

Research Article

Comparative Study on Antioxidant Activity and Cytotoxicity of Defatted Kernels of Mango (*Mangifera indica*) and Wild Mango (*Irvingia gabonensis* and *Irvingia wombolu*)

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Abstract: Defatted kernel powder of mango (*Mangifera indica*) and wild mango (*Irvingia gabonensis* and *Irvingia wombolu*) were analysed for their *in-vitro* antioxidant activity and cytotoxicity. Dry matter content of the three kernel types were in the range of 93 to 95%. On defatting, the percentage oil yield of the *Irvingia* varieties were similar and 200% higher than that of *Mangifera indica*. The antioxidant activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) technique showed that the two *Irvingia* varieties (DIGK and DIWK) had similar IC₅₀ (p>0.05) but significantly higher than that of *Mangifera indica* kernel (DMIK) (p<0.05). Likewise, results from the Ferric Reducing Antioxidant Power (FRAP) technique and Cytotoxicity assay confirmed the higher potency of DMIK over the *Irvingia* kernels.

Keywords: Antioxidant, cytotoxicity, *Irvingia gabonensis*, *Irvingia wombolu*, *Mangifera indica*

INTRODUCTION

Conventional mango (*Mangifera indica*) is a tropical fruit-bearing plants which flourishes better in Africa and Asia. Similar to many edible fruits, the pulp of the fruit is highly commercialised in form of several processed products (Arogba, 1997). The fruit is among several recommended fruits with medicinal importance. It is used in curing beriberi, wrestle heart burn, bronchial diseases, brain fatigue, mental depression and insomnia (Legesse and Emire, 2012). The kernel possesses relatively high phenolic content with effective antioxidant activity. It contains tocopherols and stigmaterol (Bandyopadhyay *et al.*, 2014). Due to the non-edible nature of the raw kernel, its application as antimicrobial or nutraceutical could be influenced by various methods of processing. Residual levels of phenolic compounds could serve as antioxidants (Arogba, 2015).

Another plant growing in wild tropical forests of family *Irvingiaceae*, is popularly called *wild mango* (*Irvingia*), African mango, bush mango, dika or ogbono. Two varieties of the *Irvingia* species are found in this region; the *Irvingia gabonensis* which has an edible yellowish pulp when ripe and the *Irvingia wombolu* with bitter inedible pulp (Anegbeh *et al.*, 2003; Asaah *et al.*, 2003). Methanolic extracts of *Irvingia gabonensis* kernel are used in the treatment of fungal and bacterial infections, similar to some other medicinal plants. However, their kernel powder have

culinary significance as soup-thickener (Matsinkou *et al.*, 2012; Arogba, 2014).

Antioxidants are compounds that can inhibit free radical reactions and restrain oxidative stress to components of cells. The word "antioxidant" is mostly used to describe natural chemicals substances found in foods and living organisms which have beneficial health effects or industrial chemicals which are capable of inhibiting oxidation (Brewer, 2011). Antioxidants in living systems have the ability to stabilize or deactivate free radicals, hence controlling their formation (Aluyor and Ori-Jesu, 2008). Natural antioxidants in foods have thus attracted considerable interest due to their safety, nutritional and therapeutic values. Consequently, phenolic antioxidants in many fruits, spices, vegetables, cereals and herbs have been examined (Pourreza, 2013; Bandyopadhyay *et al.*, 2014).

Over the years, several studies have been conducted on undefatted mango (*Mangifera indica*) and wild mango (*Irvingia gabonensis* and *Irvingia wombolu*) kernels, their testa and extracted oil (Alhassan and Arogba, 2018; Egbuonu, 2018; Irondi *et al.*, 2018; Kuyooro *et al.*, 2017; Arogba *et al.*, 2016; Arogba and Matanmisi, 2014; Arogba and Omede, 2012). However, there is dearth of information on the defatted residue of these kernels. Hence, further evaluation of the antioxidant activity and cytotoxicity of the defatted kernels in a comparative study is essential.

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MATERIALS AND METHODS

Sample collection and preparation: Ripe mango (*Mangifera indica*) fruits were plucked directly from trees at Anyigba town, Kogi State. The seeds were dissected using stainless steel knife to obtain the testa and kernel, dried at ambient temperature ($28 \pm 2^\circ\text{C}$) and pulverized using mortar and pestle into powdery form. Dry wild mango (*Irvingia gabonensis* and *Irvingia wombolu*) kernels were procured separately from Anyigba market, Kogi State. The kernels were sorted manually for wholesomeness. Like the mango kernels, they were pulverized into powdery form.

