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Assessment of *in vitro* biological properties of aqueous extracts of *Murraya koenigii* L. Spreng., *Thymus vulgaris* L., and *Ocimum gratissimum* L. leaves

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ABSTRACT

This study investigated the *in vitro* biological properties of aqueous extracts of dried leaves of *Murraya koenigii*, *Thymus vulgaris,* and *Ocimum gratissimum.* The phenolic and flavonoid contents were evaluated colorimetrically. The antioxidant capacities were assessed through scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH•), 2,2'-azinobis (3-ethylobenzothiazoline-6-sulphonate) radical (ABTS+) and nitric oxide (NO); the reducing potentials and total antioxidant capacities were also estimated. The anti-inflammatory potentials were investigated through the inhibitions of lipoxygenase activity, albumin denaturation, and proteinase activity. The inhibitory actions against α-amylase, α-glucosidase, sucrase and albumin glycation were also evaluated. The extracts contained significant amounts of phenolic compounds with the extract of *T. vulgaris* having the highest concentrations of phenolics and flavonoids; correspondingly, the extract of *T. vulgaris* had the lowest IC_{50} values for DPPH•, $ABTS^{+\cdot}$ and NO scavenging. The extract of *T. vulgaris* significantly inhibited ($p<0.05$) lipoxygenase activity, albumin denaturation and proteinase activity similar to indomethacin. The actions of the extracts against carbohydrate digesting enzymes and the formation of protein modification varied significantly ($p<0.05$). This study provides insight into the functional and medicinal properties of *M. koenigii, T. vulgaris,* and *O. Gratissimum.*

Introduction

A number of populations living in developing countries use traditional medications that are derived from plants (Bodeker et al., 2005). The recommendation of plants as the primary sources of drugs is becoming increasingly popular with the prevention, management, and treatment of various diseases. Plant-based therapies are becoming new models of therapeutic aids as many of the available synthetic agents used in the treatment of most of the diseases that are associated with metabolic disorders only provide transitory effect; besides, they also exert various side effects and contraindications (Fatemeh et al., 2018). The supposed medicinal tendencies of these plants are usually linked to the significant amount of phytochemicals that could provide a stirring prospect for the development of new drugs (Cragg and Newman, 2013).

Epidemiological studies have shown that there is a direct relationship between the consumption of plant-based foods which are also functional foods as they contain substantial quantities of secondary metabolites such as vitamins and phytochemicals and reduced risk of certain diseases (Larsson et al., 2009; Guasch-Ferré et al., 2019). Moreover, there is a growing interest in identifying medicinal plants in the effective treatment of diseases taking into consideration the concomitant side effects of synthetic drugs (De Luca et al., 2012). However, while plants are erroneously assumed to be safe, they could also be sources of exposure to toxic substances by their compositions and preparation most especially if improperly used. Consequently, there are efforts to identify plants of therapeutic importance

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which can be part of any daily diet and provide desirable functional benefits beyond basic nutrition with a relatively lesser degree of adverse effects.
Despite the available evidence on the medicinal properties of many edible plants, little is known about their biological and how they improve wellness. Therefore this study aimed at evaluating the *in vitro* antioxidant potentials, anti-inflammatory properties, carbohydrate digesting enzymes inhibitory actions, and anti-glycation effect of the aqueous extracts of dried leaves of *Murraya koenigii* (L.) Spreng (Curry), *Thymus vulgaris* L. (Thyme), and *Ocimum gratissimum* L. (Scent leaf) which are examples of edible plants and commonly used in culinary applications.

Materials and methods

Chemicals and reagents

All chemical were of analytical grade. Gallic acid, trolox, quercetin, 1, 1- diphenyl-2-picrylhydrazyl (E-Labscience, China), 3, 5 dinitrosalicylic acid (JHD, Guanghua Sci-Tech, China), Congo red (Kem Light, Indian), Potassium ferricyanide, acetic acid, Folin Ciocalteu's phenol reagent were products of Loba Chemie, India.

Plant materials

Fresh leaves of *M. koenigii*, *T. vulgaris,* and *O. gratissimum* were air-dried at room temperature of 29±1 °C. The plant materials were authenticated at the Department of Biological Sciences, McPherson University, Seriki Sotayo, Nigeria. The dried leaves were pulverized and one gram of each pulverized sample was extracted with 10 mL of water at room temperature of 29±1 °C for 24 hours and latter filtered. The filtrate of each sample was used as the corresponding extract for the subsequent analyses.

