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In Vitro Antioxidant and Antimicrobial Activities of *Aerva lanata L.*

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ABSTRACT

The present investigation has been carried out to evaluate the phytochemical, *in vitro* antioxidant and antimicrobial activities of aqueous and ethanolic leaves extracts of *Aerva lanata* L. Phytochemicals were analyzed by qualitative method and the results revealed the presence of alkaloids, phytosterols, terpenoids, flavonoids, tannins, saponins and steroids etc., *The in vitro* antioxidant activity was evaluated using total antioxidant capacity, nitric oxide scavenging activity, reducing power assay and hydrogen peroxide scavenging activity. Ethanolic extract showed the highest antioxidant activity than aqueous when compared with standard vitamin C. Antimicrobial activity was also performed by using agar disc diffusion method against *E.coli, B. subtilis, A.niger, T. viride*. Ethanol and aqueous extracts were active against both bacterial and fungal species when compared to antibiotic ciprofloxacin. As a result, ethnologic leaves extract of *Aerva lanata* L. showed high efficiency of antioxidant and antimicrobial activity due to the presence of various phytochemicals.

INTRODUCTION

Oxygen is essential for the survival of all on this earth. Free radical is a chemical compound which contains an unpaired electron spinning on the peripheral layer around the nucleus. Cell damage caused by free radicals appears to be a major contributor to aging and degenerative diseases such as cancer, cardiovascular disease, cataracts, immune system decline, liver disease, diabetes mellitus, inflammation, renal failure, brain dysfunction and stress among others[1]. Free radicals include Superoxide radical (SOR), Hydroxyl radical (OH), Hydroperoxyl radical (HPR), Alkoxyl radical (AR), Peroxyl radical (PR), Nitric oxide radical (NOR). Non-free radical includes Singlet O_2, Hydrogen peroxide (H_2O_2), Hydro chlorous acid (HOCL), Peroxynitrite ONOO-. In addition, there is another class of free radicals that are nitrogen derived called reactive nitrogen species (RNS) [2]. All these capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage. In living organisms, various ROSs can be formed in different ways including normal aerobic respiration stimulated polymorphonuclear leukocytes, macrophages and peroxisomes. Antioxidant may be defined as radical scavenger which protects the human body against free radicals that may cause pathological condition such as ischemia, anemia, asthma, arthritis, inflammation and Parkinson's disease. Antioxidant means "against oxidation" Antioxidant work to protect lipids from peroxidation by radicals. The human body has an elaborate antioxidant defense system.

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 normally like it is inevitable that we among a multitude of microbes[3, 4]. Infectious diseases also 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 certain cases, 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 a large number of antimicrobial agents used in medicinal practice are aimed at eliminating infecting microorganism are at preventing the establishment of an infection. In India, herbal medicines have been the basis

of treatment and care for various diseases. Physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha[5]. Keeping this view in our study, we evaluated the phytochemicals *in vitro* antioxidant and antimicrobial activity of *Aerva lanata* leaves.

*Aerva lanata (*Family*:*Amaranthaceae) is a woody, prostate perennial herb. It is a common weed which grows wild everywhere 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[6], anti-asthmatic[7], antiferitility[9], hypolipidemic[9], pharmacological, immunomodulatory effect[10], diuretic, anti-inflammatory[11] and *in vitro* antihelmentic[12] activity.

MATERIALS AND METHODS

Collection of plant material

The plant was collected from in and around Athamangalam, Nagai District, Tamil Nadu. The collected samples were carefully kept in polythene bags. These plant samples were authenticated by Dr.S.Johnbritto, The Director, The Rabinet Herbarium Centre for Molecular Systematic, St. Joseph's College, Tiruchirappalli and a voucher specimen was deposited in the Department of Biochemistry, S.T.E.T Women's College, Mannargudi, Thiruvarur, Tamil Nadu. The leaves were dried in the shade and stored in airtight containers until further studies.

Extraction of plant material

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

Phytochemicals analysis

Aqueous and ethanolic extracts of *Aerva lanata* Linn., were subjected to qualitative test for the identification of various plant constituents[14].

***In vitro* antioxidant activity**

The antioxidant activity of plant extracts was determined by different *in vitro* models such as total antioxidant[15], reducing power assay[16], H_2O_2 scavenging activity[17] and nitric oxide scavenging activity[18].

***In vitro* antimicrobial activity**

Selection of microorganism

Totally four pathogenic microorganisms such as *E. coli, Bacillus subtilis, Aspergillus niger* and *Trichoderma viride* were selected for this investigation.

