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RESEARCH ARTICLE

IN VITRO α -GLUCOSIDASE INHIBITORY ACTIVITY OF FOUR MEDICINAL PLANTS FROM BOUAFLÉ, CÔTE D'IVOIRE: INFLUENCE OF PLANT SPECIES AND EXTRACTION PROCEDURE

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Abstract

This preliminary study compared the in vitro α-glucosidase inhibitory activity of *Bersama abyssinica*, *Flacourtia flavescens*, *Pouteria alnifoli a* and *Sapium ellipticum* from Bouaflé, Côte d'Ivoire, using decoction, aqueous maceration and ethyl acetate maceration. Extracts were tested at final concentrations of 50, 125, 250 and 500 µg/mL against α-glucosidase from the digestive juice of *Archachatina ventricosa* using a sucrose/DNS spectrophotometric assay at 540 nm. At 500 µg/mL, *F. flavescens* showed the highest inhibition with ethyl acetate (63.72 ± 0.78%), followed by aqueous maceration (58.84 ± 0.94%), while *B. abyssinica* aqueous extract and *S. ellipticum* decoction reached 57.97 ± 1.25% and 52.21 ± 1.56%, respectively. *P. alnifolia* exhibited very weak activity (≤0.88%). Approximate graphical IC50 values were 273.47 µg/mL for *F. flavescens* (ethyl acetate), 278.15 µg/mL (aqueous maceration) and 416.28 µg/mL for *S. ellipticum* decoction. The reported LSD comparisons classified the twelve plant – extraction combinations into three activity groups; differences among species and extraction procedures are interpreted descriptively because plant species was not included as a separate factor in the original ANOVA model. No reference inhibitor was used, and the enzyme was a crude non mammalian preparation. These finding support further phytochemi cal and mechanistic investigations, although they do not establish in vivo antidiabetic efficacy.

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Introduction:-

Carbohydrate-hydrolysing enzymes are important targets in the management of postprandial hyperglycaemia. Inhibition of intestinal α-glucosidase delays the hydrolysis of dietary carbohydrates and may consequently attenuate the rapid increase in blood glucose after meals. Plant-derived α-glucosidase inhibitors have therefore received

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sustained attention as potential sources of bioactive molecules and as starting points for pharmacological research (Kumari *et al*., 2024; Benjamin *et al*., 2024; Yuca, 2024; Ashagrie *et al*., 2025). Synthetic inhibitors such as acarbose, miglitol and voglibose are used clinically, but they frequently cause gastrointestinal adverse effects, notably flatulence, abdominal pain and diarrhoea (Lebovitz, 1997), which justifies the search for alternative inhibitors of plant origin.

Recent reviews indicate that a wide chemical diversity of plant and microbial metabolites can inhibit α-glucosidase, including flavonoids, phenolic compounds, alkaloids, terpenes, saponins and related structures (Deng *et al*., 2025). The inhibitory response of a plant extract depends not only on species but also on the plant organ, extraction solvent and extraction conditions. Comparisons performed under a common enzymatic assay are therefore useful for identifying promising extracts and guiding subsequent phytochemical investigations.

Côte d'Ivoire has a rich medicinal flora and a long tradition of plant-based healthcare, including the use of plants against diabetes (Tra Bi *et al*., 2008). After a literature survey, four species were selected from the Bouaflé area (Marahoué region): *Bersama abyssinica* (stem bark decoction used against cancer and rheumatism; root bark infusion against migraine and colds; Arbonnier, 2000), *Flacourtia flavescens* (roots, twigs and leaves used against anaemia, colic, diarrhoea and dysentery; Arbonnier, 2002), *Pouteria alnifolia* (leaves and pulp used externally for yaws, wounds, rheumatism and conjunctivitis, and as an enema against childhood diarrhoea; Arbonnier, 2000) and *Sapium ellipticum* (roots, leaves and bark used as laxative, vermifuge and purgative, among other uses; Arbonnier, 2002). To our knowledge, none of these uses is specifically antidiabetic. Previous phytochemical screenings carried out in our laboratory detected polyphenols, tannins and saponins in *B. abyssinica*; alkaloids, flavonoids and anthraquinone glycosides in addition to polyphenols, tannins and saponins in *S. ellipticum* (Lagou, 2008); and only low amounts of saponins and sterols/polyterpenes in *P. alnifolia* (Monyn, 2008). The phytochemical findings cited here refer to earlier studies and not to chemical analyses performed on the extracts tested in the present work. Comparative information obtained under the same α-glucosidase assay conditions remains limited.

