IMPROVEMENT EXTRACTION OF CRUDE COMPOUNDS FROM IRAQI OLIVE LEAVES BY APPLYING WATER-BASE AND ALCOHOLIC-BASE EXTRACTION AND THEIR BIOLOGICAL APPLICATION

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ABSTRACT

Simple, green and inexpensive water and alcoholic base procedures were improved to extract the Olive leaves. The aqueous and alcoholic extracts of Olive Leaves are highly nutritious and contain several types of beneficial and curative compounds for humans. The aqueous and alcoholic extracts of Olive Leaves were prepared by taking the fresh of leaves from Iraqi Olive tree and washed under running tap water then chopped by knife to small pieces and dried by air at room temperature (25°C) and dark place to avoid the effectiveness of the high temperature and light, the chopped leaves were collected and ground to fine powder and stored in airtight dark bottles at 4°C. For preparation of aqueous extract, 50 g of dried powder was added to 150 ml distilled water "pH equal to three by using 0.1N HCl" into dark bottle then put it on shaker apparatus for a suitable moving and shaking for about one week, green slurry mixture was obtained then filtered through filter paper (wattman-5). The filtration process was repeated at least three times. The supernatant was collected to airtight ember color flask and the final volume was 90ml, then the extract was concentrated by heating gently to 40-45°C using rotary evaporator to reduce the final volume about one-tenth of the original volume. For preparation of alcoholic extract, the same procedure repeated but differ in using of 80% of ethanol solvent against water. Aqueous and alcoholic extracts of Olive leaves were diagnosed the crude compounds contents by applying the "UV- VIS" "Ultra Violet-Visible spectrophotometer and "FTIR" "Fourier Transform Infrared Spectroscopy". Aqueous and alcoholic extracts of Olive leaves were evaluated for antibacterial activity against some types of Gram-positive and Gram-negative bacteria. The in vitro antibacterial activity was
performed by agar well diffusion method and the minimum inhibitory concentration (MIC) was determined by micro-titration technique. The results indicated that, the aqueous and alcoholic extracts of Olive leaves inhibited some of Gram-positive bacteria, as well as two Gram-negative bacteria, while the alcoholic extract of Olive leaves generally showed high inhibitory active against different bacteria types, it was Inhibited Gram-positive bacteria and Gram-negative bacteria. The study provide important information for efficient and effective processing and extraction of Olive leaf crud compounds "polyphenols" for research, home and commercial use.

**KEYWORDS:** Olive tree, Olive leaves extracts, Crude compound, Antibacterial.

**INTRODUCTION**

The healing powers of Olive leaf were used in the 1880s to counteract malaria. In the early 1900s, scientists isolated Oleuropein from the Olive leaf. Leaves and drupes of Olive tree, It rich in Olive biophenols, such as oleuropein, verbascoside, ligstroside, tyrosol and hydroxytyrosol, which have exhibited antioxidant and antimicrobial,[1-3] the leaves of Olive tree are the richest source of these compounds.[4]

Back in the early 1800s it was used in liquid form as a very effective treatment for malarial infections. In the early 1900s, a useful compound was found in the Olive leaves named (Oleuropein). In 1962, an Italian researcher recorded that Oleuropein had the ability to lower blood pressure in animals. Other European researchers validated that claim and also found that use to increase blood flow in the coronary arteries, relieve arrhythmias and prevent intestinal muscle spasms. The search began for the chemical agent within Oleuropein that would be the most important medically, Dutch researcher found that the chemical compounds were elenolic acids.[5]

