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Original Research



α-Glucosidase and α-amylase Inhibitory assay of Fractions and Crude Extract of *Zingiber officinale* (Ginger) and Evaluation of Its Antioxidant (ABTS and DPPH) Activity

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Abstract:

Objectives: The objective of this work was to provide an investigation into the antioxidant, α -glucosidase, and α -amylase inhibitory properties of the crude extract and fractions of the Zingiber officinale plant.

Background: It is possible to find members of the Zingiberaceae family in both the southern and eastern regions of Asia. Plants such as Zingiber officinale (often known as ginger) and Curcuma longa (also known as turmeric) are examples of members of this family that are frequently utilized in herbal medicine. As an additional benefit, these plants are widely used as a component of polyherbal medications for the treatment of diabetes mellitus.

Methods: For the goal of assessing the antioxidant activity, a free radical scavenging assay were carried out. Furthermore, a substrate-based enzyme inhibition experiment was conducted in order to ascertain whether or not Zingiber officinale possessed inhibitory actions directed towards α -glucosidase and α -amylase.

Results: Apigenin 7-glucoside, Kaempferol 3,7-dirhamnoside, Lappaconitine and Quercetin 3,7-dirhamnoside were the primary components. The 17-(5-ethyl-6-methylheptan-2-yl) lanosta-8, as well as Lycoctonine and also 4-Hydroxyphenyl-4H-1-benzopyran-4-one (dihydroxy-3,4-phenyl)the chemical formula 7-(beta-D-glucopyranosyloxy)a compound that 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychroman-4-one, is 5-hydroxy and 4H-1-Benzopyran-4-one, -3,5,7-dihydroxychroman-4-one, 7-[[6-O-(6-deoxy-alpha-Lmannopyranosyl)-beta-D-glucopyranosyl]oxy] [7-[[6-O-(6-deoxy-alpha-L-mannopyranosyl)beta-D-glu3,3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-, (S)-;phenyl 2-O-(6deoxyhexopyranosyl) hexopyranoside, ferulic acid methyl ester, and heptyl 4methylbenzenesulfonate are the three components that make up this compound.

When compared to the standard diabetic medicine acarbose, the percentage of α -Amylase that was inhibited by the ethyl acetate and methanol fractions was shown to be considerably higher (P<0.05) than the percentage

that was attained by their respective fractions. Despite the fact that methanol and the ethanol fraction exhibited a much larger percentage of inhibition against α -glucosidase compared to acarbose, the difference did not meet the criteria for statistical significance (P>0.05). Based on the findings of the DPPH radical scavenging activity, it was observed that the ethyl acetate and ethanol fraction exhibits a significantly higher percentage of inhibition (P<0.05) compared to the standards.

Conclusion: Zingiber officinale demonstrates a robust antioxidant activity, in addition to exhibiting activity that inhibits α -glucosidase and α -amylase. On the other hand, greater research is required in order to determine the compounds that are inhibitory.

Keywords: Zingiber officinale, α-Amylase, α-Glucosidase.

Introduction:

Because of a deficiency in insulin synthesis, insulin action, or both, diabetes mellitus is a metabolic condition that is characterized by chronic hyperglycemia and a disturbance of lipid, protein, and carbohydrate metabolism [1]. Diabetes mellitus is a metabolic disorder that is characterized by chronic hyperglycemia. Diabetes mellitus is a metabolic illness that is characteristic of persistent hyperglycemia. Just diabetes is another name for diabetes mellitus, which is another name for diabetes. The presence of the enzymes alpha-glucosidase and alpha-amylase in a living organism has the potential to result in hyperglycemia from their presence. Simply put, these enzymes are responsible for the conversion of starch into simple sugars. One of the most important aspects of diabetes therapy is the procedure for controlling hyperglycemia [2]. Several individuals make use of a variety of inhibitors in order to prevent enzymes from performing their functions. There have been a number of research investigations conducted in recent years that have provided clear proof that a wide variety of plant species displayed a favorable inhibitory action toward enzymes connected to diabetes [3].

