INVITRO ANTIDIABETIC ACTIVITY AND COMPARITIVE STUDY OF ANTOXIDANT POTENTIAL OF *XIMENIA AMERICANA* AND *LINDERA COMMUNIS* EXTRACTS

K S Kulasekhar[1*], Rajasekaran.S[2]

[1] Department of Pharmacology, Research Scholar, Institute of Pharmaceutical sciences and Research centre, Bhagwant University, Ajmer, Rajasthan, India

[2] Department of Pharmacology, Professor cum HOD, Al-Azhar college of Pharmacy, Thodupuzha, Idukki, Kerala

Email: [1]kskulasekharr@gmail.com

Abstract

Oxidative stress also plays a role in beta cell death in type 2 diabetes due to insulin resistance hyperglycemia, In addition to causing difficulties in both type 1 and type 2 diabetics. In the pro-oxidant/antioxidant balance, glucose management is crucial. Some diabetes medications may, on their own, possess antioxidant characteristics unrelated to how they affect glucose regulation. The purpose of this study is to compare the methanolic extracts of Ximenia americana and Lindera communis for their protective antioxidant activity, total phenol content and antihyperglycemic properties. We came to the conclusion that Ximenia Americana and Lindera communis had potential therapeutic effects and were crucial in extending the various pharmacological actions. Both extracts have a high antioxidant capacity, according to research on their phenolic compounds' antioxidant activity. These studies demonstrated that Ximenia and Lindera both had antioxidant properties, although Lindera is more effective than Ximenia. According to the results of this investigation, Ximenia and Lindera can both be utilized to treat oxidative stress.

Keywords: *Ximenia americana*, *Lindera communis*, investigation, Antoxidant activity

1. Introduction

The normal cellular metabolism produces reactive oxygen species (ROS) and reactive nitrogen species (RNS). They play a variety of physiological activities at low to moderate doses, from cellular signal transduction to pathogen defense.[1] Under conditions of oxidative stress, there is an excess of ROS and RNS and a deficiency in the enzymatic and non-enzymatic antioxidant defense system, which leads to the breakdown of cellular components such as DNA, carbohydrates, proteins, and lipids. Currently, oxidative stress is proposed as the mechanism behind diabetes and diabetic complications [2], a disease with terrible morbidity and death that has gradually increased around the world. As a result, insulin-independent tissues take up more glucose, which increases oxidant production and weakens antioxidant defences by multiple interconnected nonenzymatic, enzymatic, and mitochondrial pathways like catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD).[4,5] Since beta-islet (insulin-releasing cell) is among the tissues with the lowest levels of intrinsic antioxidant defences and is linked to the emergence

of complications in diabetes (kidney, eye, blood vessel, and nerve damage), the level of these antioxidant enzymes has a significant impact on the susceptibility of various tissues.[6,7] Antidiabetic and antioxidant therapy can help avoid diseases associated with oxidative stress brought on by hyperglycemia and the production of free radicals from medications.[8] Butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), rutin, tertiary butylated hydroquinon, and gallic acid (GA) esters are less effective and may pose a health risk despite advances in the management of diabetes by synthetic drugs (insulin) and oxidative stress.[9] So, as a common traditional medical treatment, current research is focused on enhanced, safe, and natural antidiabetic and antioxidative plant products.[10] Herbs typically have minimal to no toxicity when taken orally over an extended period of time and are generally accessible on a large scale, especially for cultures that use them traditionally. This encourages the adoption of species with chemopreventive properties because they are more affordable and have less adverse side effects. [11,12] Ximenia americana belongs to Olacaceae family. The most common disorders treated with Ximenia americana are inflammatory. [13,14] and infectious conditions. It is a medicinal bushy, prickly shrub or small tree. Numerous illnesses, including measles, malaria, skin infections, STDs, diarrhoea, muscle cramps, and lung abscesses, have reportedly been treated using Ximenia Americana.[14,15] The twigs and leaves are also used as a mouthwash to prevent toothaches and throat infections, as laxatives, as an eye ointment, and as a cure for colds and fevers. The core genus Lindera. [15], which belongs to the Lauraceae family and has more than 100 species, is a member of the Litseeae tribe. In particular, the tropical, subtropical, and temperate regions of Asia and North America are home to many plants of the Lindera genus. Because of its exquisite scent, plants from the genus Lindera are regarded as a rich source of essential oils[9] and are frequently utilized in the manufacture of aromatic cosmetic items like soap and lubricants. Most notably, numerous Lindera plants have historically been employed in traditional medicine[10-13] for their capacity to treat a variety of health-related issues, including pain, colds, urinary tract problems, rheumatoid arthritis, gastric ulcers, abdominal pain, cholera, and beriberi.In this regard, anti-oxidant effects of two medicinal plants with proven hypoglycemic effects, Ximenia and Lindera, were investigated in order to assess their oxidative stress. [19-26] inhibitory effect.

