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# Antioxidant enzyme activities and chemopreventive potentials of *Laportea aestuans* on urinary inflammatory markers using albino rats

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**Abstract**
The study evaluated antioxidant potentials of *Laportea aestuans* and chemopreventive potentials on urinary inflammatory markers using albino rats. The rats were fed with 10%, 30% and 50% *Laportea* aestuans. Results indicates a reduction in nitric oxide levels compared to control ($P$<0.05) while concentration of polyphenols in urine were significant with increased percentage of *Laportea aestuans* supplemented diet. This study also showed reductive potencies of *Laportea aestuans* in the concentration of protein in urine. Results also showed an increase in the activities of CyP1A1 microsomal enzymes in the test groups compared to control ($P$<0.05). The activities of Glutathion S- transferase, Quinone oxidoreductase and Thioredoxin reductase increased significantly in all the test samples with increasing percentage of *Laportea aestuans* compared to control. This is indicative of the potential health benefit of *Laportea aestuans* in chemoprevention. On this basis, *Laportea aestuans* have shown to inhibit the inflammatory mechanisms underlying disease activation, that precipitate major clinical manifestations of cancer by affecting altered mechanisms of inflammation and tissue proliferation.

**Keywords:** *Laportea aestuans*, Chemoprevention, antioxidant, inflammatory, urinary

## Introduction
The potential of plants as a veritable source for pharmaceuticals and other therapeutic materials have been emphasized. Plants have formed the basis of traditional system of medicine that have been in existence for thousands of years and continue to provide human kind with medical remedies [1]. Plants have always been among the common sources of medicines, either processed as traditional preparations or used to extract pure active principles. Several studies have shown that plants provide good sources of remedy against several diseases and ailment [2]. The medicinal relevance of these plants lies in some chemical substances that produce a definite physiological action on the human body [3].

Inflammation supports the different phases of cancer development through the inflammatory modulators produced by infiltrating immune cells, resident stromal cells and cancer cells. Epidemiological studies have shown that chronic inflammation predisposes individuals to certain cancers, while anti-inflammatory and anti-oxidant agents may protect against cancer development and metastasis [4]. Major nuclear transcription factors and molecular mediators of inflammation that induce altered cell expression of adhesion molecules, proteases, and growth factors are common factors in the microenvironment leading to disease development and progression of cancer. Therefore, novel target-oriented therapies affecting altered mechanisms of inflammation, angiogenesis and tissue proliferation may similarly inhibit cancer [4]. Carcinogenesis is as a result of genomic injury leading to alterations in gene is the basic cause of all cancers. This damage occurs by abnormal changes in the genetic makeup of healthy cells through mutations [5]. These changes may also occur as a result of hereditary or environmental factors such as chemical carcinogens and ionizing radiation [6].

*Laportea aestuans*, of the family *Urticaceae* is a herbaceous plant which appears as weed in new cultivations and fallows in West India, Africa and Asia. It is used traditionally as antimicrobial, anti-inflammatory, abortifacient, febrifuge, laxative, pain-killer, in pulmonary and stomach troubles amongst others [7]. The leaf is used as an antimicrobial, laxative, in eye treatments and pain-killers [7]. It is also used in treating pulmonary and stomach troubles, diarrhea and dysentery [7].

In view of all the potential health benefit of this plant, the present work is therefore aimed at evaluating the chemopreventive potentials of *Laportea aestuans* on urinary inflammatory markers and its effects on antioxidant enzyme activities so as to ensure a holistic utilization of this plant in the treatment of diseases.

**Materials and Method**:

**Collection of plant samples**:

The plant materials used in this study were fresh leaves of Laportea aestuans harvested from a compound bush located at Okigwe, Imo State Nigeria and were identified by the Plant Science Department, Abia State University Uturu.

