



Inhibitory effect of leaves extracts of *Ocimum basilicum* and *Ocimum gratissimum* on two key enzymes involved in obesity and hypertension *in vitro*

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ABSTRACT

Aim: To evaluate the phenolics composition and inhibitory effect of the leaves extracts of *Ocimum basilicum* and *Ocimum gratissimum* on two key enzymes (pancreatic lipase [PL] and angiotensin 1-converting enzyme [ACE]) involved in obesity and hypertension *in vitro*. **Materials and Methods:** The phenolics (flavonoids and phenolic acids) were quantified using high-performance liquid chromatography coupled with diode array detection. PL and ACE inhibitory effects; DPPH* and ABTS*+ scavenging activities of the extracts were tested using spectrophotometric methods. **Results:** *O. basilicum* had the following major phenolics: Rutin, quercetin, and quercitrin (flavonoids); caffeic, chlorogenic, and gallic acids (phenolic acids); while *O. gratissimum* had the following major phenolics: Rutin, quercitrin, and luteolin (flavonoids); ellagic and chlorogenic acids (phenolic acids). "Extracts of both plants inhibited PL and ACE; scavenged DPPH* in a dose-dependent manner". *O. gratissimum* extract was more potent in inhibiting PL (IC₅₀: 20.69 µg/mL) and ACE (IC₅₀: 29.44 µg/mL) than *O. basilicum* (IC₅₀: 52.14 µg/mL and IC₅₀: 64.99 µg/mL, against PL and ACE, respectively). *O. gratissimum* also scavenged DPPH* and ABTS*+ more than *O. basilicum*. **Conclusion:** *O. basilicum* and *O. gratissimum* leaves could be used as functional foods for the management of obesity and obesity-related hypertension. However, *O. gratissimum* may be more effective than *O. basilicum*.

KEY WORDS: Angiotensin I-converting enzyme, hypertension, obesity, *Ocimum* species, oxidative stress, pancreatic lipase

INTRODUCTION

Obesity, especially visceral obesity, is the most important risk factor for the development of hypertension and other

cardiovascular diseases [1]. Population-based risk estimates studies have indicated that no less than two-thirds of the prevalence of hypertension can be directly attributed to obesity [2]. Obesity and obesity-related hypertension have

become a global public health burden; their incidence being comparable with those of diabetes mellitus and chronic kidney diseases [3].

The pancreatic lipase (PL) (EC: 3.1.1.3) plays a pivotal role in the effective digestion of lipids, as it is responsible for the hydrolysis of the bulk (50-70%) of total dietary fats [4]. It is the key enzyme that hydrolyzes triglyceride to produce glycerol and fatty acids, thereby facilitating its uptake [5]. Thus, it is widely used as an index to evaluate the potential efficacy of natural products as anti-obesity agents [6]. Due to the vital catalytic function of PL, orlistat, currently used clinically for obesity management, has been designed to inhibit PL activity; thereby reducing the absorption of triglyceride. However, this drug like other PL inhibitors has some hepatic and gastrointestinal adverse effects [7,8].

The activation of the rennin-angiotensin system (RAS) has been reported as one of the possible mechanisms through which obesity could lead to hypertension and higher cardiovascular risk [3]. In fact, experimental evidence has shown that the RAS is activated in obesity; its involvement in the pathophysiology of obesity-related hypertension has also been reported [9]. In the RAS, angiotensin 1-converting enzyme (ACE) (EC: 3.4.15.1) plays a key role in the regulation of blood pressure and normal cardiovascular function. It catalyzes the conversion of angiotensin I to angiotensin II, which is known to increase the blood pressure. Hence, inhibition of ACE is an important strategy for the treatment and management of obesity-related hypertension [10,11]. Chemically synthesized ACE inhibitors including captopril, ramipril, enalapril and fosinopril, comprise a class of drugs frequently used clinically for the treatment of hypertension [12]. However, as with the PL inhibitors, these drugs have been reported to have some adverse effects such as cough, skin rashes, hypotension, and proteinuria [13,14].

Against the backdrop of the side effects associated with the synthetic inhibitors of both PL and ACE, research has shown that plant-derived inhibitors of these two enzymes have some potential for preventing or ameliorating obesity and hypertension, without noticeable side effects [15,16]. In this regard, plant polyphenolics have been reported by several studies to inhibit either one of these two enzymes (PL and ACE) or both [4,5,17,18].

The genus, *Ocimum* (Lamiaceae), comprises 65 aromatic species, distributed in tropical and subtropical regions of the world including Africa and Asia [19]. Various *Ocimum* species, including *Ocimum basilicum* and *Ocimum gratissimum*, have important culinary and pharmacological uses. Their culinary and pharmacological values are attributed mainly to their aromatic compounds [20]. Hence, the leaves are used as a fragrance and flavoring agent in a variety of products including food, beverages, condiments, and oral care products [21]. *O. gratissimum* leaves extract possesses anti-arthritis [22], hypoglycemic [19], anticonvulsant and anxiolytic activities [23]. On the other hand, *O. basilicum* leaves are used as anti-spasmodic, carminative, galactagogue, and stomachic in folk

medicine [24]. To further explore the nutraceutical benefits of *O. basilicum* and *O. gratissimum*, this study evaluated the phenolics composition and inhibitory effects of their leaves extracts on two key enzymes (PL and ACE) involved in obesity and hypertension *in vitro*.

MATERIALS AND METHODS

Samples Collection and Preparation

About 500 g of fresh leaves samples of each of *O. basilicum* and *O. gratissimum* were collected from a local farm settlement in Akingbile area of Moniya, Ibadan, Nigeria. The samples were botanically identified and authenticated at the herbarium of the Department of Botany, University of Ibadan, Nigeria. Subsequently, they were air-dried for 7 days and later ground finely to a particle size of 0.5 mm. The powdery samples were used for the extracts preparation.