Preparation of the crude α-glucosidase and sucrose solution

The preparation of the crude α-glucosidase and sucrose solution was carried out as described by Dahlqvist (1964). The animal used was handled under the approved guidelines of the Ethical Review Committee, McPherson University, Seriki Sotayo, Nigeria. The mucosa of the small intestine of rats sacrificed under light anesthesia was carefully scraped off with a glass slide glace, homogenized with cold sodium phosphate buffer (pH 6.8), and centrifuged at 4 °C for 20 minutes at 650 ×*g*. The clear solution was used as crude of α-glucosidase and sucrose solutions.

Phytochemical analyses

Estimation of total phenolic content

The total phenolic content was estimated as described by Singleton et al. (1999). Precisely 0.1 mL of each extract was transferred into a test tube containing 0.5 mL of Folin-Ciocalteu reagent (FCR). The mixture was allowed to stand at the room temperature of 29 °C for 20 minutes and thereafter, 2.0 mL of 10% sodium carbonate ($NaCO_2$) was added and left to stand for an extra 90 minutes. The absorbance was measured at 765 nm. The total phenolic content was reported as the number of equivalents of Gallic acid (GAE).

Estimation of total flavonoids contents

The estimation was carried out as described by Zhishen et al. (1999). Exactly 0.1 mL of each extract was dispensed into a test tube containing 0.5 mL of 5% aluminum chloride ($AlCl_3$). The mixture was allowed to stand at the room temperature of 29 °C for 5 minutes and thereafter, 1.0 mL of 7% sodium nitrite and 1.0 mL of 1% sodium hydroxide was added and incubated 30 minutes. The absorbance was measured at 510 nm. The total flavonoid content was expressed as the number of equivalents of quercetin (QE).

Antioxidant properties

$DPPH^{\bullet}$ scavenging ability

The evaluation was carried out as described by Shirwaikar et al. (2006). Exactly 0.1 mL of each extract was added to a test tube containing 0.5 mL of phosphate buffer (pH). Subsequently, 2.0 mL of freshly prepared methanolic solution of 0.1 mM DPPH• was added and left to stand in the dark for 30 minutes. The absorbance was later measured at 517 nm. The percentage of DPPH• scavenging activity was calculated and the result was expressed in IC_{50} (µg/mL). The IC_{50} was determined as the inhibition concentration required to scavenge 50% of the prepared solution of DPPH•.

$ABTS^{+}$ scavenging ability

The assessment was carried out according to the method described by Re et al. (1999). Accurately 0.1 mL of each extract was added to a test tube containing 0.5 mL of ABTS+ solution prepared by adding proper amounts of ABTS (7 mM) in ammonium persulphate (2.45 mM) overnight. The absorbance was measured at 734 nm after 15 minutes The percentage ABTS+ scavenging activity was calculated and the result was

expressed in IC_{50} (µg/mL). The IC_{50} was calculated as the inhibition concentration required to scavenge 50% of the prepared solution of ABTS+.

Nitric oxide scavenging activity

The determination was carried out as described by Garrat (1964). Exactly 0.1 mL of each extract was added to 0.5 mL of 10 mM sodium nitroprusside (prepared in phosphate buffer saline (pH 7.4)) in a test tube and incubated for 60 minutes at 29 °C. Later, 0.5 mL of 20% sulphanilic acid prepared in 10% glacial acetic acid was added to the reaction solution and al stands for 5 minutes. Then, 0.5 mL of 0.1% naphthyl ethylenediamine dihydrochloride solution was added to the reaction solution. The absorbance was measured at 540 nm after 30 minutes. The percentage nitric oxide scavenging activity was calculated and the result was expressed in IC_{50} (µg/mL). The IC_{50} was calculated as the inhibition concentration required to scavenge 50% of the generated nitric oxide.

Estimation of reducing power

The estimation was carried out as described by Oyaizu (1986). Cautiously, 0.1 mL of each extract was transferred into a test tube containing 1.0 mL of 0.2M phosphate buffer, and 1.0 mL of 1% potassium ferricyanide. The mixture was allowed to stand at the room temperature 50 °C for 20 minutes and 0.05 mL of 10% trichloroacetic acid solution was added. Thereafter, the mixture was centrifuged at 650 ×*g* for 5 minutes. An equal volume of the supernatant (1.0 mL), deionized water, and 0.1% ferric chloride solution was mixed in a separate test tube and incubated for 30 minutes. The absorbance was subsequently measured at 700 nm. The reducing power of each extract was reported as the ascorbic acid equivalents (AAE).

