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Bioaccessibility of polyphenols and flavonoids in *Ocimum africanum* leaves extract

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Abstract
This study investigated the phytochemical and proximate analyses and bioaccessibility of polyphenols and flavonoids in Ocimum africanum leaves aqueous extract (OALAE) in the present and absent of digestive enzymes (in vitro). Exactly 100 g air-dried, steamed and fried samples of O. africanum were extracted with 400 ml distilled water at 45°C for 48 hours and then filtered. The phytochemical analysis of extracted samples indicated the presence of alkaloids, saponins, phenols, flavonoids, tannins, steroids and carbohydrate. A significant decrease was observed in the proximate analysis (moisture, ash and protein) of the air-dried O. africanum leave sample compared to steamed and fried samples. The moisture, ash and protein were in the following order: air dried > steamed > fried. The fat content in the different samples was in the order of fried > air-dried > steamed. Crude fibre and carbohydrate were in the following order: fried > steamed > air dried. After gastrointestinal digestion stimulation, the polyphenol compounds bioaccessibility index (PCB) (illustrated using proanthocyanidins and anthocyanins) and flavonoids bioaccessibility (FB) (such as flavonone, luteolin and flavanonols) were lower in the presence of digestive enzymes in comparison to the absence of digestive enzymes, in the air-dried, steamed, fried OALAE and quercetin standard. In conclusion, these results suggest that OALAE proved to be a significant source of bioaccessible polyphenols and flavonoids even though it is unstable and has limited bioavailability under gastrointestinal conditions.

Keywords: *Bioaccessibility, Digestion, Flavonoids, Phytochemicals, Polyphenols, Ocimum africanum*

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1. Introduction

Phenols can as well be called phenolics, which are natural compounds occurring in plant foods such as fruits, vegetables and spices (George *et al.*, 2012; George *et al.*, 2015; George *et al.*, 2019; George and Okpoghono, 2017; Okpoghono *et al.*, 2018a; Okpoghono *et al.*, 2018b; Okonta *et al.*, 2021; Otuaga *et al.*, 2020a). Bioactive compounds such as flavonoids are the most studied phenolic groups, with over 9000 identified dissimilar structures in nature (Swallah *et al.*, 2020). Bioactive compounds with varied chemical structures affect bioavailability and biological properties, while the antinutritional factor can as well decline or inhibit digestion enzymes (Septembre-Malaterre *et al.*, 2018). Therefore, to get a full understanding of absorption and metabolism of phenols inside the gastrointestinal tract, it is better to study the chemical structure of a phenol, since altered chemical structures disturb their redox potential. Comparably, polyphenols with double close hydroxyl groups are better and have free radical scavenging ability to those with a single hydroxyl group. Bioactive compounds can change metabolic progressions and stretch out positive functions such as inhibition of receptor activities, induction of enzymes and antioxidant effect (Carbonell-Capella *et al.*, 2014). The changes in bacterial microbiota in the gastrointestinal tract are associated with metabolic disorders including diabetes, obesity, or nonalcoholic fatty liver disease (Zhu *et al.*, 2010).

The *in vitro* test is repeatedly used for the determination of almost all biological activities, using polyphenol in their natural states, i.e., as it is in food without any breakdown. However, polyphenols are extensively metabolised both in tissues and by the colonic microflora. Bioavailability of polyphenols within the gastrointestinal tract depends on the phenolic

secondary microbial metabolites acting within the colons (Palafox-Carlos *et al.,* 2011). Bioavailability can be defined as the proportion of bioactive compounds that are effectively fascinated into the bloodstream for metabolic utilization (Palafox-Carlos *et al.,* 2011). However, bioaccessibility can as well be defined as the portion of bioactive compounds free from the food matrix after digestion and becoming accessible for intestinal absorption (Williamson and Clifford, 2017).

In this situation, the antioxidants will only be willingly open for absorption after effective digestion. Before absorption, the majority of dietary polyphenols are metabolised by the colonic microbiota, and a small proportion will be directly absorbed in the upper gastrointestinal tract. Hence, the microbial metabolism is a prerequisite for absorption (Williamson and Clifford, 2017). Numerous factors interfere with the absorption and release of polyphenols, and the trouble in nutrient absorption inside the intestine is due to the fluctuations in the interaction of other compounds, chemical nutrients from the food, suppressors in the food composition, and overall metabolism (Stevens and Maier, 2016).

