

## Original Research Article

# Biological Activities of three Nigerian Spices - *Laurus nobilis* Linn, *Murraya koenigii* (L) Spreng and *Thymus vulgaris* Linn

Mutiu Idowu Kazeem<sup>1</sup>, Anofi Omotayo Tom Ashafa<sup>1\*</sup> and Mikhail Olugbemiro Nafiu<sup>2</sup>

<sup>1</sup>Phytomedicine and Phytopharmacology Research Group, Department of Plant Sciences, University of the Free State, Qwaqwa Campus, Phuthaditjhaba, South Africa, <sup>2</sup>Department of Biochemistry, University of Ilorin, PMB 1515, Ilorin, Nigeria

\*For correspondence: **Email:** [ashafaat@qwa.ufs.ac.za](mailto:ashafaat@qwa.ufs.ac.za); **Tel:** +27587185313

Received: 19 May 2015

Revised accepted: 28 October 2015

### Abstract

**Purpose:** To evaluate the biological properties of polyphenol extracts of three spices – *Laurus nobilis* (bay), *Murraya koenigii* (curry) and *Thymus vulgaris* (thyme) from Lagos, Nigeria.

**Methods:** Acetone extracts of these spices were subjected to bovine serum albumin (BSA)-glucose antiglycation, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide anion radical scavenging assays. Brine-shrimp lethality and phytotoxicity tests were also performed on the extracts (10 – 1000 µg/mL).

**Results:** The extract of *T. vulgaris* had the highest antiglycation effect with 50 % inhibitory concentration (IC<sub>50</sub>) of 0.02 mg/mL, as well as antioxidant potential with IC<sub>50</sub> of 0.10 and 0.06 mg/mL for DPPH and superoxide anion radical scavenging assays, respectively ( $p < 0.05$ ). On the other hand, all the extracts exhibited weak cytotoxicity with 50 % lethal dose (LD<sub>50</sub>) ranging from 1000 – 2000 µg/mL, and for phytotoxicity, LD<sub>50</sub> ranged from 640 – 1640 µg/mL.

**Conclusion:** Acetone extracts of bay, curry and thyme displayed good antiglycation as well as antioxidant potential and are safe for consumption. However, of all the spices, thyme exhibited the best activity as an antioxidant and antiglycation agent.

**Keywords:** Glycation, Hyperglycemia, Polyphenols, Spices, Bay, Curry, Thyme

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

## INTRODUCTION

Diabetes mellitus is one of the most common non-communicable diseases [1] in the world, with 382 million people affected in 2013 [2]. The disease is accompanied by some complications which result from spontaneous reaction between the excess blood glucose and proteins, forming advanced glycation end products (AGEs). These diabetic complications include nephropathy, neuropathy and retinopathy [1]. Due to the severity of this ailment and its complications, there is the need to search for dietary agents that

can prevent and or ameliorate the condition. Spices refer to any part of plant that is used to enhance the flavour, aroma or piquancy of foods [3]. Many spices have been reported to have medicinal value due to the presence of diverse phytochemicals like polyphenols [3]. For the purpose of this study, *L. nobilis*, *M. koenigii* and *T. vulgaris* were used.

Bay (*Laurus nobilis* Linn.) belongs to the family Lauraceae and is one of the most widely used culinary spices in all Western countries. Bay leaf traditionally has been used as herbal medicine to

treat rheumatism, ear-aches, indigestion, sprains and to promote perspiration [4]. Scientific reports have revealed the antimicrobial, anticonvulsant, analgesic, anti-inflammatory [5], and wound-healing [6] activities of this plant.

The Curry tree (*Murraya koenigii* (L.) Spreng.), a tropical to sub-tropical tree of the family Rutaceae, is native to India, Sri Lanka, Bangladesh and the Andaman Islands as a food flavouring herb [7]. Its leaves are known to possess anti-inflammatory, anti-dysenteric, antioxidant and anti-diabetic activities [8]. Thyme (*Thymus vulgaris* Linn.) is an aromatic herb that is used extensively to add a distinctive aroma and flavour to food. Its leaves can be used fresh or dried as a spice. Thyme also possesses various pharmacological effects such as antiseptic, carminative, antimicrobial and antioxidative properties [9].

