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DETERMINATION ANTIOXIDANT PROPERTIES OF ALOE VERA LEAF EXTRACT

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ABSTRACT

Many diseases are caused by oxidative stress. Medicinal plants are considered as the greatest pharmaceutical stores existing on the earth as they can produce secondary phytochemicals having bioactive properties. Natural antioxidants are widely used because they are regarded as safer and causing fewer adverse. Therefore, we aimed for quantitative estimation of phytochemicals present in Aloe vera leaves and determination antioxidant activities of leaf parts of A. vera. Leaves of A. vera was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with double distilled water. The free radical scavenging activity of the aqueous (aq.) leaf extract of A. vera and standard ascorbic acid were investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. Results portrayed that aq. leaf extract of Aloe vera exhibited DPPH radical scavenging activity with an IC_{50} value of 41.59 µg/mL in comparison with the standard ascorbic acid with an IC_{50} value of 56.65 µg/mL. Furthermore, total phenolic quantity was found to be highest (28.12 mgGAE/g extract) in aq. leaf extract of Aloe vera when compared with total flavonoid quantities (2.44 mgQE/g extract). In conclusion, this study demonstrated the antioxidant potential of aq. leaf extract of A. vera, and this could be ascribed to the presence of phytochemicals such as phenolic compounds and flavonoids present in the aq. A. vera leaf extracts. Therefore, aq. leaf extracts of A. vera could be explored in the development of natural antioxidant drugs

Keywords: Antioxidant, Aq. extract, Aloe vera, Leaves, Polyphenols, Flavonoids

INTRODUCTION

Many diseases are caused by oxidative stress. Accelerated cell oxidation contributes to cardiovascular disease, tumor growth, wrinkled skin, cancer, Alzheimer's disease, and even a decline in energy and endurance.[1,2] Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effect by scavenging reactive oxygen species, activating a buttery of detoxifying proteins or preventing the generation of reactive oxygen species.[3]

Medicinal plants are considered as the greatest pharmaceutical stores existing on the earth as they can produce secondary phytochemicals having bioactive properties. These

phytochemicals work efficiently to cure various diseases and illnesses since ancient times. The use of plant in the treatment of the diseases is as old as human civilization on the earth. Although it is not clear how the primitive people discovered the phenomenon that plants could be used to cure disease. Natural product can be defined as a chemical organic substance which is produced by the living organisms found in the nature that are produced by the path way of primary and secondary metabolism.[4]

Natural antioxidants such as alpha-tocopherol and ascorbic acid are widely used because they are regarded as safer and causing fewer adverse reactions but their antioxidant activities are lower than the synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) which have been restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens.[5-7] Therefore, there is a considerable interest in finding new and safe antioxidants from natural sources to replace these synthetic antioxidants.[8,9]

Aloe vera L. is a perennial succulent plant belonging to the Aloeaceae family (Figure 1). A. vera is most widely accepted and used for various medical and cosmetic purposes.[10] A great use of aloe gel was studied to be used as antioxidant. Furthermore, recently, natural plants have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids,[11] which prevent free radical damage, reducing risk of chronic diseases. Thus, the consumption of dietary antioxidants from these sources is beneficial in preventing cardiovascular disease.[12]

Figure. Showing Aloe vera plant

With this context present study was designed for determination of antioxidant properties of A. vera leaf extract.

MATERIALS AND METHODS

Collection Leaves of A. vera

The leaves of A. vera were collected in and around district headquarter places of Karnataka. The leaves were gently and thoroughly washed with running tap water to remove the dirt particles and wiped off, and sprayed with ethanol, and then shade dried. The dried leaves were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.[13]

Extraction

Approximately 50 g of dried and coarsely powdered leaves of A. vera were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 500 mL of double

distilled water. The extracts were concentrated by distilling the solvents in a rotary flash evaporator and dried at 40ºC. The extract was preserved in airtight containers and stored at room temperature until further use.[13]

Phytochemical Screening

Phytochemical screening was carried out on the aqueous (aq.) leaf extracts of A. vera by using standard procedures to detect phytoconstituents as described by Sofora,[14] Trease and Evans[15] and Harborne.[16]

Test for alkaloids

Approximately 0.2g of aq. leaf extract of A. vera were warmed with 2% H_2SO_4 (2.0mL) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for tannins and phenolic compounds

The aq. leaf extract of A. vera in small quantity were mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green colouration indicate the presence of tannins and phenolic compounds.

Test for glycosides

About 0.6g of aq. leaf extract of A. vera were hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

Test for saponins

About 0.2g of aq. leaf extract of A. vera were shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for flavonoids

0.2g of aq. leaf extract of A. vera were dissolved in diluted 10%NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Test for steroids

2 mL of acetic anhydride was added to 0.5g of aq. leaf extract of A. vera and then added 2 mL of H_2SO_4. The change of color from violet to blue or green or red showed the presence of steroids.

Test for terpenoids

0.3g of aq. leaf extract of A. vera were mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M H_2SO_4 was carefully added to form a layer. Formation of reddish-brown coloration at the interface indicates positive results for the presence of terpenoids.

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the aq. leaf extract of A. vera was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, and its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[17] The phenolic content of the extract was determined from calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water and was expressed in mg gallic acid equivalent (GAE)/g of

extract powder (mg GAE/g).

Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination in aq. leaf extract of A. vera.[18] The flavonoid content was determined from extrapolation of calibration curve which was made by preparing quercetin solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg quercetin equivalent (QE)/g of extract powder (mg QE/g).

