Aloe barbadensis*. Unit for IC₅₀ for all the activities are μ g/ml. Data are expressed as mean \pm SD (n=3). *p< 0.0001 vs 0 μ g/ml.

IV. CONCLUSIONS

The present study showed that the presence of phytochemicals is responsible for potent anti-oxidant activity in aqueous and organic extracts of *Aloe barbadensis*. The methanolic extract showed maximum activity as compared to other organic extracts screened. Further studies could be carried out for further purification and characterization of its compounds.

V. ACKNOWLEDGMENTS

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