Consequent upon the growing calls for anti-diabetic dietary agents and as a follow-up to our previous report [10], the aim of this study was to evaluate the antiglycation and antioxidant properties of polyphenolic extracts from these aforementioned spices, as well as to determine their cytotoxic and phytotoxic potential.

## EXPERIMENTAL

### Plant materials

Bay (*Laurus nobilis*), curry (*Murraya koenigii*) and thyme (*Thymus vulgaris* L.) were obtained from the Central Spices Market in Mile 12 area, Ketu, Lagos, Nigeria. The plants were authenticated by Dr AB Kadiri of the Department of Botany, University of Lagos, Lagos, Nigeria and voucher specimens (nos. LUH 4774, 4775 and 4776) were prepared and deposited in the university herbarium. They were dried under room temperature, ground into powder and kept in plastic containers in the refrigerator prior to commencement of the study.

### Chemicals

BSA (Bovine serum albumin) was obtained from the Research Organics Cleveland USA. 1, 1-diphenyl, 2-picryl hydrazyl (DPPH), ferric chloride and trichloroacetic acid were obtained from Sigma Chemical Co. (St. Louis, MO., USA). Water-soluble tetrazolium salt (WST-1), xanthine oxidase and Williams' medium E were products of Sigma-Aldrich Chemie GmbH, Munich, Germany. All other chemicals were of analytical grade and the water was glass distilled

### Preparation of polyphenol extracts

The powdered samples were extracted with 80 % acetone (1:2 w/v) thrice each for 72 h each time at room temperature. Pooled extracts were filtered with Whatman filter paper (type 2) under vacuum and the filtrates were concentrated under reduced pressure on rotary evaporator (BÜCHI, R-3000, Switzerland) at 40 °C. The concentrated extracts were further lyophilized and then used for the experiments [11].

### Antiglycation assay

*In vitro* antiglycation activity of the spices was examined by testing the ability of the extracts to inhibit the methyl glyoxal mediated development of fluorescence of bovine serum albumin (BSA) [12]. In 96-well plate assays, each well contained 60  $\mu$ L reaction mixture of 20  $\mu$ L BSA (10  $\mu$ g/mL), 20  $\mu$ L of glucose anhydrous (50 mg/mL), magnesium oxide (14 mM) and 20  $\mu$ L test sample (0.25 – 1.0 mg/mL extract). Glycated control contained 20  $\mu$ L BSA, 20  $\mu$ L glucose and 20  $\mu$ L sodium phosphate buffer (0.1 M, pH 7.4) containing NaN<sub>3</sub> (30 mM), while blank control contained 20  $\mu$ L BSA and 40  $\mu$ L sodium phosphate buffer. The reaction mixture was incubated at 37 °C for 9 days in the presence or absence of various concentrations of the extract. After 9 days of incubation, 60  $\mu$ L trichloroacetic acid (TCA) (100 %) was added in each well and centrifuged (15,000 rpm) for 4 min at 4 °C. After centrifugation, the pellet was washed with 60  $\mu$ L 5 % TCA. The supernatant containing glucose, inhibitor and interfering substance, was removed and pellet containing advanced glycation end products (AGEs)-BSA was dissolved in 60  $\mu$ L PBS. AGE formation was measured by the fluorescence's intensity excitation (370 nm) to emission (440 nm) by using the spectrofluorometer RF-1500 (Shimadzu, Japan).

