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Antioxidant and Anti-Lipidemic Activities of Ethanol Leaf Extract of *Thevetia neriifolia* (Yellow Oleander)

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ABSTRACT

Antioxidants and anti-lipidemic are substances that play a vital role in protecting biological systems, against the deleterious effects of oxidative processes on macromolecules. The antioxidants and antilipidemic properties of *Thevetia neriifolia*, a medicinal plant, was investigated, to know if it could be used in the treatments and prevention of diseases, such as, cardiovascular diseases, inflammations, cancer, etc. The ethanol leaf extract, at various concentrations(200,100,50 and 25mg/kg b.w) were used with ascorbic acid (Vitamin C),as standard drug. Results indicated that, at higher concentrations of 100 and 200mg/kg,b.w, there were no significant differences, in total cholesterol (TC) and High density lipoprotein (HDL) levels, with values: 4.85, 4.35: 1.75, 1.3mg/dl ($p<0.05$) respectively, compared with the control values, which were 4.00,2.00.mg/dl There was significant ($p>0.05$) difference in LDL and triglycerides, at high concentrations of 100 and 200 mg/kg b.w, with values of 2.69, 2.84: 1.75, 1.05mg/dl, when compared to the control values, 1.79 and .95mg/dl. The leaf extract, at high concentration of 200 mg/kg shows a high scavenging activity in both lipid peroxidation and nitric oxide, with values 78.72 and 92.70% when compared with the standard drug value at 95.27 and 94.16% respectively.. Conclusively, the study findings indicate that *Thevetia neriifolia* possesses high antioxidant properties and low anti-lipid peroxidation properties.

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INTRODUCTION

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. They are abundantly found in the body system as a consequence of oxidative damage or stress, which leads to many human diseases particularly stroke and neurodegenerative incidents.(1) Nature has made it easy that there is a dynamic balance between the amount of free radicals generated in body and antioxidants to scavenge them thereby protecting the body against deleterious effects. The practical way to fight against degenerative diseases is to improve body's antioxidant status; which can be achieved by adding antioxidant to meals, oils, foodstuffs and other materials to prevent free radicals damage.

In recent years, a lot of plant species have been investigated for their therapeutic potential and particularly as antioxidants in reducing such free radical induced tissue damages. There are some well known and traditionally used natural antioxidants from tea, wine, fruits vegetables and spices (Schuler,1990 2) but there is still a demand to find more information on the antioxidant and anti-lipidemic potential of plants species. *Thevetia neriifolia* juss Ex. Steud plant belongs to the family Apocynaceae and commonly called Yellow Oleander.

***Thevetia neriifolia* plants (yellow oleander)**

It is a dicotyledon evergreen tropical oil seed shrub or small tree up to 10-20 feet tall (3.00-3.9m*)* *Thevetia neriifolia* flowers and fruits throughout the year with a peak in flowering during rainy season. It is a native of India, used medicinally throughout the tropics. It is usually cultivated in home gardens for use in local medicine and as ornamental. In Senegal, water in which leaves and bark were macerated has been used to cure amenorrhoea. In Mali the latex is applied to soften corns and calluses. In Cote divoire and Benin, the leaf sap is also dropped in the nostrils to revive people that have fainted and to cure colds. The seed oil is applied externally in India to treat skin infections.

Plants as gifts of nature have many therapeutic properties combined with much nutritive value, which have made their use in chemotherapy as valuable as the synthetic drugs. *Thevetia neriifolia* is a medicinal plant widely used for cardiac disorder; applied on wasp bites, the kernel is chewed for purgative effect. Chemical components of the plant are cardenolides- thevetin A and B, fixed oil and protein, Neriifolin, Cardiac glycosides, Peruvoside, Thevetoxin. It has been shown to contain significant quantities of lipids (Ejoh *el al*.,2007,3 Eleyinmi *el al*.,2008 4). Scientists have been proving that all the natural things are not good for health this apply to *T. neriifolia* because most parts of it including the latex are highly toxic, the plant must be screened and detoxified of its poisonous chemical before use.

Present study was aimed towards evaluating the antioxidant and anti-lipidemic activities of *Thevetia neriifolia* leaves extract.

