



The Antioxidant Potential and Antihyperlipidemic Activity of *Myristica fragrans* Seed (Nutmeg) Extract in Diabetic Rats

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ARTICLE INFO

Article type:

Original article

Article history:

Received: 3 March 2020

Revised: 20 April 2020

Accepted: 20 May 2020

DOI: 10.29252/jhehp.6.2.7

Keywords:

Diabetic rats
Myristica fragrans
Extract
Total phenols
Oxidative stress

ABSTRACT

Background: The present study aimed to assess the phenolic content and in-vitro antioxidant capacity of the petroleum ether extract of nutmeg, as well as its antioxidant and antihyperlipidemic activities in alloxan-induced diabetic rats.

Methods: Forty-eight male rats were allocated into six groups of eight each: non-diabetic control, diabetic control, and diabetic rats receiving 50, 100 and 200 mg/kg of the nutmeg petroleum ether extract or 100 mg/kg metformin. The in vitro antioxidant activity of the extract was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Total phenolic and flavonoid contents were estimated using Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively.

Results: The nutmeg extract contained considerable levels of phenols (112.41 mg GAE/100 g) and flavonoids (26.12 mg QE/100 g) and showed a remarkable scavenging effect on the DPPH radicals with the IC₅₀ value of 123.36 µg/ml. The administration of the nutmeg extract (100 and 200 mg/kg) to the diabetic rats caused a significant reduction in the serum levels of malondialdehyde, total cholesterol, triglycerides, and low-density lipoprotein, while a significant increase was observed in the total antioxidant capacity and high-density lipoprotein cholesterol serum levels.

Conclusion: According to the results, nutmeg extract is an abundant source of natural antioxidants, which might have beneficial effects on patients with diabetes mellitus develop educational packages.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and the deficiency of the secretion or action of insulin [1]. Oxidative stress plays a major role in the pathogenesis of diabetes and development of diabetic complications [2]. Hyperglycemia increases the production of reactive oxygen species through several pathways, including glucose autooxidation, the polyol pathway, and non enzymatic protein glycation. Free radicals could adversely affect important biomolecules such as carbohydrates, nucleic acids, and proteins [3].

Increased oxidative stress has been reported in both the humans and animals with diabetes mellitus [2].

Antioxidants play a key role in the elimination of free radicals and protection of the cells against oxidative damage. Furthermore, evidence attests to the beneficial effects of antioxidant supplementation on the prevention and control of diabetes mellitus complications [4].

In general, synthetic drugs are used for the treatment of diabetes and its complications. Despite their beneficial therapeutic effects, these drugs have unwanted side-effects such as hypoglycemia, cell death, and secondary failure [5].



Recently, special attention has been paid to complementary and alternative therapies for diabetes using medicinal plants and their bioactive compounds [6]. Evidence suggests that a high proportion of the patients with diabetes use such therapies concurrent with conventional healthcare services, and the anti-diabetic and hypoglycemic effects of some medicinal herbs have also been confirmed [4].

Myristica fragrans belongs to the Myristicaceae family and is an evergreen aromatic tree. Nutmeg is the kernel of the apricot-like fruit of the tree, and mace is the red lacy covering (aril) on the nutmeg seed. In traditional medicine, *M. fragrans* is used as an anti-bloating agent, stomach stimulator, and anti-diarrhea medicine [7-8]. Nutmeg has compounds such as lignin, flavonoid, saponin, and tannins, while it also has antifungal, hepatoprotective, anti-inflammatory, and antioxidant properties. *M. fragrans* seed (nutmeg) extract has been reported to be effective in the control of hyperglycemia in diabetic rats [9]. Moreover, the plant has been shown to reduce cholesterol and blood lipids in healthy mice [10]. Nevertheless, data are currently scarce regarding the exact role of nutmeg extract in the management of diabetes and its complications.

The present study aimed to investigate the phenolic contents and *in-vitro* antioxidant capacity of nutmeg petroleum ether extract and determine its *in-vivo* antioxidant and lipid-lowering potential in a rat model of diabetes.

