Enzyme inhibition, free radical scavenging and insecticidal activities of crude extracts and isolates from *Laportea aestuans* (Gaud)

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Abstract: *Laportea aestuans* (Gaud) belonging to the family Urticaceae is an herbaceous weed consumed as vegetable and used in traditional medicine. In continuation of our work on this plant, bioactive constituents were isolated using chromatographic technique and the structures of all isolated constituents were determined by spectroscopic techniques and comparison with literature data. Enzyme inhibition (urease and α-glucosidase inhibition activities), free radical scavenging and insecticidal activities of the crude extracts and isolates were also determined. Isolation and characterization afforded six known constituents among which are 5, 2', 4' trihydroxy 7, 8 dimethoxy flavones (LA4) (29.8± 0.15) and 15-hydroxycryptopleurine (LA6) (18.5± 0.54) that showed significant urease inhibition activity, 4-methoxy-8'-acetyl olivil (LA1) (14.9± 0.43) and 5, 2', 4' trihydroxy 7, 8 dimethoxy flavones (LA4) (14.6± 0.12) which had significant α-glucosidase inhibition activity. 5, 2', 4' trihydroxy 7, 8 dimethoxy flavones (LA4) (15.45±0.01), 3-(4-hydroxyphenyl)-4-(3-methoxy-4-hydroxyphenyl)-3,4-dehydroquinolizidine (LA5) (20.27±0.00) and 15-hydroxycryptopleurine (LA6) (28.81±0.03) were the most active in the free radical scavenging assay when activities were compared with thiourea, 1-deoxynojiromycin hydrochloride (DNJ) and butylated hydroxylanisole used as standards in the three assays. This study therefore added more information to the ethnomedicinal importance of *L. aestuans* used in traditional medicine.
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1. Introduction

Enzyme inhibition studies target human enzyme and aim to correct pathological condition. Urease and α-glucosidase inhibitors are effective against several serious infections like gastric tract syndromes and urinary tract infection. Enzyme inhibitors interact with enzymes and block their activity towards natural substrates. Blocking an enzyme's activity can correct any imbalance in metabolic activity or kill a pathogen; therefore enzyme inhibitors are used in disease treatment as drugs. There are also enzyme activators which increase enzymatic activity and enzyme substrates which bind and are converted to products in the normal catalytic cycle of the enzyme. These processes are useful in a variety of physiological conditions [1-4].

Free radicals play key role in the aging process in humans, in cardiovascular diseases, brain dysfunction, cancer, and cataracts. Free radicals are reactive oxygen containing molecules due to the presence of unpaired electrons, leading to a chain reaction. Antioxidants have the ability to completely neutralize the free radicals thereby stopping the chain reactions [5-6]. Interest in the discovery of enzyme inhibitors and antioxidants from plants is still a major concern to scientists since many plants have been reported to possess these activities.

L. aestuans is an herbaceous weed which grows in shaded area in the forest, rock crevices and farmlands. It belongs to the Urticaceae (Nettle) family and is widely distributed in Central America, West Africa and tropical Asia [7-8]. The leaves are used as vegetable, soup and as food. The stem yields fibre used for rope, thread and strings. The whole plant has been reported to have antacid, anthelminthic, and used in the treatment of oedema, migraine, gonorrhoea, cough, dysentery, urinary tract infection, asthma and fever [6, 9-14]. The whole plant of *L. aestuans* has erect and bristly glandular hairs whose tips come off when touched, transforming the hair into a needle that injects a stinging liquid, that contain acetylcholine, formic acid, 5- hydroxytrotamine, and histamine which is common to the Nettle family [15-16]. The brine shrimp lethality test, antimicrobial and antioxidant activities of *L. aestuans* crude extracts and therapeutic effect on oxidative stress have been

reported [11]. Phytochemicals such as choline, methyl esters of aliphatic acids and guanidino amino compound, (4*E*)-3,6-dimethylhep-4-en-3-ol and chrysen-2-ol derivative, methyl salicylate, fenchol, 1,2-cyclohexanedione dioxime, 1,4-octadiene and linalool have been reported in the plant [6, 9,11,15]. Phytochemical investigation of the leaves of *Cecropia schreberiana* Miq., *Urtica parviflora* Roxb., *Urtica dioica* L. and *Urtica urens* also from Urticaceae family led to the isolation of triterpenoids, flavone C-glycosides, flavan-3-ols, lignans, flavanolignans and proanthocyanidins [17,18]. Hence, this study aimed at determining the enzyme and antioxidant inhibition potentials of *Laportea aestuans* (Gaud). Insecticidal activity of the plant using maize weevil (*Sitophilus zeamais*) was also determined at lethal doses 50% and 100% concentration since the plant was reported to possess this activity.