Flacourtia flavescens is of particular interest because recent phytochemical work has documented phenolic constituents in its leaves, including caffeic acid, apigenin, luteolin, kaempferol and quercetin derivatives (Ngnokam Jouogo *et al*., 2024). Such constituents provide a plausible chemical basis for biological investigation, although their presence does not demonstrate that they are responsible for α-glucosidase inhibition. *Sapium ellipticum* has also previously shown inhibitory activity against intestinal α-glucosidase in vitro (Ighodaro *et al*., 2016).

Previous work on α-glucosidase and related activities of these species is limited and has mainly examined different plant organs or different species. An ethanol leaf extract of *S. ellipticum* inhibited intestinal α-glucosidase in vitro (Ighodaro *et al*., 2016), and its leaves were active in diabetic rats (Ighodaro and Akinloye, 2017). For *B. abyssinica*, published studies concern the leaves, with α-amylase inhibition and hypoglycaemic effects in mice (Kifle and Enyew, 2020). Fruits of *F. indica* inhibited α-glucosidase and α-amylase in vitro (Makuttan *et al*., 2022); this is a different species from *F. flavescens* and is not treated here as direct evidence for the latter. For *P. alnifolia*, previous studies have reported antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Anyam, 2011). Thus, the present study contributes a comparative assessment of four plant species and specified plant organs under the same assay conditions and across three extraction procedures. To our knowledge, α-glucosidase inhibition has not previously been reported for *F. flavescens* leaves, *B. abyssinica* stem bark or *P. alnifolia* stem bark.

The objective of the present study was to compare the in vitro α-glucosidase inhibitory activity of the four plants using three extraction procedures and four concentrations from 50 to 500 µg/mL, to identify the most active species and extraction conditions, and to give approximate IC50 values for the most active extracts.

Materials and Methods:-

Plant material:-

Plant materials were collected in June 2008 at Tibéita, in the Bouaflé area (Marahoué region), Côte d'Ivoire, and were identified by Professor Aké Assi Laurent. No voucher specimen was deposited, and no voucher number is therefore available. Four medicinal plant species and their respective organs were investigated (Table 1). The collected materials were dried under air-conditioning conditions to limit mould development, ground into powder with a mill (RETSCH SM 100), and stored in tightly closed glass containers.

Table 1. Medicinal plants and plant organs used for extraction

Species	Family	Plant part
Bersama abyssinica var. paullinoïdes Fresen	Melianthaceae	Stem bark
Flacourtia flavescens Willd.	Salicaceae (formerly Flacourtiaceae)	Leaves
Pouteria alnifolia (Bak.) Roberty	Sapotaceae	Stem bark
Sapium ellipticum (Hochst.) Pax	Euphorbiaceae	Leaves

Preparation of plant extracts:-

Three extraction procedures were used. For decoction, 10 g of powdered plant material were mixed with 100 mL of distilled water and heated to boiling for 15 min. The hot preparation was filtered through Whatman No. 1 paper, frozen and lyophilised to obtain the dry crude extract. For maceration, 10 g of powdered material were placed in 100 mL of solvent (distilled water or ethyl acetate) and mechanically agitated for 24 h. The preparations were then vacuum-filtered through Whatman No. 1 paper. The aqueous macerate was frozen and lyophilised, whereas the ethyl acetate macerate was concentrated using a rotary evaporator at 40°C, to obtain the crude extracts.

Preparation of the enzyme source:-

The enzyme source was the digestive juice of *Archachatina ventricosa*, a gastropod belonging to the family Achatinidae. The preparation procedure was adapted from Colas and Attias (1977) as reported by Soro *et al*. (2007). The snails were kept without food for three days. Their shells were then broken, the digestive tract was separated from the visceral mass, and the digestive juice was collected by successive pressure. Mucus and shell debris were removed by filtration. The collected liquid was centrifuged at 3000 rpm for 10 min, and the supernatant was stored at 4°C as the enzyme preparation. Soro *et al*. (2007) characterised an α-glucosidase from this juice, which was used here as a locally available model of α-glucosidase activity. The preparation was diluted tenfold and its activity was not standardised; its specificity may differ from that of mammalian intestinal α-glucosidase.

