NEW TECHNIQUE FOR EXTRACTION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM IRAQI VITIS-VINIFERA LEAVES

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ABSTRACT

Natural products from medicinal plants either as pure or as standardized extract provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. The aqueous extracts of Vitis-Vinifera Leaves were prepared by taking the fresh leaves from Iraqi Vitis-Vinifera tree and washed under running tap water then chopped by knife to small pieces. Air dried at room temperature(25°C) and dark place to avoid the effectiveness of high temperature and sun light. The chopped leaves were collected and ground to fine powder and stored in airtight dark bottles. For aqueous extract, 50 g of air-dried powder was added to 200ml distilled water and gently heated on slow rate rising temperature reached to 47°C for 10 hours with continuous stirring then leave on punch to cool. The mixture was collected in airtight ember color flask and filtered through filter paper (wattman-5). The filtration process was repeated at least three times. The supernatant was collected to ember color flask. The final (150 ml) was concentrated to one-tenth of the original volume by heating gently to 40-45°C using rotary evaporator. The aqueous extract was analyzed the phenolic compounds in crude extract by using Ultra Violet-Visible "UV-VIS" spectrophotometer and Fourier Transform Infrared Spectroscopy"FTIR". Aqueous extract was evaluated for antibacterial activity against some types of Gram-positive and Gram-negative bacteria. The invitro antibacterial activity was performed by agar well diffusion method and the minimum inhibitory concentration (MIC) was determined by micro-
titration technique. The results were indicated that the aqueous extract was inhibited some of Gram-positive bacteria as well as two Gram-negative bacteria.

**KEYWORDS:** Vitis-Vinifera Leaves, Polyphenolic compounds, Antibecteral agent.

**INTRODUCTION**

Vitis-Vinifera (grape) is a perennial woody vine native to Asia Minor and then introduced in Europe and other continents.[1] Among the most interesting constituents responsible for the therapeutically properties of the plant. Procyanidins were used for the treatment of microcirculatory disorders.[2] The leaves of Vitis-Vinifera (Vitaceae) was used in traditional food (dolmathes) in some Mediterranean countries and in folk medicine used for the treatment of diarrhea and vomiting. Grape leaves with antioxidant activity have been reported to treat chronic venous insufficiency for human and nephrotoxicosis induced by citrinin.[3] Vitis-Vinifera leaves contain active ingredients compounds(Polyphenolic derivatives: anthocyanins, leuco-anthocyanins, Flavonoids(rutin, quercitrin, isoquercitroside, kenferol, luteolol), Gallic tannins and Catechins) and fruits abundant like "carbohydrates (glucose) and organic acids (tartaric, malic, succinic, citric and oxalic acids)). Vitis-Vinifera seeds contain 15-20% unsaturated fatty acid (phenylacrylic acid derivatives). Anthocyanins can be found in the skin of the berries, hydroxycinnamic acids in the pulp and condensed tannins of the proanthocyanidins type in the seeds.[4]

Plant foods are rich sources of phenolics which are molecules that can act as antioxidants to prevent heart disease,[5] reduce inflammation,[6,7] decrease the incidence of cancers.[8,9] The protection afforded by the consumption of plant products such as fruits, vegetables and legumes is mostly associated with the presence of phenolic compounds. Compounds are synthesized in plants partly as a response to the ecological and physiological pressures such as pathogen and insect attack.[10,11] Flavonoids are the most widely common phenolics which distributed in plant tissues and often responsible along side the carotenoids and chlorophylls for their blue, purple, yellow, orange and red colors. The flavonoid family includes flavones, flavonols, iso-flavonols, anthocyanins, anthocyanidins, proanthocyanidins and catechins.[12,13] All flavonoids are derived from the aromatic amino acids (phenylalanine and tyrosine) and have three-ringed structures.[14]

