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RESEARCH PAPER

Total phenolic content and anti-diabetic activity of dried and fresh leaves of *Ocimum sanctum* (Linn)

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Abstract

Plants are rich sources of secondary metabolites and oils which are of medicinal importance. At present, the level of acceptance of herbal medicines is increasing and replacing modern medicines due to the plant safety, efficiency, low cost and availability throughout the world. Leaves of *Ocimum sanctum* (Linn) have been proven to possess anti -diabetic effects in experimental animals, but the mechanism of anti-diabetic remains indeterminate. Research has been conducted to investigate the total phenolic content and anti-diabetic activity of *Ocimum sanctum* (Linn) dried and fresh leaves in three different extracts. A correlation between the total phenolic content and anti-diabetic activity was established. Three types of extracts, cold water, methanol and chloroform of dried and fresh leaves of *Ocimum sanctum* (Linn), were prepared by evaporating the extraction solvent at 40°C except for cold water extract at 50°C. Stock solutions of 10 mg/mL of each type of extract were prepared using dimethyl sulfoxide (DMSO). The folin-ciocalteu method was used to determine total phenolic content, while 3, 5-dinitro salicylic acid (DNS) method was used to determine α-amylase inhibition activity by different extracts of dried and fresh leaves. Generally, all the extracts of dried leaves of *Ocimum sanctum* (Linn) contain more total phenolic content and show the highest α-amylase inhibition activity as compared to fresh leaves. The highest total phenolic content and α-amylase inhibition activity were found in the methanol extracts of both dried and fresh leaves. The total phenolic content in methanol extracts of dried and fresh leaves was 0.780 mg and 0.700 mg GAE/g extract, respectively. While the percentage of inhibition of methanol extracts for dried and fresh leaves were 47% and 39.3%, respectively. The IC_{50} of methanol extracts of dried and fresh leaves were determined using different concentrations of methanol extracts of fresh and dried leaves (3, 6, 9, 12 mg/mL). Increasing the concentration of *Ocimum sanctum* (Linn) dried and fresh leaves decrease the activity of α-amylase. The amount of dried and fresh leaves required to inhibit 50% of α-amylase activity was 10.4 and 11.8 mg/mL, respectively. A positive correlation was found between total phenolic content and α-amylase inhibition activity using Pearson correlation in SPSS 16.0 for dried and fresh leaves with R^2 = 0.994 and R^2 = 0.991, respectively. In conclusion, the methanol extract of *Ocimum sanctum* (Linn) dried leaves has a good potential to be used as an anti-diabetic agent.

1. Introduction

According to the International Diabetes Federation (IDF), 451 million individuals worldwide have diabetes as of 2017. If no effective preventative measures are taken, that number is expected to rise to 693 million by the year 2,045 (Cho *et al*., 2018). In Malaysia, the number of youngsters suffering from this disease is increasing. A nationwide survey study states that in Malaysia, 3.6 million persons (18 and older) had diabetes in 2019; 49% of these cases, and 3.7 million were undiagnosed (Akhtar *et al*., 2022). With a prevalence of 31.3%, diabetes is anticipated to impact 7 million Malaysian individuals 18 and older by 2025, creating a

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serious risk to the public's health (Institute for Public Health (IPH), National Institutes of Health, Ministry of Health Malaysia., 2021). According to papers that have been published, the prevalence of diabetes in Malaysia varies from 7.3% to 23.8% (Samsudin *et al*., 2016; Harris *et al*., 2019).

Numerous therapeutic strategies are adopted to decrease the glucose level in the body. One promising therapeutic approach to decrease hyperglycaemia is to delay and reduce the digestion and absorption of ingested carbohydrates by inhibiting the carbohydrate hydrolysing enzymes (Hasaninezhad *et al*., 2020). In clinical studies, routinely prescribed medications may affect glucose homeostasis, resulting in decreased glucose tolerance, hyperglycemia, or new-onset diabetes mellitus, or they may exacerbate glycemic control in diabetics (Fathallah *et al*., 2015).