There are around 53 genera that are classified under the Zingiberaceae family, which collectively encompasses more than 1200 distinct species [4]. Researchers are currently demonstrating a substantial level of interest in specific members of the Zingiberaceae family. This is mostly due to the fact that certain species of the Zingiberaceae family are widely used as spices and as components of herbal treatments in traditional medicine [5]. Jahe Emprit is another name for either of these two species. The ginger that is commonly used is one of the members of this family that is the most well-known. However, this flavor has been around for more than three thousand years, when it was known as the Sanskrit term srngaveram (horn root) [6]. The word "ginger" comes from the Middle English word gingivere, which means "ginger."

Ginger is actually a medicinal plant and has actually been widely used in herbal medicinal treatments in China for more than three thousand years in the Asian and East Asian regions. This part of the ginger rhizome, which is the horizontal stem from which the roots actually develop, is the root of the ginger plant. It can be said that this is due to the fact that the rhizome actually possesses useful and important characteristics and qualities such as strong flavour, quality and distinctive nutritional value. The rhizomes contain two distinct categories of substances: volatile compounds, which are responsible for the production of the essential oil, and non-volatile compounds, which include oleoresin, which is a source of pungent, as well as other phytochemicals that have biological activities that are beneficial to human health, such as flavonoids and phenolics [8]. In essence, the essential oil is composed of the volatile components. Rhizomes are the location where the non-volatile chemicals can be discovered. Many different products have been made from ginger, some of which include ginger tea, ginger beer, ginger powder, ginger sweets, and ginger juice. Ginger has also been used to make ginger candy. The food business has been able to implement these goods as a result of advancements in science and technology that are currently available.

An analytical system that is able to provide relevant information and recognize the molecules of interest is required in order to profile metabolites. This is because the system must be able to produce the information. Metabolite profiling has been utilizing a wide range of analytical techniques ever since it was first developed [9]. GC-MS is one of these techniques that offers a variety of benefits, including low cost in comparison to other analytical procedures, outstanding reproducibility, high resolution, extremely consistent mass spectrum fragmentation, and very negligible matrix effects [10]. GC-MS is one of these techniques.

In order to process the complex data sets that are obtained, multivariate statistical studies are often carried out. This is due to the fact that the chromatographic data that is obtained is frequently a set that contains several dimensions. It is possible to make the identification of patterns that exist between phenotype and the metabolite profile more transparent by employing statistical methods that take into account many variables. In order to gather metabolite profiles from a wide range of medicinal plants, it was possible to make use of GC-MS in conjunction with multivariate statistical analysis. Artemisia princeps, Cinnamomum cassia, Curcuma species, and Rehmannia glutinosa are several examples of the plants that fall under this category. In this study, we conducted an investigation into the antioxidant, α -glucosidase, and α -amylase inhibitory activities of the methanolic crude extract of Zingiber officinale and its fractions with respect to their in vitro performance.

MATERIALS and METHODS

Plant Collection and Preparation of Extract

The root of Zingiber officinale was procured from one of the commercial markets in Al-Hilla, which is located inside the Babil Governorate. Laboratory staff in the University of Babylon's College of Science were able to determine the species of the plant that had been harvested. Following the process of macerating the seeds of Zingiber officinale in methanol at a concentration of 90%, the powdered seeds were subsequently filtered. This process was carried out in a funnel that was designed for separation. After giving the mixture a thorough shake, it was decided to lay it aside for a day so that the components might become more evenly distributed. Hexane was used to perform the fractionation of the extract after it had been allowed to settle, and the residue was then mixed with ethanol. For the purpose of collecting the various fractions of ethanol, the same approach was utilized. An ethyl acetate (EA) treatment was performed on the residue that was left over after the ethanol fraction was completed. This was done in order to obtain the EA fraction. Following the completion of all the steps involved, the separation process will result in the production of a certain amount of ethyl acetate, while the remaining component will be an aqueous or water fraction.