2. Materials and Method

2.1 Plant Material, Chemical and Reagents

Methanol extract of leaves of *L. aestuans* as previously described in Oloyede and Oyelola [15], Oloyede and Ayanbadejo, [11] was used. Chemicals used were of analytical grade. Sodium nitroprusside, urease (Jack bean), α-glucosidase (*Saccharomyces cerevisiae*), and thiourea, 1-Deoxynojiromycin hydrochloride, di-nitro salicylic acid (DNS) were purchased from Sigma-Aldrich, Germany. P-nitro-phenyl-α-D-glucopyranoside (p-NPG), sodium carbonate (Na_2CO_3), sodium dihydrogen phosphate and di-sodium hydrogen phosphate were purchased from Merck, Germany. Ultra-pure water (HPLC grade, Duksan, Korea) was used throughout the experiments. Potassium phosphate buffer (100 mM), pH=7.4, was prepared in distilled water. The absorbance spectra of the solutions were obtained employing Synergy H1 Hybrid multi-mode microplate reader. The following BDH chemicals and reagents were also used: chloroform, dichloromethane, ethyl acetate, n-hexane, methanol, hydrochloric acid, ammonia solution, conc. tetraoxosulphate (VI) acid, conc. hydrochloric acid, ammonia solution, sodium potassium tartarate, potassium chloride, glacial acetic acid, were used. General purpose chemicals obtained were distilled prior to use. Dimethylsulphoxide (M & B, England), and silica gel 30 - 260 microns (Merck, Germany) and 2,2-diphenyl-1-picrylhydrazyl (DPPH), and butylatedhydroxylanisole (BHA) were obtained from Sigma Chemical Co (St Louis, MO).

2.2 Isolation of compounds from ethyl acetate fraction of Leaves of *Larpotea aestuans*.

Ethyl acetate fraction of *L. aestuans* leaves (125.0 g) obtained from the crude methanol extract was subjected to column chromatography using silica gel (60-200 mm mesh size) in the ratio 1:30 in a gradient elution technique. A total of 655 fractions (10 ml each) were collected and pooled together based on the retardation factor (R_f) from Thin Layer Chromatography (TLC). Hexane 100% (1-20), hexane: ethyl acetate 95:5 (21-40), 90:10 (41-68), 85:15 (69-90), 80:20 (91-119), 75:25 (120-133), 70:30 (134-159), 65:35 (160-185), 60:40 (186-206), 55:45 (207-235), 50:50 (236-251), 45:55 (252-273), 40:60 (274-303), 35:65 (304-333), 30:70 (334-364), 25:75 (365-389), 20:80 (390-430), 15:85 (431-468), 10:90 (469-493), ethyl acetate 100% (494-539), ethyl acetate: methanol 95:5 (540- 551), 90:10 (552-557), 85:15 (558-563), 80:20 (564-569), 75:25 (570-575), 70:30 (576-581), 65:35 (582-595), 60:40 (596-604), 55:45 (605-610), 50:50 (611-615), 45:55 (616-621), 40:60 (622-627), 35:65 (628-633), 30:70 (634-639), 25:75 (640-645), 20:80 (646-650), 15:85 (651-655), 10:90 (656-660), and methanol 100% (661-665). LA1 was obtained as white crystalline solid from 15-35% EtOAc, fractions at 40-65% ethyl acetate in hexane was labelled LA2 (white solid). LA3 was obtained from 70-90% methanol in ethyl acetate. LA4 was obtained from 100% EtOAc. LA5 was obtained from 25-40% methanol in ethyl acetate, washed with methanol and recrystallized with DCM. Fractions from 45 -50% methanol afforded LA6, which was recrystallized in ethanol.

