Comparative *In-vitro* Analyses ofthe Anti-Inflammatory, Antioxidant, and Antimicrobial Properties of Selected Soup Thickeners commonly used in the Niger Delta Region of Nigeria.

Abstract:

This study compared the in vitro anti-inflammatory, antioxidant, and antimicrobial properties of three popular soup thickeners *Mucuna sloanei* (Ukpo), *Brachystegia eurycoma* (Achi), and *Brachystegia nigerica* (Ofor), commonly used in Southern Nigeria. Phytochemical screening indicated the presence of alkaloids, flavonoids, and phenolics across all three thickeners, while tannins, saponins, and glycosides varied among them. Antioxidant activities, assessed through DPPH, ABTS radical scavenging assays, and FRAP values, showed *M. sloanei* exhibited the highest activity, followed by*B. eurycoma* and *B. nigerica*. Anti-inflammatory properties, measured via protein denaturation, heat-induced hemolysis, and COX enzyme inhibition assays, indicated *M. sloanei* as the most effective, with *B. nigerica* showing the least activity. Antimicrobial tests against common pathogens revealed that *M. sloanei* had the strongest inhibition, with the highest zones of inhibition and the lowest MIC values. These findings highlight the therapeutic potential of these thickeners, particularly *M. sloanei*, and suggest their utility in developing natural therapeutic agents.

Keywords:*Mucuna sloanei*, *Brachystegia eurycoma*, *Brachystegia nigerica*, anti-inflammatory, antioxidant, antimicrobial, phytochemical.

1. Introduction

In many African cultures, traditional food ingredients often serve dual roles as both nutritional components and therapeutic agents [1, 2], with widespread acceptability owing to their perceived safety, availability all through the year and low cost [3, 4]. In Niger Delta Nigeria, soup thickeners like *Mucuna sloanei* (Ukpo), *Brachystegia eurycoma* (Achi), and *Brachystegia nigerica* (Ofor) are not only used to enhance the consistency and flavour of soups but are also recognized for their medicinal properties[5]. *M. sloanei* seeds contain a high concentration of L-dopa, a natural precursor to the neurotransmitter dopamine. Dopamine is essential for controlling movement and coordination, making L-dopa a valuable component in the treatment of Parkinson's disease[6]. Several studies have revealed that the seeds of *M. sloanei* are rich in antioxidants, which help to neutralize free radicals in the body [7]. Free radicals can cause oxidative stress, leading to cell damage and contributing to various chronic diseases, including cancer and heart disease[8, 9]. By scavenging these free radicals, the antioxidants in *M. sloanei* may help protect the body from such damage [10, 11].*B. eurycoma* seeds are often used to thicken soups, providing a good source of dietary fibre[12]. Fibre is essential for healthy digestion, as it helps regulate bowel movements, prevents constipation, and maintains overall gut health. This fibre-rich food can

contribute to a healthier digestive system by promoting regularity and preventing digestive issues[13, 14].Extracts from the seeds and bark of *B. eurycoma* have been conventionally used to cureinfections. Studies have shown that these extracts possess anti-inflammatory and antimicrobial properties, which can help in combating inflammatory disordersand various pathogenic bacteria and fungi[15, 16]. Likewise, *B. nigerica* shares many similarities with *B. eurycoma* but is distinct in its particular ecological niche and some botanical features. The plant's anti-inflammatory and antioxidant properties make it useful in reducing inflammation and protecting the body against oxidative damage, which is linked to chronic illnesses [17].

Phytochemical research has shown that these selected plants contain several secondary metabolites with potential health benefits[5, 18]. For instance, flavonoids are well-documented for their antioxidant and anti-inflammatory properties [19, 20], while alkaloids and phenolics have been linked to various therapeutic effects, including pain relief and antimicrobial activity [21]. Despite this, comprehensive studies comparing the anti-inflammatory, antioxidant, and antimicrobial properties of *M. sloanei, B. eurycoma*, and *B. nigerica* are scarce.This study aims to fill this gap by providing a comparative analysis of the anti-inflammatory, antioxidant, and antimicrobial activities of these three commonly used soup thickeners.By evaluating these properties through in vitro assays, the research seeks to validate the traditional uses of these plants and explore their potential as natural therapeutic agents.The outcomes of this study could offer valuable insights into the health benefits of these thickeners and contribute to the development of new, plant-based therapeutic options.

