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Research Article

Effect of solvent on the extraction of constituents from the leaves of *Olea europaea* and their 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

Moeen ud Din[a], Manzar Zuhra[a], Abeera Zafar[b], Shamaim Zafar[c], Zafar Iqbal[d,*]

[a]*Department of chemistry, Lahore Gartison University, Lahore, Pakistan*
[b]*Department of Pharmacy, Hujvery University, Lahore, Pakistan*
[c]*Department of Pharmacy, Ripha International University, Lahore, Pakistan*
[d]*Applied Chemistry Research Center, PCSIR Labs. Complex, Lahore, Pakistan*

Abstract

Olive leaf extracts were prepared using the maceration method with a series of solvents of varying polarities, including n-hexane, dichloromethane, ethyl acetate, nitrobenzene, methyl ethyl ketone, acetone, methanol, and acetonitrile. Among the solvents, methanol yielded the highest extract content (26.8%), followed by nitrobenzene (18.4%), acetone (11.2%), ethyl acetate (10.4%), dichloromethane (9.2%), methyl ethyl ketone (6.8%), acetonitrile (3.6%), and n-hexane (0.96%). The antioxidant activity of these extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. At a concentration of 120 ppm, the percentage inhibition of DPPH radicals was highest for ascorbic acid (96.49%), followed by methanol (90.45%), ethyl acetate (85.58%), acetone (78.75%), and methyl ethyl ketone (66.28%). Lower inhibition was observed for acetonitrile (55.36%), nitrobenzene (39.77%), dichloromethane (38.01%) and n-hexane (28.07%). The antioxidant activity, evaluated using IC_{50} values (where lower values indicate higher antioxidant potential), identified ascorbic acid as the most potent standard (IC_{50} = 7.03 μg/mL). Among the samples, the n-hexane extract demonstrated the weakest antioxidant activity (IC_{50} 206.09 μg/mL), while the methanol extract exhibited the strongest (IC_{50} = 35.88 μg/mL). Other extracts showed IC_{50} values of 157.77 μg/mL for dichloromethane, 155.10 μg/mL for nitrobenzene, 113.18 μg/mL for acetonitrile, 80.98 μg/mL for methyl ethyl ketone, 54.18 μg/mL for acetone, and 40.29 μg/mL for ethyl acetate. These results underscore the influence of solvent polarity on both extraction efficiency and antioxidant activity, with polar solvents generally yielding higher antioxidant potential.

Keywords:
Antioxidant activity, Bioactive compounds, DPPH, Polar solvents, extraction yield.

1. Introduction

The olive tree (*Olea europea L.*), extensively cultivated in Mediterranean regions for centuries, is a key agriculture crop in Algeria and Tunisia, where around eight million hectares are devoted to its cultivation [1]. Olive industry generates a substantial volume of by-products, which can negatively impact the environment if not being managed and treated appropriately [2]. One of the primary by-products of the olive industry is olive leaves, which are generated during the pruning and harvesting of olive trees, and these leaves contain bioactive compounds that offer potential health benefits such as antioxidant activity, anti-HIV properties, anti-proliferative and apoptotic effects, protective effects against human leukemia, and lipid-lowering activity [3, 4]. A synergistic effect has been reported among natural compounds, such as carotenoids, flavonoids, and other bioactive molecules with high antioxidant activity, in preventing damage to the body´s cellular structures [5]. There is growing interest in natural antioxidants derived from plant materials. These are being explored as replacements for synthetic antioxidants [4]. Natural antioxidants are compounds that scavenge free radicals and can be derived from plants [6], microorganisms [7], or animals [8]. These antioxidants are often used to support the body´s own antioxidants [9]. Inflammation and Oxidative stress (OS) are interconnected, so their interaction plays a significant role in the development of many of the world´s leading diseases, such as neurodegenerative disorders [10], type 1 and type 2 diabetes, and cardiovascular diseases [11]. Free radicals have negative effects on public health, leading consumers to become increasingly mindful of their diet. As a result, they are adopting healthier consumption habits, including

*Corresponding Author:
zafarmayo2000@yahoo.com (Zafar Iqbal)

the intake of bioactive antioxidant compounds [12].