MATERIALS AND METHODS

Collection of plant material

The fresh leaves of *Thevetia neriifolia* were harvested from the University staff quarter of Michael Okpara, University of Agriculture, Umudike, Umuahia Abia-State of Nigeria and identified by Dr. Jimoh Mulikat. A. a plant taxonomist in the Department of plant science and biotechnology of the university.

Preparation of Plant Extract

The plant material was air dried in the plant house of Michael Okpara University of Agriculture for 28 days. It was grounded to fine powder with Thomas Wiley Mill Model ED-5 Milling machine.

50g of the fine powder of *T. neriifolia* was weighed using Triple Beam Balance MB-2610. 200ml of ethanol was added to the plant materials in a beaker. It was allowed to stand for 72hrs at room temperature and filtered using Whatman No 1 filter paper. The filtrate was concentrated at 50^0C using Uniscope waterbath, in order to evaporate the ethanol. The concentrate was stored in refrigeration until being used.

Experimental Animals

Wistar Albino rats of both sexes weighing between (70-150g) and Wistar Albino mice (30-33g) were used in the study. They were procured from the animal house of the Department of Zoology University of Nigeria Nssuka in Anambra State of Nigeria. The animals were housed in metal cages and randomly distributed. They were kept in the University animal house and allowed for acclimatization period lasting for 14days (2weeks) prior to use for any experiment. The animals have access to clean water and standard laboratory animal feed.

Determination of Antioxidant Activity of the Plant Extract

Nitric oxide inhibition activity

2.0 ml of 10mm sodium nitroprusside and 5.0 ml of phosphate buffer were mixed with 0.5ml of different concentrations of the plant extracts and incubated at 25 ˚C for 150minutes. After the incubation, 2ml of the incubation solution was added to 2ml of Greiss reagent (1% sulphanilamide, 0.1%^ -napthyl-ethylenediamine dihydrochloride and 3% phosphoric acid) and incubated at room temperature for a period of 30minutes. The absorbance of the pink chromophore formed by the diazotization of nitrite with ^ -napthyl ethylene diamine dihydrochloride was measured at 540nm. Ascorbic acid was used as standard, sodium nitroprusside as control and results were expressed as percentage inhibition of nitric oxide. All determinations were performed in triplicates.

$$\% \text{ inhibition} = \frac{\text{Ab control} - \text{Ab sample}}{\text{Ab control}} \times 100$$

Anti-lipid peroxidation activity

Anti-lipid peroxidation activity was determined by the method described by Dinakarn *et al.*, *(*2011 5). Ethanol extract of *Thevetia neriifolia* were used at various concentrates (25, 50, 100, 200^g/ml). Liver obtained from a fresh sacrificed animal was used for the determination of anti-lipid peroxidation activity. The liver was made homogenate by a mechanical grinder. 3ml of liver homogenate was added with 100^l of 15mM ferric chloride and was shaken for 30 minutes. From collected mixture, 100^l was added with 1ml of different concentrations of plant extracts individually in different test tubes. The same procedure was followed for standard and blank. Ascorbic acid was used as standard and TBARS as control. All the test tubes were incubated for 4 hours at 37c. After incubation, 20% trichloroacetic acid (TCA) was added to all the test tubes containing the mixture in 1:1 ratio and was centrifuged for 30 minutes. The supernatant liquid was collected and 0.6% thiobarbituric acid (TBA) was added in 1:1 ratio and heated for 1 hour in a water bath. The mixture was cooled and absorbance measured at 530nm. The percentage of anti-lipid peroxidation activity was calculated using the formula.

$$\% \text{ inhibition} = \frac{\text{Ab Control} - \text{Ab Sample}}{\text{A control}} \times 100$$

DPPH Radical Scavenging Activity

This was carried out according to the 2,2, diphenyl-2- picrylhydrazyl (DPPH) assasy system (Mensor *et al.*,2001). Extracts at different concentrations were mixed with 2ml of absolute ethanol (100%). 1ml of DPPH was added to the solution and shaken immediately. The mixture was allowed to stand at room temperature in the dark for 30minutes. The absorbance of the resulting mixture was measured at 518nm and converted to percentage antioxidant activity (AA%) using the formula:

$$\% \text{ inhibition} = \frac{\text{Ab control} - \text{Ab sample}}{\text{Ab control}} \times 100$$

DPPH free radical was used as control.

