



***Olea europaea* L. var frantoye grown in Algeria – extracts characterization by HPLC/MS, polyphenols and hypoglycemic activitie. *Olea europaea* L. var frantoye cultivada na Argélia – caracterização de extratos por HPLC/MS, polifenóis e atividade hipoglicemiante.**

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Abstract

This research work aims to study the different components existing in the leaves and fruits of *Olea europaea* var frantoye and test their hypoglycemic effect on albino rats administered by 50 mg/kg, 250 mg/kg, and 500 mg/kg of aqueous leaves and fruits dissolved in normal saline (N/S). The results obtained show that the extracts of the leaves and fruits of the olive tree (*Olea europaea*) contain polyphenols; the phytochemical screening showed them to be rich in different components. The quantitative study confirmed this richness and the dominant molecule is Oleuropein (781,26 and 233,83 ppm, respectively, leaves and fruits). Albino diabetic rats treated with the high dose (500 mg/kg/b.wt) as the most effective dose for significant treatment. Compared to control groups. While the standard medication (positive control Glucophage 500) is effective compared to leaf and fruit extract.

Keywords: Hypoglycemic effect. Induced Diabetes. *Olea europaea* L. Albino rats. Molecules.

Resumo

Este trabalho de pesquisa tem como objetivo estudar os diferentes componentes existentes nas folhas e frutos de *Olea europaea* var frantoye e testar seu efeito hipoglicêmico em ratos albinos administrados por 50 mg/kg, 250 mg/kg e 500 mg/kg de folhas e frutos aquosos dissolvidos em solução salina normal (N/S). Os resultados obtidos mostram que os extratos das folhas e frutos da oliveira (*Olea europaea*) contêm polifenóis; a triagem fitoquímica mostrou que são ricos em diferentes componentes. O estudo quantitativo confirmou essa riqueza e a molécula dominante é a Oleuropein (781,26 e 233,83 ppm, respectivamente, folhas e frutos). Ratos diabéticos albinos tratados com a dose alta (500 mg/kg/b.wt) como a dose mais eficaz para tratamento significativo. Comparado aos grupos de controle. Enquanto a medicação padrão (controle positivo Glucophage 500) é eficaz em comparação ao extrato de folhas e frutos.

Palavras-chave: Efeito hipoglicêmico. Diabetes Induzido. *Olea europaea* L. Ratos albinos. Moléculas.

1- Introduction

In the last decades, there has been a growing interest in the search of molecules with multiple targets as the ones from natural products, which is a strategy to achieve the real solution to prevent and/or treat complex diseases. Indeed, with an in-depth understanding, natural for further structural optimization, by increasing the potency to inhibit a specific target, decreasing the inhibition of undesired targets and/or by balancing the inhibition of some targets. Herbal medicine has been widely employed to treat or prevent diseases using plant extracts. It is part of alternative medicine, which is rich in therapeutic phytochemicals that may lead to the development of novel drugs (BABULKA, 2007). Phytocomplexes are pools of molecules contained in plants that often have an interesting biological value that justifies their use as an ingredient in food supplements or drug formulations. Their potential lies in the fact that these organic compounds bind to the same targets as drugs. In most cases, these are functional analogues without structural similarity among themselves, which makes it more complicated to identify the mechanisms underlying their effects and to represent the link between food and drugs (SANTINI et al., 2017). During the past few years some of the new bio-active drugs isolated from hypoglycemic plants showed anti-diabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy. Presently, there is an increased demand to use natural products with anti-diabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents (KIM et al., 2006). More than 400 plants with glucose lowering effect are known. The *Olea europaea* L. is a very well-known and widely used plant, especially for its nutritional qualities and its important environmental and economic impact (CHATZIKONSTANTINOOU et al., 2022). Its extracts from leaves and fruits are widely used in contrasting and preventing various pathologies. Leaves and fruits are taken orally for stomach and intestinal diseases and used as mouth cleanser (BELLAKHDAR et al., 1991). Leaves decoction has also hypotensive and anti-inflammatory activities (KIM et al., 2006). *Olea europaea* could be used as a food additive in the supplementary treatment of many diseases (ALESCI et al., 2021). The research work of (ZAKARI et al., 2023; BABU et al., 2006) mentioned that *Olea europaea* has antidiabetic activities.

