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ANTI-LIPID PEROXIDATION ACTIVITY OF *ALLMANIA NODIFLORA* (L.) R. BR.: A TRADITIONAL LEAFY VEGETABLE

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Keywords:

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ABSTRACT: *Allmania nodiflora* (L.) R. Br. is a diffuse herb, belongs to family Amaranthaceae. Fresh leaves used as a green leafy vegetable, which is rich in vitamins and iron. The present aim of this work is to evaluate the phytochemical composition and antioxidant activity of ethanol and water extracts of *A. nodiflora* leaves. The results on the phytochemical composition of the water and ethanol extracts of *A. nodiflora* revealed that water extract showed a higher amount of total phenolic (TPC) and total flavonoid (TFC) contents than ethanol extract. Regarding antioxidant activity results, both water and ethanol extracts showed a higher amount of total antioxidant capacity (TAC) and dose-dependent activity against the DPPH, hydroxyl radical and lipid peroxidation activity. In conclusion, the water extract of *A. nodiflora* leaves showed a high amount of TPC, TFC and TAC and strongly inhibited DPPH, hydroxyl radical and ferric chloride-induced lipid peroxidation in goat liver tissue homogenate. The findings of the study suggest that *A. nodiflora* leaves could be used as a potential source of natural antioxidants.

INTRODUCTION: Lipid peroxidation initiated by reactive oxygen species leads to the formation of several lipid aldehydes such as 4-hydroxy-trans-2-nonenal (HNE), acrolein and malondialdehyde (MDA). These lipid aldehydes highly reactive with cellular proteins, nucleic acids, activate signaling cascade molecules and transcription factors, which results in the up-regulation of several inflammatory mediators, thus causing inflammation [1]. Natural drugs of plant origin are the most important sources of antioxidants and anti-inflammatory compounds, which alleviate free radicals induced inflammatory cascade. Plant drugs rich in polyphenols are known to have multifunctional properties such as antioxidant, anti-inflammatory, anti-carcinogenic and *etc*. [2]

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In this connection, we selected *Allmania nodiflora* to screen anti-lipid peroxidation and antioxidant properties by using *in-vitro* methods. *Allmania nodiflora* (L.) R. Br. ex Weight is the diffuse or erect herb of family Amaranthaceae. It is widely distributed in cultivated and waste fields in Andhra Pradesh. Fresh leaves used as a green leafy vegetable along with other leafy vegetables. It is a rich source of vitamins and iron. The medicinal importance of *A. nodiflora* was well documented in the literature. The leaves traditionally used as febrifuge [3], snakebite [4] and worm infections [5], and seeds used for constipation and dysentery. Apart from these uses, the plant has been used as antidiabetic, hypolipidemic, nutritive and appetizer [6]. Pharmacologically *A. nodiflora* was reported to possess, antioxidant, anti-inflammatory, CNS depressant, analgesic and antimicrobial activities [6-9].

Based on the literature cited above, very few reports were noticed on free radical scavenging activity of *A. nodiflora* leaves by *in-vitro* methods. To the best of our knowledge, no previous report was noticed on a protective effect on ferric

chloride-induced lipid peroxidation properties of *A. nodiflora* leaf extracts by *in-vitro* methods. Hence, the present work is designed to evaluate anti-lipid peroxidation activity of alcohol (AE) and water (WE) extracts of *A. nodiflora* shade dried leaves in goat liver homogenates.

MATERIALS AND METHODS:

Plant material and Preparation of Extracts: *Allmania nodiflora* leaves were collected from agricultural fields of Ananthapuram surroundings **Fig. 1**. The voucher specimens (#RUH 08) were identified with the help of regional [10] and local floras [11] and the same was deposited in Rayalaseema University Herbarium, Kurnool, Andhra Pradesh, India.

FIG. 1: *ALLMANIA NODIFLORA* FLOWERING BRANCHES

The collected leaf samples were cleaned manually with tap water, cut into small pieces and dried under shade. The dried material was ground into fine powder by using a mixer grinder. The powdered material was successfully extracted with ethanol by using a Soxhlet apparatus for six hours. After complete extraction with ethanol, the remaining material was boiled in water and extract (WE) was concentrated on water both. The alcohol extracts (AE) was concentrated under reduced pressure to get dry powder. Both crude extracts were subjected to phytochemical and antioxidant activities.

