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Antioxidant and anti-inflammatory (*in-vitro*) properties of extract and fractions of *Syzygium guineense* leaves

Paul Toyin Olonishuwa * and Favour Nneamaka Okany

Department of Biochemistry, Science and Technology Faculty, Bingham University Karu, Nigeria.

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Abstract

The medicinal plant *Syzygium guineense* is an ethno-medicinal African plant commonly used for treating inflammatory and oxidative stress-related conditions. It is rich in phytochemicals such as flavonoids, tannins, and saponins, which are known for their therapeutic properties. The leaves of *S. guineense* are particularly noted for their antioxidant and anti-inflammatory effects. This study aimed to evaluate the *in vitro* antioxidant and anti-inflammatory activities of crude extract and various solvent fractions of *S. guineense* leaves. The leaves were extracted with ethanol and subsequently fractionated using solvents of varying polarity, including n-hexane, chloroform, ethyl acetate, n-butanol, and water. Antioxidant activity was assessed using DPPH radical scavenging and ferric-reducing antioxidant power (FRAP) assays. Anti-inflammatory activity was determined through inhibition of protein denaturation using the egg albumin denaturation method. The n-hexane fraction exhibited the highest antioxidant activity in both DPPH (100%) and FRAP (85.6%) assays, closely comparable to ascorbic acid. Anti-inflammatory assays showed that at 5 mg/ml concentration, the n-hexane and crude fractions had the highest inhibition of protein denaturation (90±0.13and 89±0.19 respectively). These findings demonstrate a strong correlation between the phytochemical constituents of *S. guineense* and its biological activities. The extract and fractions of *S. guineense* leaves possess significant antioxidant and anti-inflammatory properties, suggesting their potential as natural therapeutic agents. Further studies are recommended to isolate and characterize the bioactive compounds responsible for these effects.

Keywords: *Syzygium Guineense*; Antioxidant Activity; Anti-Inflammatory Activity; Phytochemical Fractions; Ethnomedicine

1. Introduction

Oxidative stress and chronic inflammation have been implicated in the onset and progression of numerous degenerative diseases, including cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions [1]. Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) contribute to cellular damage by attacking DNA, lipids, and proteins [2]. The body possesses antioxidant defense systems—both enzymatic and non-enzymatic—to neutralize these radicals. However, when oxidative stress exceeds the capacity of these systems, it can result in pathophysiological complications [3]. Consequently, there is growing interest in the use of plant-based antioxidants and anti-inflammatory agents as safer, natural alternatives for managing oxidative stress and related diseases.

Previous research has identified several phytochemicals in medicinal plants—including flavonoids, alkaloids, tannins, terpenoids, and phenolic acids—as having potent biological activities [4]. *Syzygium guineense*, commonly known as the water berry, is traditionally used across Africa to manage conditions such as diarrhea, infections, diabetes, and inflammation [5][6]. Studies have highlighted the plant's antioxidant and anti-inflammatory properties, as well as its antimicrobial and antidiabetic potentials [7][8]. However, while its ethnopharmacological significance is widely

* Corresponding author: Paul Toyin Olonishuwa

recognized, systematic studies that evaluate and quantify its antioxidant and anti-inflammatory properties using standardized in vitro assays remain limited.

Despite evidence supporting the therapeutic applications of *S. guineense*, most existing research is fragmented and lacks comprehensive in vitro assessment of its biochemical activities. Few studies have employed robust comparative analyses across solvent fractions or determined the extract's efficacy relative to standard pharmaceutical controls. Additionally, the phytochemical components responsible for these therapeutic effects remain largely uncharacterized. This gap limits our understanding of the full pharmacological potential of *S. guineense* and restricts its development into validated natural therapies.

The present study aims to address these gaps by preparing and evaluating the ethanol extract and solvent fractions of *S. guineense* leaves using DPPH radical scavenging, FRAP, and egg albumin denaturation assays. By comparing the antioxidant and anti-inflammatory potentials of each fraction to standard compounds such as ascorbic acid and diclofenac sodium, this research provides a quantitative evaluation of the plant's bioactivity. In doing so, it offers scientific validation for the traditional use of *S. guineense* and lays the groundwork for future pharmacological and compound isolation studies [11].

2. Materials and methods

2.1. Plant Materials and Identification

Fresh leaves of Syzygium guineense were collected from Bingham University, Karu, Nigeria. The plant material was authenticated by Mr. Akeem Lateef at the Nigerian Institute of Pharmaceutical Research and Development (NIPRD), Abuja. A voucher specimen was deposited at the NIPRD herbarium with the reference number NIPRD/H/7428.

2.2. Preparation of Crude Extract

The collected leaves were air-dried at room temperature and pulverized into a fine powder using a mechanical blender. A total of 1700 g of powdered leaves was soaked in ethanol for 48 hours with intermittent shaking to ensure maximum extraction. The mixture was filtered using muslin cloth and Whatman No. 1 filter paper. The filtrate was concentrated using a water bath set at 75°C to obtain a crude ethanol extract.

