J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 48(2): 436-455, 2024
Doi: 10.33483/jfpau.1378992
ORIGINAL ARTICLE / ÖZGÜN MAKALE

WHAT DO PEOPLE PREFER TO SUPPORT DIABETES TREATMENT IN TURKIYE? A STUDY ON OLIVE LEAF AND DIABETES

TÜRKİYE'DE DİYABET TEDAVİSİNE DESTEK OLMAK İÇİN İNSANLAR NE TERCİH EDİYOR? ZEYTİN YAPRAĞI VE DİYABET ÜZERİNE BİR ARAŞTIRMA

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ABSTRACT

Objective: *A questionnaire study was conducted to evaluate the applications of plants and herbal products consumed by diabetic patients for the treatment of chronic health conditions. Evaluation of the questionnaire confirmed that olive leaf is one of the most used herbs in the treatment of diabetes, in line with its traditional use in the literature. In vitro biological activity studies were performed to determine whether different olive leaf samples have antidiabetic effects. Additionally, the major component oleuropein was quantitively determined in the samples.*
Material and Method: *The established survey was firstly approved by the ethics committee at Gazi University then the survey was conducted at the University Hospital, Department of Endocrinology between January 2021, and July 2021. Based on the result of the survey, pharmacognostic analyses, chromatographic analyses, and inhibition on diabetes-related enzymes (α-amylase, α-glucosidase, and aldose reductase) were performed on the samples of olive leaves collected from nature, obtained from herbalists, markets and pharmacies.*
Result and Discussion: *Evaluation of the survey revealed that the patients mostly used cinnamon (29.3%) and olive leaves (21.7%) for the treatment of diabetes, and these plants were generally obtained from herbalists (51.7%). The study findings showed that aqueous and ethanolic extracts prepared from olive leaf samples contained 190.3-374.3 mg/g oleuropein. The amount of oleuropein in the ready-made olive leaf extract from herbalists was found to be much lower (50.9 mg/g) than the other olive leaf extracts. When the enzyme inhibition activity assays were evaluated, it was determined that all olive leaf samples had inhibitory effects on α-amylase, α-glucosidase, and aldose reductase enzymes. All olive leaf samples, including teas prepared by the public at home with water, were found to have capacity to decrease the blood level in other words antidiabetic activities in vitro. The oleuropein contents detected in this study once again revealed the importance of meticulous examination in herbal products.*
Keywords: *Aldose reductase, α-amylase, α-glucosidase, high pressure liquid chromatography, Olea europea*

ÖZ

Amaç: *Diyabet hastalarının tükettikleri bitki ve bitkisel ürünlerin kronik sağlık durumlarının tedavisine yönelik uygulamalarının değerlendirilmesi amacıyla bir anket çalışması yapılmıştır. Anket sonuçları, literatürdeki geleneksel kullanımına paralel olarak zeytin yaprağının diyabet tedavisinde en çok kullanılan bitkilerden biri olduğunu doğrulamıştır. Farklı zeytin yaprağı*

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Submitted / Gönderilme : 20.10.2023
Accepted / Kabul : 23.01.2024
Published / Yayınlanma : 20.05.2024

örneklerinin antidiyabetik etkisinin olup olmadığının belirlenmesi amacıyla in vitro biyolojik aktivite çalışmaları yapılmış ve ana bileşen oleuropein kantitatif olarak belirlenmiştir.

Gereç ve Yöntem: *Anket için öncelikle Gazi Üniversitesi Etik kurulu tarafından onay alınmış, Ocak 2021-Temmuz 2021 tarihleri arasında Gazi Üniversitesi Endokrinoloji Bölümü'nde uygulanmıştır. Çalışmaya 18 yaş üstü Tip 1 ve Tip 2 diyabetli 200 hasta dahil edilmiştir. Anket sonuçlarından yola çıkarak, doğadan toplanan, aktarlardan, marketlerden ve eczanelerden temin edilen zeytin yaprağı örnekleri üzerinde farmakognozik analizler (makroskopik ve mikroskobik analiz, toplam kül, kuruma kaybı), kromatografik analizler (ince tabaka kromatografisi ve yüksek basınçlı sıvı kromatografisi analizleri) ve diyabetle ilişkili enzimlerin (α-amilaz, α-glukosidaz ve aldoz redüktaz) inhibisyonu çalışmaları yapılmıştır.*

Sonuç ve Tartışma: *Anketin değerlendirilmesinde hastaların diyabet tedavisi için en çok tarçın (%29.3) ve zeytin yaprağını (%21.7) kullandığı ve bu bitkilerin genellikle aktarlardan (%51.7) temin edildiği görülmüştür. Çalışma bulguları, zeytin yaprağı örneklerinden hazırlanan sulu ve etanol ekstraktlarının 190.3-374.3 mg/g oleuropein içerdiğini göstermiştir. Aktarlardan alınan hazır zeytin yaprağı ekstraktındaki oleuropein miktarı diğer zeytin yaprağı ekstraktlarına göre çok daha düşük (50.9 mg/g) bulunmuştur. Enzim inhibisyon aktivite testleri değerlendirildiğinde, tüm zeytin yaprağı örneklerinin α-amilaz, α-glukosidaz ve aldoz redüktaz enzimleri üzerinde inhibitör etkiye sahip olduğu belirlenmiştir. Halk tarafından evde su ile hazırlanan çaylar da dahil olmak üzere tüm zeytin yaprağı örneklerinin in vitro kan seviyesini düşürme yani antidiyabetik aktiviteye sahip olduğu tespit edilmiştir. Bu çalışmada tespit edilen oleuropein içerikleri bitkisel ürünlerde titiz incelemenin önemini bir kez daha ortaya koymuştur.*

Anahtar Kelimeler: *Aldoz redüktaz, α-amilaz, α-glukozidaz, Olea europea, yüksek performanslı sıvı kromatografisi*

INTRODUCTION

Diabetes is a chronic metabolic disease characterized by high blood glucose levels, which occurs because of insufficient insulin production from the pancreas or inability to use the produced insulin effectively in the body [1]. If the blood glucose level is not controlled, it can lead to increased morbidity and mortality with serious complications.

In many countries and Türkiye various plants and plant products are used in the treatment of diabetes due to their antidiabetic effects [2-4]. In a study conducted with 453 Type 2 diabetes patients in Nigeria, 67.3% of the patients were found to use only herbal medicine, and 35.4% of them used herbal medicines together with conventional medicines. As a result of that study, *Vernonia amygdalina* Delile, *Moringa oleifera* Lam., *Ocimum gratissimum* L., *Picralima nitida* T. Durand & H. Durand plants and mixtures containing these plants were determined to be among the most preferred plants. The data showed that herbal medicine use was associated with age, education level, occupation, duration of diabetes mellitus symptoms, diabetes management style, positive history of diabetes, and presence of diabetes complications [4]. In another study conducted in Nigeria, Aloe vera, garlic, and ginger were determined to be used differently from these plants [5].

