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COMPARATIVE BIOCHEMICAL CHARACTERISTICS OF THE METHANOLIC LEAVES EXTRAC T OF *LAPORTEA AESTUANS* (LINN.) CHEW AND *LAPORTEA OVALIFOLIA* (SCHUMACH AND THONN) CHEW (URTICACEAE): ANTIOXIDANT, ANTIDIABETIC POTENTIAL AND CHEMOTAXONOMIC IMPLICATIONS

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ABSTRACT

Laportea aestuans and *Laportea ovalifolia* (Urticaceae) are stinging herbs used in West and Central African ethnomedicine, yet they resemble each other so closely that they are easily confused in the field and in the preparation of herbal remedies. This study compared the biochemical profiles of their methanolic leaf extracts as a possible chemotaxonomic complement to morphological identification. Leaves were collected on the Obafemi Awolowo University campus, Ile-Ife, were extracted by cold maceration, and the extract were assessed for percentage yield, qualitative phytochemical composition, total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP), hydroxyl radical scavenging activity (HRSA), DPPH radical scavenging, α-amylase and α-glucosidase inhibition. *L. aestuans* was the only species to test positive for steroids, it gave the higher yield (14.23%) than *L. ovalifolia* (6.81%), TPC (3.47 ± 0.01 mg GAE/g), TFC (5.15 ±

0.41 mg GAE/g), FRAP (30.08 ± 2.33 µmol Fe^{2+}/g), and it was the better hydroxy radical scavenging (IC_{50} 0.2 mg/mL) and DPPH radical scavenging (IC_{50} 0.22 mg/mL while *L. ovalifolia* did not reach the 50% inhibition). *L. ovalifolia* on the other hand had the higher TAC (133.1 ± 2.1 mg AAE/g), and inhibited α-amylase more strongly (IC_{50} 0.2 mg/mL) than acarbose (IC_{50} 0.37 mg/mL). Both extracts inhibited α-glucosidase more strongly than acarbose. The two species therefore carry distinct but complementary biochemical profiles, which supports the use of biochemical profiling as a supporting chemotaxonomic tool in *Laportea* and give laboratory backing to the traditional antidiabetic reputation of *L. ovalifolia*.

KEYWORDS: *Laportea aestuans*, *Laportea ovalifolia*, antioxidant activity, α-amylase, chemotaxonomy, Urticaceae.

INTRODUCTION

Plants remain a major reservoir of bioactive compounds, and the systematic study of species from taxonomic, ecological and biochemical standpoints continues to be central to botanical research (Essiett *et al.*, 2011). The genus *Laportea* Gaudich. (Urticaceae) comprises herbaceous, stinging plants of moist, shaded tropical habitats, several of which have long been used in indigenous pharmacopoeias across Africa, Asia and the Americas (Tsabang *et al.*, 2015; Adetunji *et al.*, 2021; Feng *et al.*, 2024). Two of them, *Laportea aestuans* (L.) Chew and *Laportea ovalifolia* (Schumach. and Thonn.) Chew, overlap in range and habitat and resemble each other closely, which has made field-level separation difficult (Essiett *et al.*, 2011).

Both species have a place in traditional medicine. *L. aestuans* is used in Nigeria for whitlow, burns, urinary complaints, diabetes and asthma (Adetunji *et al.*, 2021), and in Brazil in the management of osteoporosis (de Oliveira *et al.*, 2012). *L. ovalifolia* features prominently in Cameroonian and Ghanaian practice, where it was identified in a large ethnobotanical survey as the most credible antidiabetic plant among those assessed (Tsabang *et al.*, 2015). Its leaf extracts have lowered blood glucose and serum lipids in diabetic rats (Momo *et al.*, 2006) and have shown antioxidant activity *in vitro* and *in vivo* (Oteng Dapaah, 2015; Njina *et al.*, 2016). For *L. aestuans*, phenolic-rich extracts, isolated flavonoids and antioxidant, antimicrobial and antidiabetic effects have been reported (Oloyede and Ayanbadejo, 2014; Adeosun *et al.*, 2022; Tijani *et al.*, 2022).

