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IN VITRO ANTIFUNGAL, ANTIBACTERIAL ACTIVITIES AND NUTRITIONAL VALUE OF NINE CAMEROONIAN EDIBLE VEGETABLES AND SPICES

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ABSTRACT

Background. The high medicinal potential of plants, including edible vegetables, is well documented. Vegetables can afford significant health benefits to consumers, depending on their medicinal properties and nutritional value. This study analysed the antimicrobial activity and nutrient contents of nine commonly consumed Cameroonian vegetables and spices for which such information is at present limited.
Material and methods. The antimicrobial activity of the methanol extracts of the vegetables was evaluated by disc diffusion and microdilution methods against three tomato fungi and two pathogenic bacteria species.
Results. The inhibition zones against fungi ranged from 10–21 mm; *Irvingia gabonensis* and *Apium graveolens* showed the highest zones with dose-dependent activity against *Fusarium solani* and *F. oxysporum*. The inhibition zones against bacteria ranged from 8–12 mm with *Allium porrum* having the highest inhibition zone (12 mm). *Irvingia gabonensis* seeds had the lowest minimum inhibitory concentration (MIC) of 6.25 mg/mL against *F. solani* and also had the lowest MIC of 2 mg/mL against *S. aureus.* Proximate composition and mineral analysis were carried out on the most active antimicrobial vegetables, *I. gabonensis* seeds and *A. graveolens. I. gabonensis* seeds were rich in lipids (69.90 ±0.14%) while *A. graveolens* leaves were rich in protein (35.35 ±0.49%). For macro minerals, phosphorous had the highest concentration in *I. gabonensis* seeds (359.67 ±1.89 mg/100 g) and *A. graveolens* leaves (622.14 ±2.69 mg/100 g). Iron content was the highest of micro minerals in *I. gabonensis* seeds (276.51 ±1.41 mg/100 g) while zinc concentration was the highest in *A. graveolens* leaves (16.86 ±0.27 mg/100 g).
Conclusion. This study has shown that three of the nine Cameroonian vegetables, *I. gabonensis* seeds, *Apium graveolens* and *A. porrum*, may potentially offer both antimicrobial and nutritional benefits to consumers. Consequently, further studies should be conducted to ascertain the effect of cooking and other factors in order to maximize these benefits.

Keywords: vegetables, spices, edible, nutritional, antimicrobial

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INTRODUCTION

The burden of infectious diseases remains high and is a major contributor to global morbidity (Roser and Ritchie, 2016). This situation necessitates the constant search for suitable antimicrobials. Plants remain a major source of drugs principally derived from their secondary metabolites, which have been used to treat a wide range of diseases (Veeresham, 2012). Vegetables are part of the natural flora and a major part of the human diet consumed worldwide, not only as a source of nutrients but also vitamins, minerals, fibre or roughage (Slavin and Lloyd, 2012). Vegetables also contain various phytochemicals, which have both protective (carotenoids, flavonoids, indoles, phenols, limonene, sterols etc.) and harmful effects (aflatoxin, nitrates, goitrogens, phenolic compounds, enzyme inhibitors etc.) in the human body (Slavin and Lloyd, 2012). There are several reports on the beneficial effects of vegetable consumption. Findings show that frequent intake of vegetables is associated with a decreased incidence and mortality of chronic diseases, and may prevent cancers, cardiovascular diseases and obesity (Oguntibeju et al., 2013). There are also several reports on the antimicrobial (antibacterial, antifungal) activity of some edible vegetables and spices.

A wide variety of vegetables and spices are cultivated in Cameroon and particularly in Buea, the study area (Ngome and Foeken, 2012). The nine vegetables and spices investigated in this study are widely consumed. In most parts of Cameroon, the fresh young shoots of *Solanum nigrum* are cooked as leafy vegetable sauce and served with preparations of corn fufu, plantains and some tubers (Asseng et al., 2017). In areas where *Manihot esculenta* leaves are eaten as a relish, it is prepared by pounding and grinding of the leaves and young shoots before cooking, and consumed as a main meal or accompaniment to various cereal, root and tuber foods (Achidi et al., 2008). *Allium porrum*, *Apium graveolens* and *Petroselinum crispum* are spices used routinely as flavor enhancers when cooking sauces and vegetable meals. *Amaranthus cruentus* and *Telfairia occidentalis* are usually blanched before further preparation and eaten with boiled plantains, tubers and dough made from cereal or tuber flours. *Gnetum africanum* and *Irvingia gabonensis* are a regular part of the menu in homes, restaurants and social events (Abia et al., 2007). *Gnetum africanum* is a tough vegetable softened with *Talinum triangulare* leaves when being cooked.

