Formulation and evaluation of effect of mucilage of *Corchorus olitorius* leaves on antioxidant activity of *Spondias mombin* extract

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ABSTRACT

Background: Mucilage from medicinal plants such as *Corchorus olitorius* L. (Malvaceae) can serve as alternative sources of suspending agents as currently commercially available ones are quite costly. However, they may also affect formulation characteristics and activity of final product. This study evaluated effect of mucilage of *Corchorus olitorius* L. (Malvaceae) leaves on antioxidant activity of aqueous fraction of hydroethanolic extract of *Spondias mombin* L. (Anacardiaceae) leaves.

Methods: Standard *in vitro* antioxidant assays were used. Mucilage was investigated for its phytochemical contents. Physico-chemical evaluation using pH meter and microscopical characterization emulsion were also carried out. Repeated measures analysis of variance (ANOVA) was used to compare activity of extract versus emulsion.

Results: Terpenoids, unsaturated lactones, carbohydrates and reducing sugars were present in the mucilage. The stable emulsion formulated had a pH of 6.7 with oil droplets of average size, 9.12µm. Most droplets were small size. The extract had superior 2,2-diphenyl-1-picrylhydrazyl radical inhibition, hydrogen peroxide radical inhibition and reducing capacity ($\alpha = 0.05$) but emulsion had better metal chelating activity ($\alpha = 0.05$).

Conclusion: The mucilage significantly reduced antioxidant activity in the emulsion but could be used orally for indicated oxidative conditions.

Keywords:- Antioxidant, Emulsion, Mucilage, Inhibition, *Corchorus olitorius*

1. INTRODUCTION

Plant gums and mucilages have been an essential part of formulations since the dawn of the modern pharmaceutical industry. They have been used mainly as binders, disintegrants, wetting agents, sustained-release matrices in solid dosage forms as well as suspending agents, viscosity impacters, stabilizers and emulsifying agents in various semi-solid and liquid formulations [1]. Plant derived gums and mucilages are chemically polymeric sugars with side chains of uronic acids. These polysaccharides are known for their gummy nature which is a desirable property in formulations. However, due to their nature, they are easily prone to microbial deterioration because they are nutritional. *Corchorus olitorius* L. (Malvaceae) known commonly as Wild Jute or Jews mallow and locally as "ewedu" in South-Western Nigeria is a pantropical plant commonly grown in the tropical and temperate regions including Egypt, Mozambique, the Philippines, Senegal and tropical West Africa, Thailand and Australia [2]. The leaves are pounded to make thick soups that are eaten with local staple foods. The polysaccharide present in the mucilage consists of rhamnose, glucose, galacturonic and glucuronic acids with some extent of acetylation. The polysaccharide was also

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shown to possess proliferative activity [3]. Suspending property of mucilage from different morphological parts of the plant has been assessed. The leaf mucilage has been shown to have good suspending potential at 1.5% as well as a good emulsifying activity [1]. Increasing commercial demand of natural gums such as Tragacanth and Acacia has necessitated the search for newer and affordable substitutes. *Spondias mombin* L. (Anacardiaceae) is a deciduous plant that prospers in the tropics [4]. The plant is rich in antioxidant phytochemicals such as ascorbic acid and flavonoids. Evidence exists that show the leaves have good antioxidant activity [5]. This study is aimed at evaluation of contributory or deleterious effect of *Corchorus olitorius* mucilage to the antioxidant property of *Spondias mombin* extract in a formulated emulsion of the extract using the mucilage as emulsifier via ultraviolet spectrometric antioxidant assays. The study also assessed physical and microscopical characteristics of the formulated emulsion as well as phytochemical investigations on the mucilage. Additionally, appropriate inference was drawn on whether the emulsion would be of benefit in human therapy.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 *Equipment and Reagents*

Biomate 3 Ultraviolet spectrophotometer (Rochester, New York, USA) was used to run the assays.

Potassium ferricyanide, Trichloroacetic acid, Ferric chloride (Sigma Aldrich Germany) 1,1-diphenyl-2-picrylhydrazyl (DPPH) crystals and Orthophenanthroline (Merck KGaA, Darmstadt, Germany). Phosphate buffer (0.1M, pH 7.4), phosphate buffer (pH 6.6) and Ethanol were prepared at and obtained from Department of Pharmacognosy laboratories, University of Lagos. Absolute ethanol and Methanol (EMD Milliform Corporation, Germany). Rhodamine B dye, (Macklin Industries, Shanghai, China) while Hydrogen peroxide (20 volume) used was a standard proprietary product bought off the shelf.

