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Analysis of Hydroethanolic leaf extract of *Aerva lanata* (L.) in screening anti-oxidant activity and *in vitro* antibacterial efficacy

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ABSTRACT

The present investigation has been carried out to evaluate antioxidant and antibacterial activity of ethanolic leaf extract of *Aerva lanata* L. The extracts were screened for their in vitro antioxidant potential Inhibition of oxygen-derived free radicals, viz., assays for free radical scavenging by DPPH, SOD, OH and nitric oxide scavenging were performed. In this study, the plant showed substantial antioxidant activities as resembling standard (i.e. ascorbic acid). The highest antioxidant activity was observed and could be attributed due to the presence of flavonoids and saponins. Ethanolic extract of *Aerva lanata* L was further subjected to antibacterial assessment. It was found that plant extract showed significant antibacterial efficacy, especially on *Pseudomonas aeuroginosa* against standard Streptomycin at 10 µg. Thus, ethanolic leaf extract of *Aerva lanata* L. showed high efficiency of antioxidant and antibacterial activity due to the presence of various phytochemicals. Some of the active principles of bioactive compounds are preferred for their therapeutic purposes either singly or in combination to inhibit the life processes of microbes bacterial efficacy

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INTRODUCTION

A large number of human populations was dependant on medicinal plants for treating various illnesses as well as a source of livelihood. One of the main advantages of using medicinal plants is that these do not produce or cause any side effects when compared with synthetic drugs, because medicinal plants have a high content of antioxidant compounds. This gives protective effects against diseases without reducing their therapeutic efficacy (Baron *et al*., 1994; Linton, 1977). Medicinal herbs are used to prevent illness. Many of today's most common medicines such as ointments, syrups are developed from the components of medicinal plants. Microorganisms are in large part responsible for determining the course and human history. They are carried by air current from the earth's surface to the upper atmosphere. The condition that favours the survival for the growth of many microorganisms in those under which people usually like it is inevitable that we among a multitude of microbes (Nadkarni AK and Nadkarni KM, 1998; Vetrichelvan T and Jagadeesan M, 2002).

Infectious diseases also are known as contagious diseases or transmissible diseases and include communicable diseases comprise clinically evident illness (i.e., characteristic medical signs and/or symptoms of disease) resulting from the infection, presence and growth of pathogenic biological

agents in an individual host organism. In some instances, infectious diseases may be asymptomatic for many or their entire course. Infectious pathogens include some viruses, bacteria, fungi, protozoa, multicellular parasites and aberrant proteins known as prions. Today, there are large numbers of antimicrobial agents used in medicinal practice aimed at eliminating infecting microorganism or preventing the establishment of an infection. In India, herbal medicines have been the basis93of treatment and care for various diseases.

Aerva lanata *(*Family: Amaranthaceae) is a woody, prostrate perennial herb grows wild everywhere as a common weed in plains of India. The leaves contain alkaloids, carbohydrate, phytosterols, flavonoids, flavones, terpenoids, triterpenoids and steroids. The plant is said to be diuretic and demulcent. Its diuretic action is very effective in the treatment of urethral discharges and gonorrhea and is of value in cases of lithiasis and as an anthelmintic. Plant leaves extract possess antidiabetic, anti-asthmatic, antifertility, hypolipidemic, pharmacological, immunomodulatory effect, diuretic, anti-inflammatory and *in vitro* anthelmintic activity (Kumar *et al*., 2009; Soundararajan *et al*., 2007; Nevin and Vijayammal, 2003; Vetrichelvan *et al*., 2000; Anantha *et al*., 2010; Anonymous, 1972).

Keeping this view in our study, we evaluated the Hydroethanolic extract of *Aerva lanata* for its antioxidant and antibacterial properties.

MATERIALS AND METHODS

Preparation of plant extract

Ethanol extracts were prepared according to the methodology of Indian pharmacopeia 13. The shady dried plant materials were subjected to pulverization to get a coarse powder. The coarse powder material was subjected to Soxhlet extraction separately and successively with ethanol. The extract was concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (40-50°C). The aqueous and ethanolic extract put in airtight container and stored in a refrigerator.

Antioxidant property assay

Estimation of a Total Antioxidant assay by FTC Method

To 4 mg of plant sample added 4 ml of absolute ethanol, 8 ml of 0.05 M phosphate buffer, 4.1 ml of 2.52 % linolenic acid and 3.9 ml of water. The content was placed in a dark oven at 40°C. Taken 0.1 ml from the mixture and added 9.7 ml of 75% ethanol and 0.1 ml of 30 % ammonium thiocyanate. Added 0.1 ml of 0.02 M ferrous chloride and stood for 3 minutes. The red colour formed was measured at 500 nm. Ascorbic acid was used as positive control and water as negative control.

Evaluation of free radical scavenging status of *Aerva lanata*

DPPH radical scavenging assay

To 50 µl of plant extract was added to 5.0 ml of DPPH solution and mixed well. After 30 minutes of incubation at 37°C, the absorbance was read against control spectrometrically at 517 nm. Ascorbic acid was used as positive control.