Chemicals and Reagents

Methanol, formic acid, gallic acid, chlorogenic acid, caffeic acid, and ellagic acid were purchased from Merck (Darmstadt, Germany). Catechin, epicatechin, quercetin, rutin, apigenin, and luteolin; porcine PL, Hippuryl-histidyl-leucine, Rabbit lung ACE, DPPH, ABTS, and Trolox were acquired from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used for the analysis were of analytical grade.

Preparation of Polyphenolics Extract

Polyphenolics extracts of *O. basilicum* and *O. gratissimum* leaves were prepared as described by Kuo *et al.* [25]. A portion of the leaves powder (100 g) was extracted three successive times with 300 mL of methanol at 50°C for 3 h, and the samples were filtered after each extraction with Whatman (No. 2) filter paper. Lipids and some pigments were removed by partitioning the extract with 200 mL hexane in a separatory funnel. The aqueous phase was extracted three times with 180 mL ethyl acetate and evaporated to dryness at 45°C under reduced pressure in a rotary evaporator. The residues obtained were used for the assays.

Quantification of Flavonoids and Phenolic Acids using High Performance Liquid Chromatography Coupled with Diode Array Detection (HPLC-DAD)

Extracts of the leaves were injected by means of auto-sampler (Shimadzu, model SIL-20A) at a concentration of 15 mg/mL. Separations were carried out using phenomenex C₁₈ column (4.6 mm × 250 mm × 5 mm particle size). The mobile phase comprised solvent A (water:formic acid [98:2, v/v]) and solvent B (acetonitrile), at a flow rate of 0.6 mL/min and an injection volume of 40 µL. Gradient program was started with 95% of A and 5% of B until 2 min and changed to obtain 25%, 40%, 50%, 70% and 80% B at 10, 20, 30, 50 and 70 min, respectively, according to the method described by Boligon *et al.* [26], with slight modifications. The extracts and mobile phase were

filtered through a 0.45 μm membrane filter (Millipore) and then degassed in ultrasonic bath before use. Standards references stock solutions were prepared in the HPLC mobile phase at a concentration range of 0.025-0.300 mg/mL. Quantifications of the flavonoids and phenolic acids in the extracts were carried out by integration of the peaks using the external standard method at the following wavelengths: 254 nm for gallic acid and ellagic acid; 280 nm for catechin and epicatechin; 325 nm for caffeic acid and chlorogenic acid; 366 nm for quercetin, quercitrin, kaempferol, luteolin and rutin. The individual chromatography peaks were confirmed by comparing their respective retention time with those of reference standards and by DAD spectra (200-600 nm). Calibration curves used were gallic acid: $Y = 13581x + 1195.7$ ($r = 0.9997$), catechin: $Y = 12609x + 1187.4$ ($r = 0.9996$), epicatechin: $Y = 12719x + 1356.9$ ($r = 0.9995$), caffeic acid: $Y = 12750x + 1352.9$ ($r = 0.9999$), chlorogenic acid: $Y = 11825x + 1383.6$ ($r = 0.9998$), ellagic acid: $Y = 13192x + 1176.5$ ($r = 0.9997$), quercitrin: $Y = 12641x + 1295.7$ ($r = 0.9999$), kaempferol: $Y = 11846x + 1283.9$ ($r = 0.9994$), rutin: $Y = 11792x + 1305.8$ ($r = 0.9995$), luteolin: $Y = 13894x + 1267.1$ ($r = 0.9998$), and quercetin: $Y = 12618x + 1196.3$ ($r = 0.9996$). All chromatography operations were carried out at ambient temperature and in triplicate.

The limit of detection (LOD) and limit of quantification (LOQ) of flavonoids and phenolic acids were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as previously defined by Khaliq *et al.* [27]. LOD and LOQ were calculated as 3.3 and 10 σ/S , respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

PL Inhibition Assay

The ability of the extracts to inhibit porcine PL was determined using the method earlier reported by Eom *et al.* [28] with a slight modification. In this assay, PL hydrolyzes p-nitrophenyl butyrate to form p-nitrophenol which is measured spectrophotometrically. The enzyme solution was prepared by adding 30 μL (10 units) of porcine PL (Sigma, USA) in 10 mM morpholine propane sulfonic acid and 1 mM EDTA (pH 6.8) to 850 μL of Tris buffer (100 mM Tris-HCl and 5 mM CaCl_2 , pH 7.0). Thereafter, 100 μL of appropriate concentrations (20, 40, 60, 80 $\mu\text{g}/\text{mL}$) of the extracts or orlistat (positive control) were mixed with 880 μL of the enzyme solution and incubated for 10 min at 37°C. After incubation, 20 μL of the substrate solution (10 mM of p-nitrophenyl butyrate in dimethylformamide) was added to initiate the hydrolytic reaction, which was allowed to proceed for 20 min at 37°C. A reference test (without the extract) was included in the assay. The absorbance of the p-nitrophenol produced from the hydrolysis of p-nitrophenylbutyrate was measured at 405 nm. The inhibition of PL activity by the extracts was expressed as % inhibition thus:

$$\% \text{ Inhibition} = [(A_{405_{\text{reference}}} - A_{405_{\text{sample}}}) \div A_{405_{\text{reference}}}] \times 100.$$

Where $A_{405_{\text{reference}}}$ is the absorbance of the reference without the extract, and $A_{405_{\text{sample}}}$ is the absorbance of test containing the extract.