2.1.2 *Biological materials: Plant collection, identification and preparation*

Corchorus olitorius leaves were purchased from Mushin market, Latitude: 6° 31' 59.9988" Longitude: 3° 21' 0" in Lagos state, Nigeria in August 2019 while *Spondias mombin* leaves were purchased from Ijegun market, Latitude: 6° 27' 11.0016" Longitude: 3° 23' 44.9988" in Lagos state, Nigeria in September 2019. *Corchorus olitorius* leaves and *Spondias mombin* leaves were identified at the Department of Botany, University of Lagos where the plants were assigned voucher specimen number LUH 8047 and 8999 respectively. Voucher specimens were then deposited at the herbarium. The plant materials were processed and dried in the oven for 72h, powdered and stored in appropriate containers until when needed.

2.2 Methods

2.2.1 *Extraction of plant materials*

475.00 g of dried powdered *C.olitorius* leaves were soaked in water for 6h and then boiled for 30min and left to stand for 1h [1]. The liquid extract was separated from the marc using a multilayer muslin cloth bag. Absolute ethanol (equivalent volume to aqueous extract) was added to the liquid extract to precipitate the mucilage. The precipitated mucilage was filtered, dried in an oven in steel containers at 50°C, ground and stored in air tight containers. Maceration of 1246.55g of powdered *S.mombin* leaves was done with 10L of 70% ethanol in an amber colored Winchester bottle for 72h. This was then filtered through muslin cloth and filtrate concentrated in a rotary evaporator set at 45°C until dry. Yield of extract was calculated with reference to the dry plant material.

2.2.2 *Phytochemical investigations*

Dried mucilage was investigated for phytochemicals using standard procedures [6].

2.2.3 *Emulsion formulation*

S.mombin leaf extract ……37.5% w/v
C.olitorius leaf mucilage…… 0.5% w/v
Methylcellulose……..0.5% w/v
Mineral oil……20% v/v
Distilled water to…….100 mls

1g of the mucilage and methylcellulose were weighed, transferred into the mortar and triturated till a uniform consistent powder was obtained. 75mg of *Spondias mombin* extract was weighed, transferred into a beaker containing

little distilled water and warmed till it dissolved. The resulting mixture was filtered and filtrate transferred into the mortar in portions and triturated with 40mls of mineral oil added in aliquots until a homogenous paste was observed. This was transferred into a previously calibrated bottle and distilled water added to the mark with vigorous shaking to allow for proper formation of emulsion. This was equivalent to 0.375mg/ml (37.5%) solution. An appropriate dilution of the emulsion was prepared and 1-in-2 serial dilutions were made with methanol from this emulsion for use in the assays.

2.2.4 *Spondias mombin aqueous stock solution preparation*
200mg of the *Spondias mombin* extract was weighed and transferred into a beaker and gradually dissolved with a minute quantity of warmed distilled water to allow for dissolution. The resulting mixture was filtered, and the filtrate was made up to 200mls with distilled water. This is equivalent to 1mg/ml (100%) solution and was used as representative of the aqueous fraction of *S.mombin.* This was then refrigerated at 4^{o}C until use. An appropriate dilution of the stock was prepared and 1-in-2 serial dilutions were made with methanol from this preparation for use in the assays.

2.2.5 *Physico-chemical/Microscopical evaluation*
2.2.5.1 Microscopy
Water-soluble Rhodamine B dye (0.35g) was weighed and dissolved in 100mls distilled water. Equal volume of emulsion and dye solution was mixed and a drop of this mixture was mounted on a microscope and viewed at x 10 and x 40 objective.

2.2.5.2 Micrometry
The size of oil droplets of the previously mounted emulsion-dye mixture was measured using a calibrated eyepiece graticle (1 division=2.4µm). 10 droplets were measured and
categorized into small, medium and large sized granules. Mean size of each group was calculated and expressed as mean size ± standard error of mean (SEM).

2.2.5.3 pH measurement
A small volume of the formulated emulsion was transferred into a beaker and the pH was measured using a calibrated pH meter. Same procedure was repeated for *S. mombin* stock solution.

