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Phytochemical, Toxicity, Antimicrobial and antioxidant Screening of Extracts Obtained from *Laportea aestuans* (Gaud)

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Phytochemicals responsible for toxicity, antimicrobial and antioxidant activities of extracts obtained from *Laportea aestuans* (Gaud) were investigated. Secondary metabolites detected were alkaloids, tannins, resins, saponins and carbohydrate. Flavonoids, sterols, cardiac glycosides, phenols, glycosides were however beyond detectable limit. Brine shrimp lethality test on the partition fractions revealed that hexane, ethyl acetate and butanol fractions were toxic with a lethal dose (LC_{50}) less than $1000 \mu\text{mL}^{-1}$ while the crude methanol extract with LC_{50} greater than $1000 \mu\text{mL}^{-1}$ was non-toxic. The antimicrobial assay of the crude extract and their fractions were carried out by agar well diffusion and pour plate methods against 6 bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*) and 4 fungi (*Candida albicans*, *Aspergillus niger*, *Rhizopus stolon*, *Penicillium notatum*). All the extracts had broad spectrum antimicrobial effect at the various concentrations when compared with gentamicin and tioconazole (antibacterial and antifungal standards respectively). The antioxidant activity of *L. aestuans* was determined by three methods; scavenging effect on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), hydroxyl radical scavenging effect and ferric thiocyanate methods and it was revealed the fractions possessed significant antioxidant activity when compared with antioxidant standards butylated hydroxyl anisole (BHA), ascorbic acid and α -tocopherol used in the assay.

Key words: Toxicity, antimicrobial, antioxidant, *Laportea aestuans*, 2, 2-diphenyl-1-picrylhydrazyl radical

INTRODUCTION

The use of medicinal plants for controlling diseases in Africa and particularly in Nigeria could be traced to ancient times and despite the advances made in orthodox medicine, there has been an increasing interest in herbal medicine. The medicinal effects of plants results from the active constituents or secondary metabolites such as alkaloids, mucilage, bitters, glycosides, essential oils, flavonoids etc. Many plants contain minerals, vitamins and even antibiotic substances that can help in building our body's immune system against various harmful diseases. Medicinal plants such as *Aloe vera* have a wide array of medicinal application; for example, gel obtained from the inner part of the leaf is used to treat insect bites, burns, healing wounds and skin rashes. Another important medicinally important tree is Walnut tree as the bark and ground hull of the nut are used in the treatment of skin diseases, intestinal parasites and dysentery (Burkill, 1987; Smith *et al.*, 1996; Cragg *et al.*, 1997; Boye and Ampofo, 1990; Simson and Ogorzaly, 1995; Sofowora, 2008; Iwu, 2008; Andrew, 2009).

The study of antioxidant chemistry is on the increase in recent times because oxidation reactions can produce excess free radicals which can start chain reactions that damage cells thereby causing various pathological conditions like cancer, stroke, Parkinson's disease, atherosclerosis amongst others. Antioxidant agents have the ability to remove excess free radicals and their intermediates thereby inhibiting the free radical chain reactions. Certain polyphenols, thiols, ascorbic acid, vitamin E, superoxide dismutase, glutathione, catalase are few examples of antioxidant agents (Halliwell and Gutteridge, 1984; Bors and Saran, 1991; Sies, 1997; Koleva *et al.*, 2002).

The specie *Laportea aestuans*, a West Indian woodnettle chew belongs to the family of the Urticaceae. It has synonyms like *Fleurya aestuans* (Linn.) Miq and *Fleurya aestuans* Gaud. The plant is a weed and appears in new cultivations and fallows. It is an herbaceous plant, 1.5 m long, often growing on walls and widespread in the African and Asian tropics. It is claimed to have many medicinal uses. The crushed stem is employed as an inflammatory agent. The leaf is used as an abortifacient, laxative, pain-killer, febrifuge, eye treatment, pulmonary and stomach troubles. The phytochemical mucilage from the plant is used as adhesives. The leaf and flower parts of the plant can also be used in medicine to cure diarrhea and dysentery (Elujoba *et al.*, 2005; Smith *et al.*, 1996). The plant though used in traditional medicine extensively has not enjoyed much pharmacological research. A recent research however revealed that the essential oil from the

plant is dominated by methyl salicylate and had significant antioxidant and antimicrobial activities (Oloyede, 2011).

