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Ultrasound Assisted Extraction, Phytochemical Investigation and Antioxidant Potency of Local *Vitis Vinifera* Leaves

Noor Salman Obaid[1], Lamyaa S. Mahdi[1,*], Jihan Hameed Abdulameer [2]

[1]Department of Pharmacognosy and Medicinal Plant/College of Pharmacy/University of Kerbala, Iraq

[2]Internal Medicine, Alhassan metabolism, Endocrine and Diabetes Center, Karbala Iraq

***Corresponding Author:**

Lamyaa S. Mahdi: lamiaa.saleh@uokerbala.edu.iq

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Abstract

Background: Since many of the therapeutic claims of medicinal plants are supported by reliable scientific data, their long-standing use traditionally to treat common ailments such as fever and colds has received considerable attention. Extraction is the first step in medicinal plant research, given its importance in determining the yield and phytochemical composition of the extract through specific chemical tests, which in turn influences the results of subsequent biological tests. Ultrasound Assisted Extraction (UAE) is one of the most common modern extraction methods, due to its use of fewer solvents and shorter processing time. It is important to remember that ultrasound frequencies above 20 kHz may produce free radicals, which may cause the decomposition of sensitive phytochemicals.

Method: Vitis Vinifera leaves were weighed after being shade-dried. (UAE) was used to extract the active chemical components. Dried Vitis Vinifera leaves, weighing 15 g each, were extracted using petroleum ether (40-60°C) (500) ml and methanol: water in a 50:50 ratio subsequently. The ultrasonic treatment time was 13 minutes at room temperature using an UP400St. Chemical screening tests were performed on two different polar aqueous methanol extracts and a non-polar organic petroleum ether extract after drying. The non-polar petroleum ether extract was analysed using Gas Chromatography-Mass Spectrometry (GC-MS) method.

Results: Flavonoids, saponins, tannins, terpenes, and alkaloids were found at high concentrations in Vitis Vinifera L. extracts, according to chemical screening tests. Substances detected by the GC-MS approach included steroids, fatty acids, aldehydes, and hydroxylated compounds. The present study confirmed that the aqueous methanolic extracts at doses of 100, 200, 400,600 and 800, 1000 µg/ml were examined against oxidative compound DPPH. According to the results, the methanol/water extract showed its efficacy to scavenge free radicals in vitro and the IC50 was measured. Ascorbic acid is used reference substances.

Conclusion: Given the elevated levels of phenols and total flavonoids in the extract, its antioxidant efficacy was assessed, beginning with its capacity to scavenge free radicals in vitro. Ultrasound-assisted extraction (UAE) is a prevalent contemporary extraction technique employed effectively, owing to its reduced solvent usage and abbreviated processing duration.

الاستخلاص بمساعدة الموجات فوق الصوتية والبحث الكيميائي النباتي وقوة مضادات الأكسدة لأوراق نبات العنب المحلي

نور سلمان عبيد , لمياء صالح مهدي, جهان حميد عبد الامير

الخلاصة

المقدمة: ان العديد من التوجهات العلاجية للنباتات الطبية مدعومة ببيانات علمية موثوقة، فقد حظي استخدامها التقليدي لعلاج أمراض شائعة مثل الحمى ونزلات البرد باهتمام كبير. يُعد الاستخلاص الخطوة الأولى في أبحاث النباتات الطبية، نظرًا لأهميته في تحديد العائد والتركيب الكيميائي النباتي للمستخلص من خلال اختبارات كيميائية محددة، مما يؤثر بدوره على نتائج الاختبارات البيولوجية اللاحقة. يُعد الاستخلاص بمساعدة الموجات فوق الصوتية (UAE) من أكثر طرق الاستخلاص الحديثة شيوعًا، نظرًا لاستخدامه مذيبات أقل ووقت معالجة أقصر. من المهم تذكر أن ترددات الموجات فوق الصوتية التي تزيد عن 20 كيلوهرتز قد تُنتج جذورًا حرة، مما قد يُسبب تحلل المواد الكيميائية النباتية الحساسة.

طرق العمل: وزّنت أوراق العنب (Vitis Vinifera) بعد تجفيفها في الظل. واستُخدمت تقنية الموجات فوق الصوتية (UAE) لاستخلاص المكونات الكيميائية الفعالة. استُخلصت أوراق العنب المجففة، بوزن 15 غرامًا ، باستخدام إيثر البترول (40-60 درجة مئوية) (500 مل)، ثم ميثانول: ماء بنسبة 50:50. استغرقت المعالجة بالموجات فوق الصوتية 13 دقيقة في درجة حرارة الغرفة باستخدام جهاز UP400St. أُجريت اختبارات الفحص الكيميائي على مستخلصين مائيين قطبيين مختلفين من الميثانول ومستخلص إيثر البترول العضوي غير القطبي بعد التجفيف. حُلِّل مستخلص إيثر البترول غير القطبي باستخدام طريقة كروماتوغرافيا الغاز-مطياف الكتلة (GC-MS).