2.3. Fractionation of Extract

The dried ethanol extract was fractionated sequentially with solvents of increasing polarity, including n-hexane, chloroform, ethyl acetate, n-butanol, and distilled water, following the method described by Hostettmann [13]. For each solvent, 250 mL was refluxed with 50 g of extract in a Soxhlet apparatus for 24–48 hours. The resulting fractions were concentrated using a water bath at 70°C for organic solvents and at 100°C for aqueous fractions. All extracts were stored at room temperature until further use.

2.4. Reagents and Chemicals

All reagents used were of analytical grade. These included ethanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, FRAP assay reagents (TPTZ, $FECL_3 \cdot 6H_2O$, acetate buffer), and phosphate-buffered saline (PBS). Diclofenac sodium was used as the standard anti-inflammatory drug.

2.5. DPPH Radical Scavenging Assay

Antioxidant activity was evaluated using the DPPH radical scavenging method as described by Xu and Chang [9]. Briefly, 50 µL of extract solution (5 mg/mL) was mixed with 180 µL of 0.2 mM DPPH solution in ethanol in a 96-well microplate. Ascorbic acid was used as the positive control, while ethanol served as the negative control. The mixtures were incubated in the dark at room temperature for 60 minutes. Absorbance was measured at 492 nm using a microplate reader. Scavenging activity (%) was calculated using the formula:

$$\text{Scavenging Activity (\%)} = [1-(\text{Abs Control}/\text{Abs Sample})] \times 100.$$

2.6. Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing ability (FRAP) was determined as a measure of antioxidant capacity by using a modification of FRAP assay previously described by Jo [16]. The assay was performed on a 96 well micro titer plate by adding to 5 µl of the sample, 150 µl of FRAP reagent. The reaction was incubated for 4 mins at 37^{o}C and absorbance was read at 593 nm against reagent blank at varying time up to 30mins using Micro plate Reader. Percentage antioxidant activity was compared against positive control which was a standard solution of ascorbic acid.

Percentage inhibition = (Abs Control-Abs Sample) / Abs Control x 100.

2.7. Anti-inflammatory Activity: Egg Albumin Denaturation Assay

The anti-inflammatory activity was assessed by measuring the inhibition of egg albumin denaturation, as described by Chandra [10]. A 1% egg albumin solution was prepared using fresh hen's egg. Reaction mixtures containing 0.2 mL of egg albumin, 2 mL of extract or diclofenac sodium (standard), and 2.8 mL of PBS were incubated at 37±2°C for 30 minutes, then heated at 70±2°C for 15 minutes. After cooling, absorbance was measured at 280 nm using a UV-Vis spectrophotometer. Percentage inhibition of protein denaturation was calculated as

$$\text{Inhibition (\%)} = (\text{Abs Control} - \text{Abs Sample}/\text{Abs Control}) \times 100$$

2.8. Statistical Analysis

All experimental data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test for significance ($p < 0.05$). Results were expressed as mean ± standard deviation (SD) of triplicate determinations using GraphPad Prism version 8.0.

3. Results

3.1. Plant Identification

The plant material was identified as *Syzygium guineense*, family Myrtaceae, by Mr. Akeem Lateef at the Nigerian Institute of Pharmaceutical Research and Development (NIPRD), Abuja. The voucher specimen number assigned was NIPRD/H/7428.

3.2. Percentage Yield of Extract and Fractions

From 1700 g of dried leaf material, 127.2 g of crude ethanol extract was obtained. Subsequent fractionation using different solvents yielded various fractions with corresponding percentage yields as presented in Table 1.

Table 1 Percentage yield of samples

S/N	Sample	Before Assay (g)	% Yield
1	N-hexane	6.2	4.87
2	Ethyl Acetate	3.4	2.67
3	Butanol	3.8	2.98
4	Water	5.2	4.09
5	Crude	127.2	7.48

3.3. Antioxidant Activity

Antioxidant activities of the extracts were evaluated using DPPH and FRAP assays. The n-hexane fraction demonstrated the highest DPPH radical scavenging activity (100%), comparable to ascorbic acid (100%). Similarly, n-hexane also showed the strongest FRAP activity (85.6%) among all the fractions. The results are summarized in Figure 1 and Figure 2, respectively.

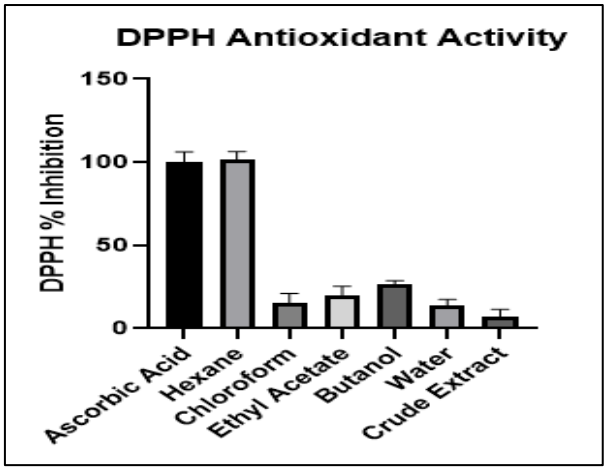

Figure 1 DPPH radical scavenging activity

The DPPH antioxidant activities of plant fractions and crude extract of *Syzyguim guineense*.