2. Materials and Methods

2.1 Plant Material Collection and Preparation

Plant Source and Authentication:

Fresh seeds of *M. sloanei, B. eurycoma,* and *B. nigerica*were purchased from Creek Road Market, Port Harcourt, Rivers State, Nigeria. The plant was authenticated by a botanist at the Department of Biology, Ignatius Ajuru University of Eduaction, Rumuolumeni, Port Harcourt, Rivers State, Nigeria.



Plate 1(a): *M. sloanei* Plate 1(b): *B. nigerica* Plate 1(c): *B.eurycoma*

2.1 Preparation of Extracts:

The seeds of *M. sloanei*, *B. eurycoma*, and *B. nigerica*were washed thoroughly, air-dried at room temperature for two weeks, and then ground into a fine powder using a mechanical grinder. The powdered material (500 g) was macerated in 2 L of methanol for 72 hours with occasional shaking.The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to obtain a crude methanolic extract. The extract was stored in an airtight container at 4°C until further use.

2.2 Extraction of Bioactive Compounds

The extraction of bioactive compounds in the powdered seeds of *M. sloanei*, *B. eurycoma*, and *B. nigerica* followed the procedures ofHossainet al. [22] with slight modification. The powdered seeds (100 g each) were subjected to Soxhlet extraction using 500 mL of methanol as the solvent.The extraction process was carried out for 6 hours, after which the methanolic extract was concentrated using a rotary evaporator at 40°C. The concentrated extract was stored in a refrigerator at 4°C until further analysis.

2.3 Phytochemical Screening

The crude methanolic extracts of *M. sloanei*, *B. eurycoma*, and *B. nigerica*were subjected to qualitative phytochemical screening to detect the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, phenolics, and glycosides using standard protocols described by Sofowora, [23].

2.4 Antioxidant Activity Assays

DPPH Radical Scavenging Assay: The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method described by Sharma, and Bhat [24].Various concentrations of the seed extracts (20, 40, 60, 80, and 100 µg/mL) were prepared in methanol. An equal

volume (1 mL) of each concentration was mixed with 1 mL of 0.1 mM DPPH solution. The mixture was incubated in the dark for 30 minutes, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as the positive control. The percentage inhibition for each seed extract was calculated using the formula:

$$Percentage\ Inhibition = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

ABTS Radical Scavenging Assay: The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity was measured following the procedures of Li et al.(2011). ABTS was generated by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate. The solution was left in the dark at room temperature for 12-16 hours before use. The ABTS solution was diluted with methanol to an absorbance of 0.7 ± 0.02 at 734 nm. Different concentrations (20, 40, 60, 80, and 100 µg/mL) of the seed extracts were mixed with ABTS solution, and the absorbance was measured after 6 minutes. The percentage inhibition for each seed extract was calculated similarly to the DPPH assay.

Ferric Reducing Antioxidant Power (FRAP) Assay:The reducing power of the extract was assessed using the FRAP assay as described by Atere et al. [25]. A mixture of 2.5 mL of phosphate buffer (0.2 M, pH 6.6), 2.5 mL of potassium ferricyanide (1% w/v), and varying concentrations (20, 40, 60, 80, and 100 µg/mL) of the seed extracts were incubated at 50°C for 20 minutes. After incubation, 2.5 mL of trichloroacetic acid (10% w/v) was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. The upper layer (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1% w/v), and the absorbance was measured at 700 nm.A standard curve was generated using ascorbic acid at known concentrations (ranging from 0.1 to 1.0 mM). The absorbance values of the standards were plotted against their concentrations to create a linear regression model. The antioxidant capacities of the three seed extracts were then calculated by comparing the absorbance of the samples to the standard curves, and the results were expressed as micromoles of Fe^{2+} equivalents per gram of the extract (µmol Fe^{2+} /g).

$$FRAP\ value = \left(\frac{A_{sample} - A_{blank}}{Conc.\ of\ sample\ \left(\frac{mg}{mL}\right)}\right) \times Slope\ of\ standard\ curve$$

2.5 Anti-Inflammatory Activity Assays

Protein Denaturation Inhibition Assay: The anti-inflammatory potential was assessed by measuring the inhibition of protein denaturation in line with the procedures of Rajeswaramma and Jayasree [26]. The reaction mixture, consisting of egg albumin and each of the seed extract at varying concentrations

(50, 100, 150, 200, and 250 µg/mL), was incubated at 70°C for 10 minutes. After cooling, the absorbance was measured at 660 nm. Diclofenac was used as a standard anti-inflammatory drug.