Olive processing residues are attractive sources of natural antioxidants. An important part of these residues is leaves of Olive tree. In addition, during Olive tree cultivation, the pruning step generates a considerable volume of Olive leaves, which are usually used as animal feed, and which could also be used for antioxidant or Olive-leaf extract production.[6-9] Olive leaves themselves have been used as a remedy against various diseases, while Olive-leaf extract has been reported to have antioxidant capacity, antimicrobial activity, anti-HIV properties, vasodilator effect, and hypoglycaemic effect.[10]
As each plant material has its unique properties in terms of phenolic extraction, it is very important to develop the optimal extraction conditions and afterwards the extract evaluation in terms of antioxidant activity and composition, as well as further utilization. Solvent extraction is a process designed to separate soluble phenolic compounds by diffusion efficiency of the solvent extraction process, such as solvent type, pH, and temperature, the number of extraction steps, solvent/solid ratio, and particle size of the solid matrix.[11, 12]

Olive leaves (solid matrix) using a solvent (liquid matrix). Many factors contribute to the more recently, during Oleuropein extraction from Olive leaves, the use of CO$_2$-supercritical fluid extraction "CO$_2$-SFE" alone had been characterized as not satisfactory, needing a polar modifier to improve yield and selectivity of the process.[13]

Solid-phase extraction (SPE) can be used to isolate analysts soluble or suspended in a liquid mixture and separated from a wide variety of matrices according to their physical and chemical properties. Conventional methods include: soxhlet extraction, maceration, percolation, extraction under reflux and steam distillation, turbo-extraction (high speed mixing) and sonication. Although these techniques are widely used, have several shortcomings: are very often time-consuming and require relatively large quantities of polluting solvents, the influence of temperature which can lead to the degradation of thermo labile metabolites.[14] Also, extraction with supercritical fluids requires higher investment but can be highly selective and more suitable for food products. This plays a mechanistic role in supercritical fluid chromatography (SFC), where it contributes to the separation of the solutes that are injected into the chromatographic system. Supercritical fluid extraction is an interesting technique for the extraction of flavouring compounds from vegetable material. It can constitute an industrial alternative to solvent extraction and steam distillation processes.[15] Pressurized solvent extraction or “accelerated solvent extraction,” employs temperatures that are higher than those used in other methods of extraction, and requires high pressures to maintain the solvent in a liquid state at high temperatures. It is best suited for the rapid and reproducible initial extraction of a number of samples.[16] Interestingly, the interest in the Olive leaf and its chemical composition has recently been increasing. In fact, the Olive leaves extract have been found to have anti-oxidative, anti-inflammatory and antimicrobial activities against bacteria and fungi.[17] Moreover, the Olive leaves extract inhibitant-viral activities against several types virus like haemorragic septicaemia rhabdovirus.[18] The main phenolic compounds in Olive leaves are Oleuropein, verbascoside, tyrosol, hydroxytyrosol
and ligstroseide. Somova et al. reported that oleuropein and its derivatives had various biochemical roles including hypotensive, coronary dilating antiarrhythmic action. All structures of phenolic compounds can be assessed; fig 1 shows the more important structures in Olive leaves supplementation. The leaves of Olive tree are rich in polyphenols, namely oleuropein, tyrosol, hydroxytyrosol, rutin, verbacoside, apigenin-7-glucoside and luteolin-7-glucoside. The polyphenols are responsible for the functional properties especially antimicrobial activity. The solvent type is the most important factor affecting the efficiency of liquid-solid extraction. The effect of solvents used in extraction on the phenolic distribution and phenol content in the Olive leaves extracts was reported by Altıok et al.

Fig 1 shows the more important structures in Olive leaf supplementation.

Olive leaves well known that a majority of health benefits credited to Olive oil are in fact due to the presence of a variety of phenolic compounds. Phenolic compounds that are mostly polar in nature find their way in Olive oil because it is pressed out and is not extracted by lipophilic solvents that are commonly used for the extraction of other cooking oils such as corn, canola and soybean oils. Oleuropein is the major component of Olive polyphenols and is extensively studied for health benefits concerning a variety of ailments such as blood pressure, cancer, heart problems and an array of viral and bacterial diseases. Oleuropein is most abundant in developing fruits but its concentration sharply declines when fruits begin to mature. On the other hand, oleuropein is the most abundant polyphenols in Olive leaves and health benefits of leaf extracts are well. Several extraction and processing methods to
optimize oil and polyphenols extractions for laboratory research and commercial preparations have been investigated.\textsuperscript{[31-35]}
	polyphenols (verbascoside, apigenin-7-glucoside, and luteolin-7-glucoside),\textsuperscript{[36]} triterpenes including oleanolic acid,\textsuperscript{[37]} and flavonoids (rutin and diosmin),\textsuperscript{[38]} constituents afford the Olive tree and its fruits and leaves resistance to damage from pathogens and insects.\textsuperscript{[39]} In 2007, researchers in Australia studying the antioxidant capacity of 55 medicinal herbs found Olive leaves extracts had the highest radical-scavenging activity of all herbs studied – more than twice that of Camellia sinensis (green tea) and Silybum marianum (milk thistle).\textsuperscript{[40]}