2. Materials And Methods

Extraction:

Preparation of methanolic extract of Lindera communis

Dried powdered material was refluxed with 500 ml of 95% methanol for 3 h. The extracts were then filtered through filter paper. The filtrates (methanol extract) were concentrated at 50ºC under reduce pressure using rotary evaporator The extract transferred to a closed container for further use and protection.

Preparation of Methanolic extract of X. americana

The leaves of X. americana were dried in an oven for 2 days at 40°C, crushed in an electrical grinder and then powdered. Extraction was performed by taking 25-g powder in 250 ml of distilled water for 18 h in a soxhlet apparatus. The extract was dried at reduced pressure and transferred to a closed container for further use and protection.

Experimental animals: Adult Wistar rats (180±10g) of either sex were obtained from animal house. The animals were housed in large, spacious polyacrylic cages at an ambient room temperature with 12h light/12h dark cycle. Rats had free access to water and rodent pellets diet. The study was approved by the Institute Animal Ethics Committee and all the animal experiments were carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

In-vitro Antidiabetic Activity-Diabetes mellitus is an endocrine disorder characterized by hyperglycemia is associated with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Plants have long been used for the treatment of diabetes, particularly in Mammalian. α-amylase is a prominent enzyme in the pancreatic juice which breaks down large and insoluble starch molecules into absorbable molecules ultimately maltose.

α-Amylase Inhibition Activity: Alpha amylase enzyme is responsible for the metabolism of polysaccharides such as starch, carbohydrate etc. The aim behind present experiment is to study the effect of α-amylase concentration on the rate of reaction and inhibition activity of ethanolic extracts of Lindera communis and Ximenia americana leaves. The present study reveals that the inhibition of α-amylase by ethanolic *Extract* **(***Lindera communis and Ximenia americana*). Metformin is α-amylase inhibitory agent as the concentration increases the time of reaction is also increases because the number of enzyme required for digestion of starch is not in sufficient, showed significant α-amylase inhibition activity which is shown by increase in reaction time i.e. the time taken by α-amylase to digest the starch. From the results, *Extract* showed good inhibition when compared to metformin.

Antioxidants analysis

Preparation of tissue homogenate- After sacrificing the rats by cervical dislocation, the liver tissue was excised immediately and washed with chilled isotonic saline. The liver tissue homogenate was prepared in 0.05 M phosphate buffer, pH 7.4 and homogenated in tissue homogenizer at 2,000 rpm for 10 min and used for analyzing antioxidant activities.

Determination of catalase activity: The catalase activity was determined by mixing 1mL of liver homogenate with 5ml of phosphate buffer, 4 mL of 0.2 M H_2O_2 in phosphate buffer and time was recorded. Exactly, After 3 minutes of H_2O_2 addition, a set of 1mL of the aforesaid reaction mixture was taken in 2mL dichromate acetic acid and then placed in a boiling water bath for about 10 minutes. The test tube containing reaction mixture was cooled under running water and reading was noted at 570nm against reagent blank using UV- visible spectrophotometer (Systronics 2201, India). Catalase activity in the liver tissue homogenate was expressed in micromoles H_2O_2 consumed/mg protein/minute.