The aims of this research work are to carry out phytochemical, toxicity, antimicrobial and antioxidant screening of extracts obtained from *Laportea aestuans*. The secondary plant metabolites present were determined by chemical methods. Toxicity test was carried out using Brine shrimp lethality test and dimethylsulphoxide (DMSO) was used as standard. The antimicrobial analysis was done using the Agar well diffusion method against ten gram negative and gram positive bacteria and fungi. Gentamicin and tioconazole were used as antibacterial and antifungal standards respectively. Lastly, antioxidant screening involved three tests, scavenging effect on 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), hydroxyl radical generated from hydrogen peroxide and ferric thiocyanate method. Ascorbic acid, butylated hydroxyl anisole and α -tocopherol were used as antioxidant standards.

MATERIALS AND METHODS

Chemicals and reagents: Hexane, ethyl acetate, methanol, butanol, chloroform, hydrochloric acid, ammonia solution, naphthol, bismuth nitrate, potassium iodide, sodium hydroxide, copper acetate, NaOH, sodium chloride, copper sulphate pentahydrate, ferric chloride, conc tetraoxosulphate (VI) acid, conc HCl, ammonia solution, sodium potassium tartarate, linoleic acid, ammonium thiocyanate, ethanol, ferrous chloride, hydrochloric acid, potassium chloride, glacial acetic acid, disodium hydrogen phosphate and dihydrogen potassium phosphate were all BDH general purpose chemicals and distilled prior to use. Dimethylsulphoxide (M and B, England), hydrogen peroxide (Merck, Germany) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, butylatedhydroxylanisole (BHA) and α -tocopherol were obtained from Sigma Chemical Co. (St. Louis, MO). Brine shrimp larvae eggs were obtained from Ocean Star International, Inc. Company, USA.

Equipment and apparatus: Soxhlet apparatus, Mettler analytical balance H80 (UK), Water Bath (Gallenkamp), Rotavapor RII0 (Buchi, England), silica gel GF₂₅₄ (precoated aluminium sheets-Merck Germany), pH meter (Jenway model), UV-Visible spectrophotometer (UVD-2960 model equipped with a UVWIN software version LABOMED INC, USA).

Plant collection and identification: Whole plant of *Laportea aestuans* was identified by Dr. L.S. Adebisi, Head of the Department of Forestry and Wildlife of the

Faculty of Agriculture University of Ibadan and were collected at Botanical Gardens, University of Ibadan, Oyo State in June, 2010.

Test organisms: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiellae pneumoniae*, *Salmonellae typhi*, *Candida albicans*, *Rhizopus stolon*, *Aspergillus niger* and *Penicillium notatum* (Microorganisms were collected from the stock of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy of University of Ibadan). The test organisms were maintained on nutrient agar slopes and kept in a refrigerator at 4°C. Nutrient broth (100 mL aliquots) were inoculated with the culture of test micro-organisms using a loop and then incubated at 37°C for 24 h.

Reference standards: Dimethylsulphoxide (DMSO) was used for toxicity study. Gentamicin at 10 mg mL⁻¹ and tioconazole (70%) were for bacteria and fungi respectively (both for antimicrobial activity); ascorbic acid, butylated hydroxyanisole (BHA) and α -tocopherol were used for antioxidant activity.

Sample preparation: Whole plant of *L. aestuans* was collected, weighed and air-dried for 3 weeks until the weight was constant and then pulverized using mill machine. The pulverized samples were weighed and kept for further analysis.