In Thailand, more than half (61%) of diabetic patients, who applied to endocrine clinic reported that they used herbal products. Patients mostly used turmeric, bitter gourd, reishi mushroom, ginseng, and cinnamon [6]. In the USA, it was reported that adults with diabetes preferred herbal treatments (56.9%) the most among complementary and alternative medicine applications [7]. In eastern Morocco, more than half (54.8%) of 279 diabetes patients used herbal supplements. The most used are *Salvia officinalis* L., *Trigonella feoenum graecum* L., *Olea europea* L., *Artemisia herba-alba* Asso, and *Origanum vulgare* L. plants/products [8]. In Iran, it was determined that 54% of 500 Type 2 diabetes patients used at least one plant and the most used plant was cinnamon (24%) [9]. In a study of 519 Type 2 diabetes patients in Serbia, 94.5% of women and 82.3% of men used herbal dietary supplements in addition to the prescribed treatment. While women mostly used garlic and St. John's Wort based products, men preferred ginseng and cinnamon based products [10].

In Turkiye, a study conducted on 453 adult diabetic patients, showed that 46.1% of the patients utilized complementary and alternative medicine applications. The most preferred application is the use of herbal products containing black cumin (26.6%), cinnamon (23.3%), and olive leaves (12.5%) [11]. In a study investigating the use of herbal products in 150 adult diabetic patients who applied to the endocrine clinic, it was determined that 22% of the patients used herbal products. It was reported that the most used herbal products were cinnamon (5.3%), lemon (4.7%), pomegranate syrup (3.3%), and green tea (2.7%). Other herbal products applied included almond, yarrow, sage, olive leaf tea and black cumin oil [12]. In a study conducted with 120 Type 2 diabetes patients, it was found that 52.1% of the patients used herbal products after being diagnosed with diabetes [13]. In a study conducted with 193 adult Type 2 diabetes patients, it was determined that this rate was 30.1% and the most used were cinnamon (25.9%) and other herbal mixture products [14].

Since Turkiye is rich in plant diversity, people living in rural areas collect plants from nature and use them in line with the knowledge from the past [15]. In cities, the way to obtain plants is usually herbalists. Many herbs are offered for sale by herbalists in Turkiye, considering that they will cure [16-18]. In a study, it was determined that 142 medicinal and aromatic plant species were sold in 20 different herbalists visited in Adana and diabetes was among the uses of these plants. In the study, it was found that the herbs were sold without a standard packaging, and without a labeling system. Moreover, recommendations for the use of herbal products do not fully coincide with the literature [16]. Studies have shown that herbalists are not academically educated on medicinal plants [19], and they mostly get information via the internet [20].

The aim of this study is to conduct a survey on Type 1 and Type 2 diabetes patients to determine the plants that were used the plants that were used among the public for the treatment of diabetes. Moreover, pharmacognostically examine the plants/plant products according to the survey results. As a result of the survey, olive leaf was determined, as one of the most used plants for diabetes in Turkiye. The comparative analyses were conducted on the olive leaf plant samples collected from nature, sold in herbalists and pharmacies. Pharmacognostic analyses (macroscopic and microscopic analysis, total ash assay, loss on drying), chromatographic analyses (thin layer chromatography and high-performance liquid chromatography assays), and *in vitro* inhibition activity assays of diabetes-related enzymes (α-amylase, α-glucosidase, and aldose reductase) were performed on the samples.

The olive leaf is the leaf of the *Olea europaea* L. plant from the Oleaceae family. Although *O. europaea* is a widely distributed plant all over the world, it is mostly grown in Mediterranean countries due to its growing conditions. Two varieties of olives present in Turkiye: *O. europaea* L. var. *europaea* Zhukovsky and *O. europaea* L. var. *sylvestris* (Miller) Lehr. which were commercially and traditionally mostly produced in Aegean and Marmara regions such as Aydın, Balıkesir, Canakkale, Hatay, Izmir, Manisa, Mersin and Mugla [21,22]. Traditionally, different parts of the plant have been used for stomach and intestinal diseases, oral hygiene, hypertension, diabetes, bronchial asthma, diarrhea, urinary tract infections, hemorrhoids, and rheumatism [23,24]. The antihypertensive, antihypercholesterolemic, cardioprotective, antidiabetic, antimicrobial, antioxidant, cytotoxic, and hepatoprotective activities of the plant and its components have been demonstrated by scientific studies [25]. In the treatment of diabetes, the leaves are consumed by brewing (infusion) and/or boiling (decoction) [26-30]. The main phytochemical components of *O. europaea* are phenolics and lipids. Phenolic components are phenolic acids (ferulic acid, gallic acid, caffeic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, sinapic acid, syringic acid, and vanillic acid), flavonoids (chrysoeriol and luteolin), phenolic alcohols (hydroxytyrosol and tyrosol), and secoiridoids (oleuropein and verbascoside) [31].

α-Amylase and α-glucosidase enzymes are involved in the breakdown of starch into small monosaccharides. α-Amylase can convert starch to 60% maltose, cleaving α-(1,4) bonds, but not α-(1,6) bonds [32]. α-Glucosidase hydrolytically cleaves disaccharides (maltose and sucrose) into monosaccharides (glucose and fructose) [33]. Inhibition of these enzymes prevents postprandial hyperglycemia by delaying the absorption and digestion of carbohydrate molecules in the gastrointestinal tract. Aldose reductase is involved in the first step of the polyol pathway in glucose metabolism. It catalyzes the conversion of glucose to sorbitol. In diabetic patients, high blood glucose activates the polyol pathway and turns into sorbitol [34]. Sorbitol accumulation causes diabetic complications such as cataracts, nephropathy, neuropathy, and retinopathy. Inhibition of the aldose reductase enzyme has been shown to prevent diabetes complications, especially cataracts and retinopathy [35].

MATERIAL AND METHOD

Materials

A total of four different olive leaf samples were used, two of which were the product containing leaves and two of which were olive leaves. The first of the leaf samples (OLE-N) were collected by the researchers from a 10–15-year-old olive tree in Dortyol, Hatay. The other leaf sample (OLE-H) was obtained from Ankara's best-known herbalist, in a packaged form. One of the samples (EH) containing olive leaves is olive leaf extract in liquid form and was purchased from an herbalist in Ankara. The other olive leaf sample (CP) is a product in capsule form containing standardized olive leaf extract and was obtained from the pharmacy. The definitions of the materials are presented in the tables as follows:

O: Oleuropein;
OLE-N.I: Olive leaf samples collected from nature- infusion extract;
OLE-N.D: Olive leaf samples collected from nature- decoction extract;
OLE-N.E: Olive leaf samples collected from nature- ethanolic extract;
OLE-H.I: Olive leaf samples obtained from herbalists- infusion extract;
OLE-H.D: Olive leaf samples obtained from herbalists- decoction extract;
OLE-H.E: Olive leaf samples obtained from herbalists- ethanolic extract;
CP: Capsule from pharmacy;
EH: Olive leaf extract from herbalist

Survey Study

The survey study is a descriptive cross-sectional study. The study was approved by Gazi University Ethics Committee (E-77082166-604.01.02-35354). It was carried out with diabetes patients who applied to Gazi University Endocrinology Department between January 27, 2021, and July 27, 2021. The study included 200 patients. While patients over the age of 18 with a definite diagnosis of Type 1 or Type 2 diabetes were included, patients with gestational diabetes and pre-diabetes were not included in the study. Questionnaire forms were filled by the researchers through face-to-face interviews.

