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Aldose Reductase Inhibitory and antioxidants Activities of the leaf Extract of *Olea europaea*

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Abstract

Plants have played a crucial role in maintaining human health and improving the quality of human life for thousands of years. The World Health Organization has estimated that 80% of the earth's inhabitants rely on traditional medicine for their health care needs, and most of this therapy involves the use of plants extracts or their active components. In the current study, we have made an attempt to screen the phytochemical components found in the leaf extract by different solvents of medicinal plant ***Olea europaea*** The results of our study indicated that *Olea europaea* is a promising source of antioxidants showing free radical scavenging activity and aldose reductase inhibitory activities. The bioactivity studies using the plant extracts clearly indicate that some of the potential compounds found in this plant species which may be explored further as lead molecules for human benefits.

Keywords: aldose reductase, phytochemicals, extract, antioxidants.

Introduction:

Plants are widely used for the treatment of human diseases. The phytochemical constituants are enriched in different parts of the plants such as leaves, stem, bark, roots and flowers. In order to evaluate the medicinal properties, The plant of ***Olea europaea* L**. were selected that belonged to the family Oleaceae,**.** belongs to the family Oleaceae have been used widely in folk medicine in European Mediterranean area, Arabia peninsula, India and other tropical and subtropical regions for diuretic, hypertensive, emollient and urinary and bladder infections [1]. It is also known to possess antihypertensive [2], vaso-dilator [3], antimicrobial [4], hypolipidemic [5], antioxidant and antidiabetic activities [6], capacity to lower blood pressure in animals [7] and increase blood flow in the coronary arteries [8].

Olea europaea L:

Table 1. Classification:

Class	**Dicots**
Kingdom	Plantae
Order	Lamials
Family	Oleaceae
Genus	Olea
Species	europea

Materials and Method:

1. Chemicals and Reagents :

2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin&clocateues phenol reagent (FCP), 2-mercaptoethanol(C_2H_6OS), 2- nicotinamide adenine dinucleotide phosphate (NADPH).

2. Plant Material Collection:

The plant part (leaf) of ***Olea europaea*** was collected from Syria, and the plant material air dried, powdered & stored in deep freezer at 20^{o}C.

3. Preparation of crude extracts of the plant:

Plant samples (20 gm) powdered individually of leaf of ***Olea europaea***,was three times extracted sequentially for 24 hours with various solvents of hexane, petroleum ether, methanol and water by Soxhlet extraction.

4. Phytochemical screening:

Phytochemical analysis of the *Olea europaea* plant:

The qualitative phytochemical screening tests were performed by using standard procedures as described by (Harborne[9], Trease and Evans [10], Sofowra [11]). The presence of bio- active compounds was screened for alkaloids, Carbohydrates, flavanoids, Phenols, terpenoids, saponins, glycosides, tannins, Proteins and Amino acids.

5. Antioxidant activity:

The analysis of antioxidant property was carried out by Non enzymatic method. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to measure the radical scavenging ability of ***Olea europaea***. Ascorbic acid was used as the standard for antioxidant activity. Antioxidant activity was defined as the amount of Ascorbic acid equivalent to the amount of crude extract that resulted in equal scavenging of DPPH.

6. Aldose reductase assay:

Isolation and purification procedure of enzyme with some modification was followed according to *Hayman* and *kinoshita*. [12]. the bovine lens was enucleated and lenses immediately dissected. The eye lenses (500 mg) were weighed and homogenised with 5 ml of 50 mM phosphate buffer (pH 7.6). Total lens protein was determined by Lowry's method using bovine serum albumin as a standard [13]. The lens Aldose reductase activity was evaluated by determining the decrease in NADPH concentration at 340 nm using a UV–visible spectrophotometer.

Results & Discussion

Phytochemical screening:

Table-1 indicates the qualitative analysis of the leaf of *Olea europaea,* the leaf extract was treated with hexane and the results showed the presence of carbohydrates, glycosides, proteins, amino acids while the other phytoconstituents were absent. The leaf extracts treated with petroleum ether extract indicated the presence of glycosides, saponin, proteins, and amino acids. Similarly, the leaf extracts in methanol indicated the presence of alkaloids, carbohydrates, glycosides, terpenoids, steroids, phenols, tannin, flavonoids, proteins, amino acids. Saponin was absent in the sample. The aqueous extract indicated the presence of alkaloids, carbohydrates, glycosides, saponin, terpenoids, steroids, phenols, tannin, proteins, and amino acids. Flavonoids were absent.