Superoxide Radical scavenging assay

Taken 1 ml of NBT solution, 1 ml of NADH solution and 1 ml of sample solution of plant extract were mixed. The reaction was started by adding 1 ml of PMS solution to the mixture. The reaction mixture was incubated at 25°C for 5 minutes and the absorbance was measured at 560 nm against the blank sample and compared with standards.

Hydroxyl Radical scavenging assay

To 0.1 ml of plant extract was added to the reaction mixture containing 0.1 ml of 3.0 mM deoxyribose, 0.5 ml of 0.1 mM $FeCl_3$, 0.5 ml of 0.1mM EDTA, 0.5 ml of 0.1 mM ascorbic acid, 0.5 ml of 1.0 mM H_2O_2 and 0.8 ml of 20 mM phosphate buffer in a final volume of 3.0 ml. The reaction mixture was incubated at 37°C for 1 hour. The TBARS formed were measured by treating with 1.0 ml of TBA and 1.0 ml of TCA (at 100°C for 20 min). After cooling the mixtures, absorbance was measured spectrometrically at 532 nm against a control.

Nitric Oxide Radical scavenging assay

Sodium nitroprusside in phosphate buffered saline was mixed with different concentrations of the extract (250-2500 µg/ml) prepared in ethanol and incubated at 25°C for 30 minutes. A control was prepared using an equivalent amount of ethanol in place of test solution. After 30 minutes, 1.5 ml of the incubated solution was taken and mixed with 1.5 ml of Griess reagent. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured spectrophotometrically at 546 nm and percentage scavenging was measured with reference to the standard.

Antibacterial activity

The antibacterial activity of *Aerva lanata* was evaluated by agar well diffusion method (Mohanasundaram *et al*., 2017). Muller Hinton agar medium was prepared and poured into the Petri dishes. Then it was inoculated with a swab of bacterial culture and spread throughout the medium

uniformly with a sterile cotton swab. Using a sterile cork borer (10 mm diameter) wells were made in the agar medium. The plant extract was introduced into the wells and all the plated were incubated at 37°C for 24 hrs. The experiment was performed five times under strict aseptic condition. Each assay was repeated for five times and the mean value was taken for analysis. In this study, standard strains of *Bacillus Subtilis, Staphylococcus aureus, Pseudomonas auruginosa, E.Coli* were used and the various concentration of extract was used for the study of the effect of inhibition zone formation in mm. The control experiment was carried out with streptomycin 10 µg.

RESULT AND DISCUSSION

The antioxidant assay by FTC method

The total antioxidant activity of leaf extracts of *Aerva lanata* was measured by the FTC method and the values are shown in Fig.1. The absorbance of plant extracts showed lesser value than negative and positive control. This result indicated a high level of antioxidant activity of the test plant extracts. The addition of the ascorbic acid can improve the total antioxidant activity of food

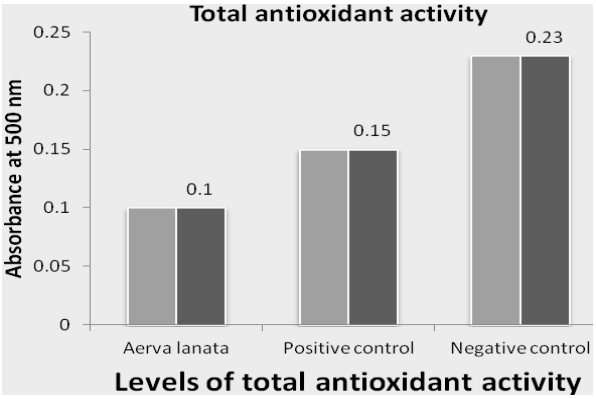

Figure 1: total antioxidant activity

DPPH radical scavenging assay

Extracts of *Aerva lanata* leaves showed scavenging activity of DPPH radical (Fig. 2), which may be attributable to its hydrogen bonding ability. Phenolic compounds of plants are active hydrogen donors making them very good antioxidants.

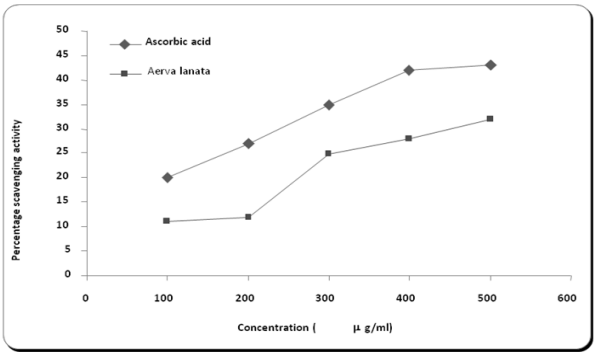

Figure 2: Scavenging activity of DPPH radical

Superoxide Radical scavenging assay

Figure 3 shows superoxide scavenging activities *of Aerva lanata* leaves extract. The results indicated that the plant extract has their superoxide scavenging activity in a concentration-dependent manner.