### Diphenylpicrylhydrazine (DPPH) radical-scavenging activity

The radical scavenging property of the extract was evaluated by assessing the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity [13]. The reaction mixture was prepared by mixing 5  $\mu$ L extract (dissolved in DMSO) with 95  $\mu$ L of DPPH (dissolved in ethanol). Varying concentrations of extract (0.125 – 0.50 mg/mL) were prepared, while the concentration of DPPH was kept as 300  $\mu$ M in all reaction mixture. These reaction mixtures were dispensed in 96-well plate and incubated at 37 °C for 30 min and the absorbance was measured at 515 nm. The radical scavenging activity was calculated as a

percentage of DPPH decolorization compared to control, as in Eq 1.

$$\text{Inhibition (\%)} = \{(Ac-Ae)/Ac\}100 \dots\dots\dots (1)$$

where Ac and Ae are the absorbance of control and extract, respectively

### Superoxide anion scavenging assay

The method of Ferda [14] was used in the determination of superoxide scavenging activity of the samples. Superoxide scavenging activity was assayed in phosphate buffer (0.1 M, pH 7.5). Xanthine oxidase (0.003 unit /well) and test samples dissolved in DMSO (0.125 – 0.50 mg/mL) were mixed in 96-well microtiter plate and pre-incubated for 10 min at room temperature, before water-soluble tetrazolium solution (WST-1) (15 µM) was added. The reaction was initiated by adding 0.1 mM of xanthine and uric acid formation was measured spectrophotometrically at 295 nm and the reduction of WST-1 was read at 450 nm by using molecular devices, spectramax 340. The inhibitory activity of the samples was determined against DMSO blank, as in Eq 2.

$$\text{Inhibition (\%)} = \{(Ac-Ae)/Ac\}100 \dots\dots\dots (2)$$

where Ac and Ae are the absorbance of control and extract, respectively

### Brine shrimp cytotoxicity assay

The test was conducted by taking half - filled hatching glass tray (22 x 32 cm) with brine solution (sea salt, 38 g/L), 500 mg eggs of brine shrimp (*Artemia salina*) (Brine Shrimp, South Africa) were sprinkled and after covering with a lid, it was incubated at 27 °C for 2 days for hatching. The brine shrimp larvae (nauplii) were collected through a light source and Pasteur pipette. The extracts were tested at concentrations of 10, 100 and 1000 µg/mL in vials containing 5 mL of brine solution and 10 nauplii in each of the three replicates. Survivors were counted after 24 h and percentage of death calculated. The data was analyzed to determine LD<sub>50</sub> values at 95 % confidence intervals [15].

### Phytotoxicity assay

This test was performed according to the modified protocol of Mclaughlin *et al* [15]. The test samples were incorporated with sterilized medium at different concentrations i.e. 10, 100, 1000 µg/mL in methanol. Sterilized conical flasks were inoculated with samples of desired concentrations prepared from the stock solution

and the samples were allowed to evaporate overnight. Each flask was inoculated with 20 mL of sterilized Williams' medium E and ten *Lemna minor*, each containing a rosette of three fronds. Other flasks were supplemented with methanol serving as negative control and a reference inhibitor (Paraquat) as positive control. Treatments were replicated three times and the flasks incubated at 30 °C in Fisons Fi-Totron 600 H growth cabinet for seven days, 56 ± 10 relative humidity and 12 h day length. Growth of *L. minor* in the sample-containing flasks was determined by counting the number of fronds (leaf-like part) per dose and growth inhibition was calculated with reference to negative control.

