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Research Article

ACTIVITY OF LIVER FUNCTION ENZYME AND ANTIBACTERIAL SUSCEPTIBILITY OF *ANNONA SENEGALENSIS* LEAF EXTRACT

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ABSTRACT

In ethnomedicine, various natural products have been implicated in the treatment of several diseases. *Annona senegalensis* has been identified as one of the plants that have the potential to cure ailments arising from microbial infections. This study however was carried out to investigate the liver function enzyme activity and *in vitro* antibacterial susceptibility of the 50% ethanol-methanol leaf extract of *A. senegalensis*. The effect of the administration of 100 mg/kg body weight of *A. senegalensis* on serum enzyme parameters and antibacterial susceptibility was investigated in albino rats. Thirty-three albino rats with average weights of 200 g were divided into two groups. Group 1 contained 30 rats and was treated with 20 mg/mL of *A. senegalensis* leaf extract while Group 2 contained three rats and served as the control. The treatment lasted for 20 consecutive days while the rats in Group 1 were daily administered with the leaf extract of *A. senegalensis.* Data were analyzed and presented using a descriptive analysis from Microsoft Excel 2016 version. The results obtained from administration of the leaf extract of *A. senegalensis* on serum enzymes parameters show a significant increase in serum enzyme activity when compared to the control. This indicates that the leaf extracts of *A. senegalensis* have significant effects on the serum enzymes parameters. The *in vitro* antibacterial susceptibility of the leaf extracts relative to the drugs (Ampicillin and Ciprofloxacin) revealed that the leaf extract of the plant holds much promise in antibacterial property when combined with antibiotics used in this study. This research revealed that there is high activity of alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase in the serum following the administration of 100 mg/kg body weight of 50% ethanol-methanol leaf extract of *A. senegalensis*. The antibacterial susceptibility test of the leaf extract carried out *in vitro* also revealed the plant antibacterial property. The combination of *A. senegalensis* leaf extract with antibiotic drugs, especially the one used in this study hold much promise in its efficacy as revealed in its synergistic interaction.

Keywords: *Annona senegalensis*, Enzyme, Antibacterial, Antibiotics, Extract, Ethanol-methanol.

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INTRODUCTION

Several plants are naturally present in our environment in which man exploit their potential for treatment of various diseases. *Annona senegalensis* Pers (*Annonaceae*) also known as "Wild Custard Apple" is a shrub which is widely distributed in Africa [1,2]. In Nigeria, it is referred to as "Abo", "Uburu ocha", "Gwandar daji", among the Yoruba's, Ibos and Hausas, respectively. It is also called "Ikpokpo" among the Idomas in the Middle Belt area of Nigeria. It is mostly found in the Savannah belt and has an immense traditional application in curing different diseases [3]. Its flowers have aromatic odor which makes it useful in flavoring food. Its fruit when ripe is yellow in color and it has a sweet edible jelly with pleasant odor [3].

The previous studies on anti-inflammatory activities of the leaf extract were determined in rats, in inflammatory models. The extract induced a significant decrease in the number of inflammatory cells. This effect was believed to be as a result of higher concentrations of tannins and phenolic compounds in the extract of the plant [4]. Anticonvulsant activities of the root bark extract on pilocarpine-induced seizures in animal model proved the efficiency of *A. senegalensis* in the treatment of epilepsy and convulsions [5]. Furthermore, analgesic, antiulcer/antacid, smooth muscle relaxant [6,7], antitumor [8,9], antiprotozoal [10], molluscicidal [11], and hormone mimetic [12] activities have also been reported. The plant has also been shown to be beneficial in the treatment of snake bite [1]. The isolation of mono-tetrahydrofuran and bis-tetrahydrofuran acetogenins [13] from this plant has also been reported. However, the aim and objectives of this research are to determine the liver function enzyme activity in serum and *in vitro* antibacterial susceptibility of the 50% ethanol-methanol leaf extract of *A. senegalensis*.

METHODS

Experimental animals

Adult male albino rats (*Rattus norvegicus*) of average weight 200 g were obtained from a commercial breeder in Markudi, Benue State, Nigeria. The rats were housed in Animal House Biochemistry Department, Kogi State University, Anyigba. The animals were maintained freely on separate cages and fed with standard rat feeds and water *ad libitum* before and during the period of the study. All animals used for this study were in compliance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals [14].