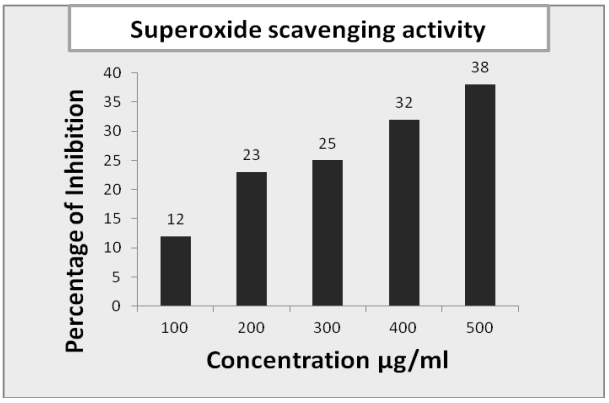

Figure 3: superoxide scavenging activities of *Aerva lanata*

Hydroxyl Radical scavenging assay

The hydroxyl radical scavenging activity found in the extracts of *Aerva lanata* were shown in Fig.4 and was found to act in a concentration-dependent manner. Hydroxyl radicals induce the peroxidation of USFA, protein and DNA in adjacent biomolecules. Plant extracts quench hydroxyl ions and prevent the propagation of lipid peroxidation which might be the cause of antioxidant activities of *Aerva lanata* leaf extracts.

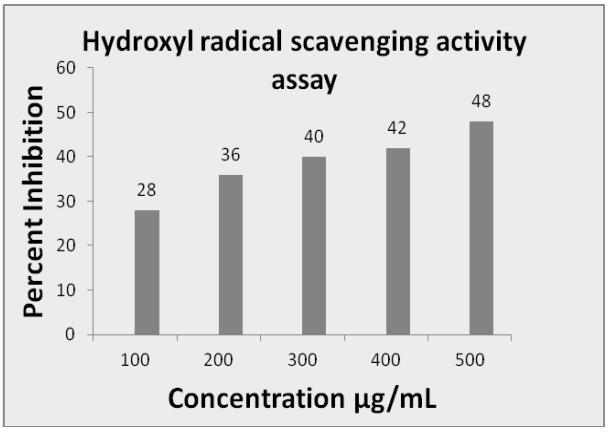

Figure 4: hydroxyl radical scavenging activity

Nitric Oxide Radical scavenging assay

The nitric oxide radical scavenging activity of 50% ethanolic extract of *Aerva lanata* leaves are shown in Fig. 5. and was found to be in a concentration-dependent manner. Nitric oxide radical inhibition assay proved that leaf extracts are potent scavengers of nitric oxide. Nitric oxide generated from sodium nitroprusside reacts with O_2 to form nitrite. Antioxidants inhibit nitrite formation by competing with oxygen to react with nitric oxide and also

inhibit its synthesis. Thus, scavengers of nitric oxide compete with O_2 leading to reduced production of nitric oxide.

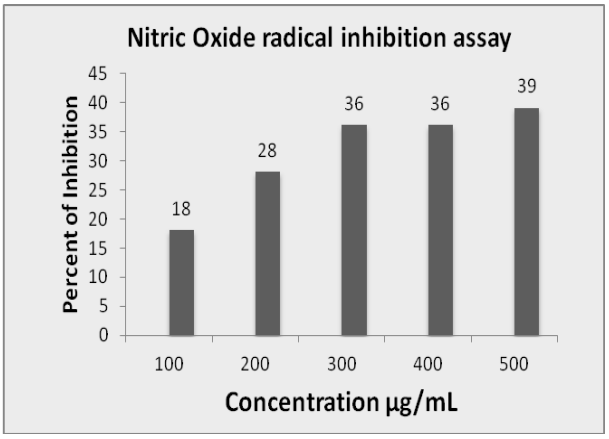

Figure 5: nitric oxide radical scavenging activity

Table 1: antibacterial activity

Concentration (µg)	Zone of inhibition (mm)			
	Bacillus subtilis	*Staphylococcus aureus*	*E.Coli*	*Pseudomonas aeruginosa*
12.5	4±0.1	6±0.1	5±0.1	12±0.2
25	11±0.2	10±0.2	9±0.2	14±0.1
50	13±0.1	12±0.1	12±0.2	18±0.2
100	16±0.1	16±0.1	15±0.1	20±0.2
Streptomycin (10µg)	17±0.1	0	16±0.1	19±0.1

Anti-bacterial assay

Ethanolic leaf extracts of *Aerva lanata* L. were evaluated for their antibacterial activity against *Staphylococcus aureus, Pseudomonas auruginosa, E.coli and Bacillus subtilis* by disc diffusion method. From the results, in the table (1) it is proved that greater activity resides in ethanolic leaf extract of the plant since when compared with antibiotic streptomycin. This may be due to the chemical constituents responsible for the antibacterial activities are more soluble in ethanol extracts.

CONCLUSION

This research showed the potential activity of a Hydroethanolic extract of *Aerva lanata* leaves and its antibacterial activity against microorganisms by its alkaloids and phenolics properties. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Palombo and Semple, 2001). Thus, the study ascertains the value of *Aerva lanata* L. used in Ayurveda, which could be of considerable interest to be the development of new drugs.

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