Antioxidant and antibacterial activity of *Aloe vera* gel extracts.

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**ABSTRACT**

The antioxidative properties of extracts of *Aloe vera* gel made in methanol (MEAG), 95% ethanol (EEAG), hexane (HEAG), acetone (AEAG) and chloroform (CEAG) were investigated employing various *in vitro* systems viz. 1, 1-diphenyl-2-picrylhydrazyl (DPPH), superoxide anion radicals scavenging, metal ion chelation, reducing power, hydroxyl radicals scavenging and total antioxidant activity in linoleic acid emulsion system. The results showed that MEAG and AEAG possessed maximum DPPH free radical and superoxide radical scavenging activities. All the extracts were effective in scavenging the hydroxyl radicals in nonsite-specific assay as well as in site specific assay. The formation of the Ferrozine-Fe$^{2+}$ complex was found to be incomplete in the presence of MEAG and AEAG, indicating their capacity in chelating iron. The AEAG was shown more reducing power than MEAG. The *in vitro* antibacterial properties of methanolic extract of *Aloe vera* gel extract were investigated against various common pathogenic bacteria. The gel extract showed significant zone of inhibition against all the pathogens studied and the results were comparable to the conventional antibiotics. Hence it is suggested that *Aloe vera* gel extract could be same as a new source of natural antioxidant with potential applications for reducing the levels of lipid oxidation and oxidative stress.

**Key Words:** *Aloe vera*; antioxidant activity; lipid peroxidation; superoxide; hydroxyl

**INTRODUCTION**

The importance of reactive oxygen species (ROS) and free radicals has attracted increasing attention over the past decade. ROS which include free radicals such as superoxide anion radicals (O$_2^-$), hydroxyl radicals (OH$^-$) and non-free radical species such as H$_2$O$_2$ and singlet oxygen (O$_2^*$) are various forms of activated oxygen. ROS is continuously produced during normal physiologic events and they can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. Under pathological conditions, ROS is overproduced and results in oxidative stress. There are a lot of antioxidants that are introduced to minimize actions of ROS. Phenol compounds can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes.$^{[1]}$ Thus efforts have been made to search for novel natural antioxidants from fruits, vegetables, herbs and spices. 

*Aloe vera* products have long been used in health foods for medical and preservative purposes. There are more than 360 different species of aloes grown in the dry regions of North American, Europe and Asia. *Aloe vera* (*Aloe barbadensis* Miller.), a member of the family Liliaceae, is a short stemmed succulent, perennial herb. A recent report has shown that the organic extracts of *Aloe vera* leaf possess potent *in vivo* antioxidant capacity.$^{[2]}$ The organic extract of *Aloe vera* leaves provided anti-inflammatory activity in the experimental rats.$^{[3]}$ *Aloe vera* has also been shown to be a potential therapeutic agent for the treatment of sepsis and hepatotoxicity.$^{[4-5]}$ Glucomannan and acemannan can accelerate wound healing, activate macrophages and demonstrate antineoplastic, antiviral effects.$^{[6-7]}$ Though the information on the physiological properties of the *Aloe vera* gel are known, its antioxidant properties are not thoroughly worked out. The present study was conducted to
investigate the antioxidant activity and antibacterial activity screening of *Aloe vera* gel extracts.

**MATERIALS AND METHODS**

**Chemicals:**
1,1-diphenyl-2-picryl-hydrozyl (DPPH), 3-(2-pyridyl)-6,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), nicotinamide adenine dinucleotide (NADH), Linoleic acid, D-deoxy ribose sugar was purchased from Sigma chemical co. Tween-20, Ferrous chloride, from Himedia Pvt Ltd. All other chemicals and reagents were analytical grade.

**Plant material and extraction:**
Specimens of *Aloe vera* were collected from Gokulum garden, identified by Prof. H.S. Prakash, Professor, Dept of Applied Botany, University of Mysore, Mysore, Karnataka, India and cultivated in the premises of our institution. A specimen of the plant was deposited at Department of Botany, University of Mysore, Mysore, India. In this study the fresh leaves of this cultivated plant were used. The gel of the *Aloe vera* leaves was scraped with sterilized spoon. The gel was blended in an electric blender. The blended sample were freeze dried and stored prior to further use. The powder (10.0 g) was extracted with solvents viz. methanol, 95% ethanol, hexane, acetone and chloroform at room temperature. The extraction process was repeated till the solvents became colorless. The extracts were then filtered using Whatman No.1 paper. The filtrates were concentrated in vacuum at 50°C ± 1°C in a rotary evaporator to obtain the crude extracts. The methanol, ethanol, acetone, hexane and chloroform form extracts of *Aloe vera* gel were named MEAG, EEAG, AEAG, HEAG and CEAG respectively.