Dry matter determination: Each sample type was weighed into dish and dried in an air-oven at 105°C to a constant weight and the percentage dry matter was calculated.

Fat extraction: Each sample type [10% (w/v)] in petroleum ether (60° to 80° grade), in triplicate, were placed in a beaker. The mixture was kept at ambient temperature of $28 \pm 2^\circ\text{C}$ and shaken periodically for 24 h. The defatted kernels were oven-dried to constant weights and stored in glass-stoppered bottles prior to analysis. The percentage oil yield was calculated. To assess efficiency of the extraction, residual oil in the defatted samples were determined using Soxhlet extraction technique as described by Williams (2007).

Antioxidant assay using DPPH: The free radical 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) was used as described by Arogba and Omede (2012).

Preparation of samples: For each sample type, 1 g was weighed into a beaker containing 50 mL of methanol and placed on orbital shaker and shaken for 2 h. The supernatant was carefully decanted to form the stock solution from which serial dilutions were made.

One mL of 0.3 mM DPPH in methanol was added to one ml of different concentrations (2000, 1000, 500, 250, 125 and $62.5 \mu\text{g/mL}$) of the extract or reference compounds, (Quercetin and Vitamin C). The mixture was vortexed and then incubated in a dark chamber for 30 min after which the absorbance was measured at 517 nm against a control containing 2 mL of methanol:

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The percentage inhibition was plotted against the Log concentration of the extracts ($\mu\text{g/mL}$) to determine the IC_{50} , as $\text{Log}^{-1}x$ ($\mu\text{g/mL}$) from the equation: $y = mx + c$ when $y = 50$.

Antioxidant assay using FRAP method: Ferric Reducing Antioxidant Power (FRAP) method as described by Benzie and Strain (1999) was modified and employed.

The sample solution was prepared by dissolving 100 mg of each sample type in 20 mL ethanol from which 1 mL was mixed with 1ml FRAP reagent [10:1:1 ratio of acetate buffer 300 mM pH 3.6 : TPTZ (2,4,6-tripyridyl-s-triazine) 10 mM in 40 mM HCl : 20mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$]. The absorbance (593 nm) was taken at 0 min and then placed in the water bath at 37°C and absorbance was again taken after 4 min. Reference sample of Vitamin C was prepared in the same manner.

Given a FRAP value of 2.0 (equivalent to 28.39 mM), FRAP value of sample = (Change in sample absorbance/Change in Reference absorbance) x 2.0

Cytotoxicity bioassay using brine shrimp larvae: This was conducted using hatched brine shrimp (*Artemia salina*) larvae as described by Arogba (2014).

Hatching brine shrimp: Equivalent sea water was prepared by dissolving 32 g of NaCl in 1000 mL of distilled water. Some 50 mg of brine shrimp eggs was added to 300 mL of the artificial sea water and incubated under bright light, connected to an air-voltage pump that aerated the mixture. It was sealed and kept for 24 h to hatch.

Preparation of sample extracts: For each sample type, 1 g was dissolved in 50 mL of distilled water to form the stock solution from which serial dilutions were made.

After incubation, 10 brine shrimp larvae were counted and transferred to different vials using a Pasteur pipette and volume was made up to 5 mL with the artificial sea water. Constant volume of 500 μL of each varied concentrations (2000, 1000, 500, 250 and $125 \mu\text{g/mL}$) of a sample type or the reference potassium dichromate was added to the vials containing the shrimps. After 24 h, the dead larvae were counted for the determination of the percentage lethality. The lethality endpoint of this bioassay is defined as the absence of controlled forward motion during 30 sec of observation.

The percentage lethality of the shrimp for each concentration was calculated as:

$$\% \text{Lethality} = \left(\frac{\text{Number of dead shrimp}}{\text{Number of surviving shrimp in control}} \right) \times 100$$

The LC_{50} of the sample's extract was determined from linear regression curve of Percentage lethality against Log (concentration) as described by Asomugha *et al.* (2015).

Statistical analysis: The statistical analysis was conducted using SEM software available at miniwebtool.com. Results were expressed as mean \pm Standard Error of Mean (SEM). Separation of mean was conducted for test of significance at ($p = 0.05$).