Extraction/partitioning procedure: The dried plant material (1 kg) was extracted with 2.5 lt of methanol using soxhlet apparatus. The extracts were collected and concentrated with the aid of a Buchi rotavapor and stored in a desiccator prior to further analysis. Thin Layer Chromatography (TLC) was employed using silica gel 60 F₂₅₄ precoated plates and solvent system: Ethyl acetate/methanol (8:2) to detect antioxidant activity by using 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) as a spray reagent. Yellow coloration on the spots on the TLC plates indicates that the methanol extract of *L. aestuans* has antioxidant activity. The crude methanol extract was partitioned in hexane, ethylacetate and butanol. Thereafter, toxicity test using Brine shrimp lethality assay, antimicrobial screening by agar well diffusion method and free radical scavenging activity test were carried out on the fractions using the following spectrophotometric experiments; scavenging effect on DPPH, scavenging effect on hydroxyl radical generated from hydrogen peroxide and peroxide oxidation by ferric thiocyanate method.

Phytochemical screening: The crude methanol extract obtained was used to test for the presence of the following plant secondary metabolites; alkaloids, flavonoids, steroids, saponins, phenols, tannins, glycosides, reducing sugars, anthraquinones, carbohydrates, resin and cardiac glycosides (Harborne, 1998).

Toxicity analysis

Brine shrimp lethality test: The brine shrimp lethality test (BST) was used to predict the toxicity of the fractions (Meyer *et al.*, 1982). The shrimp's eggs were hatched in sea water for 48 h at room temperature. The nauplii (harvested shrimps) were attracted to one side of the vials with a light source. Solutions of the extracts were made in DMSO, at varying concentrations (1000, 100 and 10 μ g mL⁻¹) and incubated in triplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the triplicate vials. Control brine shrimp larvae were placed in a mixture of sea water and DMSO only. After 24 h the vials were examined against a lighted background and the average number of larvae that survived in each vial was determined. The concentration at fifty percent mortality of the larvae (LC₅₀) was determined using the Finney computer programme (Faloje *et al.*, 1993; Oloyede *et al.*, 2010a).

Antimicrobial screening methods

Preparation of samples for antimicrobial analysis: Four samples were used for this study: crude methanol extract, n-hexane, ethyl acetate and butanol fractions of the whole plant. One gram of each sample was weighed and dissolved in 5 mL of the solvent used for the extraction to give 200 mg mL⁻¹. This was serially diluted until a concentration of 6.25 mg mL⁻¹ of the content was obtained in the sixth test tube. The seventh test tube contained the solvent of dissolution only (negative control). The eighth test tube served as the positive control and contained gentamicin for bacteria and tioconazole for fungi.

Agar diffusion: Pour plate method for bacterial: An overnight culture of each of the following organism: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiellae pneumoniae* and *Salmonellae typhi* was prepared and 0.1 mL of each of the organism was taken into 9.9 mL of Sterile Distilled Water (SDW) to give 10 mL of 1: 100 (10²) dilution. Again, 0.2 mL was taken into the prepared molten Nutrient Agar (NA) at 45°C and was aseptically poured into the sterile plates and allowed to set on the bench for

45 min. The stock was maintained on nutrient agar slant and sub-cultured in nutrient broth for incubation at 37°C prior to each antimicrobial testing. Inoculation of the test organisms on nutrient agar-prepared plates was achieved by flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms. The discs were prepared using a Grade No. 1 Whatman filter paper. A total of 100 discs were obtained by punching and putting in vials-bottles and sterilizing in an oven at 150°C for 15 min. Thereafter the cups (9 mm diameter) were aseptically bored into the solid nutrient agar using a sterile cork borer. A sterile cork-borer was used to create wells (or holes) inside the set plates. The test solutions of oils (50 µL) at concentration of 40 mg mL⁻¹ were then introduced into each of the designated cups on each plate ensuring that no spillage occurred. The same amount of the standard antimicrobial agent and solvents were introduced using syringes into the remaining cups on each plate to act as positive and negative controls respectively. The plates were left at room temperature for 2 h, allowed to diffuse into the medium, turned upside-down and thereafter incubated at 37°C for 24 h in an incubator. Clear zones of inhibition were observed. Activity or inactivity of each extract was tested in triplicate and the diameters of zones of inhibition were measured in millimeter (mm) using a transparent well-calibrated ruler. The positive control for bacteria was gentamicin at the concentration of 10 mg mL⁻¹. The analysis was done in triplicates and the average readings were calculated (Cushnie and Lamb, 2005; Larshini *et al.*, 1996; Duraipandian *et al.*, 2006).