However, there is a lack of information on the medicinal and health benefits that may be derived from the consumption of these vegetables and spices. Antibacterial activity has been recorded for *Irvingia gabonensis* bean or seed, and leaves of *Manihot esculenta* and *Solanum nigrum* vegetables grown in the West region of Cameroon (Nayim et al., 2018; Noumedem et al., 2013). However, there is no or limited information on the antibacterial and or antifungal activities of *Allium porrum*, *Amaranthus cruentus*, *Manihot esculenta* and *Petroselinum crispum* grown locally.

The preservative properties of these vegetables acting against microbial damage to foods has also not been extensively investigated. For instance, the tomato, which is widely cultivated in Cameroon, is highly susceptible to fungal attack leading to a high post-harvest loss (Fontem et al., 1999). Some fungi which attack tomato fruits threaten human health by producing mycotoxins capable of inducing mycotoxicosis following ingestion or inhalation (Wagacha and Muthomi, 2008), hence there is a need for an effective preservative for these fruits. Furthermore the nutritional value of the local varieties has not been determined. This information will reveal the percentage of recommended dietary allowance (RDA) that can be gained from them and indicate the consumption of these vegetables in order to gain optimal benefits. This study focused on Cameroonian vegetables and spices commonly consumed in the study area which have not been extensively investigated for both antimicrobial activity and their nutritional value.

MATERIALS AND METHODS

Collection, processing and preparation of extracts

The vegetables and spices, were bought from Muea market in Buea, South West Region and identified by Mr. Peter Njimba, a botanist at the herbarium of the Limbe Botanical Garden, Cameroon. The edible parts used in this study are shown in Table 1. The plant parts were chopped, air-dried for 3 weeks then ground to a fine powder and weighed. Each powder was macerated by submerging in methanol for three

Table 1. Information, yields and antibacterial activities of methanol extracts of vegetables and spices

Species voucher number	Family	Local name(s)	Edible plant part; used	Extract		
				weight g	yield %	zone of inhibition* mm
Allium porrum SCA 7762	Alliaceae	Leeks	stem and leaves; spice	0.80	1.46	12 ±0.0
Amaranthus cruentus SCA 6350	Amaranthaceae	Green, Folong	young shoot and leaves; vegetable	0.30	0.25	–
Apium graveolens SCA 356	Apiaceae	Celery	leaves; spice	2.48	6.83	10 ±0.0
Gnetum africanum SCA 4120	Gnetaceae	Eru, Okok	leaves; vegetable	2.59	2.59	7.0 ±0.0
Irvingia gabonensis SCA 4645	Irvingiaceae	Bush mango, Ogbonoh	seed; vegetable	3.49	5.82	11.0 ±0.0
Manihot esculenta SCA 3800	Euphorbiaceae	Cassava	young shoot and leaves; vegetable	1.30	1.30	8.0 ±0.0
Petroselinum crispum 25583/HNC	Apiaceae	Parsley	leaves; spice	7.50	8.82	9.0 ±0.0
Solanum nigrum SCA 4248	Solanaceae	Huckleberry, Njamanjama	young shoot and leaves; vegetable	1.85	3.36	8.0 ±0.0
Telfairia occidentalis SCA 2856	Cucumbitaceae	Oko-obong	young shoot and leaves; vegetable	2.78	5.01	9.0 ±0.0
Gentamicin (PC)						22 ±0.0

PC – positive control.
*Zone of inhibition against *S. aureus*.

days and a crude extract was prepared by filtration and rotary evaporation with the water bath temperature set at 65°C. The extracts were kept in open containers at room temperature to dry to a constant mass, which was then stored at 4°C and tested within one week (Mbah et al., 2012).