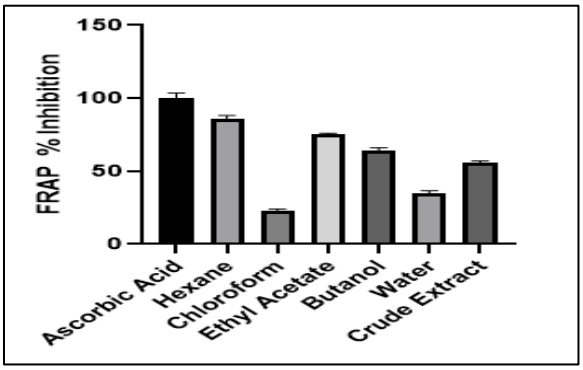

Figure 2 FRAP antioxidant activity

The FRAP antioxidant activities of plant fractions and crude extract of *Syzyguim guineense*.

3.4. Anti-inflammatory activity

The inhibition of egg albumin denaturation by the extracts was used to assess anti-inflammatory activity. At a concentration of 2.5 mg/mL, n-butanol (89±0.23%), chloroform (88±0.21%), and crude (86±0.13%) fractions showed the highest inhibition. At 5 mg/mL, n-hexane exhibited complete inhibition (90±0.13%), followed by crude extract (89±0.19%). These results are presented in Table 2**.**

Table 2 Anti-inflammatory activity (%) of plant fractions and crude extract of *Syzyguim guineense* at two concentrations

Sample	2.5 mg/mL	5 mg/mL
Standard	97±0.11	99±1.21
N-hexane	85±0.12	90±0.13
Chloroform	88±0.21	87±0.23
Ethyl Acetate	80±0.31	82±0.12
N-butanol	89±0.23	85±0.44
Water	60±0.11	70±0.17
Crude Extract	86±0.13	89±0.19

4. Discussion

The findings from this study demonstrate that Syzygium guineense leaves contain bioactive constituents with strong antioxidant and anti-inflammatory properties. These results are consistent with traditional uses of the plant in treating ailments related to oxidative stress and inflammation, such as infections, gastrointestinal disorders, and wound healing [5][7]. As noted in the introduction, oxidative damage and inflammatory pathways are critical in the development of many chronic diseases, and the identification of natural therapeutic agents is of increasing scientific interest [1][2].

The antioxidant assays (DPPH and FRAP) revealed that the n-hexane fraction exhibited the highest free radical scavenging activity (100%) and ferric reducing ability (85.6%), surpassing even the standard ascorbic acid in some instances. This indicates the presence of highly effective lipophilic antioxidant compounds in the n-hexane fraction. These findings align with earlier reports suggesting that S. guineense contains flavonoids, phenolic acids, and essential oils such as quercetin and caffeic acid, which are known to scavenge free radicals and reduce oxidative damage [12][8].

The anti-inflammatory activity, evaluated through egg albumin denaturation inhibition, further supports the therapeutic potential of the plant. At a concentration of 5 mg/mL, the n-hexane fraction showed the highest value at 90±0.13% inhibition, which was comparable to the pharmaceutical standard, diclofenac sodium. Similarly, the crude extract and n-butanol fraction also exhibited substantial inhibition at both 2.5 mg/mL and 5 mg/mL concentrations. These outcomes substantiate earlier studies indicating that *S. guineense* possesses phytochemicals like saponins, alkaloids, and terpenoids, which exert anti-inflammatory effects by modulating protein denaturation and cytokine activity [11].

The results presented here also bridge an important research gap identified in the introduction: while *S. guineense* is well known in ethnomedicine, few studies have comprehensively compared the bioactivities of its solvent fractions using standardized in vitro assays. This study provides a more complete biochemical evaluation of the plant's therapeutic potential and validates its traditional use with modern experimental evidence. Moreover, the high activity of the n-hexane fraction suggests that future research should focus on isolating the specific lipophilic compounds responsible for these effects.

5. Conclusion

The present study demonstrates that the ethanol extract and various solvent fractions of *Syzygium guineense* leaves possess significant antioxidant and anti-inflammatory properties. Among all the fractions, the n-hexane fraction exhibited the most potent activity in both DPPH radical scavenging and FRAP assays, as well as in the inhibition of protein denaturation. These findings scientifically validate the traditional use of *S. guineense* in the management of oxidative stress-related and inflammatory conditions by systematically comparing the bioactivities of each fraction and benchmarking them against standard drugs, this research fills an important gap in the current understanding of *S. guineense*. It also highlights the presence of lipophilic bioactive constituents, particularly in the n-hexane fraction, which merit further pharmacological investigation.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have declared no conflict of interest

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