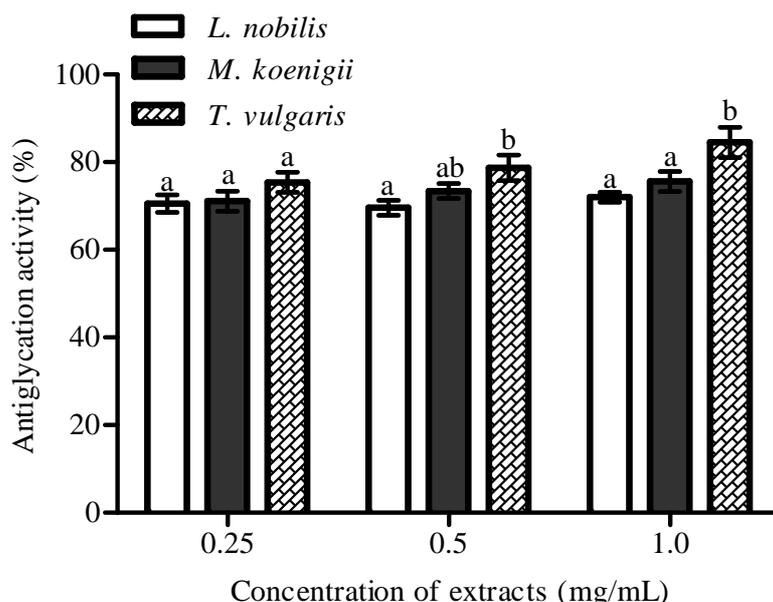
### Statistical analysis

All data were analysed by one-way analysis of variance (ANOVA) followed by Bonferroni test using GraphPad Prism 5 software (GraphPad, USA). All the results were expressed as mean ± SEM (n = 3).

## RESULTS

Figure 1 showed the result of antiglycation activity of the polyphenolic extracts of *L. nobilis*, *M. koenigii* and *T. vulgaris*. All the spices displayed very high activity (above 70 %) at all concentrations tested. However, *T. vulgaris* displayed the highest potential at all concentrations and its activity is significantly different ( $p < 0.05$ ) from the other extracts at the concentrations of 0.5 mg/mL and 1.0 mg/mL. Expectedly all the spices tested displayed very low IC<sub>50</sub> (0.02 – 0.08 mg/mL) (Table 1). Figure 2(a) depicts the ability of the various extracts of spices to scavenge diphenylpicrylhydrazyl (DPPH) radical. The ability of *L. nobilis* to scavenge the radical was significantly different ( $p < 0.05$ ) compared to *M. koenigii* at 0.125 mg/mL. At all the other concentrations tested, all the extracts displayed high activity which is not significantly different ( $p > 0.05$ ) from one another. However, the IC<sub>50</sub> shown in Table 1 depicted that *L. nobilis* had the lowest IC<sub>50</sub> value (0.02) when compared to the other extracts.

In order to further confirm the antioxidant activity of these extracts, their ability to scavenge superoxide anion radical was evaluated (Figure 2b). *T. vulgaris* showed the highest potential of scavenging superoxide anion which is significantly different ( $p < 0.05$ ) from the other extracts at all the concentrations tested. This is testified to by its IC<sub>50</sub> 0.06 mg/mL as against 0.46 mg/mL and 8.13 mg/mL for *L. nobilis* and *M. koenigii*.