**Experimental design**

Twenty four (24) male albino rats weighing between 30-45g were purchased from the animal house Department of Pharmacology, University of Nigeria Nssuka. The rats were grouped into four groups of six (6) rats each. Group I received 100% normal rat chow and served as the control. Group II received 90% normal rat chow and 10% *L. aestuans*. Group III received 70% normal rat chow and 30% *L. aestuans*. Group IV received 50% normal rat chow and 50% *L. aestuans*. All the groups were allowed free access to feed and water *ad libitum* and were exposed to normal 12 hours light and dark cycle under tropical weather conditions.

**Determination of Nitrite and Nitrate levels in Urine**

The Nitrite and nitrate levels in urine were measured by the method described by [8] following the reduction with $VCL_3$.

**CYP1A1 and CYP2B1 Determination**.

Etoxyresorufin and Pentoxyresorufin- O-deethylase (EROD and PROD) activities were monitored by the continous spectroflourimetric procedure as described by [9] while polyphonol and urine metabolites were measured with a HPLC-DAD using a 4.6x 150mm Atlantis colume in a gradient of 1% formic acid (in water) in a flow of 0.8ml/min as outlined by [10].

**Determination of Glutathione-s-transferase**.

This was determined by the method of [10] using cytosolic liver protein supernatants as source of GST and CDNB (1-chloro 2, 4-dinitrobenzol) as a substrate.

**Determination of NAD(P)H: Quinone oxidoreductase (QR).**

This was determined by the method of [11] by measuring the kinetics of NADPH- dependent menadiol-mediated reduction of 3-(4,5-dimethyl -2-thiazyl)-2,5-diphenyl -2H-tetrazolium bromide (MTT).

**Determination of Thioredoxin reductase**

This was done by the method described by [12] as outlined by [13] while glutathione (GSH) was determined using 5,5'-dithiobis (2-nitrobenzoic acid) as described by [14].

**Oxygen Radical Absorbance Capacity (ORAC) determination**

This was determined by the method of [15].

**Serum protein determination**

This was done by the method described by [16].

**Protein Concentration in Urine**

This was done by the method described by [17].

**Quantification of Polyphenol and Metabolites in urine of albino rats**

Urine was filtered with a 0.45µm filter; 1.8ml urine was diluted in 200µl 80% phosphoric acid and mixed. Afterwards, the diluted urine was centrifuged for 10 minutes at 10,000 rpm and 300µl supernatant was added onto a C18 solid phase column and eluted with methanol. The polyphenol metabolites were measured with a HPLC-DAD using a 4.6x150mm Atlantis column in a gradient of 1% formic acid (in water) in acetonitrile in flow of 0.8ml/min. a final volume of 10µl was injected and the polyphenols were detected using the respective absorption maximum.

**Statistical Analysis**

Data collected were subjected to statistical analysis using One Way Analysis of Variance(ANOVA) for a completely randomized block design and Turkey's multiple comparision test using GraphPad Prism (Version 6.0) software were used to analyse data. Values are mean of triplicate determination ± standard deviation and were considered significant when

**Results**

Table 1 shows the effect of *L. aestuans* treatment on urine parameters of albino rats. Results showed that total NO detected ($NO_2^-$ +$NO_3^-$) decreased significantly ($P$<0.05) in all the rats fed 10%, 30% and 50% *L. aestuans* supplemented diet respectively compared to control. Table 2 shows the effect of *L. aestuans* treatment on concentration of polyphenol and metabolites in urine of albino rats. Results indicate that the concentration of polyphenol in the urine of the animals expressed a dose dependent pattern. Caffeic acid, Ferulic acid and Epicatechin were detected in urine samples of animals. Animals feed with 50% *L. aestuans* and 50% feed had the highest concentration of these polyphenols. Table 3 shows the effect of *L. aestuans* treatment on protein concentration and antioxidant capacity in serum of albino rats. Findings indicates a slight significant increase in animals fed 50% *L. aestuans* while the other treatment increased non significantly ($P$>0.05). Table 4 shows the effect of *L. aestuans* treatment on liver microsomal enzyme activities (phase 1 enzymes). Results indicates that there were significant increase in the activities of CYP1A1 and amongst all the test groups compared to control Findings showed an increase in the activity of the enzyme with increase in percentage feed formulation with *L. aestuans*. The cytosolic enzymes (phase 11 enzymes) analysis shows that *L. aestuans* supplemented diet treatments increased hepatic GST activity significantly ($P$<0.05).The hepatic glutathione level increased significantly ($P$<0.05) with increasing percentage composition of feed with *L. aestuans*.