Agar diffusion: pour plate method for fungi: Molten sterile Sabouraud Dextrose Agar (SDA) was poured aseptically into the sterile plates and allowed to cool down for 45 min. Then 0.2 mL of 1:100 dilution of the organisms *Candida albicans*, *Rhizopus stolon*, *Aspergillus niger* and *Penicillium notatum* were spread on the surface using a sterile spreader. After which, a sterile cork-borer was used to create wells inside the plates. The same procedure described for anti-bacterial activity above was followed from this stage. The positive control for the fungi was 70% tioconazole. All the plates for the fungi were incubated at 28°C for 48 h unlike that of bacteria that was incubated at 37°C for 24 h. The clear zones of inhibition were observed and recorded using the same method as described in the case of bacteria (Hadecek and Greger, 2000; Bauer *et al.*, 1966).

Antioxidant activities of *L. aestuans* extracts

Scavenging effect on DPPH: The DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) free-radical scavenging method was

used to determine the antioxidant activity or the capacity to scavenge the “stable” free radical DPPH. A 3.94 mg of DPPH, a stable radical was dissolved in methanol (100 mL) to give a 100 µm solution. To 3.0 mL of the methanol solutions of DPPH was added 0.5 mL of each of the fractions with doses ranging from 1.0 to 0.0625 mg mL⁻¹ (Gulcin *et al.*, 2002; Mutee *et al.*, 2010; Oloyede *et al.*, 2010a). The decrease in absorption at 517 nm of DPPH was measured 10 min later. The actual decrease in absorption was measured against that of the control and the percentage inhibition was also calculated. The same experiment was carried out on butylated hydroxyanisole (BHA), α-tocopherol and ascorbic acid which are known antioxidants. All analysis was run in triplicates and the results obtained were averaged. The Radical Scavenging Activity (RSA) was calculated as the percentage inhibition of DPPH discoloration using the equation below:

$$\text{RSA\% or \% inhibition} = \{(A_{\text{DPPH}} - A_s)/A_{\text{DPPH}}\} \times 100$$

where, A_s is the absorbance of the solution and A_{DPPH} is the absorbance of the DPPH solution (Hatano *et al.*, 1988).

Scavenging effect on hydrogen peroxide:

Spectrophotometric determination of *L. aestuans* fractions was carried out at 285 nm. A solution of 2 mM hydrogen peroxide was prepared in Phosphate Buffered-Saline (PBS) pH 7.4. The fractions at the following concentrations; 0.1-0.00625 mg mL⁻¹ was added to the H₂O₂ solution. Decrease in absorbance of H₂O₂ at 285 nm was determined spectrophotometrically 10 min later against a blank solution containing the extract in PBS without H₂O₂. All tests were run in triplicates and averaged (Soare *et al.*, 1997; Oloyede and Farombi, 2010). The same experiment was carried out on Butylatedhydroxyanisole (BHA), ascorbic acid and α-tocopherol which are known antioxidant standards.

