PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY OF Aerva lanata (L) – AN INVITRO STUDY

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ABSTRACT

The antioxidant potential of Aerva lanata was investigated in this study. Based on the phytochemical screening aqueous, ethanol and aqueous extract were selected. The plant exhibited the most potent radical scavenging activity at a maximum concentration 2.5 mg/ml. Natural antioxidants such as Flavonoid, Total phenols, Tannin, Carotenoids and Lycopene were evaluated and also the antioxidant activity against DPPH, Super oxidant anion, Hydroxyl radical, Nitric oxide radical, Hydrogen peroxide radical, Total antioxidant capacity assay and anti-lipid peroxidation activity were evaluated. Aerva lanata showed high anti lipid peroxidation against TBA. Strong antioxidant activity showed in aqueous ethanol extracts than water and ethanol extracts, and similar to standards ascorbic acid and BHT. This plant may be explored therapeutic agent in future.

Key words: Aerva lanata, phytochemical screening, Natural antioxidant, Anti – lipid peroxidation.

INTRODUCTION

Medicinal plants have been widely used for thousand years for the treatment of fractures and joint disease. In the past, the development of herbal anti-osteoporosis formulas was mainly presumed by scientists in Asian countries. Most of the medicinal plants are allelopathic in nature has been used a popular folk and an orient medicine treat against many diseases Hypertension, Hypercholesterolemia and Gastric ulcer1. Natural therapies such as the use of plant derived products may reduce adverse side effects2 and the compounds in plants have protective effects against environmental mutagens, carcinogens and endogenous mutagens3.

Aerva lanata known as polpala (treatment for renal disease) is a prostrate to decumbent sometimes erect herb found throughout tropical India as a common weed in fields and wasteland. The plant is useful for curing diabetes. It is anemophilic, demulcent and is helpful in Lithiasis, Cough, Sore throat and Wounds4. The plant has been reported to possess anti inflammatory and nephroprotective in rats5.

Oxidative Stress imposed by reactive oxygen species may be direct or indirect cause of tissue damage and many human diseases such as Aging, Cancer, Atherosclerosis, Cardiac hypertrophy. Natural antioxidants which are commonly present in medicinal plants scavenging radicals and inhibiting lipid peroxidation and preventing oxidative damage in animal tissue or cells.

The present study is aimed at exploring the Natural antioxidant compounds and Scavenging activity of Aerva lanata in different extracts.

MATERIALS AND METHODS

Plant Collection

Fresh plants were collected from Coimbatore, Tamil Nadu, India. The plant was authenticated by Dr. G.S.Moorthy, Botanical Survey of India, TNAU Campus, Coimbatore. The Voucher No: BSI/SC/5/23/10-11/Tech/22.

Extraction

Plant powder was extracted in three different solvents Water, Ethanol, Aqueous Ethanol (1 Part Water: 1 Part Ethanol). 100g of plant powder extracted in 500ml of corresponding solvents for 24 hrs in occasional shaker at room temperature. The supernatant was collected and evaporated to make final volume one fifth of the original volume. It was stored at 4°C in air tight bottles for further studies. The dried extract thus obtained was used directly for the determination of in vitro antioxidant activities and analysis of the antioxidant compounds.

Phytochemical analysis

Preliminary phytochemical screening of the methanolic extract of J.sambac was estimated according to the method adopted by Peach and Tracey6.

Antioxidant Capacity assays

DPPH radical scavenging assay was estimated by Blois7. ABTS radical scavenging assay was estimated by Re et al. 8 Reducing power was determined by Yen et al 9. Ferric Reducing Antioxidant Power (FRAP) was estimated by Benzie et al10. Hydroxyl Radical Scavenging assay was estimated by Smirnoff et al11. Superoxide radical scavenging assay was determined by Liu et al12. Lipid peroxidation was determined by Thio Barbituric Acid method Ottolenghi13. Hydrogen Peroxide Radical Scavenging assay was determined by replacement titration method Zhang14. Nitric Oxide Scavenging assay was determined by Green et al15.

Natural antioxidant Compounds

Flavonoid content was estimated by Jia et al16. Total Phenolic content was estimated by Singleton et al17. Tannin was estimated by Robert18. Carotene and Lycopene were estimated by Ranganna19.