Olive tree is widely recognized as a species with exceptional antioxidant activity, found in its oil, fruit and leaves [13]. The medicinal and food industry applications of olive tree by-product extract are widely attributed to their rich antioxidant and phenolic compounds, preventing oxidative mutilation [14]. Polyphenols are significant class of bioactive secondary metabolites [15] that serve as anti-radical and antioxidants [16] which can also use in multiple purposes. Although much of the literature on olive polyphenols has primarily focused on olive oil as a key dietary source, olive leaves, which contain higher concentrations of phenolic compounds, are widely used in various fields, including pharmaceuticals, cosmetics, and the food industry [17]. Antioxidant compounds, such as phenolics, have shown significant effects in inhibiting the formation of Reactive Oxygen Species in organisms, thereby reducing the risk of chronic degenerative diseases like diabetics, cancer, atherosclerosis, and Alzheimer´s [18, 19]. Various traditional and innovative methods are available for extracting phenolic compounds from plant materials [20, 21]. Soxhlet, maceration, and hydro distillation are regarded as conventional and reference techniques for extracting bioactive components [21], whereas ultrasound-assisted extraction takes less than one hour and yields 6 - 35 percent more than traditional methods, which require longer extraction times [22, 23].

This research aims to investigate the antioxidant activity and extraction yield of olive leaf extract by employing a range of solvents with varying polarities, from non-polar to high polar, in order to examine the influence of solvent polarity on the efficiency of extracting bioactive compounds. By utilizing the maceration method and analyzing the antioxidant potential through DPPH radical scavenging assay, the study seeks to elucidate the relationship between the physicochemical properties of solvents and their ability to extract polar bioactive molecules, such as phenolics and flavonoids, while also determining their antioxidant efficacy. Through this approach, the research highlights the critical role of solvent polarity in optimizing extraction processes and enhancing the antioxidant potential of natural extracts.

2. Materials and Methods

2.1. Chemicals and materials

Ethanol, n-hexane, dichloromethane, ethyl acetate, nitrobenzene, and methyl ethyl ketone were purchased from Thermo fisher Scientific, USA. Acetone, methanol, acetonitrile, and 1,1-diphenyl-2-picrylhydrazyl were taken from Sigma Aldrich, Germany. A Petri dish, beaker, volumetric flask, funnel, pipette, and graduated cylinder from Pyrex were used for measuring antioxidant activity and extraction leaves. Scott bottles (Duran), Whatman filter paper No.1 (Schleicher & Schuell), and electronic balance (Shimadzu) were used.

2.1.1. Preliminary treatment of sample

The leaves were washed with tap water to remove dust and extraneous particles and then allowed to dry in the shade. The dried leaves were finely powdered using a blender at ambient temperature before being store in a polybag.

2.2. Extraction procedure

A simple maceration extraction process was acquired for these medicinal leaves. 25g of finely ground powder were macerated in 250mL of eight non-identical solvents on the basis of their ascending polarity order, in the context of their dipole moments (n-hexane 0.08, dichloromethane 1.14, ethyl acetate 1.88, nitrobenzene 3.9, methyl ethyl ketone 2.74, acetone 2.69, methanol 2.87, acetonitrile 3.44 D). The extraction was carried out under constant shaking for eight days, and the resulting extracts were filtered through Whatman filter paper to obtain an aqueous extract. The filtrates were then dried in a drying oven at 40°C, and the extraction yield was calculated using the following formula

$$Yield = \frac{W_d}{W_s} \times 100$$

W_d = weight of solvent-free extract
W_s= weight of raw material

2.3. Determination of antioxidant activity

Antioxidant activity of retrieved residues was evaluated by using 2,2-diphenyl-1-picrylhydrazyl [24]. The antioxidant activity of different extracts from olive leaves was measured using a 0.1 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol. 1mL portion of this solution was added to a flask containing 3mL of each extract from different solvents to prepare concentrations 20, 30, 40, 50, 60, and 80 μg/ml in ethanol. These solutions were kept in a dark place at room temperature for 30 minutes before measuring absorbance at 517nm using UV-Vis spectroscopy. Ascorbic acid of a similar concentration in ethanol was used as a reference standard. IC_{50} value of the extracted samples was determined using the logarithmic dose-response curve derived from the graph. A decrease in absorbance, attributed to antioxidant molecules reducing the strength of DPPH, indicated high radical scavenging activity. The percentage of free radical scavenging was calculated using the following equation [25].