The anti-atherosclerotic effect of Olive leaves extracts were also demonstrated in rabbits on a high-Lipid diet. Animals in the high-lipid diet group had higher levels of cholesterol, triglycerides, and LDL cholesterol, as well as a thick layer of lipid disposition in the aortic intima compared to those in the Olive leaves group. These results support Olive leaf’s anti-atherosclerotic effect, most likely related to suppression of inflammation.\textsuperscript{[41]}

In a human clinical trial Olive leaves extracts reduced blood pressure, blood pressure values decreased within six weeks and blood pressure remained unchanged and decreases in cholesterol, with no significant changes in other parameters.\textsuperscript{[42]} Olive leaves polyphenols have been shown to inhibit in vitro platelet function in blood was obtained by Singh I. et al.\textsuperscript{[43]}

In vitro and animal studies demonstrate a hypoglycemic effect for Olive leaves extracts decrease blood glucose levels at certain doses.\textsuperscript{[44]} In diabetic rabbits, Oleuropein was restored blood glucose values to normal and decreased diabetes-associated oxidative stress compared to no diabetic rabbits.\textsuperscript{[45]}

Olive leaves extracts given to mature male animal at certain doses which is increased T\textsubscript{3} levels in a dose-dependent manner and significantly reduced circulating thyroid stimulating hormone levels, this animal study suggests a possible use of Olive leaves extracts for hypothyroidism.\textsuperscript{[46]}

Our target in this study the crude compounds of Olive leaves are extracted with two types of solvents, and isolation can also be applied simple, reproducible, accurate and specific green and inexpensive water and alcoholic base procedures to improve the extraction of Olive
leaves. The two types of Olive leaf extracts were diagnosed by two types of analytical techniques and which could be inhibited on some types of bacteria.

EXPERIMENTAL

MATERIALS AND METHODS

Materials
The leaves of healthy Olive leaves were collected from Iraq, Baghdad in winter and then store in cold place before drying and grinding.

Methods

Sampling and Initial Processing of Samples Before Extraction
Olive leaves samples for various extractions and processing experiments were collected from Baghdad, Iraq in winter. Fully expanded leaves were handpicked and brought to the laboratory to process for different experiments. Portion of Olive leaves samples were dried at room temperature (25°C). The aqueous and alcoholic extracts of Olive Leaves are highly

Nutritious and contain several types of beneficial and curative compounds for humans. The aqueous and alcoholic extracts of Olive Leaves were prepared by taking the fresh leaves of Olive and washed under running tap water then chopped by knife to small pieces and dried by airflow at room temperature (25°C) and dark place to avoid the effectiveness of the high temperature and light, collected the dried leaves and ground it to fine powder by using grinder machine and stored in airtight dark flask at 4°C.

Preparation of Aqueous Extract
The preparation of aqueous extract was performed by weighting 50 g of air-dried powder and added to 200 ml distilled water which was adjusted the pH to three by using 0.1N HCl into dark bottle then put it on shaker apparatus for a suitable moving shake for about one week, then filtered through filter paper (wattman-5) and repeated this step at least three times. The supernatant was collected to airtight ember color flask and the volume was 90ml, then the supernatant was concentrated by slightly heating to 40-45 °C using rotary evaporator at low pressure to reduce the final volume to one-tenth of the original volume.