2.3 General Experimental Procedures and Analysis

Buchi Rotary Evaporator fitted with Vacuum pump V-700 and B-490 heating bath was used to concentrate samples and fractions. Thin layer chromatographic analysis of the samples was carried out at room temperature using Precoated TLC Aluminium sheets Silica gel 60 F_{254} (20 cm × 20 cm, 0.2 mm thick; Merck, Germany), and Alugram Sil G/UV_{254} for preparative chromatography (Macherey-Nagel MN, Germany) and microscope slides/plates (7.5 × 2.5 cm; Smethwick 40, Birmingham, England. Silica gel (230–400 mesh) was used for column chromatography (CC). The retention factor (Rf) for the polar development system was calculated. Visualization of the TLC plates was carried out under UV at 254 and 366 nm and by spraying with ceric sulfate reagent solution under heating or vanillin sulphuric acid. The compounds were further characterized by spectroscopic analysis, UV-Visible and Infra-red. Ultra violet (UV)/Visible absorption spectra of 0.01% w/v of the samples were obtained in methanol on Evoltion 300 Thermo Scientific UV – visible spectrophotometer. The Infra red (IR) spectra were recorded in KBr disc on FT-IR-8900 Fourier Transform IR Spectrophotometer Shimadzu IR spectrometer in the range 4000-400 cm^{-1}. Nuclear magnetic Resonance (NMR) ^1H recorded on AVANCE AV-400 spectrometer operating at 400 MHz for ^1H. The chemical shift values (δ) are

reported in ppm. Data from the spectrometry and comparison with literature confirmed the structures of the compounds.

2.4 Enzyme Inhibition Assays

2.4.1 Determination of urease activity

The assay solution was composed of urea (850 µL), the sample (0 -100 µL), phosphate buffer (100 mM, pH 7.4) and made up to volume of 985 µL. The mixture was incubated at 30 ^{o}C. The ammonia concentration in the enzymatic reaction was determined after 60 minutes at 37 °C when 15 µL of urease enzyme, 500 µL of solution A (0.5 g phenol and 2.5 mg of sodium nitroprusside in 50 mL of distilled water) and 500 µL of solution B (250 mg sodium hydroxide and 820 µL of sodium hypochlorite (5%) in 50 mL of distilled water) were added together. Activity of uninhibited urease was used as control activity of 100%. Modified Berthelot spectrophotometric method was used to evaluate initial urease inhibitory activity of samples and standard at 1 mg/mL. Thiourea was used as the standard. Increase in absorbance at 630 nm was measured after 50 min, using a microplate reader (Spectramax plus 384 Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 µL. The results (change in absorbance per min) were processed by using SoftMax Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). IC_{50} inhibitory activity was also calculated [4,19].

2.4.2 Determination of α-Glucosidase inhibition activity

Buffer A was prepared by mixing 0.1 mol/L potassium phosphate and 3.2 mmol/L $MgCl_2$, pH 6.8 and Buffer B was prepared by adding 0.5 mmol/L potassium phosphate and 16 mmol/L $MgCl_2$, pH 6.8. α-Glucosidase (Sigma, type 111, from yeast), 0.1 units/mL. *p*-Nitrophenyl- α-D-glucopyranoside at 6 mmol/L was used as substrate and dissolved in buffer A. Substrate (200 µL), 102 µL Buffer B, 120 µL sample solution (0.6 mg/mL in dimethylsulfoxide) at various concentration (25-500 µg), 282 µL water were mixed. Enzyme solution (200 µL) was added after the mixture was pre incubated in water bath at 37 ^{o}C for 5 minutes in a 96 well plate. The enzyme reaction then took place at 37 ^{o}C for 30 minutes and 1.2 ml of 0.4 mol/L glycine buffer (pH=10.4) was added to terminate the reaction. Absorbance of the released p-nitrophenol was measured at 410 nm using Multiplate Reader. Analysis was carried out in triplicate and 1-Deoxynojiromycin hydrochloride (DNJ) was used as standard. Negative control was mixture of reagents without test compound. Percentage Inhibitory activity and the concentration that induces inhibition halfway between the minimum and maximum response of each compound (relative IC_{50}) was determined by monitoring the inhibition effect of various concentrations of compounds in the assay [20-21].