RESULTS

Phytochemical Screening of plant materials

In table a showed Phytochemical screening of A.lanata. Aqueous and Ethanol extract having high phytochemicals than other solvents. Phytochemical screening of various ratio of aqueous ethanol extracts listed in table 1b, out of all ratios 5:5 having the presence of all phytochemicals.

<table>
<thead>
<tr>
<th>Table 1a: Phytochemical Screening of Aerva lanata.</th>
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</thead>
<tbody>
<tr>
<td>Type of Extracts</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Petroleum ether</td>
</tr>
<tr>
<td>Chloroform</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
</tr>
<tr>
<td>Ethanol Aqueous</td>
</tr>
<tr>
<td>Ethanol (1:1)</td>
</tr>
</tbody>
</table>

* Present, _Absent

Research Article
The concentration of 2.5 mg/ml. In all these three extracts, aqueous extract is similar to that of BHT standard (57.87%). Aqueous ethanol extract of A.lanata has 83.4% at the concentration 2.5 mg/ml.

Figure 1b shows the scavenging assay of ABTS. Aqueous extract of A.lanata has 88.37%.

Scavenging assay of DPPH and ABTS

Figure 1a shows the scavenging assay of DPPH. The highest scavenging activities on DPPH radicals are 82.37% for aqueous extract, 75.33% for water extract and 62.8% for ethanol extract at the concentration 2.5 mg/ml. All these three extracts have reducing activity but aqueous ethanol extract has more reducing activity than water and ethanol extract at the concentration 2.5 mg/ml.

Fig 1a : DPPH

Fig 1b : ABTS

Reducing power of water, ethanol and aqueous ethanol extract of Aerva Lanata compared to that of Butylated hydroxytoluene (BHT). Each value is expressed as mean ± standard deviation (n=3). Concentration (mg/ml) taken in X axis and % inhibition taken in Y axis.

Fig 2a : Reducing power

Scavenging assay of Hydroxyl radicals and Hydrogen Peroxide radicals

Figure 3a, all these three extracts have reducing activity but aqueous ethanol extract has more reducing activity than water and ethanol extract at the concentration 2.5 mg/ml.

Fig 3a : Hydroxyl radical scavenging assay

Table 1b: Phytochemical Screening of Aqueous ethanolic Aerva lanata.

<table>
<thead>
<tr>
<th>Phytochemical compounds</th>
<th>AL</th>
<th>SA</th>
<th>TP</th>
<th>AP</th>
<th>FL</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholysis</td>
<td>Cardiac glycosides</td>
<td>Saponins</td>
<td>Tannin</td>
<td>Phenolic compounds</td>
<td>Flavonoid</td>
<td>Steroids</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Steroids</td>
<td>Amino acids &amp; Proteins</td>
<td>Tannin &amp; Phenolic compounds</td>
<td>Saponins</td>
<td>Alkaloids</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aqueous Ethanol Extract (Ethanol : Water)</th>
<th>AL</th>
<th>ST</th>
<th>FL</th>
<th>TAN</th>
<th>AP</th>
<th>CH</th>
<th>CG</th>
<th>SA</th>
<th>TN</th>
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<tr>
<td>1:9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2:8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3:7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>4:6</td>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5:5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6:4</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

'M' Present 'L' Absent

Concentration (mg/ml) taken in X axis and % inhibition taken in Y axis.

Reducing power of water, ethanol and aqueous ethanol extract of Aerva Lanata compared to that of Butylated hydroxytoluene (BHT). Each value is expressed as mean ± standard deviation (n=3). Concentration (mg/ml) taken in X axis and Absorbance at 700nm taken in Y axis.

Fig 2b : FRAP

Ferric reducing antioxidant power of water, ethanol and aqueous ethanol extract of Aerva Lanata compared to that of Butylated hydroxytoluene (BHT). Each value is expressed as mean ± standard deviation (n=3). Concentration (mg/ml) taken in X axis and mmol FeII/mg sample taken in Y axis.

Fig 3b : FRAP

Scavenging assay of ABTS

Figure 1b shows the scavenging assay of ABTS. Aqueous extract of A.lanata has 57.87%, Ethanol extract of A.lanata has 73.62%, Aqueous ethanol extract of A.lanata has 83.4% at the concentration of 2.5 mg/ml. In all these three extracts aqueous extract is similar to that of BHT standard (58.37%).
Hydroxyl radical scavenging of water, ethanol and aqueous ethanol extract of *Aerva Lanata* compared to that of Ascorbic acid (Vit C). Each value is expressed as mean ± standard deviation (n=3). Concentration (mg/ml) taken in X axis and % inhibition taken in Y axis.