Determination of Anti-lipidemic Activity of the plant Extract

Total Cholesterol (TC), Triglyceride (TAG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) were determined by enzymatic methods as described by Stein,1987 (6),The low-density lipoprotein was calculated using the Friedewald *et al.*1997 (7).

LDL-C = TC –HDL-C + TAG/5

OR

LDL-C =TC –TAG/5 –HDL-C.

Statistical analysis

Results were expressed as the mean ± standard deviation (n=4). A one way analysis of variance (ANOVA) was used for the data analysis using SPSS (statistical package for social sciences, 17.0) software. Significant differences between groups were detected in the ANOVA, using DUNCANs multiply range test at p values less than 0.05.

RESULTS

The results of Antioxidant and anti-lipidemic activities of the ethanol extract are shown in table 1,2,3 and 4.The result is equally presented in figure 1,2,3 and 4.

TABLE 1: Result of anti-lipid peroxidation

Concentration	**Absorbance**	**% Scavenging**
Control	1.88±1.89	0 %
200mg	0.4±0.8	78.72±79. 89
100mg	0.9±0.8	52.13±57.67
50mg	1.0±1.11	46.81±41.27
25mg	1.48± 1.50	21.28±20.64
Vitamin C 100mg	0.09±0.11	95.21±94.18

TABLE 2: Result of nitric oxide scavenging activity

Concentration	Absorbance	% Mean of inhibition
Control	1.37±1.42	0 %
200mg	0.1±0.11	92.70±92.25
100mg	0.4±0.4	70.80±64.79
50mg	1.0±0.9	27.01±36.62
25mg	1.12±1.14	18.25±19.72
Vitamin C 100mg	0.08±0.07	94.16±95.07

TABLE 3: Result of the lipid profile

Total cholesterol, HDL cholesterol, LDL Cholesterol Triglyceride

Concentration	TC	HDL	LDL	Triglyceride
200mg	4.85±.0707	1.75±.0707	2.69±.0707	1.75± .0707
100mg	4.35±.707	1.30±.141	2.84±.113	1.05±.212
50mg	4.70±.566	1.75±.212	1.68±.368	1.30±0.000
25mg	4.60±.141	2.50±.141	1.95±.021	.750±.0707
12.5mg	5.10±.424	2.10±.141	2.77±.579	1.15±.0707
6.25mg	5.30±.141	2.10±.141	2.62±.169	2.20±.141
Control	4.0±.141	2.00±.283	1.79±.473	.950±.212

TABLE 4: Result of 2,2-diphenyl-1picryl hydrazyl scavenging activity

Concentration	Absorbance	% Scavenging
Control	2.65±2068	0 %
200mg	0.9±0.62	66.04±76.87
100mg	1.2±1.3	54. 72±51. 49
50mg	2.0±1.9	24. 53±29.10%
25mg	2.45±2.32	7.55±13.43
Vitamin C 100mg	0.1±0.09	96.23±96.64