Assay of Antioxidant Activity

DPPH free radical scavenging assay

The free radical scavenging activity of the aq. leaf extract of A. vera and of standard solution (ascorbic acid) were investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method as described by Karadag et al.[19] The assay mixture contained 2 mL of 1.0 mmol/L DPPH radical solution prepared in methanol and 1 mL of standard or extract solution of different concentrations (25-200 µg/mL). The solution was rapidly mixed and incubated in dark at 37°C for 20 min. The decrease in absorbance of each solution was measured at 517 nm. Ascorbic acid is a well-known antioxidant was used as positive control while DPPH radical solution with 1 mL ethanol was taken as blank. The radical scavenging (%) was calculated by the following formula:

Free radical scavenging activity (%):

Absorbance of control – Absorbance of sample/Absorbance of control X 100

The concentration of sample required to scavenge 50% of the DPPH free radical (IC_{50}) was determined from the curve of percent inhibitions plotted against the respective concentration.

RESULTS

The major phytochemicals found in aq. leaf extract of A. vera were found to be alkaloids, flavonoids, glycosides, steroids, phenolic compounds, and tannins. Whereas, phytochemicals such as saponins and terpenoids were found to be absent in aq. leaf extract of A. vera (Table 1).

Table 1: Photochemical screening of aq. leaf extracts of A. vera

Phytochemical Components	Aq. Leaf Extract of A. vera
Alkaloids	+
Flavonoids	+
Glycosides	+
Saponins	-
Steroids	+
Phenolic compounds	+

Tannins	+
Terpenoids	-

+: Present; -: Absent;

The results of quantitative estimation of phytochemicals in aq. leaf extract of A. vera was represented in Table 2. Results implied that total phenolic quantity was found to be highest (28.12 mgGAE/g extract) in aq. leaf extract of A. vera when compared with total flavonoid quantities (2.44 mgQE/g extract).

Table 2: Quantitative estimation of phytochemicals in aq. leaf extract of Aloe vera

Phytochemicals	Aq. leaf extract of Aloe vera
Total Phenolics	28.12 mgGAE/g extract
Total flavonoids	2.44 mgQE/g extract

Values are expressed mean; n=3

The aq. leaf extract of Aloe vera exhibited inhibition percentage of 7.67%, 11.00%, 18.93%, and 34.69% at a concentration range of 25 µg/mL, 50 µg/mL, 100 µg/mL, and 200 µg/mL respectively with an IC_{50} value of 41.59 µg/mL in comparison with the standard ascorbic acid with an IC_{50} value of 56.65 µg/mL (Table 3).

Table 3: Effect of aq. leaf extract of A. vera on DPPH radical-scavenging activity

Conc. of Aq. Leaf Extract of A. vera (µg/mL)	**Inhibition (%)**	**Conc. of Ascorbic Acid (µg/mL)**	**Inhibition (%)**
25	7.67	25	88.56
50	11.00	50	91.59
100	18.93	100	94.39
200	34.69	200	96.53
IC_{50} (ug/mL) = 41.59		IC_{50} (ug/mL) = 56.65	

Values were expressed as Mean; n=3

DISCUSSION

Among the ancient civilizations, India is well known for possessing an extensive array of medicinal plants. Many of the aromatic and medicinal plants found in India's forests are

harvested to be used as raw materials for the manufacturing of medications and scented products. Medicinal plants have been valued for their long history due to their diverse pharmacological effects, which are believed to be attributed to secondary plant metabolites such as tannins, alkaloids, flavonoids, glycosides, and steroids. DPPH free radical scavenging activity is an easy and widely used method for testing in-vitro antioxidant activity of natural compounds or plant extracts. DPPH is a stable free radical at room temperature, purple in color. Its reduction capability to accept an electron or a hydrogen radical from antioxidants is determined by measuring decrease in its absorbance values at 517 nm.[20] Hence in the current study we aimed to determine antioxidant activity potential of aq. leaf extract of A. vera.

Results of our study revealed that aq. leaf extract of A. vera exhibited DPPH radical scavenging activity with an IC_{50} value of 41.59 µg/mL in comparison with the standard ascorbic acid with an IC_{50} value of 56.65 µg/mL. Furthermore, total phenolic quantity was found to be highest (28.12 mgGAE/g extract) in aq. leaf extract of A. vera when compared with total flavonoid quantities (2.44 mgQE/g extract). These findings inferred that the DPPH free radical scavenging activity of aq. leaf extract of A. vera could be mainly ascribed to the presence of total polyphenolic contents.

In concurrence with our study findings literature reports evidenced that the antioxidant activity of plant materials closely correlated with the content of their phenolic compounds.[21] Moreover, Keshamma reported that antioxidant potential of methanolic leaf extract of E. scaber was mainly due to the presence of secondary metabolites viz. polyphenols and flavonoids present in it.[22] Furthermore, the reducing properties polyphenolic compounds are generally associated with the presence of reductones,[23] which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom.[24] The presence of antioxidant extracts can hinder the extent of β-carotene bleaching by acting on the linoleate-free radical and other free radicals formed in the system.[25] Saritha et al., reported that the methanol extract of A. vera gel exhibited highest antioxidant activity.[26]

CONCLUSION

In conclusion, results of our study portrayed that aq. leaf extracts of A. vera possess antioxidant activity, and this could be ascribed to the presence of phytochemicals such as phenolic compounds and flavonoids present in the aq. A. vera leaf extracts. Therefore, aq. leaf extract of A. vera could be explored in the development of natural antioxidant drugs.

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