2.2.6 *Antioxidant evaluation*
DPPH radical percentage inhibition assay
The antioxidant activity of the extract was evaluated based on the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by a slightly method employed by Blois [7]. The experiment was performed in triplicates. The extracts (0.0125-0.2%) were prepared from the stock solution using suitable dilution. This was also repeated for the emulsion. Methanol was used as blank. % inhibition was calculated using Equation 1:
Inhibition of DPPH = $(A_C - A_A)/A_C \times 100\%$.....Equation 1
Where A_C is the absorption of the control sample and A_A is the absorption of tested extract [8].

2.2.7 Hydrogen peroxide radical scavenging assay
This was carried out by a method employed by Gayathri *et al*. [9]. The experiment was performed in triplicates. The extracts (0.0125-0.2%) were prepared from the stock solution using suitable dilution. This was also repeated for the emulsion. Percentage inhibition was calculated using Equation 2:
% Inhibition = $(A_C - A_A)/A_C \times 100\%$.....Equation 2
Where A_C is the absorption of the control sample and A_A is the absorption of tested extract.

2.2.8 Ferric ion reducing capacity assay
Using a slightly modified method as employed by Adesegun *et al.* [10], ferric ion reducing capacity of the extract solution and emulsion were assessed. The experiment was performed in triplicates. The extracts (0.0125-0.2%) were prepared from the stock solution using suitable dilution. This was also repeated for the emulsion. Increased absorbance of the reaction mixture indicated increased reducing power.

2.2.9 Metal chelating activity assay

The reaction mixture contains 1 ml 0.05% orthophenanthroline in methanol, 2 ml ferric chloride 200 µM & 2 ml of various concentrations (0.0125-0.2%) of the solution and emulsion. The mixture was incubated at ambient temperature for 10 min, and then the absorbance of the same was measured at 510 nm. The chelated iron formed a red chromophore in solution and increasing absorbance indicated increasing activity. The experiment was performed in triplicate [11].

2.3 Statistical Analysis

All tests were analyzed using Microsoft Excel expressed as Mean ± Standard error of mean (SEM) and compared using Repeated Measures (RM) two-way Analysis of Variance (ANOVA) by GraphPad prism 8 Software. Bonferroni multiple comparison post-test to compare differences between the *S. mombin* solution and the emulsion. Differences between assay results were assessed at 95% confidence interval (α= 0.05).

3. RESULTS

Table 1: Yield

Samples	Dried plant material (g)	Weight of extract (g)	Percentage (%) yield
Spondias mombin	1246.55	320.94	25.75
Corchorus olitorius mucilage	475.00	16.03	3.37

Table 2: Phytochemical investigations of *C.olitorius* mucilage

Phytochemical test		Presence/Absence
Saponins		–
Alkaloids		–
Triterpenoids		+
Tannins		–
Shinoda		–
Fehling's		+
Keller killiani		–
Anthraquinones	Free	–
	O-glycoside	–
	C-glycoside	–
Kedde		+
Molisch		+

+ = present, - = absent

Table 3: Size distribution of oil globules

S/N	Size	Number of divisions	Frequency	Mean
1	Small	1-3	5	4.32±0.90
2	Medium	4-6	3	11.20±0.80
3	Large	7-9	2	18.00±1.20

Table 4: pH measurement

Sample	pH
Spondias mombin solution	3.87
Spondias mombin-Corchorus olitorius emulsion	6.70

Table 5: Statistical analysis for percentage DPPH inhibition (517nm)

Concentration(%)	0.0125	0.025	0.05	0.1	0.2
S. mombin solution	27.17±1.61	46.09±2.34	61.30±3.13	85.39±0.84	88.76±0.16
S. mombin-C.olitorius emulsion	12.18±2.39[c]	16.14±0.81[e]	14.40±1.43[e]	31.38±3.64[e]	37.65±3.61[e]

Data expressed as mean ± SEM at 5 concentrations n = 3
[e] = Significantly different from *S. mombin* solution at $p \leq 0.05$ ($p \leq 0.0001$)
[c] = Significantly different from *S. mombin* solution at $p \leq 0.05$ ($p \leq 0.01$)

Table 6: Statistical analysis for percentage hydrogen peroxide radical scavenging inhibition (230nm)