ACE Inhibition Assay

ACE inhibition was assayed by a spectrophotometric method described by Cushman and Cheung [29]. ACE from rabbit lung (EC: 3.4.15.1) and the substrate (Hippuryl-histidyl-leucine [Bz-Gly-His-Leu]) were used. In this assay, the Bz-Gly-His-Leu is cleaved by ACE to form hippuric acid, which is measured spectrophotometrically. A portion (50 μL) of appropriate concentrations of the extracts (20, 40, 60, 80 $\mu\text{g}/\text{mL}$) and 50 μL ACE solution (4 mU/mL) were pre-incubated at 37°C for 15 min. After pre-incubation, 150 μL of 8.33 mM of the Bz-Gly-His-Leu in 125 mM Tris-HCl buffer (pH 8.3) was added to the mixture to initiate the enzymatic reaction, and this was incubated at 37°C for 30 min. Next, the reaction was terminated by adding 250 μL of 1 M HCl. The hippuric acid produced by the reaction was extracted with 1.5 mL ethyl acetate. Subsequently, the ethyl acetate layer was separated from the mixture by centrifugation, and 1 mL of it was transferred to a clean test tube and evaporated to dryness in a hot-air oven. The resulting residue was redissolved in distilled water and its absorbance was measured at 228 nm. A reference test (without the extract) and a positive control test (containing 64 nmol/L of captopril) were carried out simultaneously with the test extract. 1 unit (U) of ACE activity is defined as the amount of enzyme required to catalyze the formation of 1 μmol of hippuric acid from hippuryl-histidyl-leucine per minute at 37°C. The ability of the extracts to inhibit ACE activity was expressed as percentage inhibition thus:

$$\% \text{ Inhibition} = [(A_{228_{\text{reference}}} - A_{228_{\text{sample}}}) \div A_{228_{\text{reference}}}] \times 100.$$

Where $A_{228_{\text{reference}}}$ is the absorbance of the reference without the extract, and $A_{228_{\text{sample}}}$ is the absorbance of test containing the extract.

Determination of DPPH* Scavenging Ability

DPPH* scavenging ability of the extracts was determined as described by Cervato *et al.* [30]. Appropriate dilutions (30, 60, 90, 120 $\mu\text{g}/\text{mL}$) of the extracts amounting to 1.0 mL were mixed with 3.0 mL of DPPH* (60 μM). The test mixture was kept in the dark for 30 min, after which the absorbance was measured at 517 nm. A reference test containing the DPPH* solution without the extract was carried simultaneously. The DPPH* percentage scavenging ability of the extract was calculated as follows:

$$\% \text{ scavenging ability} = [(Abs_{517_{\text{reference}}} - Abs_{517_{\text{sample}}}) \div Abs_{517_{\text{reference}}}] \times 100$$

Where $Abs_{517_{\text{reference}}}$ is the absorbance of the reference test, and $Abs_{517_{\text{sample}}}$ is the absorbance of the test containing the extract.

Determination of ABTS** Scavenging Ability

ABTS** scavenging ability of the extracts was determined according to the method described by Re *et al.* [31]. To generate the ABTS**, an equal volume of 7 mM ABTS** aqueous solution was incubated with 2.45 mM $\text{K}_2\text{S}_2\text{O}_8$ for 16 h at room

temperature in the dark; then its absorbance (at 734 nm) was adjusted to 0.7 ± 0.02 with 95% ethanol. Thereafter, appropriate dilution of the extract amounting to 0.2 mL was mixed with 2.0 mL ABTS^{•+} solution. The test mixture was kept in the dark for 15 min, after which its absorbance was measured at 734 nm. The ABTS^{•+} scavenging ability of the extracts was subsequently calculated and expressed in trolox equivalent (TE).

Statistical Analysis

Results of replicate experiments were expressed as mean \pm standard deviation (SD). *T*-test for independent samples was carried out on the result data at 95% confidence level using SPSS statistical software package, version 17. Half-maximal inhibitory and scavenging concentrations (IC_{50} and SC_{50}) were calculated from the % inhibition versus extract concentration non-linear regression curve of each extract.

RESULTS

The HPLC chromatograms of *O. basilicum* and *O. gratissimum* [Figure 1a and b] revealed the presence of gallic acid (retention time - $R_t = 10.19$ min; peak 1), chlorogenic acid ($R_t = 22.35$ min; peak 3), caffeic acid ($R_t = 26.97$ min; peak 4), epicatechin ($R_t = 37.41$ min; peak 6), rutin ($R_t = 43.86$ min; peak 7), quercitrin ($R_t = 48.13$ min; peak 8), quercetin ($R_t = 52.01$ min; peak 9), and kaempferol ($R_t = 57.69$ min; peak 10). In addition to these, catechin ($R_t = 16.07$ min; peak 2), ellagic acid ($R_t = 29.83$ min; peak 5), and luteolin ($R_t = 64.15$ min; peak 11) were also present in *O. gratissimum* [Figure 1b].

The quantitative composition of the phenolics (flavonoids and phenolic acids) is shown in Table 1. In *O. basilicum*, the flavonoids were in the order of rutin > quercetin > quercitrin > epicatechin > kaempferol, whereas the phenolic acids were in the order of caffeic acid > chlorogenic acid > gallic acid. In *O. gratissimum*, the flavonoids were in the order of rutin > quercitrin > luteolin > epicatechin > kaempferol > quercetin > catechin, while the phenolic acids were in the order of ellagic acid > chlorogenic acid > gallic acid > caffeic acid. Thus, the major flavonoids in *O. basilicum* were rutin, quercetin, and quercitrin; the major phenolic acids were caffeic, chlorogenic and gallic acids. But in *O. gratissimum* rutin, quercitrin and luteolin were the major flavonoids, whereas ellagic and chlorogenic acids were the major phenolic acids.