DNS reagent and stock solutions:-

The DNS reagent was prepared by dissolving 1 g of 3,5-dinitrosalicylic acid, 1.6 g of sodium hydroxide and 30 g of sodium potassium tartrate in 100 mL of distilled water, with heating at 37°C until complete homogenisation. The reagent was protected from light. Stock solutions of plant extracts were prepared at 1 mg/mL by dissolving 10 mg of each crude extract in 10 mL of acetate buffer (50 mM, pH 5) and were kept at 4°C.

α-Glucosidase inhibitory assay:-

The enzymatic assay was based on the method of Bernfeld (1955), as applied to the α-glucosidase preparation from *A. ventricosa* by Soro *et al*. (2007). Four final concentrations (50, 125, 250 and 500 µg/mL) were prepared for each plant extract by adding 10, 25, 50 and 100 µL of the 1 mg/mL stock solution, respectively, to the reaction mixture. Each reaction mixture (final volume 200 µL) contained 50 µL of enzyme preparation diluted tenfold, 50 µL of 2% sucrose substrate, and 100 µL of extract plus acetate buffer (50 mM, pH 5), the buffer volume being adjusted according to the extract volume. A control tube containing the reaction mixture without plant extract was prepared. For each extract and concentration, a corresponding blank without enzyme was prepared to account for background absorbance and possible interference of extract constituents with the DNS reagent. The control reaction without extract was also corrected using its corresponding blank without enzyme. The absorbance of each blank was subtracted from the absorbance of its corresponding reaction before calculating percentage inhibition. The enzyme was added last, and the mixture was immediately incubated at 37 °C for 10 min. The reaction was stopped with 200 µL of DNS reagent (1 g 3,5-dinitrosalicylic acid, 1.6 g NaOH and 30 g sodium potassium tartrate in 100 mL distilled water). The mixture was heated at 95 °C for 5 min and cooled in an ice bath, and 100 µL were transferred to a microplate well for reading at 540 nm. All assays were performed in triplicate (n = 3).

Percentage inhibition was calculated from mean corrected optical densities (OD) according to Statti *et al*. (2006):
Inhibition (%) = [1 − (mean corrected OD of the extract reaction / mean corrected OD of the control reaction)] × 100,

where the mean corrected OD of the extract reaction is the mean absorbance of the reaction containing extract after subtraction of its corresponding blank, and the mean corrected OD of the control reaction is the mean absorbance of the reaction without extract after subtraction of its corresponding blank.

IC50 and Statistical analysis:-

IC_{50} values were estimated graphically from the concentration–inhibition curves and are approximate; no confidence intervals were calculated. They are reported for the most active extract of each extraction procedure. For *P. alnifolia*, the value is given as >500 µg/mL because 50% inhibition was not reached.

For Tables 2–4, results are expressed as mean ± standard error (SE) of triplicate measurements (n = 3). The available records do not establish whether these measurements represented independent biological experiments. Data were analysed using STATISTICA 8.0 (StatSoft) by two-way analysis of variance (ANOVA), with extract concentration and extraction procedure as factors. Fisher's least significant difference (LSD) test was used for multiple comparisons, as reported in the original analysis (Dagnelie, 1999), with significance set at $P \leq 0.05$. The original statistical output is no longer available; therefore, the degrees of freedom and detailed model-term statistics could not be verified. Quadratic regression and the coefficient of determination (R^2) were used to describe concentration–inhibition relationships only; they were not used to calculate the graphical IC_{50} estimates.

Results:-

Inhibition produced by decoctions, aqueous macerates and ethyl acetate macerates:-

Inhibition generally increased with concentration (Tables 2–4; Figure 1). At 500 µg/mL, *S. ellipticum* (52.21 ± 1.56 %) and *F. flavescens* (47.78 ± 0.62 %) were the most active decoctions, and *P. alnifolia* was very weakly active. Aqueous maceration generally gave higher responses than decoction for *B. abyssinica* and *F. flavescens* (57.97 ± 1.25 % and 58.84 ± 0.94 % at 500 µg/mL), whereas *S. ellipticum* remained lower. In ethyl acetate maceration, *F. flavescens* reached 63.72 ± 0.78 %, *S. ellipticum* was at least as active as *B. abyssinica* at every concentration, and *P. alnifolia* remained weakly active.