Antioxidant activity by ferric thiocyanate method: The antioxidant activities of methanol, hexane, ethyl acetate and butanol fractions of the plant were determined by ferric thiocyanate method (Mackie and McCartney, 1989). The extract (10 mg each) was dissolved separately in 99.5% of ethanol and various concentrations (0.00625-0.8 µg mL⁻¹) were prepared. A mixture of 2 mL of sample in 99.5% ethanol, 2.0 mL of 2.51% linoleic acid in 99.5% ethanol, 4 mL of 0.05 M phosphate buffer (pH 7.0) and 2 mL of water was placed in a vial with a screw cap and placed in an oven at 60°C in the dark. To 0.1 mL of this sample solution, 10 mL of 75% ethanol and 0.1 mL of

30% ammonium thiocyanate was added. After the addition of 0.1 mL of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red colour developed was measured in 3 min at 500 nm. The control and standards were subjected to the same procedures as the sample, except that for the control, only solvent was added and for the standard, sample was replaced with the same amount of butylatedhydroxyanisole (BHA), ascorbic acid and α -tocopherol (Oloyede *et al.*, 2010b). The inhibition of lipid peroxidation in percentage was calculated using this equation:

$$\text{Inhibition (\%)} = 1 - \frac{A_1}{A_2} \times 100$$

where, A_1 was the absorbance of the test sample and A_2 was the absorbance of control reaction.

RESULTS AND DISCUSSION

The crude methanol extract of *Laportea aestuans* contained alkaloids, tannins, resins, saponins and carbohydrates. Flavonoids, sterols, cardiac glycosides, phenols, glycosides were however absent. The presence of these secondary metabolites especially alkaloids justified the use of *L. aestuans* in ethnomedicine.

Brine shrimp lethality test: The toxicity result of the extracts obtained from *L. aestuans* showed that the

fractions (hexane, ethylacetate and butanol) were toxic to brine shrimp larvae at varied degree while the crude methanol extract was not toxic having an LC_{50} (Lethal concentration) value greater than $1000 \mu\text{g mL}^{-1}$. Toxicity level as determined by Finney computer programme gave the following lethal concentration. Methanol fraction, $LC_{50} = 1330.8660 \mu\text{g mL}^{-1}$, hexane fraction, $LC_{50} = 0.0002 \mu\text{g mL}^{-1}$, ethyl acetate fraction, $LC_{50} = 57.2426 \mu\text{g mL}^{-1}$ and butanol fraction, $LC_{50} = 117.820 \mu\text{g mL}^{-1}$. The result corroborated the presence in the plant of medicinally active compounds. Toxic chemical compounds are beneficial in the therapy of some ailments involving cell or tumour growth but their usage at high dose should be properly monitored. It has also been observed by previous workers that medicinally active natural products are most times toxic to *Artemia salina* nauplii. The hexane fraction of the plant *L. aestuans* was the most toxic ($LC_{50} = 0.0002 \mu\text{g mL}^{-1}$) while the butanol fraction was the least toxic ($LC_{50} = 117.8520 \mu\text{g mL}^{-1}$).

Antimicrobial screening: *L. aestuans* whole plant extracts were screened at various concentrations for antimicrobial activity using the Agar well diffusion method. The zones of inhibition (mm) were measured in triplicate and the average results obtained is shown in Table 1-4. It was observed that all the tested samples possessed broad spectrum antimicrobial activities on both gram positive and gram negative bacteria and the fungi used.

Table 1: Antimicrobial activity of crude methanol extract of *L. aestuans*

Doses (mg mL ⁻¹)	Zones of inhibition (mm)									
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiellae pneumoniae</i>	<i>Salmonellae typhi</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>	<i>Rhizopus stolon</i>	<i>Penicillium notatum</i>
200	14	12	16	10	12	14	18	16	18	20
100	12	10	14	-	10	12	16	14	16	16
50	10	-	12	-	-	10	14	10	12	14
25	-	-	10	-	-	-	12	-	10	12
12.5	-	-	-	-	-	-	10	-	-	12
6.25	-	-	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	36	34	36	38	36	34	26	24	26

-ve: Negative control (methanol), +ve: Positive control {Gentamicin at 10 mg mL^{-1} for bacteria or Tioconazole (70%) for fungi} -: No inhibition