Concentration(%)	0.0125	0.025	0.05	0.1	0.2
S.mombin solution	51.09±1.76	39.43±7.00	1.60±1.61	-63.35±5.57	-105.21±3.11
S.mombin-C.olitorius emulsion	-37.88±5.04[d]	-101.16.70±9.79[e]	-162.68±21.10[e]	-216.92±5.44[e]	-180.18±20.10[c]

Data expressed as mean ± SEM at 5 concentrations, n = 3
[e] = Significantly different from *S. mombin* solution at $p \leq 0.05$ ($p \leq 0.0001$).
[d] = Significantly different from *S. mombin* solution at $p \leq 0.05$ ($p \leq 0.001$).
[c] = Significantly different from *S. mombin* solution at $p \leq 0.05$ ($p \leq 0.01$)

Table 7: Statistical analysis for Ferric ion reducing capacity (700nm)

Concentration(%)	0.0125	0.025	0.05	0.1	0.2
S.mombin solution	0.206±0.059	0.287±0.059	0.355±0.025	0.559±0.002	1.194±0.226
S. mombin-C.olitorius emulsion	0.333±0.056[a]	0.298±0.033[a]	0.337±0.022[a]	0.496±0.091[a]	0.354±0.036[d]

Data expressed as mean ± SEM at 5 concentrations, n = 3
[d] = Significantly different from *S. mombin* solution at $p \leq 0.05$ ($p \leq 0.001$).
[a] = Not significantly different from *S. mombin* solution at $p \leq 0.05$ ($p>0.05$)

Table 8: Statistical analysis for metal chelating activity (510nm)

Concentration(%)	0.0125	0.025	0.05	0.1	0.2
S.mombin solution	0.034±0.001[a]	0.038±0.004[c]	0.041±0.001[d]	0.056±0.003[e]	0.058±0.002[e]
S. mombin-C.olitorius emulsion	0.044±0.001	0.059±0.001	0.075±0.005	0.146±0.002	0.175±0.009

Data expressed as mean ± SEM at 5 concentrations n = 3
[e] = Significantly different from *S.mombin-C.olitorius emulsion* at $p \leq 0.05$ ($p \leq 0.0001$)
[d] = Significantly different from *S.mombin-C.olitorius emulsion* at $p \leq 0.05$ ($p \leq 0.001$)
[c] = Significantly different from *S.mombin-C.olitorius emulsion* at $p \leq 0.05$ ($p \leq 0.01$)
[a] = Significantly different from *S.mombin-C.olitorius emulsion* at $p \leq 0.05$ ($p>0.05$)

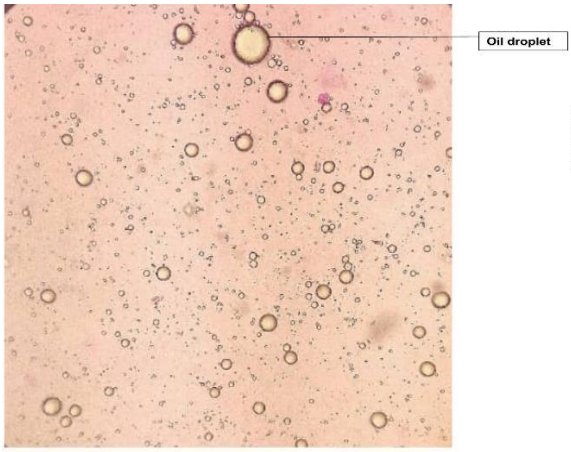

Figure 1: Microscopic view of oil-in-water

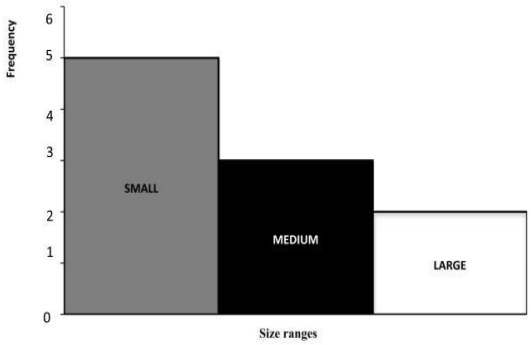

Figure 2: Size range categories of emulsion soil droplets emulsion (x 100)