2.5 Free radical Scavenging activity using 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH)

The free radical scavenging activity was measured by using the 2,2-diphenyl-1-picryl-hydrazil (DPPH) radical method. The solution of DPPH (0.3 mM) was prepared in ethanol. Five microlitres of each sample of different concentration (62.5 µg - 500 µg) was mixed with 95 µl of DPPH solution in ethanol. The mixture was dispersed in 96 well plates and incubated at 37° C for 30 min. The absorbance at 515 nm was measured by microtitre plate reader (Spectramax plus 384 Molecular Device, USA) and percent radical scavenging activity was determined by comparison with the methanol treated control. BHA was used as standard [4].

2.6 Insecticidal Activity

2.6.1 Insect: Maize weevil (*Sitophilus zeamais*) obtained and cultured in a dark incubator for 30 days at the Department of Crop Protection and Environmental Biology, Faculty of Agriculture, University of Ibadan, Ibadan, Nigeria were used.

2.6.2 Sample Preparation

Maize seeds (10.0 g) were weighed into separate containers. The methanol extract (2.0 mg) of *L. aestuans* leaves (LAL) was dissolved in 2.0 mL of methanol to make the stock solution of 100%. Half concentration (50%) was prepared from this. 2.0 mL of the prepared concentrations of extract was added to the weighed seeds and the solvent was left to evaporate at room temperature (48 hours). Positive control (Control I) was prepared by adding 2.0 mL of methanol to the seeds while negative control (control II) was 10.0 g of the maize seed without the solvent [22-23].

2.6.3 Procedure for Insecticidal Activity

The method of Jbilou *et al*, [22] was used with little modification. The jar containing the insects was refrigerated for one hour to immobilize them. The immobilized insects were added to the prepared seeds. Three male and three female insects were added to each of the container, containing the prepared seeds. The plastic containers were covered with a mesh net to prevent suffocation and escape of the insects. The insects were observed at intervals of 3, 6, 12 and 24 hours for three days. Numbers of dead insects were recorded to determine mortality at different time intervals. The test was performed in triplicate. Lethal dose at 50% and 100% concentration was determined [24].

2.7 Statistical Analysis: *Data processing & IC* $_{50}$ *Determination*

Absorbance measurements are expressed as mean absorbance ± SD of triplicate analysis. All

experimental data were analyzed statistically by one-way analysis of variance (ANOVA), processed on SPSS software package (version 15.0; SPSS, Inc., Chicago, Illinois, USA) for more than two means while Student's t-test was used for comparison between two means. Values of $P \leq 0.05$ were taken to be statistically significant. The following equation was employed; Inhibitory activity (%) = (1 − As/Ac) ×100 or Ac-As/Acx100 where, As is the absorbance in the presence of test substance or sample and Ac is the absorbance of control from which ICs_0 was calculated. The IC_{50} value was obtained from curves obtained by linear regression using GraphPad Prism 5 version 5.01 (Graph pad software, Inc., La Jolla, CA, USA.) software.

3. Results

4-methoxy-8'-acetyl olivil (LA1), white crystalline solid. Rf: 0.6 (EtOAc-Hexane, 1:3). UV [EtOH] nm (log ε): 241.52 (2.3020). IR (KBr) V_{max} cm^{-1}: 3392.5 (broad) O-H (hydrogen bonded), 2860.2 C-Hstr (methoxyl), 1464.6,1379.1 C-H (methoxyl), 1033.7 C-Ostr (methoxyl); ^1H NMR (400 MHz; $CDCl_3$): δ 9.80 (1H, s, O-H, aromatic), 6.65-7.01 (1H, s, 6xCH, aromatic), 5.1 (1H, s, CH, tetrahydrofuran), 4.75 (1H, O-H, t, alcohol), 3.99, 3.76 (2H, s, CH_2, tetrahydrofuran), 3.89 ((3H, s, OCH_3CO, methyl), 3.81 (3H, s, $3xCH_3$, methyl), 2.37 (1H, s, CH, tetrahydrofuran), 3.61, 3.33 (2H, dd, CH_2, methylene), 2.95, 2.71 (2H, s, CH_2, methylene), Molecular formulae $C_{23}H_{28}O_8$.