Analysis of the methanol fraction of Zingiber officinale (ginger) using GC-MS.

In order to carry out the (GC-MS) analysis of the methanol fraction that was derived from Zingiber officinale (ginger), an Agilent Technologies 6890 Series gas chromatograph was connected to an Agilent 5973 Mass Selective Detector. Additionally, the Agilent Chemstation software was utilized in order to carry out the analysis. This investigation was carried out with the purpose of identifying the components that make up the methanol fraction. The capillary column that was utilized was an eHP-5MS model manufactured by Agilent Technologies. It was situated on Stevens Creek Boulevard in Santa Clara, California, and it had a length of thirty meters with an internal diameter of 0.25 millimeters. At a flow rate of 0.57 milliliters per minute and a linear velocity of 27.5 centimeters per second, the ultrapure helium was utilized as the carrier gas utilizing the aforementioned parameters.

The injector was maintained at a temperature of 250 degrees Celsius throughout the experiment. The temperature of the oven began at 50.0 degrees Celsius and was programmed to gradually increase to 250 degrees Celsius. The temperature was set to gradually increase to 250 degrees Celsius. Each injection was one microliter in volume, and it was performed in the splitless mode with a split ratio of twenty to one hundred. It

was determined that the electron multiplier voltage should be set to 1859 V, and the mass spectrometer was operated in the electron ionization mode. A voltage of 70 eV was measured for the electron ionization mode. A solvent delay of four minutes, a scan range of fifty to seven hundred amu, an ion source temperature of two hundred and thirty degrees Celsius, and a quadruple temperature of one hundred fifty degrees Celsius were some of the other operating settings for the mass spectrometer. By conducting a comparative analysis of the fragmentation pattern, retention times, and mass spectral data of the unidentified constituents in the analyzed sample and those obtained from the National Institute of Standards and Technology and Wiley Libraries, which collectively possess a library of over 75,000 compounds [11], it was feasible to discern the constituents. This allowed for the identification of the constituents. After that, the characteristics of each component, including its name, molecular weight, structure, and relative fraction, were examined and confirmed.

In vitro Antidiabetic assay

Chemicals.

The gallic acid, Folin–Ciocalteu's reagent, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), methanol, acarbose, α -glucosidase from Saccharomyces cerevisiae, α -amylase from porcine pancreases, 2-chloro-4-nitrophenyl- α -D-maltotrioside (CNPG3), and p-nitrophenyl- α -D-glucopyranoside (p-NPG) were obtained from Sigma-Aldrich through the purchase. In this particular investigation, only chemicals and reagents of an analytical grade were utilized.

α-amylase inhibitory assay

For the purpose of assessing the α -amylase inhibitory activity of the extract and fractions, the standard methodology was utilized, albeit with a few minor modifications [12]. Fifty liters of phosphate buffer with a concentration of 100 millimolar and a pH of 6.8 were combined with ten liters of α-amylase at a concentration of two units per milliliter, and twenty liters of extract and fractions with varying concentrations of 0.5 milligrams per milliliter. Then, the mixture was pre-incubated at 37 degrees Celsius for twenty minutes after being transferred to a 96-well plate. The preincubation process was conducted at a setting of 37 degrees Celsius. As a substrate, twenty liters of 1% soluble starch (100 mM phosphate buffer pH 6.8) were subsequently added to the mixture, which was reintroduced into the incubator for thirty minutes at 37 degrees Celsius. After incorporating one hundred liters of DNS color reagent, the mixture was heated for ten minutes at a constant pressure. A reading of absorbance was acquired at 540 nanometers through the utilization of a Multiplate Reader (Multiska Thermo Scientific, version 1.00.40). In order to ascertain the absorbance of the resulting mélange, the measurement was conducted. To function as a standard, varying concentrations of acarbose spanning from 0.1 to 0.5 mg/ml were employed. To function as a control, a substance was prepared in parallel that had not undergone any experimental procedures (extracts and fractions). Furthermore, each experiment was replicated three times. The results were presented in the form of a percentage of inhibition, as calculated utilized formula. by the

The percentage of inhibition could be determined by applying the following formula:

% Inhibition = $(Abs_{control} - Abs_{extract}) / Abs_{control} \times 100$

Graphic representations were used to calculate the concentrations of fractions that resulted in a 50% inhibition of enzyme activity (IC₅₀).