2. Materials and Methods

2.1. Preparation of the Extract

In this study, the *M. fragrans* fruits were purchased from a local market in Tehran, Iran and authenticated by an expert taxonomist at the Department of Plant Pathology of the Islamic Azad University in Tehran, where a voucher specimen (Voucher No. IAUH- 1623) was deposited. To prepare the extract, powdered nutmeg was extracted with petroleum ether (temperature: 60-80°C) in a soxhlet extractor (Behrotest ET2, behr Labor-Technik, Dusseldorf, Germany). Following that, the obtained extract was concentrated under reduced pressure using a rotary evaporator apparatus (Rotavapor R-114, Buchi, Flawil, Switzerland). The percentage yield of the extract was 16% (w/w) [11].

2.2. Measurement of the Total Phenols

The total phenolic content of the nutmeg petroleum ether extract was evaluated using the Folin-Ciocalteu colorimetric method [12]. The aliquots of the nutmeg extract with various concentrations were mixed with five milliliters of the Folin-Ciocalteu reagent and four milliliters of 7.5% sodium carbonate. After incubation for 30 minutes at room temperature, the absorbance was read at 765 nanometers using a spectrophotometer (Shimadzu UV-1700, Japan). In addition, gallic acid was used for the calibration of the standard curve, and the total phenolic content was expressed as the milligram of the gallic acid equivalent (GAE) per 100 grams of dry weight.

2.3. Measurement of the Total Flavonoids

Total flavonoids were measured using the aluminum

chloride colorimetric method [13]. Each diluted extract sample or quercetin (2.5 ml) was added to 10% aluminum chloride solution (200 µl), followed by the addition of 200 microliters of a potassium acetate solution (1M). Ultimately, 5.6 milliliters of distilled water was added to each mixture. After 30 minutes at room temperature, the absorbance was read at 415 nanometers using a spectrophotometer (Shimadzu UV-1700, Japan). The total flavonoid content was calculated as the milligram of the quercetin equivalent (QE) per 100 grams of dry weight.

2.4. DPPH Radical Scavenging Assay

The free radical scavenging activity of the petroleum ether extracts of nutmeg was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as proposed by Blois (1958) [14]. To this end, a solution of DPPH (0.1 mM) in methanol was prepared, and 1.0 milliliter of the solution was mixed with 1.0 milliliter of various concentrations of the methanol nutmeg extract in (25-250 µg/ml). After 30 minutes, the absorbance of the mixture was read at 517 nanometers using a spectrophotometer (Shimadzu UV-1700, Japan). In addition, a DPPH methanol solution (0.01 mM) was used as the control, and butylhydroxytoluene (BHT) was used as the reference standard. The rate of the DPPH radical scavenging activity was calculated using the following equation:

$$\% \text{ DPPH Radical Scavenging Activity} = \{(A_0 - A_1)/A_0\} \times 100$$

where A_0 is the absorbance of the control, and A_1 represents the absorbance of the extractives/standard.

2.5. Animals

In total, 48 male Wistar rats (Pasteur Institute, Tehran, Iran) weighing 200-250 grams were used in the present study. All the animals were kept at the constant temperature of 22±2°C and 50-60% relative humidity within a 12-hour light/dark cycle and had access to a standard pellet diet and water *ad libitum* during the treatment period.

The study protocol was approved by the Research Ethics Committee of the Islamic Azad University, Science and Research Branch (code: IR/IAU/SRB/REC/1398/011).

2.5.1. Diabetes Induction

Diabetes was induced in the rats with overnight fasting by the single intraperitoneal injection of alloxan dissolved in normal saline at the dose of 120 mg/kg of the body weight. The animals in the control group received the same amount of saline alone. Diabetes induction was confirmed by the evaluation of the blood glucose level three days after the alloxan injection. The animals were considered diabetic with the increased blood glucose levels to more than 200 mg/dl [15].

