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ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITY OF WHOLE PLANT OF *ALLMANIA NODIFLORA* (L) R. BR EX WIGHT

KOTA PADMAJA[1,2] *, SERU GANAPATY[2], JAGADEESH PANDA[1], MARY SULAKSHNA[1], AASHIA SHEIK[1], BHAVIKA PATEL[1], MONISHA ROJA[1] AND BHAVYA[1]

[1]Raghu College of Pharmacy, Dakamarri, Bheemunipatnam (M), Visakhapatnam 531162, Andhra Pradesh, India
[2]GITAM Institute of Pharmacy, Gandhinagar, Rushikonda, Visakhapatnam 530045, Andhra Pradesh, India

ABSTRACT

Allmania nodiflora (L) R. Br. ex wight is a small herb belongs to Amaranthaceae family and is green leafy vegetable, used as folk medicine to treat constipation, decentry and febrifuge contains adequate amount of nutrients. The ethanolic extract (EEAN) of whole plant was chosen to investigate anti oxidant, antimicrobial and anti-inflammatory activity. The extract shows significant antioxidant activity evaluated by DPPH assay, Superoxide Radical and Hydroxyl radical scavenging activity compared with that of standard (ascorbic acid) and antimicrobial activity was evaluated against clinically important bacterial and fungal strains using cup plate method. Anti inflammatory activity was evaluated by Carrageenan induced rat paw edema method by the oral administration of EEAN (100, 250 mg/kg) exhibited significant and dose dependant anti-inflammatory activity.

key words: Antioxidant, Antimicrobial and Anti-inflammatory, EEAN (ethanolic extract of *Allmania nodiflora*).

INTRODUCTION

Herbs especially green leafy vegetables are taken as source of food for their nutritive values and trace elements content but they also have medicinal properties which are helpful in maintaining human health. The medicinal values of herbs can be screened from their nutritional and non nutritional properties, minerals and trace elements. The most important nutrients present in plants are carbohydrates, proteins, minerals, vitamins and phenolic compounds.[1] *Allmania nodiflora* is a diffuse herb and green leafy vegetable belongs to Amaranthaceae family is a rich source of vitamin A and commonly called as celosia.[2] The seeds used traditionally for constipation and dysentery. The leaves are used as febrifuge.[3] This plant is used as Antidiabetic, Hypolipidemic, Nutritive and appetizer.[4] The reported activities were Analgesic, Anti-inflammatory, slight CNS depressant activity[5] and Antioxidant activity by DPPH method. The phytoconstituents reported were phenolic compound, Flavanoids, carbohydrate and protein in *Allmania nodiflora* with GC-MS.[6] In this article author wants to explain detailed antioxidant activity by three methods, antimicrobial activity against two Gram+ve, two Gram-ve and two fungal strains and anti-inflammatory activity by carrageenan induced rat paw edema method which is not yet published.

MATERIAL AND METHOD

Plant material

The whole plant of *Allmania nodiflora* (L) R. Br. ex wight (Amaranthaceae) were collected from in and around cultivated and waste lands of Raghu college of pharmacy, Visakhapatnam and authenticated by Botanical Survey of India (BSI/DRC/2015-16/Tech/684), Hyderabad, Telangana (state), India.

Preparation of plant extract

Whole plants were thoroughly washed with water. The collected plants were cut into small pieces and dried under shade. The dried pieces were ground

into fine powder by using mixer grinder and extracted by maceration with ethanol for 7 days with occasional shaking and stirring. Then, the liquid was concentrated under vacuum with rotary vacuum to get dried extract.

Animals

Anti-inflammatory activity experiment was carried out using 20 albino rats of weight 200-250g male/female of age 12 weeks. All the experimental procedure and protocols used in this study are reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of Raghu College of pharmacy contributed in accordance with guidelines of the CPCSEA, government of India (1549/PO/Re/S/2011/CPCSEA).All the rats were given a period of acclimatization for 14 days before starting the experiments. Rats were fed pellet diet, and water ad libitam, temperature maintaining at 24± 2° and relative humidity 60-70%. These rats were divided into four different groups each containing five animals, the animals were marked individually.