Collection of plant materials

Fresh leaf of *A. senegalensis* was collected from Anyigba, Kogi State, Nigeria. The plant material was identified and authenticated by Dr. D. O Aina of the Department of Biological Sciences, Kogi State University Anyigba.

Chemicals and reagents

All chemicals and reagents were of analytical grade. The drug, Ciprofloxacin (BP 500 mg), was a product of Pell Tech Health Care PVT, Ltd. (India) while Ampicillin (250 mg) was a product of Laborate pharmaceuticals (India). The enzyme kits for alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were product of Randox (UK), Methanol and Ethanol were product of BDH (Poole England).

Preparation of plant extract

A. senegalensis leaf was air dried in the laboratory for 8 days and pulverized to coarse powder using a small mortar and pestle. The

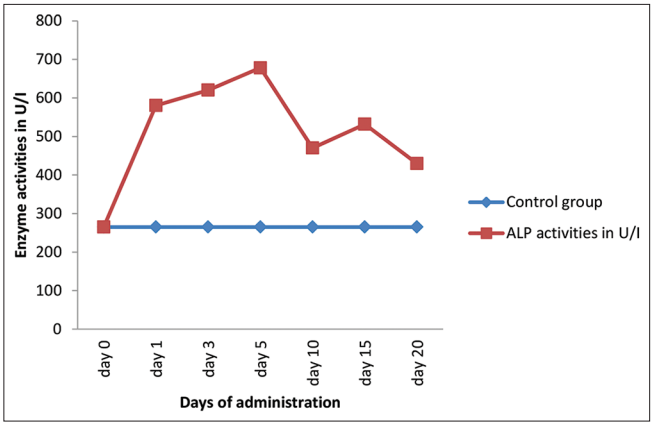

Fig. 1: The serum alkaline phosphatase activity in U/I following the administration of 100 mg/kg body weight of *Annona senegalensis* leaf extract to rats

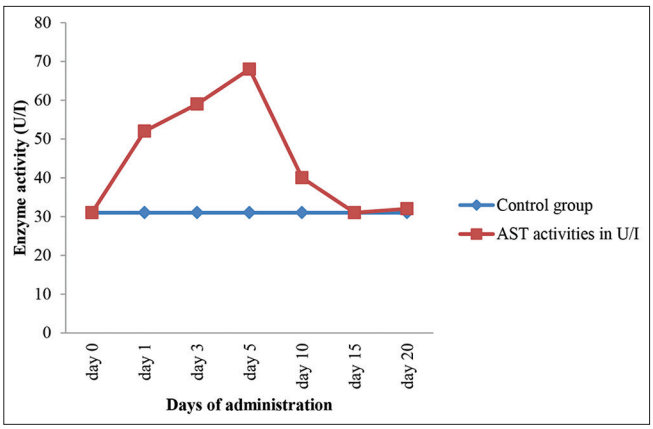

Fig. 2: Aspartate aminotransferase activity in U/I in the serum following the administration of 100 mg/kg body weight of *Annona senegalensis* leaf extract to rats

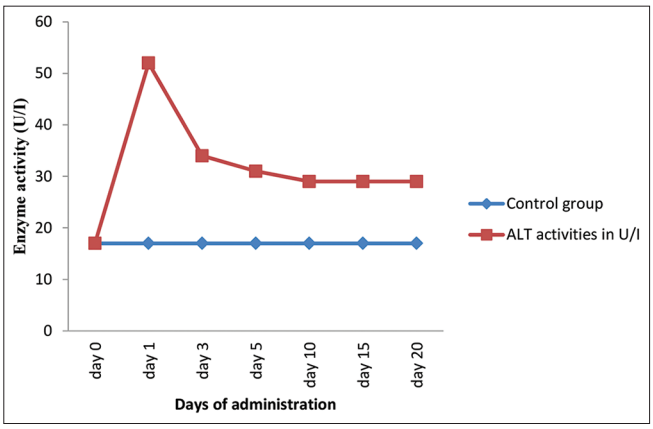

Fig. 3: Alanine aminotransferase activity in U/I in the serum following the administration of 100 mg/kg body weight of *Annona senegalensis* leaf extract to rats

powdered leaf (65.19 g) was soaked with 50% ethanol-methanol mixture both in 1:1 for 24 h and filtered with high vacuum pump connected with funnel and flask. The filtrate was then concentrated by evaporation in water bath under, reduced pressure, and yielded 9.68 g of the extract. The extract was then stored in the refrigerator at 4°C until required.