Table 2: Antimicrobial activity of n-hexane fraction of *L. aestuans*

Doses (mg mL ⁻¹)	Zones of inhibition (mm)									
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiellae pneumoniae</i>	<i>Salmonellae typhi</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>	<i>Rhizopus stolon</i>	<i>Penicillium notatum</i>
200	12	12	10	16	12	14	16	16	16	14
100	10	10	-	-	14	10	12	12	14	12
50	-	-	-	-	10	-	10	10	12	10
25	-	-	-	-	-	-	-	-	10	-
12.5	-	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	36	34	36	38	36	34	26	24	26

-ve: Negative control (hexane), +ve: Positive control {Gentamicin at 10 mg mL^{-1} for bacteria or Tioconazole (70%) for fungi} -: No inhibition

Table 3: Antimicrobial activity of ethyl acetate fraction of *L. aestuans*

Doses (mg mL ⁻¹)	Zones of inhibition (mm)									
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiellae pneumoniae</i>	<i>Salmonellae typhi</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>	<i>Rhizopus stolon</i>	<i>Penicillium notatum</i>
200	14	14	14	16	12	14	16	18	18	18
100	12	12	12	14	10	12	14	14	16	16
50	10	10	10	12	-	10	12	12	12	14
25	-	-	-	10	-	-	10	10	10	12
12.5	-	-	-	-	-	-	-	-	-	10
6.25	-	-	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	36	34	36	38	36	34	26	24	26

-ve: Negative control (ethyl acetate), +ve: Positive control {Gentamicin at 10 mg mL⁻¹ for bacteria or Tioconazole (70%) for fungi} -:No inhibition

Table 4: Antimicrobial activity of butanol fraction of *L. aestuans*

Doses (mg mL ⁻¹)	Zones of inhibition (mm)									
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiellae pneumoniae</i>	<i>Salmonellae typhi</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>	<i>Rhizopus stolon</i>	<i>Penicillium notatum</i>
200	10	10	18	14	14	12	14	14	16	12
100	-	-	16	12	12	10	12	12	14	10
50	-	-	14	10	10	-	10	10	12	-
25	-	-	10	-	-	-	-	-	10	-
12.5	-	-	-	-	-	-	-	-	-	-
6.25	-	-	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	36	34	36	38	36	34	26	24	26

-ve: Negative control (butanol), +ve: Positive control {Gentamicin at 10 mg mL⁻¹ for bacteria or Tioconazole (70%) for fungi}, -: No inhibition

Table 5: Absorbance values from scavenging effect of extracts from *Laportea aestuans* on DPPH (nm)*

Conc. (mg mL ⁻¹)	F1	F2	F3	F4	Ascorbic acid	BHA	α -tocopherol
1.0	0.128±0.011	0.090±0.003	0.181±0.003	0.132±0.012	0.217±0.001	0.053±0.001	0.205±0.001
0.5	0.104±0.006	0.083±0.003	0.076±0.0006	0.106±0.005	0.246±0.001	0.025±0.001	0.298±0.001
0.25	0.085±0.003	0.078±0.003	0.080±0.002	0.087±0.003	0.368±0.005	0.094±0.001	0.930±0.000
0.125	0.080±0.003	0.072±0.003	0.071±0.003	0.080±0.003	0.091±0.002	0.018±0.001	0.840±0.000
0.0625	0.084±0.003	0.068±0.003	0.124±0.031	0.078±0.003	0.068±0.0006	0.034±0.002	0.734±0.001

*Absorbance measurement of F1: Hexane fraction, F2: Ethyl acetate fraction, F3: Butanol fraction, F4: Crude methanol extract, Ascorbic Acid, BHA and α -tocopherol at 517 nm

It was observed that the higher the concentration, the better the activity of the extracts, the positive controls (gentamicin at 10 mg mL⁻¹ for bacteria and tioconazole (70%) for fungi) were used as the standard to compare the effect or activity of the extracts on the micro-organisms used. The methanol extract was active on *B. subtilis*, *C. albicans*, *R. stolon* and *P. notatum* at 25-200 mg mL⁻¹ which indicated a selective inhibition on fungi (Table 1). The n-hexane fraction (Table 2) inhibits *K. pneumoniae*, *C. albicans* and *R. stolon* at 50-200 mg mL⁻¹ but showed mild activity on the other microorganisms. The ethyl acetate fraction showed a broad spectrum activity on the entire microorganism at 50-200 mg mL⁻¹ but inhibits the fungi *P. notatum* better (Table 3). Table 4 showed the activity of the butanol fraction on tested microbes. It showed moderate activity for all except for *S. aureus* and *E. coli*. Overall, it was observed that the crude methanol extract and ethyl acetate fraction of whole plant of *L. aestuans* possessed broad spectrum antimicrobial activity on bacteria and the fungi tested at average concentration of 50-200 mg mL⁻¹.