Determination of glutathione peroxidase: The amounts of glutathione peroxidase in the liver homogenate were determined using standard protocol. The 0.2 ml of liver homogenate was added to the test tube containing 0.2 mL of EDTA, sodium azide, reduced glutathione, hydrogen peroxide were added, mixed thoroughly, and incubated at 37 ºC for 10 minutes. The reaction was stopped by adding 0.5 mL of TCA and centrifuged. 67 Supernatant about 0.5 mL was pipetted into test tube containing 4mL of disodium phosphate and 0.5mL of 5, 5-Dithiobis 2-nitrobenzoic acid (DTNB). The produced color was instantly read at 420nm using

UV- visible spectrophotometer (Systronics 2201, India), and the standard was handled in the same way. The glutathione peroxidase level was expressed as micromoles of glutathione utilized /milligram protein/minutes at 370C.

Determination of lipid peroxidation: The lipid peroxidation in the liver homogenate was determined by measuring the MDA using thiobarbituric acid test. Liver homogenate (0.1 mL) was taken in a test tube containing 0.2 mL of 0.1% SDS, 1.5 mL of 20% acetic acid and 1.5 mL of 0.8 N aqueous solution of TBA. The volume of reaction mixture was made up to 4 ml by adding distilled water and heated at 950C for 60 minutes. The reaction mixture was allowed to cool and 1mL of distilled water, 5 mL of mixture of n-butanol and pyridine (15:1v/v) were added and shaken well. Finally it was centrifuged at 4000 rpm for 10min and read at 532 nm; absorbance was determined using UV- visible spectrophotometer (Systronics 2201, India). In the same way, the standard malondialdehyde was treated. The degree of lipid peroxidation was measured as µmoles of MDA formed /g of wet tissue.

Estimation of lipid peroxidation- Rat tissue homogenate was used as the source of polyunsaturated fatty acids for determining extends of lipid peroxidation. Tissue was collected immediately after the sacrifice of the animals by cervical dislocation under mild ether anesthesia. The liver was homogenized with 40 mM Tris-Hcl buffer (pH 7.0) and centrifuged at 3000 rpm for 10 min to get a clear supernatant. HAEGG solution of different concentration (25 1000 g/ml) and 100 l of each of 1.5 M KCl, 15 mM FeSo4 and 6 mM ascorbic acid was incubated at 37°C for 1 hour. 1ml of 10% TCA was added to the reaction mixer and centrifuged at 3000 rpm for 20 min at 4°C to remove the insoluble proteins. In this method, malondialdehyde (MDA) and other thiobarbituric acid reactive substances (TBARS) were measured by their reactivity with thiobarbituric acid in the acidic condition to generate a pink coloured chromophore.

Assay of superoxide dismutase- Superoxide radicals react with nitroblue tetrazolium in the presence of NADH and produce formazan blue. SOD removes the superoixde radicals and inhibits the formation of formazan blue. The intensity of colour is inversely proportional to the activity of the enzyme.

Statistical Analysis - The data obtained from animal experiments are expressed as mean SEM (standard error of mean). For statistical analysis data were subjected to analysis of variance (ANOVA) followed by Students t-test. Values are considered statistically significant at $F < 0.05$ for ANOVA and $P < 0.05$ for t-test.

3. Results

Table 1: *α*-amylase inhibition activity of extract (Ximenia and Lindera)

S.No	***α*-amylase solution**	**Buffer solution pH 6.8**	**Time until starch disappearance (min.)**
1	0.5 ml of starch + 0.5% α- amylase solution	25 drops	10
2	0.5 ml of starch + 1% α-amylase solution	25 drops	13
3	0.5 ml of starch + 2% α-amylase solution	25 drops	15
4	0.5 ml of starch +0.5% metformin solution	25 drops	16
5	0.5 ml of starch + 1% metformin solution	25 drops	20
6	0.5 ml of starch + 2% metformin solution	25 drops	26
7	0.5 ml of starch + 0.5% ethanolic extract *Extract* solution	25 drops	11
8	0.5 ml of starch + 1% ethanolic extract *Extract* solution	25 drops	14
9	0.5 ml of starch + 2% ethanolic extract *Extract* solution	25 drops	17

(Each value is expressed as Mean ± S.D of three values)