Determination of the antifungal activity of extracts

Three fungal species (*Fusarium oxysporum*, *Fusarium solani*, *Colletotrichum* sp.) previously shown to cause tomato fruit spoilage postharvest were obtained from the Biotechnology Unit of the University of Buea. They had been isolated from tomatoes and characterised using molecular tools in another study (Takam et al., 2019). They were cultured and tested by disc diffusion on a Muller Hinton (MH) agar plate (containing Chloramphenicol 0.05 mg/mL) as described (Takam et al., 2019). Discs (containing 1 to 100 mg) were tested with positive (Econazole 1 mg/mL) and negative controls (Tween-80). The plates were incubated at room temperature for 4 days and zones of inhibition measured.

The minimum inhibitory concentration (MIC) was determined for extracts of *Irvingia gabonensis* and *Apium graveolens*), which were active in the disc diffusion test according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST, 2008), as described previously (Takam et al., 2019). The final extract concentration in 100 µL MH broth in a 96-well microtitre plate was 1 to 100 mg/mL in duplicate with positive (Fluconazole 200 µg/mL) and negative controls (no extract). Then, 100 µL of a McFarland 0.5 conidial spore suspension of a 5-day culture was added, the plate incubated at room temperature and the absorbance read at 595 nm (Emax-Molecular Devices Corporation, California, USA) at 0, 24, 48 and 72 h. The lowest MIC value after visual observation and confirmed using absorbances was recorded.

Determination of the antibacterial activity of extracts

Staphylococcus aureus (Gram-positive) and *Escherichia coli* (Gram-negative) were obtained from the stock of the Biotechnology Unit, University of Buea (originally isolated from clinical specimens and characterized in the medical laboratory of the Buea Regional referral hospital). They were further characterized using cultural and biochemical tests (API 20E test kit, Biomérieux, France). The disc diffusion method was used according to the Clinical and Laboratory Standards Institute procedure (CLSI, 2012), as described (Bate et al., 2018), with 4 mg discs on Muller Hinton (MH) agar and zones of inhibition recorded.

The MIC was determined (for *S. aureus* inhibited in the disc test) by the micro-dilution method (Mbah et al., 2012) with some modifications. The extract (100 µL of 0.5 to 16 mg/mL final concentration) was incubated with bacteria (100 µL of 6×10^5 CFU/mL) in a MH broth in duplicate wells in a 96-well microtitre plate at 37°C for 24 h. Negative (no extract) and positive control (20 µg/mL Gentamicin) wells were included. The MIC was the lowest concentration that showed no turbidity and was determined as for the antifungal assay above.

Determination of nutritive value. Nutrition value was determined only for *Irvingia gabonensis* seeds and *Apium graveolens* leaves which demonstrated antimicrobial activity using the standard analytical methods of the Association of Official Analytical Chemists (AOAC, 2000). All analyses were done in duplicate and are briefly described below. Fresh vegetable leaves were processed and stored in airtight containers at room temperature until they were analyzed.

Moisture content was determined in the fresh sample (2 g) in crucibles heated in an oven (Heraeus T 6060, Germany) as required and the percentage moisture content calculated (AOAC, 2000). The lipid content was estimated by soxhlet extraction from 4 g of a sample with 200 mL of hexane at 70–80°C for 16 h. The extract was concentrated by rotary evaporation, dried to completely remove the residual solvent and weighed.

For the crude fiber content, a defatted sample (2 g) was boiled with 1.25% of H_2SO_4 and bumping chips for 30 min, the mixture filtered (Whatman no. 1) and then washed with boiled distilled water to completely remove acid. The residue was re-boiled with 1.25% NaOH, filtered and washed serially with 1.25% H_2SO_4, hot distilled water and 70% ethanol. The residue was dried in a crucible (80°C for 3 h), incinerated (500°C), and the fiber content determined from the recorded weights.

The protein content was estimated by the Kjeldahl method (AOAC, 2000) following three steps: mineralization, distillation and titration of the sample to obtain the nitrogen content, which was multiplied by a conversion factor of 6.25 to obtain the crude protein content.

The ash content was determined in 2 g of the milled sample in a crucible and incinerated for 4 h at 600°C in a furnace (Kendro 1252F, Germany). The carbohydrate content was determined by subtraction after the content of all the other nutrients had been determined.