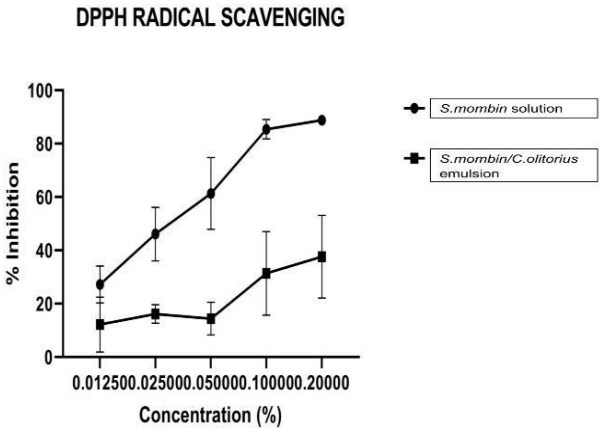

Figure 3: DPPH radical percentage inhibition

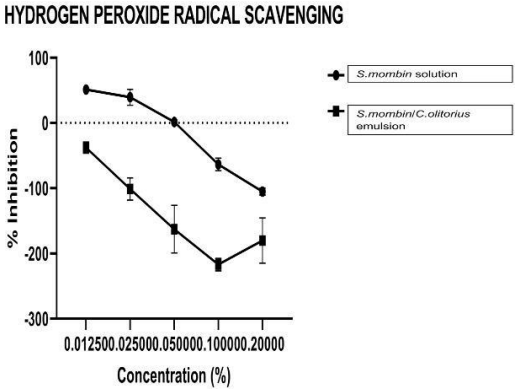

Figure 4: Hydrogen peroxide radical percentage inhibition

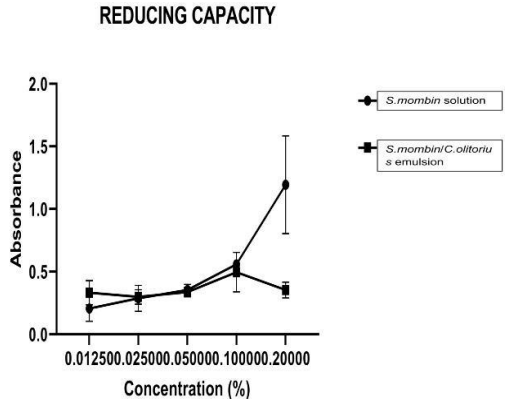

Figure 5: Ferric ion reducing capacity

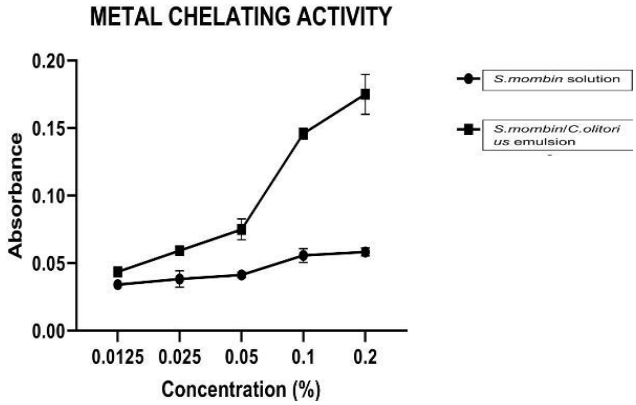

Figure 6: Metal chelating activity

4. DISCUSSION

The yield of extracts obtained of *S.mombin* and mucilage were 25.75% and 3.37% respectively. Yield of *S.mombin* leaves was quite high because the leaves possess highly polar constituents [12] which were extracted by the highly polar extracting solvent. Yield of the *S.mombin* was similar to that reported by some workers in a previous study [13]. The yield of mucilage was low compared to that obtained in a recent study which was 24.54% [1].The mucilage contained terpenoids, reducing sugars, carbohydrates and unsaturated lactones and lacked saponins, alkaloids, flavonoids, tannins, deoxysugars and anthraquinones. This was similar to results obtained by Adjatin *et al*. [14] which showed presence of carbohydrates and reducing sugars. However, this literature showed presence of tannins, flavonoids and some types of anthraquinones which were not present in this study. The use of an oil phase that will ensure a stable *S.mombin* extract emulsion is important in formulation. Mineral oil was used as the oil phase in conjunction with the dual suspending capacities of the mucilage and methyl cellulose. Total suspending agent concentration was 1% with the mucilage and methylcellulose accounting for 0.5% each. Extract-mucilage interaction may have led to the perceived reduction in antioxidant activity. This was due to formation of oil droplets with high integrity in the emulsion resulting in poor release of the active constituents in the extracts responsible for activity [15]. The resulting emulsion had good stability because the integrity was preserved on proper storage throughout the study duration. Microscopy revealed an oil-in-water emulsion with yellowish to brown droplets scattered on the pink water-soluble rhodamine dye background. The oil droplets were majorly of the small size range with average size of 4.32µm while medium and large sized droplets were observed. pH was found to be 6.7 which is close to neutral proving its suitability for use orally without causing side effects associated with increased acidity. DPPH activity increased with increasing concentration for both the *S.mombin* solution and emulsion. The extract had a superior DPPH activity than the emulsion across all concentrations (α=0.05). At the lowest concentration of 0.0125%, the extract had significantly higher DPPH activity than the emulsion ($p \leq 0.01$, α=0.05). At higher concentrations of 0.025-0.2%, the extract had significantly higher DPPH activity than the emulsion ($p \leq 0.0001$, α=0.05). Thus, it can be inferred that the mucilage may have reduced the DPPH activity of the extract in the emulsion due to entrapment of the extract globules in the emulsion matrix [15]. However, the highest DPPH activity exhibited (37.65%) may still be enough for therapeutic effectiveness. Moreover, this formulation may only delay the release of the active constituent initially and release the remnant over time. Hydrogen peroxide radical scavenging activity decreased with increasing concentration for