The questionnaire form was prepared by scanning the relevant literature. The form consists of 38 questions, which is divided into three sections. The first part consisting of 11 questions asking demographical data, patient information; the second part consisting of 9 questions about disease information; the last part consists of 18 questions, about the use of plants / herbal products. Data from the study were analysed with the Statistical Package for the Social Sciences (SPSS) program. The frequency of participant responses is shown in the tables and figures. Chi-square tests performed and crosstabs created to correlate responses. Significance was evaluated at the 95% confidence level, $p<0.05$.

Pharmacognostic Analyses

Macroscopic analysis, microscopic analysis and total ash amount determination, loss in drying experiments were performed on olive leaf samples collected from nature and obtained from herbalists. Experiments were carried out in accordance with the '*Oleae folium*' monograph in European Pharmacopoeia 8.0.

The general appearance, size and color of the samples were determined within the scope of macroscopic analysis. For microscopic analysis, olive leaf samples collected from nature and obtained from herbalists were pulverized and colors saved. Later, examined under a microscope (Leica DM500 binocular microscope, objectives x10 and x40) with chloralhydrate solution. For total ash, empty crucibles were heated in a muffle furnace at 600ºC, cooled in a desiccator and brought to constant weight. Samples weighing around 1.00 g were placed in empty crucibles and burned in a muffle furnace at 600ºC. The crucibles removed from the muffle furnace were taken to the desiccator, cooled, and weighed after constant weight. The difference between the weighings before and after combustion was calculated. 3 parallel experiments were performed for each sample. For loss on drying, samples weighing around 1.0 g were taken into cups and dried in an oven at 105 ºC for 2 hours. At the end of the period, the removed cups from the oven were cooled in the desiccator, brought to a constant weight, and weighed. The difference between the weighings before and after drying was calculated. 3 parallel experiments were performed for each sample.

Preparation of Extracts from Olive Leaf Samples

Ethanolic and aqueous extracts of *O. europaea* leaves were prepared from the leaf samples collected from Hatay-Dortyol and purchased from the herbalist. Olive leaves were ground with a grinder in large and small sizes. For the ethanolic extract, 500 ml of pure ethanol was added to 50 g of the samples and macerated for 3 days at room temperature by shaking on an orbital shaker. At the end of the maceration, the extracts were filtered and concentrated in a rotavapor under reduced pressure at 40-45°C. For infusion, 450 ml of boiling distilled water was added to 50 g of the samples, shaken, and filtered. For decoction, 450 ml of room temperature distilled water was added to 50 g of the samples and boiled. Aqueous extracts were concentrated using a lyophilizer. Olive leaf extract obtained from the herbalist as a ready-made liquid extract was first concentrated in the rotavapor. The concentrated semi-solid extract was dissolved with methanol and used in the analysis. Olive leaf extract purchased from the pharmacy as a capsule was prepared by dissolving the capsule contents with methanol.

Chromatographic Analysis

Thin Layer Chromatography (TLC)

Thin layer chromatography was carried out in accordance with the '*Oleae folium*' monograph in European Pharmacopoeia 8.0.

Test solution: Extracts obtained from olive leaves, prepared extract and capsule contents were dissolved with methanol and applied.

Reference solution: Oleuropein dissolved in methanol

Plate: TLC silica gel plate

Mobile phase: Distilled water: methanol: dichloromethane (1,5:15:85 v/v/v)

Application: as 10 µl strips

Drift: More than 10 cm

Drying: In Air

Detection: Vanillin reagent R was sprayed, heated at 100-105°C for 5 min and examined in daylight

HPLC Analysis

Oleuropein content in olive leaf samples was determined by HPLC. Agilent model instrument and C18 (150 ×4.6 mm, 5 µm) column were used for HPLC. The analysis conditions used for oleuropein quantification were determined by modifying the studies in the literature. The mobile phase was water containing formic acid (0.1%) and acetonitrile (85:15). The mobile phase flowed at a rate of 1 ml/min for 30 minutes. Analysis was carried out at 320 nm. LOD (the smallest detectable amount) and LOQ (the lowest concentration that can be measured with acceptable accuracy and repeatability) values for the analysis were calculated. The calibration equation was found for standard oleuropein. The oleuropein content of olive leaf samples were determined using this equation.

***In vitro* Activity**

The inhibition effects of different concentrations of olive leaf samples on α-amylase, α-glucosidase and aldose reductase enzymes were investigated.

For α-amylase enzyme inhibition assay, the experimental method was created by modifying the *in vitro* method used by Eruygur et al. 50 µl of amylase enzyme (0.06 M NaCl, in 0.02 M, pH 6.9 phosphate buffer) was added to the plant extracts at different concentrations and incubated at 37°C for 10 min [36]. Then, 50 µl of starch solution was added and incubated for 10 min. Starch solution was prepared in distilled water at a concentration of 0.05%. After incubation, 25 µl of HCl and 100 µl of lugol solution were added. The absorbance was measured spectrophotometrically at 540 nm. Buffer was used as a control instead of extract. The reference substance was acarbose. Inhibition levels of plant extracts on amylase enzyme were evaluated *in vitro* using the following equation:

$$\text{Inhibition (\%)} = [1 - (\text{Absorbance (sample)} / \text{Absorbance (control)}] \times 100$$

Absorbance (sample): The absorbance value of the samples at 540 nm
Absorbance (control): The absorbance value of the control at 540 nm

For α-glucosidase enzyme inhibition assay, the experimental method was created by modifying the *in vitro* method used by Eruygur et al [36]. 50 µl of α-glucosidase enzyme was added to the plant extracts at different concentrations and incubated at 37°C for 5 minutes. Then, 50 µl of p-nitrophenyl-α-D-glucopyranoside was added and incubated at 37°C for 30 minutes. The reference substance was acarbose. At the end of incubation, absorbances were read at 405 nm. The inhibition effect of plant extracts on the α-glucosidase enzyme was calculated as follows:

$$\text{Inhibition (\%)} = [1 - (\text{Absorbance (sample)} / \text{Absorbance (control)}] \times 100$$