Ten minerals were assayed. One gram of the sample was weighed and dry-ashed in a muffle furnace at 450°C, then allowed to cool in a desiccator and diluted using a mixture (400 mL conc. HCl and 133 mL of 70% HNO_3). The solution was used to measure minerals using an atomic absorption spectrophotometer at corresponding wavelengths.

Vitamin C was determined by iodometric titration as described by Dioha et al. (2011). Briefly, 5 g of the sample was macerated (in 50 mL distilled water for 2 h) and the extract centrifuged (Eppendorf 5810R, Germany). Then 1 mL of supernatant was diluted, acidified (1 mL of 2N H_2SO_4), after which 1 mL of 1% starch solution was added and rapidly titrated against sodium thiosulphate. Vitamin C content was calculated against a titrated distilled water blank.

Data analysis

All laboratory analyses were done in duplicate. Zone diameters recorded from the disc diffusion tests for antifungal activity were plotted against the amount of extract using Microsoft Excel 2010. Data for nutritive values were reported as means ±standard deviation (S.D.).

RESULTS AND DISCUSSION

Antifungal and antibacterial activities of extracts

In the disc diffusion test, three of the nine vegetable extracts showed high antifungal activity with zones of inhibition from 10 to 21 mm. *I. gabonensis* extract showed dose-dependent activity against both *Fusarium* species. *I. gabonensis* was the most active extract with the highest inhibition zones against *F. oxysporum* (21 mm) and *F. solani* (20 mm) at 100 mg/disc (Fig. 1). *Apium graveolens* and *Allium porrum* showed activity against *F. solani* at 4 mg and 20 mg extract per disc giving zones of 15 mm and 12 mm respectively. No extract was active against *Colletotrichum* sp. Meanwhile, eight extracts produced smaller zones of inhibition of 8–12 mm against *S. aureus* with no zone against *E. coli*. The highest activity (12 mm) was shown by *Allium porrum*. Only *Amaranthus cruentus* was not active. The small zones of inhibition recorded only against *S. aureus* suggests weak antibacterial activity.

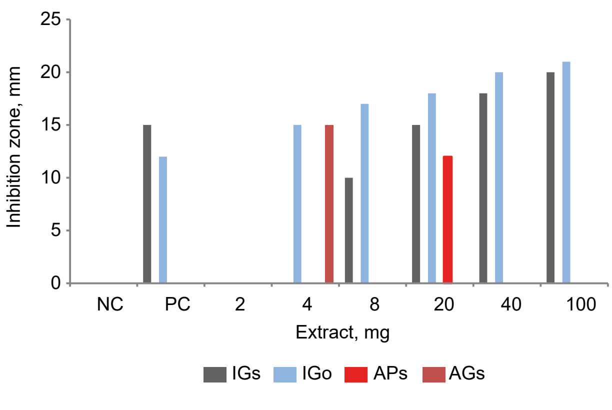

Fig. 1. Antifungal activities of methanol extracts of *Irvingia gabonensis*, *Apium graveolens* and *Allium porrum*. IGs and IGo: *I. gabonensis* activities against *Fusarium solani* and *Fusarium oxysporum* respectively. APs and AGs: *A. porrum* and *A. graveolens* activities against *F. solani* respectively. NC – negative control, Tween-80, 1 mg/mL. PC – positive control, Econazole, 1 mg/mL, against *F. solani* (PCs) and *F. oxysporum* (PCo) respectively

In the micro-dilution assays, *I. gabonensis* showed the lower MIC value (6.25 mg/mL) against *F. oxysporum*, while *A. graveolens* had a MIC of 50 mg/mL against the same fungus. However, MIC values were recorded against bacteria for only two extracts, *I. gabonensis* (2 mg/mL) and *A. porrum* (8 mg/mL). Though small zones of inhibition were only recorded against *S. aureus*, *I. gabonensis* had a lower MIC value (2 mg/mL) than in the antifungal assay (6.25 mg/mL). The quantitative MIC results are more reliable and show that *I. gabonensis* possesses antibacterial activity.

High antifungal activity was recorded against *F. oxysporum* and *F. solani*, which cause tomato spoilage. These results suggest a possible use of this extract to prevent postharvest damage of tomatoes by these fungi, which were shown in another study to attack most frequently the fruit around Buea in Cameroon (Takam et al., 2019). Preservation of tomato quality will prevent loss of its nutrients and prevent possible harm to human health when consumed.

