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Studies on the *In Vitro* Antioxidant Activity of *Laportea Aestuans* Leaf Extract

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Abstract : *Hydro – ethanolic leaf extract of Laportea aestuans L. was screened for antioxidant activity using various antioxidant models such as 1,1-diphenyl-2-picryl hydroxyl quenching (DPPH) assay, superoxide scavenging assay (O_2^{oo}), reducing power capacity (RC), scavenging capacity towards hydroxyl ion (OH^o) radical, nitric oxide (NO^o) radical inhibition, hydrogen peroxide inhibition activity (H_2O_2), lipid peroxidation (LPO) inhibition activity and 2,2'-azinobis-3-ethylbenzothiozolin-6-sulfonic acid ($ABTS^{o+}$) cation decolourization test. Total phenolic content, total flavonoid content and ascorbic acid content were also determined. The leaf extract of L. aestuans (LAE) yielded 199.3 ± 4.041mg Gallic acid/100g of sample as the total phenolic content, 90.7 ± 6.807mg Quercetin/100g of sample as total flavonoid content and 52.0 ± 5.000mg vitamin/100g of sample as ascorbic acid content. The extract exhibited high scavenging activity against DPPH, O_2, OH, NO, H_2O_2, ABTS and Lipid peroxide (LPO) with IC_{50} values of 15.0 ± 1.00, 247.0 ± 4.58, 84.3 ± 3.79, 67.3 ± 3.06, 230.7 ± 7.02, 81.0 ± 3.61 and 82.7 ± 6.43µg/ml respectively. Correlation was high between polyhenolic content and antiradical potential of different reactive oxygen species indicated that polyphenols are considered as main antioxidants.*

Key words: *Laportea aestuans, Antioxidant activity, DPPH, Reducing capacity, ABTS, Nitric oxide and Antiradical activity*

I. Introduction

Antioxidants are found useful in the prevention and treatment of many human diseases such as cardiovascular diseases, cancer, inflammation and aging related disorders which originate from free radical derived oxidative stress [1]. Natural antioxidant mechanisms can be inefficient; hence dietary intake of antioxidant compounds becomes important [2]. Studies have indicated that relationship exist between the plant antioxidants and reduction of chronic diseases [3]. These benefits are believed to result from the antioxidant components of plant origin, vitamins, flavonoids and carotenoids. Recent studies have shown that polyphenols in plants scavenge reactive oxygen species and effectively prevent oxidative cell damage [4].

Laportea aestuans belongs to the family Urticaceae which is composed of 22 species. Two species have so far been reported for Southern Nigeria. They include *Laportea aestuans* (Linn) Chew and *Laportea ovilifolia* (Schumach) Chew (male and female) [5].

Laportea aestuans is an annual herbaceous weed around waste places. The stem is erect, angular, covered with pilose hair or stinging hairs and green in colour [6]. In Gabon, Nigeria and Ghana the cooked leaves of *Laportea aestuans* are eaten as vegetables [7,8].

In West Africa, the plant finds application in ethnomedicine, in the treatment and management of headache, diuretic to cure blenorrhoea and chest problems. It is also used to deliver placenta after child birth, prevent excessive menstrual bleeding. Extracts are used to treat arthritis, anemia, hayfever, kidney problems and pain [9].

In the present study, antioxidant activity of hydro ethanolic extract of leaves of *Laportea aestuans* has been evaluated, employing different indices of antioxidant assays.

II. Materials And Methods

Plant material:

The leaves of *Laportea aestuans* (Stinging nettle) were harvested fresh from the botanic garden of Abia State University, Uturu, Nigeria and identified by Dr. S.K Chukwuka of the Plant Science Department of Abia State University. Voucher specimen was deposited in the herbarium of the Abia State University (Voucher no: ABSU 125).

Preparation of *Laportea aestuans* Leaf Extract (LAE)

The leaves of *L. aestuans* were air dried at room temperature and ground to fine powder. 1000g of the leaf powder was extensively extracted with 500ml distilled water ethanol mixture 98% [1:1(w/v)] for 10 hours, using Soxhlet apparatus. The extract was centrifuged (3000xg) thrice and the clear supernatants were filtered over Whatman No. 1 filter paper. The extract was evaporated to dryness by rotary flash evaporator (Buchi type,

Switzerland) under reduced pressure at 45^0C. Different concentrations were prepared from the resultant crude hydro ethanolic extract to determine *in vitro* antioxidant capacity.

