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Therapeutic potential of *Ocimum sanctum* flower infusion

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ABSTRACT

Infusions of different species of edible flowers have been used since ancient times as remedies for several disorders. *Ocimum sanctum* is a herb used in Ayurvedic treatment due to its bioactive composition and potential therapeutic properties. However edible flowers of this herb have been under-explored and there are no much studies which validate their health potential scientifically. Therefore the present study aims to investigate the bioactivities of *O.sanctum* flower infusions. The total phenolic content and total flavonoid content of the infusion was 68.41±0.91 µmol gallic equivalents per g and 8.32±1.44 µmol rutin equivalents per g respectively. Considering the antioxidant activity, the infusion was able to scavenge hydrogen peroxide, inhibit lipid peroxidation and expressed reducing power. The infusion also expressed good anti-diabetic property by inhibiting α-amylase, α-glucosidase and amyloglucosidase activities with IC_{50} values 2.05±0.21, 97.21±1.21 and 2.84±0.72 µg/mL respectively. The ability of the infusion to inhibit angiotensin converting enzyme and acetylcholinesterase enzyme indicates the potential of the infusion to be used as anti-hypertensive agent and anti-Alzheimer's agent respectively. *O.sanctum* infusion is a good source of phenolic compounds which could exert antioxidant, anti-inflammatory, anti-diabetic, anti-hypertensive and anti-Alzheimer's activity. Therefore, it can be used as functional ingredient with wide applications in food and pharmaceutical industry.

Keywords: Antioxidants; Edible flowers; infusion; phenolic compounds

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1. Introduction

Herbal infusions have been used since immemorial times in phyto-medication system as curative agents. They were used to treat several chronic diseases such as hypertension, diabetes, neurodegenerative disorders and cancer. Herbal infusions are also called as tisanes and prepared from variety of spices, whole herbs, leaves, roots and edible flowers (Rodrigues *et al*., 2016), by adding hot water. These are usually considered as caffeine-free teas and traditionally consumed by people from various communities to recover from general health disorders such as fever, cough, dysentery, mouth and gastric ulcers and in some ophthalmic problems (Jaggi *et al*., 2003). Apart from other plant parts which are consumed as infusions, recently edible flower infusions are gaining renewed interest due to their established therapeutic potential and unique bioactive compositions. This has sparked of the research interests of food scientist on exploring different species of edible flower infusions for their health potential and future applications. Such one widely known plant species, however underexplored for the therapeutic potential of its edible flowers is *Ocimum sanctum* (Biswas and Biswas, 2005). *O.sanctum,* belongs to the family Lamiaceae and generally known as holy basil or Tulsi. It is an aromatic medical herb, widely available in most of the tropical countries. It is referred as the queen of herbs in India, due to its vital role played in the Ayurvedic and Unani system of medicine (Biswas and Biswas, 2005). Essential oil and extracts of fresh or dried leaves of *O.sanctum* have been used as remedies for cardiovascular diseases, cold, inflammation and gastric disorders and also

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investigated by researchers to validate its health potential (Uma devi *et al*., 2000). Findings have reported the presence of rosmarinic acid, propanoic acid, apigenin, cirsimaritin, isothymusin and orientin in the leaves of *O.sanctum.* These compounds are believed to exert bioactivities such as antioxidant, anti-inflammatory, anti-diabetic and cardiac activity (Kumar *et al*., 2022). However, the edible purple flowers of this plant have not been widely investigated by researchers. Flowers are added to salads, soups and also consumed as infusions. Medicinally, fresh flowers have been used to treat bronchitis. There are no much investigations regarding the bioactivities of *O.sanctum* flower infusions and the present study aims to quantify the bioactive contents and evaluate the bioactivities of *O.sanctum* flower infusions.