$$Percentage\ inhibation = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

$A_{control}$ = DPPH and solvent
W_{sample}= DPPH and solution of extracted sample

3. Results and discussions

3.1. Solvent-dependent extraction yield

Figure 1 illustrates the extraction yields obtained using eight solvents arranged in order of increasing polarity (n-hexane to acetonitrile). The yields were influenced by the chemical nature of the solvents and their interactions with the constituents of olive leaves. Polar solvents like methanol (2.87D) and acetone dissolve polar compounds such as phenolics and flavonoids due

Table 1: Description and yield of crude extract

Extracted samples from solvents	Extraction Yield		Color
	(w/w) %	(w/w) g	
n-Hexane	0.96	0.240	Amber
Dichloromethane	9.20	2.300	Green
Ethyl Acetate	10.40	2.600	Seaweed
Nitrobenzene	18.40	4.600	Dark brown
Methyl ethyl ketone	6.80	1.700	Brown
Acetone	11.20	2.800	Greenish brown
Methanol	26.8	6.700	Blackish brown
Acetonitrile	3.60	0.900	Charcoal

to hydrogen bonding with hydroxyl and carbonyl groups. As a result, methanol provided the highest extraction (6.7g), reflecting its ability to dissolve a broad range of bioactive molecules, described in table 1. Polar solvents exhibit dipole-dipole interactions and hydrogen bonding with plant metabolites. High efficiency of methanol contrasts with acetonitrile (3.44D), which lacks hydrogen bonding capability, resulting in a lower yield (0.9 g). n-hexane (0.08D) had the lowest yield (0.24) due to its limited interaction with polar compounds while dichloromethane (1.14D) and ethyl acetate (1.88D) showed moderate yields (2.3 and 2.6 g), extracting both polar and non-polar compounds. Nitrobenzene (3.9D) produced an intermediate yield (4.6g) owing to aromatic interactions with lignins and flavonoids, despite its limited hydrogen bonding ability.

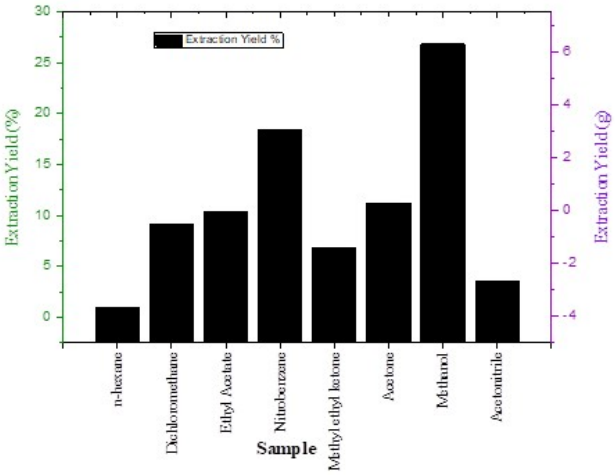

Figure 1: Yield of extraction from Olive leaves

3.2. Antioxidant activity of olive leaf extract by DPPH

The antioxidant activities of the samples were determined using DPPH assay after preparing dilutions in ethanol, ranging from 20 to 120 ppm. Ascorbic acid was used as a reference standard to compare the efficacy of the compounds. A constant volume of DPPH solution was mixed with each sample dilutions, allowing the reaction to occur. Since DPPH is a stable radical, its interaction with antioxidant leads to color change from deep violet to light yellow, indicating the scavenging activities. The absorbance of the reaction mixtures was measured at 517 nm using UV-Vis spectrophotometer to determine the extent of free radical inhibitions. Additionally, the absorbance of the crude extracts, dissolved in solvents of varying polarities from n-hexane to acetonitrile, were recorded and presented in a Table 2 to analyze solvents effects on antioxidant potential.

