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Research Article

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 ethanol 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 oxide 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 ulcer[1]. Natural therapies such as the use of plant derived products may reduce adverse side effects[2] and the compounds in plants have protective effects against environmental mutagens, carcinogens and endogenous mutagens[3].

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 anthelimintic, demulcent and is helpful in Lithiasis, Cough, Sore throat and Wounds[4]. The plant has been reported to possess anti inflammatory and nephroprotective in rats[5].

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.V.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^{o}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 Tracey[6].

Antioxidant Capacity assays

DPPH radical scavenging assay was estimated by Blois[7]. 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 al*[10]. Hydroxyl Radical Scavenging assay was estimated by Smirnoff *et al*[11]. Super Oxide radical scavenging assay was determined by Liu *et al*[12]. Lipid peroxidation was determined by Thio Barbituric Acid method Ottolenghi[13]. Hydrogen Peroxide Radical Scavenging assay was determined by replacement titration method Zhang[14]. Nitric Oxide Scavenging assay was determined by Green *et al*[15].

Natural antioxidant Compounds

Flavonoid content was estimated by Jia *et al*[16]. Total Phenolic content was estimated by Singleton *et al*[17]. Tannin was estimated by Robert[18]. Carotene and Lycopene were estimated by Ranganna[19].

RESULTS

Phytochemical Screening of plant materials

In table1a showed Phytochemical screening of *A.lanata*. Aquoeus 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 1a: Phytochemical Screening of *Aerva lanata.*

Type of Extracts	Phytochemical compounds								
	AL	ST	FL	TAN	AP	CH	CG	SA	TN
Peteroleum	-	+	-	-	-	+	+	-	-
ether	-	-	-	-	-	+	-	+	+
Chloroform	-	+	-	-	-	-	+	-	-
Ethyl Acetate	+	+	+	+	+	-	-	+	+
Ethanol	+	+	+	+	+	+	+	+	+
Aqueoue	+	-	+	+	+	+	+	+	+
Ethanol (1 :1) Water									

'+'Present **'_' Absent**

AL	Alkaloids	**CG**	Cardio glycosides
SA	Saponins	**TN**	Terpenoid
TP	Tannin & Phenolic compounds		
AP	Amino acids & Proteins		
FL	Flavonoid	**CH**	Carbohydrates
ST	Steroids		

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

Aqueous Ethanolic Extract (Ethanol : Water)	Phytochemical compounds								
	AL	**ST**	**FL**	**TAN**	**AP**	**CH**	**CG**	**SA**	**TN**
1:9	+	+	+	+	-	+	+	-	+
2:8	+	+	+	+	-	+	+	-	+
3:7	+	+	+	+	-	+	+	-	+
4:6	+	+	+	+	-	+	+	-	+
5:5	+	+	+	+	+	+	+	+	+
6:4	+	-	-	+	+	+	+	+	+

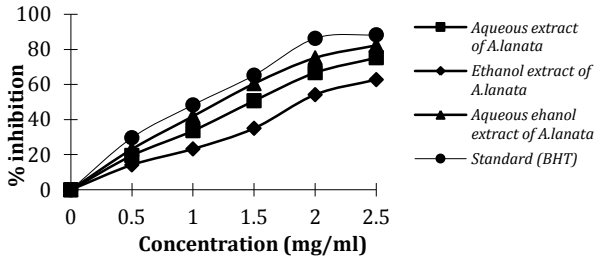
'+' Present **'_' Absent**

AL	Alkaloids	**CG**	Cardio glycosides
SA	Saponins	**TN**	Terpenoid
TP	Tannin & Phenolic compounds		
AP	Amino acids & Proteins		
FL	Flavonoid	**CH**	Carbohydrates
ST	Steroids		

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 ethanol extract, 75.33% for water extract and 62.8% for ethanol extract at the concentration of 2.5mg/ml.In all these three extracts aqueous ethanol extract is similar to that of BHT standard (88.37%).

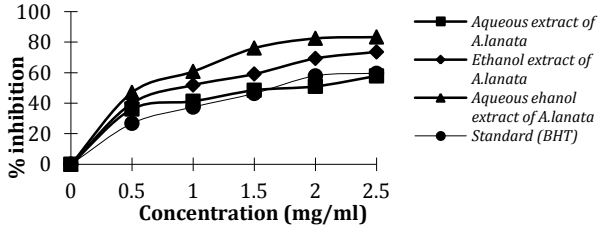
Fig 1a : DPPH



DPPH Scavenging 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.