2.5.2. Experimental Design and Treatment

The rats were randomly assigned to six groups of eight. In group one, healthy rats were administered with saline solution (non-diabetic control). The animals in group two

were diabetic control animals, which received treatment with alloxan and saline only, while the animals in groups three, four, and five were diabetic rats orally administered with the nutmeg extract at the concentrations of 50, 100, and 200 mg/kg, respectively. In group six, diabetic rats received metformin (100 mg/kg of the body weight). The mentioned doses were selected based on the previous studies as the equivalent consumed doses [9, 10]. All the treatments were administered via oral gavage once daily for three weeks [1].

2.6. Sample Collection and Biochemical Analysis

After the treatment, blood samples were collected from the tail vein of the rats and centrifuged (Centrifuge 5430R, Eppendorf, Hamburg, Germany) at 3,000 rpm for 15 minutes [3]. The obtained serum samples were used for the biochemical measurements.

2.6.1. Measurement of Serum Malondialdehyde (MDA) and Total Antioxidant Capacity (TAC)

The serum concentration of malondialdehyde (MDA) was assayed as a biomarker of lipid peroxidation. In brief, 0.5 milliliter of serum was mixed with 2.5 milliliters of 20% trichloroacetic acid in a 10-milliliter centrifuge tube. One milliliter of 0.67% thiobarbituric acid was added to the mixture, shaken, and heated in a boiling water bath for one hour. After cooling, four milliliters of n-butanol was added and mixed vigorously, and the n-butanol layer was separated by centrifugation at 3,000 rpm for 15 minutes. The serum MDA concentration was measured at 532 nanometers using a spectrophotometer against n-butanol [15] and reported as $\mu\text{mol MDA/l}$ of the serum.

The serum level of the total antioxidant capacity (TAC) was determined using the chromogenic method with a commercially available kit (Cat. No. NX 2332, Randox Laboratories Ltd., Crumlin, United Kingdom). The obtained results were expressed as nanomole of the Trolox equivalents (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) per milliliter of serum.

2.6.2. Lipid Profile Assay

Serum total cholesterol (TC), total triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were evaluated using standard colorimetric kits (Pars Azmoon kit, Pars Azmoon Inc., Tehran, Iran) in accordance with the instructions of the manufacturer. In addition, the Friedewald formula was used to estimate low-density lipoprotein cholesterol (LDL-C) in the serum samples [2].

2.7. Statistical Analysis

Data analysis was performed in SPSS version 18.0, and the obtained values were expressed as means and standard error of the mean (SEM) and mean and standard deviation (SD). One-way analysis of variance (ANOVA), Tukey's test, and LSD multiple comparisons were also used to analyze the datasets. In addition, Pearson's correlation-coefficient was applied to determine the correlation between the phenolic components and antioxidant activity. The IC_{50} values were compared using student's t-test. In all the statistical analyses, $P < 0.05$ was considered significant.

3. Results and Discussion

Medicinal plants have long been reported as a prospective source of natural antioxidant compounds, particularly the secondary metabolites of plants, such as phenolic compounds and flavonoids, which act as free radical scavengers and may be an alternative to the treatment of various diseases, such as diabetes mellitus [16, 17].

3.1. Total Phenolic and Flavonoid Contents

In this assay, the total phenolic and flavonoid contents of the petroleum ether extract of nutmeg were estimated at 112.41 ± 1.37 mg GAE/100 g dry weight and 26.12 ± 0.47 mg QE/100 g dry weight, respectively (Table 1). In this regard, Gupta *et al.* (2013) reported lower total phenolic compounds in the ethanol extract of nutmeg [18]. On the other hand, Tan *et al.* (2011) claimed that the polyphenol and flavonoid contents of *M. fragrans* fruit were within the ranges of 25.26–188.0 mg GAE/g dry weight and 117.24–1345.75 mg Rutin equivalent/g dry weight, respectively [8]. In another study, the total polyphenol content of the methanol extract of *M. fragrans* seed was determined to be 2.434 mg GAE/g dry weight, while it was 4.630 mg GAE/g dry weight in the mace extract [7]. The differences in the outcomes may be attributed to the genetic backgrounds, environmental factors, types of the extraction solvent, and methods of extraction [19].