Table 2: Inhibition of α-glucosidase by decoction extracts of the four medicinal plants at different concentrations

Species	Concentration (µg/mL)			
	50	**125**	**250**	**500**
B. abyssinica	7.08 ± 0.94	19.47 ± 1.09	24.77 ± 0.15	26.11 ± 0.15
F. flavescens	25.22 ± 1.41	31.85 ± 1.25	42.48 ± 1.41	47.78 ± 0.62
P. alnifolia	0 ± 0	0 ± 0	0.88 ± 0.94	0.88 ± 0.94
S. ellipticum	22.12 ± 0.94	23.90 ± 0.78	40.71 ± 0.47	52.21 ± 1.56

Values are expressed as mean ± standard error (SE) of triplicate measurements (n = 3).

Table 3: Inhibition of α-glucosidase by aqueous maceration extracts of the four medicinal plants at different concentrations

Species	Concentration (µg/mL)			
	50	**125**	**250**	**500**
B. abyssinica	16.37 ± 0.78	30.97 ± 1.25	47.34 ± 1.41	57.97 ± 1.25
F. flavescens	23.00 ± 0.94	38.05 ± 0.94	46.46 ± 0.78	58.84 ± 0.94
P. alnifolia	0 ± 0	0 ± 0	0.44 ± 0.94	0.88 ± 1.09
S. ellipticum	15.93 ± 0.62	23.89 ± 0.62	24.33 ± 0.94	26.55 ± 0.62

Values are expressed as mean ± standard error (SE) of triplicate measurements (n = 3).

Table 4: Inhibition of α-glucosidase by ethyl acetate maceration extracts of the four medicinal plants at different concentrations

Species	Concentration (µg/mL)			
	50	**125**	**250**	**500**
B. abyssinica	4.42 ± 2.19	14.16 ± 0.94	17.69 ± 1.25	19.47 ± 1.25
F. flavescens	12.38 ± 1.25	27.43 ± 1.41	46.46 ± 1.09	63.72 ± 0.78
P. alnifolia	0 ± 0	0 ± 0	0 ± 0	0.44 ± 1.72
S. ellipticum	13.27 ± 0.94	14.16 ± 0.94	26.55 ± 0.94	34.07 ± 0.78

Values are expressed as mean ± standard error (SE) of triplicate measurements (n = 3).

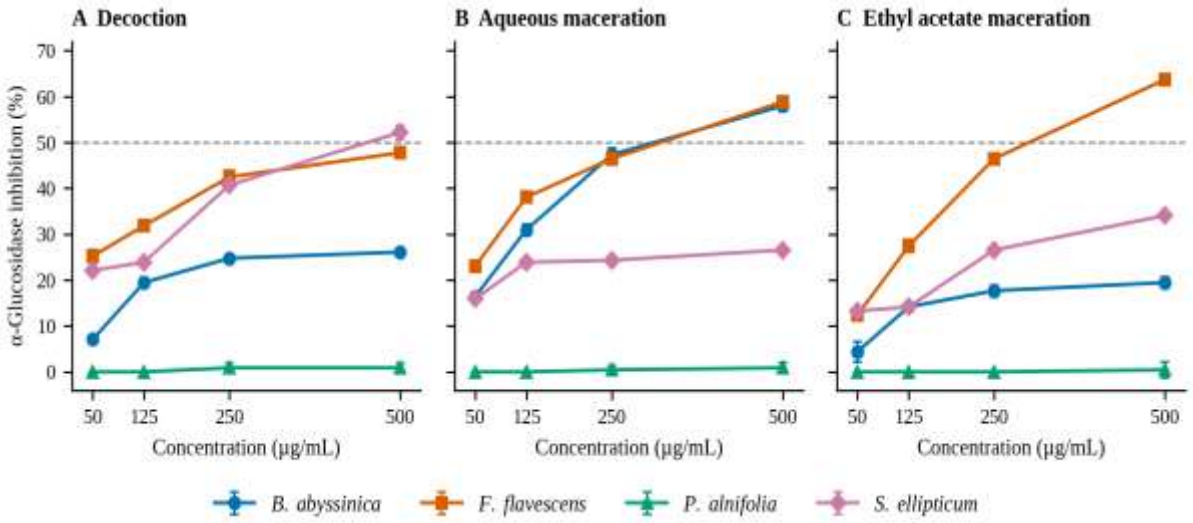


Figure 1: Concentration–inhibition curves of α-glucosidase activity for the four medicinal plants: (A) decoction, (B) aqueous maceration, (C) ethyl acetate maceration. Points represent means ± standard error (SE) of triplicate measurements (n = 3), as reported in Tables 2–4; the dashed line indicates 50% inhibition.