Inoculum preparation

The young microbial inoculum was prepared and used in the entire research period. The nutrient broth for bacteria and potato dextrose agar (PDA) for fungi were prepared and poured into tubes and sterilized. The pure microbial cultures were collected from Microbiology Department, S.T.E.T Women's College, Mannargudi and inoculated in the tube using inoculation needles or loops. The tubes were incubated at 37°C for 24 hours.

Disc diffusion method[19]

The antimicrobial activity of the leaves extracts were tested against selected bacterial and fungal strains. The 20ml of sterilized nutrient agar medium were poured into each sterile Petri plate and allowed to solidify. Test microbial cultures were evenly spread over the appropriate media by using sterile cotton swab. Then a well of 0.5cm was made in the medium using a sterile cork borer 150µl of each ethanol and aqueous. Plant extracts were transferred into separate well after these plants were incubated at 37^{o}C for 24-48 hours. After incubation period, the results were

observed and measured the diameter of inhibition zone around each well. The standard antibiotic (positive) like ciprofloxacin was also performed.

RESULTS

In the present study, the antioxidant and antimicrobial activity of aqueous and ethanolic extract of *Aerva lanata* were examined. Table 1 represents the qualitative analysis of phytochemical constituents of *Aerva lanata* L. and the results revealed that the presence of alkaloids, carbohydrate, phytosterols, terpenoids, flavonoids, protein, amino acids, volatile oils, tannins, saponins and steroids were present in aqueous and ethanol extracts. Phlobatannins and phenolic compounds were absent in both aqueous and ethanolic plant extracts.

The *in vitro* antioxidant activity of aqueous and ethanolic extracts of *Aerva lanata* L. were assayed by different *in vitro* models, including total antioxidant capacity, reducing power assay, hydrogen peroxide scavenging activity and nitric acid scavenging activity.

Table 2 showed the total antioxidant capacity of ethanolic and aqueous extract of *Aerva lanata* L. The total antioxidant capacity of ethanolic extract was 84.4% and aqueous extract showed 25.5%. Among the two extracts, ethanolic extracts showed the highest antioxidant activity which was nearer to that of standard antioxidant vitamin C (92%).

Nitric oxide scavenging activity of ethanolic and aqueous extract of *Aerva lanata* L. represented in Table 2. Ethanolic extract showed 66.7% of scavenging activity and aqueous extract having 33.5% and vitamin C showed 70.33%. Ethanolic extract exhibited moderate scavenging activity compared to vitamin C.

Reducing power assay of aqueous and ethanolic extract of *Aerva lanata* L were also represented in Table 2. Reducing power of ethanolic extract was found to be 70% and in aqueous extract, it was 44.2%. Reference compound vitamin C showed 72.23%. Ethanolic extract exhibited highest scavenging activity than aqueous extract. When compared to vitamin C ethanol extract showed moderate activity.

Table 2 also showed hydrogen peroxide scavenging activity of ethanol and aqueous extract of *Aerva lanata* L. Ethanolic extract showed 60% of hydrogen peroxide scavenging capability,

which is nearer to the value showed by reference compound vitamin C (65.3%). But aqueous extract having least potentiality (25.1%) when compared to ethanolic extract.

Antimicrobial activity

In the present study, we also analysed the antimicrobial activity of *Aerva lanata* leaves extracts. Bacteria like *E.coli, B.subtilis,* and fungi like *A.niger,* and *T.viride* were selected for antibacterial and antifungal activities of plant extracts respectively. Zone of inhibition of both extracts were represented in Table 3.

Antibacterial activity

When tested by disc diffusion method, ethanolic leaf extracts of *Aerva lanata* L. showed significant inhibitory activity against *E.coli,* (6mm) and *B.subtilis (*5mm) than aqueous leaf extract. In aqueous extract, zone of inhibition for *E.coli* and *B.subtilis* were 4mm and 3mm respectively. Antibiotic ciprofloxacin was also performed and their zone of inhibition for *E.coli* and *B.subtilis* were 2mm and 2mm respectively. Both ethanolic and aqueous extracts exhibit higher antibacterial activity than the standard drug.

Antifungal activity

Ethanolic extract of *Aerva lanata* L. showed significant antifungal activity against *A.niger* (8mm) and *T.viride* (4mm) and aqueous extract exhibited 6mm for *A.niger* and 5mm for *T.viride.* The ethanolic extract exhibited highest antifungal activity against *A.niger* whereas the aqueous extract showed maximum activity against *T.viride.* All the results were compared with standard antibiotic ciprofloxacin (3mm for *A.niger* and 3mm for *T.viride*) and both extracts showed better results than ciprofloxacin.