The objective of This work is to study the different components existing in the leaves and fruits of *Olea europaea* var frantoye and test their hypoglycemic effect on albino rats.

2- Materials and methods

2-1. Preparation of plant extract

The plant material used consists of dried fruits and leaves of *Olea europaea* L var frantoye, the plants was collected during October 2020 from wilaya of M'Sila, Northeastern Algeria. After harvest, the fruits and leaves were cleaned, washed with tap water and dried in hot air oven at 60° C, and reduced to a fine powder using an electric grinder (Moulinex). The powdered material of the leaves was exhaustively extracted with methanol /water (70/30) under the maceration process. The macerated mixture was filtered by using Whatman No1 filter paper and evaporated at room temperature to yield a solid extract. This extract was kept in the refrigerator until the analysis.

2-2. Animals

Adult male swiss albino mice (20-25 g) were obtained from animal house college institute of Paster Constantine. All animals' protocols were approved by the College Ethical Committee, of

institute Paster Constantine. All mice were fed *ad libitum* with standard laboratory pellet diet and free access to tap water. The experimental mice were maintained under a constant 12 hr light and dark cycle at room temperature. Animals were acclimatized to the new experiment environments for 5 days before the study.

2-3. Phytochemical compound determination

2-3-1. Phytochemical screening

The phytochemical screening consists in carrying out qualitative phytochemical tests, based on coloring or precipitation reactions more or less specific to each class of chemical compounds belonging to secondary metabolisms of the studied plants. 5 g of crushed olive leaves and fruit were added to a mixture of methanol and water (100 mL, 70: 30 (v / v)) for the purpose of extracting phenolic compounds. At 4° C in the dark for 5 days, the mixture was allowed to stand. It was then filtered using filter paper from Whatman (AL-QURAISHY et al., 2017). Each extract was stored until required in a dark bottle. The presence or absence of different classes of secondary metabolites contained in *Olea europaea* L extracts were qualitatively tested as follows.

2-3-2. Determination of total phenolic content

Total polyphenols measurement was carried out according to (ARNOUS et al., 2002) using the Folin-Ciocalteu method. The total polyphenols content was calculated from the standard curve of Gallic acid (0-200 mg L⁻¹) plotted by using the same procedure. Results were expressed as mg Gallic acid equivalents (GAE) g⁻¹ dried leaves and fruits.

2-3-3. Determination of total flavonoids

The total flavonoids content of the leaf and fruit extract was determined according to the colorimetric assay developed by (ZHISHEN et al., 1999). The total flavonoids content was calculated from the standard curve of quercetin (0 -100 mg L⁻¹) plotted by using the same procedure and total flavonoids was expressed as mg quercetin equivalents g⁻¹ dried leaves and fruit.

2-3-4. Determination of total condensed tannin content (TCT)

The quantification of TCT of methanolic extracts was estimated using the standard procedure (PORTER, 1986). Brief, 3 mL of a 95% solution of n butanol/HCl was added to 0.25 mL of extract, followed by 0.1 mL of a solution formed by dissolving 0.5 g of NH₄Fe (SO₄)₂.12 H₂O in 0.25 mL of 2 M HCl. The closely capped tubes were incubated at 95° C for 40 min. On a UV visible spectrophotometer, the absorbance was read at 550 nm. The results were presented as mg (mg TAE/g DW) of tannic acid equivalents. Triplicate analysis was performed.