Absorbance (sample): The absorbance value of the samples at 405 nm
Absorbance (control): The absorbance value of the control at 405 nm

The aldose reductase enzyme inhibition effect of the samples was determined by modifying the *in vitro* method used by Hayman and Knoshita [37]. Homogenizer obtained from rabbit lenses was used as the source of aldose reductase enzyme. Rabbit lenses were homogenized in 100 mM phosphate buffer (pH 6.9) and was prepared a 10% homogenizer. Centrifuged at 10,000 rpm at 4°C for 20 min. The supernatant was stored at -20°C and used in the aldose reductase inhibition experiment. Plant extracts, 25 µl of NADPH and 25 µl of homogenate were added to 100 µl of potassium buffer. The mixture was incubated at 37°C for 5 minutes. DL-glyceraldehyde was added at the end of the incubation. NADPH change was followed at 340 nm at 37°C for 15 min. Quercetin was used as a positive control. The inhibition effect of the samples on the aldose reductase enzyme was calculated as follows:

$$\text{Inhibition (\%)} = [1-(\Delta A \text{ sample/min} / \Delta A \text{ control/min})] \times 100$$

ΔA sample/min: Absorbance change of the samples at 340 nm in one minute
ΔA control/min: Absorbance change of the control at 340 nm in one minute

RESULT AND DISCUSSION

Survey Results

The mean age of 200 individuals participating in the study was 49.59±15.22 and ranged from 18 to 84. More than half of the participants are women (59.0%), most of them are married (79.5%) and they usually live in the city center (77.0%). Twenty eight percent of individuals are high school graduates and 26.0% are university graduates. The number of people having monthly income between 2000 and 5000 is more than half of the participants (55.0%). The individuals (55.0%) reported that they had chronic diseases other than diabetes, additionally 83.0% of them reported that they used regular drugs. While most of the respondents do not consume cigarettes and alcohol, only 16.5% of individuals exercise regularly. The demographic characteristics of the participants are shown in Table 1.

Of the individuals participating in the study were found to have the number of Type 1 diabetes patients is 27.0% and a Type 2 diabetes was determined as 73.0%. Nearly half of the patients reported that they used only oral antidiabetic on the other hand 18.5% reported that they used insulin as well as oral antidiabetics, 38.0% of the participants were treated in the hospital due to diabetes. Only, 12 patients stated that they did not use any medication for diabetes treatment. the most common complication was diabetic retinopathy (26.3 %). Diabetic neuropathy (24.2%), cardiovascular disease (12.6%) and diabetic foot (12.6%) are among other common diabetes complications. 53.0% of the participants reported that they received education on diabetes. Detailed information about individuals' diabetes diseases is included in Table 2.

Herbal and herbal product usage information of the participants is shown in Table 3. Only 39.0% of individuals reported that they used plants/herbal products to support diabetes treatment. The most used herbs were cinnamon (29.3%), olive leaves (21.7%) and black cumin (16.8%). Garlic, nettle, rosehip, blueberry, ginseng, bitter melon, mahaleb and fenugreek are plants used to support diabetes treatment.

Table 1. The demographic characteristics of the participants

	Number of people (n)	**Percentage (%)**
Gender		
Female	118	59.0
Male	82	41.0
Marial status		
Married	159	79.5
Unmarried	41	20.5
Education level		
Illiterate	11	5.5
Literate	7	3.5
Primary school graduate	42	21.0
Secondary school graduate	23	11.5
High school graduate	56	28.0
University graduate	52	26.0
Postgraduate (Master's/PhD)	9	4.5
Monthly income		
2000 or less	31	15.5
2000-5000	110	55.0
5000-10000	44	22.0
10000-15000	12	6.0
15000 and higher	3	1.5
Residential area		
City center	154	77.0
District	41	20.5
Other	5	2.5
Chronic disease other than diabetes		
Yes	110	55.0
No	90	45.0

Table 2. Information about the diabetes diseases of the participants

	Number of people (n)	**Percentage (%)**
Diabetes type		
Type 1	54	27.0
Type 2	146	73.0
Diabetes treatment		
Does not use medication	12	6.0
Only oral antidiabetic	96	48.0
Only insulin	55	27.5
Both oral antidiabetics and insulin	37	18.5
Have you been hospitalized due to diabetes?		
Yes	76	38.0
No	124	62.0
Do you have a complication due to diabetes?		
Yes	54	27.0
No	146	73.0

Table 2 (*continue*). Information about the diabetes diseases of the participants

	Number of people (n)	Percentage (%)
Which of the chronic complications of diabetes do you have?		
Diabetic retinopathy	25	26.3
Diabetic neuropathy	23	24.2
Cardiovascular disease	12	12.6
Diabetic foot	12	12.6
Diabetic nephropathy	11	11.6
Peripheral vascular disease	9	9.5
Liver disease	2	2.1
Cerebrovascular attack	1	1.1
Have you been trained in diabetes?		
Yes	106	53.0
No	94	47.0

Unlike these herbs, two patients reported that they mixed ginger and turmeric with yogurt for their diabetes. While 38.4% of the patients stated that they consumed these herbs every day, the rate of patients who consumed them 1-3 days a week was 26.0 %. About one-third (31.6%) of the patients using the herbs used it for one to three months. Although some participants used prescription and herbal medicines together, only 9.5% reported such use to a healthcare professional. Nearly half of the patients who preferred to use herbs determined the amount of the herbs by eye. Most patients (94.9%) did not change the dose of their current medication while using the herb. Diabetes patients learned from their neighbors and friends (28.9%) that they could use herbs for support, and more than half (51.7%) obtained herbs from herbalists and spice shops.

Table 3. Herbal and herbal product usage information of the participants

	Number of people (n)	Percentage (%)
Do you use herbs/herbal products to support diabetes treatment?		
Yes	78	39.0
No	122	61.0
Which herbs/herbal product did you use to support diabetes treatment?		
Cinnamon	54	29.3
Olive leaf	40	21.7
Black cumin	31	16.8
Garlic	14	7.6
Dead nettle	8	4.3
Rosehip	7	3.8
Blueberry	5	2.7
Ginseng	4	2.2
Bitter melon	4	2.2
Mahaleb	4	2.2
Fenugreek	2	1.1
Other	11	6.0
What is the frequency of your use of herbs/herbal products to support diabetes treatment?		
Every day	28	38.4
1-3 days a week	19	26.0
4-6 days a week	9	12.3
Biweekly	10	13.7
Sometimes	7	9.6

Table 3 (*continue*). Herbal and herbal product usage information of the participants