3.2. Antioxidant Capacity Based on DPPH Test

The in-vitro antioxidant potential of the nutmeg extract was assessed using the DPPH photometric assay, in which the reduction of DPPH free radicals was estimated. Figure 1 illustrates the free radical scavenging activity of the nutmeg extract and standard BHT. The herbal extract displayed a significant dose-dependent inhibition of the DPPH activity ($r^2 = 0.913$) with the IC_{50} value of 123.36 ± 0.76 $\mu\text{g/ml}$ (Table 1), while the effect was less significant compared to BHT ($\text{IC}_{50} = 18.51 \pm 0.37$ $\mu\text{g/ml}$). In a study in this regard, Kazeem *et al.* (2012) reported the DPPH radical scavenging activity of the polyphenol extract of *M. fragrans* seeds with the IC_{50} value of 100 $\mu\text{g/ml}$ [20], while Vangoori *et al.* (2019) reported the IC_{50} value of 52.62 $\mu\text{g/ml}$ in the ethanol extract of the mace [21]. The present study indicated a significant, positive correlation between the total phenolic content of the nutmeg extract and its DPPH radical scavenging activity ($r = 0.924$; $P < 0.05$), indicating the effectiveness of these compounds. Furthermore, Burri *et al.* (2017) have reported similar results by revealing a positive correlation between total phenols and antioxidant activity [19].

Table 1: Total phenol and total flavonoid contents and DPPH-radical scavenging activity of petroleum ether extracts of nutmeg

	Total phenols (mg/100g DW, GAE)	Total flavonoid (mg/100g DW, QE)	DPPH (IC_{50} , $\mu\text{g/ml}$)
Nutmeg extract	112.41 ± 1.37	26.12 ± 0.47	123.36 ± 0.76^a
BHT	-	-	18.51 ± 0.37^b

Values are represented as mean \pm SD ($n = 3$). Values followed by different superscript letters within the same columns are significantly different according to the Student's t-test ($P < 0.05$). GAE Gallic Acid Equivalent, QE Quercetin Equivalent

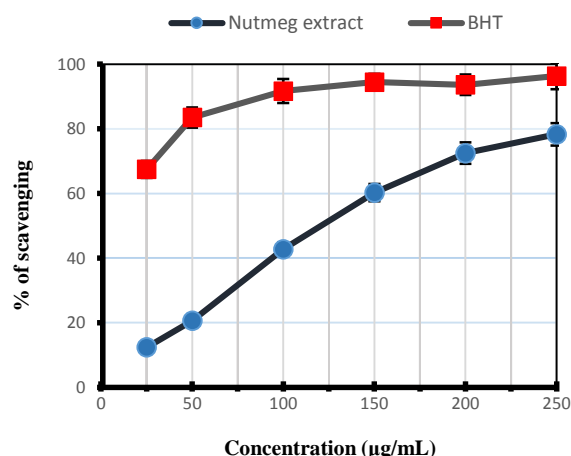


Figure 1: DPPH radical scavenging activity of nutmeg extract and Butylated hydroxytoluene (BHT). Data are presented as the means \pm SEM of triplicates

The radical scavenging activity of the nutmeg extract in the current research could be allotted to the presence of polyphenols and flavonoids. In plants, phenolic compounds are mainly produced by the secondary metabolism, and the antioxidant properties of phenolic compounds largely depends on their redox properties, which enable their action as reductive agents, hydrogen donors, and singlet oxygen quenchers [22]. Some of the natural antioxidants belonging to the flavonoid compounds (flavonols, flavones, isoflavones, and pterocarpanes) have been isolated from the *M. fragrans* seed [7]. In a research, Tan *et al.* (2013) reported epicatechin as the major flavonoid compound present in nutmeg seeds [8]. In addition, Shan *et al.* (2005) stated that the high antioxidant activity in nutmeg seed was contributed by caffeic acid and catechin [23]. Our findings suggested that nutmeg extract could be considered as a potential bioresource of antioxidants and should be investigated as a therapeutic agent in the treatment of free radical-induced diseases.