Estimation of total antioxidant capacity

The analysis was carried out as described by Prieto et al. (1999) based on the formation of a phosphomolybdenum complex. Each extract (0.1 mL) was added to 2.0 mL of a working solution containing 28 mM disodium hydrogen phosphate, 4 mM ammonium molybdate, and 0.6 M sulphuric acid in a test tube. The mixture was allowed to stand at the room temperature of 29 °C for 90 minutes. The absorbance was measured at 695 nm. The total antioxidant capacity was reported as the ascorbic acid equivalents (AAE).

Investigation of the anti-inflammatory properties

Anti-lipoxygenase activity

The determination was carried out as described by Shinde et al. (1999), using linoleic acid as the substrate and crude lipoxygenase prepared from soybean as previously reported by Oso and Karigidi (2019) as the enzyme. Accurately, 0.1 mL of each extract was added to a test tube containing 0.5 mL of 0.1 M phosphate buffer (pH 9.0) and 150 µL of the enzyme, lipoxygenase. The mixture was allowed to incubate at 29 °C for 5 minutes. Afterward, 0.5 mL of 0.6 mM linoleic acid solution was added to the mixture. The absorbance was measured at 234 nm. The percentage anti-lipoxygenase activity was calculated and the result was expressed in IC_{50} (µg/mL). The IC_{50} was determined as the inhibition concentration required to inhibit 50% of lipoxygenase activity.

Inhibition of albumin denaturation

The determination was carried out as described by Sakat et al. (2010). Precisely 0.1 mL of each extract was added to a test tube containing 0.5 mL of 1% aqueous solution of bovine serum albumin and incubated at 35 °C for 20 minutes. The temperature was subsequently increased to 50 °C and maintained for an additional 20 minutes. Afterward, the solution was cooled and the turbidity was measured at 660 nm. The percentage inhibition of albumin denaturation was calculated and the result was expressed in IC_{50} (µg/mL). The IC_{50} was determined as the inhibition concentration required to inhibit 50% of albumin denaturation.

Anti-proteinase activity

The determination was carried out as described by Sakat et al. (2010). Exactly, 0.1 mL of each extract was added to a test tube containing 0.5 mL of a solution of trypsin prepared in phosphate buffer (pH 7.4) and incubated at 35 °C for 5 minutes. Then, 0.1 ml of 1.0% casein was added and incubated for an additional 20 minutes before the reaction was terminated by adding 2.0 mL of 4.0% trichloroacetic acid solution. The reaction mixture was centrifuged for 5 minutes at 650 ×*g* and the absorbance of the supernatant was measured at 230 nm. The percentage inhibition of proteinase action was calculated and the result was expressed in inhibition concentration IC_{50} (µg/mL) required to inhibit 50% of proteinase activity.

Determination of carbohydrate digesting enzymes inhibitory properties

Inhibition of the α-amylase activity

The determination was carried out as described by Bernfeld (1955). Each (0.1 mL) extract was incubated with 0.05 mL of α-amylase solution in a test tube containing 1.0 mL of 2 mM of phosphate buffer (pH 6.9) for 20 minutes. Afterward, 0.1 mL of 1.0% freshly prepared starch solution was added and allowed to stand for 5 minutes. Then, 0.5 mL of 3, 5-dinitrosalicylic acid reagent was added to the reacting solution and kept in boiling water for 5 minutes. Afterward, the solution was chilled and the absorbance was measured at 540 nm. The percentage inhibition of α-amylase activity was calculated and the result was expressed in IC_{50} (µg/mL). The IC_{50} was determined as the inhibition concentration required to inhibit 50% of α-amylase activity.

Inhibition α-glucosidase activity

The determination was carried out as described by Kim et al. (2005). Exactly, 0.1 mL of each extract was incubated with 0.1 mL of mucosa solution in a test tube containing 1.0 mL of 2 mM of phosphate buffer (pH 6.9) for 20 minutes. Afterward, 0.1 mL of 3 mM Para-nitrophenylglucopyranoside prepared in 20 mM phosphate buffer (pH 6.9) was added allowed to stand for 15 minutes. Then, 0.5 mL of 5% sodium carbonate was added and incubated for an additional 90 minutes. The absorbance was measured at 450 nm. The percentage inhibition of α-glucosidase activity was calculated and the result was expressed in IC_{50} (µg/mL). The IC_{50} was determined as the inhibition concentration required to inhibit 50% of α-glucosidase activity.