2-3-5. HPLC analysis of fruits and leaves extracts

The HPLC analysis given in the literature was used for the identification and quantification of polyphenol compound (SAVOURNIN et al., 2001). Consequently, HPLC analysis was performed on a SHIMADZU apparatus, manual injection valve, and UV detector model SPD20A. This was done using a column of 250 x 4.60 mm with per column, packing: Thermo Scientific ODS HYPERSIL C18. Furthermore, data acquisition and quantitation were performed with SHIMADZU. The mobile phase was 92% distilled water acidified (pH = 3) with 0.10 M orthophosphoric acid (v/v, 1000: 2.30)

and 21% acetonitrile (Carlo Erba, HPLC grade) acidified with 0.10 M orthophosphoric acid (v/v, 1000: 2.30). The flow rate was 1 ml/min, and the injection volume was 20 µl.

3- Antidiabetic activity

3-1. Assessment of effect of *Olea europaea* (leaves and fruits) extract on oral glucose tolerance tests in normal rats and dose optimization

Normal fasted rats were randomly assigned to five groups (comprising of at least five animals each). Each group was administered 50 mg/kg, 250 mg/kg, and 500 mg/kg of aqueous leaves and fruits extract of OE dissolved in normal saline (N/S). The other 2 groups served as negative (administered normal saline) and positive controls (administered 0.6 mg/kg of Glucophage 500 dissolved in N/S). Thirty minutes following each administration, 3 g/kg of glucose solution was administered to each animal. Blood sugar level was measured, for each animal, at 30 min, 60 min, 90 min and 120 min intervals following glucose administration. Following this experiment, 100 mg/kg of the extract was found to be the optimum dose of the 3 doses tested and was therefore used in the subsequent tests. (CHIKA; BELLO, 2010).

3-2. Assessment of hypoglycemic activity of OE (leaves and fruits) extract in normal fasted rats

Five randomized groups of normal fasted rats were administered either 50 ,250, and 500 mg/kg of the extract, 0.6 mg/kg of Glucophage 500 or N/S. Subsequently, blood sugar levels were assessed at 30 min, 60 min, 90 min and 120 min intervals (CHIKA; BELLO, 2010).

4- Statistical analysis

The findings obtained were all presented as the mean SD. The collected data was subjected to a one-way variance analysis to determine the significant difference (ANOVA). The findings were analyzed using the SPSS 24.0 program. Statistically significant was considered a $p < 0.05$.

5- Results

5-1. Phytochemical screening

Phytochemical screening results performed on the ethanolic extracts of the 2 organs studied were shown in Table 1. These phytochemical tests carried out allowed us to have a general idea of the chemical composition of the plants studied.

Table 1 - Phytochemical screening results of *Olea europaea* L leaves and fruits ethanolic extracts

Organs	Phenolic compounds	Flavonoids	Tanin	Saponines	Free quinones	Anthraquinones	Triterpènes	Coumarins	Anthocyanins	Alcaloids	Glycosids
Leaves	+	+	+	+	+	+	+	+	-	-	-
Fruits	+	+	+	+	+	+	+	+	+	-	-

The phytochemical analysis of the ethanol extract of *Olea europaea* leaves and fruits revealed some constituents documented to have potential antidiabetic property e.g. tannins (TEOTIA; SINGH, 1997), saponins of any of these might be contributory to the observed antihyperglycaemic activity of the plant. The results of the screened phytochemicals (tannins, saponins, flavonoids, terpenoids, glycosids et alkaloids anthocyanins coumarins anthraquinones free quinones) of ethanolic extracts of leaves and fruits if the plants showed the common presence of *Phenolic compounds*, tannins, *Coumarins Free quinones* saponins, flavonoids and terpenoids. We also noted the absence of Anthocyanins, Alkaloids and Glycosids. Similar insights were found in those reported in (MEZOUAR et al., 2022; CHEURFA et al., 2022). The efficiency of extraction is affected by the chemical nature of molecules in the plant, extraction method chosen and solvent used (STALIKAS, 2007).

5-2. Quantitative studies

Yield extracts, total phenolic, flavonoids and tanins contents in the ethanolic extracts of leaves and fruits of *Olea europaea* were presented in Table 2.