Higher extraction yields of phenolics are achieved by milling the sample into smaller particle sizes thereby improve enzymatic action and extraction. Defatting processes can be applied to
remove oil from lipid-containing samples. For instance, Weidner et al.\cite{15} were defatted the ground seeds of grape to simplify phenolic extraction using hexane. Several parameters may influence on the yield of phenolics, including extraction time, temperature, solvent-to-sample ratio, the number of repeat extractions of the sample, as well as solvent types. Furthermore, the optimum recovery of phenolics is different from one sample to the other and relies on the type of plant and its active compounds. The choice of extraction solvents such as water, acetone, ethyl acetate, alcohols (methanol, ethanol and propanol) and their mixtures\cite{16}, will influence on the yields of phenolics components extract. For instance, a high yield of phenolics can be extracted from sorghum leaf using water.\cite{17} In contrast, the highest levels of phenolics are extracted from Vitis-Vinifera wastes and sun flower meal using pure methanol and 80% aqueous acetone, respectively.\cite{18,19} These differences could be due to the properties of the phenolic components of the plants concerned. There are two other important parameters that affect on the yield of phenolics extracted from plant foods: time and temperature. Normally, increasing time and temperature promote analyte solubility; however, plant phenolics are generally degraded or undergo undesirable reactions such as enzymatic oxidation by extended extraction times and high temperatures.\cite{20,21} Phenolics may bind to other sample elements such as carbohydrates and proteins.\cite{12} Acidic and alkaline hydrolysis was also employed in the isolation of phenolics from plants and plant products and important for the stability of the phenolics in the extract.\cite{22,23} Flavonoid aglycones were identified by acidic hydrolysis of the glycosidic residues bound to the flavonoid nuclei in 20 plant sources.\cite{22} Flavonoids are highly bioactive compounds found in both edible and non-edible plants. They are often extracted with methanol, ethanol, acetone, water or mixtures of these solvents using heated-reflux extraction methods.\cite{14,24-26} Following extraction, the flavonoid glycosides were hydrolyzed into the aglycone forms by applying hydrochloric acid under nitrogen enviroment. Haghi & Hatami.\cite{22} and Wu et al.\cite{27} were focused on optimization of enzymatic extraction of flavonoids from celery stalks. Proanthocyanidins are a group of polymerized polyphenols commonly referred to condensed tannins. They are found naturally in grape seed and skin, apple juice, mangosteenpericarp, berries, pine bark, chocolate, sorghum and sea bark.\cite{28} For proanthocyanadin extraction, the organic solvents are usually used ethanol as well as methanol and acetone.\cite{29} The crude extracts of phenolic compounds were prepared from the traditional plants using aqueous extraction method. The total flavonoids content was determined using Uv-Visible spectroscopy. The aqueous extracts of Vitis-Vinifera Leaves are highly nutritious and contain several types of health-promoting and curative compounds for humans, such as vitamins, tannins polyphenols and carotenoids.\cite{30}
Grapevine (Vitis-Vinifera) is one of the major fruit crops worldwide but is also very susceptible to microbial and fungal attacks which are a valuable rich source of bioactive secondary metabolites especially for polyphenolics such as phenolic acids and catechins, as well as for anthocyanins like cyanidin 3-glucoside, peonidin 3-glucoside, malvidin 3-glucoside, cyanidin 3-p-coumaroylglucose, peonidin3-p-coumaroyl glucoside and malvidin3-p-coumaroylglucoside.\(^{[31]}\)

Medicinal plants have been widely used for the treatment of diseases in traditional way for several years. An interaction between ancient medicine and biotechnological tools is to be established towards newer drug development. The interface between cell biology, structural chemistry and invitro assays will be the best way available to obtain valuable leads. The value of medicinal plants lies in the potential access to extremely complex molecular structure that would be difficult to synthesize in the laboratory. In spite of an increasing awareness and expenditure of resources, the incidence of chronic diseases like diabetes, cardiac, and cancer have not declined and in fact is rising at an alarming rate. Cancer may be the most feared disease of our time and the number of deaths continues to increase steadily. Medicinal plants represent a vast potential resource for anti-cancer drugs and continue to be subject to extensive screening worldwide in an attempt to develop still more effective anti-cancer treatment.\(^{[32]}\) Most of the therapeutic properties of the plant are attributed to phenolic compounds that have received considerable attention due to their pharmacological effects namely antioxidant activity.\(^{[33]}\) The current study was highlighted on an important information for efficient and precise process for extraction of Iraqi Vitis-Vinifera Leaves to obtain crude "polyphenols compounds" and their biological activities for research and commercial use.

