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ETHANOL EXTRACT OF IRVINGIA GABONENSIS (BUSH MANGO) SEED INCREASES MICROBIAL LOAD IN THE COLON OF WISTAR RATS

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ABSTRACT

This study assessed the microbial load of the colon of Wistar rats treated with *Irvingia gabonensis* seed extract (IGS_{ex}). Eight Wistar rats (200-250g) of both sexes were divided into two groups (n=4) thus: Normal group (received normal rat feed and water) and treated group (received 250mg/kg b.w. of IGS_{ex} in addition to normal feed and water). Treatment lasted for twenty-one days and was done twice daily via orogastric intubation. Rats were sacrificed under chloroform anaesthesia and each rat's colon was removed and put in a well labeled sample container containing normal saline. Total *anaerobes*, *bacteriodes*, *coliform* and *enterococcus* were present and their concentrations significantly ($p<0.05$) increased in colon of rats in the treated group compared with those in the normal group. *Lactobacillus* was only very slightly increased in treated group compared with normal group. Carbohydrate such as cellulose, lignin, pectin and hemicellulose were present in the colon of rats in both groups. Resin was absent in the colon of rats in the normal group but present in the treated group. There was complete absence of *fungi* and starch in the colon of rats in both groups. All these carbohydrates and starch were present in the seed extract with hemicellulose and lignin occurring highest and lowest in concentration respectively. IGS_{ex} therefore increased the colonic load as well as the fermentation activity of microbes such as *anaerobes*, *bacteriodes*, *coliform* and *enterococcus* in Wistar rats.

KEYWORDS: Carbohydrate; Colon; fermentation; *Irvingia gabonensis*; Microbe; rat.

INTRODUCTION

Irvingia gabonensis (IG) (family: Irvingiaceae), commonly called bush mango, African mango, wild mango or Dikanut is a non-timber forest product, made up of tree trunk (stem), leaves, roots and fruits.[1] It is found in rainforests and widely distributed in tropical West Africa.[2] The fruit is made up of a fleshy part and nut. The nut in turn is made up of a hard shell and seed (kernel). *Irvingia gabonensis* (IG) seeds are known as Ogbuno in Ibo and Apon in Yoruba, Nigeria. They form an important part of the diet in rural areas in Nigeria and are used as thickening agents in soups and stews.[3,4] The seeds contain a sticky wax (mucilage) that is useful for making medicinal tablets. This wax acts as a binding agent during the production of drug tablets.

Phytochemical screening of IG seeds revealed the presence of tannins, flavonoids, steroids, terpenoids, carbohydrate, volatile oils, cardiac glycosides, saponins and alkaloids.[2] IG have been reported to possess antioxidant,[5] antimicrobial, anti-diarrhoeal,[6] antifungal,[7] anti-diabetic,[8,9] radioprotective,[10] anti-poison[11] and anti-inflammatory and anti-arthritic[12] properties. IG seeds have also been reported to improve renal and hepatic functions.[13]

The major microbes in the colon of Wistar rats include: *Anaerobes, Bacteriodes, Coliform, Enterococcus and Lactobacillus*. The bacteria colon present are *Actinobacterium sp., Bacteriodes bacterium, B. dorei, B. fibrisolvas, B. sartorii, B. vulgates, Clostridium sp., Enterococcus sp., Fusobacterium sp., Lachnospiraceae sp*. and *Lactobacillus sp*.[14] The fermentation activity of these microbes in the colon of the rats is presumably increased with increase in the consumption of dietary fibre.[15] Therefore, the more the rats feed the higher the rate of fermentation in the rats' colon. Generally, the absence of intestinal bacterial flora is associated with reduction in mucosal cell turnover, vascularity, muscle wall thickness, motility, hyperactivity of digestive enzymes, baseline cytokine production and defective cell-mediated immunity.[16]