Ocimum africanum known as lime basil or curry is an annual plant native to Asia and Africa which grows under full sun to partly shady conditions in average soils (Aluko *et al.,* 2013). It is a wild herb with a distinct mint flavour, hairy leaves and scented flowers that have received considerable attention for their potential medicinal properties. The leaves have been used in traditional folk medicine for the treatment of diabetes, constipation, diarrhoea, piles and dysentery and skin diseases (Samuel *et al.,* 2020). The leaf is rich in essential oils of therapeutic importance and mostly used as a condiment for the preparation of delicious local soup, salad and tea because of its aromatic properties (Singh *et al.,* 2013). The aim of this study was to determine the in-*vitro* gastrointestinal digestion of polyphenols and flavonoids of *Ocimum africanum* leaves (curry leaves) extract.

2. Materials and methods

2.1 Collection and identification of plant

Curry leaves (*Ocimum africanum*) was purchased from Anambra State, Nigeria. The plant was identified and authenticated at forestry research institute of Nigeria Ibadan, with voucher number FHI 734512 deposited in their herbarium.

Fig 1: Picture of curry leaves (*Ocimum africanum*)

Preparation of material and sample extraction

***Sample** 1:* Fresh sliced curry leaves (200 g) was parboiled with little water (100 mL) in a pot with continuous stirring for 10 minutes. The parboiled leaves were dried open air at room temperature.

***Sample 2**:* A cooking pot was set on heat, and 100 mL of vegetable oil was added and allowed to heat before adding 200 g of fresh sliced curry leaves. This was fried for the next 10 minutes, with occasional stirring to avoid burning. The fried leaves were allowed to cool and dried open air at room temperature.

***Sample 3**:* Fresh sliced curry leaves (200 g) was dried open air at room temperature.

***Extraction of sample**:* The dried samples were ground to powdery form using electric blender. One hundred grams (100 g) of the powder samples (1, 2 and 3) was dissolved in distilled water (400 mL, 45°C) for 48 hours and then filtered (grade 1 filter paper). The water extract was evaporated under reduced temperature of 37°C using water bath until no moisture was visible.

2.2 Simulation of gastrointestinal digestion (*in-vitro*)

The *in vitro* gastro-pancreatic digestion was performed as described by Helal *et al*. (2014). This was done to mimic the cooking of the *O. africanum* leaves as closely as possible, quantities of the spice normally used in food preparation. The enzymatic reaction phase was involve, which include; artificial gastric fluid (pepsin and NaCl), and artificial intestinal fluid (pancreatin and bile extract). Digestion without enzymes was also carried out to distinguish the effect of the chemical environment from that of the digestive enzymes. Quercetin was used as a standard for comparison. Assessment of polyphenol and flavonoids were carried out on samples *(in vitro)*. Simulation of gastrointestinal digestion (*in-vitro*) was as follows:

Samples	***Treatment***
Sample extract	Before digestion
	Post-intestinal with enzymes
	Post-intestinal without enzymes
Quercetin standard	Before digestion
	Post-intestinal with enzymes
	Post-intestinal without enzymes

2.3 Bioaccessibility index

The following index was determined for better understanding the relationship between phenolic compounds and their bioaccessibility in organic systems:

Phenolic compounds bioaccessibility (PCB), which indicates the fraction of bioaccessible phenolic compounds from *O. africanum* extract was calculated using Equation (1):

$$PCB = \frac{PCa}{PCb} x \frac{100}{1} \quad (1)$$

where PCa was the concentration of phenolic compounds after *in vitro* digestion and PCb the concentration of polyphenols originally present in *O. africanum* extract.

Flavonoids bioaccessibility (FB), which indicates the fraction of bioaccessible flavonoids from *O. africanum* extract was calculated using Equation (2):

$$FB = \frac{Fa}{Fb} x \frac{100}{1} \quad (2)$$

where Fa was the concentration of flavonoid compounds after *in vitro* digestion and Fb was the concentration of flavonoid compounds initially present in *O. africanum* extract.