Preparation of Alcoholic Extract
The preparation of alcoholic extract was performed by weighting 50 g of air-dried powder was added to 150 ml 80% ethanol solvent into dark bottle then put it on shaker apparatus for
a suitable moving shake for about one week, then filtered through filter paper (wattman-5), and repeated this step at least three times. The supernatant was collected to airtight ember color flask and the volume was 70ml, then the supernatant was concentrated by slightly heating to 40-45 °C using rotary evaporator at low pressure to reduce the final volume (75ml) to one-tenth of the original volume.

**Qualitative Analysis**

All extracts were filtered through 0.45 μm filter paper before chemical analysis and biological application of aqueous and alcoholic extracts.

**Infrared (FTR) Analysis**

**Aqueous Extract Infrared Spectra**

Aqueous extract Infrared spectra was applied on a Fourier Transform Infrared Spectroscopy (FTIR) (Shimadzu -8400S, Japan). A few drops were placed on disc and scanned from 650 - 4000 cm⁻¹ with a resolution of 4 cm⁻¹. Each recorded spectrum was the result of coated scans. The chromatogram of FTIR-spectra, cm-1: 836.5 (C-H) deformation oscillations of olefins), 1000 (C-O the valence oscillations), 1376 (C-H vibrations of CH₃), 1448 (C-H) vibrations CH₃ & CH₂), 1666.7 (C=C), 2853 (CH at CH₂), 2918.66 (CH at CH₂, CH₃), 2916.86 (CH₃), 3321.96 (the polymer associates). The result was shown in fig. 2.

![FTIR-spectra of polyphenolic crude compounds of aqueous extract of Olive leaves.](image)

**Fig 2:** FTIR-spectra of polyphenolic crude compounds of aqueous extract of Olive leaves.
Alcoholic extract: FTIR-spectra

Alcoholic extract Infrared spectra were collected on a Fourier Transform Infrared Spectroscopy (FTIR), (Shimadzu -8400S, Japan). A few drops were placed disc and scanned from 650 - 4000 cm$^{-1}$ with a resolution of 4 cm$^{-1}$. Each recorded spectrum was the result of coated scans. The chromatogram of FTIR-spectra, cm$^{-1}$: 836.5 (C-H deformation oscillations of olefins), 1000 (C-O the valence oscillations), 1376 (C-H vibrations of CH$_3$), 1448 (C-H) vibrations CH$_3$ & CH$_2$, 1666.7 (C=C), 2853 (CH at CH$_2$), 2918.66 (CH at CH$_2$, CH$_3$), 2916.86 (CH$_3$), 3321.96 (the polymer associates). The result was shown in fig. 3.

![FTIR spectrum](image)

**Fig 3:** FTIR -spectra of polyphenol crude compounds of alcoholic extract of Olive leaves.

Aqueous extract Ultra Violet Analysis

Ultraviolet spectra were collected on a UV/VIS spectrometer (Cary 1000 Con., Australia). Polyphenol crude compounds of aqueous extract was diluted to 5, and 10 times and scanned in 3.5 mL quartz cuvettes. UV-spectra were collected from 200 to 400 nm at 240 nm/min with a 2nm slit width. A minimum of five replicates were collected. The result was shown in fig 4.
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**Fig 4:** UV-Visible analysis of polyphenol crude compound of Olive Leaves aqueous extract.

**Alcoholic extract Ultra Violet Analysis**

Ultraviolet spectra of alcoholic extract were collected on a UV/VIS spectrometer (Cary 1000 Con., Australia). Polyphenol crude compounds of alcoholic extract to 5, and 10 times and scanned in 3.5 mL quartz cuvettes. UV spectra were collected from 200-400 nm at 240 nm/min with a 2 nm slit width. A minimum of five replicates were collected. The result was shown in fig. 5.