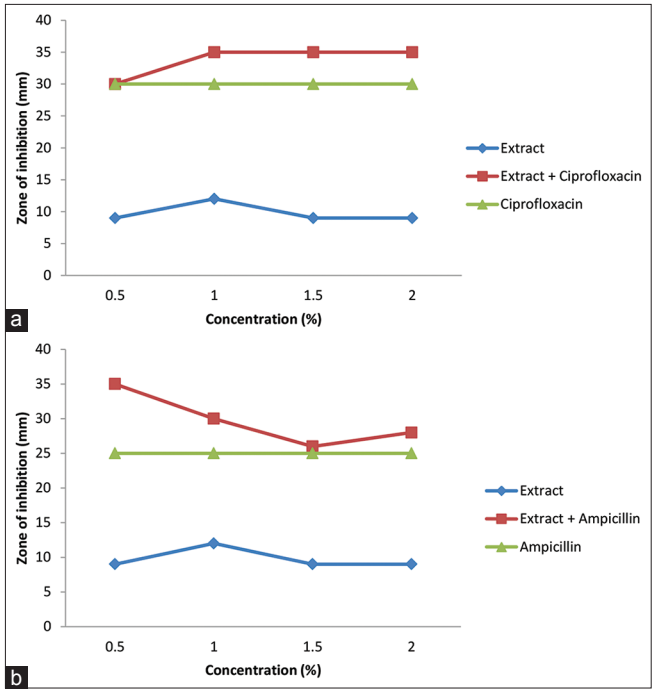

Fig. 4: (a) The zone of inhibition of *Escherichia coli* to *Annona senegalensis* leaf extract, extract plus Ciprofloxacin, and Ciprofloxacin alone in millimeters. (b) The zone of inhibition of *Escherichia coli* to *Annona senegalensis* leaf extract, extract plus Ampicillin, and Ampicillin alone in millimeters

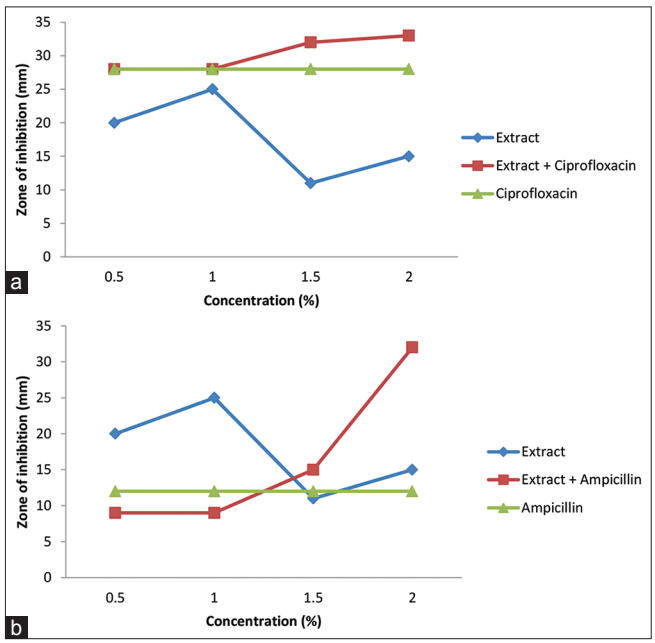

Fig. 5: (a) The zone of inhibition of *Salmonella enterica* to *Annona senegalensis* leaf extract, extract plus Ciprofloxacin and Ciprofloxacin alone in millimeters. (b) The zone of inhibition of *Salmonella enterica* to *Annona senegalensis* leaf extract, extract plus Ampicillin, and Ampicillin alone in millimeters

Preparation of drugs for *in-vitro* experiment

About 0.5% of Ampicillin and 0.5% of Ciprofloxacin were prepared in equal volumes and each concentration of the extracts (0.5%, 1.0%, 1.5%, and 2.0%) was added to the two antibiotics and was tested on the animal, *Escherichia coli* and *Salmonella enterica.*

Treatment of animal
Thirty-three albino rats with average weights of 200 g were divided into two groups and designated as Group 1 and 2. Group 1 served as the test group and contained 30 rats, while Group 2, served as the control and contains three rats.