Plants are the primary source of medicines. Medicinal plants are considered to be very rich sources of secondary metabolites and oils which are of therapeutic importance. The important advantages of medicinal plants in various treatments are their safety, being less expensive, efficacy and availability throughout the world (Hosseinzadeh *et al*., 2015). According to Twaij and Hasan (2022), among the diverse bioactive compounds, phenolics, which are plant secondary metabolites, have been proven to exhibit many health-protective effects.

Among the plants known for medicinal value, the plants of genus *Ocimum* belonging to the family *Lamiaceae* are very important for their therapeutic potential. *Ocimum sanctum* (Linn) is known as “The Queen of Herbs”, “The Incomparable One” and “The Mother Medicine of Nature” (Joshi *et al*., 2012). In Malaysia it is called as “Kemangi” or “Selasi jantan” (Kadian and Parle, 2012). In addition to Ayurveda and Siddha, *O. sanctum* (Linn) also has several therapeutic uses in the Roman, Greek and Unani systems of medicine (Triveni *et al*., 2013). Experimental animals have demonstrated hypoglycaemic effects from *O. sanctum* leaves (Singh *et al*., 2010).

Although many traditional medicine practitioners have been widely using *O. sanctum* (Linn) plant for treating various diseases from ancient times, there is not much scientific evidence to verify and validate the anti-diabetic properties of the plant. This research is done to determine the potency of different *O. sanctum* (Linn) leaves extracts and in vitro evidence for the anti-diabetic activities and to find the relationship between total phenolic compound and the carbohydrate hydrolyzing enzymes inhibition activity of this plant.

2. Materials and methods

2.1 Preparation of plant leaves for extraction

The fresh leaves of *Ocimum Sanctum* (Linn) were collected in Rawang, Selangor Darul Ehsan. The extraction and collection of crude extract procedures were carried out as described by Mittal *et al*. (2018) with some modifications. The fresh leaves of *O. Sanctum* (Linn) were washed under running tap water, surface sterilized using 1% ethanol and washed using distilled water. For dried leaves extractions, the leaves were dried at room temperature for three days and ground into powder. For fresh leaves extractions, the leaves were dried at room temperature for three hours to remove the water on the surfaces of the leaves and ground them into a paste. Approximately 50 g of each dried leaves powder and fresh leaves paste were soaked in 1000 mL solvents (1:20 w/v) of decreasing degree of polarity. The solvents used in sequential order are cold water, methanol and chloroform. All the solutions were left at room temperature for three days and continuously stirred except for cold water extraction, which was placed in a chiller at 4°C.

2.2 Preparation of plant extract

The plant materials were separated from the solvent by filtration. A rotary evaporator was used to evaporate filtered cold water extract at 50°C until the volume was reduced by approximately 10% of the original volume. The collected extract was kept in a freezer at -20°C and then subjected to a freeze-drying process. The methanol and chloroform extracts were evaporated at 40°C until the crude extracts of methanol and chloroform were collected. All the crude extracts obtained were kept at 4° C. Before use, a stock solution of plant extract with a concentration of 10 mg/mL in 50.0 mL of dimethyl sulfoxide (DMSO) was prepared.

2.3 Determination of total phenolic contents in the plant extracts

Determination of total phenolic content was done according to the Folin-ciocalteu method as described by Phang *et al*. (2011) with some modifications. In brief, the reaction mixture contains 0.02 mL of each leaf extract respectively, 1.58 mL of distilled water, 0.1 mL of Folin-ciocalteu reagent and 0.3 mL of 35% saturated sodium carbonate (Na_2CO_3) solution. A blank was prepared for each plant extract respectively. After 30 minutes of reaction at 40°C in a water bath, the absorbance at 765 nm was measured and used to calculate the phenolic contents using gallic acid as a standard. The phenolic content in sample extracts was expressed as mg gallic acid equivalent (GAE) per gram of plant extract.