**DPPH free radical scavenging activity:**
The antioxidant activity of *Aloe vera* gel freeze dried extracts viz. MEAG, EEAG, AEAG, HEAG and CEAG and the standard compound BHA was measured in terms of hydrogen donating radical scavenging ability using the stable DPPH method.\(^{18}\) 0.1 ml of extract was added to 2.9 mL of methanol solution. After centrifugation, the supernatant is collected 50 µmol L\(^{-1}\) of DPPH solution is added. Kept in the dark for 45 min and the resulting decrease in absorbance at 517 nm were recorded against blank using a UV-Vis Spectrophotometer. Amongst test samples, MEAG and AEAG showed the maximum scavenging activity and hence were used for all the subsequent studies. The radical scavenging activity on DPPH was expressed as:

\[
\text{Scavenging effect (\%) = } \left( \frac{A_o - A_1}{A_o} \right) \times 100
\]

Where \(A_o\) is the absorbance of control and \(A_1\) is the absorbance of sample extract or standard.

**Superoxide anion (O\(_2^{-}\)) radical scavenging activity:**
Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the radiation of NBT.\(^{9}\) In these experiments, the superoxide radicals were generated in 16 mmol L\(^{-1}\) Triss-HCl buffer (pH 8.0) containing 50 µmol L\(^{-1}\) NBT, 78 µmol L\(^{-1}\) NADH and 100-300 µg mL\(^{-1}\) of sample extract. The reaction was started by adding 10µmol L\(^{-1}\) PMS to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560 nm was measured against blank samples. L-Ascorbic acid was used as a control. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The inhibition percentage of superoxide anion generation was calculated by using the following formula:

\[
\text{% Inhibition } = \left( \frac{A_o - A_1}{A_o} \right) \times 100
\]

Where \(A_o\) is the absorbance of L-ascorbic acid and \(A_1\) is the absorbance of sample extract or standard.

**Metal chelating activity:**
The extracts, at the concentration of 200-1000 µg mL\(^{-1}\) was added to a solution of 2 mmol L\(^{-1}\) FeCl\(_2\). The reaction was initiated by the addition of 5 mmol L\(^{-1}\) ferrozine and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was measured spectrophotometrically at 562 nm.\(^{10}\) The percentage of inhibition of Ferrozine-Fe\(^{2+}\) complex formation was calculated from:

\[
\text{% Inhibition } = \left( \frac{A_o - A_1}{A_o} \right) \times 100
\]

Where \(A_o\) is the absorbance of control and \(A_1\) is the absorbance of sample extract or standard.

**Reducing power:**
The sample extract at the concentration of 2-10 mg mL\(^{-1}\) were mixed with 0.2 mol L\(^{-1}\) phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. 10% trichloroacetic acid was added to the mixture, which was then centrifuged 650 x g at
room temperature for 10 min. The upper layer of solution was mixed with distilled water and 0.1% FeCl$_3$, and the absorbance was measured at 700 nm. The increase in absorbance of the reaction mixture indicated reducing power.

**Hydroxyl (OH$^-$) radical scavenging activity:**
The hydroxyl radical scavenging activity of MEAG and AEAG was measured by the competition between deoxyribose and MEAG or AEAG for the hydroxyl radicals generated from the Fe$^{3+}$/ascorbate/EDTA/H$_2$O$_2$ system (nonsite-specific assay) or Fe$^{3+}$/ascorbate/H$_2$O$_2$ (site-specific assay).