Drugs and chemicals

The chemicals used for these experiments were procured from various sources i.e. DPPH (CDH Pvt. Ltd. New Delhi), Ascorbic acid (Yarrow chem Mumbai), NBT (CDH Pvt. Ltd. New Delhi), Riboflavin (CDH Pvt. Ltd. New Delhi), EDTA (CDH Pvt. Ltd. New Delhi), Methanol (Fisher scientific Mumbai), sodium di hydrogen phosphate (Yarrow chem Mumbai), di sodium hydrogen phosphate (Yarrow chem Mumbai), de oxy ribose (CDH Pvt. Ltd. New Delhi), potassium di hydrogen phosphate (Yarrow chem Mumbai), ferric chloride (Fisher scientific Mumbai), hydrogen peroxide (Yarrow chem Mumbai), Thio barbituric acid (CDH Pvt. Ltd. New Delhi), tri chloroacetic acid (Fisher scientific Mumbai), Carrageenan (sigma), Indomethacin (Recon, Bangalore)

Antioxidant activity

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay:[7]

The free radical scavenging activity of ethanolic extract of *Allmania nodiflora* was measured by DPPH (1, 1-diphenyl-2-picryl-hydrazil) employing the method described by Blois, 1958. 0.1 mM solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml of various concentrations (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, µg/ml) of ethanolic extract. After 30 min, absorbance was measured at 517 nm. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Ascorbic acid was used as a reference compound. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{ DPPH scavenging activity} = (\text{Abs Control} - \text{Abs test}) / \text{Abs Control} \times 100$$

Where Abs Control is the absorbance of the control reaction and Abs test is the absorbance in the presence of the sample of the extracts/standard. The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%.

Determination of Superoxide Radical Scavenging Activity: [8]

Super oxide is biologically important as it can form singlet oxygen and hydroxyl radical. Over production of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. Super oxide anion are generated in PMS-NADH system by the oxidation of NADH and assayed by the reduction of NBT resulting in the formation of blue formazan. 100 µl of Riboflavin solution [20 µg], 200 µl EDTA solution [12mM], 200 µl methanol and 100µl NBT (Nitro-blue tetrazolium) solution [0.1mg] were mixed in test tube and reaction mixture was diluted up to 3 ml with phosphate buffer [50mM]. The absorbance of solution was measured at 560 nm using phosphate buffer as blank after illumination for 5 min. This is taken as control. 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, µg/ml of different concentrations of extract as well as standard preparation were taken and diluted up to 100 µl with methanol. To each of these, 100 µl Riboflavin, 200 µl EDTA, 200 µl methanol and 100 µl NBT was mixed in test tubes and further diluted up to 3 ml with phosphate buffer.Absorbance was measured after illumination for 5 min. at 560 nm on UV visible spectrometer Shimadzu, UV-1601, Japan. The superoxide anion scavenging activity was calculated according to the following equation.

$$\% \text{ inhibition} = (\text{Abs Control} - \text{Abs test}) / \text{Abs Control} \times 100$$

Where Abs Control is the absorbance of the control reaction and Abs test is the absorbance in the presence of the sample of the extracts/standard. The IC50 values for EEAN as well as standard preparation were calculated.[9, 10]

Hydroxyl radical scavenging activity: [11]

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and EEAN for hydroxyl radical generated by Fe^{3+}-Ascorbate–EDTA– H2O2 system (Fenton reaction). The reaction mixture contained in a final volume of 1.0 ml, 100 µl of 28 mM 2-deoxy-2- ribose in 20 mM KH_2PO_4-KOH buffer of pH 7.4, 500 µl of the selected concentrations of extract (0.1, 0.5, 1, 5, 10, 20, 50, 100, 150, 200, 250, 300, 350, 450, 500, 700, 1000 µg/ml) in KH_2PO_4-KOH buffer (20 mM, pH 7.4), 100 µl of 1.04 mM EDTA, 100 ml 200 mM $FeCl_3$, 100 µl of 1.0 mM H_2O_2 and 100 µl of 1.0 mM ascorbic acid was incubated at 37 ^oC for 1 h. 1.0 ml of thio barbituric acid (1%) and 1.0 ml of trichloroacetic acid (2.8 %) were added to the test tubes and were incubated at 100 ^oC for 20 min. After cooling, absorbance was measured at 532 nm against control containing deoxy ribose and buffer. Ascorbic acid was used as a positive control. Reactions were carried out in triplicate. The percentage inhibition was determined by comparing the results of the test and control compounds by using following formula.

$$\text{Hydroxyl scavenging activity (\%)} = (\text{Abs Control} - \text{Abs test}) / \text{Abs Control} \times 100$$

Where Abs Control is the absorbance of the control reaction and Abs test is the absorbance in the presence of the sample of the extracts/standard.

Calculation of 50% inhibition concentration

The graph was plotted by taking concentration on X-axis and percentage inhibition on Y-axis, the graph was extrapolated to find the 50% inhibition concentration of the sample.