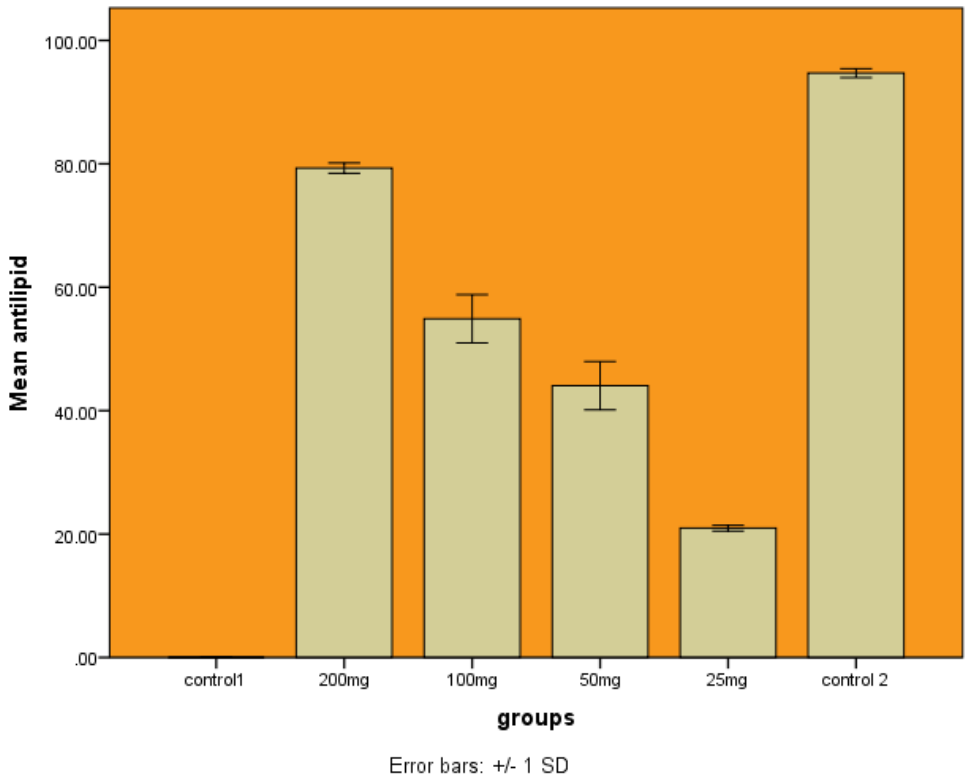

Figure 1: Anti-lipid peroxidation activity

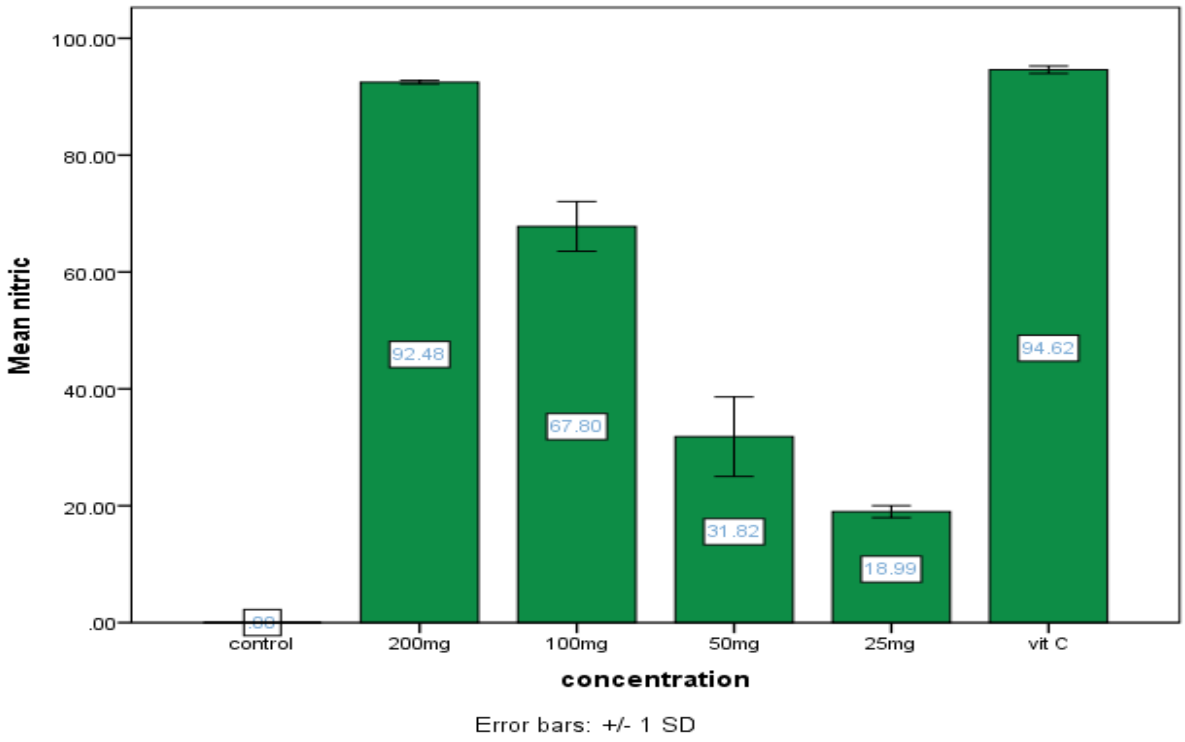

Figure 2: Nitric oxide inhibition activity

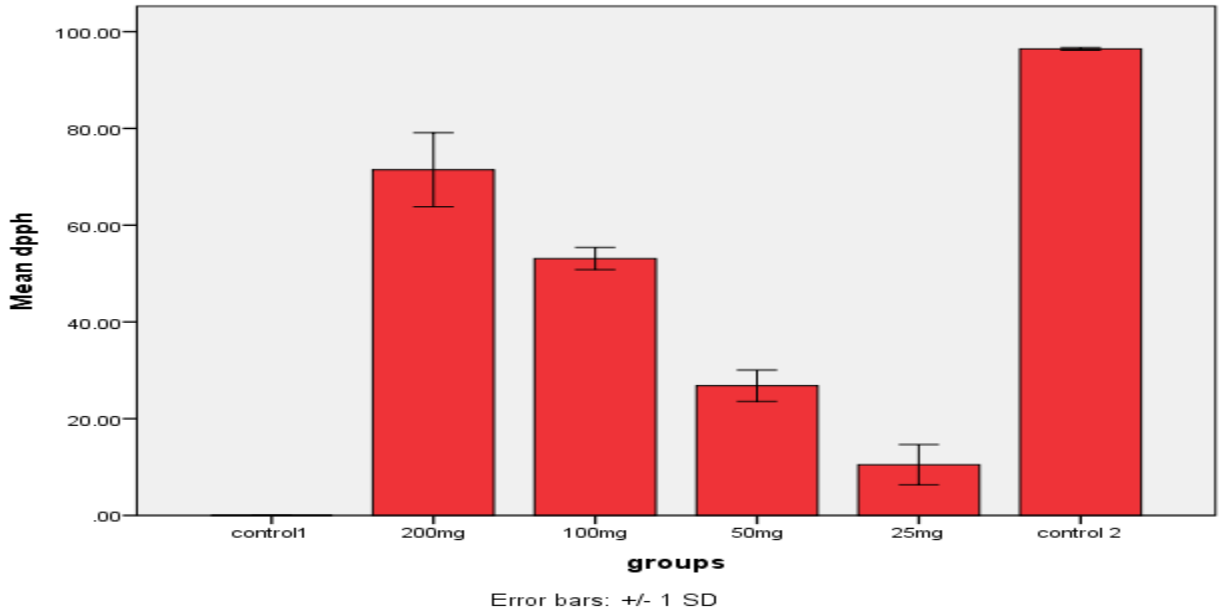

Figure 3: 2,2 Diphenyl-1-picyrl hydrazyl scavenging activity

DISCUSSION

The analysis of data in Table 1, confirms that anti-lipid peroxidation activity was maximum at 200mg. From table 2,it was found that the scavenging of nitric oxide was decreased in dose dependent manner and exhibited maximum inhibition at 200mg.The maximum concentration of inhibition was found to be 94.16% for ascorbic acid and 92.70% for the extract, which shows that the extract possess anti-oxidative agents like tannins, flavonoids and other polyphenolic compounds which have the ability to scavenge free radicals; therefore, acting as anti-oxidants (.8, 9). It has been established that anti-oxidants help to prevent cardiovascular diseases by interfering with the oxidation of very low density lipoproteins which are the chief engineers of artherosclerosis. (10). Increase in concentration of Triacylglycerol during the course of study indicated that the plant had an ability to increase the rate of lipid break down; lipolysis leading to the accumulation of Triacyiglycerol. The increase in rate of lipid break down may also have been responsible for the increase in the concentration of Total Cholesterol.

The results on lipid profile in table 3, indicated that at higher concentration of 100 and 200 mg/kg; there was no significant difference on the Total Cholesterol and HDL but significant difference on LDL and TAC values were observed. The extract significant increase LDL and TAC ($p>0.05$), LDL-cholesterol transport cholesterol to the arteries where they can be retained

in artheria protoglycans starting the formation of plaques,(11) LDL-cholesterol presents a risk of cardiovascular disease when it invades endothelium and oxidized since the oxidized form is more retained by the proteoglycan, thus increase of LDL-cholesterol is associated with artherosclerosis, heart-attack, stroke, peripheral vascular disease (12.13). The results indicated that, the extract does not contain anti-lipidemic properties.

CONCLUSION

This present study has clearly revealed that ethanolic extract of *Thevetia neriifolia* has been found to be anti-oxidant in rats. The information obtained from these work shows that the plant extract could be useful in improving and management of complications associated with patients suffering from cardiovascular diseases due to the myriad of phytochemicals inherent in it.

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