Briefly for the nonsite-specific hydroxyl radical system, the reaction mixture are in the following order: 200 µmol L$^{-1}$ of FeCl$_3$ and 1.04 mmol L$^{-1}$ of EDTA (v/v), 28 mmol L$^{-1}$ of 2-deoxy-D-ribose, 1 mmol L$^{-1}$ of H$_2$O$_2$, 50-300 µg/ml of sample extracts in 0.02 mol L$^{-1}$ of KH$_2$PO$_4$-K$_2$HPO$_4$ (pH 7.2), 0.1ml of 1 mmol L$^{-1}$ of ascorbic acid were added. For the site-specific hydroxyl radical system, EDTA was replaced by phosphate buffer. The extent of deoxyribose degradation was measured by thioarbituric acid (TBA) method. The reaction solution was incubated at 37°C for 1 hr. Then add TBA prepared in 1% of 50 mmol L$^{-1}$ NaOH and 5% TCA into the reaction tubes, which were placed into a temperature-controlled water bath at 100°C for 20 min. The tubes were cooled and then the absorbance was measured at 532 nm against blank sample. The inhibition percentage of hydroxyl radical scavenging activity was calculated by using the following formula:

% Inhibition = \[
\frac{(A_o - A_1)}{A_o} \times 100
\]

Where $A_o$ is the absorbance of control and $A_1$ is the absorbance of sample extract or standard.

**Antioxidant activity in linoleic acid model system:**
The mixture of linoleic acid and tween 20 in phosphate buffer (pH 7.2) were homogenized and the extracts were added at the final concentration of 0%, 0.005%, 0.01%, 0.02% and 0.05% wt/vol. The BHA at the level of 0.01% was used as a control. The mixture was incubated at 37°C and 80°C for 12 d and 10 h, respectively. The course of oxidation was monitored by measuring the conjugated dienes formation (CD). The antioxidative activity at the end of assay time was expressed for each indicator as reduction percent for peroxidation (RP %) with a control containing no antioxidant being 0%.

$$\text{RP\%} = \frac{[(\text{peroxidation indicator value without antioxidant}) – (\text{peroxidation indicator value with antioxidant}) / (\text{peroxidation indicator value without antioxidant})] \times 100.}$$

**Conjugated dienes:**
Aliquots of extracts were taken at different intervals (12 d and 10 h) during incubation. After incubation, 60% methanol in deonized water was added, and the absorbance of the mixture was measured at 233 nm. The concentration of conjugated dienes was expressed in ml/mg in each sample. The results were calculated as CD = B x vol/wt; where B is the absorbance reading, vol denotes the volume (ml) of the sample and wt is the mass (mg) of emulsion measured.

**Antibacterial activity:**
The methanolic extract of *Aloe barbadensis* was assayed for five strains of Gram (+) ve bacterial strains viz, *Bacillus cereus* ATCC1457, *Bacillus subtilis* DFR13, *Enterococcus faecalis* (lab culture), *Listeria monocytogenes* ATCC13932, *Staphylococcus aureus* and Gram (-) ve bacterial strains viz, *Aeromonas hydrophius* MTCC646, *Escherichia coli* ATCC14028, *Psudomonas aeruginosa* DFR219, *Salmonella enteritidis* ATCC14028, *Shigella boydii* MTCC1457, *Vibrio parahaemolyticus* ATCC01: K25. Antibacterial activity was determined by agar-well diffusion method as reported earlier. All the microbes were inoculated in nutrient broth and were incubated at 37°C for 24 hours. Methanolic extract was dissolved in water to a final concentration of 20% and sterilized by filtration through a 0.22 µm membrane filter. Hundred µl of suspension containing 10$^8$ colony forming units ml$^{-1}$ of bacteria was spread on nutrient agar medium. Wells (8 mm) were punched out of the solid of respective medium using sterile cork borer. 100 µl of different concentrations of the extracts were filled into each well. Petri dishes were incubated at 37°C for 24 hrs. At the end of the incubation period, inhibition zone formed on the medium were measured in mm. The assays were performed in duplicate and inhibition zone was also compared with ampicilline as a reference standard. Antimicrobial activities of the gel extracts were expressed by – (no zone of inhibition), + (zone of inhibition ≤ 8 mm in diameter) and ++ (zone of inhibition ≥ 8 mm in diameter).
Statistical analysis:
The statistical processing of the data obtained from all studies is expressed as Mean ± standard deviation (SD) of three separate experiments using the computer programme Excel.