Antimicrobial activity

Test micro organism and inoculums preparation

The test organisms used in this study were as follows: *Bacillus subtilis, Staphylococcus aureus(Gram +ve), Escherichia coli, Proteus vulgaris (Gram-ve)* and two fungal strain *Aspergillus niger* , *Candida albicans* for the antimicrobial tests, procured from MTCC (Microbial type culture collection), IMTECH (Institute of Microbial Technology), Chandigarh, (Punjab), maintained on nutrient agar slants (oxide) at 4 ^{o}C., a loop-full culture of all micro organism was inoculated into nutrient broth under sterile conditions, and incubated at 37 °C for 24 h in rotary shaker.

Antimicrobial activity: [12]

The agar disc diffusion method was used to evaluate the antibacterial potentiality of EEAN against various Gram+ve, Gram-ve, and fungal strain. A 100 µL of freshly prepared inoculum was spread onto the surface of sterile Mueller Hinton agar using sterilized glass spreader. A sterile borer was used to prepare the cups of 6mm diameter in the agar medium spread with micro-organism and 0.1 ml of inoculums was spread on the agar plate by spread plate technique. Accurately measured (0.05m1) solution of each concentration and reference standard (Ciprofloxacin 50 µg/ml) was added to the cups with a micropipette.All the plates were kept in a refrigerator at 2°C to 8°C for effective diffusion of test compounds and standards for 2 hours. Later, they were incubated at 37 °C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control (DMSO 4.0%, v/v) was run simultaneously, which was used as vehicle. The diameter of the zone of inhibition was measured and recorded.

Anti-inflammatory activity

Acute toxicity

The acute toxicity of *Allmania nodiflora* was assessed by using up and down method.[13]After the administration of one single dose of EEAN (5, 50, 300, 2000 mg/kg) the survival of animals was observed during 24 hrs. If an animal survived at any given dose, the dose for the next animal was logarithmically increased; if it died, the dose was decreased.

Anti-inflammatory activity: [14]

Male or female Albino rats are starved overnight and grouped into five. To ensure uniform hydration, the rats receive pure drinking water. At the time of experiment they were not allowed access to both feed and water. The rats are subjected to intra peritoneal injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the right hind

paw. The three groups of rats an hour post intra peritoneal (IP) administration were subjected to 100, 250 mg/kg of test extract and 20 mg of standard. The standard and test drug is dissolve or suspended in the same volume. The vernier calipers [RSK, Mumbai] was used to measure paw width and thickness before and at 1st , 2nd , 3rd , 4th, 5th and 6th hour after injection of carrageenan the paw volume was then calculated from width (a) and thickness (b) measurement using the following Equation:

$$\text{Volume} = \pi \times a^2 \times b$$

The percentage inhibition of paw edema was calculated for each group with respect to its vehicle treated control group by using the formula:

$$\text{Percentage inhibition of edema} = (1 - Vt / Vc) \times 100$$

STATISTICAL ANALYSIS

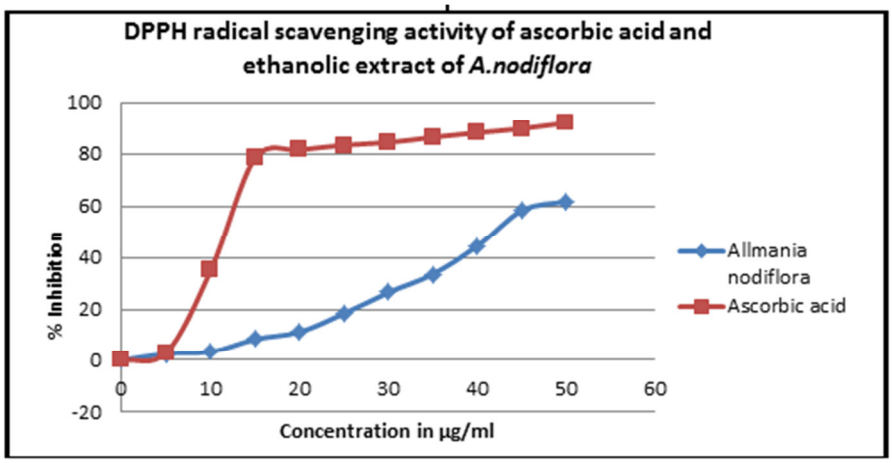
The result were expressed as the mean ± SD (standard deviation) for three parallel measurements using graph pad prism version 6.0 for windows and subjected to statistical analysis by student t-test for comparison between groups. In all the cases $P < 0.05$ was considered as statistically significant. Data were computed for statistical analysis by using graph PAD prism.