Morphologically similar species within a genus are difficult to separate using gross morphology alone, particularly at the vegetative stage, and chemotaxonomy, the use of secondary-metabolite profiles as a complementary identification tool, has been proposed for exactly this situation (Feng *et al.*, 2024). Misidentification also matters in practice, because substituting one species for another in a herbal preparation may change its biochemical and therapeutic profile. Each species has been studied independently, but standardized, side-by-side biochemical data generated under identical conditions are scarce. In particular, the strong traditional antidiabetic reputation of *L. ovalifolia* has rested largely on whole-extract blood-glucose data rather than on enzyme-inhibition assays.

This study therefore compared the methanolic leaf extracts of *L. aestuans* and *L. ovalifolia* for percentage yield, qualitative phytochemical constituents, total phenolic, flavonoid and antioxidant content, free-radical scavenging and reducing power, and inhibition of the carbohydrate-digesting enzymes α-amylase and α-glucosidase, and interpreted the differences from a botanical and chemotaxonomic perspective.

MATERIALS AND METHODS

Plant materials

Fresh, healthy, mature leaves of *Laportea aestuans* and *Laportea ovalifolia* were collected from naturally growing populations on the Obafemi Awolowo University campus, Ile-Ife, Osun State, Nigeria with the GPS 7º31′8″N 4º31′35″E. Diseased or damaged leaves and foreign matter were discarded, and the samples were transported to the laboratory in labelled polyethylene bags. Identification and authentication were carried out in the Department of Botany, Obafemi Awolowo University by IFE Herbarium curator and vouch number was given (IFE 18632).



Figure 1: *Laportea aestuans* (L.) Chew.



Figure 2: *Laportea ovalifolia* (Schumach and Thonn) Chew.

Preparation of plant extracts

Dried and pulverized leaves of *Laportea aestuans* and *Laportea ovalifolia* were soaked separately in 1 L of 70% methanol. After 7 days of macerating, the extracts were filtered using cheesecloth, then air-dried in Petri dishes, until a constant weight was achieved. The percentage yields of the obtained crude extracts were determined using the formula.

$$Yield = \frac{Weight\ of\ concentrated\ extract}{Total\ amount\ of\ extract} \times 100$$

The obtained crude extracts were stored in sealed jars for further laboratory analyses.

Determination of Total Phenolic Content

The extracts total phenolic content (TPC) was determined following the Folin-Ciocalteu colorimetric method (Singleton *et al*., 1999). A standard gallic acid solution (0.1 g/mL) was prepared, and varying volumes (0.2 - 1 mL) were diluted to 1 mL. Each test tube received 10% Folin-Ciocalteu reagent (0.2 mL), vortexed for 5 minutes, followed by 7% Na_2CO_3 (1 mL) and this was made up 2.5 mL with distilled water and then incubated at room temperature for 90 minutes. The absorbance was read at 750 nm. Samples were run in triplicate. The TPC of the extracts was expressed as mg gallic acid equivalents (GAE) per gram dry weight (mg/g) using the formula.

$$C = \frac{c \times v}{m}$$

Where

(C) = Total phenolic content (mgGAE/g dry extract)

(c) = Gallic acid concentration from calibration curve (mg/mL)

(v) = Volume of extract (mL)

(m) = Weight of crude extract (g)

Determination of Total Flavonoid Content

Total flavonoid content was determined using the aluminium chloride colorimetric metho (Zhilen *et al*., 1999). Standard quercetin solutions (0.2 - 1.0) mg/mL were prepared. To each assay, the extract or the standard (0.1 mL) was added distilled water (0.4 mL), followed by the addition of 5% sodium nitrite (0.1 mL). This was left for 5 minutes after which 10% aluminium chloride (0.1 mL) and sodium nitrite (0.2 mL) were added, and the volume was adjusted to 2.5 mL with distilled water. Samples were run in triplicate, and absorbance was taken at 510 nm against a blank. The flavonoid content was expressed as milligram quercetin equivalents (mgQE/g) using the formula.

$$X = \frac{q \times v}{w}$$

Where:

(X) = Total flavonoid content (mgQE/g)

(q) = Quercetin concentration from calibration curve (mg/mL)

(v) = Volume of extract (mL)

(w) = Weight of crude extract (g)