**Table 1:** Effect of *L. aestuans* treatment on urine parameters on albino rats

| Treatment | $NO^{2-}/NO^{3-}$ | Protein (µg/mg creatinine) |
|---|---|---|
| 100% feed (control) | 2.20±0.02 | 3.95±0.05 |
| 90%feed+10% *L. aestuans* | 2.09±0.01*** | 3.95±0.05 |
| 70%feed+30% *L. aestuans* | 1.90±0.01*** | 3.86±0.04* |
| 50% feed +50% *L. aestuans* | 1.80±0.01*** | 3.80±0.03*** |

Mean ± standard deviation, Data normalized to creatinine, *** strongly significant ($P$<0.05)

**Table 2:** Effect of *Laportea aestuans* on polyphenols and metabolite in urine of albino rats

| Treatment | Caffeic acid | Ferulic acid | Epicatechin |
|---|---|---|---|
| 100% feed (control) | 11.78±2.05 | 24.06±2.23 | 145.20±2.95 |
| 90%feed+10%*L aestuans* | 15.50±0.97 | 32.10±1.09*** | 190.80±4.33 |
| 70%feed+30%*L aestuans* | 24.76±1.21*** | 70.66±2.19*** | 529.80±2.49*** |
| 50%feed+50%*L aestuans* | 234.5±4.44*** | 459.30±1.99*** | 2654.00±53.67*** |

Mean ± standard deviation, Data normalized to creatinine, ******* strongly significant ($P$<0.05)

**Table 3:** Effect of *L. aestuans* treatment on protein concentration and antioxidant capacity in serum of albino rats.

| Treatment | Protein(mg/ml) | ORAC (units/µg) |
|---|---|---|
| 100% feed | 35.00±2.92 | 11.70±0.08 |
| 90% feed +10%*L aestuans* | 37.24±1.45 | 11.90±0.05 |
| 70% feed +30%*L aestuans* | 37.64±0.82 | 11.70±0.04 |
| 50% feed +50%*L aestuans* | 38.96±2.66 | 12.10±0.12*** |

Mean ± standard deviation, Data normalized to creatinine, *** strongly significant ($P$<0.05)

**Table 4:** Effect of *L. aestuans* treatment on liver microsomal enzyme of albino rats (phase 1 enzymes)

| Treatment | EROD(CYP1A1) nmol/min/mg | PROD(CYP2B1) nmol/min/mg |
|---|---|---|
| 100% feed(control) | 17.28±0.08 | 8.28±0.08 |
| 90% feed+ 10% *L. aestuans* | 18.80±0.07*** | 7.92±0.11*** |
| 70% feed+30%*L.aestuans* | 21.52±0.08*** | 7.64±0.05*** |
| 50% feed+50%*L.aestuans* | 41.28±0.08*** | 7.48±0.04*** |

Mean± standard deviation ($P$<0.05). Data normalized to protein (mg)

**Table 5:** Effect of *L. aestuans* treatment on liver cytosolic enzyme activities of albino rats (phase 11 enzymes)

| Treatment | GSH(nmol/min/mg) | QR(nmol/min/mg) | TrxR(nmol/min/mg) |
|---|---|---|---|
| 100% feed | 1740±0.90 | 33.7±0.28 | 10.2±0.11 |
| 90% feed | 1745±1.6*** | 35.6±0.39*** | 12.7±0.08*** |
| 70% feed | 1832±1.46*** | 38.1±0.19*** | 15.4±0.11*** |
| 50% feed | 1990±0.86*** | 43.2±0.18*** | 20.8±0.07*** |