Estimation of Total Phenolic Content: Total phenolic content of *A. nodiflora* leaf alcoholic (AE) and water (WE) extracts was estimated by FCA reagent method [12]. Different concentrations of *A. nodiflora* leaf alcoholic and water extracts were incubated for 5-8 min with 100 µL of Folin-Ciocalteau (FCA) reagent and 580 µL of double distilled water at room temperature (RT). To this solution, 300 µL of 20% sodium carbonate solution was added and volume was made up to 2 mL and again incubated at room temperature for 2 h.

The absorbance of the solution was measured at 765 nm against the blank using a UV spectrophotometer. The total phenol content of *A. nodiflora* leaf extracts was calculated from the calibration curve of gallic acid and expressed as milligrams of gallic acid equivalents per gram dry weight (mg GAE/g dwt).

Estimation of Total Flavonoid Content: Total flavonoid content (TFC) of the plant extracts was estimated by aluminum chloride method [13]. Briefly, different aliquots of (50 and 100 µL) the extracts were mixed with 5% sodium nitrite and 10% aluminum chloride and incubated for 5 min at room temperature. After that, the samples were treated with 1M sodium hydroxide and incubated for 15 min at room temperature. The absorbance of the reaction mixture was measured at λ 510 nm. Quercetin was used as a standard and the results were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g dwt).

Estimation of Total Antioxidant Capacity: Different concentrations of *A. nodiflora* leaf alcohol and water extracts were incubated with 1mL of ammonium molybdate reagent in the dry bath at 95 °C for 90 min. After that, the samples were cooled to RT and the absorbance of the solution was measured at 695 nm against blank in UV spectrophotometer [14]. The amount of total antioxidant capacity was calculated from the calibration curve of ascorbic acid and expressed as milligrams of ascorbic acid equivalents per gram dry weight (mg AAE/g dwt).

DPPH Scavenging Activity: Different concentrations of plant extracts were taken and 1mL of methanolic DPPH solution was added followed by incubation for 30 min in dark at room temperature. After incubation period absorbance of the control and test samples, OD was measured at 517 nm against blank in UV spectrophotometer [15].

Hydroxyl Radical Scavenging Activity: Hydroxyl radical scavenging property of plant extracts was measured by the Fe^{3+}- EDTA ascorbic acid and H_2O_2 system according to method [16].

Anti-lipid Peroxidation Activity in Goat Liver Tissue Homogenate: The effect of *A. nodiflora* leaf alcoholic and water extracts on ferric chloride-induced lipid peroxidation in goat liver tissue homogenate was tested by using the TBARS method. The detailed method used in this study was followed as described in Vidya *et al*., 2014 [17].

Statistical Analysis: The protective effect of different extracts against DPPH and the percentage of inhibition of lipid peroxidation was calculated as follows:

% inhibition = Control OD - Sample OD × 100 / Control OD

Ascorbic acid and gallic acid were used as reference compounds. All the results were expressed as mean ± standard deviation (S.D.).

RESULTS AND DISCUSSION:

Total Phenolic Content, Total Antioxidant Capacity and Extract Yield: Total phenolic content (TPC) and total antioxidant capacity (TAC) of *A. nodiflora* leaf AE and WE were assayed by spectrophotometer methods. TPC of *A. nodiflora* leaf AE and WE extracts were estimated by FCA reagent methodand expressed as milligrams of gallic acid equivalents per gram dry weight (mg GAE/g dwt). Among the test extracts, WE showed higher amount of phenolic content than AE extract **Fig. 2**. Higher phenolic content of WEs is due to the solubility of wide range of phenolic compounds in water [18].

Total flavonoids content (TFC) of the aqueous and ethanol extracts of *A. nodiflora* leaf was quantified based on the linear equation obtained from quercetin standard curve and expressed as milligrams of quercetin equivalent per gram dry weight of the sample (mg QE/g dwt). Flavonoids are large sub group of phenolic compounds occur in food plants and are found to act as free radical terminators and potential antioxidants. The results of TFC of *A. nodiflora* leaf extracts revealed that WE showed higher amount of flavonoid content than ethanol extract **Fig. 2**. This could be due to the breakdown of the cell wall at high temperature and release of trapped flavonoids into the extracts. Presence of high quantities of phenolic and flavonoid content in water extracts made them to have strong free radical scavenging activity. The high temperature of water while extracting plant tissues increases the solubility of phenolics and flavonoids, facilitate their to release into cell-free extracts is known [18]. Our results suggest that, *A. nodiflora* leaves subjected to domestic processes at high temperatures can have a higher amount of TPC and TFC compounds.