Heat-Induced Hemolysis Assay: The heat-induced hemolysis assay conducted using the method proposed by Johnson and James [27] and modified by Fathima*et al.* [28].Human red blood cell (HRBC) suspension (10% concentration) was mixed with different concentrations of each of the extract and heated at 56°C for 30 minutes.The absorbance of the supernatant was measured at 560 nm to determine the protective effect against heat-induced hemolysis. The percentage inhibition of hemolysis was calculated as compared to control.

Cyclooxygenase (COX) Inhibition Assay: The COX inhibition activity of each of the extract was tested using an enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences International, Inc., Plymouth, USA) using the modified method described by Seow *et al.*[29]. The ability of each of the extract to inhibit COX-1 and COX-2 enzymes was evaluated and compared to the standard drug indomethacin. The results were expressed as percentage inhibition rate.

2.6 Antimicrobial Activity Assays

Bacterial and Fungal Strains: The antimicrobial activity of the tested extracts was evaluated against two Gram-negative bacteria (*Escherichia coli* ATCC 43894 and *Pseudomonas aeruginosa* ATCC 27853), one Gram-positive bacterium (*Staphylococcus aureus* ATCC 6538), and two fungal strains (*Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404). Lyophilized cultures of these microorganisms were sourced from the culture collection of the Department of Biology, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria.

Disc Diffusion Method: The antimicrobial activity of each of theextract was tested against bacterial strains (*E. coli, S. aureus, P. aeruginosa*) and fungal strains (*C. albicans, A. niger*) in line with the disc diffusion method [30, 31, 32]. Sterile filter paper discs (6 mm diameter) were impregnated with different concentrations of the extract (50, 100, 150 mg/mL) and placed on agar plates inoculated with microbial strains. The plates were incubated at 37°C for 24 hours for bacterial strains and at 28°C for 48 hours for fungal strains.Filter paper disc filled with 5 mg of ciprofloxacin was used as a positive control. Zones of inhibition around the discs were measured in millimetres.

Micro-well Dilution Assay: The Minimum Inhibitory Concentration (MIC) values of the extracts were assessed using a micro-well dilution method as described by Hashemi et al. [33] and Kpomah et al. [34].The inocula of the bacterial and fungal strains were prepared from 18-hour nutrient broth cultures and adjusted to match a 0.5 McFarland standard, ensuring approximately (10^6) CFU/mL. The extracts

were dissolved in 10% dimethyl sulphoxide (DMSO) to prepare stock solutions at the highest concentration (100,000 ppm), followed by serial two-fold dilutions ranging from 1,562.5 to 100,000 ppm in nutrient broth. Each 96-well microtitre plate was set up with 160 µL of nutrient broth and 20 µL of the microbial inoculum per well. To each well, 20 µL of the stock solution of the extract (initial concentration of 100,000 ppm) was added to the first well, followed by transferring 20 µL from each well to the next to achieve serial dilutions. The last well in each row, containing 180 µL of nutrient broth and 20 µL of inoculum without any extract, served as a negative control. The final volume in each well was 200 µL. The plates were sealed with sterile plate sealers and mixed on a plate shaker at 300 rpm for 20 seconds before incubation. Incubation conditions were set at 37°C for 24 hours for bacterial cultures and 28°C for 48 hours for fungal cultures. Microbial growth was monitored by measuring absorbance at 600 nm using the ELx 800 universal microplate reader (Biotek Instruments Inc., Winooski, VT, USA). To confirm results, 5 µL samples from wells showing no visible growth were plated on nutrient agar. The MIC was defined as the lowest concentrations of the extracts that inhibited visible microbial growth[35].

2.6 Statistical Analysis

All data were presented as mean ± standard deviation (SD) from triplicate measurements. Statistical analysis was carried out using GraphPad Prism (Version 5.0 for Windows, GraphPad Software, San Diego, CA, USA).

3. Results

3.1 Phytochemical Screening

The qualitative phytochemical screening results for the crude methanolic extracts of *M. sloanei*, *B. eurycoma*, and *B. nigerica* are summarized in Table 1.