α-Glucosidase Inhibitory Assay

An analysis was conducted to determine the α -glucosidase inhibitory activity of the extract and fractions. The analysis was conducted using the standard method, with some minor modifications [13]. In a 96-well plate, a reaction mixture was preincubated at 37 degrees Celsius for fifteen minutes. The reaction mixture included fifty liters of phosphate buffer with a concentration of one hundred millimolar and a pH of six.8; ten liters of alpha-glucosidase with a concentration of one unit per milliliter; and twenty liters of various extract and fractions with a concentration of half a milligram per milliliter. At a temperature of 37 degrees Celsius, the preincubation was carried out. Following that, twenty liters of P-NPG with a concentration of five millimolar was added to the mixture as a substrate, and the mixture was then incubated at 37 degrees Celsius for an additional twenty minutes. The process was stopped by adding fifty liters of a sodium carbonate solution with a concentration of 0.1 M. Through this test an actual evaluation of the absorption of freshly released nitrophenol was carried out with the help of a multiplate reader at a wavelength of 405 nm. Meanwhile, acarbose was found in the examined sample at a concentration of 0.5 mg/mL, and at the same time was used as a standard measurement. Hence, each of these experiments was conducted approximately three times, in addition to conducting a control that did not include the actual chemical being tested in parallel as a comparison to the read results.

The studies were carried out three times to ensure accurate results, and the α -glucosidase inhibitory activity was measured in terms of the percentage of inhibition using the following expression:

% Inhibition = (Abs_{control} - Abs_{extract}) /Abs_{control} × 100

Where the absorbances of the control and fractions, respectively, are denoted by the symbols A control and A extract. Graphic representations were used to calculate the concentrations of fractions that resulted in a 50% inhibition of enzyme activity (IC₅₀).

In vitro antioxidant assay

2,2-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid radical determination

The procedure that has already been decided upon was used to evaluate how well the fractions were able to scavenge the ABTS cation chromophore that was produced during the oxidation of the ABTS solution with potassium persulfate. Free radicals are being fought against by a variety of activities. To evaluate the antioxidant activity of the crude extract of the plant as well as its fractions, the ABTS free radical scavenging activity was utilized [14]. This was done in order to determine the level of antioxidant activity. For the aim of this experiment, one hundred liters of plant sample and one hundred liters of ABTS (0.1 mM) were combined. After that, the combination was left to react at room temperature and in the dark for a period of thirty minutes as the experiment was carried out. Following the completion of the experiment, the absorbance was determined by using a spectrophotometer at a wavelength of 517 nm. The percentage of inhibition was then determined by employing the procedure that is described in the following paragraphs. The sample of the plant was dissolved in DMSO at a concentration of fifty percent, and this solution formed the control for the experiment. In order to establish a standard for antioxidant activity, quercetin was investigated.

% Inhibition = (Abs_{control} - Abs_{extract}) /Abs_{control} × 100

The half maximal inhibitory concentration (IC₅₀) value was calculated and obtained from the linear regression equation using y = mx + c, where y is the percentage activity and equals 50, m is the slope, c is the intercept, and x is the IC₅₀ value.