Sucrase inhibitory activity

The determination was carried out as described by Honda and Hara (1993). Exactly 0.1 mL of each extract was incubated with 0.1 mL of mucosa solution in a test tube containing 1.0 mL of 2 mM of phosphate buffer (pH 6.9) for 20 minutes. Afterward, 0.1 mL of 60 mM sucrose solution was added and allowed to stand for 5 minutes. Then, 0.5 mL of 3,5-dinitrosalicylic acid reagent was transferred into the test tube and allowed to incubate in boiling water for 5 minutes. The test tube was cooled and the optical density was read at 540 nm. The percentage inhibition of sucrase activity was calculated and the result was expressed in IC_{50} (µg/mL). The IC_{50} was determined as the inhibition concentration required to inhibit 50% of sucrase activity.

Assessment of glycation-induced attenuation properties

In vitro glycation of albumin

The preparation of glycated albumin was carried out according to the procedure described by Safari et al. (2010). The solution contained bovine serum albumin (0.1 g/mL) prepared in 0.1 M phosphate buffer (pH 7.4) containing 0.01% sodium azide, D-glucose (10 mg/mL) and the extract mixed in ratio 3:2:1 and incubated for 72 hours.

Estimation of anti-glycation capacity

The determination was carried out as described by Furth (1988). Precisely, 0.5 mL of 10% trichloroacetic acid was added to a test tube containing 1.0 mL of glycated sample. The solution was centrifuged at 650 ×*g* for 5 minutes. Then, 1 mL phosphate buffer and 0.5 mL of 0.3 N oxalic acids were added to the sediment and kept in a boiling water bath for 60 minutes. The solution was cooled and 0.5 mL of 10% 4 % trichloroacetic acid solution and 0.5 mL of 0.05 M thiobarbituric acid were added and kept in boiling water for 10 minutes. The solution was centrifuged at 650 ×*g* for 5 minutes and the absorbance of the supernatant was measured at 443 nm. The results were expressed in percentage.

Determination of inhibition of glycation-induced oxidation of protein thiol groups

The determination was carried out as described by Ellman (1959). Accurately, 1.0 ml of 0.5 mM 5, 5'-dithiobis (2-nitrobenzoic acid) in 0.1 M phosphate buffer (pH 7.4) was added to a test tube containing 1.0 mL of glycated sample and incubated at room temperature of 29 °C for 15 minutes. The absorbance was measured at 412 nm. The concentration of thiol groups was calculated using ε= molar extinction =1.34 × $10^4 M^{-1} cm^{-1}$. The results were reported as nmol/mg protein.

Determination of Inhibition of β-amyloid aggregation

The determination was carried out as described by Klunk et al. (1999). Precisely, 0.1 mL of 1% Congo red prepared in phosphate buffer with 10% ethanol was added to a test tube containing 1.0 mL of glycated sample. The solution was incubated for 30 minutes and

the absorbance was measured at 530 nm. The results were expressed in percentage.

Statistical analysis

The results were analyzed using a one-way analysis of variance (ANOVA) for mean differences among the various extracts followed by Duncan's multiple comparison tests for posthoc comparisons at $p<0.05$ and presented as means ± standard deviation of three determinations.

Results

Total phenolics and total flavonoids

The results presented in Fig. 1 showed the estimated quantities of total phenolics and total flavonoids of the extracts expressed in mg/100 g of the dried weight of the leaves. All the extracts demonstrated significant amounts of total phenolics and total flavonoids. Comparatively, the highest concentration of the total phenolics and flavonoids found in the extract of *T. vulgaris* followed by the aqueous extract of *O. gratissimum*.

Antioxidant activities

The determination of antioxidant properties of the extracts was performed by investigating the scavenging potential of the extracts against DPPH•, $ABTS^{+\cdot}$ and NO (Table 1). The results revealed a significant decrease in the absorbance of the chromogenic radicals when incubated in the presence of the extracts. It was observed that the extracts of *T. vulgaris* and *O. gratissimum* exhibited significantly ($p<0.05$) higher DPPH• scavenging activity than the extract of *M. koenigii*. The $ABTS^{+}$ scavenging potential of the extract of *T. vulgaris* was statistically equivalent to that of Trolox, a reference standard, but significantly higher than the $ABTS^{+}$ scavenging potentials of *O. gratissimum* and *M. koenigii*. A similar trend was observed in the nitric oxide scavenging capacities of the extracts, reducing potential and total antioxidant capacities (Table 1, Fig. 2).