2. Material and Methods

2.1. Sample collection and preparation of infusion

Fresh *O.sanctum* flowers were collected, cleaned and washed with distilled water. The drained floral parts were frozen for 24 hours at -20°C prior to freeze drying at -35°C for 30 hours at 46µbar (Alpha 1-2 L Dplus, Germany) and reduced to fine powder (0.7 mm) and stored in amber glass bottles at -20°C until analysis. About 2 g of flower sample was added in to commercial tea bags and the infusion was prepared using the domestic procedure where each bag was steeped for 5 minutes in 200 mL of boiling water. The bioactive content and bioactivity of the obtained extract was evaluated as described below.

2.2. *Phytochemical analysis*

Total phenolic content (TPC) and total flavonoid content (TFC) of the extracts were determined by Folin- Ciocalteau method and aluminum chloride method respectively as described by Janarny and Gunathilake (2020). TPC was expressed as mg gallic acid equivalents per g (mg GAE/g) and TFC was expressed as mg rutin equivalents per g (mg RE/g).

2.3. Determination of antioxidant activity

2.3.1. Hydrogen peroxide scavenging assay

The method described by Ali *et al*., (2019), was followed to determine the hydrogen peroxide scavenging activity of the samples. Hydrogen peroxide solution (40 mM, 600 µL) was mixed with 400 µL of the samples and made up to 2 mL with 50 mM sodium phosphate buffer (pH 7.4). The mixture was incubated at 30°C for 40 minutes and the absorbance of the mixture was measured at 230 nm using a UV/visible spectrophotometer (840-210800 Thermo Fisher Scientific, USA).

2.3.2. Inhibition of lipid peroxidation

Thiobarbituric acid reactive substances (TBARS) assay described by Ohkowa *et al*., (1979) was used to measure the ability of the flower extracts to inhibit lipid peroxides in egg yolk homogenates. Accordingly, 500 µL of 10% egg homogenate was mixed with 100 µL of samples. Then, distilled water (400 µL) and 50 µL of 70 µM ferrous sulphate was added and mixed well. The resulting mixture was incubated for 30 minutes at room temperature. After incubation, 20% acetic acid (1.5 mL), 0.8% (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulphate (1.5 mL) and 20% trichloroacetic acid (50 µL) were added and the mixture was vortexed well. The resulting mixture was heated at 95 °C for 60 minutes. After cooling, 5 mL of butanol was added and centrifuged at 528 g for 10 minutes. Control was prepared by replacing the sample with the solvent. Absorbance of the mixture was read at 532 nm and percentage inhibition of lipid peroxidation was calculated as follows,

$$\% \text{ Inhibition} = \frac{A\ control - A\ sample}{A\ control} \times 100 \quad (1)$$

2.3.4. Ferric reducing power

The ferric reducing power of the prepared extracts was determined according to the method described in Janarny and Gunathilake (2020). Samples (1mL) was added with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 1% (w/v) potassium ferricyanide solution (2.5 mL). The mixture was incubated at 50 °C for 20 minutes and then 2.5 mL of 10% (w/v) trichloroacetic acid solution was added. The homogenized mixture was centrifuged at 1000 g for 10 minutes. From the supernatant 2.5 mL was separated and mixed with 2.5 mL of distilled water and 500 µL of 0.1% (w/v) ferric chloride solution. Absorbance of the reaction mixture was read using UV/visible spectrometer at 700 nm and the results are expressed as µmol ascorbic acid equivalents (AAE) per g DW of flowers.

2.4. *Determination of anti-inflammatory activity*

2.4.1. Inhibition of denaturation of bovine serum albumin

Protein denaturation assay described by Gambhire *et al*., (2009) was followed to evaluate the anti-inflammatory properties. Accordingly, 200 µL of 1 % bovine serum albumin (BSA), 4.78 mL of phosphate buffered saline (PBS, pH 6.4) and 20 µL of samples were mixed together and was incubated at 37°C for 15 minutes in a water bath. After incubation the reaction mixture was heated at 70°C for 5 minutes. After cooling the absorbance of the solution was measured at 660 nm using UV/visible microplate reader. PBS without sample was used as the control and the percentage inhibition of protein denaturation was calculated.