1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity

Based on the scavenging activity of the stable DPPH free radical, the free radical scavenging activity of the fractions derived from Zingiber officinale (ginger) was determined. In the beginning, a microtiter plate with 96 wells was used. Separately, 150 microliters of plant fraction, which had a concentration of 0.5 milligrams per milliliter, was mixed to 150 microliters of a DPPH solution that was 0.004% methanolic. After a period of thirty minutes, the absorbance at 517 nm was determined by employing a 96-well microplate reader manufactured by Bio-Rad and manufactured in Japan. In order to determine the percentage inhibitory activity, the absorbance of the control (A0) was divided by the absorbance of the fraction or standard (A1), and the resulting value was then multiplied by 100. Specifically, the absorbance of the control or standard is denoted by the letter A0 in this equation.

The half maximal inhibitory concentration (IC₅₀) value was calculated and obtained from the linear regression equation using y = mx + c, where y is the percentage activity and equals 50, m is the slope, c is the intercept, and x is the IC₅₀ value.

Statistical analysis

GraphPad Prism 5, a statistical package developed in the United States by GraphPad Software, was utilized in the statistical study. After doing an analysis of variance (ANOVA) using a single factor, the data were subjected to the Bonferroni test. The results of the in vitro IC_{50} were presented as the mean value. The activities of scavenging free radicals were expressed as a percentage, while the quantification of phytochemicals was expressed as a mean with a standard deviation. When P was less than 0.05, statistical significance was assumed to exist.

Results and Discussion:

Analysis of the methanol fraction obtained from Zingiber officinale (ginger) using gas chromatography and mass spectrometry.

There are over twenty-one chemicals that have been found in the methanol fraction of Zingiber officinale, which accounts for 99.9% of the total contents of the methanol fraction. The major constituents were Apigenin 7-glucoside, Kaempferol 3,7-dirhamnoside, Quercetin 3,7-dirhamnoside, Lappaconitine, Lanosta-8,24-dien-3-ol, 17-(5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-2-(3,4-dihydroxyphenyl)-7-(beta-Dol. Lycoctonine, 4H-1-benzopyran-4-one, glucopyranosyloxy)-5-hydroxy, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychroman-4-one, 4H-1-Benzopyran-4-one, 7-[[6-O-(6-deoxy-alpha-L-mannopyranosyl)-beta-Dglucopyranosyl]oxy]-2,3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-, (S)-, 3,5dihydroxy-4-[3-(3-hydroxy-4-methoxyphenyl)propanoyl]phenyl 2-0-(6deoxyhexopyranosyl)hexopyranoside, Ferulic acid methyl ester, and Heptyl 4methylbenzenesulfonate.

According to the findings of the GC-MS analysis of the fraction, the phytoconstituents of the fraction have been linked to a variety of biological activities that have substantial therapeutic applications [15]. It is possible that the phytol in it is specifically responsible for its anti-oxidant and anti-diabetic capabilities, which were strongly elicited in this investigation. Although it has been shown that 2-hexadecyloxirane and stearic acid have good potentials as antioxidants,[16] the antioxidant and hypoglycemic actions of phytol have also been described [17]. It is possible that the existence of these identified bioactive elements in Zingiber officinale (ginger) is to blame for the evoked potential seen in this investigation. The glycosyloxyflavone known as apigenin 7-O-beta-D-glucoside is a glycosyloxyflavone that has had a beta-D-glucopyranosyl moiety attached to position 7 of the apigenin molecule via a glycosidic linkage. In addition to being a metabolite and an

antibacterial agent, it also functions as a non-steroidal anti-inflammatory medication. It is a dihydroxyflavone, a glycosyloxyflavone, and a monosaccharide derivative in addition to being a beta-D-glucoside. It shares some of the same properties as an apigenin.

According to the findings of our research on the antimicrobial activity of ginger, it is possible to assert that ginger possesses notable antibacterial characteristics [18-21]. In the course of developing this study, it was discovered that ginger has a large number of bioactive chemicals, some of which can be acquired in the form of an essential oil, an extract, or an oleoresin. Because of the large antibacterial and antifungal inhibitory range that several of these compounds possess, they have the potential to inhibit the most significant pathogens that are linked with foodborne illnesses. According to the findings of the study, ginger possesses powerful antibacterial activity and has the potential to be utilized in a variety of research fields, including the pharmaceutical and food industries.