Wistar rats can be treated with plant extracts using methods designated as enteral administration. This involves the introduction of substances into the gastrointestinal tract of an animal via the mouth. These

extracts can be administered by mixing them with the food or water of the animal (dietary administration) or by passing the extracts in their dissolved form directly into the gastrointestinal tract of the animal using a gavage (intragastric administration).[17] The administration of an extract over a period of time will allow the effects of its phytochemical contents to play out, which may alter the microbial activity in the colon of these rats, as well as affect the microbes themselves. *Irvingia gabonensis* seed extract (IGS_{ex}) is one of such extracts that can be administered to Wistar rats (within the limits of the lethal dosage) and then the effects on the microbial flora in the rats' colon can be investigated. There is knowledge on ground already on the usefulness of IGS_{ex} for treating fungal infections.[18] There have been native claims that the microbial flora of the human large intestine (colon) is affected by IG seeds when consumed as food. But there are little or no scientific proofs to these claims which makes the seeds unidentifiable as suitable sources of drugs to control colon microbes. The present study therefore sought to investigate the credibility of these claims by evaluating the effect of *Irvingia gabonensis* seed extract on the microbial load of the colon of Wistar rats.

MATERIALS AND METHODS

Animal Preparation

Eight Wistar rats of both sexes (200-250g body weight) collected from the Animal House of Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Nigeria were used for the study. The animals were handled according to Helsinki's.[19] laid down principles. They were housed in well ventilated wooden cage with wire merge at the top and kept under normal environmental conditions. The animals were allowed access to rat feed and water *ad libitum* and exposed to 12/12-hour light/dark cycle. They were allowed for seven days to acclimatise before commencement of treatment. Care was taken to use sterile containers for feeding the rats, and the feed and water were changed daily and the cages cleaned daily in order to maintain good hygiene.

Preparation of *Irvingia gabonensis* seed extract (IGS_{ex})

Fresh fruits of *Irvingia gabonensis* (IG) were carefully plucked from its tree in the University of Calabar farm. The *Irvingia gabonensis* tree was identified by Dr. Mike Eyo, a botanist in the University of Calabar. The fresh fruits were opened to remove the seeds. The seeds were later dried under the sun, and a manual hand grinder was used to grind the dried seeds to powdery form. The entire sample extraction process was carried out in the Endocrinology and Medicinal Plant Research Laboratory of the Department of Biochemistry, University of Calabar. About 100 grams of the powdered IG seed sample was soaked in 300mls of ethanol and covered in an air-tight container for 48 hours. Afterwards, the mixture was sieved with a sieve cloth to get the filtrate. Further filtration was done by pouring the filtrate into a filter cloth, and finally into a filter paper to obtain the main IGS_{ex}

Experimental design and extract administration

The eight Wistar rats (200-250g) were randomly assigned into two groups (n=4) thus: Normal group: received normal rat feed and water. Treated group: received 250mg/kg body weight of IGS_{ex} in addition to normal rat feed and water. The extract administration lasted for twenty-one days and was done twice daily (8am and 3pm) via orogastric intubation. The dosage of the extract used falls comfortably below the lethal dosage which is $\geq$ 2500 mg/kg body weight.[20]

Collection of Wistar rat's colon

At the end of the 21 days of administration, all the 8 Wistar rats were sacrificed under chloroform anaesthesia. They were placed on a dissecting slab where a longitudinal cut was first made abdominally to the rib cage followed by transverse cut to the limbs using surgical scissors. Each rat's colon was removed and put in a well labeled sample container containing normal saline and taken to a microbiology laboratory for microbial evaluation.

Microbial evaluation of wistar rat's colon

0.5 gram of fresh colon content of each rat was obtained from each colon and was well labeled. The viable microbial cells in each colon were then determined via the following three steps:

Step 1: Serial dilution of the fresh colon content.

Step 2: Plating on selective media and Columbia blood agar.

Step 3: Aerobic or anaerobic incubation at 37^0C for 14 days, followed by microbial identification and count using a microscope.

Aerobic incubation required normal laboratory atmosphere while anaerobic incubation required an atmosphere of 10% CO_2, 10% H_2, and 80% N_2 in the incubation chamber. The following media and incubation models were used:

a. Endo agar incubated aerobically for *Escherichia coli* and *Enterococci*.
b. De Man, Rogosa and Sharpe (MRS) agar incubated aerobically for *Lactobacillus spp.*
c. Columbia blood agar incubated aerobically for whole numbers of aerobes and anaerobically for anaerobes.
d. Columbia blood agar with Neomycine incubated anaerobically for *Bacteroides* group.