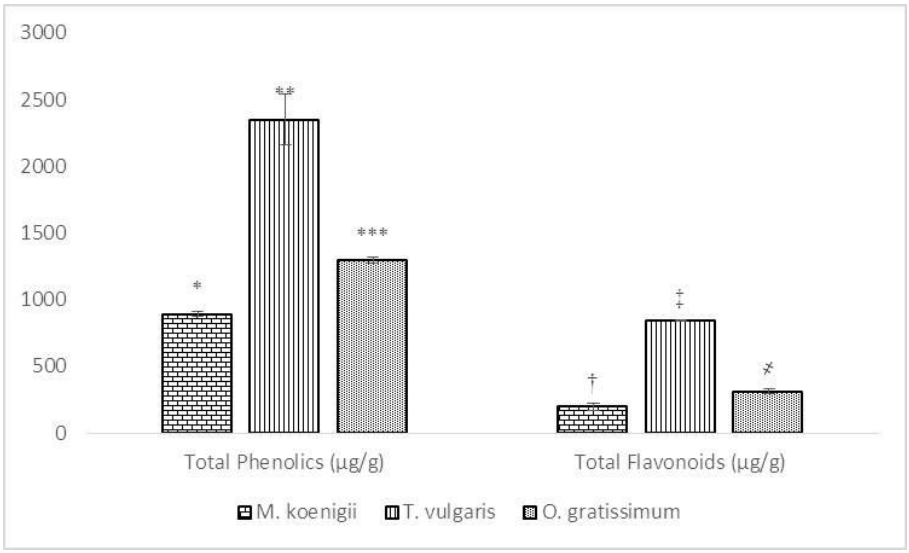

Fig. 1. Total phenolics and total flavonoid contents of the aqueous extracts of dried leaves of *M. koenigii, T. vulgaris,* and *O. gratissimum*. Different symbols indicate significant differences ($p<0.05$) among the extracts

Table 1. Average value of Antioxidant parameters of the aqueous extracts of dried leaves of *M. koenigii*, *T. vulgaris*, and *O. gratissimum*

	DPPH• Scavenging (IC_{50}, µg/mL)	$ABTS^{+}$ Scavenging (IC_{50}, µg/mL)	NO Scavenging (IC_{50}, µg/mL)
M. koenigii	355.72 ± 41.79^{a}	382.19 ± 20.18^{a}	118.38 ± 32.34^{a}
T. vulgaris	110.56 ± 23.51^{b}	43.45 ± 14.62^{b}	78.33 ± 21.64^{b}
O. gratissimum	136.19 ± 10.34^{b}	149.17 ± 26.94^{c}	131.05 ± 22.96^{c}
Trolox	67.39 ± 0.15^{c}	41.41 ± 0.68^{b}	69.33 ± 5.37^{d}

Different letters within the column designate significant differences ($p<0.05$).

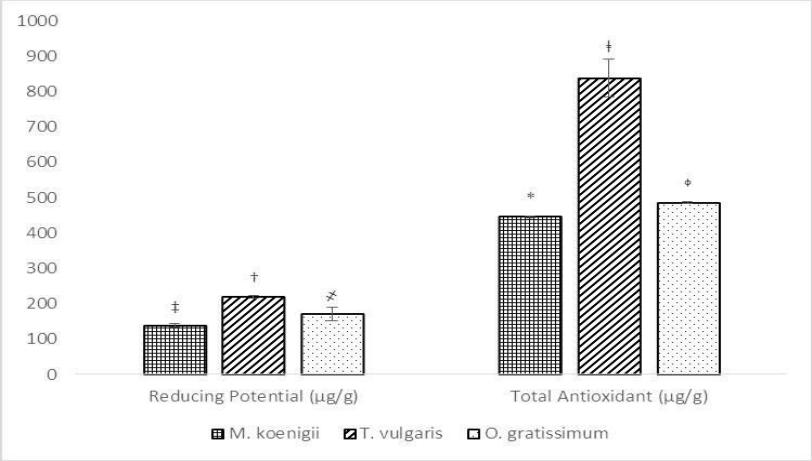

Fig. 2. Reducing potentials and total antioxidant capacities of the aqueous extracts of dried leaves of *M. koenigii, T. vulgaris,* and *O. gratissimum*. Different symbols indicate significant differences ($p<0.05$) among the extracts

Table 2. Average value of anti-inflammatory parameters of the aqueous extracts of dried leaves of *M. koenigii, T. vulgaris,* and *O. gratissimum*

	Lipoxygenase (IC_{50}, µg/mL)	Albumin Denaturation (IC_{50}, µg/mL)	Proteinase Activity (IC_{50}, µg/mL)
M. koenigii	76.33±15.17^a	91.22±21.64^a	275.49±35.09^a
T. vulgaris	51.06±8.33^b	57.67±12.81^b	209.12±37.96^b
O. gratissimum	74.14±14.25^c	81.73±18.36^c	416.04±99.09^c
Indomethacin	45.90±0.89^b	46.23±0.16^b	79.83±1.28^d

Different letters within the column designate significant differences ($p<0.05$).