Murtadha M Hussein a Kadhim / α -Glucosidase and α -amylase Inhibitory assay of Fractions and Crude Extract of Zingiber *officinale* (Ginger) and Evaluation of Its Antioxidant (ABTS and DPPH) Activity



Inhibitory potency of *Zingiber officinale* (ginger) root fractions against α -amylase and α - glucosidase activity

The measured inhibitory activity of the screened methanol fraction and the ethyl acetate fraction derived from Zingiber officinale against α -amylase and α -glucosidase, respectively, are shown in Figures 1 and 2, respectively. Hence, the results obtained in reality from the actual enzyme inhibitor test showed that the inhibitory activity of Zingiber officinale fractions against α -amylase and α -glucosidase was simultaneously dependent on both the dose and the calculated fraction. According to the data obtained, the maximum studied

dose actually resulted in significant observed inhibition, while the lower dose resulted in the least amount of measured inhibition. According to the type of extract (Crude, Ethyl acetate fraction, Ethanol fraction, Hexane fraction, Water fraction and Acarbose as standard) recorded (113.07 ± 0.36 , 59.00 ± 0.13 , 36.98 ± 0.07 , 57.12 ± 0.99 , 67.00 ± 0.16 and 17.03 ± 1.35) respectively inhibitory potency against α -amylase. While recorded (57.44 ± 0.60 , 29.92 ± 0.51 , 52.07 ± 0.34 , 30.61 ± 0.34 , 21.59 ± 0.34 , and 05.00 ± 0.01) respectively inhibitory potency against α -glucosidase activity. The percentage of -amylase that was inhibited by the Methanol and Water fraction was found to be significantly higher (P 0.05) than that achieved by the usual diabetic medication acarbose. While methanol and the ethanol fraction showed a substantially higher percentage of inhibition against α -glucosidase than acarbose, the difference was not statistically significant (P > 0.05).

Radical scavenging activities of Zingiber officinale (ginger) fractions for in vitro

antioxidant assays

The in vitro antioxidant capacities of methanol fractions derived from the root of *Zingiber officinale* were illustrated in Figures 3 and 4. According to the type of extract (Crude, Ethyl acetate fraction, Ethanol fraction, Hexane fraction, Water fraction and Quercetin as standard) recorded (81.95 ± 2.06 , 104.12 ± 3.05 , 71.05 ± 2.00 , 62.00 ± 1.05 , 26.00 ± 0.22) respectively 2,2-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid radical scavenging activity. While recorded (70.04 ± 2.00 , 91.86 ± 2.67 , 75.00 ± 2.05 , 50.97 ± 1.00 , 40.00 ± 0.92 and 17.10 ± 0.02) respectively 1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity.

Indeed, compared to the results of samples with standard acarbose, the calculated percentage of inhibition against α -amylase that was actually demonstrated by the use of the ethyl acetate and methanol fractions was significantly higher (P < 0.05). From this we find that despite the fact that the methanol and the ethanol fraction showed a significantly greater percentage of inhibition against α -glucosidase compared to acarbose, the difference did not meet the criteria for statistical significance (P > 0.05).

The results presented in Figure 3 indicate that the calculated percentage of inhibitors of ethyl acetate and methanol fractions against ABTS scavenging activities was indeed significantly higher (P < 0.05) than that of the standards, which consisted of quercetin. Hence, the results of the DPPH radical scavenging activity assay are presented in Figure 4, which shows that the percentage of DPPH radical scavenging activity that is inhibited by the ethyl acetate and ethanol portion is much higher (P 0.05) than the percentage actually found in the standards.