النتائج: اظهرت الدراسة وجود الفلافونويدات، والسابونينات، والعفص، والتربينات، والقلويدات بتركيزات عالية في مستخلصات نبات العنب (Vitis Vinifera L.)، وذلك وفقًا لاختبارات الفحص الكيميائي. وشملت المواد التي تم الكشف عنها باستخدام تقنية كروماتوغرافيا الغاز-مطياف الكتلة (GC-MS) الستيرويدات، والأحماض الدهنية، والألدهيدات، والمركبات الهيدروكسيلية. وأكدت هذه الدراسة أن المستخلصات الميثانولية المائية بجرعات 100، 200، 400، 600، و800، 1000 ميكروغرام/مل قد فُحصت ضد المركب المؤكسد DPPH. ووفقًا للنتائج، أظهر مستخلص الميثانول/الماء فعاليته في التخلص من الجذور الحرة في المختبر، وتم قياس (IC50). واستخدم حمض الأسكوربيك كمواد مرجعية.

الاستنتاج: نظرًا للمستويات المرتفعة من الفينولات والفلافونويدات الكلية في المستخلص، تم تقييم فعاليته المضادة للأكسدة، بدءًا من قدرته على التخلص من الجذور الحرة في المختبر. يعد الاستخلاص بمساعدة الموجات فوق الصوتية (UAE) تقنية استخلاص معاصرة شائعة يتم استخدامها بشكل فعال، نظرًا لاستخدامها المنخفض للمذيبات ومدة المعالجة المختصرة.

1. Introduction

Natural bioactive compounds utilized in the dairy, cosmetic, and pharmaceutical industries primarily come from plants. The antioxidant potency of the medicinal plants determines their health advantages (Shang *et al*., 2022; Xu *et al*., 2017). Grapevine, one of the most widely cultivated plants globally, produced approximately 80 million tons around the world in 2018 (Madadian, Rahimi *et al.,* 2022). Woody vines known as grapes are part of the Vitaceae family. Among the over 700 species in this genus, the majority thrive in tropical and subtropical climates, although some are also found in cooler regions. There are more than 50 species within the genus Vitis. Occasionally, certain grape cultivars are employed in gardening. Two species commonly used in horticulture are Vitis vinifera and Vitis Coignetiae (Singh *et al*., 2004). The grapevine is used for various purposes, including table grapes, wine, or juice. It is regarded as a significant source of bioactive chemicals, primarily polyphenols (Gülcü, Ghafoor *et al.,* 2020). Grape leaves are a widely used plant material known for their antioxidant properties, and they have a long history of use in herbal medicine to address liver issues. Most research on bioactive compounds from grapevines has primarily focused on the grapes themselves, while the vegetative parts of the plant, particularly the leaves, are often overlooked as an underutilized by product of the industry. Common plant materials such as grape leaves, which are rich in antioxidants, have traditionally been used in herbal therapy to help treat liver problems related to diabetes, inflammation, leakage, and vomiting (Tiwari *et al*., 2009). Numerous physiochemical investigations have observed the presence of phenolics, stilbenoids, anthocyanins, tannins, terpenoids, and proteins (Gambuti *et al*., 2004). The main physiologically active components of grape leaves consist of catechins, flavonoids, tannins, malic acid, silicic acid, citric acid, tartaric acid, succinic acid, and resveratrol (Garidini *et al*., 2005). Many studies have shown that the phenol content of grapes is greatly affected by geographic and climatic conditions, as well as the specific type of grape (Li, Wei *et al*. 2022).the medicinal importance for human of leaves attributed to the presence of secondary metabolites, specially phenolics, so it is used in the treatments of many diseases and prevent progression such as diabetes, cancer, aging, and cardiovascular problems (Cosme, Pinto *et al*., 2017, Ali, Benfante *et al*., 2023).

2. Materials and Methods

2.1. Chemicals

Petroleum Ether from: Srlchem (SRL), India and Methanol From: Chem-Labs, Belgium was used to extract the non-polar and polar component Vitis vinifera leaves respectively. Sodium hydroxide, lead oxide and potassium iodide are from: Srlchem (SRL), India. Mercuric chloride and Lead acetate were from: Srlchem (SRL), India. GC-MS n-Hexane of analytical grade (HPLC-Grade) was acquired from England , DPPH and ascorbic acid was purchased from Market, TLC silica gel 60, GF 254 nm , 0.25 mm, 20 x 20 cm aluminum

cards obtained from Merk;Germany . The Vitis vinifera leaves collected from Karbala city /Iraqi garden in summer, July 2024.

2.2. Extraction of The Plant

2.2.1. Aqueous Methanol Solvent

Ultrasound-assisted extraction was used extracting method. First, Vitis vinifera course powder leaves (15 g) were extracted using Methanol: water (1:1), (250 mL) as a solvent in a sonication UP400St ultrasonic processor for 15 min and the mixture was cooled until it reaches the room temperature. Then, the samples were filtered to remove solid materials. The supernatants were dried at room temperature, and the dried residue was collected and weighed (5.17 g). (Rodríguez-Pérez *et al*., 2013).