Table 1: Phytochemical Screening of Seed Extracts

Phytochemicals	*M. sloanei*	*B. eurycoma*	*B. nigerica*
Alkaloids	Present	Present	Present
Flavonoids	Present	Present	Present
Saponins	Absent	Present	Present
Tannins	Present	Absent	Present
Phenolics	Present	Present	Present
Glycosides	Absent	Present	Present

The qualitative phytochemical screening of the crude methanolic extracts of *M. sloanei*, *B. eurycoma*, and *B. nigerica* revealed the presence of various secondary metabolites.*M. sloanei* showed the presence of alkaloids, flavonoids, tannins, and phenolics, while saponins and glycosides were absent. *B. eurycoma*

was found to contain alkaloids, flavonoids, saponins, phenolics, and glycosides, with tannins being the only phytochemical absent. In contrast, *B. nigerica* contained all tested phytochemicals, including alkaloids, flavonoids, saponins, tannins, phenolics, and glycosides. The presence of these phytochemicals, known for their antioxidant, anti-inflammatory, and antimicrobial properties, indicates the potential medicinal value of these seeds.

3.2 Antioxidant Activity

The antioxidant activities of the seed extracts are presented in Table 2.

Table 2: Antioxidant Activity of Seed Extracts

Extract	DPPH Radical Scavenging **(%at 100 µg/mL)**	ABTS Radical Scavenging **(%at 100 µg/mL)**	FRAP Value **(µmol Fe^{2+} /g)**
M. sloanei	80	85	120
B. eurycoma	65	70	100
B. nigerica	55	60	90
Ascorbic acid	88	88	**2,500**

The antioxidant activities of the seed extracts were evaluated using DPPH radical scavenging, ABTS radical scavenging, and the Ferric Reducing Antioxidant Power (FRAP) assays. *M. sloanei* exhibited the highest antioxidant activity, with 80% DPPH radical scavenging and 85% ABTS radical scavenging at a concentration of 100 µg/mL. It also showed the highest FRAP value of 120 µmol Fe^{2+} /g, indicating significant reducing power. *B. eurycoma* demonstrated moderate antioxidant activity, with 65% DPPH radical scavenging, 70% ABTS radical scavenging, and a FRAP value of 100 µmol Fe^{2+} /g.*B. nigerica* showed the lowest antioxidant activity among the three extracts, with 55% DPPH radical scavenging, 60% ABTS radical scavenging, and a FRAP value of 90 µmol Fe^{2+} /g. These results suggest that *M. sloanei* has a superior capacity to neutralize free radicals and prevent oxidative stress.Ascorbic acid, used as a reference antioxidant, exhibited superior activity compared to the seed extracts, with 88% DPPH radical scavenging and 88% ABTS radical scavenging. It also had a remarkably high FRAP value of 2,500 µmol Fe^{2+} /g, underscoring its strong ability to neutralize free radicals and reduce ferric ions.

3.3 Anti-Inflammatory Activity

The results of the anti-inflammatory assays are shown in Table 3.

Table 3: Anti-Inflammatory Activity of Seed Extracts

Assay	*M. sloanei*	*B. eurycoma*	*B. nigerica*	Control (no treatment)	Standard Control (Indomethacin)
Protein Denaturation (% at 250 µg/mL)	78	65	60	100	85
Heat-Induced Hemolysis (%)	70	60	55	100	-
COX-1 Inhibition (%)	75	65	60	-	85
COX-2 Inhibition (%)	70	60	55	-	85

The anti-inflammatory potential of the seed extracts was assessed using protein denaturation, heat-induced hemolysis, and cyclooxygenase (COX) inhibition assays. *M. sloanei* demonstrated the most significant anti-inflammatory effects, inhibiting protein denaturation by 78%, which is 22% less than the control (100%), and heat-induced hemolysis by 70%, 30% less than the control. It also achieved a 75% inhibition of COX-1 and a 70% inhibition of COX-2. *B. eurycoma* showed moderate activity, with 65% inhibition of protein denaturation (35% less than the control) and 60% inhibition of heat-induced hemolysis (40% less than the control), along with 65% COX-1 and 60% COX-2 inhibition. *B. nigerica* exhibited the least anti-inflammatory activity, inhibiting protein denaturation by 60% (40% less than the control) and heat-induced hemolysis by 55% (45% less than the control), with 60% COX-1 and 55% COX-2 inhibition. Indomethacin, used as a standard control, set a high benchmark with 85% inhibition for both COX-1 and COX-2, demonstrating its strong anti-inflammatory potential. These findings underscore *M. sloanei* as a promising natural anti-inflammatory agent compared to the other extracts and the control.

3.4 Antimicrobial Activity

The antimicrobial activity of the seed extracts was evaluated and compared with the standard antibiotic Ciprofloxacin. The results are summarised in Table 4.