Figure 1. IC₅₀ values of α-amylase inhibition by *Zingiber officinale* fractions compared with Acarbose (standard)



Figure 2. IC₅₀ values of α-glucosidase inhibition by *Zingiber officinale* fractions compared with Acarbose (standard)



Figure 3. IC₅₀ values of 2,2-azino-bis 3-ethylbenzothiazoline)-6-sulfonic acid radical scavenging activity of crude extract and fractions compared with Quercetin (Standard).



Figure 4. IC₅₀ values of 1-Diphenyl-2-picrylhydrazyl radical scavenging activity of crude .(extract and fractions compared with Quercetin (Standard

Conclusion:

The present study has provided evidence that the methanolic extract and its fractions derived from Zingiber officinale exhibit antioxidant characteristics, in addition to inhibiting the activities of α -glucosidase and α -amylase. The plant possesses not only a significant antioxidant capacity but also the capability to inhibit the activity of α -glucosidase and α -amylase. Additionally, investigation should be conducted to isolate and identify the bioactive molecules accountable for these effects.

References:

- Crichton, M., Marshall, S., Marx, W., McCarthy, A.L. and Isenring, E. (2019). Efficacy of ginger (*Zingiber officinale*) in ameliorating chemotherapy-induced nausea and vomiting and chemotherapyrelated outcomes: A systematic review update and meta- analysis. Journal of The Academy of Nutrition and Dietetics, 119(12), 2055–2068.
- 2. Danwilai, K., Konmun, J., Sripanidkulchai, B.O. and Subongkot, S. (2017). Antioxidant activity of ginger extract as a daily supplement in cancer patients receiving adjuvant chemotherapy: A pilot study. Cancer Management Research, 9, 11–18.
- 3. El-Gayar, M.H., Aboromia, M.M.M., Ibrahim, N.A. and Abdel-Hafiz, M.H. (2019). Effects of ginger powder supplementation on glycemic status and lipid profile in newly diagnosed obese patients with type 2 diabetes mellitus. Obesity Medicine, 14, 100094.
- 4. El-Ghorab, A.H., Nauman, M., Anjum, F.M., Hussain, S. and Nadeem, M.A. (2010). Comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*). Journal of Agricultural Food Chemistry, 58(14), 8231–8237.
- 5. Ahn, E. K. & Oh, J. S. 2012. Inhibitory effect of galanolactone isolated from Zingiber officinale Roscoe extract on adipogenesis in 3T3-L1 Cells. Journal of Korean Society for Applied Biological Chemistry, Vol.55, pp.63–68.