3β,19α-dihydroxy-30-norurs-12-ene (LA2): white crystalline solid. Rf: 0.5 (EtOAc-Hexane, 1:4). UV [EtOH] nm (log ε): 216.07 (1.5120). IR (KBr) V_{max} cm^{-1}: 3381.2 (O-H), 2967.2, 2865.8 C-Hstr (methyl), 2909.56, 2814.5 C-Hstr (methylene), 1031.2 (s) C-Ostr (alcohol), 837.85 C=C (cyclohexne) ^1H NMR (400 MHz; $CDCl_3$): δ 5.26 (1H, CH, t, cyclohexene), 4.61 (1H, O-H, s, alcohol), 4.79 (1H, O-H, s, alcohol), 1.37-3.18 (1H, 4xCH, s, cyclohexane), 1.26-2.07 (2H, m, $10xCH_2$, cyclohexane), 0.99-1.31 (3H, $7xCH_3$, s, methyl), Molecular formulae $C_{29}H_{48}O_2$.

2α,3β,21β,23,28-penta hydroxyl 12-oleanene (LA3): white crystalline solid. Rf: 0.65 (EtOAc-Hexane, 1:4). UV [EtOH] nm (log ε): 219.84 (1.6120). IR (KBr) V_{max} cm^{-1}: 3391.8 (broad) O-H (hydrogen bonded), 2956.6, 2871.2 C-Hstr (methyl), 2867.25, 2853.4 C-Hstr (methylene), 1415.2,1311.6 C-H (methyl), 1033.7 C-Ostr (alcohol), 862.15 C=C (cyclohexene); ^1H NMR (400 MHz; $CDCl_3$): δ 5.29 (1H, CH, s, cyclohexene), 4.81 (1H, 3x O-H, s), 4.78 (1H, 2xO-H, s), 1.39-3.23 (1H, 6xCH, s), 1.34-3.56 (2H, $10xCH_2$, s), 0.99-1.30 (3H, $6xCH_3$, s), Molecular formulae $C_{30}H_{50}O_5$.

5, 2', 4' trihydroxy 7, 8 dimethoxy flavones (LA4): yellowish solid. Rf: 0.3 (EtOAc-Hexane, 1:4). UV [EtOH] nm (log ε): 261.54 (2.658). IR (KBr) V_{max} cm^{-1}: 3391.8 (broad) O-H (hydrogen bonded), 2867.3, 2817.3 C-Hstr (methoxyl), 2853.4 C-Hstr (methylene), 1566.6,1345.9 C-H (methoxyl), 1033.7 C-Ostr, 937.85 C=C (cyclohexene); ^1H NMR (400 MHz; $CDCl_3$): δ 11.85 (1H, 2xO-H, s, aromatic), 9.89 (1H, O-H, s, aromatic), 6.18-7.04 (1H, 4x C-H, benzene), 3.93 (3H, s $2xCH_3$), 6.54 (1H, C=H, chromene). Molecular formulae $C_{17}H_{14}O_7$.

3-(4-hydroxyphenyl)-4-(3-methoxy-4-hydroxyphenyl)-3,4-dehydroquinolizidine (LA5): white crystalline solid. Rf: 0.5 (EtOAc-Hexane, 1:3). UV [EtOH] nm (log ε): 218.43 (1.6120). IR (KBr) V_{max} cm^{-1}: 3394.7 (broad) O-H, 2856.8, 2860.2 C-Hstr (methoxyl), 2853.4 C-Hstr (methylene), 1464.6,1379.1 C-H (methoxyl), 1287.5 (C-N str), 1031.7 C-O str, 839.85 C=C (cyclohexene); ^1H NMR (400 MHz; $CDCl_3$): δ 9.83 (1H, O-H, s), 9.43 (1H, O-H, s), 6.70-7.33 (1H, m, 7xC-H, benzene), 2.88 (2H, m, CH_2, tetrahydropyridine). 2.51 (2H, m, $2xCH_2$, piperidine), 3.83 ((3H, m, CH_3, methoxyl), 2.44 (1H, m C-H, tetrahydropyridine), 2.16, 1.90 (2H, m C-H, tetrahydropyridine), 1.49-1.55 (2H, m, $2xCH_2$, piperidine), Molecular formulae $C_{22}H_{25}NO_3$.