IC50 values:-

IC50 values were estimated for the most active extract of each extraction procedure (Table 5). The lowest values were obtained for *F. flavescens* with ethyl acetate and aqueous maceration, and for *S. ellipticum* with decoction, whereas *P. alnifolia* did not reach 50% inhibition at the highest concentration tested whatever the extraction procedure.

Table 5: IC_{50} values of the most active extracts against α-glucosidase

Species	Extraction	IC50 (µg/mL)
F. flavescens	Aqueous maceration	278.15
F. flavescens	Ethyl acetate maceration	273.47
S. ellipticum	Decoction	416.28
P. alnifolia	All three procedures	>500

IC_{50} values were estimated graphically from the concentration–inhibition curves.

Statistical classification:-

The original analysis included Fisher's least significant difference (LSD) comparisons of the twelve species × extraction combinations (Table 6). Group a comprised the aqueous macerates of *F. flavescens* and *B. abyssinica*, the ethyl acetate macerate of *F. flavescens*, and the decoctions of *S. ellipticum* and *F. flavescens*; group b comprised the remaining extracts of *S. ellipticum* and *B. abyssinica*; and group c comprised the three extracts of *P. alnifolia*. The highest reported mean inhibition (42.87 ± 4.94%) was obtained with the aqueous macerate of *F. flavescens*, although it did not differ significantly from the other group a extracts. The observed differences among plant species and extraction procedures are described as patterns in the data; they should not be interpreted as separate species effects or species × extraction interactions statistically established by the original ANOVA, because plant species was not included as a separate factor. Concentration–inhibition relationships were described by quadratic regression (R^2 = 0.858–1.000), with $R^2 \geq 0.90$ for ten of the twelve extracts; the exceptions were the decoction of *P. alnifolia* (R^2 = 0.898) and the aqueous macerate of *S. ellipticum* (R^2 = 0.858).

Table 6: Classification of plant extracts according to their overall α-glucosidase inhibitory activity and statistical comparison using Fisher's LSD test.

Species/extraction	Mean inhibition ± SE	Group
F. flavescens – aqueous	42.87 ± 4.94	a
B. abyssinica – aqueous	40.15 ± 5.95	a
F. flavescens – ethyl acetate	39.10 ± 7.24	a
S. ellipticum – decoction	36.95 ± 4.45	a
F. flavescens – decoction	35.18 ± 3.52	a
S. ellipticum – aqueous	23.67 ± 1.59	b
S. ellipticum – ethyl acetate	22.07 ± 2.89	b
B. abyssinica – decoction	20.19 ± 2.70	b
B. abyssinica – ethyl acetate	15.93 ± 2.15	b
P. alnifolia – decoction	3.21 ± 1.33	c
P. alnifolia – aqueous	1.94 ± 0.55	c
P. alnifolia – ethyl acetate	1.83 ± 0.71	c

Values are expressed as mean ± standard error (SE). Means followed by the same letter are not significantly different according to Fisher's least significant difference (LSD) test ($P \leq 0.05$). $F = 4.5701$; $P < 0.001$

Discussion:-

The comparative design provides a descriptive overview of α-glucosidase inhibition across four plant species, three extraction procedures and four concentrations. *F. flavescens* showed the most consistent inhibitory profile, with values of 47.78, 58.84 and 63.72% at 500 µg/mL for decoction, aqueous and ethyl acetate macerates, respectively, and the two lowest graphical IC50 estimates. Its three extracts were classified in the most active LSD group. This profile suggests that inhibitory constituents of *F. flavescens* may be recovered by both water and ethyl acetate, although the present experiment does not identify the compounds responsible. Overall, the reported means varied across extraction procedures; these patterns are descriptive and should not be interpreted as proof of an independent extraction effect for each species.

The phytochemical literature supports the interest of *F. flavescens*. Ngnokam Jouogo *et al*. (2024) reported a phenolic glycoside and several known phenolic constituents from its leaves, including caffeic acid and flavonoids. Phenolic compounds and flavonoids are among chemical classes frequently reported as α-glucosidase inhibitors (Kumari *et al*., 2024; Deng *et al*., 2025). These findings come from earlier work and not from the extracts tested here, which were not chemically characterised. Tannins and other polyphenols can also inhibit digestive enzymes through non-specific binding to proteins (Bruneton, 1999), so part of the inhibition observed with crude extracts may be non-specific. The present study cannot attribute the inhibition to any specific compound; bioassay-guided fractionation and direct testing of isolated constituents would be necessary.