Anti-inflammatory properties

The inhibitions of lipoxygenase activity, albumin denaturation, and proteinase activity by the extracts are presented in Table 2. The results showed that the inhibitory potential of the extract of *T. vulgaris* showed the strongest inhibitory effect against lipoxygenase activity, albumin denaturation, and proteinase activity comparable to indomethacin, a reference standard followed by the activities of the extracts of *O. gratissimum.*

Inhibition of carbohydrate digesting enzymes

The α-amylase inhibitory activities showed IC_{50} value of 185.96 ± 29.65, 104.12 ± 15.87 and 140.84±29.79 µg/ml for the extracts of *M. koenigii*, *T. vulgaris* and *O. gratissimum* (Table 3). The best activity was observed in the extract of *T. vulgaris* with an IC_{50} value of 104.12±15.87 µg/mL. There are significant differences ($p<0.05$) between the IC_{50} values of all extracts and acarbose, a reference standard. The extract of *M. koenigii* showed the least inhibitory potential against α-glucosidase activity while the α-glucosidase inhibitory capacities of extracts of *T. vulgaris* and *O. gratissimum* were comparable to the inhibitory potential of acarbose. The sucrose inhibitory potential ranking of the extracts was *T. vulgaris*> *O. gratissimum,*> *M. koenigii*, with IC_{50} values of 79.00 ± 18.50, 116.3 3 ± 1.67, 3480 and 152.67 ± 21.22 µg/mL, respectively.

Table 3. Carbohydrate digesting enzymes inhibitory potentials of the aqueous extracts of dried leaves of *M. koenigii, T. vulgaris* and *O. gratissimum.*

	α-Amylase (IC_{50}, µg/mL)	α-Glucosidase (IC_{50}, µg/mL)	Sucrase (IC_{50}, µg/mL)
M. koenigii	185.96±29.65^a	147.41±41.83^a	152.67±21.22^a
T. vulgaris	104.12±15.87^b	65.57±2.27^b	79.00±18.50^b
O. gratissimum	140.84±29.79^c	71.69±15.29^b	116.33±1.67^c
Acarbose	62.61±1.89^d	62.03±1.054^b	30.74±1.42^d

Different letters in the column indicate significant differences ($p<0.05$) among the extracts.

Table 4. Anti-glycation properties of the aqueous extracts of dried leaves of *M. koenigii, T. vulgaris* and *O. gratissimum*

	Inhibition of albumin glycation (%)	Inhibition of oxidation of Thiol groups (nmol/mg protein)	Inhibition of β-amyloid aggregation (%)
M. koenigii	50.00±2.31[a]	0.82±0.06[a]	15.67±2.96[a]
T. vulgaris	48.00±2.89[a]	2.35±0.19[b]	33.33±4.16[b]
O. gratissimum	55.33±3.76[a]	1.56±0.15[c]	36.00±4.58[b]

Different letters within the column designate significant differences ($p<0.05$).

Attenuation of glycation-induced protein modifications

The aqueous extract of the selected plants inhibited the albumin glycation properties, though there was no significant difference ($p<0.05$) among the mean percentage inhibition (Table 4). Relatively, the extract of *T. vulgaris* showed the highest inhibitory capacity of glycation-induced oxidation of albumin thiol groups followed by *O. gratissimum*. The highest inhibitory effect of the extracts on β-amyloid fibril formation on glycated albumin was observed for the extract of *T. vulgaris*. There was no statistical difference between the effects of the extracts of *M. koenigii* and *O. gratissimum* on the relative (%) amounts of the protein aggregates.

Discussion

The interplay among chronic diseases encourages a need to explore therapeutic strategies that could identify polyfunctional agents to combat the pathological effects that are interrelated in metabolic disorders. Recently, there has been much interest in functional foods, especially from edible plants to enhance and supplement the actions of enzymatic and non-enzymatic endogenous antioxidant actions thus limiting the burden of oxidative damage. The health benefits of these functional foods could be assumed to be linked to their corresponding antioxidant compounds, most especially the phenolics and flavonoids. The assessment of the phytochemical compositions of the aqueous extracts of dried leaves of *M. koenigii, T. vulgaris* and *O. gratissimum* revealed that each contained significant amounts of phenolics and flavonoids estimated by the reduction of FCR to a mixture of blue oxides and development of flavonoid-aluminum complex, respectively. These results indicated that the extract of *T. vulgaris* had a high content of phenolic compounds. The protecting effect of these food materials could be accredited to the presence of the antioxidant compounds.