Figure 2 shows minimum absorbance value indicates higher free radical scavenging activity, meaning that the antioxidant present in the sample effectively neutralize the free radicals, reducing the absorbance of the DPPH solution. The less the DPPH remains in its radical form, the higher the antioxidant activity of the samples. To quantify this activity, the percentage inhibitions were calculated and presented in the table.

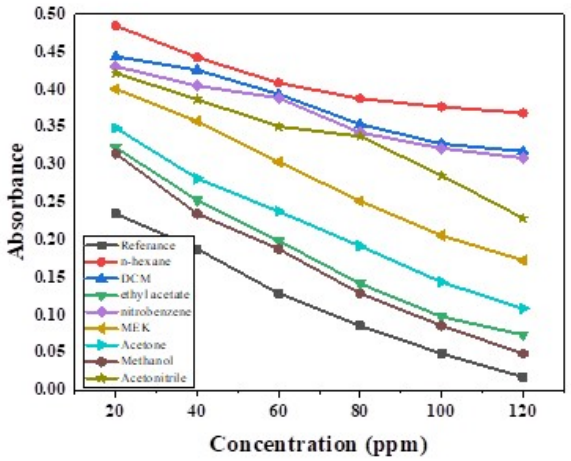

Figure 2: Absorbance for the determination of percentage inhibition

The percentage inhibition of DPPH radicals was determined using the standard equation having concentrations from 20 to 120 ppm as shown in Table 3, reflecting the antioxidant capacity of different solvent extracts based on their chemical properties. The observed trends align with the polarity principle in solvent extraction, where polar solvents like methanol, ethyl acetate and acetone demonstrate higher radical scavenging activity due to their ability to dissolve and extract polar antioxidant compounds, such as flavonoids and phenolics, through hydrogen bonding and dipole interactions. In contrast, non-polar solvents like n-hexane and nitrobenzene exhibited lower inhibition percentages due to Van der Waals interactions, depicted in Figure 3.

3.3. Oxidative stress-reducing properties of the extracts

The antioxidant activity of olive leaves extract was evaluated using solvents of varying polarities, as listed in Table 4: n-hexane, dichloromethane, ethyl acetate, nitrobenzene, methyl ethyl ketone, methanol, and acetonitrile. A standard antioxidant, ascorbic acid, was used for comparison with an IC_{50}

Table 2: Description and yield of crude extract

Conc. (ppm)	Ascorbic Acid	*n*-hexane	DCM	Ethyl acetate	Nitrobenzene	MEK	Acetone	Methanol	Acetonitrile
20	0.235	0.485	0.444	0.323	0.431	0.401	0.349	0.315	0.422
40	0.188	0.443	0.426	0.253	0.405	0.358	0.282	0.235	0.387
60	0.129	0.409	0.394	0.199	0.389	0.304	0.238	0.188	0.351
80	0.086	0.388	0.354	0.142	0.343	0.252	0.192	0.129	0.338
100	0.049	0.377	0.328	0.098	0.322	0.206	0.144	0.086	0.286
120	0.018	0.369	0.318	0.074	0.309	0.173	0.109	0.049	0.229

Table 3: Percentage inhibition of samples

Conc. (ppm)	Ascorbic Acid	n-hexane	DCM	Ethyl acetate	Nitrobenzene	MEK	Acetone	Methanol	Acetonitrile
20	54.19	5.46	13.45	37.04	15.98	21.83	31.97	38.60	17.74
40	63.35	13.65	16.96	50.68	21.05	30.21	45.03	54.19	24.56
60	74.85	20.27	23.20	61.21	24.17	40.74	53.61	63.35	31.58
80	83.24	24.37	30.99	72.32	33.14	50.88	62.57	74.85	34.11
100	90.45	26.51	36.06	80.90	37.23	59.84	71.93	83.24	44.25
120	96.49	28.07	38.01	85.58	39.77	66.28	78.75	90.45	55.36