DPPH Radical Scavenging Activity

The plant extracts antioxidant activities were evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method (Brand-Williams *et al.*, 1995). Different concentrations of the extracts and standard (ascorbic acid) ranging from 0.1 to 0.5 mg/mL were mixed with 1 mL of 0.3 mM DPPH in methanol. These were incubated in the dark for 30 minutes after which the absorbance was read at 517 nm. The percent inhibition was calculated using the formula:

$$\%\ Inhibition = \frac{Abs(blank) - Abs(sample)}{Abs(blank)} \times 100$$

Where;

Abs(blank) = Absorbance of the control

Abs (Sample) = Absorbance of test compound

The IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was determined from the plot of percentage inhibition against extract concentration.

Evaluation of Total Antioxidant Capacity

Total antioxidant capacity of the extracts was determined following the phosphomolybdenum method (Prieto *et al*., 1999). To 0.3 mL of extract (5 mg/mL) or standard (ascorbic acid, 1 mg/mL), 3 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) was added. The tubes were capped, incubated at 95 °C for 90 minutes and cool at room temperature. The absorbance was read at 695 nm against a blank containing distilled water in place of extract. Antioxidant activity was expressed as ascorbic acid equivalents.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was determined based on the metal-chelating ability of antioxidants under Fenton-like reaction conditions, following the method of Chang and But (1987). A reaction mixture containing phosphate buffer (1.2 mL) at pH 7.4 and extract (200 µL) was combined with 200 µL each of 10 mM $FeSO_4 \cdot 7H_2O$, 10 mM EDTA and 10 mM 2-deoxyribose. Thereafter, 200 µL of 10 mM H_2O_2 was added. The mixture was incubated at 37 °C for 3 hours after which 1 mL each of 1% thiobarbituric acid (TBA) and 2% trichloroacetic acid (TCA) were added. This was heated in a water bath for 10 minutes and allowed to cool to room temperature before the absorbance was read at 532 nm. The percentage scavenging activity was calculated using the formula;

$$\%\ Inhibition = \frac{Abs(blank) - Abs(sample)}{Abs(blank)} \times 100$$

Where:

Abs(blank) = Absorbance of the control reaction

Abs (Sample) = Absorbance of test compound

Sample concentration providing 50% Scavenging activity (IC_{50}) was calculated from the graph of percentage scavenging activity against concentration.

Ferric Reducing Antioxidant Power Assay

The determination of ferric reducing antioxidant power (FRAP) of the extracts was carried out following the method of Iris and Strain (1999). The working FRAP reagent comprising acetate buffer (300 mM) at pH 3.6, tripyridyltriazine (10 mM), and ferric chloride (20 mM) in a ratio 10:1:1 was prepared. To 2 mL of this reagent, 20-100 µL of extract (5 mg/mL) or standard ascorbic acid solution (1 mg/mL) was added. Samples were prepared in triplicates and absorbance was read at 593 nm using a reagent blank containing distilled water. The

antioxidant capacity was expressed as ascorbic acid equivalent concentration, defined as the concentration of antioxidant that produces a ferric reducing ability equivalent to that of the ascorbic acid standard.

Statistical Analysis

Data were expressed as percentages. Replicate values (n=3) were expressed means ± standard errors.

RESULTS

Extracts Yield

Table 1 below shows the percentage extract yields of *Laportea aestuans* extract and *Laportea ovalifolia* extract. The result showed that *L. aestuans* had higher extract percentage yield than *L. ovalifolia.*

Table 1: Yield of the methanolic extract of *Laportea aestuans* and *Laportea ovalifolia.*

Methanolic Extract	Yield (%)
Laportea aestuans	14.23
Laportea ovalifolia	6.81

Table 2: Qualitative phytochemical screening of methanolic leaf extracts of *Laportea aestuans* and *Laportea ovalifolia.*

Qualitative Screening	*L. aestuans*	*L. ovalifolia*
Flavonoids	−	−
Phenols	−	−
Tannins	−	−
Terpenoids	−	−
Steroids	+	−
Anthraquinones	−	−
Cardiac glycosides	−	−

+ = present; − = not detected.