Table 2 - Yield of extracts, TPC, TFC and TCT contents in leaves and fruits of *Olea europaea*

Organs	Yield (%)	Phenolics content ± SD	Flavonoid content ± SD	Tannin content ± SD
		(mg GAE/g DW)	(mg QAE/g DW)	(mg TAE/g DW)
Leaves	30.58	282.5±0.27	70.77± 0.06	98.30± 0.06
Fruits	27.44	197.5±0.11	19.7 ± 0.02	60.05± 0.08

The results mentioned in this table show that the extract of the leaves represents the highest content of polyphenols (282.5 mg GAE/g DW) than fruits (197.5mg GAE/g DW). One-way analysis of variance reveals the existence of a highly significant ($P = 0.008$). The results show that the leaf extract represents the highest flavonoid content with (70.77 mg QAE/g DW) than fruits (19.7 mg QAE/g DW). The analysis of variance (AVI), it emerges from the differences highly significant ($P = 0.003$) The tannin contents show that the extract of the leaves continuously represents the highest tannin content (98.30 mg TAE/g DW) compared to fruit (60.05 mg TAE/g DW). One-way analysis of variance (AVI) shows significant differences ($P = 0.024$) between tannin contents in both leaf and fruit organs. A highly significant correlation ($R^2 = 0.913^*$; $R^2 = 0.926^{**}$, $R^2 = 0.900^*$) between the extraction yield and total polyphenol content, flavonoid content and tannin content, respectively. Several works have shown that the contents of total phenol, flavonoids and tannin are higher than that of fruits (PALMERI et al., 2022; XIE et al., 2015; SILVA et al., 2006). Mebirouk-Boudechiche et al. (2014) and Dekdouk et al. (2015) showed that *Olea europaea* is rich in tannin.

5-3. Results of HPLC analysis of fruits and leaves extracts

The total phenolic content measured by the Folin-Ciocalteu procedure does not give a full picture of the qualification or quantification of the phenolic constituents in plant matrices (WOJDYŁO et al., 2007). Olive fruit and leaves extracts were analyzed by HPLC-MSn. Quantitative data were calculated from their respective calibration curves. Ethanol extract allowed to tentatively identify 04 constituents in fruits and 7 in leaves the chromatogram is reported in Figure 1 and Table 3.