both the *S.mombin* solution and emulsion. The extract had a superior activity than the emulsion across all concentrations (α=0.05). At the lowest concentration of 0.0125%, the extract had significantly higher activity than the emulsion ($p \leq 0.001$, α=0.05). At higher concentrations of 0.025-0.1%, the extract had significantly higher activity than the emulsion ($p \leq 0.0001$, α=0.05). At the highest concentration of 0.2%, the extract had significantly higher activity than the emulsion ($p \leq 0.01$, α=0.05). This suggests that the mucilage may have reduced the hydrogen peroxide radical scavenging activity of the extract in the emulsion. Both extract and emulsion had very low activities in this assay hence may not be suitable for use in scavenging hydrogen peroxide radicals. Reducing capacity increased with increasing concentration for both the *S.mombin* solution and emulsion. The extract and emulsion exhibited similar activity at 0.0125-0.1% ($p > 0.05$, α=0.05). However at the highest concentration of 0.2%, the extract had significantly higher activity than the emulsion ($p \leq 0.05$, α=0.05). It can be inferred that at lower concentrations, both extract and emulsion had similar activity, but activity tended to be higher for extract at higher concentrations. This suggests that the mucilage may have reduced the ferric ion reducing capacity of the extract in the emulsion. However, the emulsion still exhibited sufficient reducing capacity that may be therapeutically effective. There was a concentration-dependent increase in metal chelating activity. The extract and emulsion exhibited similar activity at 0.0125% ($p > 0.05$, α=0.05). However, the emulsion had superior activity compared to the extract at the higher concentrations (α=0.05). The emulsion had better activity at 0.025% ($p \leq 0.01$, α=0.05), 0.05% ($p \leq 0.001$, α=0.05), 0.1-0.2% ($p \leq 0.0001$, α=0.05). This infers that the mucilage may have increased the metal chelating activity of the extract in the emulsion.

5. CONCLUSION

This study provides evidence that *Corchorus olitorius* mucilage may have reduced the antioxidant activity of the aqueous fraction of *Spondias mombin* hydroethanolic leaf extract though the formulated, stable, oil-in-water emulsion still exhibited some activity. The emulsion may be safe for oral therapy of indicated oxidative conditions. The mucilage may serve as a reliable source of excipients for pediatric formulations which require reduced antioxidant concentration.

Acknowlegdement

The authors appreciate the technical support offered by Mr. H.O. Yusuf and Mr. T.I. Adeleke of the Department of Pharmacognosy laboratories, University of Lagos and Mr. S. Adenekan of the Department of Biochemistry laboratories, College of Medicine of the University of Lagos during the duration of the study.

Conflict of interest
The authors declare an absence of any potential conflict of interest.

Contribution of authors
JOO, AAO and OAO were responsible for conception and design of the work with support from MOI. JOO and OKR did the experiments and collected data. JOO carried out data analysis and interpretation. JOO drafted the article then OAO, AAO, MIO and OKR carried out critical revision of the article while OAO and MOI gave final approval of the version to be published.

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