The abilities of the *O. basilicum* and *O. gratissimum* leaves extracts to inhibit porcine PL and ACE were expressed in terms of their half-maximal inhibitory concentrations (IC_{50}), and the results are presented in Table 2. The IC_{50} of *O. gratissimum* against PL ($20.69 \pm 2.14 \mu\text{g/mL}$) was significantly ($P < 0.05$) lower than that of *O. basilicum* ($52.14 \pm 3.96 \mu\text{g/mL}$). However, the IC_{50} ($3.48 \pm 0.13 \mu\text{mol/g}$) of orlistat, the reference PL inhibitor, was lower than those of *O. gratissimum* and *O. basilicum*. Similarly, the IC_{50} of *O. gratissimum* against ACE ($29.44 \pm 3.98 \mu\text{g/mL}$) was significantly ($P < 0.05$) lower than that of *O. basilicum* ($64.99 \pm 5.04 \mu\text{g/mL}$) but that of captopril ($19.32 \pm 2.23 \mu\text{mol/g}$), the reference ACE inhibitor,

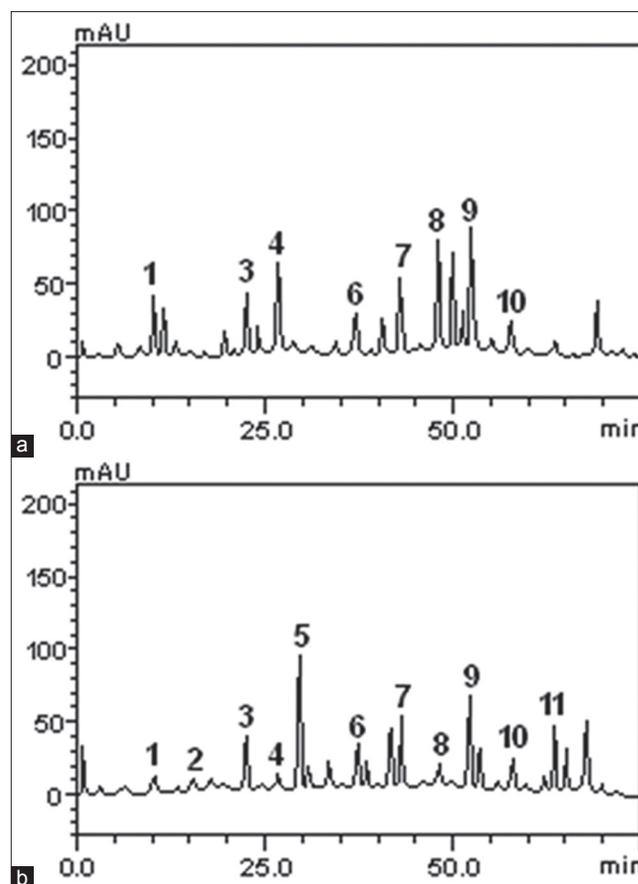


Figure 1: Representative high performance liquid chromatography chromatograms of *Ocimum basilicum* and *Ocimum gratissimum* extracts; detection ultraviolet was at 325 nm. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epicatechin (peak 6), rutin (peak 7), quercitrin (peak 8), quercetin (peak 9), kaempferol (peak 10), and luteolin (peak 11). (a) *O. basilicum*, (b) *O. gratissimum*

Table 1: Flavonoids and phenolics acids composition of *O. basilicum* and *O. gratissimum* leaves extracts

Phenolic compounds (mg/g)	<i>O. basilicum</i>	<i>O. gratissimum</i>	LOD $\mu\text{g/mL}$	LOQ $\mu\text{g/mL}$
Gallic acid	2.07 ± 0.03^a	0.53 ± 0.01^b	0.024	0.079
Catechin	ND	0.51 ± 0.02	0.013	0.042
Chlorogenic acid	2.10 ± 0.01^a	1.79 ± 0.01^b	0.008	0.026
Caffeic acid	2.73 ± 0.01^a	0.48 ± 0.03^b	0.019	0.061
Ellagic acid	ND	3.96 ± 0.02	0.026	0.085
Epicatechin	1.35 ± 0.04^a	1.32 ± 0.01^a	0.011	0.034
Quercitrin	2.49 ± 0.01^a	2.07 ± 0.01^b	0.020	0.066
Quercetin	3.18 ± 0.01^a	0.54 ± 0.03^b	0.027	0.089
Rutin	3.25 ± 0.03^a	2.26 ± 0.04^b	0.014	0.047
Kaempferol	1.06 ± 0.01^a	1.03 ± 0.01^a	0.008	0.026
Luteolin	ND	2.01 ± 0.02	0.017	0.056

Results are expressed as mean \pm SD of three determinations. Values followed by different superscript letters along the same row differ significantly at $P < 0.05$. LOD: Limit of detection, LOQ: Limit of quantification, *O. basilicum*: *Ocimum basilicum*, *O. gratissimum*: *Ocimum gratissimum*, SD: Standard deviation

was lower than both. The patterns of PL and ACE inhibition by *O. basilicum* and *O. gratissimum* were both dose-dependent

as depicted by their % inhibition versus extract concentration curves [Figures 2 and 3] respectively.

The free radical scavenging activities of *O. basilicum* and *O. gratissimum* leaves extracts, as determined using DPPH* and ABTS** scavenging assays, are presented in Table 3. *O. gratissimum* had a significantly ($P < 0.05$) stronger DPPH* scavenging ability (SC_{50} , $84.76 \pm 1.06 \mu\text{g/mL}$) than *O. basilicum* (SC_{50} , $104.72 \pm 1.85 \mu\text{g/mL}$). However, ascorbic acid, the reference antioxidant, had much stronger DPPH* scavenging ability (SC_{50} , $7.29 \pm 0.34 \mu\text{g/mL}$) than both *O. gratissimum* and *O. basilicum*. However, both leaves extracts scavenged DPPH* in a dose-dependent [Figure 4]. Similarly, *O. gratissimum* had a stronger ABTS** scavenging ability ($1.82 \pm 0.02 \text{ mmol TE/g}$) than *O. basilicum* extract ($0.52 \pm 0.01 \text{ mmol TE/g}$).