Total Phenolic Content

Total Phenolic Content in *Laportea aestuans* and *Laportea ovalifolia* extracts (Table 3) showed that *L. aestuans* exhibited a higher total phenolic content compared to *L. ovalifolia*.

Table 3: Total Phenolic Content (TPC) of Andrographis paniculata and Petiveria alliacea extracts.

Extracts	Total Phenolic Content (mgGAE/g)
Laportea aestuans	3.47 ± 0.01
Laportea ovalifolia	1.34 ± 0.01

Values are mean ± standard error; where n = 3

Total Flavonoid Content

Table 4 presents the results of the analyses of total flavonoid content in *L. aestuans* and *L. ovalifolia* extracts expressed as quercetin equivalent (mgQE/g). *L. aestuans* showed a higher total flavonoid content than *L. ovalifolia*.

Table 4: Total Flavonoid Contents of *Laportea aestuans* and *Laportea ovalifolia* extracts.

Extract	Total Flavonoid Content (mgQE/g)
Laportea aestuans	5.15 ± 0.41
Laportea ovalifolia	2.27 ± 0.28

Values are mean ± standard error; where n = 3

Total Antioxidant Content

The results of the analyses of *Laportea aestuans* and *Laportea ovalifolia* extracts for total antioxidant capacity expressed as mg ascorbic acid equivalent (mgAAE/g) are shown in Table 5. The total antioxidant capacity was higher in *L. ovalifolia* extract than that of *L. aestuans*

Table 5: Total Antioxidant Content of *Laportea aestuans* and *Laportea ovalifolia* extracts.

Extract	Total Antioxidant Content (mgAAE/g)
Laportea aestuans	78.8 ± 6.4
Laportea ovalifolia	133.1 ± 2.1

Values are mean ± standard error; where n = 3

Ferric Reducing Antioxidant Potential

Table 6 below shows the Ferric reducing antioxidant potential of *L. aestuans* and *L. ovalifolia* methanolic extracts expressed as Ascorbic acid equivalent (mgAAE/g). *Laportea aestuans* displayed a stronger ferric reducing power than *Laportea ovalifolia*.

Table 6: Ferric Reducing Antioxidant Potential of *Laportea aestuans* and *Laportea ovalifolia* extract.

Extract	Ferric Reducing Antioxidant Potential (mgAAE/g)
Laportea aestuans	30.08 ± 2.33
Laportea ovalifolia	18.02 ± 0.16

Values are mean ± standard error; where n = 3

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activities of *L. aestuans* and *L. ovalifolia* extracts are presented in Figure 3 and Figure 4. The two extracts were close to one another across the range, and the IC_{50} values obtained from the curves were 0.2 mg/mL for *L. aestuans* and 0.3 mg/mL for *L. ovalifolia* (ascorbic acid, 0.245 mg/mL).

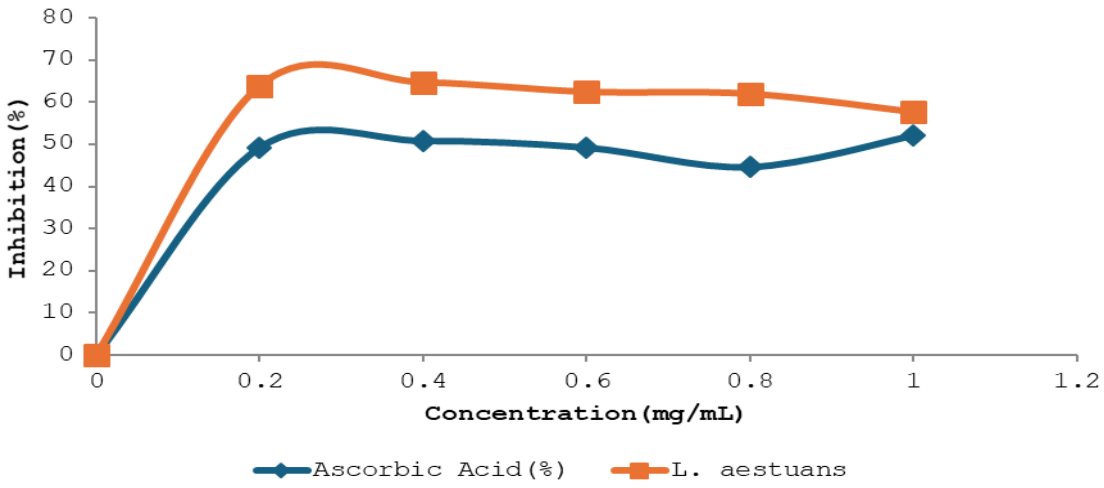


Figure 3: Hydroxyl Radical Scavenging Activity of Methanolic Extracts of *Laportea aestuans.*