Determination of carbohydrates by spectrophotometry

The concentrations of the carbohydrates in the colon of all the rats and in the *Irvingia gabonensis* seed extract were determined by first extracting the carbohydrates as follows:

1. 1 gram each (W1) of finely crushed *Irvingia gabonensis* seed and sample of fresh colon content

of all the rats were measured into separate 250 ml Erlenmeyer flasks.
2. 1-3g $CaCO_3$ was added to neutralize each sample.
3. 25 ml of 85% ethanol was added to each sample
4. The flasks were then capped with aluminum foil and placed on shaking water bath at 60^0C for 1h.
5. Each sample was removed from the shaking water bath and the solution was immediately filtered through a pre-weighed filter paper into a 250 ml flat bottom flask.

The procedure above was repeated with 25ml of boiling 85% ethanol three times. Then the residues were dried on filter paper with 2 × 25 ml acetone and put in an autoclave at 105^0C for 10 minutes. They were then cooled in a desiccator and weighed (W2). The carbohydrates in each sample were hydrolyzed using protease and amyloglucosidase enzymes, requiring sodium phosphate buffer, termamyl, NaOH, HCl and 95% ethanol. Then each product was centrifuged at 3000 revolutions per minute for 15 minutes. 1:20 sample dilution with distilled water was carried out for each supernatant and 0.15ml of each product was mixed with 2.5ml of PAHBAH solution. Each solution was boiled for 5 minutes, cooled in a dessicator, weighed (W3) and read at 410nm using a spectrophotometer. The carbohydrate content was determined against normal standards.

Calculations

$$Carbohydrate\,(g/100g) = \frac{\%\,Total\,carbohydrates \times 0.9 \times W2}{W3 \times W1}$$

Where
W1 = weight of sample
W2 = weight of carbohydrate extracted
W3 = weight of carbohydrate taken for analysis

Determination of fermentation end products

Soluble fermentation end products were determined by high-performance liquid chromatography procedures.[21] An aminex ion-exclusion column (HPX-87H; Bio-Rad Laboratories, Richmond, Calif.) at 35^oC, which was eluted with 0.0013N H_2SO_4 (0.55mL/min, 77kg. cm^2) was used. Compounds were detected by refractive index, identified and quantified with a C-R3A integrator (Shimadzu Scientific, Baltimore, Md.) using the absolute calibration curve method.

The amounts of end products were determined by external standards with reference to internal standards. Formate, acetate, propionate, butyrate, lactate, succinate and ethanol were measured in colonic samples using the Megazyme biochemical assay kits according to the manufacturer's instructions

Statistical Analysis

Results are presented as mean ± standard error of mean (SEM). Computer software, SPSS (version 21) was used for data analysis with Student's T-test used for comparing the means between the two groups. $p<0.05$ was considered statistically significant

RESULTS

Comparison of concentration of microbes between normal and treated groups

Figure 1 below shows *anaerobes*, *bacteriodes*, *coliform*, *enterococcus*, and *lactobacillus* to be present in the colon of albino Wistar rats in both the normal and treated groups. These microbes occurred in significantly higher ($p<0.05$) concentrations in the colon of rats in the treated group compared with rats in the normal group. But *lactobacillus* was not significantly different between the two groups. There was complete absence of *fungi* in the colon of rats in both groups.

