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

Abstract: This study comparatively evaluated the in vitro α-glucosidase inhibitory activity of *Bersama abyssinica*, *Flacourtiaflavescens*, *Pouteria alnifolia* and *Sapiumellipticum* 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 *Archachatinaventricosa* 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%). The lowest IC50 values were recorded for *F. flavescens* with ethyl acetate (273.47 µg/mL) and aqueous maceration (278.15 µg/mL). Significant differences were observed among extracts ($F = 4.5701$; $P < 0.001$). *F. flavescens* was the most consistent species, being the only one whose three extracts all belonged to the most active statistical group, which highlights the influence of both plant species and extraction procedure. These findings support further phytochemical and mechanistic investigations, although they do not establish in vivo antidiabetic efficacy.

Key words:-
α-glucosidase; medicinal plants; *Flacourtiaflavescens*; plant extracts; Côte d'Ivoire

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Introduction:- Carbohydrate-hydrolyzing enzymes are important targets in the management of postprandial hyperglycemia. 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 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), *Flacourtiaflavescens* (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 *Sapiumellipticum* (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 *F. flavescens* and *S. ellipticum*; and only low amounts of saponins and sterols/polyterpenes in *P. alnifolia* (Monyn, 2008; Lagou, 2008). However, comparative information obtained under the same α-glucosidase assay conditions remains limited.

Flacourtiaflavescens is of particular interest because recent phytochemical work has documented phenolic constituents in its leaves, including caffeic acid, apigenin, luteolin, kaempferol and quercetin derivatives (NgnokamJouogo*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. *Sapiumellipticum* has also previously shown inhibitory activity against intestinal α-glucosidase in vitro (Ighodaro*et al*., 2016).

The objective of the present study was to compare the in vitro α-glucosidase inhibitory activity of the four medicinal plants using three extraction procedures (decoction, aqueous maceration and ethyl acetate maceration) and four concentrations ranging from 50 to 500 µg/mL. The comparative design was intended to identify the most active species and extraction conditions and to provide IC50 estimates 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 AkéAssi Laurent. 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 organsused for extraction

Species	**Family**	**Plant part**
Bersama abyssinica var. *paullinoïdes*Fresen	Melianthaceae	Stem bark
*Flacourtiaflavescens*Willd.	Salicaceae (formerly Flacourtiaceae)	Leaves
Pouteria alnifolia (Bak.) Roberty	Sapotaceae	Stem bark

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Sapiumellipticum (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 lyophilized 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 lyophilized, 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 *Archachatinaventricosa*, 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) characterized an α-glucosidase from *A. ventricosa* digestive juice, supporting the use of this biological source for the present assay.

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 homogenization. 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 and a blank containing sucrose and buffer were also prepared. The enzyme solution was added last, after which the reaction mixture was immediately incubated in a water bath at 37°C for 10 min. The reaction was stopped by adding 200 µL of DNS reagent. The mixture was heated at 95°C for 5 min, cooled in an ice bath, and 100 µL was transferred to a microplate well. Optical density (OD) was measured at 540 nm. The experiments were repeated. The DNS procedure is based on colorimetric determination of reducing sugars released during carbohydrate hydrolysis (Bernfeld, 1955). Percentage inhibition was calculated from the mean optical densities according to Statti*et al*. (2006) as:

$$\text{Inhibition (\%)} = [1 - (\text{mean OD extract} / \text{mean OD control})] \times 100,$$

where OD extract is the optical density in the presence of plant extract and OD control is the optical density of the control reaction.

IC50 determination:-

The concentration producing 50% inhibition (IC50) was estimated graphically from the concentration–inhibition curves. IC50 values are reported for the most active extract of each extraction procedure. For *P. alnifolia*, the IC50 was reported as >500 µg/mL because 50% inhibition was not reached at the highest concentration tested.

Statistical analysis:-

Data were analysed with STATISTICA version 8.0 (StatSoft). The influence of extract concentration and of the extraction procedure on percentage inhibition was assessed by two-way analysis of variance (ANOVA). The mean inhibition values of the twelve species × extraction combinations were then compared with Fisher's least significant difference (LSD) test

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(Dagnelie, 1999), and differences were considered significant at $P \leq 0.05$. The relationship between extract concentration and inhibitory response was examined by regression analysis, and the coefficient of determination (R^2) was calculated for each extract; $R^2 \geq 0.90$ was taken as indicating a strong correlation.

Results:-

Inhibition produced by decoctions:-

The α-glucosidase inhibitory activity of the decoction extracts varied according to both plant species and extract concentration (Table 2; Figure 1A). Overall, the inhibitory activity increased with increasing concentration for most species. *S. ellipticum* showed the highest inhibition at 500 µg/mL (52.21 ± 1.56%), followed by *F. flavescens* (47.78 ± 0.62%), whereas *P. alnifolia* exhibited very weak activity across the tested concentration range.

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

Inhibition produced by aqueous macerates:-

Aqueous maceration generally produced higher inhibitory responses than decoction for *B. abyssinica* and *F. flavescens*, whereas the response of *S. ellipticum* remained comparatively lower (Table 3; Figure 1B). At 500 µg/mL, *F. flavescens* (58.84 ± 0.94%) and *B. abyssinica* (57.97 ± 1.25%) were the most active extracts.

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

Inhibition produced by ethyl acetate macerates:-

The inhibitory activity of the ethyl acetate extracts also varied markedly among species and concentrations (Table 4; Figure 1C). *F. flavescens* exhibited the highest response, reaching 63.72 ± 0.78% inhibition at 500 µg/mL, *S. ellipticum* was at least as active as *B. abyssinica* at every concentration, and *P. alnifolia* remained weakly active.