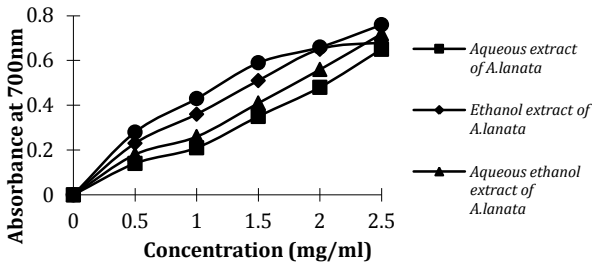
Figure 1b shows the scavenging assay of ABTS. Aqueous extract of *A.lanata* has 57.87%, Ethanol extract of *A.lanata* has 73.62%, Aqueous ethanol extract of *A.lanata* has 83.4% at the concentration of 2.5mg/ml. In all these three extracts aqueous extract is similar to that of BHT standard (57.87%).

Fig 1b : ABTS



ABTS radical Scavenging 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

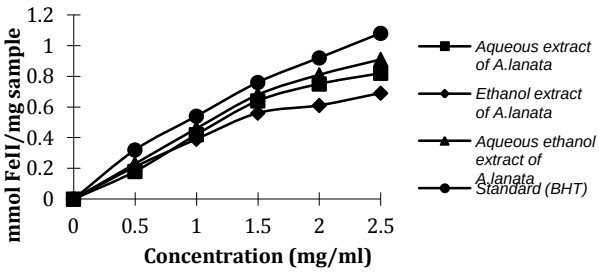


Reducing power of water, ethanol and aqueous ethanol extract of *Aerva Lanata* compared to that of Butylated hydroxytoluene (BHT). Each value is expressed as mean ± standard deviation (n=3). Concentration (mg/ml) taken in X axis and Absorbance at 700nm taken in Y axis.

Scavenging assay of Reducing Power and FRAP

Figure 2a, 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.

Fig 2b : FRAP

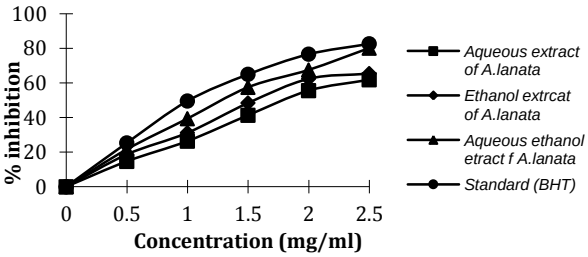


Ferric reducing antioxidant power of water, ethanol and aqueous ethanol extract of *Aerva Lanata* compared to that of Butylated hydroxytoluene (BHT). Each value is expressed as mean ± standard deviation (n=3). Concentration (mg/ml) taken in X axis and mmol FeII/mg sample taken in Y axis.

Scavenging assay of Hydroxyl radicals and Hydrogen Peroxide radicals

Figure 3a, all these three extracts water- 61.86% , ethanol- 65.45% and Aqueous ethanol – 80.12% at maximum in 2.5mg/ml, have hydroxyl scavenging capacity but the scavenging of aqueous ethanol extract is similar to that of ascorbic acid standard(82.67%).

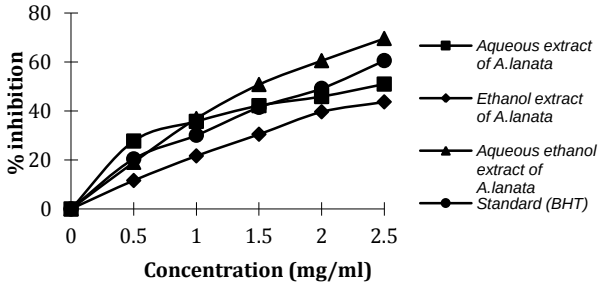
Fig 3a : Hydroxyl radical scavenging assay