Figure 3b, all these three extracts water - 50.96%, ethanol- 43.70% and Aqueous ethanol - 69.57% at maximum in 2.5mg/mL have hydrogen peroxide radical scavenging capacity but the scavenging of aqueous ethanol extract is similar to that of ascorbic acid standard (60.57%).

![Fig 3b: Hydrogen peroxide radical scavenging assay](image)

**Hydrogen peroxide radical scavenging of water, ethanol and aqueous ethanol extract of *Aerva Lanata* compared to that of Ascorbic acid (Vit C). Each value is expressed as mean ± standard deviation (n=3). Concentration (mg/ml) taken in X axis and % inhibition taken in Y axis.**

Scavenging assay of Super oxide radicals and Nitric oxide radicals

Figure 4a, all these three extracts water - 48.19%, ethanol- 37.25% and Aqueous ethanol – 52.19% at maximum in 2.5mg/mL have super oxide radical scavenging capacity but the scavenging of aqueous ethanol extract is similar to that of ascorbic acid standard (55.87%).

Super oxide anion scavenging of water, ethanol and aqueous ethanol extract of *Aerva Lanata* compared to that of Ascorbic acid (Vit C). Each value is expressed as mean ± standard deviation (n=3). Concentration (mg/ml) taken in X axis and % inhibition taken in Y axis.

![Fig 4a: Superoxide radical scavenging assay](image)

**Fig 4a : Superoxide radical scavenging assay**

Super oxide anion scavenging of water, ethanol and aqueous ethanol extract of *Aerva Lanata* compared to that of Ascorbic acid (Vit C). Each value is expressed as mean ± standard deviation (n=3). Concentration (mg/ml) taken in X axis and % inhibition taken in Y axis.

Figure 4b, all these three extracts water - 55.47%, ethanol- 63.54% and Aqueous ethanol – 81.91% at maximum in 2.5mg/mL have nitric oxide radical scavenging capacity but the scavenging of aqueous ethanol extract is similar to that of ascorbic acid standard (82.61%).

![Fig 4b: Nitric oxide radical scavenging assay](image)

**Fig 4b : Nitric oxide radical scavenging assay**

Nitric oxide scavenging of water, ethanol and aqueous ethanol extract of *Aerva Lanata* compared to that of Ascorbic acid (Vit C). Each value is expressed as mean ± standard deviation (n=3). Concentration (mg/ml) taken in X axis and % inhibition taken in Y axis.

Estimation of Anti-Lipid peroxidation

In table 2 shows the anti-lipid peroxidation of *Aerva lanata*, all these three extracts water, ethanol and Aqueous ethanol extract at maximum in 2.5mg, have FTC carbonyl compound scavenging capacity but the scavenging of aqueous ethanol extract is similar to that of ascorbic acid standard.

**Table 2: Anti-lipid peroxidation of Water, Ethanol, and Aqueous Ethanol Extracts of *Aerva lanata* by Ferric thiocyanate method (TBA)**

<table>
<thead>
<tr>
<th>Solvents</th>
<th>A. lanata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>45.46 ± 0.157</td>
</tr>
<tr>
<td>Ethanol</td>
<td>35.42 ± 0.209</td>
</tr>
<tr>
<td>Aqueous Ethanol</td>
<td>42.77 ± 0.103</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>50.72 ± 0.117</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n = 3).

Estimation of Natural antioxidants

In table 3 Shows the natural antioxidants (Flavonoid, Phenolics, Tannins, Carotenoids and Lycopene) in water, Ethanol and Aqueous ethanol extracts. In all these three extracts Water and Aqueous ethanol extract have similar value then compared with ethanol extract.