Table 1
IC 50% value of invitro antioxidant activities of Allmania nodiflora:

s.no.	sample	DPPH radical scavenging activity	Hydroxyl radical scavenging activity	Superoxide radical scavenging activity
1	*Allmania nodiflora*	42±0.06	8±1.64	44±0.07
2	Ascorbic acid	12±0.05	1±1.18	23±1.04

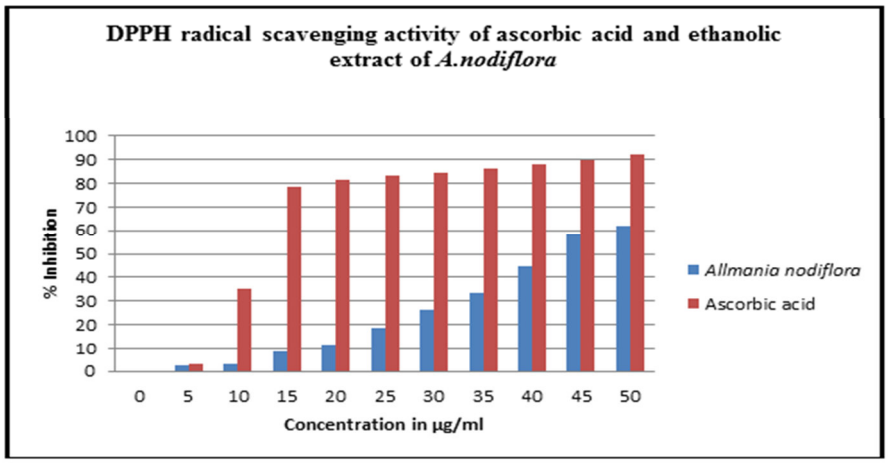
Data are presented as mean±SD

Graph 1



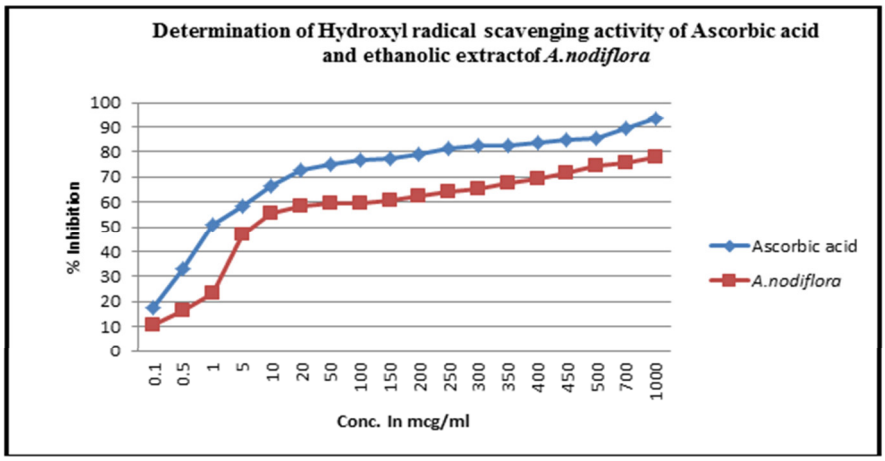
DPPH radical scavenging activity of ethanolic extract of A.nodiflora and Ascorbic acid
Data are presented as the percentage of DPPH radical scavenging, Mean±SD

Graph 2



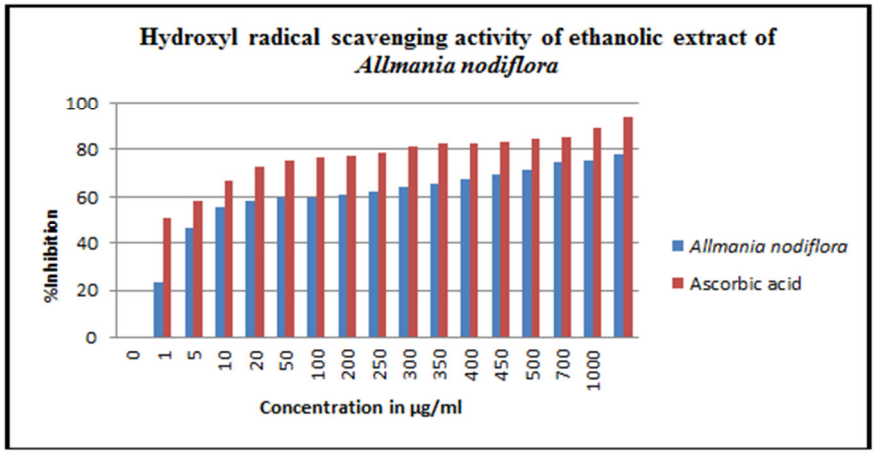
DPPH radical scavenging activity of ethanolic extract of A.nodiflora and Ascorbic acid Data are presented as the percentage of DPPH radical scavenging, Mean±SD