2.4 α-amylase inhibition assay

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This assay was carried out using a modified procedure by Mechchate *et al*. (2021). A total of 0.5 mL of different leaf extracts of 10 mg/mL concentration was placed in different tubes respectively, and 0.5 mL of 0.1 M acetate buffer containing a-amylase solution (0.01 mg/mL) was added in each tube. These solutions were pre-incubated at 37°C for 10 min, after which 0.5 mL of 1% starch solution was added to each tube and then further incubated at 37°C for 15 min. The reaction was terminated by adding 1.0 mL of 3, 5-dinitro salicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 15 mL of distilled water and the absorbance was measured at 540 nm using a spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase inhibitory activity was calculated as percentage inhibition:

$$\% \text{ inhibition} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right) \times 100$$

2.5 Determination of the IC_{50} values of solvent extract that shows the highest inhibition activity on α-amylase enzyme

The IC_{50} values of the plant extracts were determined by performing the assay as stated in the α-amylase inhibition assay with different concentrations (3, 6, 9, 12 mg/mL) of the plant extracts that showed the highest inhibition activity. Graph of percentage inhibition against different concentrations of extracts was plotted to determine IC_{50} values for dried and fresh leaf extracts that show the highest inhibition activity (De *et al*., 2013).

2.6 Statistical analysis

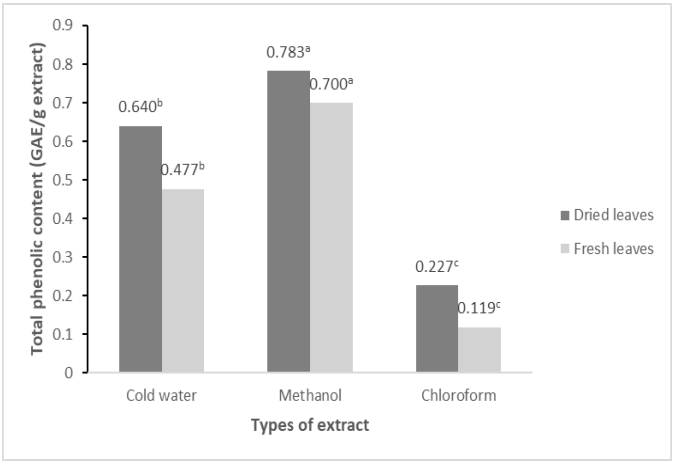
All analyses were performed in triplicate (n = 3), and SPSS 16.0 software was used to perform all the analyses. The result obtained from total phenolic content and α-amylase inhibition activity were analysed using one-way ANOVA. The correlation between total phenolic content and α-amylase inhibition activity of extracts was analysed using Pearson correlation.

3. Results and discussion

The results for the total phenolic content for three different plant extracts of dried and fresh leaves of *Ocimum sanctum* (Linn) are shown in Figure 1. For percentage of α-amylase inhibition by the three different plant extracts of dried and fresh leaves of *O. sanctum* (Linn) is shown in Figure 2. The IC_{50} value for methanol leaf extracts was obtained from the graph of the percentage of inhibition of α-amylase activity against the different concentrations of methanol extract in Figure 3 (A) for dried leaves and Figure 3 (B) for fresh leaves.