The antifungal and antibacterial activities recorded for *I. gabonensis* reflect the high antimicrobial potential of the seed, which may be protective against some microbial infections when consumed in the diet. The activities of *Apium graveolens* and *Allium porrum* also indicate potential antimicrobial health benefits. Few studies have been carried out on the antimicrobial properties of *I. gabonensis* seeds. Interestingly, a study done in Nigeria also reported higher antifungal than antibacterial activity for *I. gabonensis* seeds (Dosumu et al., 2012). A study conducted in Cameroon on the leaves of *I. gabonensis* documented their weak antibacterial activity (Nayim et al., 2018). Edziri et al. (2012) reported higher antifungal activity (MIC = 0.08 to 0.31 mg/mL) for *A. graveolens* against three clinical Candida species. On the contrary, Shad et al. (2011) observed no activity for the methanol extract of *Apium graveolens* against *F. solani* in Pakistan. This could be due to differences in the varieties found in Pakistan and Cameroon.

A second important finding was that bioactive vegetables were found to be good sources of some nutrients: *I. gabonensis* was rich in lipids, iron, phosphorus and potassium, while *Apium graveolens* was rich in protein, calcium, phosphorus, potassium, zinc and vitamin C (Table 2 and Table 3). The nutritional importance of *I. gabonensis* and *A. graveolens* was established, since they showed the highest antifungal activities. The proximate analysis showed the highest macro nutrient content in *I. gabonensis* to be for lipids (69.90%) and protein (35.35%) in *A. graveolens*. The rest of the components were present to varying degrees. The lipid content of *I. gabonensis* is greater than the value reported by Dosumu et al. (2012) and confirms reports by Fokou et al. (2004), which state that *I. gabonensis* seeds are richer in fat content than *Cucumeropsis mannii* (egusi; 42–57%) and *Glycine max* (soya beans; 45%). Both *A. graveolens* and *I. gabonensis* have a higher lipid content than *Moringa oleifeira* seeds (13.35%), as stated in the report by Ijarotimi et al. (2013), and the protein content of *A. graveolens* is higher than the value (17.94%) reported by Salem (2014) in Pakistan, thus showing these two plants to be good sources of lipids and proteins respectively. The moisture content (15%) of *A. graveolens* is much lower than the 81.00% reported by Qureshi et al. (2014) for species from Pakistan; it shows that the Cameroonian varieties of *A. graveolens* can be easier to store. *A. graveolens* had lower ash content (13%) than the 23.19% reported by Salem (2014), but a little more than *Vernonia amygdalina* (bitter leaf; 11.7%) and *Moringa oleifera* leaves (12.23 ±0.03%; Karuna and Rajni, 2014). *A. graveolens* leaves had lower (10.96%) crude fiber than the 19.28% reported by Qureshi et al. (2014), but higher than *I. gabonensis*, which is not unexpected, since *A. graveolens* leaves were used. However, the fiber for *I. gabonensis* (8.25%) was similar to that (10.4%) reported by Onojah et al. (2018). Since *I. gabonensis* has a much higher lipid content (69.90%) than *A. graveolens* (17.90%), it is proved to be a better energy source when consumed.

Table 2. Proximate composition of *Irvingia gabonensis* and *Apium graveolens*

Property	*Irvingia gabonensis* %	*Apium graveolens* %
Moisture	3.23 ±0.08	15.23 ±2.22
Ash	2.48 ±0.01	13.03 ±1.68
Lipid	69.90 ±0.14	17.90 ±0.14
Fiber	8.25 ±0.21	10.96 ±0.64
Protein	10.12 ±0.39	35.35 ±0.49
Carbohydrates	6.01 ±1.51	7.40 ±0.12

Table 3. Macro minerals, micro minerals and vitamin C content of *Irvingia gabonensis* and *Apium graveolens*

Minerals	*Irvingia gabonensis* seeds mg/100 g	*Apium graveolens* leaves mg/100 g
Macro minerals		
Sodium	7.39 ±0.09	0.19 ±0.00
Potassium	180.94 ±2.64	276.51 ±1.41
Calcium	18.55 ±0.59	51.05 ±1.42
Phosphorous	359.67 ±1.89	622.14 ±2.69
Magnesium	13.25 ±0.33	0.47 ±0.057
Micro minerals		
Manganese	8.06 ±0.08	0.02 ±0.014
Zinc	5.42 ±0.12	16.86 ±0.27
Iron	276.51 ±1.41	1.04 ±0.06
Copper	2.9 ±0.88	0.13 ±0.028
Iodine	0.48 ±0.014	0.06 ±0.028
Vitamin C	0.49	58.04 ±0.75