Graph 3



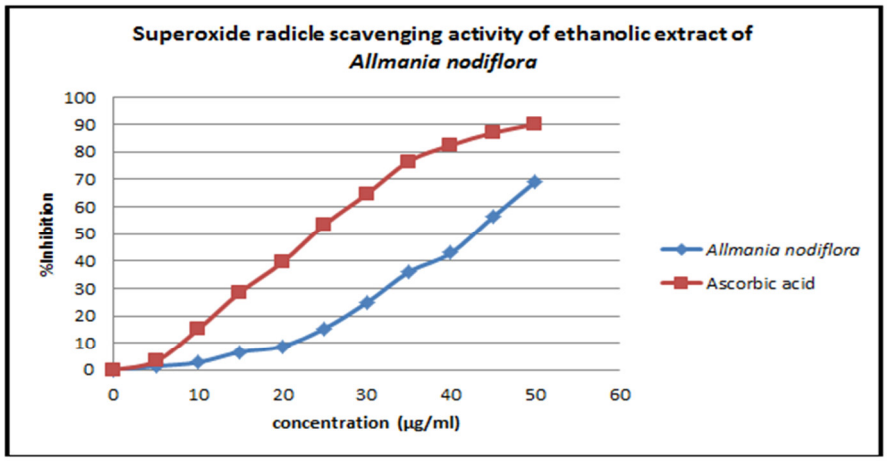
Determination of Hydroxyl radical scavenging activity of ethanolic extract of A.nodiflora and Ascorbic acid, Data are presented as the percentage of hydroxyl radical scavenging, Mean±SD

Graph 4



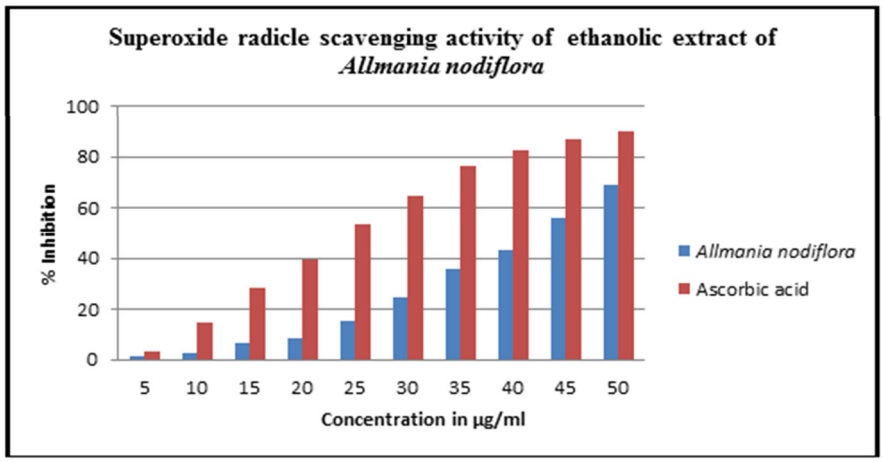
Determination of Hydroxyl radical scavenging activity of ethanolic extract of A.nodiflora and Ascorbic acid, Data are presented as the percentage of hydroxyl radical scavenging, Mean±SD

Graph 5



Determination of superoxide radical scavenging activity of ethanolic extract of A.nodiflora and Ascorbic acid, Data are presented as the percentage of superoxide radical scavenging, Mean±SD