2.4 Proximate and phytochemical analyses

The moisture, ash, crude fats, proteins and carbohydrates of all the samples (*in-vitro*) were carried out using standard Association of Official Agricultural Chemists (AOAC) method (1990). The qualitative test was carried out following the method described by Trease and Evans (2002).

2.5 Proanthocyanidin

Proanthocyanidin was determined according to the method of Sun *et al.* (1998). Sample (0.5 ml), 4% vanillin solution in methanol (3 ml) and concentrated HCl (1.5 ml) were mixed together in test tube. The mixture was allowed to stand for 15 min, and absorption was taken at 500 nm against methanol as a blank. Catechin solution was used for the preparation of calibration curve. The amount of proanthocyanidin was expressed as mg (+) - catechin /g DW.

2.6 Flavanones

Flavanones estimation were accomplished using spectrophotometric method proposed by Nagy and Grancai (1996). Flavanones in the sample react with 2,4-Dinitrophenylhidrazin (DNP) and potassium hydroxide solution. The absorbance was measured at 486 nm. Pinocembrin was used as the reference standard. Flavanones was expressed as µg of pinocembrin equivalent per milligram of sample extract (µg PNE/mg).

2.7 Flavanonols (Dihydroquercetin) and Flavones (Luteolin)

Dihydroquercetin amd flavones (Luteolin) were determined according to the method of Struchkov *et al.* (2018). Zero point three millilitres (0.3 ml) of 5% $NaNO_2$ aqueous solution and 5 ml of water were added to 0.1 ml of sample extracts (steamed dried, fried and air-dried), and after 5 min, 0.9 ml of acetic acid and 0.2 ml of 10 % $AlCl_3$ were added followed by 1.5 ml of 1M NaOH. UV spectrum was set at the range of 300–600 nm against the blank. Dihydroquercetin or luteolin was calculated using Equation (3):

$$X\ (mg/mL) = \frac{10\ x\ V\ x\ A}{p\ x\ E} \quad (3)$$

where X is the total content of dihydroquercetin or luteolin, V is total volume of the reaction (8 ml), A is the absorbtion at 517 nm for luteolin and 507 nm for dihydroquercetin, p is the sample volume (ml), E is the dihydroquercetin specific absorbance (134.5 at 507 nm) or luteolin specific absorbance (63.9 at 517 nm).

2.8 Anthocyanins

Determination of total anthocyanins in steamed dried, fried and air-dried samples was based on the method described by Iland *et al*. (1996). Sample (200 µl) was mixed with 3.8 ml of 1 M HCl and incubated at room temperature for 3 hours. The absorbance (A) of the acidified diluted extract was read at 520 nm against 1 M HCl as blank solution. Anthocyanin's concentration in mg/mlwas calculated using the absorbance (B) of 1% w/v malvidin-3-glucoside solution as follows:

$$Anthocyanins\ (mg/ml) = A \times Dilution\ factor \times \frac{1000}{B} \quad (4)$$

3. Results and discussion

3.1 Proximate analysis and phytochemicals of *O. africanum* leaves

Table 1 shows the result of the proximate analysis of *O. africanum* leaves. Significant decreases were observed in the moisture, ash, and protein in air-dried *O. africanum* leave sample compared to steamed and fried samples. The