Administration of extract
Group 1 (test group) which contained 30 rats were administered with plant extract of 20 mg/mL of 100 mg/kg bodyweight. The 30 rats (groups 1) were administered with their appropriate dosage of 1 mL of the extract per day for 1 day, 3 days, 5 days, 10 days, 15 days, and 20 days, respectively, for observation of the effect of the plant extract while Group 2 (control) were given distilled water only and allow free access to feed for 20 days.

Animal sacrifice and sample collection
Animal sacrifice was carried out in appropriate days corresponding to daily doses represented by day 1, 3, 5, 10, 15, and 20. Five rats were randomly selected from the test group and sacrificed on appropriate days. The first sacrifice representing day 1 was done 24 h after the first administration and the blood sample was collected through the jugular veins and was transferred immediately into plain sample bottle and then centrifuge at 1000 rpm for 10 min to obtain a clear supernatant which was stored at –20°C until required for biochemical assays.

Biochemical assays
The activity of alkaline phosphate (ALP), AST, and ALT were determined following the method described by Reitman and Frankel [15].

Antibacterial susceptibility test
The agar well-diffusion method was adopted for the antibacterial susceptibility test as described by Perez *et al.*, [16]. The test organisms (*S. enterica* and *E. coli*) were obtained from the Microbiology Laboratory of Kogi State University and were sub-cultured on Nutrient broth for 6 h before the test. Mueller Hinton Agar (Fluka Spain) was prepared according to manufacturer's instruction and poured aseptically into sterile petri dishes. The plates were kept for the medium to gel. Agar wells were created in the medium using 6 mm cork borer. 0.1 mL of the broth culture of the test organisms were introduced into the plates. Sterile cotton swab was employed to evenly distribute the inoculums over the surface of the medium and the plates were allowed to stand for 5 min.

Four concentrations of *A. senegalensis* plant extract were prepared (0.5%, 1.0%, 1.5%, and 2.0%). Furthermore, 0.5% of Ampicillin and 0.5% of Ciprofloxacin were prepared in equal volumes and each concentration of the extracts was added to the two antibiotics and also tested on the organisms. The antibiotics were introduced into separate well as controls. Each plate had five wells. 0.1 mL of the extracts, the extracts plus (+) antibiotics, and antibiotics were introduced into the properly labeled wells in the medium. The plates were incubated at 37°C for 18–20 h. Antibacterial activity was determined by measuring the diameter of zone of exhibition in millimeters around the wells with a standard ruler. The environment took for the isolation is a blood sample of a typhoid patient.

Statistical analysis
All data were analyzed and presented using a descriptive analysis from Microsoft Excel 2016 version. The level of significance was set at $p<0.05$.

RESULTS

ALP

Serum ALP activities

Following the administration of *A. senegalensis* leaf extract (100 mg/kg body weight) to rats (Table 1), there was a steady significant increase in concentration of ALPs at day 1, 3, and 5 followed by a gradual decrease on day 10, with further administration, leading to a slight increase on day 15. Subsequent administration led to a significant decrease on day 20. All values compared with the control were significantly higher than the control value (Fig. 1).

AST

Serum AST activities

On the administration of *A. senegalensis* leaf extract (100 mg/kg body weight) to rats (Table 2), it was observed that there was a progressive increase in serum AST concentration from days 1, 3, and 5. However, enzyme activity on day 10 was decreased significantly followed with subsequent decrease in values tending toward the control group at day 15 and 20 (Fig. 2).

ALT

Serum ALT activity

Following the administration of *A. senegalensis* leaf extract (100 mg/kg body weight) to rats (Table 3), there was a significant increase from day 1 compared with the rats in control group. Further administration led to a down regulation of the enzyme activities up to day 5. There was a comparative decrease in activity between days 10 and 20; but activity values remain constant from day 10 to 20, though higher significantly when compared with the control (Fig. 3).