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



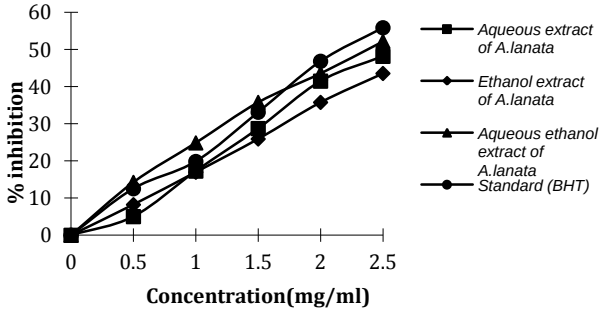
Hydrogen peroxide radical scavenging of water, ethanol and aqueous ethanol extract of *Aerva Lanata* compared to that of Ascorbic acid (Vit C). Each value is expressed as mean ± standard deviation (n=3).Concentration (mg/ml) taken in X axis and % inhibition taken in Y axis.

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.

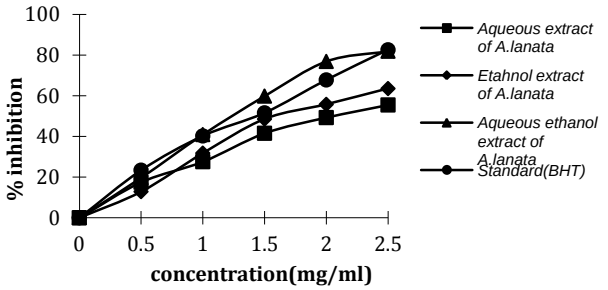
Fig 4a : Superoxide radical scavenging assay



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



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 *A.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 thio cyanide method (TBA)

Solvents	*Aerva lanata* Antioxidant activity / 5mg
Water	45.46 ± 0.157
Ethanol	35.42 ± 0.209
Aqueous Ethanol	42.77 ± 0.103
Ascorbic acid (Std)	50.72 ± 0.117

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*.

Parameters	Solvents		
	Water	Ethanol	Aqueous Ethanol
Total Flavonoid (mg/g)[a]	15.23 ± 0.305	11.83 ± 0.251	14.64 ± 0.262
Total Phenols (mg/g)[b]	64.27 ± 0.305	24.23 ± 0.252	48.3 ± 0.360
Tannins (mg/g)[a]	5.13 ± 0.352	2.37 ± 0.305	3.73 ± 0.208
Total Carotenoids (mg/g)	21.83 ± 0.208	9.5 ± 0.3 9.83 ± 0.252	15.43 ± 0.351
Lycopene (mg/100g)	16.56 ± 0.208		13.37 ± 0.503

Values are expressed as mean ± SD (n = 3).
[a]mg of catechin/g [b]mg of catechol/g

DISCUSSION

Antioxidant activity has become one of the studies on mechanisms of the nutraceutical and therapeutical effects of traditional medicines, there is numerous antioxidant activities[20]. 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 cardioglycoside, carbhohydrate are present in aqueos 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[21]. The highest scavenging activities on DPPH radicals are 82.37% for aqueous ethanol extract, 75.33% for water extract and 62.8% for ethanol extract at the concentration of 2.5mg/ml. In previous study, DPPH scavenging activities of *Drymaria diandra* showed more than 70% inhibition[22]. 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 redox reaction antioxidants actas reductant. In this context assay an easily reducible oxidant Fe (III) – 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 H_2O_2 , OH, Peroxy nitrate 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. H_2O_2 can across the membrane rapidly, once inside the cell, It can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of its toxic effects[27]. 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[28]. 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 nitrate 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*[30].

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[31]. 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[32]. Hence an attempt was made to determine the putative antioxidant componens 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[33]. 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[35]. Studies have shown that the polyphenols found in dietary and medicinal plants could inhibit oxidative stress by antioxidant mechanism[36]. 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 [37]. 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[38]. In this study suggest plant extract contain carotenoid in water (21.83mg/100g) and aqueous ethanol (15.43mg/100g) extracts.

Tannins are astringer, bitter plant polyphenols that either bind and precipitate or shrink proteins and various other organic compounds including amino acids and alkaloids. Tannins have shown potential antiviral , antibacterial and antiparasitic effects[39]. In the past few years tannins have also been studied for their potential effects against cancer through different mechanisms[40]. In this study suggest plant extract contain tannin in water (5.13 mg/g) and Aqueous ethanol (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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