

Pharmacognostic Specifications of Leaves of *Olea europaea* Collected from Iraq

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ABSTRACT

Olea europaea L. is used traditionally as hypotensive, emollient, laxative, diuretic, febrifuge, skin cleanser, and also used for the treatment of urinary infections, gallstones, bronchial asthma and diarrhoea. Several phytoconstituents have been reported from different parts of the plant such as glycosides, secoiridoid, flavonoids and poly-unsaturated fatty acids. As the herb is used widely in the traditional systems of medicine, it was thought worthwhile to undertake the standardization. The present study designed for determination of physico-chemical parameters, phytochemical screening and also its antioxidant activity. The quality control parameters like extractive of plant with different solvents, ash values, foreign organic matter, loss on drying and pH of aqueous solution were determined. The antioxidant activity was determined by DPPH free radical scavenging method. The results obtained from preliminary pharmacognostic standardization of leaves of *O. europaea* are very helpful in determination of quality and purity of the crude drug and its marketed formulation. The ethanolic, hydro-alcoholic and aqueous extracts of leaves of *O. europaea* showed potent DPPH free radical scavenging activity.

Keywords: *Olea europaea*, Oleaceae, Antioxidant activity, Quality standards, WHO guidelines.

INTRODUCTION

Olea europaea (syn. Zaytoun, Jetun) belonging to the family Oleaceae is a small evergreen tree, from 12 to 20 feet high, with hoary, rigid branches, and a grayish bark. *O. europaea* preparations have been used widely in folk medicine in European Mediterranean area, Arabia peninsula, India and other tropical and subtropical regions, as

diuretic, hypotensive, emollient and for urinary and bladder infections¹. Olive oil represents an important component of the Mediterranean diet whose intake is greatly growing in developed and developing countries for its known healing effects. Several phytoconstituents have been isolated and identified from different parts of the

plant belonging to the category glycosides, secoiridoid, flavonoids and poly-unsaturated fatty acids². *O. europaea* have been reported for its antihypertensive³, vaso-dilator⁴, antimicrobial⁵, hypolipidemic⁶, antioxidant and antidiabetic activities⁷. The present study deals with development of quality standards and antioxidant activity of leaves of *O. europaea* as per WHO guidelines⁸.

MATERIALS AND METHODS

Plant material and chemicals

The leaves of *Olea europaea* L. were collected from the Shaqlawa, Iraq. The sample was identified and a voucher specimen (PRL/2013/05) of the plant was kept for future reference. Ascorbic acid and DPPH (2, 2-diphenyl-1-picrylhydrazyl) were obtained from Sigma Chemicals Co., St. Louis, MO, USA. All other solvents and chemicals were of analytical grade.

Preliminary phytochemical screening

The qualitative chemical tests were performed for different extracts according to the methods described by Farnsworth *et al.*,⁹ with slight modifications.

Determination of alkaloids

The alkaloids were extracted by refluxing the sample with sufficient amount of water for about 2 hr. The extract was concentrated on a rotor vapor, basified with NH_4OH and was extracted with CHCl_3 (three times). Then the content was concentrated and 2 drops were spotted separately on a thin layer chromatography (TLC) plate. After development the plate was dried, Dragendorff's reagent was sprayed onto them. Alkaloids give an orange color with Dragendorff's reagent.

Determination of steroid glycosides

The extracts were dissolved in equal volumes of acetic anhydride and CHCl_3 . The mixture was transferred to a dry test tube and

conc. H_2SO_4 acid was added at the bottom of the tube. Formation of a reddish brown or violet brown ring at the interface of the 2 liquids indicates presence of steroids.

Determination of polyphenolics

Two to three drops of 1 % FeCl_3 solution was added to 2 ml portions (1 %) of each extract. Phenolic compounds produce a deep violet color with ferric ions.

Determination of saponins

The extract is taken in test tube with small amount of water and shaken vigorously for one minute and observed for formation of rich lather, which is stable for more than ten minutes.

Determination of flavonoids

The extracts were dissolved in methanol (50 %, 1-2 ml) by heating. Then metal magnesium and 5-6 drops of concentrated hydrochloride acid (HCl) were added. The solution turns red when flavonoids are present.