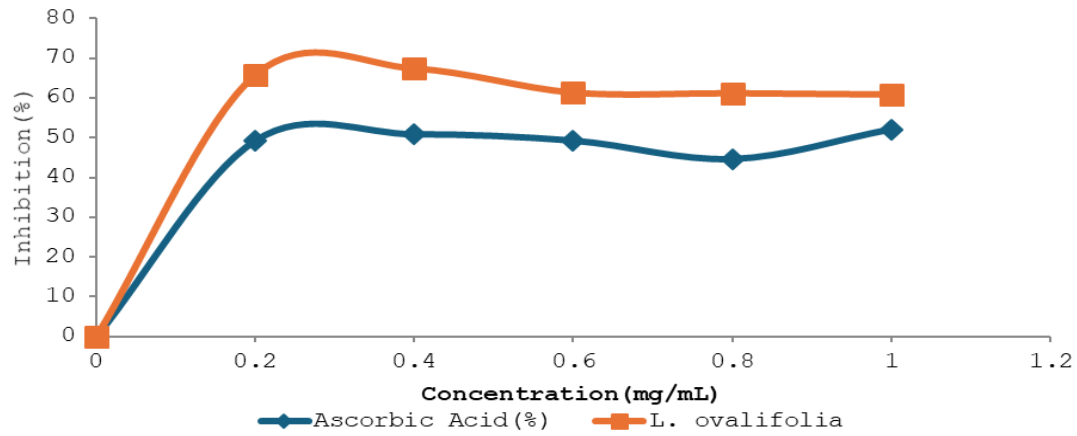


Figure 4: Hydroxyl Radical Scavenging Activity of Methanolic Extracts of *Laportea ovalifolia.*

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Scavenging Activity

Figure 5 and Figure 6 presents the DPPH assay, *L. aestuans* showed clear dose-dependent scavenging and an IC_{50} of 0.22 mg/mL, whereas *L. ovalifolia* reached only 29.91% at 0.5

mg/mL and did not attain 50% inhibition within the range tested. Ascorbic acid had an IC_{50} of 0.125 mg/mL.

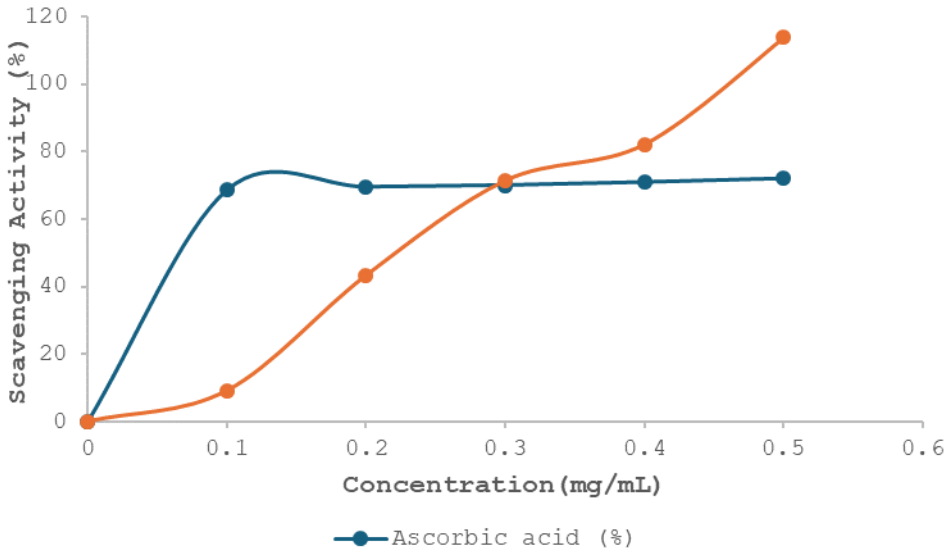


Figure 5: DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Scavenging Activity of *Laportea aestuans* Extracts.