3.3. Effects of the Extract on the Serum MDA and TAC

Figure 2 shows the serum MDA and TAC levels in the experimental groups. In the diabetic control group, the serum MDA concentrations elevated significantly (Figure 2-A), while the serum TAC levels decreased (Figure 2-B) compared to the non-diabetic group. Evidence attests to the key role of oxidative stress in the development of diabetes mellitus and the associated complications [24]. The increased levels of MDA and diminished total antioxidant capacity observed in the present study may indicate the occurrence of oxidative stress in the rats administered with alloxan.

According to the current research, treatment with the nutmeg extract at the concentrations of 100 and 200 mg/kg significantly decreased the serum MDA levels (Figure 2-A) and increased the serum TAC levels (Figure 2-B) in the diabetic rats. The improvement of the serum oxidative status in the rats administered with 200 mg/kg of the extract was considered more significant compared to the metformin group. The antioxidant potential of the nutmeg extract in the diabetic groups could be attributed to the natural phenolic substances of this plant as previously described [20-25].

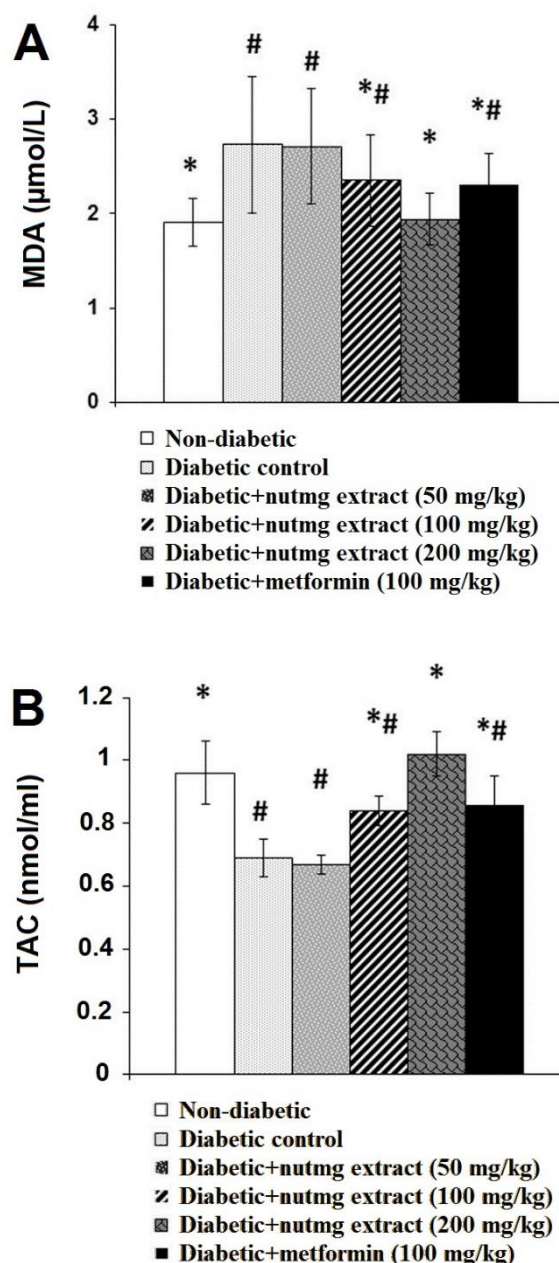


Figure 2: Effects of nutmeg extract supplementation for 21 days on (A) MDA and (B) TAC levels in the serum of experimental groups. * $P < 0.05$ as compared with the non-diabetic group; # $P < 0.05$ as compared with the diabetic control group

In this regard, compounds such as eugenol and β -caryophyllene could be considered effective contributors to the antioxidant activity of the extract owing to the relatively easy abstraction of the atomic hydrogen from their benzylic and/or allylic positions by peroxy radicals, which are formed under oxidative stress conditions [7]. Furthermore, it has been reported that eugenol stimulates the activity of some antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase [18]. The findings of the current research suggested that the nutmeg extract could exert protective effects against diabetes-induced oxidative stress through antioxidant activity.