Graph 6



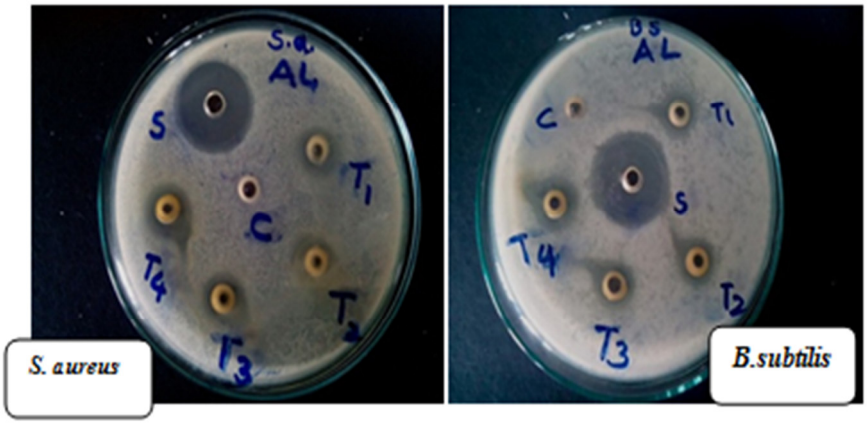
Determination of superoxide radical scavenging activity of ethanolic extract of A.nodiflora and Ascorbic acid, Data are presented as the percentage of superoxide radical scavenging, Mean±SD

Table 2

Zones of growth inhibition (mm) showing antibacterial activity of ethanolic extract of A.nodiflora; well diameter 8.0mm

Name of the organism	Standard (Ciprofloxacin 50µg/ml)	T_1 (50mg of EEAN)	T_2 (100mg of EEAN)	T_3 (150mg of EEAN)	T_4 (200mg of EEAN)	Control
S. aureus (Gram + ve)	24±0.11	12±0.12	14±0.11	14±0.12	14±0.11	-
B. subtilis (Gram +ve)	25±0.23	10±0.13	12±0.14	12±0.22	14±0.13	-
P.vulgaris (Gram -ve)	24±0.12	12±0.12	12±0.14	13±0.23	14±0.11	-
E.coli (Gram -ve)	25±0.22	11±0.11	12±0.14	12±0.16	15±0.16	-

EEAN=Ethanolic extract of A.nodiflora, data are presented as Mean±SD



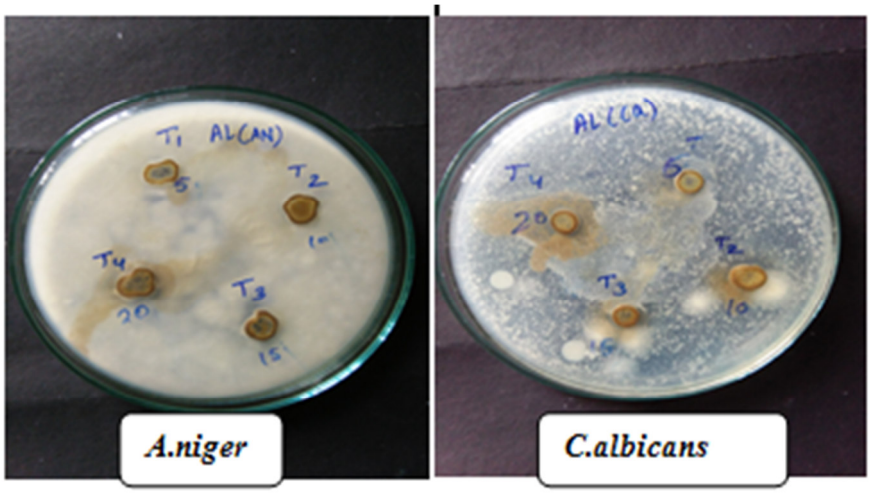
T_1=50mg EEAN;T_2=100mg EEAN;T_3=150mg EEAN; T_4=200mg EEAN; S=standard (Ciprofloxacin 50µg/ml); C=Control (DMSO)

Figure 1
Antibacterial activity of ethanolic extract of A.nodiflora

Table 3
Zones of growth inhibition (mm) showing Antifungal activity of ethanolic extract of A.nodiflora; well diameter 8.0 mm

Name of the organism	Standard Fluconazole (0.1 mg/ml)	TI (50mg of EEAN)	T2 (100mg of EEAN)	T3 (150mg of EEAN)	T4 (200mg of EEAN)	CONTROL
A.niger	16±0.11	12±0.05	11±0.12	11±0.17	11±0.04	-
C.albicans	16±0.16	10±0.06	11±0.05	11±0.05	11±0.07	-

