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IN-VITRO ANTI-OXIDANT AND ANTI-DIABETIC ACTIVITIES OF ETHANOL EXTRACT OF CITRUS LIMON

Dr. Famobuwa O. E.*[1], Akinsiku R. O.[2], Amuho U. E.[1], Adekunbi E. A.[1] and Ogungbemi T. S.[1]

[1]Department of Chemistry, Adeyemi Federal University of Education, Ondo Nigeria.
[2]Department of Chemical Sciences, School of Science, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria.

***Corresponding Author: Dr. Famobuwa O. E.**

Department of Chemistry, Adeyemi Federal University of Education, Ondo Nigeria.

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ABSTRACT

Citrus limon is a medicinal plant containing valuable bioactive compounds that functions as antioxidants, combating oxidative stress. It holds potential as an alternative treatment for various medical conditions like diabetes. This study was aimed at the evaluating the in-vitro antioxidant and anti-diabetic activities of the ethanol extract of Citrus limon. The extract was analyzed for its potential antioxidant activities using Ferric reducing antioxidant assay (FRAP) and DPPH radical scavenging activity. The anti-diabetic activities of the extract were determined using α-amylase inhibition assay and β-glucosidase inhibition assays. The FRAP of the ethanoic extracts was 9.68 mgFe^{2+}E/100g while the maximum DPPH inhibition activity of 52.83% was reported at a concentration of 0.4 mg/ml and the minimum inhibition of 29.89% was reported at a concentration of 0.1 mg/ml. The α-amylase inhibition activity of the extract shows a maximum and minimum inhibition of 35.87% and 18.24% at a concentration of 0.2 mg/ml and 0.8 mg/ml respectively. The β-glucosidase inhibition activity of the extract shows a maximum and minimum inhibition of 60.92% and 23.11% at a concentration of 0.8 mg/ml and 0.2 mg/ml respectively. This result suggests the potential use of this plant species as a source of natural antioxidant and also in the management of diabetes, through its inhibitory effect on the activity of β-glucosidase.

KEYWORDS: Citrus limon; anti-oxidant; anti-diabetic; β-glucosidase.; α-amylase.

INTRODUCTION

Medicinal plants constitute an effective source of both traditional and modern medicine. These plants have been shown to have genuine utility and about 80% of the rural population depend on them as primary health care.[1] Plants have been used as sources of remedies for the treatment of many diseases since ancient times and people of all continents especially Africa have this old tradition. Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants.[11] However, plants used in traditional medicine are still under-studied. In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population use medicinal plants as remedies.[9]

Citrus limon, commonly known as the lemon, is a species of evergreen tree native to South Asia, particularly in the northeastern regions of India.[10] This small to medium-sized tree belongs to the Rutaceae family and famous for its vibrant, ellipsoidal fruits, which are prized for their tart flavor and versatile culinary applications. Lemons have a long and illustrious history, with records of their cultivation and use dating back centuries.[10] It is believed that lemons were first domesticated in India and subsequently spread to other parts of Asia, the Middle East, and eventually Europe through ancient trade networks. This extensive history has contributed to the lemon's reputation as a globally recognized fruit.

One of the distinctive features of Citrus limon is its remarkably high vitamin C content.[10] This nutrient-rich fruit not only adds a burst of flavor to various dishes and beverages but also serves as a natural preservative due to its acidity, which has made it invaluable in food preservation and pickling.

MATERIALS AND METHODS

Collection of plant material: Fresh matured leaves of lemon plant were collected from plantations in Ondo, South Igodan Lisa Okitipupa Local. Government Area of Ondo State, Nigeria. The leaves were identified and authenticated by a Botanist in the Department of

Biology, Adeyemi Federal University of Education, Ondo, by comparing with voucher specimens deposited at the Herbarium of the Department of Crop Protection and Pest Management, Federal University of Technology, Akure, Nigeria.[6] Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

EXTRACTION OF PLANT MATERIAL

Solvent Extraction: The solvent and chemicals used for this work were of analytical grade. Thoroughly washed plant parts were dried in shade for five days and then powdered with the help of blender. The powdered plant parts were extracted successively with ethanol in Soxhlet extractor for 48hr. The residue was evaporated at 45°C to dryness and extract yield was determined using the formula below:

$$\% \text{ Extract yield} = \frac{\text{gram of extract}}{\text{initial gram}} \times 100$$

The concentrated extract was thereafter stored in a desiccator.

Reconstitution of Extract for Analysis: The extract (0.02g) was weighed and transferred into a universal bottle. It was dissolved with 2 ml of dimethyl sulphoxide and made up to 20 ml with distilled water. It was kept in the refrigerator until further use.