3.4. Effect of the Extract on the Serum Lipid Profile

Table 2 shows the changes in the serum lipid parameters of the study groups. Accordingly, a significant increase was observed in the serum TC, TG, and LDL-C levels, while HDL-C reduced in the diabetic control animals compared to the non-diabetic group. These findings are consistent with the results of the previous studies in this regard [26, 27]. The prominent role of lipids in the development of diabetes has been confirmed, and a strong interaction has also been reported between carbohydrates and lipid metabolism. As such, the impairment of carbohydrate metabolism leads to disrupted lipid metabolism [28].

According to the information in Table 2, the administration of the nutmeg extract significantly decreased the serum levels of TC (concentrations of 100 and 200 mg/kg), TG (concentration of 200 mg/kg), and LDL-C (concentrations of 100 and 200 mg/kg), while increasing the serum HDL-C levels (concentrations of 100 and 200 mg/kg) compared to the diabetic control group. The improvement of dyslipidemia observed in the treatment group

administered with the extract concentration of 200 mg/kg was comparable with the metformin group, with the exception of the TC and TG parameters. These findings are in line with the previous studies that have confirmed the hypolipidemic and hypocholesterolemic effects of nutmeg on non-diabetic mice [10].

The lipid-lowering effects of polyphenols (especially flavonoids) have been reported in several studies in this regard [27,28], which could be attributed to their antioxidant properties that protect the pancreatic islets against diabetes-induced oxidative damage, thereby improving insulin secretion [24]. According to the literature, insulin regulates hepatic apolipoprotein synthesis, lipoprotein lipase activity, and cholesteryl ester transfer protein gene expression, which are the underlying causes of dyslipidemia in diabetes [29]. The beneficial impact of the extract on dyslipidemia may also be mediated by the inhibitory effects of the polyphenol and flavonoid contents of nutmeg on HMG CoA reductase, which could reduce the cholesterol levels or through the stimulating effects of glucose utilization in the peripheral tissues [30].

Table 2: Lipid profile of alloxan induced diabetic rats treated with different levels of nutmeg extract

Groups	Serum levels			
	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Non-diabetic	88.28 ± 0.91 ^e	72.33 ± 1.50 ^c	40.13 ± 0.54 ^a	33.56 ± 0.25 ^d
Diabetic control	144.42 ± 1.58 ^a	111.37 ± 1.67 ^a	23.42 ± 1.06 ^d	98.47 ± 1.24 ^a
Treated groups	nutmeg 50 mg/kg	136.07 ± 2.12 ^a	105.12 ± 2.04 ^a	25.18 ± 1.32 ^d
	nutmeg 100 mg/kg	119.47 ± 0.47 ^b	101.80 ± 1.34 ^{ab}	30.38 ± 0.23 ^c
	nutmeg 200 mg/kg	103.69 ± 0.57 ^c	91.64 ± 1.32 ^b	38.27 ± 0.67 ^{ab}
	metformin	95.17 ± 2.06 ^d	75.19 ± 1.71 ^c	36.62 ± 0.72 ^b

All values are expressed as mean ± SEM.; n = 8

Values followed by different superscript letters within the same columns are significantly different according to the Tukey's test ($P < 0.05$). TC; total cholesterol, TG; triglycerides, HDL-C; high density lipoprotein cholesterol and LDL-C; low density lipoprotein cholesterol

4. Conclusion

According to the results, the petroleum ether extract of the *M. fragrans* seeds (nutmeg) could be a potent source of natural antioxidants owing to its considerable phenolic and flavonoid contents and remarkable scavenging effects on DPPH. Our findings also indicated a strong correlation between the total phenolic content and antioxidant activity, while also suggesting considerable values in terms of the in-vivo antioxidant and antidiabetic activities of the nutmeg extract. The treatment of the alloxan-induced diabetic rats with the extract at the doses of 100 and 200 mg/kg could enhance the oxidative stress and lipid profile parameters. In conclusion, nutmeg could be used as an achievable source of natural antioxidants with beneficial properties in the treatment of diabetes and the associated complications.