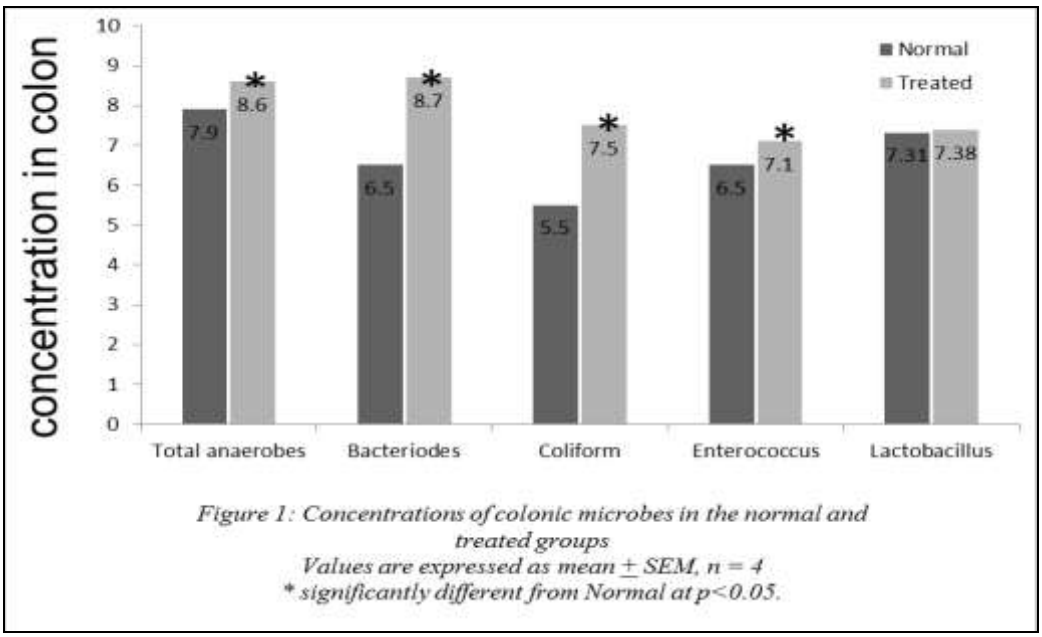

Figure 1: Concentrations of colonic microbes in the normal and treated groups
Values are expressed as mean ± SEM, n = 4
** significantly different from Normal at p<0.05.*

Comparison of Colonic Carbohydrate Concentration between the Normal and Treated Groups

Figure 2 shows cellulose, lignin, pectin and hemicellulose to be present in the colon of Wistar rats in both the normal and treated groups. Concentrations of cellulose, pectin and hemicellulose were significantly ($p<0.05$) decreased in the treated group compared with the normal group. The concentration of lignin was significantly ($p<0.05$) increased in the treated group compared with the normal group. But resin occurred only in the colon of rats in the treated group, as it was completely absent in the colon of rats in the normal group. Distinctively, there was complete absence of starch in the colon of both groups of rats.