DISCUSSION

The various health benefits of medicinal plants are attributed to the presence of secondary metabolites such as phenolic acids and flavonoids [32]. In this study, therefore, we quantified the flavonoids and phenolic acids constituents of *O. basilicum* and *O. gratissimum* leaves, and evaluated their inhibitory effects against PL and ACE, as well as their free radicals scavenging abilities *in vitro*. Flavonoids and phenolic acids are prominent for their diverse health benefits including anti-diabetic [33], anti-hypertensive and antioxidative [34]; hence, they are regarded as being pharmacologically important [35]. Interestingly, the level of caffeic acid (2.73 mg/g) we quantified in *O. basilicum* in this study is about 40% higher than the 1.65 mg/g reported earlier by Harnafi et al. [36].

The inhibition of PL, the most important enzyme in the digestion of dietary triglycerides, is one of the possible

Table 2: IC₅₀ of *O. basilicum* and *O. gratissimum* leaves extracts against PL and ACE activities

Extract	PL IC ₅₀	ACE IC ₅₀
<i>O. basilicum</i> (μg/mL)	52.14 ± 3.96 ^a	64.99 ± 5.04 ^a
<i>O. gratissimum</i> (μg/mL)	20.69 ± 2.14 ^b	29.44 ± 3.98 ^b
Orlistat eq. (μmol/g)	3.48 ± 0.13	NA
Captopril eq. (μmol/g)	NA	19.32 ± 2.23

Results are expressed as mean ± SD of replicate analysis. Values followed by different superscript letters along the same column vary significantly at $P < 0.05$. NA: Not applicable, *O. basilicum*: *Ocimum basilicum*, *O. gratissimum*: *Ocimum gratissimum*, SD: Standard deviation, PL: Pancreatic lipase, ACE: Angiotensin 1-converting enzyme

Table 3: DPPH* SC₅₀ and ABTS scavenging ability of *O. basilicum* and *O. gratissimum* leaves extracts**

Antioxidant activity	<i>O. basilicum</i>	<i>O. gratissimum</i>	Ascorbic acid
DPPH* SC ₅₀ (μg/mL)	104.72 ± 1.85 ^a	84.76 ± 1.06 ^b	7.29 ± 0.34 ^c
ABTS** scavenging ability (mmol TE/g dw)	0.52 ± 0.01 ^b	1.82 ± 0.02 ^a	NA

Results are presented as mean ± SD of triplicate determinations. Values with different lowercase superscript letter along the same row vary significantly at $P < 0.05$. NA: Not applicable, *O. basilicum*: *Ocimum basilicum*, *O. gratissimum*: *Ocimum gratissimum*, SD: Standard deviation, PL: Pancreatic lipase, ACE: Angiotensin 1 converting enzyme

approaches to retard the uptake of fat, and consequently, reduce weight and obesity [37]. Polyphenols, including flavonoids and phenolic acids, from natural origin are regarded as a major class of the PL inhibitors [38,39]. However, flavonoids, especially the catechin-types, are presumed to possess more PL inhibitory and anti-obesity effect. The presence of galloyl moiety in the structural backbone of the polyphenolics plays an important role in their ability to inhibit PL. Possibly, the more the galloyl moieties, the stronger the PL inhibitory effect, as Nakai et al. [40] demonstrated that the PL IC₅₀ values of (-)-epigallocatechin 3,5-digallate with two galloyl groups, epigallocatechin gallate

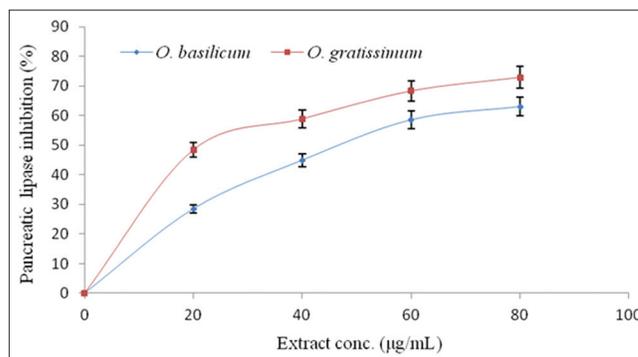


Figure 2: Pancreatic lipase % inhibition - extract concentration curve of *Ocimum basilicum* and *Ocimum gratissimum* leaves extracts

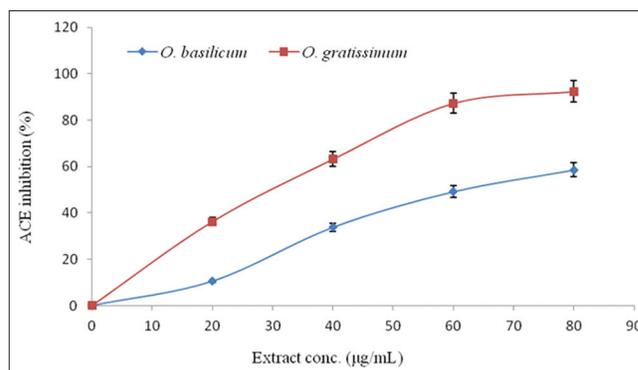


Figure 3: Angiotensin I-converting enzyme % inhibition - extract concentration curve of *Ocimum basilicum* and *Ocimum gratissimum* leaves extracts