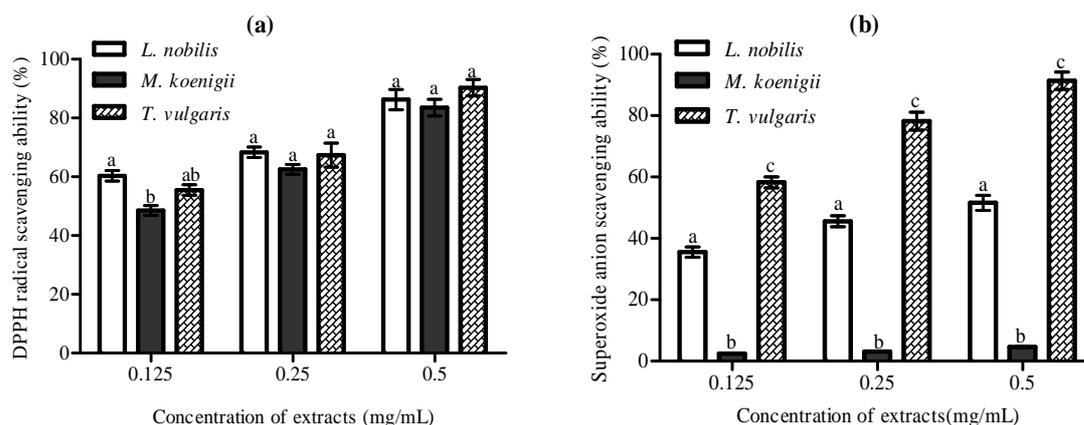


**Figure 1:** Inhibitory capacities of the polyphenolic extracts of *Laurus nobilis*, *Murayya koenigii* and *Thymus vulgaris* on the formation of advanced glycation end products (AGEs). Values are expressed as means  $\pm$  SEM ( $n=3$ ). Means carrying different superscript at the same concentration are significantly different from each other ( $p < 0.05$ )

**Table 1:** IC<sub>50</sub> values of the extracts of *Laurus nobilis*, *Murayya koenigii* and *Thymus vulgaris* in antiglycation and antioxidant assays

Spices	IC <sub>50</sub> (mg/mL)		
	Antiglycation	DPPH Assay	SAS Assay
<i>Laurus nobilis</i>	0.08 $\pm$ 0.00 <sup>a</sup>	0.02 $\pm$ 0.00 <sup>a</sup>	0.46 $\pm$ 0.12 <sup>a</sup>
<i>Murayya koenigii</i>	0.05 $\pm$ 0.01 <sup>a</sup>	0.15 $\pm$ 0.02 <sup>b</sup>	8.13 $\pm$ 0.85 <sup>b</sup>
<i>Thymus vulgaris</i>	0.02 $\pm$ 0.00 <sup>b</sup>	0.10 $\pm$ 0.01 <sup>b</sup>	0.06 $\pm$ 0.01 <sup>c</sup>

Test values down the vertical columns carrying different superscripts are significantly different from each other ( $P < 0.05$ )



**Figure 2:** Inhibitory capacities of the polyphenolic extracts of *Laurus nobilis*, *Murayya koenigii* and *Thymus vulgaris* on the formation of (a) DPPH and (b) superoxide anion radicals. Values are expressed as means  $\pm$  SEM ( $n=3$ ). Means carrying different superscript at the same concentration are significantly different from each other ( $p < 0.05$ ).

Figure 3(a) shows the cytotoxic effect of various extracts on brine shrimp (*Artemia salina*). At all the concentrations tested, the activity of *M.*

*koenigii* extract was the lowest. At 10 and 1000  $\mu$ g/mL, *M. koenigii* extract had a significantly decreased activity ( $p < 0.05$ ) compared to the

other extracts. The activity of *T. vulgaris* extract was significantly higher ( $p < 0.05$ ) when compared to the others at 100  $\mu\text{g/mL}$ . However, *M. koenigii* extract has the highest LD<sub>50</sub> of 2000  $\mu\text{g/mL}$  (Table 2).

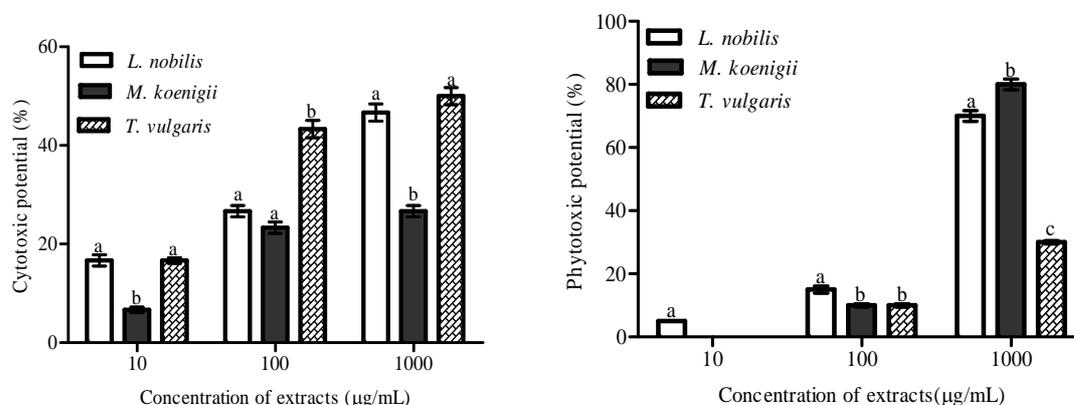
The phytotoxic effect of polyphenolic extracts of *L. nobilis*, *M. koenigii* and *T. vulgaris* on the growth of plant, *Lemna minor*, is presented in Figure 3(b). It shows that at very low concentration (10  $\mu\text{g/mL}$ ), both *M. koenigii* and *T. vulgaris* were not toxic to the plant while *L. nobilis* displayed very low potential. This trend continues at 100  $\mu\text{g/mL}$  where *L. nobilis* also displayed the highest potential which is significantly different ( $p < 0.05$ ) from the other extracts. However at high concentration (1000  $\mu\text{g/mL}$ ) *M. koenigii* displayed the highest toxic potential against the plant while *T. vulgaris* had the lowest both, of which are significantly different ( $p < 0.05$ ) to other spice.