Determination of Total Phenolic (TP) Content

The total phenolic content was determined using Folin – Ciocalteu method [10]. 1ml of diluted extract was mixed with diluted Folin – Ciocalteu reagent (1N. 1ml). The mixture is allowed to react for 3min at room temperature. The absorbance was read at 765nm using UV – Visible Spectrophotometer (Shimadzu, Kyoto, Japan). The analysis was done in triplicate. The TPC was expressed as mg gallic acid equivalent from gallic acid standard curve (mg GAE/100g fresh material $R^2 = 0.9968$).

Measurement of Total Flavonoid (TFC)

The content of total flavonoid iin *L. aestuans* leaf extract was based on the [11] method. 0.5ml of 2% $AlCl_3$ ethanol solution was measured after 1 hour at room temperature using UV – Visible spectrophotometer (Shimadzu, Kyoto, Japan). The TFC was determined from a quercetin standard curve and the results were expressed as (mg quercetin equivalent mg QE/100g fresh material $R^2 = 0.992$).

Measurement of Ascorbic Acid (AA)

The amount of ascorbic acid is estimated using the method of [12]. 5ml of the standard solution of Vitamin C were taken into 100ml conical flask. 10ml of 4% oxalic acid was added and titrated against the dye. Pink colour that appears and persists for few minutes signifies end point. The amount of the dye consumed is equivalent to the amount of ascorbic acid. Extract the sample (0.5 – 5g depending on sample) in 4% oxalic acid and make up to a known volume (100ml) and centrifuge. A volume of 5ml of the supernatant and 100ml of 4% oxalic acid were taken and titrated against the dye. Amount of ascorbic acid was calculated and expressed as mg/100g sample.

Antioxidant Activity Determination

The antioxidant activity of hydro – ethanolic extract was evaluated using DPPH radical quenching assay, reducing capacity, scavenging capacity towards hydroxyl ion (OH^o) radicals, scavenging capacity towards nitric oxide (NO^o), $ABTS^{o+}$ cation decolorization test, hydrogen peroxide (H_2O_2) scavenging capacity and lipid peroxidation assay TBARS.

DPPH Radical Quenching Activity

DPPH radical scavenging activity was adopted from those previously described with slight modification [13]. Various concentrations (20 - 100µg/ml) of sample were mixed with 5ml of 0.1mM ethanol solution of DPPH and vortexed. The tubes were allowed to stand at room temperature for 20min. the control was prepared as above without any extract and ethanol was used for the baseline correction. Changes in the absorbance of the samples were measured at 517nm. Mean values were obtained from triplet experiments. Butylated hydroxyl toluene (BHT) was taken as reference standard. The percentage quenching of DPPH was calculated as follows:

$$\text{Inhibition of DPPH (\%)} = \frac{\text{Control 517nm} - \text{Sample 517nm}}{\text{Control 517nm}} \times 100$$

Where sample 517nm was absorbance of the sample and control 517nm was absorbance of control. The results were expressed as IC50, which means the concentration at which $DPPH^o$ radical were quenched by 50%.

Reducing Capacity

The ferric reducing power of the leaves of *L. aestuans* extract was quantified according to the method of [14]. Different concentrations of the extract (200 - 1000µg/ml) were prepared. To all the extracts in test tubes 2.5ml of sodium phosphate buffer followed by 25ml of 1% potassium ferricyanide [$K_2Fe(CN)_6$] solution was added. The contents were vortexed well and incubated at 50^0C for 20mins. After incubation, 2.5ml of 10% trichloroacetic acid (TCA) was added to all the tubes and centrifuged at 3000g for 10mins. Afterwards, to 5ml of the supernatant, 5ml of deionized water was added. To this about 1ml of 1% ferric chloride was added to each test tube and incubated at 35^0C for 10mins. The absorbance was read at 700nm. Mean values were obtained from triplet experiments.

The reducing power of the extract was linearly proportional to the concentration of the sample. Increased absorbance of the reaction mixture indicated increased reducing power. Butylated hydroxyl toluene (BHT) was taken as reference standard.

Scavenging Capacity towards Superoxide Radical (O_2^o)

Superoxide anion radical (O_2^o) generated in the phenazine methosulfate – reduced form of nicotinamide adenine dinucleotide (PMS – NADH) system by oxidation of NADH and assayed by the reduction of nitro blue tetrazolium chloride (NBT) by the extract with some changes [15].

The O_2^o were generated in 1.25ml of Tri –HCl (16mM, pH 8.0), 0.25ml of NBT (150µM), 0.25ml of NADH (468µM) and different concentrations (100 -1000µg/mg) of LAE and standard. The reaction was initiated by addition of 0.25ml of Phenazine methosulfate (60µM) to the mixture. Following incubation at ambient temperature for 5mins, the absorbance was read at 560nm. Blank was used as the same was using ethanol instead of sample. BHT was used for comparison. The percentage scavenging of O_2^o was calculated as follows:

$$\text{Inhibition of } O_2^o\ (\%) = \frac{\text{Control 560nm} - \text{Sample 560nm}}{\text{Control 560nm}} \times 100$$

Where sample 560nm was absorbance of sample and control 560nm was absorbance of control. IC_{50} was calculated at 50% reduction in absorbance brought about by sample compared with blank.