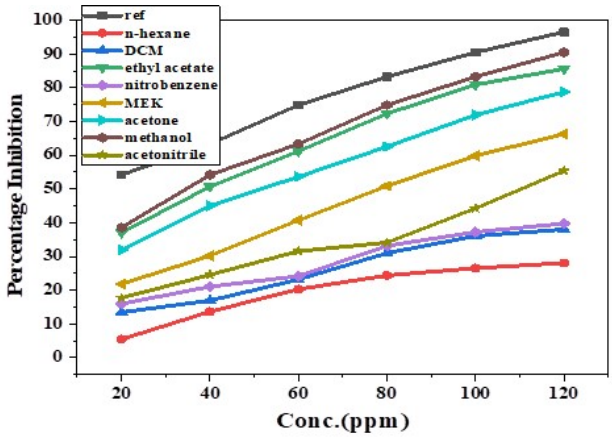

Figure 3: Percentage Inhibition

Table 4: IC_{50} Values of reference and samples

Compound extracted from solvents	IC_{50} Value (μg/mL)
Ascorbic Acid	7.03
n-Hexane	206.09
DCM	157.77
Ethyl acetate	40.29
Nitrobenzene	155.10
MEK	80.98
Acetone	54.18
Methanol	35.88
Acetonitrile	113.18

value of 7.03 μg/mL. Among the solvents, shown in Figure 4, methanol (IC_{50}=35.88 μg/mL), acetone (IC_{50}=54.18 μg/mL) and ethyl acetate (IC_{50}=40.29 μg/mL) were exhibited strongest antioxidant activity, because of its ability to extract a wide range of polar bioactive compounds such as phenolic and flavonoids. Dichloromethane and nitrobenzene (IC_{50}= 157.77 and 155.10 μg/mL) were founded low antioxidant activity due to their moderately polar compounds like alkaloids and aromatic metabolites. Methyl ethyl ketone (IC_{50}=80.98 μg/mL) and acetonitrile (IC_{50}=113.18 μg/mL), despite high dipole moment of acetonitrile, showed lower efficiency compared to ascorbic acid. In contrast, non-polar solvents like n-hexane were yielded weak antioxidant activity (IC_{50}=206.09 μg/mL).

4. Conclusion

The results emphasize the importance of solvent selection in maximizing the yield and bioactivity of plant extracts. Polar solvents, due to their hydrogen bonding and dipole-dipole interaction capabilities, are more effective in extracting antioxidant-rich compounds. Methanol, most polar solvent used, achieved the highest extraction yield (26.8%) and the strongest antioxidant activity (IC_{50} = 35.88$\mu g/mL$). In contrast, non-polar solvents such as n-hexane resulted both lower extraction yields and

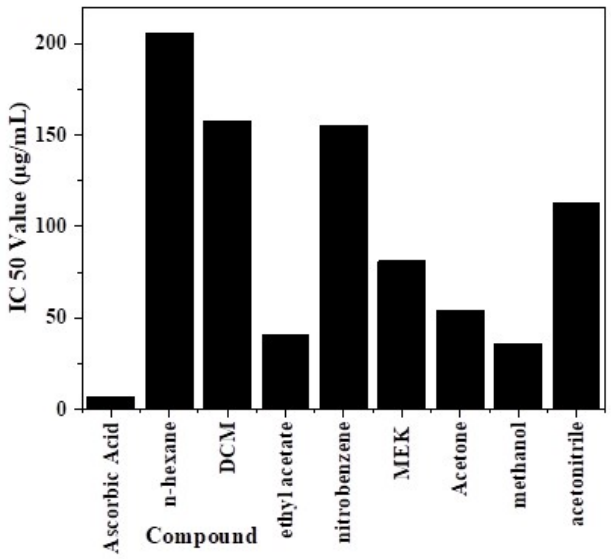

Figure 4: $IC_{50} values in \mu g/mL$

weaker antioxidant activities. These findings confirm that polar solvents are more efficient in extracting phenolic compounds and flavonoids, which are key contributors to the antioxidant potential of olive leaves. The study underscores the potential of optimizing solvent systems to enhance the recovery of bioactive compounds for applications in pharmaceuticals, nutraceuticals, and functional foods.

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