15-Hydroxycryptopleurine (LA6), white crystalline solid. Rf: 0.8 (EtOAc-Hexane, 1:4). UV [EtOH] nm (log ε): 211.09 (2.1250). IR (KBr) V_{max} cm^{-1}: 3394.7 (broad) O-H, 2856.8, 2860.2 C-Hstr (methoxyl), 2853.4 C-Hstr (methylene), 1464.6,1379.1 C-H (methoxyl), 1287.5 (C-N str), 1031.7 C-Ostr, ^1H NMR (400 MHz; $CDCl_3$): δ 7.33-7.53 (1H, m, 5xC-H, phenanthrene), 5.17 (1H, O-H, s), 4.73 (1H, m, C-H, methine), 4. 11, 4.01 (2H, m, CH_2, methylene). 3.83 (3H, m, $3xCH_3$, methyl), 2.70 (1H, m, C-H, piperidine), 2.51, 2.41 (2H, m, CH_2, piperidine), 1.49-1.59 (2H, m, $3xCH_2$, piperidine), Molecular formulae = $C_{24}H_{27}NO_4$.

The IC_{50} (μM) values of the isolated compounds in the urease, α-glucosidase and free radical scavenging inhibitory assays is shown in Table 1. 15-hydroxycryptopleurine (LA6) (18.5± 0.54) showed significant urease inhibition activity while 5, 2', 4' trihydroxy 7, 8 dimethoxy flavones (LA4) (14.6± 0.12) had significant α-glucosidase inhibition activity. In the free radical scavenging screening, 5, 2', 4' trihydroxy 7, 8 dimethoxy flavones (LA4) (15.45±0.01) was the most active The mortality rate at 100% concentration was 17% in the insecticidal screening. Due to the insignificant activity, the isolates were not screened for insecticidal activity.

4. Discussion

The infra red spectroscopic data showed absorption peaks around 3390 cm^{-1}, 2800 cm^{-1} and

1287.5 cm^{-1} which are indicative of hydroxyl (OH), methoxyl (OCH_3) and cyano groups (C-N) for the isolated compounds. 4-methoxy-8'-acetyl olivil (LA1) is a lignan and showed broad, hydrogen bonded hydroxyl (O-H) band at 3392.5 cm^{-1}. Peaks at 2860.2 cm^{-1} and 1033.7 cm^{-1} represent C-H and C-O stretch of methoxyl respectively. The proton NMR peak at δ 9.80 was assigned to aromatic O-H and 4.75 to alcohol O-H. 3β,19α-dihydroxy-30-norurs-12-ene (LA2) and 2α,3β,21β,23,28-penta hydroxyl 12-oleanene (LA3) are triterpenoids with IR bands in the range of 2967.2, 2865.8 for C-H stretch (methyl), 2909.56, 2814.5, C-Hstr (methylene), 3381.2 O-H, 1031.2 C-O, and 837.85 C=C (cyclohexne) respectively. Peaks were observed at δ 5.26-5.29 for protons in cyclohexene, 4.61 - 4.79 for O-H, 1.26-2.07 for CH_2 and 0.99-1.31 for CH_3. The flavonoid, 5, 2', 4' trihydroxy 7, 8 dimethoxy flavones (LA4) with characteristic IR bands at 3391.8 cm^{-1} represents broad, hydrogen bonded O-H, 2867.3 cm^{-1} and 2817.3 cm^{-1} for C-H stretch of methoxyl, 2853.4 cm^{-1} for C-H stretch of methylene, and 1033.7 cm^{-1} for C-O stretch; The ^{1}H NMR peaks at δ 11.85 and 9.89 were assigned to the aromatic O-H, and 6.54 to the C=H of chromene. The two alkaloids obtained from the plant namely 3-(4-hydroxyphenyl)-4-(3-methoxy-4-hydroxyphenyl)-3,4-dehydroquinolizidine (LA5) and 15-hydroxycryptopleurine (LA6) gave peaks in the IR at 3394.7 cm^{-1} for O-H, 2856.8 cm^{-1} and 2860.2 cm^{-1} for C-H stretch of methoxyl and peaks in the range of 1287.5 cm^{-1} were observed for C-N stretch of alkaloids. Also the ^{1}H NMR peaks in the range of δ 9.83 and 9.43 were assigned to O-H signals, 3.83 for CH_3 of methoxyl and 1.49-1.55 for CH_2 of the piperidine ring, Data obtained in this study compared favourably well with those previously reported in literature [9, 16- 18, 25-27]. In addition to the compound isolated in this present study, (4*E*)-3,6-dimethylhep-4-en-3-ol and chrysen-2-ol derivative has been previously reported from the extract of *L. aestuans* [15].