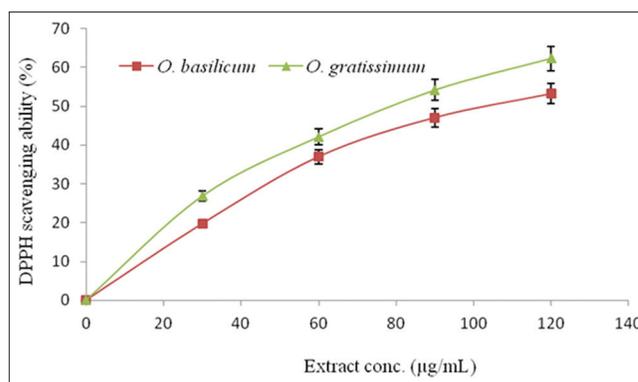


Figure 4: DPPH* % scavenging ability-extract concentration curve of *Ocimum basilicum* and *Ocimum gratissimum* leaves extracts

with one galloyl group and (–)-epigallocatechin with no galloyl group were 0.098, 0.349 and 20 μM , respectively, in their study in which they evaluated the inhibitory activities of 54 tea polyphenols against PL. The galloyl moieties may exert their inhibitory effect on PL by competing with its substrate for the active site, at least, at a substrate concentration below the K_m value [38]. Therefore, it is possible that the presence of catechin in *O. gratissimum* accounted for its stronger ability to inhibit PL than *O. basilicum* that had no catechin [Table 1].

ACE catalyzes the cleavage of angiotensin I (a decapeptide) to angiotensin II (an octapeptide) and inactivates bradykinin, a vasodilator and hypotensive peptide [41]. Angiotensin II stimulates vasoconstriction, increases sodium and water reabsorption, and elevates blood pressure. Consequently, the excessive action of ACE leads to hypertension [42]; for this reason, inhibition of ACE has emerged as an important treatment and management strategy for hypertension [10,11]. Plant extracts rich in flavonoids and phenolic acids have been shown to inhibit ACE activity by previous studies [18,34]. The flavonoids are the largest group of polyphenolic compounds found in higher plants [43] and are regarded as an excellent source of functional anti-hypertensive products. Guerrero *et al.* [44] observed this in their study in which they evaluated the structure-activity relationship of inhibition of ACE activity by flavonoids. The combination of sub-structures on the flavonoid skeleton, including the catechol group in the B-ring, the double bond between C2 and C3 at the C-ring, and the cetone group in C4 at the C-ring, increases the ACE inhibitory activity of the flavonoids. Similarly, the phenolic acids are effective ACE inhibitors. Their ability to inhibit ACE has been attributed to the overall contribution of their functional groups, including carboxyl and hydroxyl groups; their ability to form charge-charge interactions with the zinc ion present in the active site of ACE, through the oxygen atom of their carboxylate moiety; and their interaction with the amino acids residues at the active site of ACE, to give a stable complex between the phenolic acid molecule and ACE. Thus, the ACE inhibitory activity of phenolic acids may be due the effect of this interaction with the zinc ion and the subsequent stabilization by other interactions with amino acids in the active site [45]. In this study, *O. gratissimum* had stronger ACE inhibitory activity than *O. basilicum* [Table 2]. This may be attributed to the presence of luteolin (flavonoid) in *O. gratissimum* (2.01 mg/g) and ellagic acid (phenolic acid), which were not detected in *O. basilicum* [Table 1]. This is supported by the findings of Guerrero *et al.* [44], who reported that luteolin had the highest ACE inhibitory activity, relative to other sixteen flavonoids in their study.

Systemic oxidative stress is a common feature of obesity [46], and by extension, obesity-related hypertension. Oxidative stress arises due to increased generation of free radicals and reactive oxygen species (ROS), with attendant reduction in the antioxidant defense system, both enzymic and non-enzymic, in the obese. Angiotensin II also induces oxidative stress, which plays a key role in the development of hypertension [47,48]. Free radicals and ROS are notorious for their ability to damage both intra- and extra-cellular macromolecules, including the DNA, lipids, and protein [49]. However, several previous studies

have demonstrated that plant extracts rich in flavonoids and phenolic acids can scavenge free radicals [18,34]. The efficiency of flavonoids and phenolic acids as antioxidant polyphenols is attributable to their unique molecular structures. The hydroxyl groups of flavonoids, especially at the 3' OH and 4' OH of their three-carbon chain, make them potent electrons donors and terminators of chain reactions [50,51]; hence their antioxidant effect. On the other hand, the antioxidant activity of phenolic acids has been attributed to the ability of the phenolic ring in their structure to stabilize and delocalize unpaired electrons [52]. The ability of the *O. basilicum* and *O. gratissimum* leaves extracts to scavenge free radicals (DPPH* and ABTS**) is an indication that they could help prevent and/or alleviate oxidative stress in obesity and obesity-related hypertension. This may complement the overall antihypertensive activity of the extracts, as Rodriguez-Rodriguez and Simonsen [53] reported that the anti-oxidative effects of ACE inhibitors contributed to their therapeutic effects in patients suffering from cardiovascular complications.

CONCLUSION

The leaves extracts of *O. basilicum* and *O. gratissimum* inhibited PL and ACE; and scavenged DPPH* and ABTS**. These bioactivities could be due to the combined effects of the flavonoids and phenolic acids present in the leaves. Hence, *O. basilicum* and *O. gratissimum* leaves could be used as functional foods for the management of obesity and obesity-related hypertension, and preventing the oxidative stress that characterizes both disorders. However, *O. gratissimum* may be more effective due to its stronger inhibitory effect on PL and ACE.