Antioxidant activity: The antioxidant activities of the methanol, hexane, ethyl acetate and butanol extracts of *L. aestuans* were determined by three methods: scavenging effect on 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), hydroxyl radical generated from hydrogen peroxide and ferric thiocyanate (FTC) method. The results are presented in Table 5-7.

Scavenging effects on DPPH: DPPH is known to be a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soare *et al.*, 1997). The reduction in absorbance of DPPH at 517 nm caused by the samples was measured in triplicate after 10 min. At 517 nm, the absorbance of the DPPH solution was 0.933 nm. The tested samples showed good activity when compared to the standard used; ascorbic acid, butylatedhydroxylanisole (BHA) and α -tocopherol (Table 5). There was decrease in absorption at 517 nm indicating that the fractions had hydrogen donating ability or can scavenge free radical. The fractions gave %inhibition of 80-92% at

Table 6: Absorbance values from scavenging effect of extracts from *Laportea aestuans* on H₂O₂ (nm)*

Conc. (mg mL ⁻¹)	F1	F2	F3	F4	Ascorbic acid	BHA	α -tocopherol
0.1	0.497±0.000	0.245±0.001	0.076±0.000	0.225±0.000	0.1952±0.001	0.0413±0.016	0.0321±0.045
0.05	0.226±0.000	0.171±0.000	0.067±0.002	0.234±0.003	0.2078±0.012	0.0617±0.019	0.0633±0.032
0.025	0.190±0.000	0.127±0.000	0.064±0.004	0.096±0.000	1.2645±0.119	0.0740±0.015	0.1552±0.061
0.0125	0.097±0.000	0.096±0.000	0.046±0.013	0.093±0.000	2.7586±0.049	0.0947±0.003	0.1807±0.015
0.00625	0.102±0.000	0.118±0.000	0.070±0.027	0.079±0.000	2.9236±0.211	0.1126±0.014	0.4940±0.017

*Absorbance measurement of F1: Hexane fraction, F2: Ethyl acetate fraction, F3: Butanol fraction, F4: Crude methanol extract, Ascorbic Acid, BHA and α -tocopherol at 285 nm

Table 7: Peroxide oxidation of *Laportea aestuans* Extracts at 500 nm using the Ferric thiocyanate method*

Conc. (mg mL ⁻¹)	F1	F2	F3	F4	Ascorbic acid	BHA	α -tocopherol
0.8	0.049±0.034	0.329±0.014	0.035±0.001	0.746±0.003	0.173±0.008	0.326±0.006	0.133±0.004
0.4	0.061±0.003	0.362±0.064	0.037±0.001	0.742±0.001	0.173±0.008	0.375±0.008	0.164±0.006
0.2	0.064±0.048	0.394±0.119	0.042±0.003	0.697±0.101	0.245±0.008	0.431±0.008	0.184±0.009
0.1	0.066±0.002	0.407±0.044	0.045±0.001	0.684±0.007	0.275±0.006	0.616±0.005	0.195±0.023
0.05	0.085±0.000	0.426±0.022	0.046±0.002	0.679±0.037	0.287±0.050	0.647±0.004	0.294±0.004
0.025	0.091±0.005	0.494±0.009	0.056±0.003	0.667±0.065	0.367±0.004	0.653±0.008	0.340±0.069
0.0125	0.093±0.002	0.564±0.030	0.057±0.002	0.651±0.154	0.516±0.008	0.747±0.003	0.360±0.005
0.00625	0.097±0.009	0.739±0.003	0.063±0.001	0.545±0.126	0.668±0.002	0.750±0.001	0.377±0.008