Authors' Contributions

A.P., S.M., and P.M., designed the study; A.P., performed the experiments; S.M., and P.M., performed data acquisition; A.P., drafted the manuscript; S.M., and P.M., supervised data analysis and edited the manuscript.

Conflict of Interest

The Authors declare that there is no conflict of interest.

Acknowledgments

Hereby, we extend our gratitude to the Science and Research Branch of the Islamic Azad University for assisting us in this research project (Project No. IR/IAU/SRB/2539).

References

- Baiga VP, Kumar K, Yadav S, Kumar P, Shukla D, Mani M. Evaluation of Antidiabetic Activity of Leucomeris Spectabilis Extract in Alloxan-Induced Diabetic Rats. *J Drug Deliv Ther*. 2018; 8(5): 273-9.
- Ramachandran S, Rajasekaran A, Manisenthilkumar KT. Investigation of Hypoglycemic, Hypolipidemic and Antioxidant Activities of Aqueous Extract of Terminalia paniculata Bark in Diabetic Rats. *Asian Pac J Trop Biomed*. 2012; 2(4): 262-8.
- Haidari F, Omidian K, Rafiei H, Zarei M, Mohamad Shahi M. Green Tea (Camellia Sinensis) Supplementation to Diabetic Rats Improves Serum and Hepatic Oxidative Stress Markers. *Iran J Pharm Res*. 2013; 12(1): 109-14.
- Soni LK, Dobhal MP, Arya D, Bhagour K, Parasher P, Gupta RS. In Vitro and in Vivo Antidiabetic Activity of Isolated Fraction of Prosopis Cineraria Against Streptozotocin-Induced Experimental Diabetes: A Mechanistic Study. *Biomed Pharmacother*. 2018; 108: 1015-21.
- Suji G, Sivakami S. Approaches to the Treatment of Diabetes Mellitus: An Overview. *Cell Mol Biol*. 2003; 49: 635-9.
- Tahrani AA, Piya MK, Kennedy A, Barnett AH. Glycaemic Control In Type 2 Diabetes: Targets and New Therapies. *Pharmacol Ther*. 2010; 125: 328-61.