Table 2: Effect of Lindera Extract on antioxidant levels in diabetic rats

Treatment group	**GSH (µ/mg/ protein)**	**TBARS (µ/mg/ protein)**	**LPO (µg/mg/ protein)**	**SOD (µ/mg/ protein)**	**Catalase (µ/mg protein)**
Normal Control	12.52±2.15	2.51±2.12	0.51±1.26	15.8± 1.25	165.2±2.34
Diabetic control	5.22±2.32	7.95±2.25	0.93±1.32	8.22± 1.23	114.5±2.16
60 mg/kg STZ + 500 mg/kg of *Extract*	10.41±2.24	3.52±2.14	0.58±1.17	13.12±1.35	155.6±2.39
60 mg/kg STZ	9.85±2.36	4.42±2.38	0.62±1.15	12.16±1.18	150.5±2.26
60 mg/kg STZ + 50 mg/kg metformin	11.94±2.18	3.01±2.27	0.57±1.28	13.91±1.25	158.2±2.19

(Each value is expressed as Mean ± S.D of five values)

Table 3: Effect of Ximenia Extract on antioxidant levels in diabetic rats

Treatment group	**GSH (µ/mg/ protein)**	**TBARS (µ/mg/ protein)**	**LPO (µg/mg/ protein)**	**SOD (µ/mg/ protein)**	**Catalase (µ/mg protein)**
Normal Control	8.52±2.23	0.51±2.06	0.51±1.26	13.8± 1.11	145.2±2.22
Diabetic control	3.22±2.03	6.92±2.13	0.93±1.32	6.03± 1.51	102.5±2.04
60 mg/kg STZ + 500 mg/kg of *Extract*	8.41±2.36	1.52±2.	0.58±1.03	11.12±1.22	131.6±2.26
60 mg/kg STZ	6.85±2.25	4.42±2.38	0.44±1.02	8.16±1.12	126.5±2.13
60 mg/kg STZ + 50 mg/kg metformin	8.94±2.03	3.01±2.27	0.42±1.14	11.91±1.09	114.2±2.05

(Each value is expressed as Mean ± S.D of five values)

4. Conclusion

It was found that the methanolic extract of Lindera communis and Ximenia americana had significant potential anti-inflammatory and anti-diabetic activities. This scientific data can act as a crucial foundation for the ongoing development of natural medicines that are both safe and efficient. The development of powerful medications derived from plants that can replace clinically hazardous ones might be assisted by the elucidation of the molecular mechanisms at play and the isolation of the bioactive molecules implicated. Therefore, additional research must be done to fully identify and characterise the chemicals that give Lindera communis and Ximenia americana their antioxidant and antidiabetic properties. However, Lindera communis extract could be proposed as a more recommendable herbal supplement for diabetic patients because of better anti- oxidant capacity.

References

[1] Luo JZ, Luo L. American ginseng stimulates insulin production and prevents apoptosis through regulation of uncoupling protein-2 in cultured b Cells. Evid Based Complement Alternat Med 2006; 3:365-372.

[2] Valko M, Leibfritz D, Moncol J, Cronin MT. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007, 39:44-84.

[3] Halliwell B, John MC Gutteridge. Free Radicals in Biology and Medicine. 4th ed. Oxford University press; London,UK; 1989.

[4] King GL, MR Loeken. Hyperglycemia-induced oxidative stress in diabetic complication. Histochem Cell Biol 2004; 122:333-8.

[5] Mehta JL, Rasouli N, Sinha AK, Molavi B. Oxidative stress in diabetes: A mechanistic overview of its effects on atherogenesis and myocardial dysfunction. Int J Biochem Cell Biol 2006; 38:794-803.

[6] Grodsky GM, Anderson CE, Coleman DL, Craighead JE, Gerritsen GC, Hansen CT, et al Metabolism and underlying causes of diabetes mellitus. Diabetes 1982; 31(Suppl 1 Pt 2): 45-53.

[7] Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. J Biol Chem 2004; 279:42351-4.

[8] Balasubramanian, K., & Ragunathan, R. (2012). Study of antioxidant and anticancer activity of natural sources. Journal of Natural Product and Plant Resources, 2(1), 192-197.