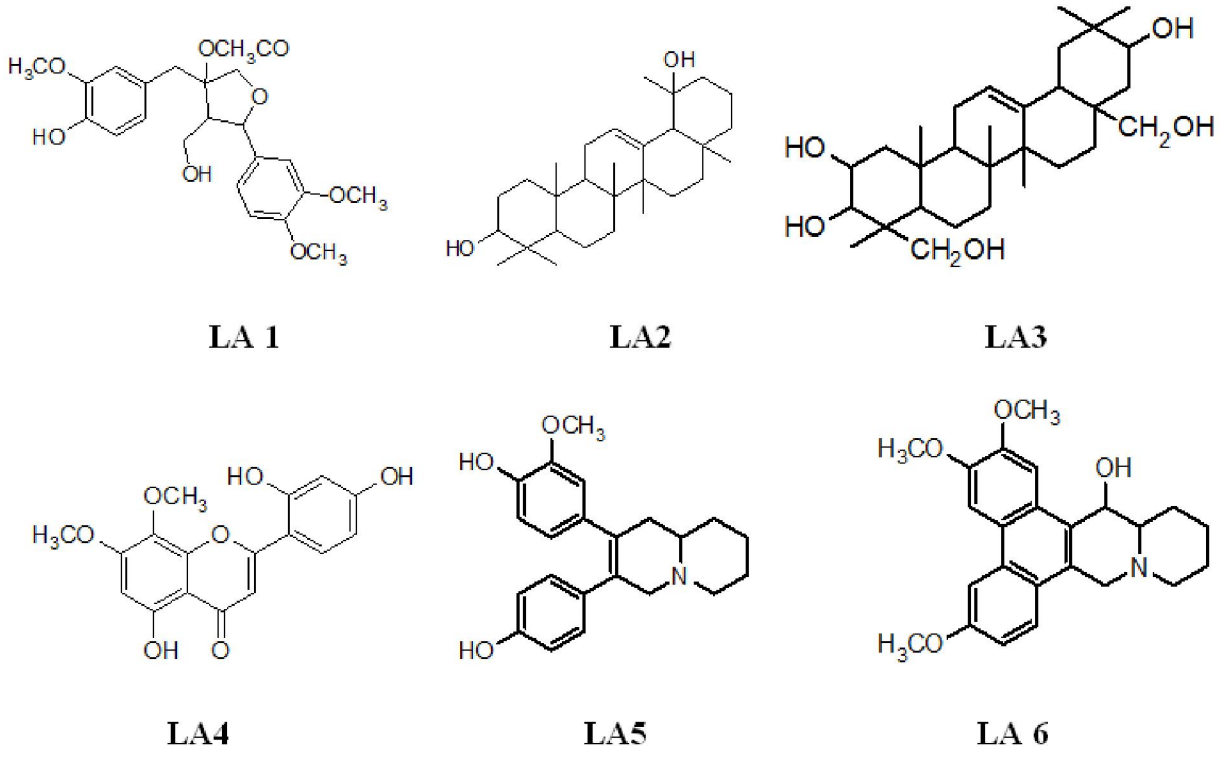

Figure 1: Structures of 4-methoxy-8'-acetyl olivil (LA1), 3β,19α-dihydroxy-30-norurs-12-ene (LA2), 2α,3β,21β,23,28-penta hydroxyl 12-oleanene (LA3), 5, 2', 4' trihydroxy 7, 8 dimethoxy flavones (LA4), 3-(4-hydroxyphenyl)-4-(3-methoxy-4-hydroxyphenyl)-3,4-dehydroquinolizidine (LA5), 15-Hydroxycryptopleurine (LA6)