**Fig 5:** UV-Visible analysis of polyphenol crude compound of Olive Leaf alcoholic extract.
The in vitro antibacterial activity was examined for aqueous and alcoholic extracts of Olive Leaves used by traditional healers. Microorganisms were obtained from Almustansiryha University, College of Sciences, Department of Biology.

For aqueous and alcoholic extracts an agar well diffusion method was applied, and antibiotic assay by the agar well diffusion method was used in biological application. For alcoholic extract, the molten Mueller Hinton Agar was inoculated with the 100 μl of the inoculum and poured into the Petri plate (Hi-media). For agar disc diffusion method, the disc (Hi-Media) was saturated with 100 μl of the test compound, allowed to dry and was introduced on the upper layer of the agar plate. For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer (0.85cm). 100 μl of the test compound was introduced into the well. The plates were incubated overnight at 37°C. Bacterial growth was determined by measuring the diameter of inhibition zone. For each bacterial strain controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter (Table-1).

RESULTS
The chromatogram of FTIR-spectra of phenolic crude compounds of aqueous and alcoholic extract were explained that the regions cm\(^{-1}\): 836.5 (C-H deformation oscillations of olefins), 1000 (C-O the valence oscillations of ally alcohol), 1376 (C-H deformation vibrations of CH\(_3\)), 1448 (C-H deformation vibrations CH\(_3\), CH\(_2\)), 1666.7 (C=C), 2853 (CH at CH\(_2\)), this data was shown in figs. 2 & 3.

The UV spectra of the phenolic crude aqueous extract dissolved in water and the phenolic crude compounds of alcoholic extract in alcohol solvent were recorded using UV/VIS spectrophotometer. The UV spectra were similar for water extracts. The maxima absorption was noted on 336 nm and 340 nm. The strong absorption bands at 330-340 nm can be caused by the presence of phenolic crude compounds in the extracts which was shown in figs. 4 & 5. The data reported in Table 1 presents the antibacterial activities of the phenolic crude compounds aqueous extract and the phenolic crude compounds of alcoholic extract of Olive leaves. The results indicate that the two extracts from the medicinal plants studied showed inhibition of growth of some of the tested microorganisms with to various degrees.
The aqueous and alcoholic extracts were found to be the effective antibacterial on some types of microorganisms. The inhibitory activities of the two extracts reported in Table 1 are comparable with standard antibiotics.

**Table 1: The aqueous extract of Olive leaves exhibited remarkable activity against some microorganisms. Staphylococcus aureus, Micrococcus, pseudomonas aeruginosa and E.coli. (Zone=mm)**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Crude compounds concentrations</th>
<th>100%</th>
<th>50%</th>
<th>100%</th>
<th>50%</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Alcoholic extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>staphylococcus aureus</strong></td>
<td></td>
<td>14</td>
<td>-</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td><strong>pseudomonas aeruginosa</strong></td>
<td></td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td><strong>E.coli</strong></td>
<td></td>
<td>10</td>
<td>-</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td><strong>Micrococcus</strong></td>
<td></td>
<td>10</td>
<td>6</td>
<td>8</td>
<td>-</td>
</tr>
</tbody>
</table>

The phenolic crude compounds aqueous extract and the phenolic crude compounds of alcoholic extract of Olive Leaves exhibited remarkable activity against some microorganisms.

Staphylococcus aureus and Micrococcus were the most susceptible gram-positive bacteria followed the least susceptible gram-positive bacteria. It was the most resistant gram-negative bacterial strain followed pseudomonas aeruginosa and E.coli. against the two extracts were the most susceptible gram-negative bacteria. These results were shown in fig. 6.