2.4.2. Inhibition of proteinase denaturation

Modified method of Sakat *et al*., (2010) was followed to evaluate the inhibitory effect of samples against proteinase. Briefly, 0.06 mg trypsin, 1 mL of 20 mM Tris-HCl buffer (pH 7.4), 20 µL of samples and 980 µL of ethanol were mixed, and the reaction mixture was incubated at 37°C for 5 minutes, and then 1 mL of 0.8% (w/v) casein was added. The mixture was incubated further for an additional 20 minutes. About 2 mL of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged, and the absorbance of the supernatant was read at 210 nm against buffer as blank. Phosphate buffer solution without sample was used as the control.

2.5. *Determination of anti-diabetic activity*

2.5.1. *Inhibition of α-glucosidase activity*

The α-glucosidase inhibition was determined using the method of Elya *et al*.,(2012). Briefly, 200 µl of 67 mM sodium phosphate buffer (pH 6.8) and 120 µl of 10 mM *p*-nitrophenyl α-D-glucopyranoside was added to 40 µl of samples. The homogenized mixtures were pre incubated for 15 minutes at 37°C. After incubation, 40 µl of 0.1 U α-glucosidase from *Saccharomyces cerevisiae* was added to the mixture. The reaction mixture was incubated for 15 minutes at 37°C. Reaction was terminated by adding 200 mM sodium carbonate (800 µl). The hydrolysis of α-D-glucopyranoside to *p*-nitrophenol was measured at 405 nm. Acarbose was used as the standard compound for all the anti-diabetic assays.

2.5.2. *Inhibition of α-glucosidase activity*

In vitro α amylase activity was measured using the method described in Xiong *et al*., (2020). Samples of 40 µL were mixed with 100 µl of the α-amylase enzyme (27.5 mg of enzyme in 100 mL of 20 mmol sodium phosphate buffer, pH 6.9). Then the reaction mixture was pre-incubated at 37 °C for 20 minutes. At the end of incubation, 100 µl of 1% starch solution was added into the mixture and further incubated at 37 °C for 10 minutes. After incubation, 200 µl of dinitro salicylic acid solution was added for colour development and the reaction was terminated by placing the samples in a boiling water bath for 5 minutes. Absorbance was read at 540 nm by UV/visible microplate reader and the percentage inhibition was calculated.

2.5.3. *Inhibition of amyloglucosidase activity*

The *in-vitro* amyloglucosidase inhibition activity of samples was evaluated as described by Chiranthika *et al*., (2021) with slight modifications. Samples (40 µL) were mixed with equal volume of freshly prepared amyloglucosidase (6.5 U/ mL in 0.1M sodium phosphate buffer, pH 6.9) and incubated in a water bath at 37 °C for 20 minutes. Subsequently, 60 µL of 1% starch solution was added as the substrate to each reaction mixture and incubated at 37 °C for 10 minutes. Then 100 µL of dinitro salicylic acid was added and incubated in a boiling water bath for 5 minutes. Then reaction mixtures were cooled in an ice-water bath before adding 2.5 mL of distilled water to dilute the reaction mixture. The absorbance of the resultant mixture was measured at 540 nm using a UV/Visible microplate reader. A blank was prepared by replacing amyloglucosidase and 1% starch solution with sodium phosphate buffer. Control was prepared by replacing the amyloglucosidase enzyme with sodium phosphate buffer.

2.6. *Determination of anti-hypertensive activity*

2.6.1. *Inhibition of angiotensin converting enzyme (ACE) activity*

The ACE inhibitory property of the samples was assayed by the modified method of Das and De (2013), using hippuryl-histidyl-leucine (HHL) as a substrate. ACE from rabbit lung was prepared in 200 mM borate buffer (pH 8.3) at a concentration of 100 $mUmL^{-1}$. A reaction mixture containing 250 µL of 7 mM HHL in pH 8.3 borate buffer (200 mM), 200 µL NaCl (2 M), 20 µL distilled water, 15 µL of samples and 15 µL of prepared ACE was incubated at 37 °C for 30 minutes. At the end of incubation, the reaction was terminated by adding 250 µL of 1N HCl. The hippuric acid liberated from the HHL by ACE was extracted with ethyl acetate (1.5 mL). An aliquot of the extract (1.3 mL) was evaporated to dryness and the residue was dissolved in 400 µL of distilled water. The control contained 15 µL of distilled water instead of sample. The hippuric acid concentration was determined by measuring the absorbance spectrophotometrically at 228 nm against a blank solution similarly prepared by adding buffer instead of ACE. The percentage inhibition of ACE activity was calculated.