RESULTS AND DISCUSSION

Dry matter and oil yield: The percentage dry matter content of the three test samples (Table 1) were comparable, within the range of 93.5-94.7%. These values were similar to those reported by Arogba and Omede (2012) and Arogba (2014). The high dry matter content of these test samples showed that the kernels were properly dried and would not easily promote mould growth or early sprouting.

On defatting, the oil yield of 22% from *Mangifera indica* kernel in this study was similar to that reported by Arogba (2015) as the variety was obtained from the same locality. The oil yields from *Irvingia gabonensis* and *Irvingia wombolu* kernels were also similar and agree with report of Bamidele *et al.* (2015). However, those of *Irvingia* varieties were 200% higher than that of *Mangifera indica* kernel and describing the former as oil-seeds.

The low residual oil yields from the three test samples using Soxhlet extraction technique implied that the cold extraction technique at ambient temperature range of 26-30°C for 24 h was adequate for optimal oil extraction in large-scale experiments.

Antioxidant activity assay: Antioxidants are crucial in preventing degenerative diseases that result from oxidative stress. In living organisms, complex natural substances (glutathione, vitamins C and E) and enzymes (superoxide dismutase, glutathione peroxidase, catalase) and foods of plant origin provide total antioxidant capacity for the system (Arogba, 2014).

From the DPPH result in Table 2, the two *Irvingia* kernels varieties (DIGK and DIWK) had the relatively similar IC₅₀ (p>0.05) but higher than that of DMIK. *Mangifera indica* kernel (DMIK) possessed relatively higher antioxidant activity than *Irvingia* kernels and

was attributed to the high phenolic content of *Mangifera indica* kernel (Arogba, 2014; Bandyopadhyay *et al.*, 2014). The tannin constituents of *Mangifera indica* kernel were identified and quantified in a previous report (Arogba, 2000).

The values of IC₅₀ for DMIK and DIGK in this study were higher than those studied without defatting by Arogba and Omede (2012) and Arogba (2015). The defatting process therefore, had depleted the fat soluble polyphenolic component of the kernels. This could account for lower antioxidant activity of the test samples compared with the reference Quercetin and Vitamin C.

The FRAP results in Table 3 were comparable with those obtained by DPPH technique. *Irvingia* kernel samples (DIGK and DIWK) had similar FRAP values but differed significantly from DMIK (p<0.05), confirming the high potency of DMIK over the *Irvingia* kernels. The FRAP value of DMIK in this study supports the similar work of Arogba (2015). Furthermore, the study has shown that the test samples could be used to provide higher levels of natural antioxidants to reduce the risk of oxidative stress, if undefatted.

Cytotoxicity assay: Brine Shrimp larvae are considered as suitable samples for preliminary cytotoxicity assay in pharmacology (Quazi *et al.*, 2017; Montanher *et al.*, 2002). In Table 4, the reference Potassium dichromate (K₂Cr₂O₇) gave 100% lethality at 2000 µg/mL while test samples gave between 73-80%. This indicated that the test samples were less toxic than the reference sample. The observation was supported by the LC₅₀ of 285 µg/mL for the reference and 458-553 µg/mL of the test samples. Among the test samples, DMIK had relatively higher toxicity than the DIGK or DIWK (p<0.05). This observation implied that the *Irvingia*

Table 1: Percentage dry matter, oil yield and residual oil content of the kernel samples

Samples	Dry matter (%)	Oil yield (%)	Residual oil (%)
<i>Mangifera indica</i>	93.5±0.55	21.9±1.68	0.9±0.19
<i>Irvingia gabonensis</i>	94.7±1.60	68.3±2.20	2.5±0.39
<i>Irvingia wombolu</i>	94.4±1.62	65.7±2.14	3.1±0.29

Values are expressed as mean ± SEM (n = 3); the two *Irvingia* kernels had higher oil and residual oil content than *Mangifera indica*

Table 2: Percentage inhibition of DPPH in different concentrations of defatted kernel samples