**Fig. 6: Biological application of the aqueous and alcoholic of Olive leaves extracts exhibited remarkable activity against some microorganisms. Staphylococcus aureus, Micrococcus, pseudomonas aeruginosa and E.coli. (Zone = mm).**
DISCUSSION
Successful prediction of potential compounds from the Olive leaves is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioners make use of water primarily as a solvent, but other studies showed that methanol and ethanol extracts of these leaves were certainly much better and powerful due to the better solubility of the active components in organic solvent.\(^\text{[47]}\) These observations can be rationalized in terms of the polarity of the compounds being extracted by aqueous solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. The growth media also seem to play an important role in the determination of the antibacterial activity.\(^\text{[48]}\) The data reported that Muller-Hinton agar appears to be the best medium to explicate the antibacterial activity and the same was used in the present study. Amongst the gram-positive and gram-negative bacteria, gram-positive bacterial strains were more susceptible to the extracts as compared to gram-negative bacteria shown in fig. 6 and table 1. This is in agreement with previous reports that plant extracts are more active against gram-positive bacteria than gram-negative bacteria.\(^\text{[49]}\) The results of present study support the traditional usage of the studied plants and suggests the phenolic crude compounds of aqueous extract and alcoholic extract of Olive Leaves possess compounds with antibacterial properties that can be used as antibacterial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antibacterial and carry out further pharmacological evaluation.

The phenolic crude compounds aqueous extract and alcoholic extract of Olive Leaves in this study was compared between Gram-positive and Gram-negative bacteria, with inhibited the most of Gram-positive bacteria, as well as the Gram-negative outer membrane acting as a barrier to two Gram-negative bacteria Yersinia and Escherichia many environmental substances, including E.coli. Similar results of antibacterial activity of aqueous antibiotics.\(^\text{[50]}\) These Olive leaves extracts are rich in phenolic compounds which are found by Sparker & kramul.\(^\text{[51]}\) The Gram-negative bacteria seemed to be more resistant to phenolic crude compounds aqueous extract of Olive Leaves. The all of these compounds have antibacterial activity, aqueous and alcoholic extracts of the Olive leaves against Gram-negative especially against Gram-positive bacteria. Our results for inhibition of different types of bacteria were remarkable clearly; many workers, have supported, to a certain degree, the traditional
medicinal generally reported that water extracts of Olive leaves do not use of the Olive leaves appraised for human disease therapy have much activity against bacteria especially diseases caused by Gram positive bacteria such as S. aureus which is involved in several humans’ The alcoholic extracts of Olive leaves also have antibacterial properties could be generally showed high inhibitory activity against different used to increase the shelf-life of food bacteria. It inhibited Gram-positive bacteria and some antifungal activity results showed no activity of any Gram-negative bacteria such as Pseudomonas, aqueous extract against.

Yeast strains tested, however, aeruginosa, Acinetobacter baumannii and Proteus, further the absence of against Gram-positive than Gram-negative bacteria. active compounds and many reasons could explain the conflicting results were found by Iqbal and Arina, lack of antifungal activity, containing the parts of the who reported that the alcoholic extract of Olive leaves, used method of extraction and the type of solvent leaves showed antibacterial activity against Gram-positive and possibly the time of collection and Gram-negative bacteria. In general, these Olive leaves extracts inhibited the Gram-positive bacteria better than the Gram-negative ones.

CONCLUSION

The study give successful prediction of potential compounds from the Olive leaves is largely dependent on the type of solvent used in the extraction procedure. The traditional healers or practitioners make use of water primarily as a solvent, but other studies showed that methanol and ethanol extracts of these leaves were certainly much better and powerful. This may be due to the better solubility of the active components in organic solvent. The solvent type is the most important factor affecting the efficiency of liquid-solid extraction. Our results for antibacterial extracts were remarkable clearly that have supported, to a certain degree, the traditional medicinal generally reported that water extracts of Olive leaves do not use of the Olive leaves appraised for human disease therapy have much activity against bacteria, especially diseases caused by Gram positive bacteria such as S. aureus which is involved in several humans. The alcoholic extracts of Olive leaves also have antibacterial properties could be generally showed high inhibitory activity against different uses one is to increase the shelf-life of food bacteria. It inhibited Gram-positive bacteria and some antibacterial activity results showed no activity of any Gram-negative bacteria such as Pseudomonas.

The study provide important information for efficient and effective processing and extraction of Olive leaf crud compounds "phenols" for research, home and commercial use.
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