## DISCUSSION

Hyperglycemia is regarded as a key factor in the development of diabetic complications [16]. Chronic exposure of tissues to high levels of blood glucose can lead to adverse intracellular effects, leading to what is known as glucose

toxicity [17]. Several major mechanisms have been proposed for hyperglycemia induced-tissue damages, including increased polyol pathway flux, increased de-novo diacylglycerol synthesis with resultant activation of protein kinase C isoforms, hexoamine pathway and AGEs formation [16-18]. A large body of evidences point to glycation as a key molecular basis of diabetic complications. AGEs contribute to diabetic complications through a series of pathological changes such as increased atherogenicity of low density lipoprotein (LDL), increased basement membrane permeability and decreased insulin binding to its receptors [16]. AGEs also play an important role in diabetic micro- and macroangiopathy where it deposits under endothelial cells [19]

The extracts of all the spices tested displayed good antiglycation ability which increases with the increase in concentration, though thyme extract showed highest potential at all concentrations tested. Glycation and AGE-induced toxicity are known to be associated with increased free radical production [20]. Therefore, agents that possess good antioxidant activity by mopping up free radicals can simultaneously inhibit the formation of advanced glycation end products [21].



**Figure 3:** Effects of polyphenolic extracts of *Laurus nobilis*, *Murayya koenigii* and *Thymus vulgaris* on the growth of (a) *Artemia salina* and (b) *Lemna minor*. Values are expressed as means  $\pm$  SEM ( $n=3$ ). Means carrying different superscript at the same concentration are significantly different from each other ( $p < 0.05$ )

**Table 2:** LD<sub>50</sub> values of polyphenolic extracts of *Laurus nobilis*, *Murayya koenigii* and *Thymus vulgaris* cytotoxicity and phytotoxicity assays

Spices	LD <sub>50</sub> ( $\mu\text{g/mL}$ )	
	Cytotoxicity	Phytotoxicity
<i>Laurus nobilis</i>	1100 $\pm$ 4.92 <sup>a</sup>	700 $\pm$ 5.62 <sup>a</sup>
<i>Murayya koenigii</i>	2000 $\pm$ 11.15 <sup>b</sup>	640 $\pm$ 3.75 <sup>a</sup>
<i>Thymus vulgaris</i>	1000 $\pm$ 7.25 <sup>a</sup>	1640 $\pm$ 9.52 <sup>b</sup>

Test values down the vertical columns carrying different superscripts are significantly different from each other ( $p < 0.05$ )

Free radicals especially reactive oxygen species (ROS) have been implicated in many degenerative diseases such as diabetes and cardiovascular diseases. Overproduction of ROS can directly attack the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation [22]. Antioxidants carry out their protective properties on cells either by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body [23].