EEAN=ethanolic extract of Allmania nodiflora, data are presented as Mean±SD



T_1=50mg EEAN;T_2=100mg EEAN;T_3=150mg EEAN; T_4=200mg EEAN

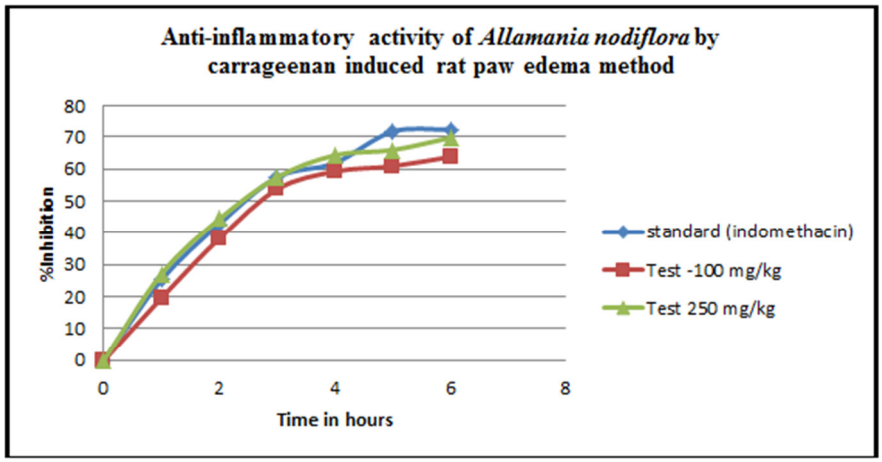
Figure 2
Anti fungal activity of ethanolic extract of A.nodiflora

Table 4
Anti-inflammatory activity of Allmania nodiflora carrageenan induced rat paw edema method

s.no	Name of the sample	Dose (mg)	% inhibition of paw volume at various time intervals (hr)					
			1	2	3	4	5	6
1	Control	-------	0	0	0	0	0	0
2	Standard (Indomethacin)	20	25.31±0.47	42.90±0.45	57.5±0.45	61.95±0.44	71.74±0.45	72.35±0.45
3	Test extract -100 (EEAN)	100	19.71±0.13	38.27±0.15	53.75±0.56	59.31±0.17	60.96±0.66	63.85±0.67
4	Test extract -250 (EEAN)	250	27.17±0.14	44.44±0.13	57.5±0.18	64.32±0.19	65.99±0.14	69.84±0.48

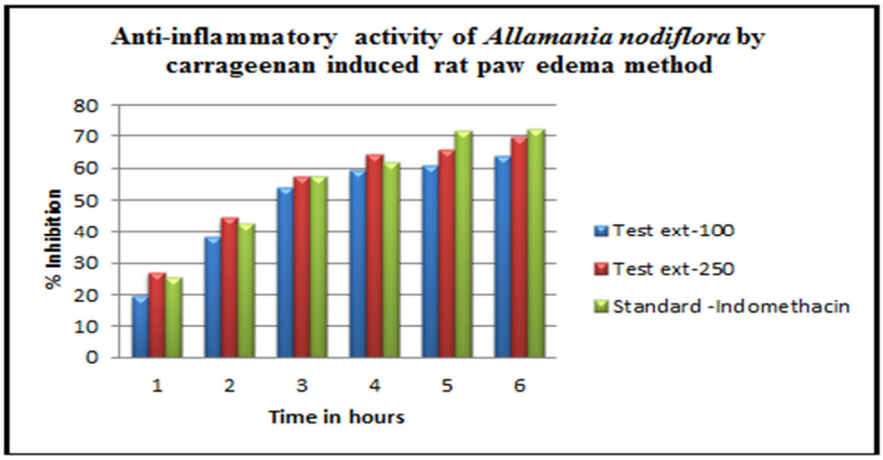
EEAN=Ethanolic extract of Allmania nodiflora, each value is presented as Mean±SD of three values

Graph 7



Anti-inflammatory activity of Allmania nodiflora by carrageenan induced rat paw edema method, data is represented as mean±SD

Graph 8



Anti-inflammatory activity of Allmania nodiflora by carrageenan induced rat paw edema method, data is represented as mean±SD

RESULT AND DISCUSSION

Antioxidant activity

In this study, the antioxidant activity of EEAN was compared with ascorbic acid and shown in a various invitro tests i.e. DPPH scavenging activity, Superoxide Radical Scavenging Activity and hydroxyl radical scavenging activity.

DPPH scavenging activity

The stable radical DPPH has been widely used in the determination of primary antioxidant activity, i.e. the free radical activity of the pure antioxidant compound (standard) and the extract. The assay was based on the reduction of DPPH radicals in methanol which cause the decreasing absorbance at 517 nm. The scavenging effect increased with increasing with the increasing concentrations of the test compounds. The IC_{50} was calculated for the extract as well as standard and summarized in table: 1 and graphically represented in graph no: 1&2. The IC_{50} value of the extract by DPPH scavenging activity was 42 and ascorbic acid was 12, which was comparatively lower than the standard. It means ethanolic extract of *Allmania nodiflora* at higher concentration captured more free radicals formed by DPPH resulting into decrease in absorbance and increase in IC 50 value.