	Number of people (n)	**Percentage (%)**
How long did you use a herbs/herbal product for supporting diabetes treatment?		
Less than one month	22	28.9
One-three months	24	31.6
Three-twelve months	8	10.5
More than one year	22	28.9
How did you adjust the dose of the herbs/herbal product you use to support diabetes treatment?		
Sense of proportion	36	46.2
Teaspoon	20	25.6
Handful/ pinch	11	14.1
Tablet/capsule/pill	6	7.7
Sensitive scales	2	2.6
Other	3	3.8
Where do you get the plant/herbal product you use for supporting diabetes treatment?		
Herbalist, Spice	62	51.7
Village/Country	19	15.8
Pharmacy	15	12.5
I gathered it myself	8	6.7
Market, supermarket	8	6.7
Internet, television	7	5.8
Other	1	0.8
From whom/where did you learn that the herbs/herbal products you use can be used in your disease?		
Neighbor, friend	37	28.9
Internet, television	35	27.3
Relatives, family elders	29	22.7
Pharmacist	16	12.5
Doctor	5	3.9
Health personnel	4	3.1
Other	2	1.6
Have you made any changes in the dosage of the current medications you use while using herbs/herbal products for support of diabetes treatment?		
Yes	4	5.1
No	75	94.9
Do you think that side effects occur in your body depending on the herbs/herbal product you use to support diabetes treatment?		
Yes	5	6.3
No	74	93.7
Have you shared with your doctor that you are using herbs/herbal products?		
Yes	19	24.1
No	60	75.9

Patients were also asked about diabetes-related wounds and diabetic foot, one of the common and important complications of diabetes (Table 4). Most of the patients (80.3%) reported that they would consult a doctor in case of inflamed wounds on their feet. The patients who stated that they could use herbs/herbal products in case of foot wounds constituted only 9.2% of the respondents. Approximately one-third (30.3%) of patients with diabetes-related wounds used herbs/herbal products to treat their wounds. The most preferred ones are St. John's Wort oil and olive oil. Patients generally used the herbals they preferred every day (40.9%) and for less than one month (68.2%).

Table 4. Questions about diabetic wound

Question	Number of people (n)	Percentage (%)
What do you do if you have an inflamed wound on your foot?		
I will go to doctor	192	80.3
I apply/use herbs/herbal products	22	9.2
I apply vaseline	14	5.9
I apply ice to my feet	5	2.1
Other	6	2.5
Do you have sores on your feet, mouth or any part of your body?		
Yes	43	21.5
No	157	78.5
Have you used herbs/herbal products to treat wounds on your body?		
Yes	23	30.3
No	53	69.7
Which herbs/herbal products did you use to treat the wounds on your body?		
St. John's Wort oil	16	37.2
Olive oil	10	23.3
Aloe vera gel	7	16.3
Rosemary oil	4	9.3
Calendula flower	3	7.0
Bitter melon	1	2.3
Pomegranate peel	1	2.3
Coconut oil	1	2.3
What is the frequency of use of herbs/herbal products for your wounds on your body?		
Every day	9	40.9
1-3 days a week	6	27.3
Biweekly	5	22.7
Other	2	9.1
How long did you use the plant/herbal product for your wounds on your body?		
Less than one month	15	68.2
One-three months	6	27.3
More than one year	1	4.5

As a result of the cross-statistical analysis, the characteristics of the patients who used the plant for diabetes diseases were determined (Table 5). Gender, type of diabetes and number of years with diabetes were effective in plant use (p<0.05) (Figure 1). Women and Type 2 diabetes patients applied to plants more. Patients turned to plants in the first years after the diagnosis of diabetes, and interest in plants decreased in the following years ($p=0.05$). Individuals who are married, high school graduate, income between 2000-5000, living in the city center, using only oral antidiabetic and without diabetes-related complications used plants more. On the other hand, these cases were not found statistically significant (p>0.05). The distribution of the use of plants determined to be used because of the survey according to diabetes type is given in Table 6. It has been determined that cinnamon, olive leaves and black cumin, which are the most used plants, are often preferred by Type 2 diabetes patients.

Table 5. Characteristics of patients using plants for the treatment of diabetes

		Number and percentage of people using plants n (%)	***p value***
Gender	Female	54(69.2%)	*p*<0.05
	Male	24(30.8%)	
Marital status	Married	67(85.9%)	*p*>0.05
	Unmarried	11(14.1%)	
Education level	Illiterate	1(1.3%)	*p*>0.05
	Literate	4(5.1%)	
	Primary school graduate	19(24.4%)	
	Secondary school graduate	9(11.5%)	
	High school graduate	22(28.2%)	
	University graduate	18(23.1%)	
	Postgraduate (Master's/PhD)	56.4(%)	
Monthly income	0-2000	11(14.1%)	*p*>0.05
	2000-5000	41(52.6%)	
	5000-10000	20(25.6%)	
	10000-15000	6(7.7%)	
	15000 and higher	0	
Residential area	City center	55(70.5%)	*p*>0.05
	District	21(26.9%)	
	Other	2(2.6%)	
Diabetes type	Type 1	15(19.2%)	*p*<0.05
	Type 2	63(80.8%)	
Diabetes duration	0-5 years	24(30.8%)	*p*=0.05
	5-10 years	23(29.5%)	
	10-15 years	12(15.4%)	
	15-20 years	9(11.5%)	
	20-25 years	4(5.2%)	
	25-30 years	6(7.7%)	
Diabetes treatment	Does not use medication	2(2.6%)	*p*>0.05
	Only oral antidiabetic	44(56.4%)	
	Only insulin	16(20.5%)	
	Both oral antidiabetics and insulin	16(20.5%)	
Presence of complications	Yes	24(30.8%)	*p*>0.05
	No	54(69.2%)	

Table 6. Distribution of the use of plants determined to be used as a result of the survey according to diabetes type

	Diabetes type n(%)	
Plant name	**Type 1**	**Type 2**
Cinnamon (n=54)	13(24.1%)	41(75.9%)
Olive leaf (n=40)	6(15.0%)	34(85.0%)
Black cumin (n=31)	5(16.1%)	26(83.9%)
Garlic (n=14)	4(28.6%)	10(71.4%)
Dead nettle (n=8)	1(12.5%)	7(87.5%)
Rosehip (n=7)	1(14.3%)	6(85.7%)
Blueberry (n=5)	1(20.0%)	4(80.0%)
Ginseng (n=4)	4(100.0%)	0
Bitter melon (n=4)	1(25.0%)	3(75.0%)
Mahaleb (n=4)	0	4(100.0%)
Fenugreek (n=2)	1(50.0%)	1(50.0%)