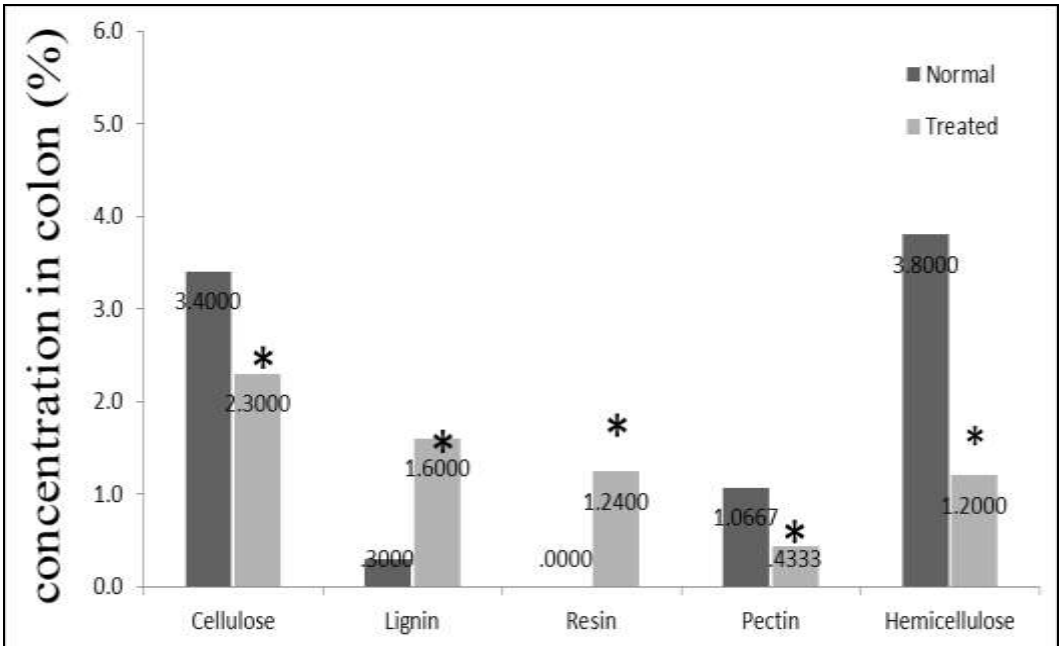

Figure 2: Comparison of concentration of carbohydrates between the normal and treated groups

Values are expressed as mean ± SEM, n =4
** significantly different from Normal at p<0.05.*

Comparison of Fermentation End Products between the Normal and Treated groups

Table 1 shows acetate (umol/g), propionate (umol/g), butyrate (umol/g), formate (umol/g), lactate (umol/g), ethanol (umol/g) and succinate (umol/g) to be present in the colon of Wistar rats in both the normal and treated groups. Apart from lactate and succinate which were significantly ($p<0.05$) decreased in the colon of rats in the treated group compared with rats in the normal group, all other fermentation end products were significantly ($p<0.05$) increased in the colon of rats in the treated group compared with those in the normal group.

Table 1: Comparison of fermentation end products (umol/g) between the normal and treated group.

	Acetate	Propionate	butyrate	Formate	Lactate	Ethanol	Succinate
Normal	44.48	3.10	3.30	1.10	48.00	0.71	4.80
	±0.31	±0.07	±0.04	±0.07	±0.71	±0.01	±0.08
Treated	77.75	8.50	14.65	5.35	31.85	0.82	1.60
	±0.46*	±0.04*	±0.06*	±0.06*	±0.50*	±0.00*	±0.04*

Values are expressed as mean ±SEM, n = 4.
** = significantly different from normal at p<0.05*

Concentrations of carbohydrates in *Irvingia gabonensis* seed extract (IGS_{ex})

Table 2 shows cellulose, lignin, resin, starch, pectin and hemicellulose present in the IGS_{ex} with hemicellulose and lignin occurring highest and lowest in concentration respectively.

Table 2: Concentrations of carbohydrates in *Irvingia gabonensis* seed extract.

Carbohydrates in extract (%)	Mean ± SEM
Cellulose	6.30±0.12
Lignin	1.33±0.07
Resin	1.37±0.03
Starch	5.37±0.18
Pectin	2.17±0.12
Hemicellulose	14.30±0.10

Values are expressed as mean ± SEM, n =4

DISCUSSION

Irvingia gabonensis (IG) has been reported to possess several medicinal properties. This study was carried out to assess the impact of *Irvingia gabonensis* seed extract (IGS_{ex}) on microbial load of the colon of Wistar rats. IGS_{ex} contains carbohydrates such as cellulose, lignin, resin, starch, pectin and hemicelluloses.[22] These carbohydrates add to the bulk already present in the normal rat chow on which the Wistar rats were fed on. Starch was completely absent in the colon of Wistar rats treated with *Irvingia gabonensis* seed extract as well as in the colon of the normal group rats. But from Table 2, starch was found to be abundantly present in *Irvingia gabonensis* seed extract. This increased amount of starch in *Irvingia gabonensis* seed extract probably explains the increase fermentation in the colon of the treated rats as evidenced in the result in table 1 where fermentation end products were significantly increased in the treated group compared with rats in the normal group. It is known that starch undergoes fermentation in the colon. The fermentation processes in the colon are largely controlled by the amounts and types of substrates, particularly complex carbohydrates such as starch, accessible to the microbes in the colon.[23] This suggests that the starch component of the rats' food and that of the seed extract were completely fermented in the rats' colon.