Table 1: IC_{50} (µM) values of compounds in the U

Compounds and standard	Urease Inhibition	α-Glucosidase Inhibition	Free radical scavenging assay
LA1	54.8± 0.67	14.9± 0.43	45.3± 0.61
LA2	> 500	24.8± 0.87	89.2± 0.84
LA3	> 500	29.6± 0.90	> 200
LA4	29.8± 0.15	14.6± 0.12	15.45±0.01
LA5	32.5± 0.21	27.5± 0.30	20.27±0.00
LA6	18.5± 0.54	18.6± 0.53	28.81±0.03
BHA	-	-	44.2 ± 0.09
Thiourea	21.6 ± 0.12	-	-
DNJ	-	3.9 ± 0.08	-

rease, α-Glucosidase and Free radical inhibitory assays.
* BHA-Butylatedhydoxyanisole, DNJ-1-Deoxynojiromycin hydrochloride

Table 1 shows the IC_{50} (µM) values of the isolated compounds in the urease, α-glucosidase and free radical scavenging inhibitory assays. 5, 2', 4' trihydroxy 7, 8 dimethoxy flavones (LA4) (29.8± 0.15) and 15-hydroxycryptopleurine (LA6) (18.5± 0.54) showed significant urease inhibition activity when compared with thiourea used as standard. 4-methoxy-8'-acetyl olivil (LA1) (14.9± 0.43) and 5, 2', 4' trihydroxy 7, 8 dimethoxy flavones (LA4) (14.6± 0.12) had significant α-glucosidase inhibition activity. Activity was compared with 1-deoxynojiromycin hydrochloride (DNJ). Lastly, 5, 2', 4' trihydroxy 7, 8 dimethoxy flavones (LA4) (15.45±0.01), 3-(4-hydroxyphenyl)-4-(3-methoxy-4-hydroxyphenyl)-3,4-dehydroquinolizidine (LA5) (20.27±0.00) and 15-hydroxycryptopleurine (LA6) (28.81±0.03) were the most active in the free radical scavenging assay when activity was compared with butylated hydroxylanisole. Mortality rate of 17% at 100% concentration was recorded for the crude extract of *L. aestuans* in the insecticidal screening. This showed that the plant had little or no activity as an insecticidal agent. Since the crude extract did not show activity as insecticidal agent, the isolates were not investigated.

Plants have been reported to be used for the treatment of *H. pylori* [28], due to the presence of secondary metabolites. In developing countries, the application of antibiotics is still under poor management as a whole and there is a growing need for finding new anti urease agents from nature that can eradicate the invasion and presence of survived *H. pylori* strains to avoid relapse of some ailments especially gastric ulcer. These activities may be mediated by direct inhibition of bacterial urease (*H. pylori* urease) and their subsequent anti- *H. pylori* activity or indirect action as an anti-oxidant, anti-inflammatory agents or pH-mediator compounds through their active chemical constituents [29]. Diseases such as cancer, stroke, diabetes, Parkinson's disease and other neurodegenerative diseases have been linked to oxidation reactions. Natural antioxidants and enzyme inhibitors will therefore serve as a remedy for emerging diseases.

Conclusion

Isolation of bioactive constituents from *Laportea aestuans* afforded six constituents namely 4-methoxy-8'-acetyl olivil, 3β,19α-dihydroxy-30-norurs-12-ene, 2α,3β,21β,23,28-penta hydroxyl 12-oleanene, 5, 2', 4' trihydroxy 7, 8 dimethoxy flavones, 3-(4-hydroxyphenyl)-4-(3-methoxy-4-hydroxyphenyl)-3,4-dehydroquinolizidine, and 15-Hydroxycryptopleurine. Enzyme inhibition (urease and α-glucosidase inhibition activities), and free radical scavenging activities of the isolates showed that some of the them possessed moderate to significant activity when activities were compared with thiourea, 1-deoxynojiromycin hydrochloride (DNJ) and butylated hydroxylanisole used as standards in the three assays. It can therefore be concluded that bioactive compounds from *Laportea aestuans* could serve as lead in emerging natural therapies.

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