The phosphomolybdenum method is commonly used to evaluate the total antioxidant capacity (TAC) of vegetables and medicinal plants. The total antioxidant capacity of *A. nodiflora* leaf AE and WE were expressed as milligrams of ascorbic acid equivalents per gram dry weight (mg AAE/g dwt). TAC of *A. nodiflora* leaf extracts was found to be high in water extract (60.34 ± 0.76 mg AAE/g dwt) than ethanol extract **Fig. 2**. This may be due to the extractability of the high amount of phenolic content of WE. The higher amount of phenolic content of WE correlates with higher antioxidant capacity [18].

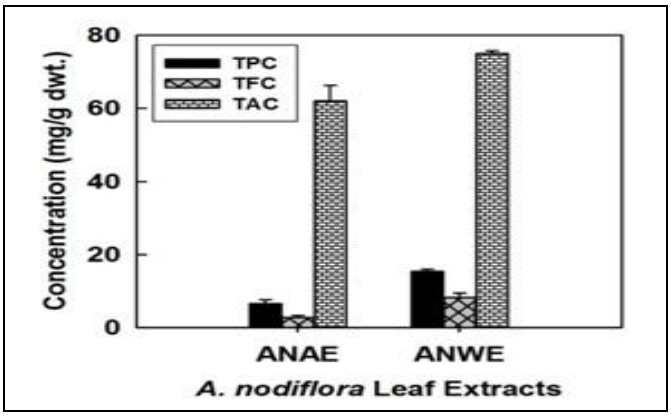

FIG. 2: TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND TOTAL ANTIOXIDANT CAPACITY OF ALCOHOLIC AND WATER EXTRACTS OF *A. NODIFLORA* LEAVES

DPPH Scavenging Activity: DPPH scavenging activity of *A. nodiflora* leaf AE and WEs was presented in **Fig. 3**. The results revealed that both extracts showed concentration-dependent DPPH scavenging activity. WE extract exhibited 80% and AE extract showed 70% as maximum inhibition to DPPH. Both the tested extracts expressed lower IC_{50} value (1.28 and 1.32 mg dwt/mL) to reduce DPPH purple color to 50%. AE extracted less amount of phenolic content than WE extract, even though it showed potential DPPH scavenging activity by expressing very low IC_{50} value **Table 1**. Potential DPPH scavenging activity of medicinal plant extracts and herbal formulations were well reported [19-23].

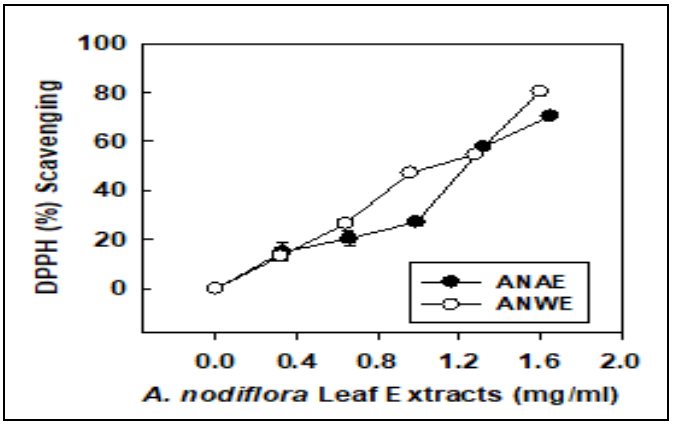

FIG. 3: DPPH REDUCING CAPACITY OF ALCOHOLIC AND WATER EXTRACTS OF *A. NODIFLORA* LEAVES

Hydroxyl Radical Scavenging Activity: Hydroxyl radicals generated through oxidation of H_2O_2 in the presence of transitional metals by Fenton's reaction are known to be the most reactive free radical species capable of damaging almost every biological molecule found in living cells. These species are considered to be rapid initiators of the lipid peroxidation process by taking the hydrogen atoms from unsaturated fatty acids.