**Table 3: Bioactive Compounds of Water, Ethanol, and Aqueous Ethanol Extracts of *Aerva lanata*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Water (mg/g)</th>
<th>Ethanol (mg/g)</th>
<th>Aqueous Ethanol (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Flavonoid</td>
<td>15.23 ± 0.305</td>
<td>11.83 ± 0.251</td>
<td>14.64 ± 0.262</td>
</tr>
<tr>
<td>Total Phenols</td>
<td>64.27 ± 0.208</td>
<td>24.23 ± 0.252</td>
<td>34.8 ± 0.360</td>
</tr>
<tr>
<td>Tannins (mg/g)</td>
<td>5.13 ± 0.157</td>
<td>2.37 ± 0.252</td>
<td>15.43 ± 0.351</td>
</tr>
<tr>
<td>Total Carotenoids</td>
<td>0.352 ± 0.208</td>
<td>0.305 ± 0.252</td>
<td>13.37 ± 0.503</td>
</tr>
<tr>
<td>Lycopene (mg/g)</td>
<td>21.83 ± 0.252</td>
<td>9.83 ± 0.252</td>
<td>0.305 ± 0.252</td>
</tr>
<tr>
<td>(mg/100g)</td>
<td>16.56 ± 0.208</td>
<td>9.83 ± 0.252</td>
<td>0.305 ± 0.252</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n = 3).

**DISCUSSION**

Antioxidant activity has become one of the studies on mechanisms of the nutraceutical and therapeutic effects of traditional medicines, there is numerous antioxidant activities. Due to the
complexity of the oxidation – anti oxidation processes. It is obvious that no single method is capable of providing a comprehensive picture of the antioxidant profile of a studied sample. In phytochemical screening of A. lanata, aqueous, ethanol and aqueous ethanol extract having high phytochemical content than other extracts. The reason for choosing aqueous ethanol extract, some phytochemical constituents such as cardiologyside, carbohydrate are present in aqueous extract but not present in ethanol extract. For this reason aqueous ethanol extract is also included. In the present study evaluate antioxidant properties of Aqueous, Ethanol and Aqueous Ethanol extracts and quantitative content of bioactive compounds of Aerva Lanata by using a range of testing system in vitro

The scavenging activity of DPPH, a stable free radical is a widely used index and a quick method to evaluate antioxidant activity. The highest scavenging activities on DPPH radicals are 82.37% for aqueous ethanol extract, 75.33% for water extract and 62.98% for ethanol extract at the concentration of 2.5mg/ml. In previous study, DPPH scavenging activities of Drymaria diandra showed more than 70% inhibition. ABTS is an excellent tool for determining antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidants. Aqueous extract of A. lanata has 57.87%, Ethanol extract of A. lanata has 73.62%, Aqueous ethanol extract of A. lanata has 83.4% at the concentration of 2.5mg/ml.

The reducing power capacity of compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition, Metal ion catalysts, Decomposition of peroxides, Prevention of continued hydrogen abstraction. In this study, all these three extracts have reducing activity but aqueous ethanol extract has more reducing activity than water and ethanol extract at the concentration 2.5mg/ml. In this present study related to Mau et al[23] mentioned reducing activity of Ling chin mushroom at 5mg/ml.

In Ferric reducing Antioxidant power, Non enzymatic antioxidants reacts with prooxidants and inactive them. In this reox reaction antioxidants acts reductant. In this context assay an easily reducible oxidant Fe (II) – TPTZ complex by antioxidant to formed Fe (II) – TPTZ (24). In our results indicate all the three extracts having hydrogen donating capacity, which suppress the formation of free radicals. In this study is similar to that of antioxidant potential of tea extract[25].

Super oxide anions damage bio molecules directly or indirectly by forcing H2O2, OH, Peroxy nitrite or Singlet Oxygen. Super oxide has also been observed to directly initiate lipid peroxidation. In this study, Super oxide scavenging activity of all these three extracts, Aqueous ethanol is similar to that of Vitamin C as standard. This results is revealed the same by Super oxide scavenging of Cyperus rotundus[26].

Hydrogen Peroxide is a weak oxidizing agent produced from fenton reaction. H2O2 can cross the membrane rapidly, once inside the cell, It can probably react with Fe2+ and possibly Cu2+ ions to form hydroxyl radicals and this may be the origin of its toxic effects. By this study, all these three extracts have hydroxyl scavenging activity, aqueous ethanol is similar to that of Vitamin C as standard. This results is revealed the same by Hydrogen peroxide scavenging of Cyperus rotundus[26].

Hydroxyl radical is the most reactive oxygen species and it induces several damage in adjacent biomolecule[26]. In this present study, hydroxyl radical scavenging effect of Aerva Lanata in a concentration of 2.5mg/ml was found to be more in aqueous ethanol extract (80%) compared with water and ethanol extracts. In related study, Scavenging effects of S. Pinnata were found to be 60% at the concentration 200µg/ml.