7. Assa JR, Widjanarko SB, Kusnadi J, Berhimpon S. Antioxidant Potential of Flesh, Seed and Mace of Nutmeg (*Myristica Fragrans* Houtt). *Int J Chem Tech Res.* 2014; 6(4): 2460-8.
8. Tan KP, Khoo HE, Azrina A. Comparison of Antioxidant Components and Antioxidant Capacity in Different Parts of Nutmeg (*Myristica fragrans*). *Int Food Res J.* 2013; 20(3): 1049-52.
9. Somani RS, Singhai AK. Hypoglycaemic and Antidiabetic Activities of Seeds of *Myristica Fragrans* in Normoglycaemic and Alloxan-Induced Diabetic Rats. *Asian J Exp Sci.* 2008; 22(1): 95-102.
10. Arulmozhi DK, Kurian R, Veeranjanyulu A, Bodhankar SL. Antidiabetic and Antihyperlipidemic Effects of *Myristica fragrans* in Animal Models. *Pharm Biol.* 2007; 45(1): 64-8.
11. Hossain MM, Mondal M, Morad RU, Uddin N, Das A, Hossain MS, et al. Evaluation of Bioactivities of Methanol and Petroleum Ether Extracts of Cassia Renigera Seed. *Clin Phytoscience.* 2018; 4(33): 2-10.
12. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *J Agric Food Chem.* 1998; 46: 4113-17.
13. Wang SY, Jiao H. Correlation of Antioxidant Capacities to Oxygen Radical Scavenging Enzyme Activities in Blackberry. *J Agric Food Chem.* 2000; 48: 5672-6.
14. Blois MS. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature.* 1958; 181: 1199-200.
15. Tagang AT, Ayo JO, Kpukple A, Oladipo OO. Amelioration of Hyperglycaemia, Oxidative Stress and Dyslipidaemia in Alloxan-Induced Diabetic Wistar Rats Treated with Probiotic and Vitamin C. *Nutrients.* 2016; 8(5): 151.
16. Tungmunthum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines.* 2018; 5(3): 93.
17. Phuyal N, Jha PK, Raturi PP, Rajbhandary S. Total Phenolic, Flavonoid Contents, and Antioxidant Activities of Fruit, Seed, and Bark Extracts of *Zanthoxylum Armatum* DC. *Sci World J.* 2020; 2020: 7. Available from: URL: <https://pubmed.ncbi.nlm.nih.gov/32256249/>
18. Gupta AD, Bansal VK, Babu V, Maithil N. Chemistry, Antioxidant and Antimicrobial Potential of Nutmeg (*Myristica fragrans* Houtt). *J Genet Eng Biotechnol.* 2013; 11: 25-31.
19. Burri SCM, Ekholm A, Hakansson A, Tornberg E, Rumpunen K. Antioxidant Capacity and Major Phenol Compounds of Horticultural Plant Materials Not Usually Used. *J Funct Foods.* 2017; 38: 119-27.
20. 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(9): 727-32.
21. Vangoori Y, Dakshinamoorthi A, Kvamani S. Prominent Pancreatic Lipase Inhibition and Free Radical Scavenging Activity of a *Myristica Fragrans* Ethanolic Extract in Vitro. Potential Role in Obesity Treatment. *J Clin Med.* 2019; 14(3): 254-9.
22. Hosseini M, Jamshidi A, Raeisi M, Azizzadeh M. The Antibacterial and Antioxidant Effects of Clove (*Syzygium Aromaticum*) and Lemon Verbena (*Aloysia Citriodora*) Essential Oils. *J Hum Environ Health Promot.* 2019; 5(2): 86-93.
23. Shan B, Cai YZ, Sun M, Corke H. Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. *J Agric Food Chem.* 2005; 53: 7749-59.
24. Tarique A, Sharma M, Pillai KK, Haque SE, Alam MM, Zaman MS. Protective Effect of Bezfi Brate on Streptozotocin-Induced Oxidative Stress and Toxicity in Rats. *Toxicology.* 2007; 229(1-2): 165-72.
25. Chatterjee S, Niaz Z, Gautam S, Adhikari S. Antioxidant Activity of Some Phenolic Constituents from Green Pepper (*Piper Nigrum* L.) and Fresh Nutmeg Mace (*Myristica fragrans*). *Food Chem.* 2007; 101: 515-23.
26. Belhekar SN, Chaudhari PD, Saryawanshi JS, Mali KK, Pandhare RB. Antidiabetic and Antihyperlipidemic Effects of *Thespesia Populnea* Fruit Pulp Extracts on Alloxan-Induced Diabetic Rats. *Indian J Pharm Sci.* 2013; 75(2): 217-21.
27. Koshy AS, Anila L, Vijayalakshmi NR. Flavonoids from *Garcinia Cambogia* Lower Lipid Levels in Hypercholesterolemic Rats. *Food Chem.* 2001; 7(3): 289-94.
28. Dhulasavant V, Shinde S, Pawar M, Naikwade NS. Antihyperlipidemic Activity of *Cinnamomum Tamala* Nees. on High Cholesterol Diet Induced Hyperlipidemia. *Int J Pharm Tech Res.* 2010; 2(4): 2517-21.
29. Yang MY, Peng CH, Chan KC, Yang YS, Huang CN, Wang CJ. The Hypolipidemic Effect of *Hibiscus Sabdariffa* Polyphenols Via Inhibiting Lipogenesis and Promoting Hepatic Lipid Clearance. *J Agric Food Chem.* 2010; 58: 850-9.
30. Bhaskar A, Kumar A. Antihyperglycemic, Antioxidant and Hypolipidemic Effect of *Punicagranatum* L Flower Extract in Streptozotocin Induced Diabetic Rats. *Asian Pac J trop Biomed.* 2012; 1764-9.