Table 1: **Qualitative phytochemical screening of *Aerva lanata* L.**

S.No	Phytochemicals	Results	
		Ethanol	Aqueous
1.	Alkaloids	+	+
2.	Carbohydrate	+	+
3.	Phytosterol	+	+
4.	Steroids	+	-
5.	Terpenoids	+	+
6.	Triterpenoids	+	+
7.	Flavonoids	+	+
8.	Protein	+	+
9.	Amino acids	+	+
10.	Glycosides	+	+
11.	Volatile oils	+	+
12.	Tannin	+	+
13.	Phenolic	-	-
14.	Saponin	+	+
15.	Phlobatannins	-	-

+ = Presence - = Absence

Table 2: *In vitro* antioxidant activity of *Aerva lanata* L.

S. No	Antioxidant Models	Ethanolic extract	Aqueous extract	Vitamin C
1	Total antioxidant capacity	84.4%	25.5%	92%
2	Nitric oxide scavenging activity	66.7%	33.5%	70.33%
3	Reducing power assay	70%	44.2%	75.23%
4	Hydrogen peroxide scavenging activity	60%	25.1%	65.3%

Table 3: In *vitro* antimicrobial activity of *Aerva Lanata L.*

S. No	Name of the species	Zone of inhibition		
		Plant extracts		Standard Ciprofloxacin
		Ethanol	Aqueous	
Bacteria				
1	*Escherichia coli*	6mm	4mm	2mm
2	*Bacillus subtilis*	5mm	3mm	2mm
Fungi				
3	*Aspergillus niger*	8mm	6mm	3mm
4	*Trichoderma viride*	4mm	5mm	3mm

DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases[20]. Flavonoids, which are well known antioxidants and free radical scavengers such as kaempferol 3 - rhamnoside and kaempferol 3 - rhamnogalactoside have been reported to be present in *Aerva lanata* L.[21]. The significant hepatoprotective, nephroprotective and antioxidant effect of *Aerva lanata* L. may be due to presence of alkaloids and its inhibitory effect on microsomal Cyt P_{450} enzyme or on lipid peroxidation[11]. Reactive oxygen species are generated in the human body cause oxidative damage and responsible for many degenerative diseases such as coronary heart disease, atherosclerosis, diabetes, aging and cancer[22]. Many antioxidant compounds occurring naturally in plants sources have been identified and proved as free radical scavenger both *in vitro* as well as *in vivo*. *Aerva lanata* L. has been used as a traditional medicine for various ailments which are

closely associated with free radical formation. Hence in the present investigation, we have evaluated the free radical scavenging activity of *Aerva Lanata* L.

Total antioxidant capacity of the ethanolic extracts of *Aerva lanata* L. was more when compared to aqueous extract. The total antioxidant capacity of the extract was calculated based on the formation of phosphorus molybdenum complex which was measured at spectrometrically at 695nm[15]. Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and involved in regulation of various physiological processes[23]. Excess concentration of NO is associated with several diseases[24]. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals[25, 26]. In the present study, the extract competes with oxygen to react with nitric oxide and thus inhibits the generation of the anions.

For measurements of ethanolic and aqueous extract of *Aerva lanata* L. the reducing ability, the Fe^{3+} to Fe^{2+} transformation was investigated in the presence of *Aerva lanata* L. The reducing capacity of a 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, reductive capacity and radical scavenging[27, 28]. The result showed that *Aerva lanata* L. consists of alkaloids and flavonoids that causes the greater reducing power. Hydrogen peroxide is generated *in vivo* by several oxidase enzymes. In this method, when an antioxidant is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide is measured spectrophotometrically[1]. Hydrogen peroxide is a weak oxidizing agent which inactivates enzymes by oxidation of the essential thiol (SH^-) groups. It rapidly transverses cell membranes and once inside the cell interior interacts with Fe^{2+} and Cu^{2+} to form hydroxyl radical, which is harmful to the cell[29]. The extracts showed good scavenging effect.

Antimicrobial activity

Plants are an important source of potentially useful structures for the development of new chemotherapeutic agents. On this basis, ethanolic and aqueous leaves extracts of *Aerva lanata* L. were evaluated for their antimicrobial activity against bacterial pathogens *E.coli* and *B.subtilis*

and fungal pathogens live *A.niger*, *T.viride* by disc diffusion method. From the results, it dictated the greater activity resides in ethanolic leaves extract of plant since other aqueous extract did not effectively inhibit the growth of the bacteria when compared with antibiotic ciprofloxacin. This may be due to the chemical constituents responsible for the antibacterial activity are more soluble in ethanol extracts. It can be interpreted that the antimicrobial activity against microorganisms is due to anyone or more alkaloids of the plants[30]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants[31].

In conclusion, the results of the present study showed that the ethanolic extract of *Aerva lanata* L. leaves exhibited the greatest antioxidant and antimicrobial activity may be due to the presence of active constituents. Thus, the study ascertains the value of *Aerva lanata* L. used in ayurveda, which could be of considerable interest to be development of new drugs.

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