ANTIOXIDANT ROPERTY

The Ferric Reducing Antioxidant property: Ferric Reducing Ability of Plasma (FRAP) of the extract was done using the method of Benzie and strain.[5] The FRAP working reagent was freshly prepared by mixing solutions of Acetate buffer (pH 3.6), TPTZ solution, and ferric chloride in ratio 10:1:1 and warmed at 37 °C before use. Samples (0.2 ml) were mixed with 2.80 ml of the FRAP reagent and the mixtures were kept in the dark for 30 min at room temperature. The absorbance was read at 593nm and FRAP was evaluated from ferrous sulphate standard curve and expressed as (mg Fe^{2+}E/100g). This procedure was carried out in triplicate.

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) Scavenging Activity

Procedure: The 1, 1-diphenyl-2-picrylhydrazyl scavenging activity of the extract was done using the Gyamfi method with slight modification.[6] Appropriate dilutions of 1.0 ml (0.1-0.4 mg/ml) sample were added to 4 ml of DPPH solution (40 mg/l) prepared in methanol. The samples were mixed thoroughly and left in the dark for 30 minutes. The absorbance was read at 520 nm. The inhibition percentage was calculated as;

Inhibition percentage of DPPH = {(Abs control- Abs Sample)/ (Abs Control)} × 100

DPPH solution without sample served as control. This procedure was carried out in triplicate.

DETERMINATION OF ANTIDIABETIC ACTIVITY

Alpha-Amylase Inhibition (AI) Assay: Alpha-amylase inhibition of the extract was determined using the method of Worthington with slight modification.[12] After appropriate dilution, 500 µl (0.2-0.8 mg/ml) of samples and 500 µl (0.02M) of sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 1mg/ml α-amylase solution were incubated at room temperature for 10 min. Thereafter, 500 µl (1 %) of starch solution prepared with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added. The reaction mixture was then incubated at 25°C for 10 minutes. The reaction was halted with 0.1 ml (44 mm) of dinitrosalicylic acid. The test tubes were then incubated in a boiling water bath for 5 min and allowed to cool. The absorbance was read at 540 nm. The inhibition percentage was calculated using the formular:

Inhibition percentage = {(Abs Control- Abs Sample)/ (Abs Control)} × 100

Reaction mixture without sample was used as a control. This procedure was carried out in duplicate.

Beta-Glucosidase Inhibition (GI) Assay: Glucosidase Inhibition of the extract was done using the method of Apostolidis with slight modification.[2] After appropriate dilution, 500 µl (0.2 – 0.8 mg/ml) of sample was suspended in 1000µl β-glucosidase solution (1.0 U/mL) prepared in of 0.1 M phosphate buffer (pH 6.9) and pre-incubated for 10 minutes at 25° C. After pre-incubation, 500 µl of 5 mM nitrophenyl-glucopyranoside solution prepared in 0.1 M phosphate buffer (pH 6.9) was added. The reaction mixture was incubated in boiling water for an hour. The absorbance of the reaction mixture was measured at 405 nm. The percentage inhibition was calculated using the formula:

Inhibition percentage = {(Abs Control- Abs Sample)/ (Abs Control)} × 100

Reaction mixture without sample was used as a control. This procedure was carried out in duplicate.

RESULTS AND DISCUSSION

Extract Yield: The yield of the crude ethanol extract of Citrus limon after concentration and drying was 0.73g of 30g (2.43%).

Antioxidant activity

Table 1 and 2 show the results of ferric reducing antioxidant potential the DPPH activity of ethanol extracts of Citrus limon respectively.

Table 1: Ferric reducing antioxidant potential (FRAP) of ethanol extracts of Citrus limon.

Assay	Absorbance	Concentration
FRAP (mg Fe^{2+}E/100g)	0.166±0.009	9.68

Values represent Mean ± Standard deviation of three replicates.

Ferric reducing antioxidant potential (FRAP): FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ). The free radical chain breaking takes place through donating a hydrogen atom (Benzie and Strain, 1996). FRAP was expressed as mg Fe^{2+}E/100g. FRAP values of the ethanol extract of Citrus limon is 9.68 mgFe^{2+}E/100g. This result was compared to what was reported by Azrina[4] on Citrus limon peel who reported the ethanol extract of Citrus limon to be 38.10±0.01 mgFe^{2+}E/100g. This result shows that both the leaves and peels of citrus limon contains antioxidant properties. The variation in concentration could be due to the part of the plant used.

Table 2: DPPH radical scavenging activity (% inhibition) of ethanol extract of Citrus limon.