RESULTS AND DISCUSSION
DPPH free radical scavenging activity:
Percentage yield of extracts of Aloe vera gel was found to be the maximum in the methanol extract (Table 1). The models of scavenging the stable DPPH radical are widely used to evaluate the free radical scavenging ability of various samples. [16-19] The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517nm induced by antioxidants. The extracts were able to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine (Figure 1). The scavenging effect of the Aloe vera gel extracts and BHA using the DPPH radical were in the following order: BHA (94.56%) > MEAG (93.14%) > AEAG (74.03%) > CEAG (57.68%) > EEAG (57.21%) > HEAG (23.59%). The methanol extract showed better radical scavenging activity than the other extracts.

Table 1. Percentage yield of extracts of Aloe vera gel in different solvents

<table>
<thead>
<tr>
<th>Sample extracts</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>28.14%</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>15.41%</td>
</tr>
<tr>
<td>Hexane</td>
<td>15.02%</td>
</tr>
<tr>
<td>Acetone</td>
<td>12.13%</td>
</tr>
<tr>
<td>Chloroform</td>
<td>03.05%</td>
</tr>
</tbody>
</table>

Superoxide (O₂⁻) radical scavenging activity:
Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species. [20] The superoxide radical is known to be produced in vivo and can result in the formation of H₂O₂ via dismutation reaction. Moreover, the conversion of superoxide and H₂O₂ into more reactive species, e.g., the hydroxyl radical, has been thought to be one of the unfavorable effects caused by superoxide radicals. [21] (Figure 2) shows the inhibitory effect of MEAG and AEAG on superoxide radical generation. MEAG was markedly a more potent scavenger of superoxide anion than AEAG. However, the reference compound, BHA exhibited higher superoxide scavenging activity than the Aloe vera extracts.

Metal chelating activity:
The method of metal chelating activity is based on chelating of Fe²⁺ ions by the reagent ferrozine which is a quantitative formation of a complex with Fe²⁺ ions. [22] Ferrozine can quantitatively form complexes with Fe²⁺. In the presence of other chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of the rate of colour of reduction therefore allows estimation of the chelating activity of the coexisting chelator. [23] (Figure 3) shows that the formation of the Ferrozine- Fe²⁺ complex is not complete in the presence of MEAG and AEAG, indicating their capacity in chelating iron. The absorbance of Ferrozine- Fe²⁺ complex decreased linearly in a dose dependent manner (200-1000 µg mL⁻¹). MEAG showed more chelating ability than AEAG. However, the chelating ability was relatively lower than that of EDTA. In this assay both the extracts, MEAG and AEAG and standard EDTA compounds interfered with the formation of ferrous complex with the reagent ferrozine, suggesting that it has chelating activity and captures the ferrous ion before ferrozine. EDTA is used for comparison as it is a known metal ion chelator. This indicates that the extract has good capacity for iron binding, suggesting its action as an antioxidant relating to its iron binding capacity.
Reducing power:
In the reducing power assay, the presence of reductants in the sample would result in the reducing of Fe$^{3+}$ to Fe$^{2+}$ by donating an electron. Amount of Fe$^{2+}$ complex can then be monitored by measuring the formation of Perl’s blue at 700 nm. (Figure 4) shows the reducing power of MEAG and AEAG and the reference compound, α-tocopherol. Here, the AEAG was shown more reducing power than MEAG. However, the reducing power of α-tocopherol was relatively more pronounced than that of sample extracts. Earlier authors [24-25] have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties 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 hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The reducing power of bioactive compounds has been reported to be associated with their antioxidant activity. [26-27] Osur data on the reducing power of the extracts suggest that it is likely to contribute significantly towards the observed antioxidant effect.