Furthermore, figure 2 shows that resin was completely absent in the colon of rats in the normal group but was present in the colon of rats in the treated group. From Table 2, resin was found to be present in *Irvingia gabonensis* seed extract. It therefore follows that the bulk of resin in the colon of rats treated with the seed extract were derived from the IGS_{ex} administered to the rats. More so, figure 2 shows that cellulose, lignin, pectin and hemicelluloses were present in both the normal and treated groups. But these carbohydrates occurred significantly in higher concentrations in the colon of rats in the treated group. It is known that these carbohydrates cannot be digested by the rats, and they also escape fermentation in the colon.[23] Table 2 shows that these carbohydrates were present in IGS_{ex}. Thus since more of these carbohydrates were introduced into the treated rats' digestive system by the administration of IGS_{ex}, they therefore occurred in increased concentration in the colon of these rats.

Figure 1 shows that *anaerobes*, *bacteriodes*, *coliform*, *enterococcus*, and *lactobacillus* were present in the colon of both groups of Wistar rats. But, these microbes occurred significantly in higher concentrations in the colon of rats treated with IGS_{ex}, except for *lactobacillus* which was only very slightly higher in concentration. All these microbes are either gram positive or gram negative bacteria,[14] and they ferment starch for their nutrition and survival. This implies that these microbes fermented the starch in the food and *Irvingia gabonensis* seed extract administered to the rats, leading to its complete absence from the colon of all the rats. The increased concentration of starch supplied to the colon of the rats in the treated group as a result of additional starch from IGS_{ex} will make room for more fermentation in the colon of this group of rats,[15] which will in turn cause higher survival chances for the microbes in the colon of these rats and encourage their speedy reproduction. Our result for fermentation end products shows that acetic acid, propionic acid, butyric acid, formate, lactic acid, ethanol and succinic acid fermentations occurred in the colon of the rats in both groups. IGS_{ex} increased these fermentations as observed in our result where the various fermentation end products except lactate and succinate were significantly increased in the treated group compared with the normal group (Table 1). The significant decrease in lactate in the treated group compared with the normal group implies that IGS_{ex} decreased lactic acid fermentation. This may mean that the extract decreased the activity of lactic acid bacteria. Lactic acid is mostly used in food and pharmaceutical processes and is produced from glucose by homofermentative lactic acid bacteria like *Lactobacillus,*[24] The level of *Lactobacillus* was not significantly different between the normal and treated groups as observed in our study.

Fungi are not capable of fermentation and thus have no chance of survival in the colon of both groups of rats.[18,25]

CONCLUSION

The major microbial activity in the colon of Wistar rats is fermentation. *Irvingia gabonensis* seed extract increased the load of microbes such as *anaerobes*, *bacteriodes*, *coliform* and *enterococcus* contained in the colon of Wistar rats and enhanced their fermentation activity but had no significant effect on *lactobacillus*. Fungi are absent in the colon of Wistar rats.