A. nodiflora leaf extracts were tested to scavenge $FeCl_3$/EDTA/H_2O_2/ascorbic acid - mediated hydroxyl radical generation. Water and alcoholic extract of *A. nodiflora* leaf showed dose-dependent activity with maximum scavenging activity of 95.75 % at 192 µg dwt/mL and 87.75% at 92.4 µg dwt/mL respectively **Fig. 4**. IC_{50} values for WE and AE was found to be 51.2 µg dwt/mL and 52.8 µg dwt/mL respectively **Table 1**.

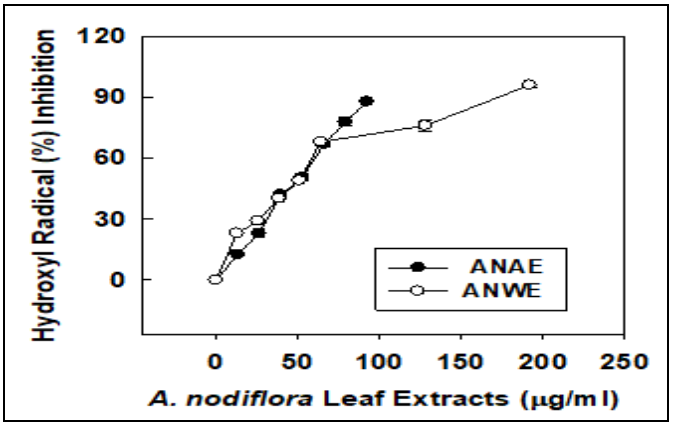

FIG. 4: HYDROXYL RADICAL SCAVENGING CAPACITY OF ALCOHOLIC AND WATER EXTRACTS OF *A. NODIFLORA* LEAVES

Anti-lipid Peroxidation Activity with Goat Liver Tissue Homogenate: The oxidation of unsaturated fatty acids present in biological membranes is a key step in the initiation of peroxidation, which results in the formation of malondialdehyde, well known mutagenic and carcinogenic product. Ethanol and water extracts showed dose-dependent inhibition to ferric chloride-induced lipid peroxidation in goat liver **Fig. 5** tissue homogenates.

Water extract showed maximum inhibition as 96% to liver lipid peroxidation than ethanol extract. But both the tested extracts showed strong inhibition to liver lipid peroxidation by expressing the lowest IC_{50} value (224 and 264 µg dwt/mL) **Table 1**.

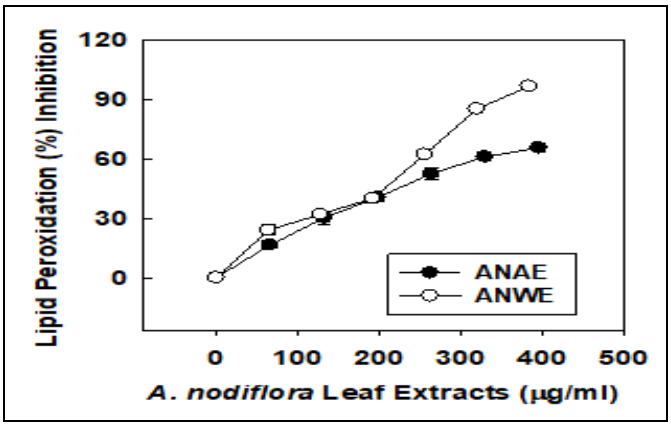

FIG. 5: ANTI-LIPID PEROXIDATION ACTIVITY OF ALCOHOLIC AND WATER EXTRACTS OF *A. NODIFLORA* LEAVES

TABLE 1: IC_{50} VALUES (µg/mL) OF THE TEST EXTRACTS AGAINST DPPH, HYDROXYL AND LIPID PEROXIDATION

Activity	IC_{50} values (µg/ml)	
	AE	WE
DPPH	1.32	1.28
Hydroxyl radical	52.8	51.2
Lipid peroxidation	264	240

CONCLUSION: The present study results indicated that the water extract of *A. nodiflora* leaf showed a high amount of TPC, TFC and TAC and strongly inhibited DPPH, Hydroxyl radical and ferric chloride-induced lipid peroxidation in rat liver tissue homogenate. The findings of the study suggest that *A. nodiflora* leaves could be used as a potential source of natural antioxidants.

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CONFLICTS OF INTEREST: Authors don't have a conflict of interest.

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