2.7. *Determination of anti-Alzheimer's activity*

2.7.1. *Inhibition of acetylcholinesterase (AChE) activity*

The method described by Jukic *et al*., (2007) was used to evaluate the inhibitory property of the samples against AChE activity. The reaction mixture was prepared by adding 1 mL of 0.05 M Tris-hydrochloric acid buffer at pH 7.8; 10 µL of α-naphthyl acetate, at a final concentration of 0.53 mM; and 20 µL of a sample solution. The homogenized mixture was pre-incubated at 37 °C for 5 minutes. The reaction was initiated by adding 20 µL of AChE, at a final assay concentration of 95 mU/mL and absorbance at 321 nm was taken after 15 minutes of incubation at 37 °C. Control was prepared by replacing the samples with the solvent. Percentage inhibition was calculated using the equation and Galanthamine was used as the standard.

2.8. *Statistical analysis*

All the assays were conducted in triplicates, and the results were expressed as mean± standard deviation. SPSS version 16.0 software was used for statistical calculations, and the statistical differences were determined with one-way ANOVA. Mean separation was carried out by Tukey's multiple variance test, and the $p < 0.05$ was considered as significantly different.

3. Results and Discussion

3.1. Phytochemical analysis

The TPC of the *O.sanctum* flower infusion was quantified using the Folin-Ciocalteau method, which is a widely used simple and reproducible method. The assay relies on the transfer of electrons from the phenolic compounds to the Folin-Ciocalteau reagent in alkaline media. In the present study it was found that the TPC of the infusion was 68.41±0.91 µmol GAE /g DW. Reshma and Brindha (2014), has reported that the TPC of aqueous *O.sanctum* leaf extracts was 70.53 µmol GAE/g whereas Chaudry *et al*., (2020) has reported that the TPC of butanol extracts of *O.sanctum* leaves was 1.24 µmol GAE/g which is a significantly lower value compared to the data from the present work. Though there are no much studies based on the polyphenol profile of flowers of *O.sanctum* which contributes to its TPC value, researchers have documented the presence of certain bioactive compounds from this plant. Rosmarinic acid, chlorogenic acid, caffeic acid and ursolic acid have been detected in the leaves and stem of *O.sanctum* which could also be present in the flowers (Mondal *et al*., 2010). The TFC of the infusion was measured using the aluminum chloride colorimetric assay. This method utilizes Al (III) as a chelating agent to form complexes with the flavonoids which is characterized by the absorption band at 500 nm (Shraim *et*

al., 2021). According to the present work the TFC of the infusion was noted to be 8.32±1.44 µmol RE/g DW. Vastrad *et al*., (2015) have reported the TFC of ethanolic extracts of *O.sanctum* leaves as 156 nmol RE/g DW which indicates that *O.sanctum* flower infusions are better sources of flavonoids compared to ethanolic extracts of leaves. Chromatographic profile of methanolic extracts of leaves and stem of *O.sanctum* investigated by Ullah *et al*., (2022) has revealed the presence of flavonoids such as apigenin, kaempferol, rutin and quercetin which could also be extracted from the flowers of *O.sanctum.*

3.2. Antioxidant activity

In comparison to the other parts of the plant, antioxidant compounds play a crucial role in flowers as they prevent senescence of the petals caused by the action of free radicals in the cell membranes leading to the loss of ions and changing the osmotic pressure of the flowers. *O.sanctum* flower infusion could exert antioxidant activity through multiple mechanisms depending on its bioactive composition and their chemo properties. Owing to the complex characteristics of the bioactive compounds the antioxidant potential of the infusion cannot be evaluated using a single assay. Therefore, the present study employed three assays: hydrogen peroxide scavenging assay, assay to evaluate the inhibition of lipid peroxidation and ferric reducing power assay. The results obtained are summarized in Table 1.