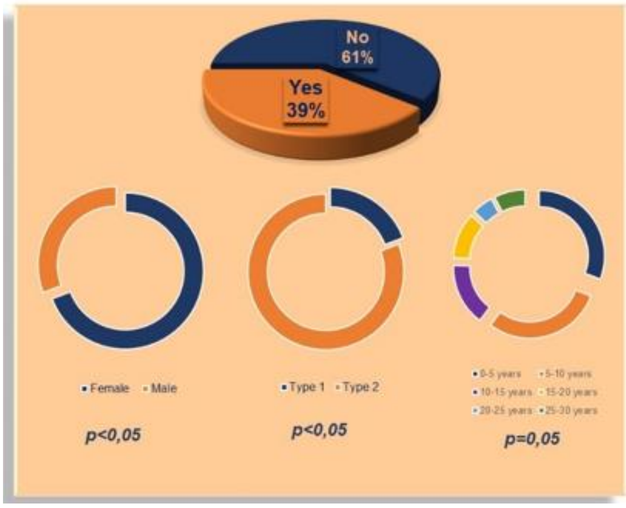

Figure 1. Relationship between plant use and gender, type of diabetes, number of years with diabetes

Pharmacognostic Analysis Results

Olive leaf samples collected from nature and purchased from the herbalist have similar characteristics in terms of general appearance (Figure 2). The leaves have a long thin appearance of 5-6 cm long, 0,5-1 cm wide. The leaves are lanceolate. The upper surface of the leaves is dark green, the lower surface is grayish and has a hairless and skinny structure. The leaf edges are curled due to drying.

It was determined that the colors of the powdered olive leaf samples for microscopic analysis were yellowish green. As stated in the European Pharmacopoeia 8.0, abundant peltate trichomes were observed in the microscope examination of the samples examined with chloralhydrate. Other elements detected microscopically are epiderma and parenchymatic parts, and stone cells like sclerenchyma bundles. Microscope images are presented in Figure 3.

The total ash content and the loss on drying results of olive leaf samples are presented in Table 7. According to European Pharmacopoeia 8.0, the total ash content of olive leaf samples should be at most 9%, and the loss on drying of olive leaf samples should be at most 10%.

Figure 2. Olive leaf sample (A. Olive leaf from natura, B. Olive leaf from herbalist)

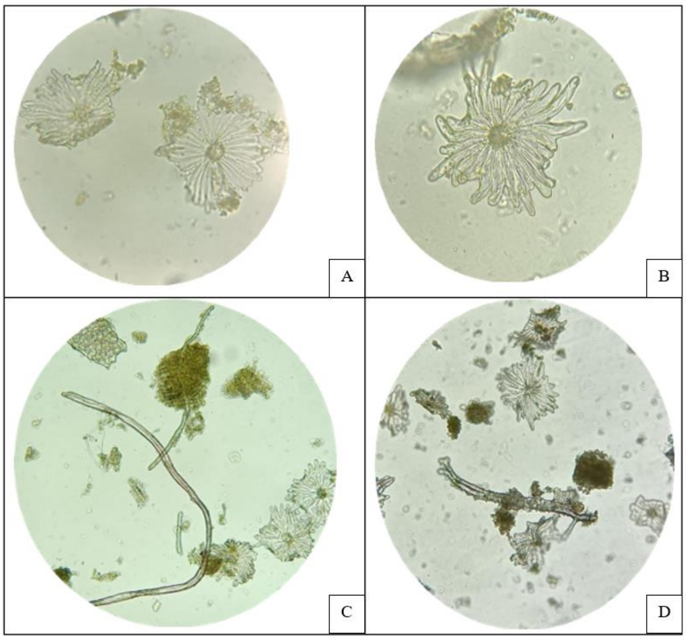

Figure 3. Peltate trichomes (A, B), and stone cells like sclerenchyma bundle found together with parenchymatic fragments (C, D) detected in olive leaf samples

Table 7. Analysis findings of total ash and loss on drying amount of olive leaf samples

Sample	Total ash content±standard deviation	Loss on drying+ standard deviation
Olive leaf samples collected from nature (OLE-N)	5.57±0.00	3.57±0.07
Olive leaf samples obtained from herbalists (OLE-H)	6.32±0.16	4.69±0.31

Chromatographic Analysis

Thin Layer Chromatography Results

Brownish oleuropein stains were detected because of the reaction developed by spraying the vanillin reagent on the plate after the drift on the plate was completed. The resulting image of the plaque is shown in Figure 4.

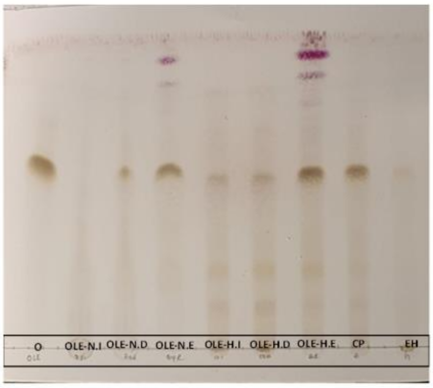

Figure 4. TLC plate image of olive leaf samples

*O: Oleuropein; OLE-N.I: Olive leaf samples collected from nature- infusion extract; OLE-N.D: Olive leaf samples collected from nature- decoction extract; OLE-N.E: Olive leaf samples collected from nature- ethanolic extract; OLE-H.I: Olive leaf samples obtained from herbalists- infusion extract; OLE-H.D: Olive leaf samples obtained from herbalists- decoction extract; OLE-H.E: Olive leaf samples obtained from herbalists- ethanolic extract; CP: Capsule from pharmacy; EH: Olive leaf extract from herbalist

HPLC Analysis

The major component oleuropein was detected by HPLC analysis. The equation to be used for oleuropein quantification was found to be y = 0.6576x-2.6542 (R^2 = 0.99). LOD and LOQ values determined as 1.63 and 4.94 respectively.

The results of oleuropein assay analysis of olive leaf samples are shown in Table 8 and Figure 5. As a result of the analysis, the amount of oleuropein in olive leaf extracts varied between 19-38%. The amount of oleuropein in the ethanolic extracts of olive leaves was higher than in the aqueous extracts. Olive leaf capsules obtained from the pharmacy have higher oleuropein content than olive leaves prepared by infusion method. Compared to olive leaf extracts, the oleuropein content of the liquid olive leaf extract obtained from herbalists is quite low.