It is well known that nitric oxide has an important role in various inflammatory processes sustained levels of production of this radical are directly toxic to tissues and contribute to the vascular collapse associated with septic shock, whereas chronic expression of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, Multiple Sclerosis, Arthritis and Ulcerative colitis[29]. The toxicity of NO increases greatly when it reacts with superoxide radical is forming the highly reactive peroxy nitrite anion (ONOO−). The plant extract inhibits nitrite formation by directly competing with oxygen in the reaction with nitric oxide. In this study, nitric oxide radical scavenging effect of Aerva Lanata in a concentration of 2.5mg/ml was found to be more in aqueous ethanol extract (85.87%) compared with water and ethanol extracts. In this results revealed the same that of nitric oxide scavenging effect of S. Pinnata[26].

Antioxidant activity is measured to inhibit lipid peroxidation by TBA method. A later stage of lipid peroxidation, peroxide decomposes to form carbonyl compounds that are measured by TBA method. In general, the antioxidant by TBA method is higher than that of FTC method. This might suggest that the amount of peroxide in the initial stage of lipid peroxidation is less than the amount of peroxide in the secondary stage[30]. In this study, anti lipid peroxidation of Aerva Lanata in a concentration of 5mg/ml was found to be more in water (45.46%) compared with ethanol and aqueous ethanol extracts.

Natural antioxidant compounds in plants

Recently, these have been increasing interest in discovery of natural antioxidants, especially those of plant origin. Natural antioxidants derived from plants chiefly phenolics are of considerable interest as dietary supplements or food preservatives. Hence an attempt was made to determine the putative antioxidant components of Aerva Lanata.

Flavonoid is one of the main groups of Phenolic compounds and widely distributed Flavonoid, Flavones and Flavonols. Many flavonoids and related compounds are reported to possess strong antioxidative characteristics. In this study suggest plant extract contain catechin in water (15.23mg/g) and aqueous ethanol (14.64mg/g) extracts. In this results revealed the same that of Antioxidant Capacity of Macaronesian Traditional Medicinal Plants 34.

Phenolic compounds are known to be a powerful chain breaking antioxidants, they possess scavenging ability due to their hydroxyl groups. Studies have shown that the polyphenol content in dietary and medicinal plants could inhibit oxidative stress by antioxidant mechanism. So also, In the present study, the pronounced antioxidant activity of the extract of Aerva Lanata as inhibition of LPO, Scavenging of hydroxyl and Superoxide radical was possibly due to its high Phenolic content.

Carotenoids are the potent antioxidant and free radical scavengers. In vitro studies using radical generating systems have documented the capacity of β-Carotene to quench free radicals by mechanisms that include addition of the radical; to the carotenoid, hydrogen, abstraction and electron transfer. In this study suggest plant extract contain carotenoid in water (21.83mg/100g) and aqueous ethanol (15.43mg/100g) extracts.

Tanins are astringer, bitter plant polyphenols that either bind and precipitate or shrink proteins and various other organic compounds including amino acids and alkaloids. Tanins have shown potential antiviral, antibacterial and antiparasitic effects. In the past few years tanins have also been studied for their potential effects against cancer through different mechanisms. In this study suggest plant extract contain tannin in water (5.13 mg/g) and Aerva lanata (3.73mg/g) extracts than ethanol extract (2.37mg/g).

CONCLUSION

The Water, Ethanol and Aqueous ethanol extracts of Aerva Lanata and of various known antioxidants showed concentration – dependent antioxidant activity by virtue of inhibiting LPO, Scavenging Hydroxyl, Super oxide radicals, Nitric oxide radicals, Hydrogen peroxide radicals, reducing power when compared with different standards (BHT and Ascorbic acid). The plant extract contain a perceptible amount of total phenols, Flavonoid, tannins, Carotenoids and Lycopene, All of which probably contributed to the observed antioxidant activity. The result of the present study
suggest that an all these three different extracts of *Aerva lanata*. Aqueous ethanol extract has high scavenging than water and ethanol extract and similar to that of standards (BHT and Ascorbic acid). So the plant could serve as an easily accessible item of natural antioxidants even as a pharmaceutical agent.

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**REFERENCES**