Scavenging Capacity towards Hydroxyl ion (OH^o) Radical

The OH^o scavenging activity of the LAE was determined according to the method described by Kleim *et al,* (1981). Different concentrations (50 - 250µg/ml) of extract were added with 1.0ml of iron – EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of EDTA solution (0.018%), and 1.0ml of DMSO (0.85% v/v in 0.1M phosphate buffer, pH 7.4). the reaction was started by adding 0.5ml of ascorbic acid (0.22%) and incubated at 80 – 90^{0}C for 15mins in a water bath. The reaction was terminated by the addition of 0.1ml of icd – cold TCA (17.5%v/v). the milliliters of Nash reagent (75.0g of ammonium acetate, 3.0ml of glacial acetic acid and 2ml of acetyl acetone were mixed and raised to 1L with distilled water), was added and left at room temperature for 15mins. The intensity of the colour formed was measured at 412nm against reagent blank. The hydroxyl radical scavenging reference standard was also used for comparison. The radical scavenging activity was calculated by the following formular:

$$\text{HRSA } (\%) = 1 - \frac{\text{difference in absorbance of sample}}{\text{Difference in absorbance of blank}} \times 100$$

Scavenging Capacity towards Nitric Oxide (NO)

Sodium nitroprusside (SNP) in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griss Illosvoy reaction [17]. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. The reaction mixture (3ml) containing SNP (10nM, 2ml) phosphate buffer saline (0.5ml) and the LAE at different concentrations and standard (50 - 250µg/ml) were incubated at 25^{0}C for 15mins. After incubation, 0.5ml of the incubated solution containing nitrite was pipette and mixed with 1ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5mins for completing diazotization, then, 1ml of N–1–naphthyl ethylene diamine at 25^{0}C. The absorbance was measured at 540nm. BHT was used for comparison. The percentage scavenging of NO was calculated as follows:

$$\text{Inhibition of NO } (\%) = \frac{\text{Control 540nm} - \text{Sample 540nm}}{\text{Control 540nm}} \times 100$$

Where sample 540nm was absorbance of the sample and control 540nm was absorbance of control.

Hydrogen Peroxide (H_2O_2) Scavenging Activity

The hydrogen peroxide scavenging activity of the extract was determined by the method of [18]. The extract at different concentrations (100 - 500µg/mg) was dissolved in 3.4ml of 0.1M solution of H_2O_2. The absorbance value of the reaction mixture was recorded at 230nm and BHT was considered as the standard. The percentage scavenging of H_2O_2 was calculated as follows:

$$\text{Inhibition of } H_2O_2\ (\%) = \frac{\text{Control 230nm} - \text{Sample 230nm}}{\text{Control 230nm}} \times 100$$

Where, sample 230nm was absorbance of sample and control 230nm was the absorbance of control.

2,2' – Azinobis (3-ethylbenzothiazoline sulphonic acid) ($ABTS^{o+}$) Cation Decolouration Test

The $ABTS^{o+}$ radical cation was produced by oxidizing $ABTS^{o+}$ with potassium persulphate ($K_2S_4O_8$) [19]. The solution (7mM) was oxidized with $K_2S_4O_8$ (2.4mM) for 12 hours at room temperature in the dark. The $ABTS^{o+}$ solution was then diluted by mixing 990µl $ABTS^{o+}$ solution with 60ml ethanol to obtain an absorbance of 0.706 ± 0.001 at 734nm. LAE and BHT at various concentrations (100 - 500µg/ml) were allowed to react with 1.0ml of the $ABTS^{o+}$ solution and absorbance was measured at 734nm. After 7mins BHT was added for comparison. Percentage inhibition was calculated as $ABTS^{o+}$ radical scavenging activity

$$= \frac{\text{Control 734nm} - \text{Sample 734nm}}{} \times 100$$

Control 734nm

Where, sample 734nm was absorbance of the sample and control 734nm was absorbance of control.