Other chemical tests for phytoconstituents were performed as per approved methods.

Determination of physico-chemical parameters

Physicochemical parameters were determined for leaves of *O. europaea* according to methods described in WHO guidelines.

Determination of total ash

The powdered material (2g) was accurately weighed and placed in a crucible. The material was spread in an even layer and it was ignited to a constant weight by gradually increasing the heat to 500-600°C until it was white indicating the absence of carbon. The residual ash was allowed to cool in a desiccator. The content of total ash (in

mg/g) of air-dried material was calculated as follows:

$$\text{Total ash (\% w/w)} = \frac{(\text{weight of ash}) \times 100}{\text{weight of sample}}$$

Determination of acid insoluble ash

HCl (2 N; 25 mL) was added to the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the rinsed contents were added to the crucible. The acid insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing acid insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The residue was allowed to cool in a desiccator and weighed. The content of the acid insoluble ash (in mg/g) of air-dried material was calculated as follows:

$$\text{Acid insoluble ash (\% w/w)} = \frac{(\text{weight of ash}) \times 100}{\text{weight of sample}}$$

Determination of water soluble ash

Water (25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water and added to the crucible. The water insoluble matter was collected on an ash less filter paper and washed with hot water. The filter paper containing the water insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The water soluble ash content was calculated using the following equation.

$$\text{Water soluble ash (\% w/w)} = \frac{(\text{total ash} - \text{water insoluble residue in total ash}) \times 100}{\text{weight of sample}}$$

Determination of alcohol soluble extractive

Accurately weighed powdered material (4g) and was placed in a glass stoppered round bottle flask (RBF). Ethanol (100 ml) was added to the RBF and then, it was shaken well and allowed to stand for 1 h. A reflux condenser was attached and boiled gently for 1 h, and then it was cooled and filtered. The flask was shaken well and filtered rapidly through a dry filter paper. After that, 25 ml of the filtrate was transferred to a tarred flat bottomed dish and evaporated to dryness on a water bath. Then the dish was dried at 105°C for 6 h and cooled in a desiccator and weighed. The content of extractable matter (% w/w) air-dried material was calculated as follows:

$$\text{Alcohol soluble extractives (\% w/w)} = \frac{(\text{weight of residue}) \times 4 \times 100}{\text{weight of sample}}$$

Determination of hydro-alcohol and water soluble extractives

The same procedure as described for the ethanol soluble extractive matter was followed for the determination of hydro-alcoholic and water soluble extractive matter using ethanol-water (1:1) and distilled water instead of ethanol.

Foreign matter analysis

Foreign matter presence may be due to faulty collection of crude drug or due to deliberate mixing. It was separated from the drug so that results obtained from analysis of the drug gives accuracy. Its percentage in the crude drug was calculated.

Determination of moisture content

The powdered material (10 g) was placed in a moisture dish and dried to a constant weight in an oven at 100-105°C. The loss of weight (in mg/g) of air dried was calculated as follows:

$$\text{Moisture content (\% w/w)} = \frac{(\text{Initial weight of sample} - \text{final weight of sample}) \times 100}{\text{weight of sample}}$$

Determination of pH

The pH of 1 and 10 % aqueous solution were determined by making appropriate concentration of powdered drug in aqueous solution, filtered and checked the pH of the filtrate with a standardized glass electrode.

Antioxidant (DPPH scavenging) activity of *Olea europaea*

The ability of leaf extracts of *O. europaea* to scavenge DPPH free radicals was assessed by the standard method^{10,11} adopted with suitable modifications. The ethanolic, hydro-alcoholic and aqueous extracts are reconstituted in methanol. The stock solution of all extracts were prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions 1000, 500, 250, 125, 62.5 and 31.25 µg/ml were prepared by serial dilution method. Diluted solutions (1 ml each) were mixed with 1 ml of methanolic solution of DPPH (1 mg/ml). After 30 min incubation in darkness at room temperature (25°C), the absorbance was recorded at 517 nm. Control sample contained all the reagents except the plant extract. Percentage inhibition was calculated using equation given below:

$$\% \text{ Inhibition} = \frac{A_{CO} - A_t}{A_{CO}} \times 100$$

Where, A_{CO} is absorbance of the control and A_t is absorbance of the samples.