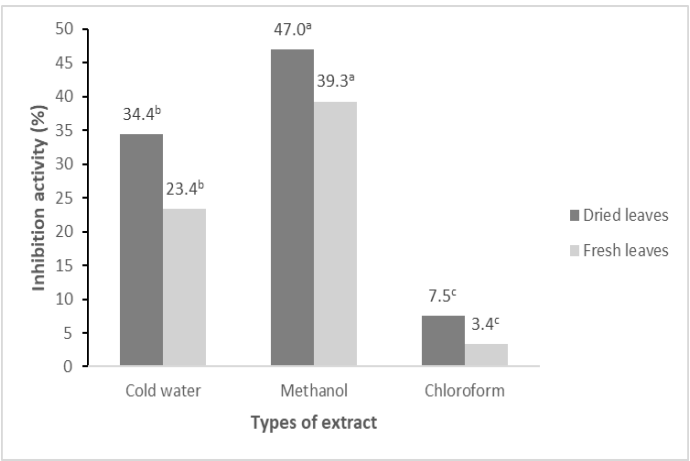
In this study, the dried leaf extracts of *O. sanctum* (Linn) obtained from the polar solvent, which is cold water and methanol, contained high amount of total phenolic content as compared to an extract obtained from a non-polar solvent, which is chloroform. Among the two polar extracts of methanol and cold water, the methanol extract has the highest amount of phenolic compounds compared to the cold water extract. The total phenolic content of methanol extract and cold water extract was 0.783 mg GAE/g extract and 0.640 mg GAE/g extract of dried leaves, respectively. The lowest total phenolic content was found in the chloroform extract, which contains only 0.226 mg GAE/g extract.

For fresh leaf extracts of *O. sanctum* (Linn), the



*Total phenolic content (mg GAE/g of extract) was expressed as the mean of three replication ± standard deviation. Abbreviations (^{a,b,c}) represent statistical comparisons made for total phenolic content among each extract in each sample. The mean with the different letters for each extract is significantly different ($p < 0.05$).

Figure 1. Total phenolic content of three extracts for dried leaves and fresh leaves of *Ocimum sanctum* (Linn).



*Percentage of inhibition was expressed as a mean of three replication ± standard deviation. Abbreviations (^{a,b,c}) represent statistical comparisons made for the percentage of α-amylase inhibition activity among each extract in each sample. The mean with the different letters for each extract is significantly different ($p<0.05$).

Figure 2. Percentage of α-amylase inhibition activity of three extracts of dried leaves and fresh leaves of *Ocimum sanctum* (Linn).

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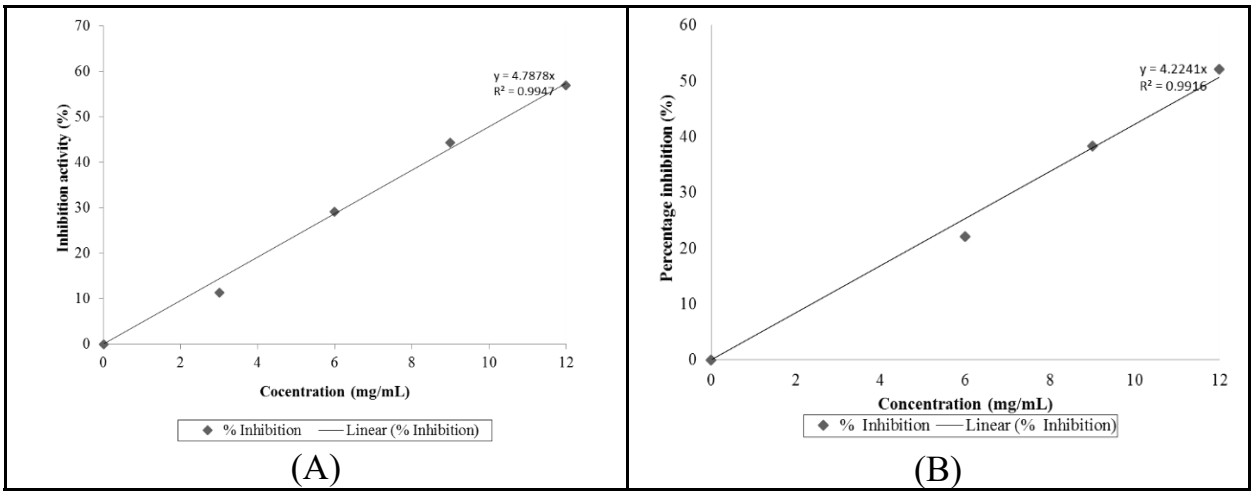

Figure 3. Percentage of inhibition of α-amylase activity at different concentrations of *Ocimum sanctum* (Linn) dried leaves (A) and fresh leaves (B) methanol extract to determine IC_{50} value.