The ability of the extracts to act as a radical scavenger or reducing agent was revealed by DPPH• and $ABTS^+$ radical-scavenging activity assay. The extract of *T. vulgaris* had the highest radical capacities. The radical scavenging pattern was similar to the NO scavenging potential. The synthesis of NO by the enzyme nitric oxide synthase (NOS) had been shown to play important roles as a biological mediator in a variety of biological processes (Khazan and Hdayati, 2015). It employs a biphasic response; NO production plays beneficial roles at low concentrations, for instance, it can function as a neurotransmitter and anti-inflammatory agent. It can inflict pathological conditions such as antiapoptotic and immunostimulatory functions at high concentrations or in the presence of free radicals such as hydrogen peroxide (H_2O_2) or superoxide anion ($O_2^{\cdot -}$) forming peroxynitrite which can be mutagenic and exacerbate inflammatory response and tissue injury (Lee and Pfeifer, 2007). Different diseases such as cardiovascular diseases, cancer, rheumatoid arthritis, diabetes, asthma, atherosclerosis, and thyroid disorders had been associated with excessive release of NO (Khazan and Hdayati, 2015). Regulations of the activities of NOS through endogenous mechanisms or inhibitors are well-controlled to promote the rapid clearance of NO and limit tissue damage (Peeri et al., 2013). This was beneficial in the treatment and management of NO-induced pathologies (Pacher et al., 2007). However, non-enzymatic production of NO by one-electron reduction of nitrite could greatly increase under hypoxic conditions and contribute to the physiological elevated concentration of NO (Pereira et al., 2011). Ferric ion reducing the potential of the extracts demonstrated the ability of the reductones in the extracts to reduce ferric ion. The observed properties are linked with the electron transferability of the extracts. Thus, they indicate the antioxidant activities of the extracts. Thus, the extract of *T. vulgaris* possesses reducing agents that can prevent peroxide and radical accumulation and increase termination of chain reaction.

A relationship has been reported to exist between oxidant species and inflammatory response (Oso et al., 2018). Inhibition of the activity of pro-inflammatory mediators prevents tissue injury that is associated with chronic inflammation, a complication of prolonged oxidative stress. Inflammatory disorders are initiated by a wide array of reactions involving the release of pro-inflammatory mediators, activation of enzymes,

stimulation of immune cells, and alteration of protein structure. Derivatives of plant-based natural materials have been shown to prevent complications of diabetes that are related to inflammatory disorders (Lucarini et al., 2013).

The relative IC_{50} values of lipoxygenase inhibitory effects of the extracts of *M. koenigii, T. vulgaris* and *O. gratissimum,* and indomethacin, showed that the extract of *T. vulgaris* had appreciable inhibitory effects when compared with indomethacin, a non-steroidal anti-inflammatory drug, which is commonly used as an anti-inflammatory agent. It may be due to the presence of more chemical constituents such as thymol, 1,8 cineole, and carvacrol which had previously been reported to be probable functional agents in the extract (Ocaña and Reglero, 2012).

Alteration of the secondary structure and tertiary structure through denaturation could alter the antigenicity of these proteins and initiate inflammatory responses. Protein denaturation has been detected in patients and rats with chronic inflammatory lesions and the conventional nonsteroidal anti-inflammatory drugs such as diclofenac and indomethacin had been theorized to confer anti-inflammatory effects through prevention of denaturation of proteins in addition to blocking cyclooxygenases and inhibiting the biosynthesis of eicosanoids (Kim et al., 2008).

Additionally, neutrophils are known to be a rich source of proteinases that are involved in the mediation of pro-inflammatory responses. There were significant differences among all the extracts as regards the inhibition of proteinase activity. Remarkably, the extract of *T. vulgaris* displayed the highest percentage inhibition and this demonstrated by the IC_{50} value which was the lowest when compared to other extracts. This suggests that the active constituent inhibiting the enzyme could be most in the extract of *T. vulgaris*. This is following previous accounts that the extract of *T. vulgaris* could provide a significant level of protection as proteinase inhibitors (Ocaña and Reglero, 2012; Oliveira et al., 2017). These results suggest that extracts of *M. koenigii, T. vulgaris,* and *O. gratissimum* leaves could be explored in the development of anti-inflammatory formulation.