Table 1. Antioxidant, anti-inflammatory and anti-diabetic activity of O.sanctum flower infusion. Data are given as means ± S.D. (n = 3).

Bioactivity	***O.sanctum* infusion**
Antioxidant activity	
Hydrogen peroxide scavenging assay (IC_{50} in µg/mL)	73.45±1.73
Inhibition of lipid peroxidation (IC_{50} in µg/mL)	121.75±0.55
Ferric reducing power (µmol AAE/g DW)	0.47±0.53
Anti-inflammatory assays	
Inhibition of bovine serum albumin denaturation (IC_{50} in µg/mL)	101.54±0.84
Inhibition of proteinase activity (IC_{50} in µg/mL)	780.03±1.12
Anti-diabetic assays	
Inhibition of α-amylase activity (IC_{50} in mg/mL)	2.05±0.21
Inhibition of α-glucosidase activity (IC_{50} in µg/mL)	97.21±1.21
Inhibition of amyloglucosidase activity (IC_{50} in mg/mL)	2.84±0.72

Considering the hydrogen peroxide scavenging assay, the infusion was able to scavenge 50% of hydrogen peroxide at a concentration of 73.45±1.73 µg/mL. Hydrogen peroxide is a non-radical reactive oxygen species with biological significance. It is produced during the regular aerobic metabolism and the production is enhanced with exposure to radiation and stressful conditions. Excess levels of hydrogen peroxide are harmful to the body as it can oxidize the thiol groups in enzymes and inactivate them. It can also be converted to other more harmful free radicals such as hydroxyl radicals and affect cellular functions (Guzik *et al*., 2003). Therefore the ability of *O.sanctum* infusion to remove hydrogen peroxide indicates that it can act as strong antioxidant and combat oxidative stress. As reported by Reshma and Brindha, (2014) IC_{50} value for hydrogen peroxide scavenging activity of aqueous extracts of *O.sanctum* leaves was 157.7 µg/mL, which is higher compared to the findings of the present study. This indicates that *O.sanctum* flower infusion is effective scavenger of hydrogen peroxide. Studies conducted on infusions of other flower species were also in line with the present findings. For instance, *Crataegus monogyna* extracts were able to scavenge 40% of hydrogen peroxide at 200 µg/mL (Keser *et al*., 2012). Generally, hydrogen peroxide scavenging activities by phenolic compounds, is correlated with the number of hydroxyl groups bonded to the aromatic ring and the position at which hydroxyl groups are present in the aromatic rings (Kumar *et al.,* 2022). It was observed that stronger radical and hydrogen peroxide scavenging activities were shown by compounds with an ortho and para system of hydroxyl substitution in relation to each other, while compounds with hydroxyl groups in a meta position exert lower scavenging activities.

Ability of extracts to inhibit lipid oxidation is another mechanism which measures the antioxidant potential of the extracts. Low-density lipoproteins modification by oxidation has been proposed as a major factor for the development of atherosclerosis and the deterioration of the nutritional value of foods. Unsaturated fatty acids, which make up the majority of bio membranes, are vulnerable to oxidation, which affects the osmotic fragility and viscosity of cellular membranes (Félix *et al*., 2020). Since antioxidants have shown to control these modifications, the antioxidant capacity of the infusion has been assessed by measuring the bioactives' capacity to prevent lipid peroxidation. In the current study, the percentage inhibition of lipid peroxidation caused by the infusion was determined using the thiobarbituric acid assay. Egg yolk, which contains linoleic and linolenic acids, is used as the lipid substrate. These fatty acids combine with oxygen to form malonaldehyde, which turns pink when combined with thiobarbituric acid. As a result, a lower color seen after the reaction denotes a higher level of inhibition. Data of the present study indicates that the infusion was able to inhibit 50% of the lipid oxidation at a concentration of 121.75±0.55 µg/mL. Studies by Geetha *et al*., (2004) has reported that aqueous and alcoholic extracts of *O.sanctum* leaves inhibited lipid peroxidation with IC_{50} values of 80 µg/mL and 16 µg/mL respectively, which emphasizes that leaves exhibit better inhibition than flowers. Reducing power assay is a potential indicator of antioxidant capacity and involves the presence of reductants, which can reduce oxidative damage by donating an atom of hydrogen to break the chain of free radicals. The oxidation chain reaction can be stopped by reducing the oxidized intermediates into the stable form using antioxidants with a high reducing power, which are regarded as excellent electron donors. In the present work reducing power of the infusion was noted to be 470.00 ±0.53 nmol AAE/g DW. As reported by Kelm *et al*., (2000) antioxidant directed extraction of leaves and stems have yielded compounds cirsimaritin, circirsilineol, isothymusin, apigenin, isothymonin, rosmarinic acid and eugenol which could also be present in the flowers of *O.sanctum* and contribute to its antioxidant activity.