*Absorbance measurement of F1: Hexane fraction, F2: Ethyl acetate fraction, F3: Butanol fraction, F4: Crude methanol extract, Ascorbic acid, BHA and α -tocopherol at 500 nm

1.0-0.0625 mg mL⁻¹. The activity was however lower than that of BHA but better than that of ascorbic acid and α -tocopherol. The ethylacetate fraction had better activity than the other fractions at all concentrations. The presence of secondary plant metabolites in plants has been found to be responsible for antioxidant activity (Yen and Duh, 1994; Miller, 1996).

Scavenging effects on Hydrogen peroxide (H₂O₂):

Scavenging effects on H₂O₂ was measured in triplicates after 10 min of incubation at 285 nm. It has been observed that H₂O₂ through the Fenton reaction is an active-oxygen specie and has the potential to produce the highly reactive hydroxyl radical which is often involved in free radical chain reactions (Namiki, 1990; Lugasi *et al.*, 1999). Table 6 shows the absorbance measurement of fractions and standards.

Hydroxyl radical scavenging ability of the fractions from *L. aestuans* as seen in Table 6 at 0.1-0.0065 mg mL⁻¹ showed that the fractions had high scavenging activities when compared to standards. The % inhibition was between 86-98% at all the concentrations used. Activity was found to be better especially with the butanol fraction. This is to be expected since butanol extracts contain highly polar constituents. *L. aestuans* scavenged the highly reactive hydroxyl radicals therefore is a source of antioxidant compounds.

Antioxidant activity by ferric thiocyanate method: The FTC method was used to determine the amount of peroxide which oxidized ferrous chloride (FeCl₂) to a reddish ferric chloride (FeCl₃) pigment. In this method, the concentration of peroxide decreases as the antioxidant activity increases. Hexane, ethyl acetate, butanol and

crude methanol extract at various concentration (0.00625- 0.8 mg mL⁻¹), showed antioxidant activities in a concentration dependent manner. However, butanol extracts at all the concentration, showed antioxidant activity (91-96%) better than the activities of all the reference compounds, ascorbic acid, BHA and α -tocopherol. It has been observed that the extract exhibited strong activity with the increase in polarity (with reference to organic solvent), indicating that highly polar organic compounds may play important roles in the activities (Wolf, 2005).

CONCLUSION

Alkaloids, tannins, resins, saponins and carbohydrates were the major secondary plant metabolites found in *L. aestuans*. Toxicity level as determined by Brine shrimp lethality test showed that the hexane fraction of the plant *L. aestuans* was the most toxic (LC₅₀ = 0.0002 μ g mL⁻¹) while the butanol fraction was the least toxic (LC₅₀ = 117.8520 μ g mL⁻¹), ethyl acetate fraction was moderately toxic (LC₅₀ = 57.2426 μ g mL⁻¹). The result corroborated the presence in the plant of medicinally active compounds. *L. aestuans* whole plant was also found to possess broad spectrum antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiellae pneumoniae*, *Salmonellae typhi*, *Candida albicans*, *Rhizopus stolon*, *Aspergillus niger* and *Penicillium notatum*. The activity was however selective on the fungi strains used in this study. This result supported its local use as an antifungal and/or as an antibiotics because of its toxicity to bacteria and fungi. The high antioxidant activity of the plant at low concentration especially the

butanol fraction shows that it could be very useful for the treatment of ailments resulting from oxidative stress such as Parkinson's disease, Alzheimer's disease, cancer, cardiovascular disorders, bacterial and viral infections, inflammation, coronary heart disease and stroke.

RECOMMENDATION

This current study revealed the medicinal importance of the plant *Laportea aestuans* especially as an antimicrobial and antioxidant agent. Further work therefore needs to be carried out on the plant in order to determine the active chemical constituents responsible for the observed activities.

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