*L. nobilis* exhibited the lowest IC<sub>50</sub> for the DPPH radical scavenging, which suggests that aqueous extract possessed the best DPPH radical scavenging abilities. The mopping of DPPH radical by this extract mitigates oxidative stress [24]. *T. vulgaris* displayed the lowest IC<sub>50</sub> for superoxide radical scavenging activity, which also signified that it possessed the best activities and prevents harm caused to the cellular components by superoxide radicals [25]. The antiradical activity of polyphenols is principally based on the redox properties of their hydroxyl groups and the structural relationships between different parts of their chemical structure [26]. The free radical scavenging ability of all these extracts is an indication that these spices promise to be excellent dietary source of antioxidants.

Our result indicated that polyphenolic extract of *M. koenigii* is the least cytotoxic of the three spices. This is attested to by the difference in their LD<sub>50</sub> values (2000, 1100 and 1000 µg/mL for *M. koenigii*, *L. nobilis* and *T. vulgaris* respectively) (Table 2). However, all the extracts can still be considered safe due to their high LD<sub>50</sub> values. Anderson *et al* [27] stated that extracts which showed LD<sub>50</sub> higher than 100 µg/mL in the brine shrimp lethality test can be considered inactive, and so safe for consumption. From the LD<sub>50</sub> values generated from their phytotoxic study, it also showed that all the extracts had high LD<sub>50</sub>, the implication of which is that they are non-toxic to the plant except at very high concentrations. According to Khan *et al* [28], a number of plants, their extracts or their purified active constituents can act as allelochemicals to other plants. These spices are not suitable for such purpose due to their inactivity.

## CONCLUSION

Acetone extract of the leaves of bay, curry and thyme were tested for their antioxidant and antiglycation potential *in-vitro*. Out of the three spices, extract from *T. vulgaris* displayed the highest antioxidant and antiglycation potential. However, all the spices were not cytotoxic and

phytotoxic. It can be concluded from this study that the leaves of *L. nobilis*, *M. koenigii* and *T. vulgaris* could prevent and or ameliorate the complications suffered by diabetics due to their strong antiglycation and antioxidant activities. Therefore, they may be suitable for inclusion in the diets of diabetic patients.

## ACKNOWLEDGEMENT

The authors hereby acknowledge the National Research Foundation (NRF), South Africa for the NRF Freestanding Postdoctoral Fellowship granted Dr. M. I. Kazeem (Grant no. 88342) tenable at the Phytomedicine and Phytopharmacology Research Unit of the Department of Plant Sciences, University of the Free State, Qwaqwa Campus, Phuthaditjhaba, South Africa. Our gratitude equally goes to the UFS-Qwaqwa Research Committee for the funding of the work (211427604). The support of Lagos State University, Lagos, Nigeria is also gracefully acknowledged.

## REFERENCES

1. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001; 414: 782-787.
2. International Diabetes Federation. *Diabetes Atlas, 6th Edition, Brussels, Belgium, 2013.*
3. Shobana S, Naidu KA. Antioxidant activity of selected Indian spices. *Prostaglandins, Leukot Essens Fatty Acids* 2000; 62: 107-110
4. Heinerman J. *The complete book of spices: their medical, nutritional and cooking uses.* CT: Keats publishing Inc., New Canaan, 1983.
5. Sayyah M, Saroukhani G, Peirovi A, Kamalinejad M. Analgesic and anti-inflammatory Activity of the Leaf Essential oil of *Laurus nobilis* Linn. *Phytother Res* 2003; 17: 733-736
6. Nayak S, Nalabothu P, Sandiford S, Bhogadi V, Adogwa A. Evaluation of wound healing activity of *Allamanda cathartica*. L. and *Laurus nobilis*. L. extracts on rats. *BMC Compl Altern Med* 2006; 6: 1-6
7. Prajapati ND, Purohit SS, Sharma AK, Kumar T. A handbook of medicinal plants. Agrobios, Jodhpur, India 2003.
8. Iyer D, Uma DP. Phytopharmacology of *Muraya koenigii* L. *Pharmacog Rev* 2008; 2: 180.
9. Baranauskiene R, Venskutonis PR, Viskelis P, Dambrauskiene E. Influence of nitrogen fertilizers on the yield and composition of thyme (*Thymus vulgaris*). *J Agric Food Chem* 2003; 51: 7751-7758.
10. Kazeem MI, Akanji MA, Hafizur Rahman M, Choudhary MI. Antiglycation, antioxidant and toxicological potential of polyphenol extracts of alligator pepper,