Mean± standard deviation ($P$<0.05). Data normalized to protein (mg). GST: Glutathion-S-transferase, QR: Quinone oxidoreductase, TrxR (NADPH): Thioredoxin reductase

**Table 6:** Effect of *L. aestuans* treatment on liver GSH levels of albino rats

| Treatment | GSH(nmol GSH/mg) |
|---|---|
| 100% feed | 99.8±0.11 |
| 90% feed | 105.0±0.17*** |
| 70% feed | 110.0±0.22*** |
| 50% feed | 139.0±0.13*** |

Mean ± standard deviation ($P$<0.05). Data normalized to protein (mg)

## Discussion

Chemoprevention is usually associated with plant-based diets [18]. The bioactive phytochemicals contained in plant foods are the critical components of their potential chemo-preventive actions due to their effects on certain macromolecules and some detoxification enzymes [19]. [7] have reported the phytochemical composition of *Laportea aestuans*. Nitric oxide is produced by several different types of cells including endothelial cells and macrophages. Findings from this study showed significant decrease in the level of $NO^2/NO^3$ with increasing concentration of *Laportea aestuans*. Although early release of nitric oxide is important in maintaining the dilation of blood vessels, increased concentration of nitric oxide by inducible nitric oxide synthase in macrophages can result in oxidative damage. These reductive potentials of *Laportea aestuans* may be attributed to the bioactive constituents of the plant such as quercetin and curcumin [7]. Protein concentration in urine decreased significantly with increased percentage of *Laportea aestuans.* Increase in protein level in urine is associated with nephrotoxicity and is common in diabetes and inflammation hence the reduction in protein concentration in urine with increased percentage of *Laportea aestuans* compared to control is considered as an anti-inflammatory property of *Laportea aestuans*. Results also showed increased level of polyphenols and metabolites such as caffeic acid, ferulic acid and epicatechin in urine with increased percentage of *Laportea aestuans*. This increase in the level of these polyphenols in urine is a chemopreventive mechanism of *Laportea aestuans*. Phytochemicals such as phytosterol, caroteniod, lycopene, terpenes, flavonoid, coumarin, querctin, isoflavonoid etc are known to produce chemopreventive effects on animal models [20, 21]. The antioxidant properties of *Laportea aestuants* have also been reported by [22]. One of the major components of the chemopreventive potentials of plant foods is their antioxidants capacity and their effect on antioxidant enzymes. Protein concentration as well as ORAC value increased with increasing concentration of *Laportea aestuans*. The etiology of numerous diseases such as cancer and cardiovascular diseases lies in the generation of reactive oxygen species (ROS).The result of this study showed increased activities of the antioxidant enzymes with increasing concentration of the plant sample. Metabolic activation is performed by cytochrome P450 (CYP) proteins. Findings from this study revealed significant decrease in both CYP1A1 and CYP2B1 enzyme activities in the test group compared to control. Reduction in the induction of phase 1 enzymes is considered favourable in chemoprevention [23]. The liver microsomal enzymes CYP1A1 and CYP2B1 play critical role in the metabolic activation of carcinogenic compounds leading to toxicity and colorectal cancer [23]. The cytosolic enzymes GSH, QR and TrxR levels increased with increasing percentage of *Laportea aestuans*. Cellular mechanisms of defense against reactive oxygen species rely heavily on GSH which also participate in several other physiological processes [24]. Findings from this study also showed an increase in the of protein concentration and ORAC values with increased percentage of *Laportea aestuans* Hence a novel therapeutic approach using *Laportea aestuans* as a chemopreventive agent can be of great benefit to man in the fight against cancer.

## Conclusion

The study revealed that *Laportea aestuans* exhibits chemopreventive properties. This property is based mainly on the positive effect *Laportea aestuans* exhibits on the

antioxidant and detoxifying system. This could be due to its bioactive components as reported by [7]. Therefore, the use of *Laportea aestuans* is advocated in order to inhibit the activities of cancer causing agent.

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