REFERENCES

1. Ekundayo FO, Oladipupo OA, Ekundayo EA. (Studies on the effects of microbial fermentation on bush mango [*Irvingia gabonensis*] seed cotyledons). Afr J Microbiol Res, 2013; 7(34): 4363-7.
2. Obianime AW, Uche FI. (Effects of aqueous extracts of *Irvingia gabonensis* seeds on the hormonal parameters of male guinea pigs). Asian Pacific Journal of Tropical Medicine, 2010; 200-4.
3. Ekpe OO, Umoh IB, Eka OU. (Effect of a typical rural processing method on the proximate composition and amino acid profile of bush mango seeds [*Irvingia gabonensis*]). African J Food Agric Nut Dev, 2007; 7(1): 1-12.
4. Ndjouenkeu R, Goycoolea FM, Morrisa ER, Akingbala JO. (Rheology of okra [*Hibiscus esculentus* L.] and dika nut [*Irvingia gabonensis*] polysaccharides). Car. Polym, 1996; 29(3): 263-9.
5. Wong SK, Lim YY, Chan EWC. (Antioxidant properties of Hibiscus: species variation, altitudinal change, costal influence and floral colour change). J Tro For Sci, 2009; 21(4): 307-15.
6. Okolo CO. (The analgesic effects of *Irvingia gabonensis* stem bark extract). J Ethno Pharmacol, 1995; 45(2): 125-9.
7. Khan M, Wassilew SW. Natural pesticides from the neem tree and other tropical plants. Schmutterer H and Ascher KRS (eds.). Germany; Digitalverlag GmbH, 1987; 645-650.
8. Singh N, Gupta M. (Effect of ethanolic extract of *Syzygium cumuni* [Linn] seed powder on pancreatic islets of alloxan diabetic rats). Indian J Exp Biol, 2007; 45(10): 861-7.
9. Agbor LO. (Marketing trends and potentials for *Irvingia gabonensis* products in Nigeria. Conference on I. gabonensis. Ibadan Nigeria, May 1994.
10. Jagetia GC, Baliga MS, Venkatesh P. (Influence of seed extracts of *Sygygium. cumini* [Jamun] on mice exposed to different doses of gamma-radiation). J Radiat Res, 2005; 46(1): 59-65.
11. Ayuk ET. (Uses, management and economic potential of *Irvingia gabonensis* in humid low lands of Cameroon). Forest Ecology and Management, 1999; 113: 1-9.
12. Kumar A, Ilavarasan R, Jayachandran T, Deecaraman M, Kumar RM, Aravindan P, Padmanabhan N, Krishan MRV. Anti-inflammatory activity of *Syzygium cumini* seed. Afr J Biotechnol, 2008; 7(8): 941-3.
13. Bassey SO, Udefa AL, Ekpe OO, Essien NM, Eteng MU. (Ethanol extract of *Irvingia gabonensis* [bush mango] seed improves renal and hepatic functions in wistar rats). IOSR Journal of Pharmacy and Biological Science, 2018; 13(3): 46-50.
14. Miller TL, Wolin MJ. (Enumeration of *Methanobrevibacter smithii* in human feces). Arch. Microbiol, 1982; 131(1): 14-8.
15. Maczulak AE, Wolin MJ, Miller TL. (Amounts of viable anaerobes, methanogens, and bacterial fermentation products in feces of rats fed high-fiber or fiber-free diets). Applied and Environmental Microbiology, 1993; 59(3): 657-62.
16. Canny GO, McCormick BA. (Bacteria in the intestine, helpful residents or enemies within)? Infect and Immun, 2008; 76(8): 3360-73.
17. Claassen V. Neglected factors in pharmacology and neuroscience research. Amsterdem; Elsevier: 1994.
18. Kuete V, Wabo GF, Ngameni B, Mbaveng AT, Metuno R, Etoa FX, Ngadjui BT, Beng VP, Meyer JJ, Lall N. (Antimicrobial activity of the methanolic extract, fractions and compounds from the stem bark of *Irvingia gabonensis* [Ixonanthaceae]). J Ethnopharmacol, 2007; 114(1): 54-60.
19. Helsinki. World Medical Association Declaration of Helsinki. Adopted by the 18th WMA General Assembly, Helsinki, Finland, 1964.
20. Kothari SC, Shivarudraiah P, Venkataramaiah SB, Gavara S, Soni MG. (Subchronic toxicity and mutagenicity/genotoxicity studies of *Irvingia gabonensis* extract [IGOB131]). Food Chem Toxicol, 2012; 50(5): 1468-79.
21. Ehrlich GG, Goerlitz DF, Bourell JH, Eisen GV, Godsy EM. (Liquid chromatographic procedure for fermentation product analysis in the identification of anaerobic bacteria). Appl Environ Microbiol, 1981; 42(5): 878-85.
22. Li BW. (Determination of sugars, starches, and total dietary fiber in selected high-consumption foods). J AOAC Int, 1996; 79: 718-23.
23. Macfarlane GT, Macfarlane S. (Fermentation in the human large intestine: its physiologic consequences and the potential contribution of prebiotics). J Clin Gastroenterol, 2011; 45: 120-7.
24. Müller V. Bacterial Fermentation. Encyclopedia of Life Sciences, Nature Publishing Group. Ludwig-Maximilians- Universität München, Munich, Germany, 2001.
25. Fadare DA, Ajaiyeoba EO. (Phytochemical and antimicrobial activities of the wild mango-*Irvingia gabonensis* extracts and fractions). Afr J Med Med Sci, 2008; 37(2): 119-24.