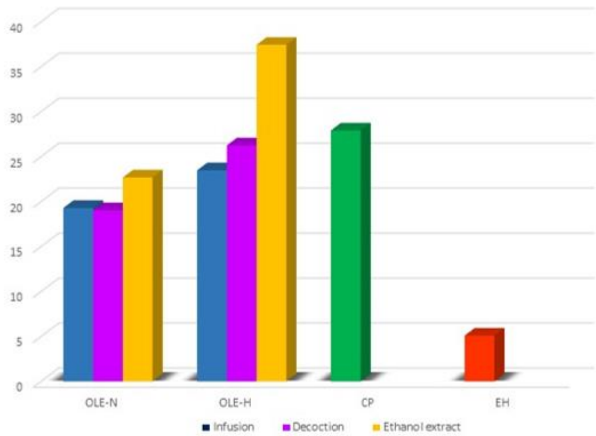

Figure 5. Percent oleuropein content of olive leaf samples

Table 8. Results of oleuropein assay analysis of olive leaf samples

Sample name		The amount of ppm oleuropein found in 100 ppm extract	mg amount of oleuropein (mg/g) in 1 gram extract
Olive leaf samples collected from nature (OLE-N)	Infusion	19.29±1.96	192.90±19.62
	Decoction	19.03±3.05	190.31±30.54
	Ethanolic extract	22.67±4.67	226.75±46.70
Olive leaf samples obtained from herbalist (OLE-H)	Infusion	23.47±6.04	234.76±60.47
	Decoction	26.24±2.98	262.45±29.86
	Ethanolic extract	37.42±4.20	374.29±42.09
Capsule from pharmacy (CP)		27.90±3.52	279.04±35.20
Olive leaf extract from herbalist (EH)		5.09±1.84	50.95±18.45

In vitro Enzyme Inhibition Effects

All the olive leaf samples showed high α-amylase enzyme inhibition comparable to the reference substance. Analyses are shown in Table 9. Extracts of olive leaves collected from nature and obtained from herbalists exhibited similar degree of inhibition of α-amylase enzyme. Ethanolic extracts provided higher inhibition than aqueous extracts prepared with different techniques. The olive leaf capsule obtained from the pharmacy and the olive leaf extract obtained from the herbalist in liquid form showed similar inhibition to the aqueous extracts.

The results of α-glucosidase enzyme inhibition of olive leaf samples are shown. According to the results of the analysis, all the extracts exhibited very high α-glucosidase enzyme inhibition. Although the solvent used in the preparation of the extracts affected the inhibition rate, large differences were not detected between the results. In addition to the extracts, the capsule obtained from the pharmacy and the liquid extract obtained from the herbalist were also found to be effective on the α-glucosidase enzyme.

The aldose reductase enzyme inhibition findings of olive leaf samples are given Although the extracts of olive leaf samples provided lower inhibition than quercetin, the activities of especially ethanolic extracts were high enough to compare with quercetin. While the lowest inhibition was observed in the infusion extracts of the samples, the activity of the capsule obtained from the pharmacy and the extract obtained from the herbalist on aldose reductase enzyme was also found to be lower than the infusions of the extracts.

Results showed that olive leaf inhibited enzymes associated with diabetes. The mechanism of action of olive leaf on diabetes is presented in Figure 6.

Table 9. Results of enzyme inhibition assays of olive leaf samples

		α-amylase enzyme inhibition (%Inhibition+Standard deviation)	*α*-glucosidase enzyme inhibition (%Inhibition+Standard deviation)			Aldose reductase enzyme inhibition (%Inhibition+Standard deviation)	
Sample name		**0.625 µg/ml**	**2.5 µg/ml**	**5 µg/ml**	**10 µg/ml**	**25 µg/ml**	**50 µg/ml**
Olive leaf samples collected from nature (OLE-N)	Infusion	41.04± 2.57	58.66±0.54	74.04±0.20	82.43±2.83	41.38±0.12	76.02±0.36
	Decoction	48.86±1.77	87.63±0.81	92.97±0.18	95.66±0.38	55.73±0.65	77.40±0.11
	Ethanolic	50.11± 1.42	90.54±0.54	95.07±2.13	95.19±0.21	66.49±0.0	80.90±1.35
Olive leaf samples obtained from herbalists (OLE-H)	Infusion	34.34±0.86	69.47±2.55	71.79±1.50	86.10±1.26	45.50±0.18	68.55±0.26
	Decoction	47.29±1.70	89.48±0.05	94.67±0.13	96.03±1.04	56.75±1.94	75.73±0.01
	Ethanolic	49.35± 2.17	89.34±1.25	95.21±0.22	97.10±0.01	79.95±0.63	90.69±0.80
Capsule from pharmacy (CP)		41.29±3.22	84.19±1.46	90.55±2.47	95.47±0.74	42.64±1.45	71.73±0.70
Olive leaf extract from herbalist (EH)		33.15± 1.05	83.07±0.16	91.18±0.29	95.59±0.13	38.89±0.95	69.88±2.77
Reference substance		21.80± 3.40 (Acarbose)	88.37±1.72 (Acarbose)	96.15±0.93 (Acarbose)	99.99±0.01 (Acarbose)	87.46±0.01 (Quercetin)	98.87±0.10 (Quercetin)

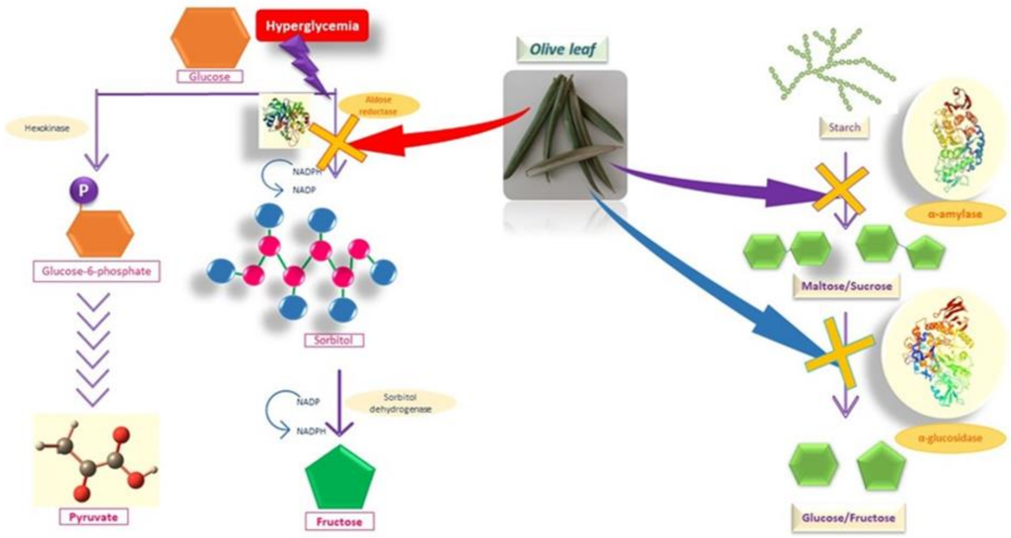

Figure 6. The mechanism of action of olive leaf on diabetes

Diabetes is a chronic disease that affects the whole world, the incidence of which is increasing day by day. After choosing this common disease as a subject, the plants to be used were determined by a survey conducted with diabetes patients. Based on the data of the survey study, the plants and the analyses to be made on them were decided.

Type 2 diabetes, which is the most common type of diabetes among all diabetes types, is also the most detected type among the survey study participants. This may be due to factors such as modern lifestyle, dietary habits, more limited physical work due to increased mental workloads, and sedentary life. The fact that most of the participants (83.5%) do not exercise regularly proves these possibilities.