Antibacterial susceptibility of *E. coli* to *A. senegalensis*
From Table 4, it was discovered that when the leaf extract of *A. senegalensis* sensitivity was tested on the microorganism *E. coli*, the zone of inhibition in millimeters at 1.0% was 3 mm higher than that of 0.5%, 1.5%, and 2.0%. Then, when the sensitivity of the organism to the drug (ciprofloxacin) was tested, a higher zone of inhibition than that of the extracts and Ampicillin was observed (Fig. 4a). Furthermore, when the sensitivity of the bacteria to the drug (Ampicillin) was tested,

Table 1: The serum alkaline phosphatase activity in U/I following the administration of 100 mg/kg body weight of *Annona senegalensis* leaf extract to rats

Days of administration of extracts	Enzyme activities (U/I)
0/control group	264.96
1	580.45
3	620.20
5	678.25
10	470.12
15	531.76
20	429.64

Table 2: The serum aspartate aminotransferase activity in U/I following the administration of 100 mg/kg body weight of *Annona senegalensis* leaf extract to rats

Days of administration of extracts	Enzyme activities (U/I)
0/control group	31.0
1	52.0
3	59.0
5	68.0
10	40.0
15	31.0
20	32.0

Table 3: The serum alanine aminotransferase activity in U/I following the administration of 100 mg/kg body weight of *Annona senegalensis* leaf extract to rats

Days of administration of extracts	Enzyme activities (U/I)
0/control group	17.0
1	52.0
3	34.0
5	31.0
10	29.0
15	29.0
20	29.0

the zone of inhibition was higher than that of leaf extracts but lower with 5 mm than that of Ciprofloxacin (Fig. 4b). However, when the extracts plus Ciprofloxacin were tested on the bacteria, a higher zone of inhibition than that of the Ciprofloxacin alone, Ampicillin alone, and extracts alone was observed (Fig. 4a). Furthermore, when the sensitivity of Ampicillin plus extracts were tested on the organism, the zone of inhibition at 0.5% extracts was as high as that of Ciprofloxacin plus extracts at 1.0%, 1.5%, and 2.0%, while it was 5 mm lower at 1.0% of extract plus Ciprofloxacin, 9 mm lower at 1.5%, and 7 mm lower at 2.0% of extracts plus Ciprofloxacin (Fig. 4b).

Antibacterial susceptibility of *S. enterica* to *A. senegalensis*

From Table 5, it was observed that when the sensitivity of the bacteria *S. enterica* was tested on the leaf extract of *A. senegalensis,* it shows a significant zone of inhibition at 0.5% and 5 mm higher at 1.0% and a lower zone of inhibition at 1.5%, and a 4 mm higher than at 1.5% on 2.0% (Fig. 5a). When the sensitivity of the bacteria to Ciprofloxacin was tested, it shows a higher zone of inhibition than the extracts and Ampicillin. When Ampicillin alone was tested, it shows a lower zone of inhibition compared to the extract and Ciprofloxacin (Fig. 5b). Furthermore, when the organism was tested on extract plus Ciprofloxacin, a higher zone of inhibition was observed than when it was tested with Ampicillin plus extract (Fig. 5b).

DISCUSSION

In this study, the effect of 50% ethanol-methanol extract of *A. senegalensis* leaf extract was investigated on serum enzyme parameters. Liver enzymes such as AST, ALT, and ALP are important biomarkers of the liver damage normally found in large quantities in either plasma or serum when there is hepatic cellular membrane permeability alteration or cellular injury [17]. The activities of these enzymes in the serum of the experimental rats were examined and all the results obtained showed that the enzymes level in the serum increased significantly after the 1st–5th day. There are many conditions that may result into increase in the activities of liver function enzymes. Certain drugs may also cause increase in ALT levels in the serum. ALT is more specific for hepatic diseases than AST [18]. In general, there was an increase in the liver function enzymes following the administration of the leaf extract. The observed increase may be an indication of hepatic toxicity, cirrhosis, and cellular degeneration reported by Adisa *et al*. [19]. In contrast to this observation, the hydroacetonic extract of root barks from *A. senegalensis* has been shown to have no significant effects on plasma levels of liver enzymes (ALT, AST, and ALP) following intraperitoneal administration of high dose of the extract ranging from 600 to 5000 mg/kg [20]. However, the data obtained from this finding were similar to the report of Adisa *et al*. [19] who also observed increase in the serum liver enzymes following the administration of ethanol extracts (150 mg/kg) of the plant stem bark to Wistar albino rats for 28 days. This observation also suggests that tissue compromise may occur if rats were administered with ethanol-methanol leaf extract of *A. senegalensis* at the observed dosage.