Lipid Peroxidation (LPO) Assay

A modified thiobarbituric acid – reactive species (TBARS) assay was used to measure the lipid peroxide formed using egg yolk homogenate as lipid rich medium [20]. Malondialdehyde (MDA), a secondary end product of oxidation of polyunsaturated fatty acids. Thiobarbituric acid (TBA) yielding a pinkish red chromogen with an absorbance maximum at 532nm. Egg homogenate (0.5ml of 10% v/v) and 0.1ml of extract were added to a test tube and made up to 1ml with distlled water. 0.005ml of $FeSO_4$ (0.07M) was added to induce lipid peroxidation and incubated for 30mins. Then 1.5ml of 20% acetic acid (pH 3.5), 1.5ml of (w/v) TBA (0.8%) in 1.1% sodium dodecyl sulfate (SDS) and 0.5ml TCA (20%) were added. The resulting mixture was votexed and then heated at 95^{o}C for 60mins. After cooling, 5.0ml of butn-n-ol was added to each test tube and centrifuged at 3000rpm for 10mins. The absorbance of the organic upper layer was read at 532nm.
Inhibition of lipid peroxidation by the extract was calculated according to:

$$\frac{1 - E}{C} \times 100$$

Where, C is the absorbance value of the fully oxidized control and E is the (Abs 532 = TBA – Abs 532 – TBA).

STATISTICAL ANALYSIS OD DATA

The experimental data were expressed as mean ± SD of 3 independent measurements. Linear regression analysis was used to calculate the inhibition concentration (IC_{50}) values. One way analysis of variance (ANOVA) and Tukeys's multiple range tests were carried out. The P values less than 0.05 were adopted as statistically significant. Regression analysis was used to determine correlation between the antioxidant properties and polyphenolic content. GraphPad Prism (Version 6.0) was the software used for the statistical analysis.

III. Results And Discussion

Table 1showed the results of total phenolic compound, total flavonoids and ascorbic acid content of hydro – ethanolic leaf extracts of *L. aestuans*. Total phenolic content was found to be 199.3 ± 4.041mg/100g of Gallic acid equivalent (GAE), the total flavonoids content was 90.7 ± 6.807mg/100g of quercetin equivalent (QE), whereas the ascorbic acid content was found to have 52.0 ± 5.00mg/100g of Vitamin C equivalent. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [21]. Polyphenols are present in a variety of plants utilized as important components of both humans and animal diets [22].

Table 1: Polyphenols and ascorbic acid content of *L. aestuans* leaf extract.

Parameters	Mg/100g
Total Phenols[a]	199.3 ± 4.041
Total Flavonoid[b]	90.7 ± 6.807
Ascorbic acid[c]	52.0 ± 5.00

Each value in the table was obtained by calculating the average of three experiments ± standard deviation (n = 3). [a]mg Gallic acid/100g of sample. [b]mg Quercetin/100g of sample. [c]mg Vitamin C/100g of sample.

The hydro – ethanolic extract of leaves of *L. aestuans* was tested for their antioxidant properties in a range of *in vitro* assays to determine their ability to scavenge reactive oxygen species (ROS). The extract exhibited a concentration – dependent antiradical activity by quenching DPPH radical and the DPPH scavenging activity was comparable to that of BHT (Fig.1).

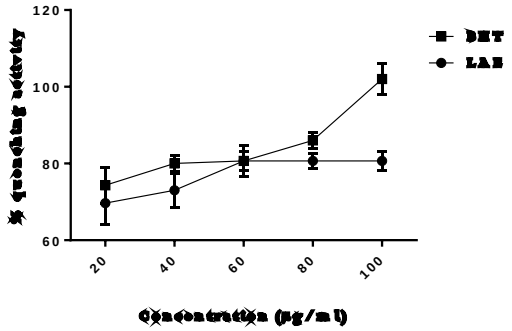

Fig. 1: DPPH quenching activity of *L. aestuans* hydro – ethanolic extract (LAE) and butylated Hydroxy Toluene (BHT).

The reduction of the ferric ion (Fe^{3+}) to ferrous ion (Fe^{3+}) is measured by the intensity of the resultant blue – green solution which absorbs at 700nm, an increase in absorbance shows higher reducing power. The dose – dependent reducing capacity of LAE was shown in Fig. 2.

It caused a significant elevation of reducing power with OD value of 1.18 ± 0.015 at 1000µg/ml as compared to that of BHT which was 1.130 ± 031 at 1000µg/ml. the reducing power of a compound may serve as a significant indicator of its potent antioxidant activity [23].