methanol extract contains the highest amount of phenolic content as compared to cold water and chloroform extract. The chloroform has the least amount of phenolic content as compared to cold water and methanol extracts. The total phenolic content of methanol extract was 0.700 mg GAE/g extract of fresh leaves while the amount of phenolic content in cold water and chloroform extracts were 0.447 mg and 0.119 mg GAE/g extract of fresh leaves of *O. sanctum* (Linn). The total phenolic content of cold water and methanol fresh leaves extracts was lower as compared to dried leaves in cold water and methanol extracts.

The different polarity of total phenolic compounds in cold water, methanol and chloroform extracts could be the result of the various quantities of the phenolic compounds in different solvents. *O. sanctum* (Linn) leaves contain more polar phenolic compounds as there is more total phenolic content in methanol and cold-water extracts as compared to chloroform extract.

The total phenolic content of fresh leaves of *O. sanctum* (Linn) was less as compared to dried leaves of the plant in all three extracts. The drying process makes the tissue of the leaves brittle which enhance the breakdown of the cell wall during the grinding process, and more phenolic compound are released during vigorous agitation of the plant sample (Rabeta and Lai, 2013)

The reason for choosing cold water extract instead of doing the extraction at room temperature was to reduce the loss of phenolic compounds, particularly in water extract of fresh leave due to the presence of active enzymes such as polyphenol oxidases that degrade the compounds in the fresh leaves during extraction (Hossain *et al*., 2010). This problem does not occur in dried leaf samples as the enzyme will be deactivated as the water activity decreases due to the effect of drying. These enzymes are also inactivated in the methanol and chloroform solvent for fresh leaf extracts (Prashant *et al*., 2011).

Glucose is the only monosaccharide that will be absorbed into the bloodstream. Rapid degradation of dietary starch leads to elevated post-prandial hyperglycaemia. Hence, the slowdown of starch digestion by inhibiting the activity of α-amylase plays an important role in controlling diabetes (Li *et al*., 2022). Although the consumption of synthetic drugs was efficient in maintaining post-prandial blood glucose levels under control in many patients, these drugs altered side effects (Padhi *et al*., 2020). When these medications are used, they have unpleasant side effects such as diarrhoea, stomach distention, flatulence, hypoglycemia and weight gain, which are potentially dangerous for patients with liver disorders (Maideen, 2019; Wang *et al*., 2019).

The extraction of these plant leaves using solvents with different polarity results in determining the characteristics of the compounds of this plant that has the inhibition activity against α-amylase to reduce post-prandial hyperglycaemia. In this study, methanol and cold water extracts of the dried and fresh leaves of *O. sanctum* (Linn) shows good anti-diabetic activity by inhibiting the α-amylase from catalysing the breakdown of starch. The percentage inhibition of α-amylase by cold water, methanol and chloroform extracts of dried and fresh leaves of *O. sanctum* (Linn) were shown in Figure 2.

Methanol extract from dried leaves of *O. sanctum* (Linn) shows the highest inhibition of α-amylase activity. The second highest inhibitions of α-amylase were found in the cold-water extract, and the lowest

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inhibition was found in the chloroform extract. The percentage of inhibition of methanol and cold water were 47.0% and 34.4%, respectively. The chloroform extract shows the least inhibition activity, which was 7.5%.

The trend of α-amylase inhibition by fresh leaves of *O. sanctum* (Linn) was similar to the dried leaves of the plant. The methanol extract has the highest inhibition activity against α-amylase, followed by cold water extract and finally, the chloroform has the least inhibition activity. From the result obtained it is clear that methanol extracts contain the most compounds that are responsible for the inhibition of α-amylase. The percentage of inhibition by methanol and cold-water extracts were 39.3% and 23.4%, respectively. The percentages of inhibition shown by chloroform extract, which was only 3.6%, indicating that it contains the least compounds that inhibit the α-amylase and is not suitable to be used as an α-amylase inhibitor. The methanol and cold-water extracts of dried leaves show better inhibition activity against α-amylase as compared to methanol and cold-water fresh leaves extract. This is due to the number of compounds extracted from the leaves of *O. sanctum* (Linn). Fresh leaves extract generally contains fewer bioactive compounds as compared to dried leaf extract.