Infusions from medicinal plants have been employed for centuries in traditional medical systems in the treatment and management of most diseases with apparent features of disorders such as diabetes. Studies on the antidiabetic properties of these plants using the rat model revealed the plants possess hypoglycemic and anti-hyperglycemic properties (Ekoh et al., 2014). The inhibition of the carbohydrate digesting enzymes is a central target in the maintenance of a lower or slower rise in blood glucose in diabetic patients.

The assessment of the α-amylase and α-glucosidase inhibitory properties showed that the aqueous extracts of *T. vulgaris* and *O. gratissimum* had significant inhibitory potentials against the enzymes. The IC_{50} value of *T. vulgaris* and *O. gratissimum* extracts are virtually similar to that of acarbose, an anti-diabetic drug that inhibits the hydrolysis of 1,4-glycosidic linkages of polysaccharides. Thus, consumption of the leaves of *T. vulgaris* and *O. gratissimum* could limit carbohydrate digestion and absorption and could be beneficial in the management of diabetes mellitus type II.

Additionally, the extracts proved to have sucrase inhibitory effects. Sucrase is a key enzyme in the brush borders of the small intestine promoting the hydrolysis of sucrose to glucose and fructose. Phenolic compounds could be responsible for the sucrase inhibitory property (Kaewnarin and Rakariyatham, 2017).

The glycoside hydrolases inhibitors in these extracts could reasonably be effective in regulating blood glucose levels. Although the oligosaccharides-like structure of acarbose is responsible for the high-affinity binding of acarbose to the glycoside hydrolases and the presence of an imino bridge responsible for the inability of the digestive enzymes to hydrolyze the acarbose (Wehmeier, 2003). Naturally occurring non-oligosaccharide substances without an imino bridge such as flavonoids and phenolics had been demonstrated to have inhibitory properties against amylases and glucosidase (Proença et al., 2017). Moreover, consumption of these botanicals as functional foods containing phenolics and flavonoids might reduce the effective dose of clinically used synthetic inhibitors such as acarbose, miglitol, voglibose, etc required in the regulation of starch digestion thus controlling post-prandial blood glucose levels in diabetic patients. Nonetheless, further studies could be needed to identify the constituents of these plants that are responsible for hydrolases inhibitory activity.

Besides, modifications in the structural conformation of protein induced by hyperglycemia may result in impairments of the biological functions of many proteins. Glycation of biomolecules is associated with the pathology of various diseases, but a continuous elevation of blood glucose could exaggerate the steady formation of these glycated compounds in various body tissues through a spontaneous non-enzymatic reaction of reducing ends of glucose with amino acids, nucleic acids or lipids generating Amadori products which could form advanced glycation end products (AGEs) (Negre-Salvayre et al., 2009; Gkogkolou and Böhm, 2012). The AGEs could induce altered intracellular signaling. Studies have shown that plant extracts could prevent excessive glycation of macromolecules in the biological system thus

attenuating the formation of AGEs. This is in agreement with the earlier claim that natural products that inhibit protein glycation may be beneficial in preventing secondary complications that are related to diabetes (Yamagishi et al., 2008). The aqueous extract of *T. vulgaris* was more potent in inhibiting the formation of glycation products as compared to the extracts of *M. koenigii* and *O. gratissimum*.

The protective capacity of the extracts against AGEs formation was further validated through the inhibition of thiol oxidation and β-fibril formation. The aqueous extract of leaves of *M. koenigii*, *T. vulgaris*, and *O. gratissimum* could contain agents that could prevent the advancement of diabetic complications. The aqueous extracts of *T. vulgaris* and *O. gratissimum* significantly decreased the extent of glycation-induced oxidation of albumin thiol groups, a marker of the protein oxidation mediated by glycation.

Glycation induces the refolding of globular protein into β-amyloid structures which can be detected by the binding of Congo red to the hydrophobic region between the antiparallel β-strands (Girych et al., 2016). Many plant-based natural products are known to be effective against amyloid aggregation which is considered to be linked with many complications such as Alzheimer's and Parkinson's disease (Khan et al., 2017). Amyloid beta and reactive oxygen species induced toxicities had been implicated in the pathogenesis of Alzheimer's disease. The observed biological effect of these extracts could be related to the estimated quantities of the phytochemicals. The extracts could contain phytochemicals with anti-amyloidogenic properties that can bind to various amyloid species and lead to changes in the structure of a β-sheet assembly of the proteins to form non-toxic aggregates (Bu et al., 2016).

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