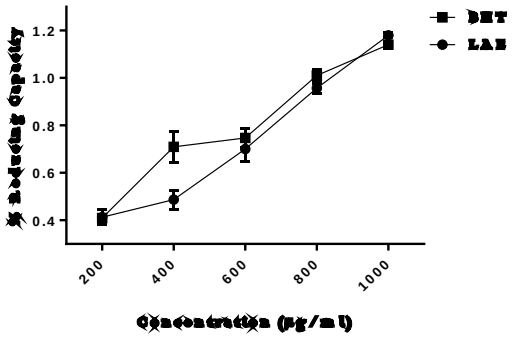

Fig. 2: Reducing Capacity of *L. aestuans* hydro – ethanolic extract (LAE) and butylated hydroxyl toluene

Superoxide radical is the main source for the formation of other ROS such as hydroxyl radical and hydrogen peroxide [24]. Result of the O_2^{oo} scavenging activity of LAE measured by PMS – NADH – NBT superoxide generating system is shown in Fig. 3. The scavenging effect of extract at a concentration of 500µg/ml is 79.7%.

In the PMS – NADH – NBT system O_2^{oo} derived from dissolved oxygen by PMS – NADH coupling reaction reduces NBT. The decrease of absorbance at 560nm with antioxidants indicates the consumption of O_2^{oo} in the reaction mixture. The result was comparable with standard BHT which was found to be 80.7% inhibition at 500µg/ml.

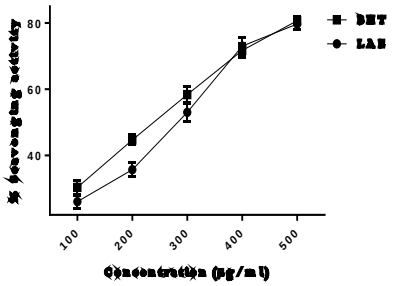

Fig. 3: Superoxide (O_2^{oo}) Scavenging activity of *L. aestuans* hydro – ethanolic extract (LAE) and butylated hydroxyl toluene BHT

Hydroxyl radicals conjugate with nucleotides in DNA, cause strand breakage and lead to carcinogenesis, mutagenesis and cytotoxicity [25].

The marked antioxidant activity of LAE shown in Fig. 4; scavenged hydroxyl radicals by 71.0% at 250µg/ml which is comparable with standard BHT, which showed 73.3% inhibition at the same concentration.

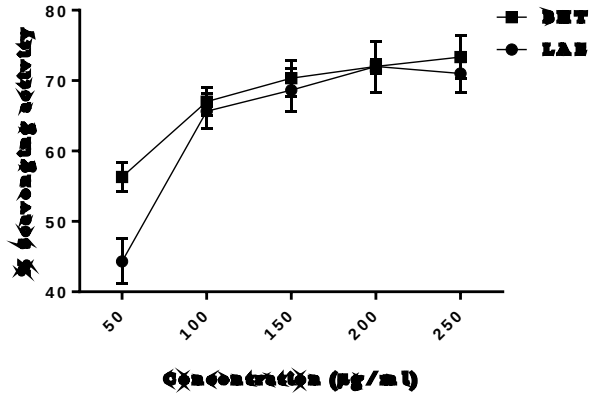

Fig. 4: Hydroxyl Radical (OH^*) scavenging activity of *L. aestuans* hydro – ethanolic extract (LAE) and butylated hydroxyl toluene BHT

Extent of nitric oxide radical scavenged was determined by the decrease in intensity of pink coloured chromophore at 540nm.

The extract exerted compatible inhibitory 72.7% potential at the concentration of 250µg/mg against nitric oxide generation.

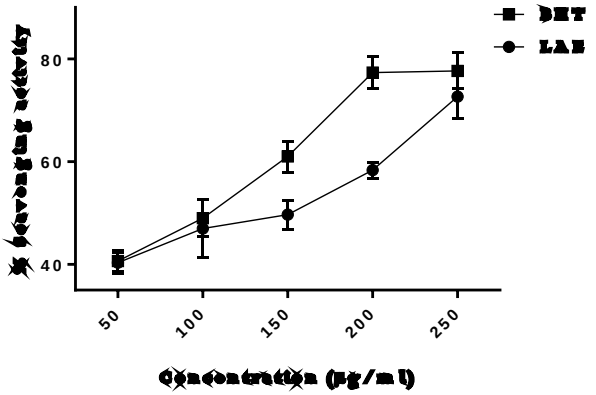

Fig. 5: Nitric Oxide (NO^*) scavenging activity of *L. aestuans* hydro – ethanolic extract (LAE) and butylated hydroxyl toluene BHT

The antioxidant activity was compared with BHT which has 77.7% inhibition. The study demonstrated the potent nitric oxide scavenging activity of the *L. aestuans.*

Removal of hydrogen peroxides from cells or food systems is very important for antioxidant defense, since H_2O_2 may give rise to hydroxyl radicals which are toxic to cells [26].

Fig. 6 showed that hydrogen peroxide decomposition activity has a concentration dependent pattern. The LAE had 35.0% at 100µg/ml and 70.7% at 500µg/ml; while BHT had 47.3% at 100µg/ml and 74.0% at 500µg/ml. the result showed that the extract possessed inhibition capacity.