Among the bioactive compounds that have been reported for the ability to inhibit α-amylase are flavonoids, tannin and triterpenoids (oleanane, ursane and lupane) (Sales *et al*., 2012). These compounds are also found in the leaves of *Ocimum* spp. (Flegkas *et al*., 2019; Dev Sharma *et al*., 2022). Therefore, it is necessary to isolate, purify and characterise compounds from methanol and cold-water extracts which show good inhibition activity to use the compounds from these extracts as an anti-diabetic agent.

3.1 Determination of IC_{50} for extracts with the highest inhibition α-amylase activity

From the result obtained in Figure 2, the highest inhibition of α-amylase activity was discovered in the methanol extracts of both dried and fresh leave extracts. Different concentrations of methanol extract from dried and fresh leaves were prepared to determine the IC_{50} for each type of extract.

After plotting the graph of percentage inhibition against the different concentrations of *O. sanctum* (Linn) fresh and dried leaves methanol extract, the IC_{50} value for dried and fresh leaves methanol extract were 10.4 mg/mL and 11.8 mg/mL, respectively.

Increasing the concentration of *O. sanctum* (Linn) dried and fresh leaves decrease the activity of α-amylase. The value of IC_{50} for dried leave methanol extract was lower as compared to IC_{50} for fresh leave methanol extract. This means that a higher concentration of fresh leave methanol extract was needed to inhibit 50% of α-amylase activity as compared to dried leave methanol extract.

3.2 Determination of correlation between total phenolic content and α-amylase inhibition activity of cold water, methanol and chloroform extract

According to Aleixandre *et al*. (2022), certain plant phenolic can inhibit the activity of α-amylase due to its structure and plays important role in the dietary management of Type 2 diabetes. No studies on the relationship between total phenolic content and α-amylase inhibition were done using *O. sanctum* (Linn). Through this study, the correlation between total phenolic content and α-amylase inhibition of *O. sanctum* (Linn) leaves has been revealed. Based on statistical analysis using SPSS 16.0 (Pearson correlation) there is a positive strong relationship between total phenolic content and inhibition activity of α-amylase by extracts of dried and fresh leaves of *O. sanctum* (Linn). The increase of the total phenolic content in the extract increases the inhibition rate of α-amylase activity. The R^2 obtained from this analysis for dried leaf extracts was 0.994, while for fresh leaf extracts was 0.991. The correlation between total phenolic content and α-amylase inhibition activity for dried and fresh leaves was significant at $\alpha = 0.05$. From the correlation value obtained, the bio-compounds that are responsible for the α-amylase are phenolic compounds. Further phenolic compound isolation, purification, and characterization from the three extracts of *O. sanctum* (Linn) leaves have to be done to confirm the specific types of phenolic compounds which are responsible for the α-amylase inhibiting activity. Extracts of *O. sanctum* (Linn) leaves need to be investigated to find out the type of inhibition mechanism exerted by the phenolic compound of this plant on α-amylase.

Conclusion

Through this study, methanol extract from dried leaves was found to have good potential to be used as an anti-diabetic agent. A positive strong correlation was found between the total phenolic content and α-amylase inhibition activity. In conclusion, this study has revealed the anti-diabetic activity of *O. sanctum* (Linn) fresh and dried leaves against α-amylase. This research is helpful for pharmaceutical companies to consider the easily available *O. sanctum* (Linn) plant to develop new products and replace the synthetic drugs that produce side effects. Therefore, health supplements or beverages may be produced from this plant to treat or prevent the occurrence of diabetes.

Conflict of interest

https://doi.org/10.26656/fr.2017.7(S4).18

We have no conflicts of interest to disclose.

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