moisture, ash, and protein were in the following order: air-dried > steamed> fried. The low level of moisture in the fried *O. africanum* leaves suggests that the fried *O. africanum* leaves could be stored for a longer period compared to air-dried and steamed samples because a higher water activity could enhance microbial attack that may lead to spoilage (Salami *et al.*, 2019). The values of ash observed in the entire sample are an indicator that the samples are good sources of minerals. This is in accordance with the study of Salami *et al.* (2021), who stated that ash content measures the minerals present within a food. The high protein values recorded for air-dried *O. africanum* leaves suggest that the leaves can be a potential source of plant protein and it could be used as a protein supplement in diets. The results show no significant differences in fat and crude fibre contents of the entire samples. The fat trends were fried > air dried > steamed. Crude fibre result was as follows: fried > steamed > air dried. Generally, the low-fat content of the samples proposes that the plant may be of low sources of vegetable oil but may be beneficial for personnel on weight-reducing diets. Study has shown that plant with low fat could be used in weight-reducing diets (Emebu and Anyika, 2011). Significant increases were observed in carbohydrate content of fried and steamed samples compared to the air-dried sample. Total carbohydrate levels in the leave were relatively high. The trends in carbohydrate were found as: fried > steamed > air-dried sample. The result of the qualitative phytochemical analysis of *O. africanum* leaves is shown in Table 2. Alkaloids, phenol, saponin, flavonoids, steroids, tannins and carbohydrate were present in all the samples. Flavonoids and phenols are well known antioxidants subsiding free radical induced tissue injury (Swallah *et al.*, 2020; Okpoghono *et al.*, 2018a; George and Okpoghono, 2017; Otuaga *et al.*, 2020b). Tannins are known to have antibacterial, antidiabetic and antitumor properties via carbohydrate modulation on gastrointestinal tract (Salami, 2021). Alkaloids and tannins are known to play protective role in animal. The antimicrobial activity of tannins may be due to their partially hydrophobic nature (Salami *et al.*, 2021).

Table 1: Proximate analysis of *O. africanum* leaves

Proximate composition (%)	Steamed dried	Fried	Air-dried
Moisture	11.12±3.10 [a]	8.30±1.60 [a,b]	12.50±2.87 [a]
Ash	1.50±0.30 [b]	1.00±0.30 [b]	4.30±0.60 [a]
Protein	9.50±2.20 [a]	7.39±0.90 [a,b]	10.10±0.90 [a]
Fat	5.10±1.30 [a]	6.31±0.80 [a]	6.30±1.50 [a]
Crude fibre	15.10±5.10 [a]	16.40±2.80 [a]	13.50±1.60 [a]
Carbohydrate	57.54±12.31[a]	60.50±5.10 [a]	53.30±4.50 [a,b]

Triplicates values are represented in mean ± SD. Different superscript letters of mean values in the same horizontal row differ significantly at $p < 0.05$.

Table 2: Qualitative phytochemical analysis of *O. africanum* leaves

	Steamed			Fried			Air-dried		
Phytochemicals	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Tannins	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+
Flavonoids	++	+	+	++	+	+	++	+	+
Phenols	++	+	+	++	+	+	++	+	+
Carbohydrate	+	+	+	+	+	+	++	+	+

++ = Highly present; += Moderately present; A= Before digestion; B= Post-intestinal with enzymes; C= Post-intestinal without enzymes

Table 3: Polyphenol and flavonoids contents and bioaccessibility of *O. africanum* leaves aqueous extract after simulated gastrointestinal digestion (*in-vitro*)