Hydroxyl (OH·) radical scavenging activity:
The sugar deoxyribose on exposure to hydroxyl radicals, generated by the Fenton reaction model system degrades into fragments and generates a pink chromogen on heating with TBA at low pH. [28] It was observed that all the extracts were effective in scavenging the hydroxyl radicals in nonsite-specific assay as well as in site specific assay (Figures 5 and 6). However, the change was greater in the nonsite specific than in the site-specific assay indicating their weak chelating power. However, the MEAG shows high effectiveness in the non-site specific assay at the concentration of 300 µg mL$^{-1}$ (94.55%) than that of AEAG (91.64%). The site-specific assay, AEAG at the concentration of 300 µg mL$^{-1}$ (65.59%), was comparatively higher than MEAG (58.11%). Therefore the extracts were efficient in competing with deoxyribose for OH radicals produced free in solution from a Fe$^{2+}$ - EDTA chelate. [29] Among the oxygen radicals specifically, the hydroxyl radical is the most reactive which severely damages adjacent biomolecules such as proteins, DNA, PUFA, nucleic acid and almost any biological molecule it touches. This damage causes aging, cancer and several diseases. [30] In addition; this radical species is considered as one of the quick initiators of the lipid oxidation process, abstracting hydrogen atoms from unsaturated fatty acids. [31] Therefore, the removal of hydroxyl radical is probably one of the most effective defenses of a living body against various diseases.
Antioxidant activity in linoleic acid model system:
In order to assay the effect of temperature on the antioxidant effect of the MEAG and AEAG extract, the experiments for inhibiting the peroxidation of the linoleic acid were conducted at two temperatures 37° and 80°C (Figures 7-10). An intensive formation of conjugated dienes was observed on day 4 of incubation of the linoleic acid at 37°C, and the maximum was reached on day 10. Of the five concentrations of the MEAG and AEAG, the highest antioxidant activity was at 0.1% level. However, the antioxidant activity was high in MEAG than in AEAG. The MEAG and AEAG at the concentration of 0.005%, 0.01%, 0.02% and 0.05% showed a weaker effect in inhibiting lipid peroxidation. Where as, BHA at 0.01% showed comparatively higher antioxidant activity than the extracts. At 80°C, a peak in conjugated dienes formation was observed at 5 h of storage, although the level of conjugated dienes was lower than that at 37°C. At the same time, efficiency was the maximum at the 0.1% concentration.

Atibacterial activity:
Since microorganisms become resistant over the period of time against antibodies, there is a need to explore the natural antibiotic sources for the prevention and treatment of several diseases. In
the last decade, Aloe vera has been used extensively in health drinks, topical creams, toiletries and cosmetics. [32] This is a systematic study on the antibacterial properties of Aloe vera leaf gel extract. In general, Aloe vera extract had inhibitory activity against Gram (+) ve bacteria exception of B. cerus and B. subtilis also had inhibitory activity against Gram (-) ve bacteria with the exception of P. aeruginosa( Table 2).

Table 2. Antibacterial activity of Aloe vera gel methanolic extract of against Gram (+) ve and Gram (-) ve bacterial strains

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Aloe vera extract Inhibition zone (mm)</th>
<th>Antibiotics Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 ug</td>
<td>200 ug</td>
</tr>
<tr>
<td>Bacillus cereus ATCC1457</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Bacillus subtilis DFR13</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Enterococcus faecalis (lab culture)</td>
<td>3.75 ± 0.25</td>
<td>04.75 ± 0.25</td>
</tr>
<tr>
<td>Listeria monocytogenes ATCC1393</td>
<td>9.50 ± 0.50</td>
<td>10.75 ± 0.25</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5.50 ± 0.50</td>
<td>06.50 ± 0.50</td>
</tr>
<tr>
<td>Aeromonas hydrophius MTCC646</td>
<td>3.00 ± 0.00</td>
<td>03.50 ± 0.00</td>
</tr>
<tr>
<td>Escherichia coli ATCC14028</td>
<td>6.75 ± 1.25</td>
<td>04.25 ± 0.25</td>
</tr>
<tr>
<td>Psudomonas aeruginosa DFR219</td>
<td>2.15 ± 0.15</td>
<td>01.00 ± 0.00</td>
</tr>
<tr>
<td>Salmonella enteritidis ATCC14028</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Shigella boydii MTCC1457</td>
<td>5.05 ± 0.05</td>
<td>10.50 ± 0.50</td>
</tr>
<tr>
<td>Vibrio paraahemolyticus ATCC01:K25</td>
<td>8.05 ± 0.05</td>
<td>05.00 ± 0.00</td>
</tr>
</tbody>
</table>

CONCLUSION

For the purpose of characterizing antioxidant activity of plant extracts, it is desirable to subject it to a battery of tests that evaluates the range of activities such as DPPH, superoxide anion radicals scavenging, metal ion chelation, reducing power, hydroxyl radicals scavenging and total antioxidant activity in linoleic acid emulsion system. The in vitro antioxidant activities of the methanol and acetone extracts indicated the efficacy of the gel as a source of natural antioxidants or nutraceuticals which will have application towards reducing lipid peroxidation /oxidative stress with consequent health benefits.

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