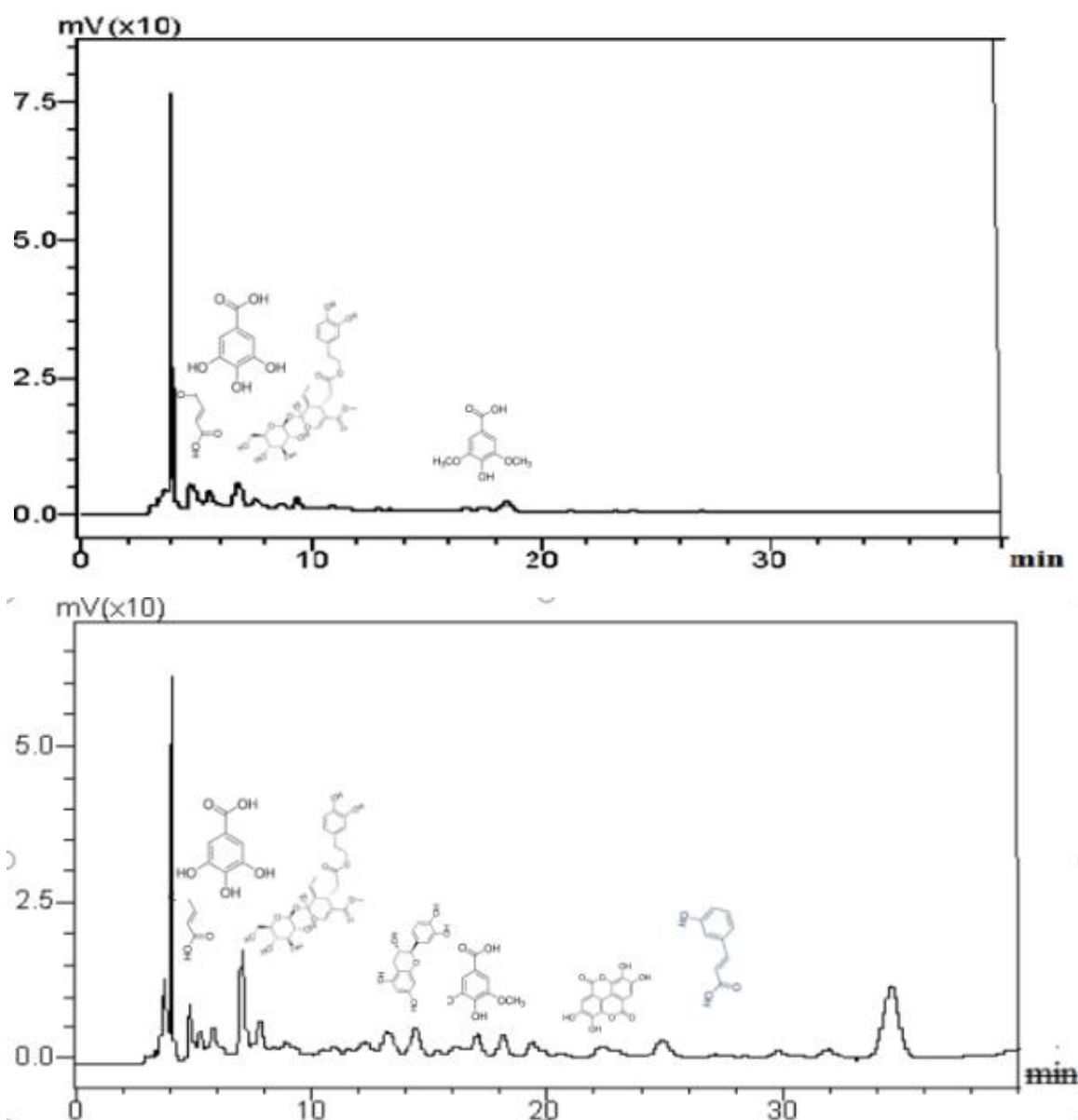


Figure 1 - HPLC phenolic profile of fruits and leaves extract of *Olea europaea* Var frantoye Subsequently if we compare our extracts with the references of Phenolic Standard, we will find that these molecules appear in the different run time of (Table 3).

Table 3 - Quantity of phenolic components of fruits and leaves extract of *Olea europaea* var frantoye

	Components	Run time	Quantity ppm	Typ
Leaves	Fumaric acid	5,249	43,47	Organic compound
	Galic acid	5,789	0,667968	Trihydroxybenzoic acid
	Oleuropein	7,772	781,26	Phenolic compound
	Catechin	13,171	4,7732	Flavonol
	Syringic acid	17,033	0,78581	Phenolic compound
	Ellagic acid	22,347	0,513486	Polyphenol
	T-3 hydroxy cinnamic acid	29,814	0,387828	Phenolic compound
Fruits	Fumaric acid	5,1	12,06	Organic compound
	Galic acid	5,57	0,396466	Trihydroxybenzoic acid
	Oleuropein	7,565	233,83	Phenolic compound
	Syringic acid	17,693	0,1128	Phenolic compound