IC_{50} values were estimated from the % inhibition versus concentration plot using a non-linear regression algorithm.

RESULTS

The results of standardization parameters are:

Phytochemical Screening

The results of phytochemical screening of leaves of *Olea europaea* were depicted in Table 1.

Physico-chemical parameters

Results of physico-chemical parameters of leaves of *Olea europaea* were summarized in Table 2.

Antioxidant (DPPH scavenging) activity of leaves of *Olea europaea*

The antioxidant activity of ethanolic, hydro-alcoholic and aqueous extracts of leaves of *O. europaea* were determined using a methanol solution of DPPH reagent. The antioxidant activity of *O. europaea* was expressed in terms of percentage of inhibition (%). Parallel to examination of the antioxidant activity of the extract, the values for standard ascorbic acid was obtained and compared with the antioxidant activity of all extract of *O. europaea*. The plot of % inhibition verses concentration given for ascorbic acid and extract is in Figure 1 was used to calculate IC_{50} values. The maximum inhibition produced by ethanolic, hydro-alcoholic and aqueous extracts of leaves of *O. europaea* were 67.78±4.75, 79.86±4.28 and 86.37±3.23 %, respectively at 1 mg/ml concentration level. The IC_{50} values and % inhibition at each concentration level of Vitamin C, and different extracts of *O. europaea* where depicted in Table 3.

DISCUSSION

Standardization of crude drug is an integral part of establishing its correct identity. The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. Phytochemical screening revealed the presence of glycosides, alkaloids, flavonoids and amino acids in ethanolic, hydro-alcoholic and aqueous extracts mainly. The physicochemical analysis of plant drugs is an

important for detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity and quality of drugs. The ash value was determined by 3 different methods, which measured total ash, acid insoluble ash, and water soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition¹². The total ash usually consists of carbonates, phosphates, silicates and silica, which include both physiologic ash and nonphysiologic ash. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing. Acid insoluble ash indicates contamination with silica, for example, earth and sand. Comparison of this with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug. Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water soluble salts in the drug. Extractive values are representative of the presence of the polar or nonpolar extractable compounds in a plant material. Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Insufficient drying leads to spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles¹³. All these parameters, which are being reported, could be useful in identification of distinctiveness features of the crude drug and used for establishing quality of leaves of *O. europaea*. In conclusion, the results obtained from phytochemical screening studies and physico-chemical parameters can be used to standardize leaves of *O. europaea*.

The antioxidant (DPPH scavenging) activity of different extract of leaves of *O. europaea* were determined and compared with ascorbic acid. The maximum inhibition was produced by aqueous extract (86.37±3.23 %) at concentration level 1 mg/ml. Thus the

aqueous extract of *O. europaea* can be used as natural source of antioxidant.

CONCLUSION

The results of physico-chemical analysis, phytochemical screening and *in-vitro* antioxidant activity is useful in determining quality, safety and efficacy of *O. europaea* leaves for its use as potential drug candidate.

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Table 1. Results of phytochemical screening

Extract constituents	Ethanollic extract	Hydro-alcoholic extract	Aqueous extract
Glycosides	+	++	++
Alkaloids	+	+	+
Carbohydrates	+	+	+
Tannin	+	+	+
Phenolics	+	+	-
Flavonoids	+	++	+
Proteins & amino acids	+	+	+
Resins	+	+	-
Lipids/fats	+	++	+

(Where, – absent and + present)

Table 2. Summary of results of physico-chemical parameters

Parameters	Results (n= 3, Mean ± SD)
Ash values	
Total ash (% w/w)	8.75±1.13
Acid insoluble ash (% w/w)	1.82±0.26
Water soluble ash (% w/w)	4.27±0.85
Extractive values	
Alcohol soluble extractives (% w/w)	7.43±1.56
Hydro-alcoholic extractives (% w/w)	12.93±1.28
Water soluble extractives(% w/w)	17.67±2.18
Foreign organic matter (% w/w)	3.62±1.04
Loss on drying (% w/w)	12.41±1.82
pH values of aqueous solution	
pH of 1 % aqueous solution	6.81±0.25
pH of 10 % aqueous solution	5.63±0.28