Group		Proanthocyanidins (mg catechin /ml)	PCB (%)	Anthocyanins (mg/ml)	PCB (%)	Flavonone (µgPNE/mg)	FB (%)	Luteolin (mg/ml)	FB (%)	Flavanonols (mg/ml)	FB (%)
1 (Steamed)	Before digestion	12.47 ± 0.35^{a}		0.82 ± 0.29^{a}		12.10 ± 1.95^{a}		2.01± 0.52^{a}		4.33 ± 1.17^{c}	
	Post-intestinal with enzymes	5.42 ± 0.96^{b}	43.46	0.23 ± 0.15^{b}	28.05	6.20 ± 2.06^{b}	51.23	0.68 ±0.32^{b}	33.83	0.80 ± 0.43^{b}	18.47
	Post-intestinal without enzymes	9.44 ± 0.96^{a}	75.70	0.50 ± 0.311^{c}	60.97	10.20 ± 2.20^{a}	84.29	1.05 ± 0.42^{a}	52.23	2.16 ± 0.28^{a}	49.88
2 (Fried)	Before digestion	10.23 ± 2.03^{a}		0.61 ± 0.300^{c}		6.21 ± 0.95^{b}		1.16 ± 0.35^{a}		2.21 ± 0.94^{a}	
	Post-intestinal with enzymes	5.26 ± 0.99^{a,b}	51.41	0.24 ± 0.11^{b}	39.34	2.26 ± 0.25^{c}	36.39	0.50 ± 0.20^{b}	43.10	0.42 ± 0.30^{b}	19.00
	Post-intestinal without enzymes	7.21 ± 1.00^{a}	70.77	0.51 ± 0.32^{c}	83.60	4.43 ± 0.55^{b}	71.33	0.80 ± 0.30^{b}	68.96	1.25 ± 0.70^{a}	56.56
3 (Air dried)	Before digestion	16.22 1.94^{c}		1.01 ± 0.21^{a}		14.50 ± 2.09^{a,d}		4.10 ± 1.05^{c}		5.37± 0.25^{c}	
	Post-intestinal with enzymes	8.84 ± 3.91^{a}	54.50	0.41 ± 0.95^{c}	40.59	5.33 ± 2.01^{b}	36.75	1.20 ± 0.20^{a}	29.26	2.23 ± 0.63^{a}	41.52
	Post-intestinal without enzymes	9.49 ± 0.76^{a}	58.51	0.81± 0.10^{a}	80.19	9.53 ± 2.22^{a}	65.72	2.10 ± 0.36^{a}	51.21	3.24 ± 1.00^{a}	60.33
4 (Quercetin)	Before digestion	25.12 ±5.17^{d}		8.17 ± 2.01^{d}		22.26 ± 2.15^{e}		14.16 ± 5.20^{d}		12.33 ± 0.57^{d}	
	Post-intestinal with enzymes	18.25 ± 2.08^{c,d}	72.65	4.40 ± 1.01^{c}	53.85	17.33 ± 3.25^{d}	77.85	10.33 ± 2.75^{c}	72.95	6.85 ± 0.65^{c}	55.56
	Post-intestinal without enzymes	20.23 ± 5.94^{d}	80.53	4.97 ± 0.11^{c}	60.80	15.87 ± 3.25^{d}	71.29	11.16 ± 3.25^{c}	78.81	6.33 ± 1.05^{c}	51.33

Triplicate values are given in mean ± SD. n = 5. Mean values in the same column with different letter differ at p < 0.05.
Phenolic compounds bioaccessibility (PCB), Flavonoids bioaccessibility (FB)

3.2. Polyphenol and flavonoids content and bioaccessibility of *O. africanum* leaves

Table 3 summarizes the polyphenol and flavonoids contents and bioaccessibility of *O. africanum* leaves aqueous extract (OALAE). There were significant decreases in proanthocyanidins, anthocyanins, flavonone, luteolin and flavanonols of OALAE in post intestinal digestion with enzymes and post-intestinal without enzyme when compared to before digestion in Groups 1, 2, 3, and 4 (air-dried, steamed, fried samples and quercetin standard). However, these parameters were lower in the present of digestive enzymes in comparison to the absent of digestive enzymes. The trends were: quercetin standard > air-dried > steamed > fried sample. The significant decrease in flavonoids such as flavonone, luteolin, flavanonols and their bioaccessibility percentage in post intestinal with enzyme compared to post-intestinal without enzyme may be attributed to the different compounds of the phytoconstituents of OALAE that may resist the digestive process. Some authors have reported values between 0 and 14% for bioaccessibility of flavonoids and phenol compounds from the seeds of *Opuntia albicarpa cv. Reyna* and *O. ficusindica cv. Rojo Pelon* (Ramírez-Moreno *et al.*, 2011). These values are very low, considering those obtained in this present work. Alminger *et al.* (2014) reported that for solid matrices, such as plant extracts, phenolic compounds bioaccessibility may vary from 30 to 100%. These values range are in line with that obtained in this study and that reported by Garbetta et al. (2014) who stated that bioaccessibility range from 30 to 100% may be due to the action of digestive enzymes, which in turn, may impair the stability and integrity of phenolic molecules.

4. Conclusions

In conclusion, most polyphenols and flavonoids consumed in food cannot be absorbed intact after consumption due to the fact that they undergo a series of reactions by digestive enzymes. Despite the decrease in polyphenols and flavonoids of OALAE after digestion, the plant may also prove to be a significant source of bioaccessible polyphenols and flavonoids. However, continuing investigation is needed to enhance the bioaccessibility and subsequent efficacy of polyphenols and flavonoid of edible plants.

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