3.3. Anti-inflammatory activity

Inflammation plays a vital role in the development of various diseases, such as rheumatoid arthritis, and atherosclerosis, which are highly prevalent world-wide. The pro-inflammatory mediators such as nitric oxide, tumor necrosis factor-α, and interleukins are produced as a result of the inflammatory reactions, which in turn

causes the activation of circulating leukocytes. The development of several chronic inflammatory diseases is linked to a high production of pro-inflammatory mediators. Therefore, one most convenient way to lower the effects brought by excessive inflammation is to inhibit excessive inflammatory response, particularly through diet. The present study has employed two assays to evaluate the anti-inflammatory potential of the infusion through two different mechanisms. Denaturation of tissue proteins is a well-documented phenomena associated with inflammation. It has been reported that denatured proteins lead to the formation of antigens which initiates type III hypersensitive reaction causing inflammation (Heendeniya *et al*., 2018). Hence, compounds which are capable of preventing protein denaturation can acts as potential anti-inflammatory drugs. This principle is applied in evaluating the anti-inflammatory potential of flower infusion through inhibition of BSA denaturation. From the present work it was noted that the 50% of denaturation was inhibited by the infusion at a concentration of 101.54±0.84 µg/mL. Sharma et al., (2022), has compared the anti-inflammatory activity of three different extracts of *O.sanctum* and reported that ethanolic extracts inhibited protein denaturation better than methanol and acetone extracts.

Proteinases have been associated with arthritic reactions. Neutrophils, in their lysosomal granules, carry many serine proteinases and proteinases of leukocytes play a significant role in the development of tissue damage during inflammatory processes. Hence, a significant level of protection against inflammation is provided by proteinase inhibitors (Gunathilake *et al*., 2018). *O.sanctum* infusion investigated in the present work was able to inhibit 50% of proteinase activity at a concentration of 780.03±1.12 µg/mL indicating potential anti-inflammatory activity.

Anti-inflammatory activity of various parts of *O.sanctum* has been attributed to the presence of phenolic acids and flavonoids detected in this herb. For instance, apigenin has been reported to inhibit nitric oxide production specially, due to their C-2,3 double bond and substitution of hydroxyl groups on A and B rings. It also plays a vital role in inhibiting the expression of cytokine genes and their associated protein kinases. Chlorogenic acid has been reported to control inflammatory actions by inhibiting the secretion of interleukins and by reducing the expression of mRNA. This mechanism is associated with the presence of catechol groups (Pandi and Kalappan, 2021).