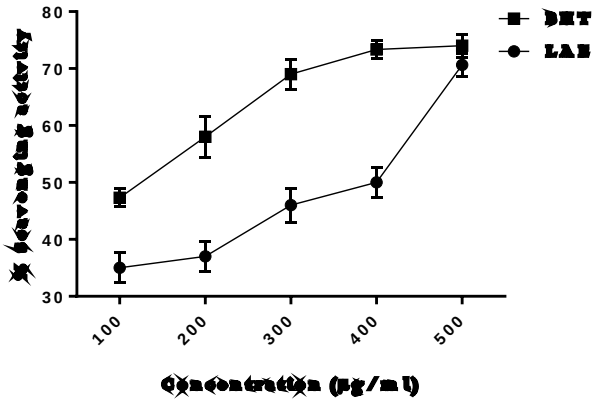

Fig. 6: Hydrogen Peroxide scavenging activity of *L. aestuans* hydro – ethanolic extract (LAE) and butylated hydroxyl toluene BHT

The $ABTS^{o+}$ scavenging assay is used to indicate the antioxidant activity of the extract. The LAE demonstrated as an effective scavenger to that of BHT. Fig. 7 showed the extract expressed maximum scavenging activity of 81.3% as against that of BHT which was 80.7%.
The results are considered to be useful when compared to that of previous studies on medicinal plants [27].

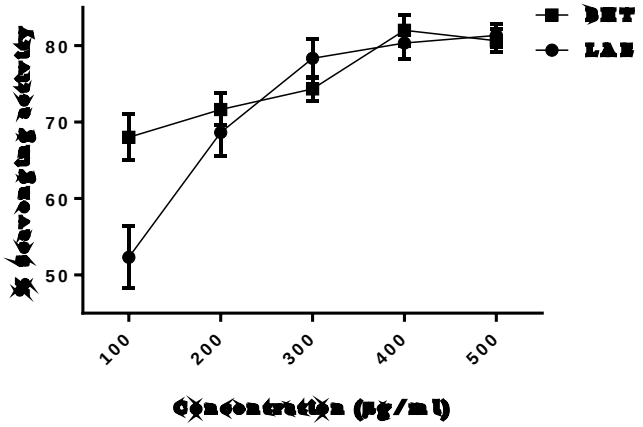

Fig. 7: ABTS^{o+} Cation scavenging activity of *L. aestuans* hydro – ethanolic extract (LAE) and butylated hydroxyl toluene BHT

The effect of LAE on peroxidation of lipids in the presence of ferrous sulphate is shown in Fi. 8. It showed an inhibition of 73.7% at a concentration of 500µg/ml.
The LAE inhibited generation of lipid peroxides in a concentration dependent manner. Therefore, LAE has a strong resistance on peroxidation.

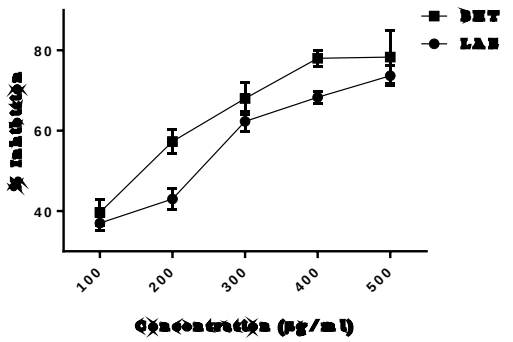

Fig. 8: Lipid Peroxide inhibition by *L. aestuans* hydro – ethanolic extract (LAE) and butylated hydroxyl toluene BHT

IC50 values and multiple correlations

Table 2 showed the IC_{50} values (µg extract/ml). LAE exhibited IC50 values in DPPH; O_2^{oo}, OH^o, NO, H_2O_2, $ABTS^{o+}$ and LPO antiradical activities as 15.0 ± 1.00, 247.0 ± 4.58, 84.3 ± 3.79, 67.3 ± 3.06, 230.7 7.02, 81.0 ± 3.61 and 82.7 ± 6.43µg/ml respectively.

DPPH was found to have better inhibiting capacity among all the assays,, with 15.0 ± 1.00µg/ml. Table 2 also showed the IC_{50} values of standard BHT.