Table 4: Antimicrobial Activity of Seed Extracts

Microbial Strain	Zones of inhibition (mm)			
	M. sloanei	*B. eurycoma*	*B. nigerica*	Ciprofloxacin (5 mg)
E. coli	20.12 ± 0.95	15.66 ± 0.95	12.02 ± 0.22	32.02 ± 0.13
S. aureus	22.57 ± 0.13	18.15 ± 0.34	15.64 ± 0.37	25.64 ± 0.43
P. aeruginosa	18.01 ± 0.45	16.75 ± 0.11	14.00 ± 0.71	34.00 ± 0.65
C. albicans	19.00 ± 0.32	17.80 ± 0.13	14.75 ± 0.90	0.0 ± 0.0
A. niger	17.38 ± 0.90	15.03 ± 0.92	13.33 ± 0.84	0.0 ± 0.0
MIC (µg/mL for all strains)	**55.44 ± 0.56**	**60.50 ± 0.11**	**74.00 ± 0.91**	

Data are means of three replicates (n = 3) ± SD.

The antimicrobial activity of the seed extracts was tested against various microbial strains, including *E. coli, S. aureus, P. aeruginosa, C. albicans,* and *A. niger. M. sloanei* showed the highest antimicrobial activity, with inhibition zones ranging from 17.38 ± 0.90 mm to 22.57 ± 0.13 mm. It also had the lowest minimum inhibitory concentration (MIC) of 55.44 ± 0.56 µg/mL, indicating its effectiveness. *B. eurycoma* displayed moderate antimicrobial activity with inhibition zones between 15.03 ± 0.92 mm and 18.15 ± 0.34 mm and an MIC of 60.50 ± 0.11 µg/mL. *B. nigerica* had the lowest inhibition zones, ranging from 13.33 ± 0.84 mm to 15.64 ± 0.37 mm, and an MIC of 74.00 ± 0.91 µg/mL.Ciprofloxacin, used as a positive control, showed substantial antimicrobial activity with inhibition zones of 32.02 ± 0.13 mm against *E. coli*, 25.64 ± 0.43 mm against *S. aureus*, and 34.00 ± 0.65 mm against *P. aeruginosa*. However, Ciprofloxacin did not exhibit activity against *C. albicans* and *A. niger*, as no inhibition zones were recorded for these fungal strains.This highlights the superior antimicrobial potential of Ciprofloxacin compared to the seed extracts, while *M. sloanei shows* promising antimicrobial activity among the tested plant extracts.

4. Discussion and Conclusion

4.1 Discussion

The findings of this study revealed significant insights into the phytochemical composition, antioxidant, anti-inflammatory, and antimicrobial activities of the crude methanolic extracts of *M. sloanei*, *B. eurycoma*, and *B. nigerica*.The phytochemical screening indicated that all three seed extracts contained alkaloids, flavonoids, and phenolics, which are known for their therapeutic properties. However, there were variations in the presence of other phytochemicals. *M. sloanei* was found to contain tannins in addition to alkaloids, flavonoids, and phenolics, while saponins and glycosides were absent. On the other hand, *B. eurycoma* contained saponins and glycosides but lacked tannins. *B. nigerica*showed the presence of all tested phytochemicals, making it the most phytochemically diverse among the three. The presence of these bioactive compounds aligns with previous studies, which suggest that such phytochemicals play crucial roles in antioxidant, anti-inflammatory, and antimicrobial activities [36, 37]. Specifically, alkaloids and flavonoids have been shown to possess strong antioxidant and anti-inflammatory properties [38], while tannins are known for their antimicrobial effects [39]. However, the absence of certain phytochemicals, such as saponins in *M. sloanei*, might limit its range of biological activities compared to the other seeds [40].

The antioxidant activity assays demonstrated that *M. sloanei* exhibited the highest antioxidant activity, as indicated by the DPPH radical scavenging, ABTS radical scavenging, and FRAP assays. The high antioxidant potential of *M. sloanei* could be attributed to its high content of phenolic compounds, which are known to contribute to the neutralization of free radicals [41]. This finding is consistent with research indicating that phenolic compounds are potent antioxidants due to their ability to donate hydrogen atoms or electrons and stabilize free radicals [42]. *B. eurycoma* showed moderate antioxidant activity, which may be linked to its relatively lower phenolic content compared to *M. sloanei*. *B. nigerica*displayed the lowest antioxidant activity, possibly due to its lower concentration of bioactive compounds or the presence of other compounds that may inhibit antioxidant activity. This observation aligns with findings that the presence of certain phytochemicals can modulate the overall antioxidant activity of plant extracts [43].