As a result of the survey study, it was determined that the patients who use plants for the treatment of diabetes mostly prefer cinnamon, olive leaves and black cumin. These plants preferred by patients showed similarities with previous survey studies [9-12]. Analyses showed that there was a significant relationship between plant use and gender, type of diabetes, and duration of diabetes. Like previous studies, women preferred plants more than men in this survey study [10]. The fact that diabetic patients prefer plants more in the first years of their diabetes made us think that plants are the first-choice treatment. On the other hand, it has been determined that individuals who do not have complications prefer plants more. Considering that diabetes complications occur over time because of high blood sugar values, the survey data are compatible among themselves.

The reason for the patients who did not prefer plants was that they thought that they were ineffective. On the other hand, patients using herbal medicine mostly did not inform their doctors about their use, determined the number of herbs they used with the rule of thumb, and did not change the doses of their conventional drugs. The reason why patients cannot share their herbal use with their doctors may be because they think that herbs are completely harmless or because they are afraid of a negative attitude from the doctor. But the common point that can be deduced from all these data diabetes patients do not have enough correct information about herbs/herbals. The fact that diabetic patients learned the use of herbs from their neighbors and friends rather than from health professionals revealed that not only the patients but also the individuals around them should be educated about herbs. Similarly, the fact that herbs are frequently procured from herbalists/spices has once again revealed that herbalists/spices need to be under stricter control.

According to the results of the analysis, the leaves of the olive plant are the second most used plant for the treatment of diabetes and the oil obtained from the olive plant was used in patients with diabetes wounds as complications. In addition to these, the olive plant was chosen as the study subject because it grows in Turkiye. Since the plants used by the patients are mostly obtained from herbalists, pharmacognostic analyses such as macroscopic, microscopic, TLC, total ash amount determination, and loss on drying were carried out on both samples collected from nature and obtained from the herbalist.

When the pharmacognostic analysis results of olive leaf samples were evaluated, it was determined that olive leaf samples collected from nature and obtained from herbalists had similar macroscopic and microscopic results, and these results were in accordance with the '*Oleae folium*' monograph in the European Pharmacopoeia 8.0. Similarly, the determination of the total ash amount of the samples and the loss findings on drying are below the maximum values specified in the monograph and are following the pharmacopoeia.

Oleuropein content of olive leaf samples were determined to be between 19-38%. It is known that the oleuropein content of olive leaves varies depending on factors such as collection time. Olive leaf methanol extract was found to contain 40.33% oleuropein in the study of Hayes et al [38]. The oleuropein amounts of the olive leaf samples in this study are not contrary to the literature findings. On the other hand, the oleuropein content of the liquid olive leaf extract obtained from herbalists is lower than a quarter of the extracts prepared by the infusion method of other samples.

Chigurupati et al. investigated the activity of *O. europaea* leaves extracted using ethanol on α-amylase and α-glucosidase enzymes [39]. As a result of the analyses, the IC_{50} value of the reference substance acarbose for the α-amylase assay was determined as 20.06±0.19 μg/ml and the IC_{50} value of the ethanolic extract was 37.99 μg/ml. In the analysis results of Ahamad et al., olive leaf extract showed inhibitory activity close to acarbose (acarbose IC_{50}: 91.04±2.16 μg/ml and extract IC_{50}: 121,8±3,18 μg/ml) [40]. Javed et al. reported that olive leaf extract also has inhibitory effect on α-glucosidase enzyme, but this effect is lower than amylase (Acarboz IC_{50}: 116.5±2.17 μg/ml and extract IC_{50}: 165.04±5.27 μg/ml). Like this study, olive leaves showed lower but similar activity than acarbose in our study.

When the literature was examined, only two studies were found about the aldose reductase enzyme inhibition effect of olive leaves. Elimam et al. reported that, methanolic (70%) extract of olive leaf showed an inhibitory effect on the aldose reductase with an IC_{50} value of 65 μg/ml [41]. However, Elimam et al. did not use a reference substance in their study and therefore did not determine the effect of olive leaf compared to the reference substance. Considering that the results of *in vitro* experiments show serious differences according to the working conditions, it would not be correct to make a comparison with this study. Papoti et al. prepared infusion, decoction, and ethanolic extracts of olive leaf in their studies but gave results only for infusion extract (IC_{50}: 26 ± 1 μg/ml). Unlike our study, Papoti et al. used sorbinil, a chemical aldose reductase inhibitor, as a reference substance and found that olive leaf had much lower activity than sorbinil [42]. In our study, the reference substance has a much higher inhibitory effect than olive leaf infusion extracts, but there is a difference in the reference substance used between these studies.

Conclusion

From a general perspective, olive leaf samples were found to have *in vitro* antidiabetic activities. Higher activity of ethanolic extracts in enzyme experiments showed that ethanolic is a good solvent for all three enzymes, and it is effective in revealing the components that cause enzyme inhibition in plant content. On the other hand, it was understood that the tea prepared by the people at home using water was not ineffective. It was determined that the extracts of the plants prepared using only ethanol or water, as well as the samples obtained from herbalists and pharmacies in different forms, had antidiabetic effects *in vitro*.

Although the ready-made liquid extract olive leaf extract showed *in vitro* enzyme inhibition levels close to the other extracts, the amount of oleuropein contained was much lower than the others. These data question the reliability of ready-made preparations obtained from outside the pharmacy. On the other hand, the extracts prepared by us from the olive leaves obtained from the most well-known herbalist in Ankara showed as strong results as the extracts prepared from the olive leaves collected from the nature. Moreover, the content determination results of these samples are like those of the samples collected from nature. The olive leaves samples obtained from the herbalist were beautiful looking, brightly colored, and carefully packaged. This showed that plant selection is also important. The preparations obtained from the pharmacy were food supplements and were not approved by the

Ministry of Health. Despite this, it showed inhibitory activity on diabetes enzymes and there was no problem with its content. This situation suggests that the products sold in pharmacies are chosen more carefully than the products sold outside the pharmacy, even though they are not approved by the Ministry of Health, and that the products offered to the patient/consumer by health workers, namely pharmacists, may be more reliable. Although our current study is sufficient to contribute to the literature and to create a general impression about the importance of the issue with data, it can be expanded with more examples.

AUTHOR CONTRIBUTIONS

Concept: M.M., U.K.C.; Design: M.M., U.K.C.; Control: U.K.C.; Sources: M.M., U.K.C.; Materials: M.M., U.K.C.; Data Collection and/or Processing: M.M., U.K.C.; Analysis and/or Interpretation: M.M., U.K.C.; Literature Review: M.M., U.K.C.; Manuscript Writing: M.M., U.K.C.; Critical Review: M.M., U.K.C.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The study was approved by Gazi University Ethics Committee (E-77082166-604.01.02-35354).

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