- ginger and nutmeg from Nigeria. *Asian Pac J Trop Biomed* 2012; 2: 727–732
11. Chu Y, Sun J, Wu X, Liu RH. Antioxidant and antiproliferative activity of common vegetables. *J Agric Food Chem* 2002; 50: 6910–6916.
  12. Matsuda H, Wang T, Managi H, Yoshikawa M. Structural requirements of flavonoids for inhibition of protein glycation and radical scavenging activities. *Bioorg Med Chem* 2003; 11: 5317–5323.
  13. Gyamfi MA, Yonamine M, Aniya Y. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. *Gen Pharmacol* 1999; 32: 661- 667.
  14. Ferda C. Effect of *Rhus coriaria* L (Anacardiaceae) on superoxide radical scavenging and xanthine oxidase activity. *J Enzyme Inhib Med Chem* 2003; 18: 59–62.
  15. Mclaughlin JL, Chang CJ, Smith DL. “Bench-Top” bioassays for the discovery of bioactive natural products; an update. In: *Studies in Natural Products Chemistry*, Atta-ur-Rahman (Ed.), Elsevier Science Publishers, Amsterdam, 1991.
  16. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; 414: 813–820
  17. Robertson RP, Harmon JS. Diabetes, glucose toxicity and oxidative stress: A case of double jeopardy for the pancreatic islet beta cell. *Free Radic Biol Med* 2006; 41: 177–184.
  18. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocrinol Rev* 2002; 23: 599–622.
  19. Stitt AW, Frizzell N, Thorpe SR. Advanced glycation and advanced lipoxidation: possible role in initiation and progression of diabetic retinopathy. *Cu Pharma Res* 2004; 10: 3349 – 3360.
  20. Nakagawa T, Yokozawa T, Terasawa K. Protective activity of green tea against free radical and glucose-mediated protein damage. *J Agric Food Chem* 2002; 50: 2418–2422.
  21. Ahmad MS, Ahmed N. Antiglycation properties of aged garlic extract: possible role in prevention of diabetic complications. *J Nutr* 2006; 136: 796–799.
  22. Elmegeed GA, Ahmed HA, Hussein JS. Novel synthesized amino steroidal heterocycles intervention for inhibiting iron-induced oxidative stress. *Euro J Med Chem* 2005; 40: 1283–1294.
  23. Oboh G, Rocha JBT. Distribution and antioxidant activity of polyphenols in ripe and unripe tree pepper (*Capsicum pubescens*). *J Food Biochem* 2007; 31: 456–473.
  24. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V. Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argentea* Desf Ex DC), sage (*Savia triloba* L.), and black tea (*Camellia sinensis*) extracts. *J Agric Food Chem*. 2000; 48, 5030-5034
  25. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of *Spondias pinnata*. *BMC Complement Altern Med* 2008; 8: 1-10
  26. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. *Trends Plant Sci* 1997; 2: 152–155.
  27. Anderson JE, Goetz CM, Mclaughlin JL, Suffness MA. A blind comparison of simple bench-top bioassays and human tumour cell cytotoxicities as antitumor prescreens. *Phytochem Analysis* 1991; 2: 107.
  28. Khan T, Zahid M, Asim M, Shahzad-UI-Hussan IZ, Choudhary MI, Ahmad VU. Pharmacological activities of crude acetone extract and purified constituents of *Salvia moorcraftiana* Wall. *Phytomed* 2002; 9: 749–752.