REFERENCES

1. Sironi AM, Gastaldelli A, Mari A, Ciociaro D, Positano V, Buzzigoli E, *et al.* Visceral fat in hypertension: Influence on insulin resistance and beta-cell function. *Hypertension* 2004;44:127-33.
2. Krauss RM, Winston M, Fletcher BJ, Grundy SM. Obesity: Impact on cardiovascular disease *Circulation* 1998;98:1472-6.
3. Narkiewicz K. Obesity and hypertension - The issue is more complex than we thought. *Nephrol Dial Transplant* 2006;21:264-7.
4. Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: Unexplored potential. *Drug Discov Today* 2007;12:879-89.
5. Sakulnarmrat K, Konczak I. Composition of native Australian herbs polyphenolic-rich fractions and *in vitro* inhibitory activities against key enzymes relevant to metabolic syndrome. *Food Chem* 2012;134:1011-9.
6. Sugiyama H, Akazome Y, Shoji T, Yamaguchi A, Yasue M, Kanda T, *et al.* Oligomeric procyanidins in apple polyphenol are main active components for inhibition of pancreatic lipase and triglyceride absorption. *J Agric Food Chem* 2007;55:4604-9.
7. Filippatos TD, Derdemezis CS, Gazi IF, Nakou ES, Mikhailidis DP, Elisaf MS. Orlistat-associated adverse effects and drug interactions: A critical review. *Drug Saf* 2008;31:53-65.
8. Viner RM, Hsia Y, Tomsic T, Wong IC. Efficacy and safety of anti-obesity drugs in children and adolescents: Systematic review and meta-analysis. *Obes Rev* 2010;11:593-602.
9. Rahmouni K, Correia ML, Haynes WG, Mark AL. Obesity-associated hypertension: New insights into mechanisms. *Hypertension* 2005;45:9-14.
10. Sharma AM. Is there a rationale for angiotensin blockade in the management of obesity hypertension? *Hypertension* 2004;44:12-9.
11. Villiger A, Sala F, Suter A, Butterweck V. *In vitro* inhibitory potential of *Cynara scolymus*, *Silybum marianum*, *Taraxacum officinale*, and