The contents of fumaric acid are 43,47 ppm and 12.06 ppm. respectively, for leaves and fruits of frantoye, also (AVI) showed a THS difference, and the coefficient of variation is very high 59.34%. As for the contents of Galic acid are of the order of 0,66 ppm and 0,39 ppm, respectively, for leaves and fruits of frantoye. The leaves of *Pistacia lentiscus* are rich in secondary metabolite, noticing the presence of Catechin, Ellagic acid and T-3 hydroxy cinnamic acid. High-Performance Liquid Chromatography (HPLC) is commonly used to analyze the chemical composition of plant materials such as the leaves and fruits of *Olea europaea* (olive tree). This method helps in identifying and quantifying various bioactive compounds, including phenolic compounds, flavonoids, and other. If we specify the important components detected; in leaves and fruits of *Olea europaea* var frantoye appears to have data. Chemicals are present as the main component fumaric acid, Oleuropein, gallic acid and Syringic acid. Fumaric acid is a naturally occurring organic acid found in various plants, including *Olea europaea* (olive tree). Pereira et al. (2007) are mentioned that fumaric acid is present in different parts of the olive tree, including the leaves, fruits, and oil. Oleuropein is highly concentrated in olive leaves compared to fruits and oil. It is a secoiridoid glycoside that contributes significantly to the bitter taste of the leaves (BENAVENTE-GARCÍA et al., 2000; BOUAZIZ; SAYADI, 2005). Dekdouk et al. (2015) and Balasundram et al. (2006) also mentioned the presence of galic acid, syringic acid and Oleuropein in fruit of *Olea europaea* L. Balasundram et al. (2006) mentioned the presence of this molecule in leaves and fruits of *Olea europaea* L.

5-4. Oral glucose tolerance tests in normal rats

The blood glucose level significantly ($P < 0.05$) changes in normal mice administered the glucose. The oral glucose tolerance test of the leaves and fruits extracts were evaluated in the fasting. Administration of 500 mg/kg the leaves and fruits extracts showed a significantly improved glucose tolerance ($p < 0.05$), In normal rat's leaves extract at 500 mg/kg improved glucose tolerance (51,85 % maximum reduction in blood glucose at 120) more than fruits extracts (50,66%) and Glucophage 500 (42,6%) at the same time. However, of the 2 other doses tested, 250 mg/kg of extracts leaves and fruits were found to be the effective dose (44,29% and 39,39% leaves and fruits, respectively). In all other times (30, 60, 90 min) the leaves have had the most important power compared to the fruits and Glucophage 500 (Table 4).

Table 4 - Effect of fruits and leaves extract of *Olea europaea* on oral glucose tolerance test

		Blood glucose concentration (mg/dL)				
Treatment		0 min	30 min	60 min	90 min	120 min
Fruits	500 mg/kg	97±1.80	150±0.07	135±2.05	89±2.48	74±0.12
	250 mg/kg	92±0.01	165±0.80	145±0.55	112±3.52	100±0.77
	50 mg/kg	93±2.25	169±1.52	166±2.15	135±1.48	112±0.47
Leaves	500 mg/kg	98±0.33	135±0.98	124±0.02	92±0.28	65±0.83
	250 mg/kg	96±0.08	149±1.20	135±0.58	106±1.17	83±0.85
	50 mg/kg	91±0.85	155±2.58	133±0.83	124±0.58	109±0.66
Witness	Negative control	93±0.05	175±0.85	164±1.11	149±1.22	135±0.41
	Glucophage 500	90±0.52	157±1.36	142±0.30	101±0.02	90±1.85

SD (n = 5)

5.5. Hypoglycemic activity in fasting normal mice

Also, fasting blood glucose test in normal rats revealed that *Olea europaea* var frantoye. Don leaves and fruits extracts given at 500 mg/kg produced a significant ($p < 0.05$) fall in blood glucose

at the time 120 min after administration (Table 5), though the effect was more than that produced by Glucophage 500, the highest activity was recorded of leaves extracts (49 ± 0.24 mg/dL) compared to the fruits extracts (54 ± 0.67 mg/dL) with reduction of (50,50% and 44,89 %) we compare them with Glucophage 500 (72 ± 1.22 mg/dL) with reduction of 27,08%.