Table 4: The zone of inhibition of *Escherichia coli* to *Annona senegalensis* leaf extract and drugs in mm

Drugs and/extract zone of inhibition (mm)	Concentration (%)				
	0.5	1.0	1.5	2.0	Drugs (0.5)
Extract	9	12	9	9	-
Ciprofloxacin	-	-	-	-	30
Ampicillin	-	-	-	-	25
Extract+Ciprofloxacin	30	35	35	35	-
Extract+Ampicillin	35	30	26	28	-

Table 5: The zone of inhibition of *Salmonella enterica* to *Annona senegalensis* leaf extract and drugs in mm

Drugs and/extract zone of inhibition (mm)	Concentration (%)				
	0.5	1.0	1.5	2.0	Drugs (0.5)
Extract	20	25	11	15	-
Ciprofloxacin	-	-	-	-	28
Ampicillin	-	-	-	-	12
Extract+Ciprofloxacin	28	28	32	33	-
Extract+Ampicillin	9	9	15	32	-

The antimicrobial susceptibility test has three categories of susceptibility, namely: Resistance, intermediate, and sensitivity. For Ampicillin, any zone less than or equal to 13 mm is resistance, any zone between 14 mm and 16 mm is intermediate, and any zone greater than or equal to 17 mm is sensitive while for Ciprofloxacin, any zone less or equal to 15 mm is resistance, any zone that is between 16 mm and 20 mm is intermediate, and any zone that is greater than 21 mm is sensitive. The zone inhibition observed in this study suggests that the plant leaf extract when combined with antibiotics used in this study is more resistant to bacteria (*E. coli* and *S. enterica*). *A. senegalensis* plant extract has been shown to possess some antimicrobial [21], anticancer [22], trypanocidal [2] activities. The methanol stem bark extract of *A. senegalensis* which has been investigated using both *in vivo* and *in vitro* models by oral application of effective dose (5000 mg/kg) shows that *A. senegalensis* is a potent phytomedicine for diarrhea [23]. The cytotoxic activity of *A. senegalensis* and anti-parasitic activity against *Trypanosoma brucei*, *Leishmania donovani,* and *Leishmania* has also been previously documented [24]. Alawa *et al*. [25] investigated the efficiency of the extract of *A. senegalensis* against *Haemonchus contortus* eggs and were shown a significant reduction in the egg hatch and larval recovery as the concentration increases. However, this research has revealed the antibacterial property of *A. senegalensis* leaf extract when used in synergy with other antibiotics like those used in this study.

CONCLUSION

A. senegalensis or African custard-apple is one of the potent medicinal plants generally used traditionally in the treatment of many diseases. The 50% of ethanol-methanol effects of *A. senegalensis* leaf extract on serum enzymes parameter investigated in this study shows that the plants have some significant effects on the system. This research revealed that there is high activity of ALP, AST, and ALT in the serum following the administration of 100 mg/kg body weight of 50% of ethanol-methanol leaf extract of *A. senegalensis*. The antibacterial susceptibility test of the leaf extract carried out *in vitro* also revealed the plant antibacterial property, although this property may not be as potent as the antibiotic drugs used which may be attributed to low dosage of the plant extract used. However, the combination of *A. senegalensis* leaf extract with antibiotic drugs, especially the one used in this study hold much promise in its efficacy as revealed in its synergistic interaction.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

All animal experiments were approved by the quality control unit of Kogi State University, Anyigba, Kogi State, Nigeria.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

All data and materials used for this research work are available from the corresponding author on reasonable demand

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AUTHORS' CONTRIBUTIONS

J.R.J. and A.V.J. proposed the idea for this researched work. J.R.J., O.O.J., and A.V.J. conducted experiments and analysis and contributed to the design and discussion of the results. A.V.J. drafted this work and contributed to the original writing, while O.O.J. and J.R.J. carried out review and proofreading of this researched work. O.O.J. critically revised the manuscript. All authors read and approved the final manuscript.

COMPETING INTEREST

Authors declare no competing interest and give consent to the publication of this work.

FUNDING

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