3.4. *Anti-diabetic activity*

Diabetes mellitus is a chronic endocrine disease characterized by high fasting plasma glucose concentration of ≥126 mg/dl. There are two types of diabetes mellitus: Type 1 (insulin dependent) diabetes mellitus and Type 2 (non-insulin dependent) diabetes mellitus. One of the strategies for the management of Type 2 diabetes is to decrease postprandial hyperglycemia through inhibiting carbohydrate digestive enzymes. In the present study it was noted that the infusion was able to inhibit 50% of α-amylase, α-glucosidase and amyloglucosidase at concentrations of 2.05±0.21, 97.21±1.21 and 2.84±0.72 µg/mL respectively. Several investigations conducted previously by different authors produced outcomes which were consistent with the findings from the present work. Zhao *et al*., (2015) has reported that *Edgeworthia gardneri* flowers are good sources of α-amylase and α-glucosidase inhibitors, and their inhibitory activity has attributed to the coumarins and their derivatives present in these flowers. Aqueous extracts of *Chenopodium album* flowers have been reported to inhibit 3.82% of α-amylase activity which is similar to the findings from the current study. Findings of Sharma et al., (2022), has reported that acetone extracts of *O.sanctum* species were able to inhibit 60% α-amylase activity which was less than the value obtained from the present study.

α-amylase and α-glucosidase enzymes are two main enzymes involved in the carbohydrate metabolism. α-amylase breaks down the glycosidic linkages of α-D-(1,4) in carbohydrates to produce oligosaccharides, which are further cleaved to monosaccharide glucose by α-glucosidase. The enzyme inhibitory activity of polyphenols extracted in to the infusion is highly related to its molecular structure and binding interaction between the enzyme and polyphenols (Sun *et al*., 2020). For example, for flavonoids such as quercetin and apigenin, the presence of hydroxyls (-OH) at 5-, 6-, 7-positions of ring A and at 4′ -position of ring B are able to enhance the inhibitory activity. Considering the structure activity relationship of phenolic acids in enzyme inhibition, chlorogenic acid has been identified to effectively inhibit both the enzymes in a mixed mode of inhibition. Reports also confirm that phenolic acids and starch digestive enzymes interact by non-covalent interactions, being responsible of their inhibitory activity. Particularly by hydrogen binding, salt bridge interactions or electrostatic forces. It has also been reported that interactions of α-amylase with phenolic acids are able to cause conformational changes of the enzyme molecule, no matter the resulted inhibition is reversible or irreversible (Aleixandre *et al*., 2022).

3.5. *Anti-hypertensive activity*

Hypertension is one of the extensively reported cardiovascular risk factors which leads to the pathological conditions such as cardiac failure, renal disorders and stroke. ACE is an exopeptidase enzyme involved in the renin–angiotensin–aldosterone hormonal system of human body to regulate blood pressure. The key functions of ACE are catalyzing the conversion of angiotensin I to angiotensin II and degrading bradykinin, and other vasoactive peptides (Hussain *et al*., 2018). These two functions make ACE inhibition a goal in the treatment of conditions including high blood pressure, heart failure and nephropathy. In the present assay, ACE catalyzes the degradation of the substrate HHL to hippuric acid and therefore inhibitory activity can be assessed using the increase in the absorbance at 228 nm during the reaction (Xie and Zhang, 2012). Based on the outcomes of the study as illustrated in Fig 1, the *O.sanctum* infusion inhibited 50% of ACE activity at a concentration of 65.47±0.91 µg/mL. Kwon *et al*., (2006) has reported that aqueous extracts of Rose flowers strongly inhibited ACE activity by 133% and methanolic extracts of *Musa paradisiaca* flowers inhibited 68.63% of ACE activity at a concentration of 15 µg/mL which were better inhibitors than *O.sanctum* infusion.

Investigations have highlighted two major mechanisms by which phenolic compounds inhibit the activity of ACE enzyme. One is that the compounds lead to conformational rearrangement and reduces the ability of the enzyme to accommodate the substrate and secondly, flavonoids such as apigenin bind with the zinc atom of the enzyme and generate chelates thereby reducing the activity of ACE enzyme (Ali *et al*., 2019).