Table 2: IC_{50} values of *L. aestuans* Hydro – ethanolic leaf extract (LAE) and standard Butylated Hydroxyl Toluene (BHT)

Assay models	IC_{50}µg/ml	
	LAE	LAE
DPPH antiradical activity	15.0 ± 1.00^a	11.0 ± 1.00^a
O_2^{oo} antiradical activity	247.0 ± 4.58^g	57.0 ± 3.00^d
OH^o antiradical activity	84.3 ± 3.79^e	45.0 ± 2.00^b
NO antiradical activity	67.3 ± 3.06^c	34.7 ± 2.52^c
H_2O_2 antiradical activity	230.7 ± 7.02^f	52.3 ± 5.86^d
$ABTS^{o+}$ antiradical activity	81.0 ± 3.61^b	42.7 ± 2.89^b
LPO inhibition	82.7 ± 6.43^d	44.3 ± 3.51^c

Each value in the table was obtained by calculating the average of three experiments ± standard deviation (n=3). Values in a column with different superscripts indicate significantly different at P<0.05.

It was observed that a strong correlation exist between total flavonoid (TF) and $ABTS^{o+}$ test ($R^2 = 0.982$). Lipid peroxidation and TF exhibited a perfect correlation (R^2=1.0). Lower IC_{50} values indicate higher antioxidant activity of the extracts [28].

Linear regression method was used to demonstrate a direct correlation among the antioxidant test.

Table 3: Correlation coefficient of total phenolic and flavonoids with different antioxidant assays

Parameters	TP	TF
TP	1	
TF	1	1
DPPH	0.832	0.848
O_2	0.986	0.986
OH	0.899	0.899
NO	0.946	0.946
RC	0.988	0.988
H_2O_2	0.936	0.938
ABTS	0.978	0.978
LPO	1	0.934

Supportive reports established the positive correlatin between phenolic content and antioxidant activity [29]. A positive correlation between antioxidant activity and polyphenol content was found. This suggests that the antioxidant properties of the plant extract is due to the polyphenols it contains [30]. These results showed that the antioxidant capacity of the leaf extract of *L. aestuans* is due to the quality of the phenolic compounds and flavonoids it contains.

References

[1]. G. Kaur, R. Tuli and M. Chintamaneni, "Antioxidant Potential of Methanolic and Hydrolyzed Extracts of *Eclipta alba*", *Pharmacologyonline,* vol. 2, pp. 947 – 956, 2009.

[2]. J.C. Espin, C. Soler – Rivas, and H.J. Wichers, "Characterization of the total free radical scavenger capacity of vegetable and oil fractions using 2, 2'-diphenyl-1-picrylhydrazyl radical". *J. Agric. Food Chem.*, vol. 48, pp. 648 – 656, 2000.

[3]. R.H. Lieu, "Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals", *Am. J. Clin. Nutr.*, vol. 78, pp. 517 – 520, 2003.

[4]. R.L. Prior, "Fruits and vegetables in the prevention of cellular oxidative damage". *Am. J. Clin. Nutr.*, vol. 78, pp. 570 – 578, 2003.

[5]. W.L. Chew, "A monograph of *Laportea* (Urticaceae)". *Gard. Bull. Singapore,* vol. 21, pp. 178 – 195, 1969.

[6]. I. Cheryl, "Laportea aestuans". *J. Ethnobiol. and Medicine,* vol. 3, pp. 13, 2007.

[7]. J.F. Morrison, and S.K. Twumasi, "Comparative studies on the *in vitro* antioxidant properties of methanolic and hydro – ethanolic leafy extracts from eight leafy vegetables of Ghana". *Afr. J. Biotech.*, vol. 9, no. 32, pp. 5177 – 5184, 2010.

[8]. U.A. Essiett, N.I. Edet, and D.N. Bala, "Phytochemical and physiological analysis of leaves of *Laportea aestuans* (Linn) Chew and *Laportea ovalifolia* (Schumach) Chew (male and female)". *Asian J. Plant Science and Res.*, vol. 1, no. 2, pp. 35 – 42, 2011.