- Chang, W. T., Thissen, U., Ehlert, K. A, Koek, M. M., Jellema, R. H., Hankemeier, T., van der Greef, J. & Wang, M. 2006. Effects of growth conditions and processing on *Rehmannia glutinosa* using fingerprint strategy. Planta Medica, Vol.72, pp.458–467.
- Chareonkla, A., Pohmakotr, M., Reutrakul, V., Yoosook, C., Kasisit, J., Napaswad, C. & Tuchinda, P. 2011. A new diarylheptanoid from the rhizomes of Zingiber mekongense. Fitoterapia, Vol.82, pp.534– 538.
- D. Shrestha, T. Pokhrel, K. Dhakal, A. Pandey, P. Sharma, and S. Sapkota, "α-Glucosidase and αamylase inhibition study and in silico analysis of Mimosa pudica L. of Nepalese origin," Current Bioactive Compounds, vol. 18, pp. 2–8, 2022.
- 9. A. B. Olokoba, O. A. Obateru, and L. B. Olokoba, "Type 2 diabetes mellitus: a review of current trends," Oman Medical Journal, vol. 27, no. 4, pp. 269–273, 2012.
- 10. C. Lankatillake, T. Huynh, and D. A. Dias, "Understanding glycaemic control and current approaches for screening an- tidiabetic natural products from evidence-based medicinal plants," Plant Methods, vol. 15, no. 1, p. 105, 2019.
- 11. A. I. Martinez-Gonzalez, 'AG. D'1az-S'anchez, L. A. Rosa, C. L. Vargas-Requena, I. Bustos-Jaimes, and E. Alvarez-Parrilla, "Polyphenolic compounds and digestive enzymes: in vitro non-covalent interactions," Molecules, vol. 22, no. 4, p. 669, 2017.
- 12. D. Shrestha, P. Sharma, A. Adhikari, A. K. Mandal, and A. Verma, "A review on Nepalese medicinal plants used traditionally as alpha-amylase and alpha-glucosidase inhibitors against diabetes mellitus," Current Traditional Medicine, vol. 7, no. 5, p. 1, 2021.
- 13. T. Pokhrel, D. Shrestha, K. Dhakal, P. M. Yadav, and A. Adhikari, "Comparative analysis of the antioxidant and antidiabetic potential of Nelumbo nucifera gaertn. And nymphaea lotus L. Var. pubes1," Journal of Chemistry, vol. 2022, Article ID 4258124, 5 pages, 2022.
- 14. Y. M. Kim, M. H. Wang, and H. I. Rhee, "A novel α-glucosidase inhibitor from pine bark," Carbohydrate Research, vol. 339, no. 3, pp. 715–717, 2004.
- 15. M. Toeller, "α-Glucosidase inhibitors in diabetes: efcacy in NIDDM subjects," European Journal of Clinical Investigation, vol. 24, no. S3, pp. 31–35, 2010.
- 16. N. Saito, H. Sakai, S. Suzuki, H. Sekihara, and Y. Yajima, "Efect of an α-glucosidase inhibitor (voglibose), in combi- nation with sulphonylureas, on glycaemic control in type 2 diabetes patients," Journal of International Medical Research, vol. 26, no. 5, pp. 219–232, 1998.
- 17. K. Dhakal, D. Shrestha, T. Pokhrel et al., "Antioxidant, an- tibacterial, antidiabetic potential, and in silico analysis of rhus chinensis from western Nepal," Current Topics in Medicinal Chemistry, vol. 22, no. 26, pp. 2145–2151, 2022.
- 18. Z. X. Zhu, J. H. Wang, Y. C. Cai, K. K. Zhao, M. J. Moore, and H. F. Wang, "Complete plastome sequence of Erythropalum scandens (Erythropalaceae), an edible and medicinally important liana in China," Mitochondrial DNA Part B, vol. 3, no. 1, pp. 139-140, 2018.
- 19. D. T. Vu and T. A. Nguyen, "Te neglected and underutilized species in the Northern mountainous provinces of Vietnam," Genetic Resources and Crop Evolution, vol. 64, no. 6, pp. 1115–1124, 2017.
- 20. S. Sutha, A. Maruthupandian, V. R. Mohan, and T. Athiperumalsami, "Anti-infammatory activity of leaf of Erythropalum scandens bl., bijdr against carrageenan induced paw edema," Int J PharmTech Res CODEN, vol. 3, no. 1, pp. 24–26, 2011.
- S. Kupina, C. Fields, M. C. Roman, and S. L. Brunelle, "Determination of total phenolic content using the Folin-C assay: single-laboratory validation, frst action 2017.13," Journal of AOAC International. 2018; 101 (5): 1466–1472.
- 22. C.-C. Chang, M.-H. Yang, H.-M. Wen, and J.-C. Chern, "Estimation of total favonoid content in propolis by two complementary colometric methods," Journal of Food and Drug Analysis. 2020; 10(3).

- 23. L. L. Mensor, F. S. Menezes, G. G. Leitão. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method," Phytotherapy Research. 2001; 15(2): 127–130.
- 24. M. Telagari and K. Hullatti, "In-vitro α-amylase and α-glucosidase inhibitory activity of Adiantum caudatum Linn. and Celosia argentea Linn. extracts and fractions," Indian Journal of Pharmacology. 2015; 47(4):425–429.