**EXPERIMENTAL**

**MATERIALS AND METHODS**

**Materials**

The leaves of healthy Vitis-Vinifera was collected from (Iraq, Baghdad region) in winter and store in cold place before drying and grinding.

**Methods**

**Plant material**

Fresh Vitis-Vinifera leaves were collected randomly from the Baghdad region in Iraq. Whole plant of Vitis-Vinifera leaves was taken for extraction and investigation of phenolics as
antibacterial property. Fresh leaves of Vitis-Vinifera was washed under running tap water, choped by knife to small pieces, air dried at room temperature and dark place to avoid the effectiveness of high temperature and light then ground to a fine powder and stored in airtight ember color flask.

**Preparation of aqueous extract**
For aqueous extract, 50 g of air-dried powder was added to 200ml distilled water and gently heated on slow rate rising temperature reached to 47°C for 10 hours with continuous stirring then leave on punch to cool. The mixture was collected in airtight ember color flask and filtered through filter paper (wattman-5). The filtration process was repeated at least three times. The supernatant was collected to ember color flask. The final (150 ml) was concentrated to one-tenth of the original volume by heating gently to 40-45 °C using rotary evaporator under reducing pressure using rotary evaporator apparatus (Rotavpor - R-210, BUCHI, Switzerland) then continus heating at the same condition until to obtain about 2ml of final volumn and stored at 4°C.

**Qualitative Analysis**

**Ultra Violat (UV)Spectrum**
UV spectra of the aquous extract was assied by dilute a small amount of concentrated extract with water then recorded using (Cary – 1000, Australia) spectrophotometer. The UV spectra were similar for water extract (Figure 1). The maximum absorption was appeared on 336 nm and 340 nm. The strong absorption bands at 330-340 nm can be caused by the presence of phenolic compounds in aqueous extract.

**FITR Spectrum**
Infrared spectra were collected on a Fourier Transform Infrared Spectroscopy (FTIR) (Shimadzu, 8400S, Japan). A few drops were placed on the FTIR system and scanned from 650 to 4000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\). Each recorded spectrum was the result of coadded scan. The chromatogram of FTIR-spectra, 839 cm\(^{-1}\) (C-H deformation oscillations of olefins), 987 cm\(^{-1}\) (C-O the valence oscillations of allyl alcohol), 1394 cm\(^{-1}\) (C-H deformation vibrations of CH\(_3\)), 1429 cm\(^{-1}\) (C-H deformation vibrations CH\(_3\), CH\(_2\)), 1661 cm\(^{-1}\) (C=C), 2848 cm\(^{-1}\) (CH at CH\(_2\)), 2912 cm\(^{-1}\) (CH at CH\(_2\), CH\(_3\)), 2919 cm\(^{-1}\) (CH\(_3\)) and 3322 cm\(^{-1}\) (the polymer associates).
Biological Aplication
In vitro antibacterial activity was examined for aqueous extract of Vitis-Vinifera used by traditional healers. Microorganisms were obtained from Almustansirya University, College of Sciences, Department of Biology. For aqueous extract an agar well diffusion method was performed: An Antibiotic assay by the agar well diffusion method was used for biological application. 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. The bacterial growth was determined by measuring the zone diameter of inhibition. 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.

RESULTS
The UV spectra of the crude extracts dissolved in water were recorded using Cary – 1000 spectrophotometer. The UV spectra were similar for aqueous extract (Figure 1).

![UV-Visible analysis of polyphenol crude compound of Vitis-Vinifera leaves extract.](image)

The maximum absorption was appeared on 336 nm and 340 nm. The strong absorption bands at 330-340 nm can be caused by the presence of phenolic compounds. The chromatogram of FTIR-spectra was explained that, 836.5 cm⁻¹ (C-H deformation oscillations
of olefins), 1000 cm\(^{-1}\) (C-O the valence oscillations of allyl alcohol), 1376 cm\(^{-1}\) (C-H deformation vibrations of CH\(_3\)), 1448 cm\(^{-1}\) (C-H deformation vibrations CH\(_3\), CH\(_2\)), 1666.7 cm\(^{-1}\) (C=C), 2853 cm\(^{-1}\) (CH at CH\(_2\)), 2912 cm\(^{-1}\) (CH at CH\(_2\), CH\(_3\)), 2919 cm\(^{-1}\) (CH\(_3\)) and 3322 cm\(^{-1}\) (the polymer associates).