The macro minerals revealed that both vegetables are richer in phosphorous and potassium than the other minerals (Table 3). However, phosphorous and potassium were lower than reported for *Moringa olefeira* leaves (Asante et al., 2014). Nevertheless, consumption of about 224 g and 129 g of *I. gabonensis* seeds and *A. graveolens* leaves respectively can meet the RDA for the two minerals. The two vegetables are not very good sources of sodium. However, this is not of great consequence, since it is commonly obtained from table salt or common salt.

Of the micro minerals analysed in the two vegetables, iron was highest in *I. gabonensis*, while zinc was highest in *A. graveolens*. Iron deficiency anaemia is still a public health problem, so good food sources of iron cannot be overemphasized. The iron content (276.51 mg/100 g) of *I. gabonensis* seed in this study is higher than the 101.01 mg/kg reported by Dosumu et al. (2012) and the 11.85 mg/100 g for *Moringa oleifeira* seeds reported by Anjorin et al. (2010). *A. graveolens* leaves had higher zinc content than *I. gabonensis* but was less than the 305.2 µg/g reported for wild celery by Qureshi et al. (2014). Recommended dietary allowance – RDA for iron is 8 mg/day for adults males, 18 mg/day for adult females of child bearing age and a little more for pregnant and lactating women. Both vegetables can more than meet the RDA for iron and thus can be good sources of iron when consumed. Zinc is now in focus as an essential mineral for sexual reproduction, cell repair and wound healing. It is a component of many enzymes. In this study, its content in both plants can provide more than 50% of RDA (10 mg). The two vegetables can also meet the RDA for manganese but *I. gabonensis* seeds are better sources of manganese. Copper and iodine were both higher in *I. gabonensis* than in *A. graveolens*. RDAs for copper and iodine for adults are about 1 mg/day and 150 µg/day respectively and these two minerals can meet more than the RDA for 100 g consumption per day. This is important considering the role of copper in metabolism and cretinism in permanent damage caused by iodine deficiency in infants and children. The two vegetables have appreciable levels of phosphorous and iron, so they can be incorporated in complementary food, because of their importance in the bones and blood, as well as energy metabolism.

Vitamin C is used in the human body for collagen synthesis in teeth, bone and connective tissues of blood vessels, where it plays the role of an enzyme cofactor. Vitamin C is also an antioxidant. A deficiency in it can lead to scurvy, which is the breakdown of skin, blood vessels and teeth. The vitamin C content of *I. gabonensis* seeds was much lower than the value 76.04 mg/100 g reported by Olayiwola et al. (2013). In this study, *A. graveolens* leaves contain more vitamin C than *I. gabonensis* seeds, but with a lower content than the level of 60.35 mg/100 g that was reported by Qureshi et al. (2014). The RDA for vitamin C for adults is 60–70 mg/day and 45 mg for children. This can be met by consuming about 103–120 g and 77.5 g of *A. graveolens* leaves respectively. Moreover, consuming 110–128 g and 82.72 g of *I. gabonensis* seeds will meet the RDA in adults and children respectively. The absorption of nutrients, minerals and vitamin C may not be affected, because antinutrients like oxalate and phytate which can reduce their absorption have not been reported in vegetables and spices.

CONCLUSION

This study has shown that the vegetables selected possess antimicrobial activity with considerable antifungal activity in *I. gabonensis* and *Apium graveolens*. The antifungal activity of *I. gabonensis*, *A. graveolens*, and *Allium porrum* indicates a potential preservative effect in the tomato postharvest. *I. gabonensis* seeds and *A. graveolens* both have high lipid, protein, vitamin C, zinc, copper and phosphorous content. Manganese and iron were also high in *I. gabonensis* seeds. Therefore consumption of even moderate quantities of *I. gabonensis* seeds and *A. graveolens* leaves can meet the RDA for several nutrients for both adults and children and also provide protective antimicrobial action.

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