B. abyssinica also showed substantial activity, particularly after aqueous maceration, which reached 57.97% inhibition at 500 µg/mL. The lower responses of its decoction and ethyl acetate extract suggest that extraction conditions affect recovery or stability of active constituents; the contrast between aqueous maceration at ambient temperature and decoction at boiling point could reflect the degradation of heat-labile constituents, but this was not tested. This supports the value of comparing extraction procedures under identical assay conditions.

S. ellipticum showed a distinct profile. Its decoction reached 52.21% inhibition at 500 µg/mL and produced an IC50 of 416.28 µg/mL, whereas aqueous and ethyl acetate macerates were less active. Ighodaro *et al*. (2016) previously reported inhibition of intestinal α-glucosidase by an ethanol leaf extract of *S. ellipticum*. The magnitude of inhibition should not be directly compared because solvent, concentration and assay conditions differed.

The very weak response of *P. alnifolia* was consistent across the three procedures, remaining below 1% at 500 µg/mL. This emphasises that traditional medicinal use does not necessarily predict strong inhibition of a particular enzyme target. Earlier phytochemical screening of this species detected only low amounts of saponins and sterols/polyterpenes (Monyn, 2008), which may be consistent with its weak activity, although the present dataset does not establish a causal relationship.

To our knowledge, this is the first report of α-glucosidase inhibition by *F. flavescens* leaves, *B. abyssinica* stem bark and *P. alnifolia* stem bark. For *B. abyssinica* and *S. ellipticum*, earlier studies used leaf extracts (Kifle and Enyew, 2020; Ighodaro *et al*., 2016; Ighodaro and Akinloye, 2017). Inhibition generally increased with extract concentration, consistent with concentration–response behaviour commonly reported for plant-derived α-glucosidase inhibitors (Benjamin *et al*., 2024; Yuca, 2024). The observed inhibition profiles varied across species and extraction procedures. However, these differences are interpreted descriptively because plant species was not included as a separate factor in the original ANOVA model.

The main strengths of the study are the comparative assessment of four species under common assay conditions, three extraction procedures and four concentrations. Its limitations should be acknowledged. The assay is an in vitro enzyme model and does not establish intestinal bioavailability, toxicity or antidiabetic efficacy in vivo. The enzyme source, the digestive juice of *A. ventricosa*, differs from purified mammalian intestinal α-glucosidase, and its activity was not standardised. No reference inhibitor such as acarbose was included, so the potency of the extracts could not be benchmarked against a standard. Although corresponding blanks were used to correct extract and control absorbances, no independent alternative assay was performed to fully exclude interference by extract constituents with the DNS reaction. IC50 values are approximate graphical estimates without confidence intervals. The plant material was collected in 2008, no voucher specimen was deposited, and extraction yields and the exact maceration temperature were not recorded. Only crude extracts were tested and no direct chemical profiling of the tested extracts was performed. Further work should include a reference inhibitor, standardised enzyme activity, assays using mammalian α-glucosidase, an independent assay to assess DNS interference, phytochemical profiling, fractionation, testing of isolated compounds and toxicity studies.

Conclusion:-

The four medicinal plants displayed different in vitro α-glucosidase inhibitory profiles under the conditions tested. *F. flavescens* showed the most consistent activity, with inhibition of 63.72 ± 0.78% at 500 µg/mL for the ethyl acetate macerate and 58.84 ± 0.94% for the aqueous macerate, together with approximate graphical IC50 values of 273.47 and 278.15 µg/mL, respectively. *B. abyssinica* aqueous macerate and *S. ellipticum* decoction also showed substantial inhibition, whereas *P. alnifolia* was weakly active. The observed profiles varied across botanical sources and extraction procedures, but these patterns should be interpreted descriptively because plant species was not included as a separate factor in the original ANOVA model. *F. flavescens* merits further phytochemical and mechanistic investigation, including identification of active compounds and assessment using mammalian enzyme systems and complementary assays. These results constitute preliminary in vitro evidence and do not demonstrate antidiabetic efficacy.

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No external funding was reported for this study.

Conflict of Interest:-

The authors declare no conflict of interest.

Data Availability:-

The data supporting the findings of this study are included in the article (Tables 2–6 and Figure 1). The original raw data files and statistical output from the 2013 experiments are no longer available. Other information is available from the corresponding author on reasonable request.

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