This data was shown in fig. 2.

![FTIR Analysis](image1.png)

**Fig. 2:** FTIR analysis of polyphenolic crude compound of *Vitis-Vinifera* Leaves extract.

The aqueous was found to be the effective antibacterial on some types of microorganisms. The aqueous extract of *Vitis-Vinifera* 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*. These results were shown in fig. 3 & fig. 4.

![Bacterial Samples](image2.png)

**Fig. 3:** Biological application of the aqueous extract of *Vitis-Vinifera* leaves exhibited remarkable activity against *pseudomonas aeruginosa* & *E.coli*.
Fig. 4: Biological application of the aqueous extract of *Vitis-Vinifera* Leaves. exhibited remarkable activity against *Staphylococcus aureus* & *Micrococcus*.

The data reported in Table 1 presents the antibacterial activity of the aqueous extract of *Vitis-Vinifera* leaves. The results indicate that the extract from the medicinal plants studied showed inhibition of growth of some of the tested micro-organisms with to various degrees. The inhibitory activities of the extract reported in Table 1 are comparable with standard antibiotics.

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

<table>
<thead>
<tr>
<th>Strains</th>
<th>Vitisvinifera extract in different % concentrations</th>
<th>100% (zone=mm)</th>
<th>50% (zone=mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>staphylococcus aureus</td>
<td>12</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>pseudomonas aeruginosa</td>
<td>10</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>E.coli</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Micrococcus</td>
<td>7</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Successful prediction of potential compounds from plant material is largely dependent on the type of solvent extraction procedures. The traditional healers or practitioners make use of water primarily as a solvent. These observations can be rationalized in terms of the polarity of the compounds being extracted by aqueous solvent, in addition to their intrinsic bioactivity, by their ability to dissolving or diffuse in the different media used in the assay. The growth media seem to play an important role in the determination of the antibacterial activity these result is in agree to Sarker.^[34]
The data reported that Muller-Hinton agar appears to be the best medium to explicate the antibacterial activity and used in the present research amongst the gram-positive and gram-negative bacteria when gram-positive bacterial strains were more susceptible to the extract as compared to gram-negative bacteria. This is in agreement with previous reports that plant extract are more active against gram-positive bacteria than gram-negative bacteria. The results were supported the traditional usage of the studied plants and suggests that some of the plant extracts 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 aqueous extract of Vitis-Vinifera was explained the comparism 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, including E. coli. Similar results of antibacterial activity of aqueous antibiotics.

The Gram-negative bacteria appeared to be more myricetin, ellagic acid, quercetin, gallic acid, resistant to the aqueous extract. The activity of the all of these compounds have antibacterial activity and the results of biological activity of aqueous extract of the Vitis-Venifera against Gram-negative especially against-Gram-positive bacteria was agreed to Souàda, et al. In general, the Vitis-Venifera leaves extracts inhibited the Gram-positive bacteria better than the Gram-negative ones which is agreement with previous reports.

CONCLUSION

The study gives successful prediction of crude compounds from the Vitis-Vinifera leaves is largely dependent on the types of solvents extraction procedures. The practitioners make use of water primarily as a solvent but other studies were 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 were remarkable clearly that have supported to a certain degree, the traditional medicinal generally reported which is aqous extract of Vitis-Vinifera leaves have much activity against bacteria, especially diseases caused by Gram-positive bacteria such as S. aureus. It inhibited Gram-positive bacteria and some antibacterial activity results were showed no activity of any Gram-negative bacteria such as Micrococcus. The current study was highlighted on an important
information for efficient and precise process for extraction of Iraqi Vitis-Vinifera Leaves to obtain crud "polyphenols compounds" and their biological activities for research and commercial use.

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REFERENCES