Table 5 - Effect of fruits and leaves extract of *Olea europaea* on normal mice

		Blood glucose concentration (mg/dL)				
	Treatment	0 min	30 min	60 min	90 min	120 min
Fruits	500 mg/kg	98 ± 4.22	93 ± 4.58	66 ± 3.20	60 ± 0.58	54 ± 0.67
	250 mg/kg	101 ± 0.33	99 ± 2.97	98 ± 2.25	96 ± 1.05	95 ± 1.85
	50 mg/kg	95 ± 0.02	96 ± 2.20	94 ± 0.44	93 ± 2.55	93 ± 2.89
Leaves	500 mg/kg	99 ± 0.41	84 ± 0.08	64 ± 0.01	52 ± 2.25	49 ± 0.24
	250 mg/kg	89 ± 0.00	85 ± 0.20	84 ± 0.50	83 ± 0.12	82 ± 0.70
	50 mg/kg	95 ± 0.21	95 ± 0.81	94 ± 0.07	92 ± 0.02	91 ± 0.57
Witness	Negative control	94 ± 1.20	92 ± 0.12	90 ± 0.10	90 ± 1.15	89 ± 0.54
	Glucophage 500	96 ± 0.20	94 ± 0.78	87 ± 0.80	83 ± 0.11	70 ± 1.22

SD (n=5)

In this study, the hypoglycemic exertion and oral glucose tolerance test of the OE in dieted normal mice delved at 30, 60, 90 and 120 min. In normal rats, the extent of improvement of glucose tolerance by leaves and fruits of *Olea europaea* extract is either comparable or less than that produced by. This suggests that the plant acts by Glucophage 500. However, the observation that aqueous extract of OE reduced blood sugar level in diabetic rats more potently than Glucophage 500 indicates the possibility of additional extra pancreatic mechanism of effect. Diabetes mellitus is probably the fastest growing metabolic disorder in the world, and it is a major source of morbidity in developed countries. Medicinal plants play an important role in the management of diabetes mellitus, especially in developing countries where resources are meager. Zakari et al. (2023) shows that olive (*Olea europaea*) could play a key role in the management of diabetes. The study of Wainstein et al. (2012) assessed the effects of 500 mg of olive leaves extract on diabetic patients and diabetic rats. That revealed significantly lower fasting plasma insulin levels and HbA1c; however, the postprandial plasma insulin levels did not differ. Its results are confirmed by Alesci et al. (2021) and Wainstein et al. (2012). The phytochemical analysis of the ethanol extract of *Olea europaea* leaves and fruits revealed some constituents documented to have potential antidiabetic property tannins (TEOTIA; SINGH, 1997), saponins (PANDEY et al., 2022) any of these might be contributory to the observed antihyperglycaemic activity of the plant. The main active component in olive leaf and fruits and its extract is Oleuropein, a natural product of the secoiridoid group. Several studies have shown that Oleuropein possesses a wide range of pharmacologic and health-promoting properties (HASSEN et al., 2015; SEDEF; KARAKAYA, 2009). Oleuropein was reported to have an antihyperglycemic, lipid-regulating, and cardioprotective effects especially in cell culture and animal models (KHAN et al., 2007).

6- Conclusion

The *in vitro* analyses of *Olea europaea* leaves and fruits revealed that the extracts obtained through leaves and fruits powder maceration are rich in natural bioactive substances. *Olea europaea* var frantoye is recognized as a valuable medicinal plant globally. Our study initially demonstrated

that leaves and fruits extracts exhibit high levels of polyphenols, which contribute to their potential antidiabetic. The results of biological activity assays, antidiabetic activity, indicated that frantoye leaves and fruits extracts possess considerable antidiabetic power across all two extracts studied. In conclusion this study suggests that the extracts of leaves and fruits of *Olea europaea*. Don has potential antidiabetic property, thereby justifying the traditional claim. This activity is due to the presence of phenolic components specifically Oleuropein.

Conflict of interest

There was no conflict of interest between the authors.

Contribution of authors

All authors contributed equally to this work.

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