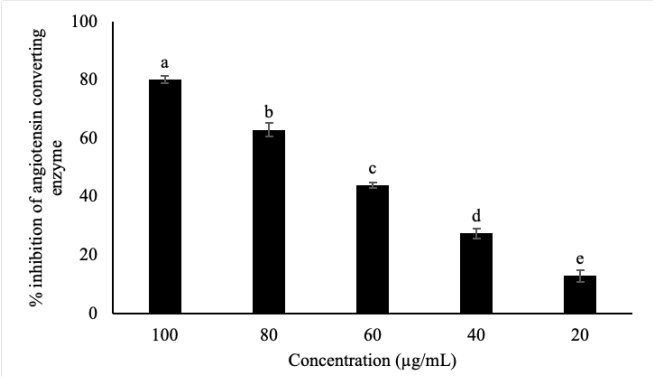

Fig. 1. Inhibition percentage of angiotensin converting enzyme activity by *O.sanctum* flower infusion at different concentrations. Data are given as means ± S.D. (n = 3).

3.6. *Anti-Alzheimer's activity*

Alzheimer's disease (AD), is the most common form of dementia and is a progressive age-related disorder that is identified by the degeneration of neurological function (Adewusi *et al*., 2011). Epidemiological investigation reveals that AD is primarily due to the drop in the levels of the neurotransmitter acetylcholine, in the brains of the elderly people. Acetylcholinesterase inhibitors (AChEIs) have been shown to function by increasing acetylcholine within the synaptic region, thereby restoring deficient cholinergic neurotransmission. Selective cholinesterase inhibitors, free of dose-limiting side effects, are not currently available, and current compounds may not allow sufficient modulation of acetylcholine levels to elicit the full therapeutic response (Felder *et al*., 2000). Therefore, the search for new AChEIs, particularly from natural products, with higher efficacy continues. In the present study, the colorimetric method was used to determine the inhibitory activity of the samples based on the increase in absorbance at 321 nm that occurs with the hydrolysis of α-naphthyl acetate to α-naphthol. *O.sanctum* infusion was able to inhibit 50% of the AchE activity at a concentration of 140.21±1.68 µg/mL (Fig 2). Calendula flowers have been reported with 50% AchE inhibitory activity at a dosage of 223.9 µg/mL and the activity has been correlated to its flavonoid content and phenylpropanoids (Olennikov *et al*., 2017).

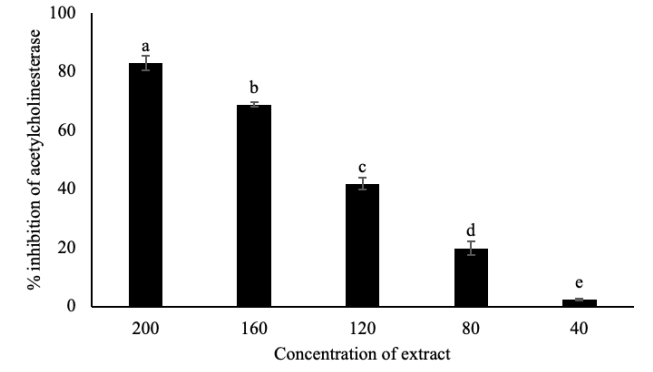

Fig. 2. Inhibition percentage of acetylcholinesterase activity by infusion of *O.sanctum* flowers at different concentrations. Data are given as means ± S.D. (n = 3).

Only a limited number of studies have been conducted to date regarding the molecular mechanism of acetylcholine esterase inhibition by polyphenols. It has been identified that presence of methoxy group in the phenolic acids enhances the inhibitory activity as it can orientate towards the peripheral anionic site of the enzyme and form a connection with it and inhibit the enzyme activity. Flavonoids such as apigenin has been reported to inhibit AChE by blocking the active site of the enzyme rather than interfering with their tertiary structure. This is achieved by the formation of hydrogen bonds between the amino acid residues of the anionic sub site of the enzyme and the carbonyl moiety or -OH group of the carbon ring (Jukic *et al*., 2007).

4. Conclusion

Infusion of edible flowers of *O.sanctum* are rich sources of phenolic compounds. These compounds are capable of exerting antioxidant, anti-inflammatory, anti-diabetic, anti-hypertensive and anti-Alzheimer's activity. Therefore, the infusion can be used for potential applications in functional food and pharmaceutical industry.

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Conflict of interest

The authors declared that they have no conflict of interest.

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