Table 3. Antioxidant (DPPH scavenging) activity of leaf extracts of *O. europaea* and Vitamin C

Conc. (µg/ml)	Vit. C	<i>Olea europaea</i>		
		Ethanollic extract	Hydro-alcoholic extract	Aqueous extract
1000	85.79±3.26	67.78±4.75	79.86±4.28	86.37±3.23
500	84.07±2.65	64.15±5.17	77.15±4.58	81.85±4.34
250	70.28±3.31	57.96±3.05	65.01±3.52	71.27±3.67
125	56.99±2.42	40.61±4.06	47.88±2.28	53.89±1.16
62.5	44.69±2.92	26.65±3.03	32.53±2.04	32.79±4.21
31.25	23.94±2.38	17.25±3.19	22.27±1.47	22.27±1.48
IC₅₀ value (µg/ml)	82.42±5.12	204.27±19.74	140.48±14.34	122.38±8.51

(Data where presented as mean±SD, n=3)

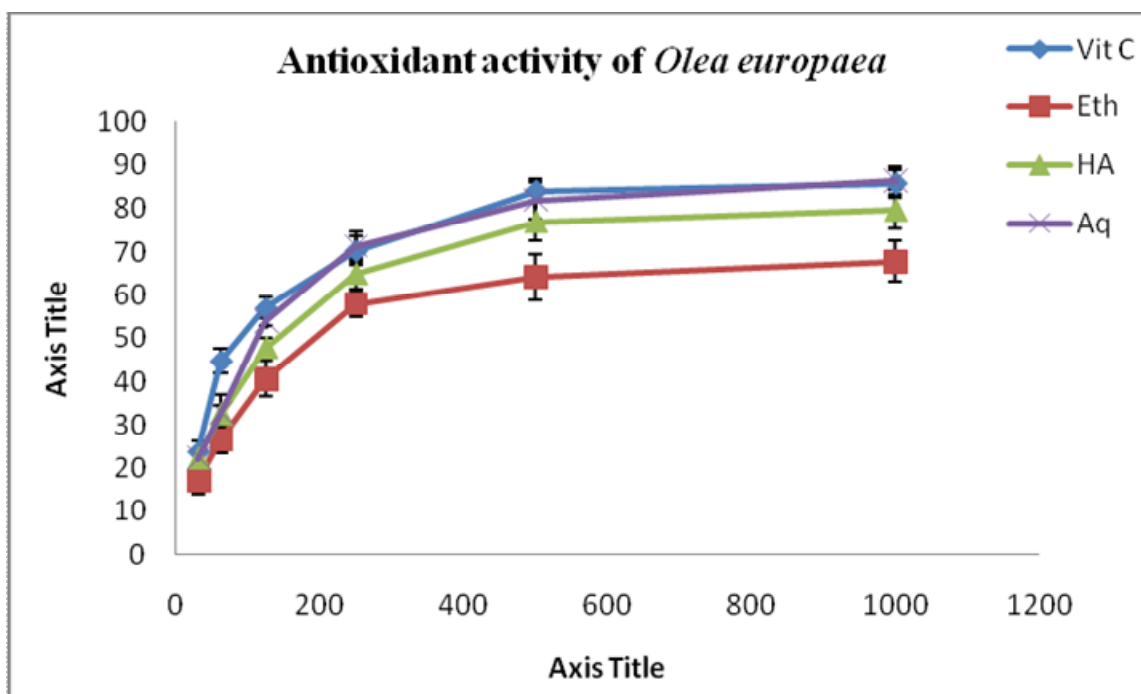


Figure 1. Antioxidant (DPPH scavenging) activity of *Olea europaea* (Data where presented as mean \pm SD, n=3, Vit C (vitamin C), Eth (ethanolic extract), HA (hydro-alcoholic extract) and Aq (aqueous extract) of *O. europaea*)