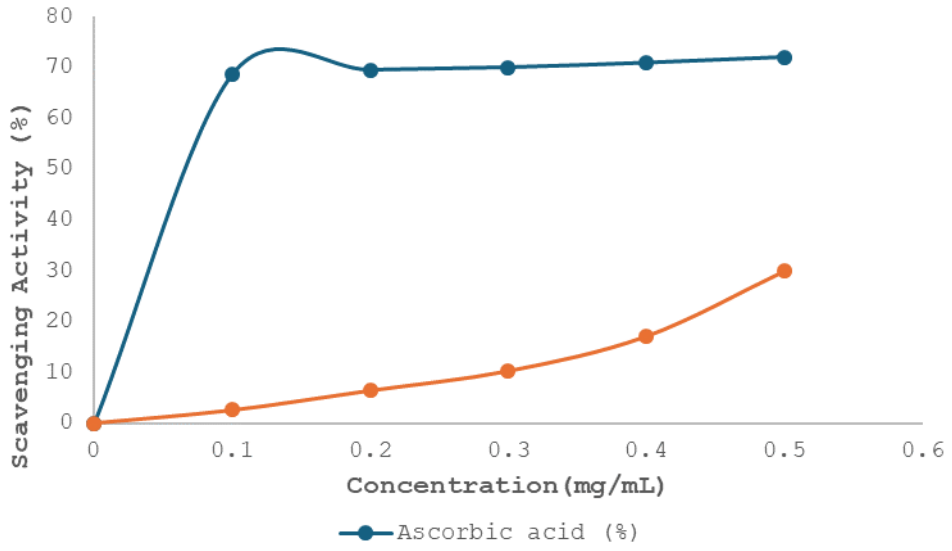


Figure 6: DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Scavenging Activity of *Laportea ovalifolia* Extracts.

α-amylase Enzyme inhibitory activity

The α-amylase enzyme inhibitory activities of *L. aestuans* and *L. ovalifolia* extracts are presented in Figure 7. *L. ovalifolia* inhibited α-amylase more strongly than acarbose and its

reported IC_{50} of 0.20 mg/mL was lower than that of acarbose (0.37 mg/mL) and of *L. aestuans* (0.39 mg/mL).

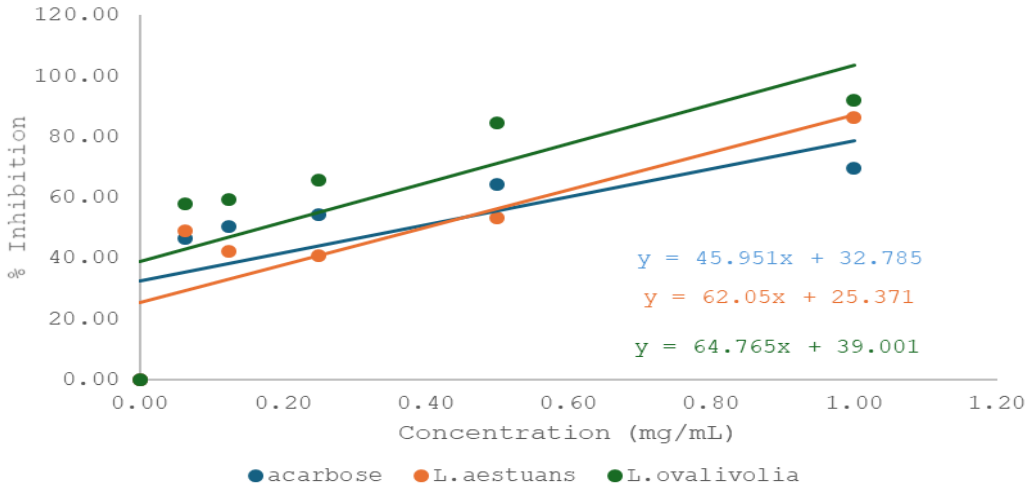


Figure 7: α-amylase enzyme inhibitory activity of *Laportea aestuans* and *Laportea ovalifolia* Extracts.