[9]. A.A. Sofowora, Medicinal Plants and Traditional Medicine in West Africa, 2nd Edition, John Willey and Sons Ltd. New York, 1993, Pp: 289.
[10]. V.L. Singleton, R. Orthofer, and R.M. Lamuela – Raventos, "Analysis of total phenols and other oxidation substrates and antioxidants by means folinciocalteu reagent method". *Enzymol*., vol. 299, pp 152 – 178, 1997.
[11]. A.A.L. Ordonez, J.D. Gomez, M.A. Vattuone, and M.J. Isla, "Antioxidant activities of *Sechium edule* (Jacq) swart extracts". *Food Chem.*, Vol. 9, pp. 452 – 458, 2006.
[12]. S. Sadasivam and A. Manickan, *Biochemical Methods*. New Delhi: New-age International (P) Limited, 1996, Pp: 193 – 194.
[13]. R.P. Singh, K.N. Chidambara-Murthy, and G.k. Jayaprakash, "Studies on antioxidant activity of Pomegranate peel and seed extracts using *in vitro* models". *J. Agric. Food Chem.*, vol. 5, pp.. 86 – 89, 2002.
[14]. M. Oyaizu, "Studies on product of browning reaction prepared from glucose amine". *Jpn. J. Nut.*, vol. 44, pp. 307 – 315, 1986.
[15]. H. Yu, X. Liu, R. Xing, S. Liu, Z. Guo, P. Wang, C. Li, and P. Li, "*In vitro* determination of antioxidant activity of proteins from jellyfish *Rhopilema esculentum". Food Chem.*, vol. 95, pp. 123 – 130, 2006.
[16]. S.M. Klein, G. Cohen, and A.I. Cederbaum, "Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical generating systems", *Biochemistry Journal*, vol. 20, pp. 6006 – 6012, 1981.
[17]. D.C. Garret, The Quantitative Analysis of Drugs. 3rd Ed. Chapman and Hall Ltd, Japan, 1964, Pp: 456 – 458.
[18]. R.T. Ruch, S.J. Cheng, and E. Klawnig, "Prevention of cytotoxicity and inhibition of intercellular communication by antioxidants catechins isolated from Chinese green tea". *J. Carcinogenesis*, vol. 10, pp. 1003 – 1008, 1989.
[19]. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, "Antioxidant activity applying an improved ABTS radical cation decolourization assay". *Free Rad. Bio. and Med.*, vol. 26, pp. 1231 – 1237, 1999.
[20]. G. Ruberto, M.T. Barrata, S.G. Deans, and H.J.D. Dorman, " Antioxidant and antimicrobial activity of *Foeniculum vulgarae* and *Crithumum maritimum* essential oils". *Planta. Med.*, vol. 66, pp. 687 – 693, 2000.
[21]. A. Parr and G.P. Bolwell, "Phenols in plant and in man: The potential for possible nutritional enhancement of the diet by modifying the phenol content or profile". *J. Sci. Food Agric.*, vol. 80, pp. 985 – 1012, 2000.
[22]. L. Bravo, "Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance", *Nutr. Rev.*, vol. 56, pp. 317 – 333, 1998.
[23]. S. Meir, J. Kanner, B. Akiri, and S.P. Hades, "Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves". *J. Agric. Food Chem.*, vol. 4, pp. 1813 – 1817, 1995.
[24]. P.G. Pieta, "Flavonoids as antioxidants". *J. Nat. Prod.*, vol. 63, pp. 1035 – 1042, 2000.
[25]. M.M. Naidu, G. Sulochanamma, S.R. Sampathu, and P. Srinivas, "Studies on extraction and antioxidant potentials of green coffee". *Food Chem.*, vol. 107, pp. 377 – 384, 2008.
[26]. B. Halliwell, "Reactive oxygen species in living systems: sources, biochemistry and role in human disease", *Am. J. Med.*, vol. 9, pp. 14 – 22, 1991.
[27]. J.M. Sasikumar, V. Maheshu, G.S.|B. Aseervathan, and D..T.P. Darsini, "*In vitro* antioxidant activity of *Hedyotis corymbosa* (L) Lam. aerial parts". *Indian J. Biochem. Biophy.* Vol. 47, pp. 49 – 52, 2010.
[28]. M.E.G.C. Silva, A.L. Guimaraes, A.P. Oliveira, S. Araujo, J.A.S. Filho, A.P. Fontana, K.F. Damasceno, C.R.C. Branco, A. Branco, and J.R.G.S. Almeida, "HPLC-DAD analysis and antioxidant activity of *Hymenaea martiana* Hayne (Fabaceae)". *J. Chem. and Pharm. Res.*, vol. 4, no. 2, pp. 1160 – 1166, 2012.
[29]. L. BuØieova and Z. Reblova, "Czech medicinal plants as possible sources of antioxidants", *Czech J. Food Sci.*, vol. 26, pp. 132 – 138, 2008.
[30]. Y. Kiselova, D. Ivanova, T. Chervenkov, D. Gerova, B. Galunska, and T. Yankova, "Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs". *Phythother. Res.*, vol. 11, pp. 961 – 965, 2006.

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Elekwa, I., Okereke, S.C and Ekpo, B.O (2008). Pre;iminary phytochemical analysis and proximate composition of the roots and leaves of *Rauwolfia vomitoria. Pakistan Journal of Nutrition, 7* (5): 1 – 3.

Elekwa, I., Okereke, S.C and Ekpo, B.O (2009). Preliminary phytochemical and antimicrobial investigation of the stem bark and leaves of *Psidium guajava* L. *Journal of Medicinal Plant Research, 3* (1): 45 – 48.

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