Superoxide Radical Scavenging Activity

Superoxide radical is one of the strongest ROS among the free radical and gets converted to other harmful reactive oxygen species such as hydrogen peroxide and hydroxyl radical damaging biomolecules which results in chronic diseases.[15] These radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560 nm with the plant extract (EEAN) and the reference compound ascorbic acid indicates their abilities to quench superoxide radicals in the reaction mixture. The IC 50 value of extract is 44 and standard is 23 respectively shown in table:1 and graph:5&6.

Hydroxyl radical scavenging activity

The scavenging ability of EEAN on hydrogen peroxide is shown in figure: 3&4, and compared with ascorbic acid as standard. The EEAN was capable of scavenging hydrogen peroxide in an amount dependent manner. At a concentration of 1mg/ml, the scavenging activity of extract and the standard was found to be 77.72±0.052915 and 93.62±0.23072 respectively. The IC 50 value of extract was (8) was found to be more effective in quenching the hydroxyl radicals produced in the reaction mixture. The hydroxyl radical can induce oxidative damage to DNA, lipids and proteins.[16] Thus removing the H_2O_2 is very important for antioxidant defense in cell by various food materials like fruits, green leafy vegetables etc. The hydroxyl radical scavenging ability of extract was determined by its ability to compete with deoxyribose for hydroxyl radical. The extract diminishes the chromogen formation by compeating with deoxyribose. In this assay 2-deoxy-2-ribose was oxidized when exposed to hydroxyl radicals generated by Fenton-type reaction. The oxidative degradation can be detected by heating the product with TBA under acidic conditions to develop a pink chromogen (thiobarbituric acid reactive species) with a maximum absorbance at 532 nm.[17]

Anti-microbial activity

The antimicrobial activities of the plant extract against four bacterial strains and two fungal strains were examined and assessed by the presence or absence of inhibition zones. The inhibition zones of the extract tested for antibacterial and antifungal activity is given in table no: 2, 3 and fig: 1, 2 shows potential Gram+ve and Gram-ve activity but very less antifungal activity. *S.aureus* causes infections including superficial skin lesion, localized abscesses, and food poisioning.[18] *B. subtilis* causes meningitis, endocarditis, pneumonia, wound infection and lesions.[19] *E.coli* is Gram negative bacteria causing severe type of food poisoning and also causes liver abscess, infra abdominal obstetric and gynecologic. Enteropathogenic strains of *E.coli* cause acute diarrhea in children below 2 years and may cause acute death due to the dehydration and electrolyte imbalance[20] and *P.vulgaris* causes wound infection and urinary tract infection.[21] These infections can be treated with many standard drugs but not all strains can be successfully treated by these drugs, further evaluation on this plant is needed. The extract shows moderate activity against microbes. This could be the beginning for further research on the screening approach by taking in to consideration the extract preparation and the mechanism of action.

Anti-inflammatory activity

Inflammation can be defined as a reaction of a living cell or tissue to injury, infection, irritation or infiltration. It is characterized by pain, swelling, redness and heat/fever. Prostaglandins and leukotrienes are released by a host of mechanical, thermal, chemical, bacterial and other insults, and they contribute importantly to the signs and symptoms of inflammation.[22] Mast cell which is very rich in histamine has membrane receptors both for special class of antibody (IgE) and for complements components-C3a and C5a mast cell can be activated to secrete inflammatory mediators through these receptors and also by direct physical damage. A trans membrane protein CD40, expressed on monocytes, neutrophylls and platelets also plays roles in inflammation.[23] Our results clearly show anti-inflammatory activity of the *Allmania nodiflora*. Generally, the effect of the ethanolic extract of *Allmania* with inhibition ranging from 63.85 to 69.84 but lower than the standard Indomethacin. Mechanism possibly involved in this anti-inflammatory activity include inhibition of the action of inhibitory mediators such as histamine, prostaglandins, nitric oxide, platelets activating factors and substance P, effect on adreno carticoid hormone and immune supression.
This work is at present is limited to only animals, further clinical trials must required.

CONCLUSION

The results of the present study revealed that *A.nodiflora* extract possess potent free radical scavenging ability, anti-inflammatory and antimicrobial activity. The activity may be due to the presence of flavonoids, phenols and triterpenoids. Further we conclude that this plant can be used as natural anti oxidant, anti inflammatory and anti microbial purpose.

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