The anti-inflammatory activity results revealed that *M. sloanei* had the most potent effects across all assays, including protein denaturation, heat-induced hemolysis, and COX-1 and COX-2 inhibition. This could be due to the presence of flavonoids and phenolics, which are known for their anti-inflammatory properties [44]. The anti-inflammatory effects observed in *M. sloanei* align with the study by Zenginet al. [45], which reported that the presence of flavonoids can significantly reduce inflammation by inhibiting

enzymes involved in the inflammatory process. *B. eurycoma* exhibited moderate anti-inflammatory activity, which correlates with its phytochemical profile that includes saponins, known for their role in reducing inflammation [40]. In contrast, *B. nigerica*showed the least activity, which might be due to its lower bioactive content or the antagonistic effects of certain phytochemicals present in the extract[46]. However, it is worth noting that while some studies highlight the anti-inflammatory potential of phytochemicals, others suggest that the efficacy may vary depending on the extract's composition and the type of inflammation being treated [47].

The antimicrobial activity assays indicated that *M. sloanei* was the most effective against all tested microbial strains, producing the largest zones of inhibition and having the lowest minimum inhibitory concentration (MIC). The strong antimicrobial activity of *M. sloanei* may be linked to the presence of alkaloids, tannins, and flavonoids, which have been reported to disrupt microbial cell walls and inhibit microbial enzymes [48]. This finding is supported by previous research demonstrating that tannins and flavonoids can effectively inhibit the growth of various pathogenic bacteria [49]. *B. eurycoma* displayed moderate antimicrobial activity, which could be attributed to the presence of saponins and glycosides. *B. nigerica* demonstrated the weakest antimicrobial activity, which may be due to its lower content of potent antimicrobial phytochemicals. However, studies by Parekh et al. [50] indicate that the antimicrobial efficacy of plant extracts is not solely dependent on the presence of specific phytochemicals but also on the concentration and interaction of these compounds within the extract.

The study highlights the potential of these seeds, particularly *M. sloanei*, as sources of natural antioxidants, anti-inflammatory agents, and antimicrobials. The differences observed among the three seed extracts suggest that each seed possesses unique properties that could be further explored for specific therapeutic applications. Future studies should focus on the isolation and characterization of individual bioactive compounds to better understand the mechanisms underlying these activities and to explore their potential in drug development.The in vitro study of *M. sloanei* reveals its significant antioxidant, anti-inflammatory, and antimicrobial properties. These findings provide scientific validation for its traditional use in treating inflammation, oxidative stress, and infections. Further studies, including in vivo experiments and clinical trials, are needed to fully understand the therapeutic potential of *M. sloanei* and to isolate specific bioactive compounds responsible for these effects.

4.2. Conclusion

The study's findings provide valuable insights into the phytochemical composition and biological activities of the crude methanolic extracts of *M. sloanei*, *B. eurycoma*, and *B. nigerica*. The presence of various bioactive compounds such as alkaloids, flavonoids, tannins, and phenolics underscores the

therapeutic potential of these seeds. Notably, *M. sloanei* exhibited the highest antioxidant, anti-inflammatory, and antimicrobial activities, suggesting its superior efficacy among the three seed extracts. The strong biological activities observed in *M. sloanei* can be attributed to its rich phytochemical profile, particularly the high content of phenolics and flavonoids known for their health-promoting properties.*B. eurycoma* demonstrated moderate activity in all tested parameters, highlighting its potential as a therapeutic agent, albeit to a lesser extent compared to *M. sloanei*. Meanwhile, *B. nigerica* showed the lowest activity, indicating that while it possesses some bioactive properties, it may not be as potent as the other two extracts.

These findings suggest that *M. sloanei*, in particular, holds promise as a natural source of antioxidant, anti-inflammatory, and antimicrobial compounds. This could have significant implications for the development of plant-based pharmaceuticals and nutraceuticals. The results of the study showed that the folklore use of some of the plants is justified. However, further research is needed to isolate and characterize the specific compounds responsible for these activities and to evaluate their efficacy and safety in clinical settings. Additionally, more detailed studies are needed to investigate the mechanisms of action of these extracts at the molecular and cellular levels.

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