[9] Fusco, D., Colloca, G., Monaco, M. R. L., & Cesari, M. (2007). Effects of antioxidant supplementation on the aging process. Clinical Interventions in Aging, 2(3), 377–387

[10] Steenkamp, V., Mathivha, E., Gouws, M. C., & Van Rensburg, C. E. J. (2004). Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. Journal of Ethnopharmacology, 95(2), 353-357.

[11] Martin, A. (1996). The use of antioxidants in healing. Dermatologic Surgery, 22(2), 156-160.

[12] Di Mascio, P., Murphy, M. E., & Sies, H. (1991). Antioxidant defense systems: The role of carotenoids, tocopherols, and thiols. The American Journal of Clinical Nutrition, 53(1), 194S-200S

[13] Almeida M.L.B., de Souza Freitas W.E., de Morais P.L.D., Sarmento J.D.A., Alves R.E. Bioactive compounds and antioxidant potential fruit of Ximenia americana L. *Food Chem.* 2016;192:1078–1082.

[14] Sarmento J.D.A. 7. Bioactive compounds and antioxidant activity of Ximenia americana coming from different collection sites. *Archivos Latinoamericanos de Nutrición.* 2015; 65(4).

[15] Hunde D. 2012. Uses and Management of Ximenia Americana, Olacaceae in Semi-arid East Shewa, Ethiopia.

[16] Maikai V., Maikai B., Kobo P. Antimicrobial properties of stem bark extracts of Ximenia americana. *J. Agric. Sci.* 2009; 1(2):30.

[17] Tsui H.-P. A study on the system of Lindera. *Acta Phytotax. Sin.* 1987; 25:161–171.

[18] Han H., Xu B., Amin A., Li H., Yu X., Gong M., Zhang L. Quercetin-3-O-α-L-rhamnopyranoside derived from the leaves of Lindera aggregata (Sims) Kosterm. Evokes the autophagy-induced nuclear factor erythroid 2-related factor 2 antioxidant pathway in human umbilical vein endothelial cells. *Int. J. Mol. Med.* 2019; 43:461–474.

[19] Noda Y., Mori A. Antioxidant activities of Uyaku (Lindera strychnifolia) leaf extract: A natural extract used in traditional medicine. *J. Clin. Biochem. Nutr*. 2007; 41:139–145.

[20] Joshi S.C., Verma A.R., Mathela C.S. Antioxidant and antibacterial activities of the leaf essential oils of Himalayan Lauraceae species. *Food Chem. Toxicol*. 2010; 48:37–40.

[21] Kim, Y.S.; Kim, E.K.; Dong, X.; Park, J.S.; Shin, W.B.; Kim, S.J.; Lim, B.O. Lindera glauca (Siebold et Zucc.) Blume Stem Extracts protect against tert-Butyl hydroperoxide-induced oxidative stress. J. Med. Food 2019, 22, 508–520.

[22] Feyssa D. H., Njoka J. T., Asfaw Z., Nyangito M. M. Uses and management of *Ximenia americana*, olacaceae in semi-arid east Shewa, Ethiopia. *Pakistan Journal of Botany*. 2012; 44(4):1177–1184.

[23] Joshi, S.C.; Mathela, C.S. Antioxidant and antibacterial activities of the leaf essential oil and its constituent's furanodienone and curzerenone from Lindera pulcherrima (Nees.) Benth. ex hook. f. Phcog. Res. 2012, 4, 80–84. 36.

[24] Kim, Y.U.; Yun, J.M. Antioxidative and Antiproliferative Effects of Lindera glauca Blume on Human Colorectal Cancer Cells. J. Korean Soc. Food Sci. Nutr. 2015, 44, 635–640.

[25] Huh, G.W.; Park, J.H.; Kang, J.H.; Jeong, T.S.; Kang, H.C.; Baek, N.I. Flavonoids from Lindera glauca Blume as low-density lipoprotein oxidation inhibitors. Nat. Prod. Res. 2014, 28, 831–834.

[26] Lucilania M. B. A., Wallace F. D. S. L., Patrícia L. D. M. Dárcio J. S. A., Elesbão R. "Bioactive compounds and antioxidant potential fruit of ximenia Americana L. *Food Chemistry*. 2016; 192:1078–1082.