α-Glucosidase Enzyme inhibitory activity

The α-Glucosidase Enzyme inhibitory activities of *L. aestuans* and *L. ovalifolia* extracts are presented in Figure 8. Both extracts inhibited α-glucosidase more strongly than acarbose. The IC_{50} values were 0.57 mg/mL for *L. aestuans*, 0.78 mg/mL for *L. ovalifolia* and 1.15 mg/mL for acarbose.

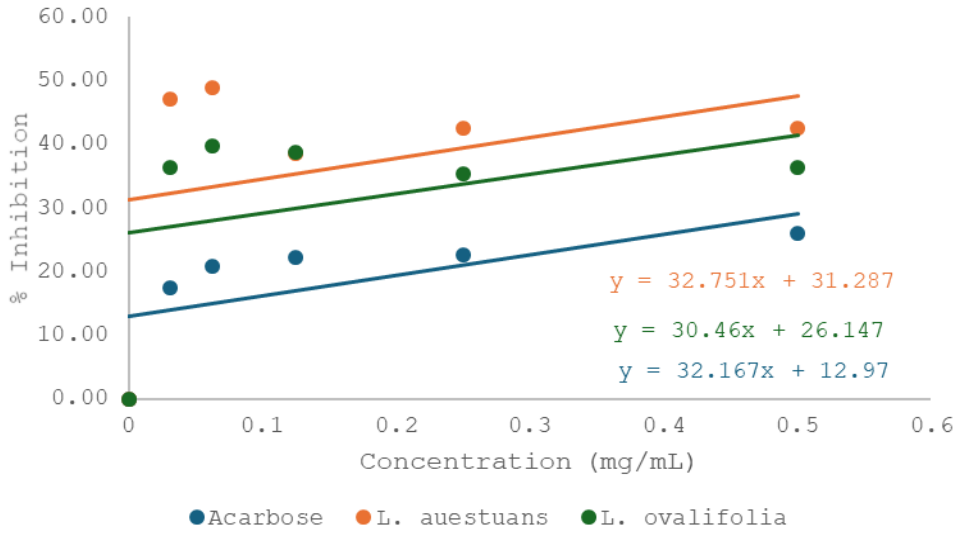


Figure 8: α-glucosidaes enzyme inhibitory activity of *Laportea aestuans* and *Laportea ovalifolia* Extracts.

DISCUSSION

The yield difference (14.23% against 6.81%) simply says that more of the *L. aestuans* leaf dissolves in methanol. That fits the richer phenolic and flavonoid profile usually reported for the species (Essiett *et al.*, 2011), and it may also have something to do with growth form: *L. aestuans* is a broad-leaved, fleshy, erect herb, *L. ovalifolia* a smaller-leaved creeper (Tsabang *et al.*, 2015), and the two could well differ in how much of their leaf tissue is freely extractable. We have not tested that idea, so it remains a suggestion.

The color tests detected steroids in *L. aestuans* only. Steroids are precursors of phytosterols and steroidal saponins, groups associated with anti-inflammatory, analgesic and antimicrobial activity (Trease and Evans, 2002). Their presence in one species and not the other suggests that the two differ in the secondary-metabolite pathways they express, despite their morphological similarity. The negative results for phenols and flavonoids, set against measurable TPC and TFC in the quantitative assays, are best explained by sensitivity: a color test shows a result only above a concentration threshold, whereas the Folin-Ciocalteu and aluminium chloride assays quantify much smaller amounts. The contents recorded here (1.34 mg GAE/g for *L. ovalifolia* – 3.47 mg GAE/g for *L. aestuans*; 2.78 mg QE/g for *L. ovalifolia* – 5.15 mg QE/g for *L. aestuans*) are modest. Njina *et al.* (2016) reported higher values for an aqueous extract of *L. ovalifolia* (13.33 mg GAE/g; 5.27 mg catechin equivalent/g), but the solvent, extraction procedure and standards differ, so the figures are not directly comparable. A negative screening result should not be read as proof that a compound class is absent, which is why quantitative assays were paired with the screening.