Aldose Reductase Activity:

The effects of the leaf extracts of plants samples were estimated with the aldose reductase enzyme using DL-glyceraldehyde as a substrate. Their inhibitory potencies and IC50 values on the AR enzyme were determined. The average inhibitory activities of the leaf extracts were calculated and shown in **Table-2**. The effect of leaf extracts of ***Olea europaea***, It is evident that from the data that when the extracts of *Olea europaea* were tested with methanol at different concentrations (10 µg/ml to 50 µg/ml), the percent AR inhibiting activity increased. In case of Petroleum ether extract the % ARI activity followed a similar trend up to 40 µg/ml. However the results indicated that the % ARI activity was low with hexane leaf extracts. The maximum % ARI activity was observed with methanol extract (93.33±07 µg/ml) at 50 µg/ml in O***lea europaea***. AR inhibition was 94.66±2 at a concentration of 40 µg/ml with Petroleum ether extract while the inhibitory activity of the leaf of ***Olea europaea*** in the hexane extract was not significant when compared with negative control.

Antioxidant activity:

The DPPH (1- diphenyl-2-picrylhydrazy) scavenging activity was performed using a solution of 0.1 mM DPPH in methanol solution. The average antioxidant activities of the leaf extracts ***Olea europaea*** **are illustrated in Table-3**. It is evident from the data that when the leaf extracts of ***Olea europaea*** were tested with methanol at different concentrations (10 µg/ml to 50 µg/ml), the maximum percent antioxidant activity was 64.66±02 at a concentration of 50 µg/ml while it was minimum (9.66±33) at the concentration of 10 µg/ml.

Discussion:

The results obtained in our studies demonstrated that under in-vitro conditions, the % ARI activity of the methanolic leaf extracts of ***Olea europaea*** resulted in significant ARI activity as compared to the control. It is also interesting to know that the ARI activity of the methanol extracts of the leaf of ***Olea europaea***, was high as compared to the solvents like hexane & Pet ether. The differential response of the ARI activity can be attributed to solubility property of the leaf extracts in methanol as compared to other solvents. Moreover, the effect can also be due to the presence of flavonoids and phenols. There are many reports of AR inhibiting activity of natural products [14]. Some sulfated flavonoids in Polygonum hydropiper were discovered to show potent inhibition against bovine lens aldose reductase [15]. Other studies showed that flavonoid, glycosides [16], isoflavonoids [17], flavanone, glucosides (myrciacitrins III) [18] Plants which are rich in polyphenols and bioflavonoids are reported to reduce the AR activity. That methanol extract of the leaf of *Olea europaea.* Had a good antioxidant activity that is very probably attributed to their high phenolic compounds and flavonoids. This antioxidant activity could be useful in prevention of atherosclerotic and heart diseases. The antioxidant activity may attributed to terpenoid content [19], as well as phenolic compounds (tannins) and flavonoids [20, 21] that are found to be present in the extracts. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triple oxygen, or decomposing peroxides [19]. The DPPH free radical method determines the number of studies reported that a relation exists between the antioxidant activity and the reducing power [22-24].

Table 1: **Screening of phytochemical constituents of leaf extracts of *Olea europaea***

Phytochemical	Hexane	Pet ether	Methanol	Aqueous
alkaloids	-	-	+	+
carbohydrates	+	+	+	+
glycosides	+	+	+	+
Saponin	-	+	-	+
Terpenoids	-	-	+	+
Steroids	-	-	+	+
phenolics	-	-	+	+
Tannins	-	-	+	+
Flavonoids	-	-	+	-
proteins	+	+	+	+
amino acids	+	+	+	+

Note: + indicates presence of phytoconstituents, - Indicates absence of phytoconstituent

Table-2: Effect of crude leaf extracts on AR inhibitory activity of *Olea europaea L*

% ARI activity						
Olea europaea						
Solvent	**10µg/ml**	**20µg/ml**	**30µg/ml**	**40µg/ml**	**50µg/ml**	**IC_{50}**
Hexane	60.00±25	64.00±18	74.33±19	75.33±12	65.00±30	11.83±18
Pet ether	46.66±18	65.33±22	76.33±14	94.66±02	91.33±03	15.00±18
Methanol	82.66±03	84.33±04	88.33±07	90.66±06	93.33±07	05.33±01
Aqueous	79.00±02	82.66±05	64.00±25	85.66±10	86.66±09	05.83±01

Table -3: Antioxidant activities of the leaf extracts of *Olea europaea*

% DPPH radical	
Concentration(µg/ml)	***Olea europaea*** **Methanol extract**
10	39.66±33
20	43.66±22
30	55.66±18
40	58.00±10
50	64.66±02
IC_{50}	06.83±01

Reference:

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