Extract	Concentration (mg/ml)	Absorbance 520 nm	% Inhibition of DPPH activity
Control	-	0.920±0.032	-
ECL	0.1	0.645± 0.016*	29.89
ECL	0.2	0.540±0.007*	41.30
ECL	0.3	0.489±0.009*	46.85
ECL	0.4	0.434±0.009*	52.83

ECL represents Ethanol extract of Citrus limon
Each value represents the mean ± standard deviation of three experiments.
Experimental group were compared with control *p<0.05 is considered to be significant.

DPPH

Scavenging activity of DPPH is based on one-electron reduction which represents the free radical reducing activity of antioxidants. The result in Table 2 shows the percentage inhibition of DPPH radical by the ethanol extract of Citrus limon at different concentrations. The percentage inhibition of DPPH increases as the concentration increases. The result shows that the ethanol extract of Citrus limon extracts have the highest inhibition activity of 52.83% at concentration 0.4 mg/ml and the lowest inhibition activity of 29.89% at concentration 0.1 mg/ml. The result shows the Citrus limon leaf is a good source of antioxidant.

ANTIDIABETIC ACTIVITIES OF ETHANOL EXTRACTS OF CITRUS LIMON

Tables 4 and 5 show the α-amylase inhibition activity of ethanol extract and β-glucosidase inhibition activity of ethanol extract of Citrus limon respectively.

Table 4: α-amylase inhibition of ethanol extract of Citrus limon.

Extract	Concentration (mg/ml)	Absorbance 540 nm	% Inhibition of α-amylase activity
Control	-	0.329±0.004	-
ECL	0.2	0.211±0.025*	35.87
ECL	0.4	0.246±0.011*	25.23
ECL	0.6	0.256± 0.011*	22.19
ECL	0.8	0.269±0.018*	18.24

ECL represents Ethanol extract of Citrus limon
Each value represents the mean ± standard deviation of three experiments.
Experimental group were compared with control *p<0.05 is considered to be significant.

α-amylase is an enzyme that catalyzes the hydrolysis of starch and ultimately producing glucose. Controlling the catalytic activity of this enzyme reduces glucose production in the postprandial stage, which could be a therapeutic benefit for people with diabetes.[8] The percentage inhibition of ethanol extract of Citrus limon in Table 4 shows that amylase activity decreases as the concentration increases, with minimum inhibition of 18.24% reported at concentration 0.8 mg/ml and maximum inhibition of 35.87% at concentration 0.2 mg/ml. The difference between the results may be due to difference in the concentration. In conclusion, the result suggests that Citrus limon when administered at lower concentration inhibits α-amylase better than when administered at higher concentration.

Table 5: β-glucosidase inhibition of ethanol extract of Citrus limon.

Extract	Concentration (mg/ml)	Absorbance 405 nm	% Inhibition of β-glucosidase activity
Control	-	0.476±0.014	-
ECL	0.2	0.366±0.008*	23.11
ECL	0.4	0.259±0.003*	45.59
ECL	0.6	0.218±0.016*	54.20
ECL	0.8	0.182±0.006*	60.92

ECL represents Ethanol extract of Citrus limon

Each value represents the mean ± standard deviation of three experiments.
Experimental group were compared with control *p<0.05 is considered to be significant.

β-glucosidase enzymes catalyze the hydrolysis of starch to simple sugars. Inhibition of β-glucosidase can have various applications, including in agriculture to control pests or in pharmaceuticals to combat diseases.[3] The percentage inhibition of ethanol extract of Citrus limon in Table 5 shows that glucosidase activity increases as the concentration increases, with minimum inhibition of 23.11% reported at concentration 0.2 mg/ml and maximum inhibition of 60.92% at concentration 0.8 mg/ml. This result suggests that Citrus species has high inhibition activity against β-glucosidase enzyme when administered at higher concentration.

CONCLUSION

Citrus limon leaves have demonstrated remarkable potential in the realm of antioxidant and anti-diabetic activity. The rich presence of bioactive compounds, such as flavonoid and polyphenols, in these leaves contributes to their powerful antioxidant properties, which help combat oxidative stress and protect cells from damage. Furthermore, studies have shown that citrus limon leaf extracts exhibit promising anti-diabetic effect by improving insulin sensitivity and regulating blood glucose levels. These findings suggest that citrus is a natural source of therapeutic agents for managing oxidative stress and diabetes. Further research and clinical studies are essential to unlock the full potential of this botanical resource for promoting health and well-being

RECOMMENDATION

The data obtained from this research could serve as leverage for further research on the possible use of this plant as a natural source of antioxidant and anti-diabetics therapy in the management of diseases caused by oxidative stress such as cancer, cardiovascular diseases, neurodegenerative disease, and aging and also in the management of diabetes.

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