The antioxidant assays gave assay-dependent rather than uniform results. TPC, TFC and FRAP, which track phenolic and flavonoid content (Benzie and Strain, 1996), all favoured *L. aestuans*. TAC favoured *L. ovalifolia*. The phosphomolybdenum assay responds to a wide range of reductants, not phenolics alone (Prieto *et al.*, 1999), so the high TAC of *L. ovalifolia* may draw on non-phenolic constituents, possibly including the cerebroside and ceramide isolated from its leaves (Tazoo *et al.*, 2007). This is a hypothesis that the present data cannot test. In DPPH scavenging, *L. aestuans* was far more active than *L. ovalifolia*, whose activity was low within the range tested. That is consistent with Njina *et al.* (2016), who found the DPPH activity of aqueous *L. ovalifolia* extract to differ significantly from that of vitamin C, although Oteng Dapaah (2015) reported appreciable DPPH scavenging for a methanol leaf extract, so extraction conditions probably matter. Both extracts scavenged hydroxyl radicals,

the most reactive of the reactive oxygen species (Pham-Huy *et al.*, 2008), at least as strongly as ascorbic acid, and were close to each other in this assay. Overall, the picture partly agrees with the broader literature that places *L. aestuans* ahead as an antioxidant (Tijani *et al.*, 2022; Marpaung *et al.*, 2026), but it also shows that *L. ovalifolia* is not uniformly weaker.

The enzyme-inhibition results are the most notable. *L. ovalifolia* inhibited α-amylase more strongly than acarbose at all concentrations, which gives *in vitro* enzymatic support to its reputation as an antidiabetic plant (Tsabang *et al.*, 2015) and complements the glucose and lipid-lowering effects seen in alloxan-diabetic rats (Momo *et al.*, 2006). Both extracts also exceeded acarbose in α-glucosidase inhibition, consistent with the α-glucosidase inhibitory constituents previously isolated from *L. aestuans* (Essiett *et al.*, 2011). Because the inhibition by the extracts in this assay was flat across the concentration range and the IC_{50} values lie beyond it, the α-glucosidase ranking between the two species should be treated cautiously. Inhibition of both enzymes is considered useful for limiting post-meal glucose rise, so the two species may be complementary in this respect. Acarbose is a purified drug and the extracts are crude mixtures, so these findings indicate potential and do not establish therapeutic equivalence.

Taken together, *L. aestuans* is marked by higher yield, phenolic and flavonoid content, FRAP and DPPH activity and the presence of steroids, while *L. ovalifolia* is marked by higher TAC and stronger α-amylase inhibition. These consistent differences, in two species that are hard to separate on morphology, support the use of biochemical profiling as a complementary identification aid in *Laportea* (Essiett *et al.*, 2011; Feng *et al.*, 2024). The study has limits that should be kept in view: one collection site and season, leaves only, *in vitro* assays only, and a descriptive rather than statistical comparison between species. A single biochemical profile is not by itself a diagnostic character, and it should be combined with morphological and, ideally, molecular evidence.

CONCLUSION

The methanolic leaf extracts of *Laportea aestuans* and *Laportea ovalifolia* have distinct and complementary biochemical profiles. *L. aestuans* gave the higher yield, phenolic and flavonoid content, ferric reducing power and DPPH scavenging, and was the only species with detectable steroids. *L. ovalifolia* had the higher total antioxidant capacity and inhibited α-amylase more strongly than acarbose. These differences support biochemical profiling as a complementary tool for distinguishing the two species and lend experimental support to the

antidiabetic use of *L. ovalifolia*. Chromatographic profiling (HPLC or GC-MS), *in vivo* validation, other plant parts, and DNA barcoding alongside the biochemical markers are recommended, with collection season, plant part and solvent standardized across species.

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AUTHORS' CONTRIBUTIONS

AAO: Collection of plant materials, data collection and curation, drafting the manuscript. **IMO:** Formal analysis, writing, reviewing and editing. **AKF:** Methodology, visualization, formal analysis, supervision. **FAE:** Conceptualization, collection of plant materials, formal analysis, supervision, review and editing.

POTENTIAL CONFLICT OF INTEREST

None.

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