Conc. (µg/mL)	DMIK (%)	DIGK (%)	DIWK (%)	Quercetin (%)	Vitamin C (%)
2000	88.8±2.17	82.6±1.88	84.6±2.66	95.2±0.97	92.8±1.97
1000	75.5±1.85	72.6±2.17	74.9±1.99	86.5±1.73	84.5±1.20
500	67.9±0.42	65.2±2.23	66.7±0.27	81.1 ±1.99	76.1±0.82
250	59.2±2.23	50.9±2.82	51.0±2.35	74.5±2.24	63.0±2.05
125	45.6±0.98	41.2±0.85	41.6±1.97	60.8±1.48	47.0±1.31
62.5	29.1±1.85	25.3±1.65	28.5±2.17	31.1±0.95	30.0±1.11
IC ₅₀	181.4 ^c	241.3 ^d	219.3 ^d	97.9 ^a	150.7 ^b
SEM	25.38				

Percentage inhibition. Values are expressed as mean±SEM (n = 3); Values with the same superscripts on the same row are not significantly different at p>0.05; 1: DMIK = Defatted *Mangifera indica* kernel; 2: DIGK = Defatted *Irvingia gabonensis* kernel; 3: DIWK = Defatted *Irvingia wombolu* kernel; 4: SEM = standard error of mean; The IC₅₀ for each sample were obtained from the following equations (y = 50); 1: DMIK: y = 37.8x-35.373, R² = 0.9833; 2: DIGK: y = 37.619x-39.629, R² = 0.9885; 3:DIWK: y = 37.724x-38.312, R² = 0.9931; 4: Quercetin: y = 38.495x-26.63, R² = 0.8859; 5: Vitamin C: y = 41.867x-41.193, R² = 0.9764; Decreasing order of IC₅₀ observed was DIGK ≅ DIWK > DMIK > Vitamin C > Quercetin

Table 3: Antioxidant activity of the defatted kernel samples using FRAP method

Sample type	FRAP value	(Vitamin C equivalent in mM)
DMIK	2.73±0.05 ^d	38.81±0.74 ^d
DIGK	1.78±0.02 ^a	25.19±0.33 ^a
DIWK	1.93±0.04 ^{ab}	27.40±0.47 ^{ab}
Vitamin C	2.00±0.00 ^{bc}	28.39±0.00 ^{bc}
SEM	0.21	3.03

Values are expressed as mean±SEM (n = 3). Values with the same superscripts on the same column are not significantly different at p>0.05; 1: DMIK = Defatted *Mangifera indica* kernel; 2: DIGK = Defatted *Irvingia gabonensis* kernel; 3: DIWK = Defatted *Irvingia wombolu* kernel; 4: SEM = standard error of mean; DMIK had higher potency compared with the *Irvingia* kernel samples (DIGK and DIWK)

Table 4: Brine shrimp lethality assay of defatted kernel samples

Conc. (µg/mL)	DMIK (%)	DIGK (%)	DIWK (%)	K ₂ Cr ₂ O ₇ (%)
2000	80.0	73.3	76.7	100
1000	66.7	63.3	63.3	90.0
500	56.7	53.3	50.0	70.0
250	40.0	33.3	36.7	60.0
125	16.7	16.7	20.0	13.3
LC ₅₀	457.6 ^b	552.5 ^c	517.7 ^c	284.6 ^a
SEM	59.49			

Percentage inhibition. Values with the same superscripts on the same row are not significantly different at p>0.05. Values are expressed as mean (n = 3); 1: DMIK = Defatted *Mangifera indica* kernel; 2: DIGK = Defatted *Irvingia gabonensis* kernel; 3: DIWK = Defatted *Irvingia wombolu* kernel; 4: SEM = standard error of mean; The LC₅₀ for each sample was obtained from the following equations (y = 50): DMIK: y = 41.867x - 41.193, R² = 0.9764; DIGK: y = 47.733x - 80.9, R² = 0.9774; DIWK: y = 46.667x - 76.66, R² = 0.9977; K₂Cr₂O₇: y = 67.8x - 116.4, R² = 0.9074; Decreasing order of LC₅₀ between the test samples were DIGK ≡ DIWK > DMIK

kernel varieties (DIGK and DIWK) are safer for consumption than the *Mangifera indica* kernel (DMIK). If defatting is combined with other treatments such as blanching, the kernel sample could be as safe.

CONCLUSION

This study has shown that defatting process removed 96% of the oil content of the *Irvingia* and *Mangifera* kernels along with fat-soluble polyphenolic substances. The effect of the latter caused the relatively lower antioxidant activity of *Irvingia* kernel samples than *Mangifera indica* sample, which also exhibited relatively higher toxicity on Brine shrimp larvae.

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