- Peumus boldus* on key enzymes relevant to metabolic syndrome. *Phytomedicine* 2015;22:138-44.
12. Lan X, Liao D, Wu S, Wang F, Sun J, Tong Z. Rapid purification and characterization of angiotensin converting enzyme inhibitory peptides from lizard fish protein hydrolysates with magnetic affinity separation. *Food Chem* 2015;182:136-42.
 13. Thurman JM, Schrier RW. Comparative effects of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers on blood pressure and the kidney. *Am J Med* 2003;114:588-98.
 14. Vysoulis GP, Karpanou EA, Papavassiliou MV. Side effects of anti-hypertensive treatment with ACE inhibitors. *Am J Hypertens* 2001;14:14-5.
 15. Mayer MA, Höcht C, Puyó A, Taira CA. Recent advances in obesity pharmacotherapy. *Curr Clin Pharmacol* 2009;4:53-61.
 16. Tanzadehpanah H, Asoodeh A, Saberi MR, Chamani J. Identification of a novel angiotensin-I converting enzyme inhibitory peptide from ostrich egg white and studying its interactions with the enzyme. *Innov Food Sci Emerging Technol* 2013;18:212-9.
 17. Slanc P, Doljak B, Kreft S, Lunder M, Janes D, Strukelj B. Screening of selected food and medicinal plant extracts for pancreatic lipase inhibition. *Phytother Res* 2009;23:874-7.
 18. Ironi EA, Agboola SO, Oboh G, Boligon AA, Athayde ML, Shode FO. Guava leaves polyphenolics-rich extract inhibits vital enzymes implicated in gout and hypertension *in vitro*. *J Intercult Ethnopharmacol* 2016;5:122-30.
 19. Casanova LM, da Silva D, Sola-Penna M, Camargo LM, Celestrini Dde M, Tinoco LW, *et al.* Identification of chicoric acid as a hypoglycemic agent from *Ocimum gratissimum* leaf extract in a biomonitoring *in vivo* study. *Fitoterapia* 2014;93:132-41.
 20. Bhuvaneshwari K, Gokulanathan A, Jayanthi M, Govindasamy V, Milella L, Lee S, *et al.* Can *Ocimum basilicum* L. and *Ocimum tenuiflorum* L. *in vitro* culture be a potential source of secondary metabolites? *Food Chem* 2016;194:55-60.
 21. Al Abbasy DW, Pathare N, Al-Sabahi JN, Khan SA. Chemical composition and antibacterial activity of essential oil isolated from Omani basil (*Ocimum basilicum* Linn.). *Asian Pac J Trop Dis* 2015;5:645-9.
 22. Madhu KD, Harindran J. Antiarthritic potential of *Ocimum gratissimum* L. in collagen induced arthritic Sprague-Dawley rats. *Biomed Aging Pathol* 2014;4:191-6.
 23. Okoli CO, Ezike AC, Agwagah OC, Akah PA. Anticonvulsant and anxiolytic evaluation of leaf extracts of *Ocimum gratissimum*, a culinary herb. *Pharmacognosy Res* 2010;2:36-40.
 24. Sajjadi SE. Analysis of the essential oils of two cultivated basil (*Ocimum basilicum* L.) from Iran. *Daru* 2006;14:128-30.
 25. Kuo C, Kao E, Chan K, Lee H, Huang T, Wang C. *Hibiscus sabdariffa* L. extracts reduce serum uric acid levels in oxonate-induced rats. *J Funct Foods* 2012;4:375-81.
 26. Boligon AA, Piana M, Kubiś TF, Mario DN, Dalmolin TV, Bonez PC, *et al.* HPLC analysis and antimicrobial, antimycobacterial and antiviral activities of *Tabernaemontana catharinensis* A. DC. *J Appl Biomed* 2015;13:7-18.
 27. Khaliq A, Sabir SM, Ahmad SD, Boligon AA, Athayde ML, Jabbar A, *et al.* Antioxidant activities and phenolic composition of olive (*Olea europaea*) leaves. *J Appl Bot Food Qual* 2015;88:16-21.
 28. Eom SH, Lee MS, Lee EV, Kim YM, Kim TH. Pancreatic lipase inhibitory activity of phlorotannins isolated from *Eisenia bicyclis*. *Phytother Res* 2013;27:148-51.
 29. Cushman DW, Cheung HS. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochem Pharmacol* 1971;20:1637-48.
 30. Cervato G, Carabelli M, Gervasio S, Cittera A, Cazzola R, Cestaro B. Antioxidant properties of oregano (*Origanum vulgare*) leaf extracts. *J Food Biochem* 2000;24:453-65.
 31. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999;26:1231-7.
 32. Ajila CM, Brar SK, Verma M, Tyagi RD, Godbout S, Valéro JR. Extraction and analysis of polyphenols: Recent trends. *Crit Rev Biotechnol* 2011;31:227-49.
 33. Ironi EA, Oboh G, Akindahunsi AA, Boligon AA, Athayde ML. Phenolics composition and antidiabetic property of *Brachystegia eurycoma* seed flour in high-fat diet, low-dose streptozotocin-induced type 2 diabetes in rats. *Asian Pac J Trop Dis* 2015;5 Suppl 1:S159-65.
 34. Oboh G, Ademiluyi AO, Akinyemi AJ, Henle T, Saliu JA, Schwarzenbolz U. Inhibitory effect of polyphenol-rich extracts of Jute leaf (*Corchorus olitorius*) on key enzyme linked to type-2 diabetes (α -amylase and α -glucosidase) and hypertension (angiotensin I converting) *in vitro*. *J Funct Food* 2012;4:450-8.
 35. Ironi AE, Oboh G, Akindahunsi AA, Boligon AA, Athayde ML. Phenolic composition and inhibitory activity of mango and horse-eye bean seeds extracts against key enzymes linked to the pathology and complications of type 2 diabetes. *Asian Pac J Trop Biomed* 2014;4:903-10.
 36. Harnafi H, Ramchoun M, Tits M, Wauters J, Frederich M, Angenot L, *et al.* Phenolic acid-rich extract of sweet basil restores cholesterol and triglycerides metabolism in high fat diet-fed mice: A comparison with fenofibrate. *Biomed Prev Nutr* 2013;3:393-7.
 37. Kaisoon O, Konczak I, Siriamornpun S. Potential health enhancing properties of edible flowers from Thailand. *Food Res Int* 2012;46:563-71.
 38. Rahim AT, Takahashi Y, Yamaki K. Mode of pancreatic lipase inhibition activity *in vitro* by some flavonoids and non-flavonoid polyphenols. *Food Res Int* 2015;75:289-94.
 39. Lunagariya NA, Patel NK, Jagtap SC, Bhutani KK. Inhibitors of pancreatic lipase: State of the art and clinical perspectives. *EXCLI J* 2014;13:897-921.
 40. Nakai M, Fukui Y, Asami S, Toyoda-Ono Y, Iwashita T, Shibata H, *et al.* Inhibitory effects of oolong tea polyphenols on pancreatic lipase *in vitro*. *J Agric Food Chem* 2005;53:4593-8.
 41. Eriksson U, Danilczyk U, Penninger JM. Just the beginning: Novel functions for angiotensin-converting enzymes. *Curr Biol* 2002;12:R745-52.
 42. Lee BH, Lai YS, Wu SC. Antioxidation, angiotensin converting enzyme inhibition activity, nattokinase, and antihypertension of *Bacillus subtilis* (natto)-fermented pigeon pea. *J Food Drug Anal* 2015;1-8. Available from: <http://www.dx.doi.org/10.1016/j.jfda.2015.06.008>.
 43. Croft KD. The chemistry and biological effects of flavonoids and phenolic acids. *Ann N Y Acad Sci* 1998;854:435-42.
 44. Guerrero L, Castillo J, Quiñones M, Garcia-Vallvé S, Arola L, Pujadas G, *et al.* Inhibition of angiotensin-converting enzyme activity by flavonoids: Structure-activity relationship studies. *PLoS One* 2012;7:e49493.
 45. Al Shukur N, Van Camp J, Gonzales GB, Staljanssens D, Struijs K, Zotti MJ, *et al.* Angiotensin-converting enzyme inhibitory effects by plant phenolic compounds: A study of structure activity relationships. *J Agric Food Chem* 2013;61:11832-9.
 46. Mohamed S. Functional foods against metabolic syndrome (obesity, diabetes, hypertension and dyslipidemia) and cardiovascular disease. *Trends Food Sci Technol* 2014;35:114-28.
 47. Sprague AH, Khalil RA. Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochem Pharmacol* 2009;78:539-52.
 48. Virdis A, Duranti E, Taddei S. Oxidative stress and vascular damage in hypertension: Role of angiotensin II. *Int J Hypertens* 2011;2011. Available from: <http://www.dx.doi.org/10.4061/2011/916310>.
 49. Takemoto K, Tanaka M, Iwata H, Nishihara R, Ishihara K, Wang DH, *et al.* Low catalase activity in blood is associated with the diabetes caused by alloxan. *Clin Chim Acta* 2009;407:43-6.
 50. Kim JK, Lee SY, Chu SM, Lim SH, Suh SC, Lee YT, *et al.* Variation and correlation analysis of flavonoids and carotenoids in Korean pigmented rice (*Oryza sativa* L.) cultivars. *J Agric Food Chem* 2010;58:12804-9.
 51. Cho JG, Song NY, Nam TG, Shrestha S, Park HJ, Lyu HN, *et al.* Flavonoids from the grains of C1/R-S transgenic rice, the transgenic *Oryza sativa* spp. japonica, and their radical scavenging activities. *J Agric Food Chem* 2013;61:10354-9.
 52. Goufo P, Trindade H. Rice antioxidants: Phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ -oryzanol, and phytic acid. *Food Sci Nutr* 2014;2:75-104.
 53. Rodriguez-Rodriguez R, Simonsen U. Measurement of nitric oxide and reactive oxygen species in the vascular wall. *Curr Anal Chem* 2012;8:1-10.

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