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

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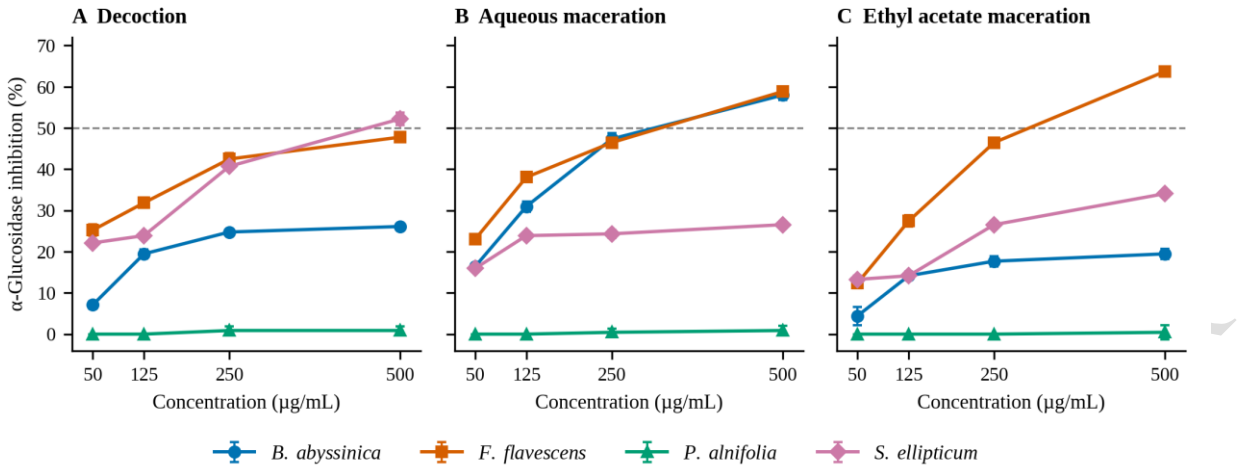


Figure 1: Concentration–inhibition curves of α-glucosidase activity for the four medicinal plants: (A) decoction, (B) aqueous maceration, (C) ethyl acetate maceration. Points are means ± error as in Tables 2–4; the dashed line indicates 50% inhibition.

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

Statistical classification:-

Two-way ANOVA revealed highly significant differences among extracts ($F = 4.5701$; $P < 0.001$). The LSD test grouped the twelve species × extraction combinations into three statistical groups (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 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. Within each plant, the extraction procedure significantly affected the inhibition ($P < 0.001$): *F. flavescens* remained highly active whatever the procedure, *B. abyssinica* was most active with aqueous maceration, *S. ellipticum* with decoction, and *P. alnifolia* showed very weak activity with all procedures. Overall, aqueous macerates gave the highest mean activities. Concentration–inhibition relationships were well 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: Mean α-glucosidase inhibition (%, mean ± SE) of the plant extracts and statistical comparison (LSD test; extracts sharing the same letter do not differ significantly; A: highest activity, B: intermediate, C: weak)

Species/extraction	Mean inhibition ± SE	Group
F. flavescens – aqueous	42.87 ± 4.94	A

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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

Discussion:-

The comparative design reveals a clear species- and extraction-dependent pattern of α-glucosidase inhibition. *F. flavescens* was the most consistent inhibitor, with high activity under the three extraction conditions (47.78, 58.84 and 63.72% at 500 µg/mL for decoction, aqueous and ethyl acetate macerates, respectively) and the two lowest IC50 values. It was the only species whose three extracts all belonged to the most active statistical group, although some of its extracts did not differ significantly from the most active extracts of *B. abyssinica* and *S. ellipticum*. This profile suggests that inhibitory constituents of *F. flavescens* are efficiently recovered by water as well as by ethyl acetate, although the present experiment does not identify the compounds responsible. Overall, aqueous macerates gave the highest mean activities, which is compatible with the extraction of polar constituents such as phenolic compounds and tannins by water.

The phytochemical literature supports the interest of *F. flavescens*. NgnokamJouogo*et al.* (2024) reported a phenolic glycoside and several known phenolic constituents from its leaves, including caffeic acid and flavonoids, and earlier screening in our laboratory detected alkaloids, polyphenols, flavonoids, tannins and saponins in its leaves and stem bark (Lagou, 2008). Phenolic compounds and flavonoids are among chemical classes frequently reported as α-glucosidase inhibitors (Kumari *et al*., 2024; Deng *et al*., 2025). However, 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 emphasizes 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.

Overall, inhibition generally increased with extract concentration, consistent with the concentration-response behavior commonly reported for plant-derived α-glucosidase inhibitors (Benjamin *et al.*, 2024; Yuca, 2024). The statistical

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differences among extracts further show that concentration alone does not explain the response: activity depended on the combination of species and extraction procedure.

The main strengths of the study are the direct comparison of four species under common assay conditions, three extraction procedures and four concentrations. Its limitations should be acknowledged. It is an in vitro enzyme model that 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. No reference inhibitor such as acarbose was included, so the potency of the extracts could not be benchmarked against a standard, and possible interference of extract constituents with the DNS reaction was not specifically evaluated. Finally, only crude extracts were tested. Further work should include a reference inhibitor, enzyme kinetics, assays on mammalian α-glucosidase and on other digestive enzymes such as α-amylase, phytochemical profiling, fractionation, testing of isolated compounds and toxicity studies.

Conclusion:-

The four medicinal plants displayed markedly different in vitro α-glucosidase inhibitory profiles. *F. flavescens* was the most promising species, 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 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 findings show that both botanical source and extraction procedure influence α-glucosidase inhibition. *F. flavescens* merits further phytochemical and mechanistic investigation, including the identification of the active compounds and the assessment of its effect on other digestive enzymes. These results should be considered preliminary in vitro evidence rather than proof of antidiabetic efficacy.

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