2.2.2. Petroleum Ether Solvent

Vitis vinifera course powder leaves (15 g) were extracted using Petroleum Ether (40–60 °C), (250 mL) as a solvent in a sonication UP400St ultrasonic processor for 15 min and the mixture was cooled until it reaches the room temperature. Then, the samples were filtered to remove solid materials. The supernatants were dried at room temperature, and the dried residue was collected weighed (1.72 g) (Rodríguez-Pérez *et al*., 2013).

2.2.3. Chemical Analysis Tests

The aqueous methanol and petroleum ether crude extract underwent phytochemical screenings to determine the abundance or absence of specific plant phytochemicals, including flavonoids, saponins, tannins, terpenoids, and alkaloids.

2.2.4. Flavonoid Test

Reaction with sodium hydroxide: the extract (leaves) was combined with a 1% NaOH solution. The mixture was examined for the formation of yellow, which is regarded as a successful outcome.

2.2.5. Saponin Test

Froth test: vitis vinifera course powder leaves (2 g) were extracted dissolved with 20 ml of water then heated for 3min. Filtered the mixture when it’s hot then take 10ml of filtered and mix with 5ml of water and shaking, fume formation shows positive result.

2.2.6. Tannins Test

Lead acetate test: Few drops of freshly prepared %1 lead acetate were added to the extract (1 mL) in a test tube. Yellow precipitate shows Positive result.

2.2.7. Alkaloids Test

Mayer's test: Few drops of ethanol, (2 drops) dilute HCl (10%) and (2 drops) Mayer's reagent was added to the extract (1 mL) and mixed. Yellow precipitate shows a positive result.

2.2.8. Terpenoids Test

Petroleum ether extract (5 mL) was mixed carefully with chloroform (2mL) and concentrated sulfuric acid (3 mL) presence of terpenes was indicated by formation of reddish-brown coloration of the solution. (Sofowora *et al*. 1993, Sharma *et al*. 2010)

2.3. Gas Chromatography- Mass Spectrometry Analysis

A GC (Agilent Technologies 7890A) paired with a mass-selective detector (MSD, Agilent 7000) that had a polar Agilent HP-Sms (5%-phenyl methyl polysiloane) capillary column (30 m x 0.25 m i.d. and 0.25 um film thickness) was used to analyze organic extracts. The carrier gas (Helium) was used with a linear velocity of 1 milliliter per minute. The temperature of the injector was 200°C and the detector was 280 °C. One microliter of the sample was injected. The following were the MS operational parameters:

The acquisition mass range is S0-800, the interface temperature is 250 °C, and the ionization potential is 70 eV. The components were identified by computer matching with the NIST and WILEY libraries, comparing their mass spectra and retention times with those of the real compounds, and Compare the Mass Spectral data's fragmentation pattern with those documented in the literature (Balamurugan *et al*., 2015).

2.4. Anti-Oxidant Potency

DPPH (2,2-Diphenyl-1-picrylhydrazyl) the stable free radical, scavenging method used to examine the anti-oxidant activity of the polar leaves extract. Initially, samples were made in ethanol at different concentrations (0.2, 0.4, 0.6, 0.8, and 1 mg/mL). Next, 2.6 mL of ethanol containing DPPH radicals was added to 0.4 mL of each solution. The reaction mixture was then kept for 1 hour at laboratory temperature in the dark. The absorbance of each concentration was measured at a wavelength of 516 nm; the results are averages of three separate measurements. The following formula was used to determine the percentage scavenging activity of the sample: [(control sample - test sample) / control sample] * 100 = RSA (%) where (control sample) is the absorbance of DPPH, (test sample) is the absorbance of the test sample, and RSA is the radical scavenging activity. A (positive control) was ascorbic acid (Acero *et al*., 2025).

3. Results

3.1. Chemical Screening Tests

After the extraction of *Vitis vinifera* leaves, both extracts were subjected to some screening tests for the abundance of active phytochemical compounds and the results mentioned in Table1.

Table1: Chemical Tests Results of Both Solvents

Test	Solvent	Result
Flavonoids	Aqueous methanol	+++
Alkaloids	Aqueous methanol Petroleum ether	+ +
Saponin	Aqueous methanol	++
Terpenes	Petroleum ether	++
Tannins	Aqueous methanol	+++

3.2. Gas Chromatography Mass Spectroscopy

The petroleum ether leaves extract rich in fatty acids and their esters as identified by GC MS analysis technique. Seven phytochemicals found in organic extract of Vitis vinifera leaves they were n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z), trans-13-Octadecenoic acid, cis-Vaccenic acid, Glycidyl palmitate, Cyclohexane, 1,3,5-triphenyl, 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester. As shown in Fig.1 and Fig.2, and Table2.

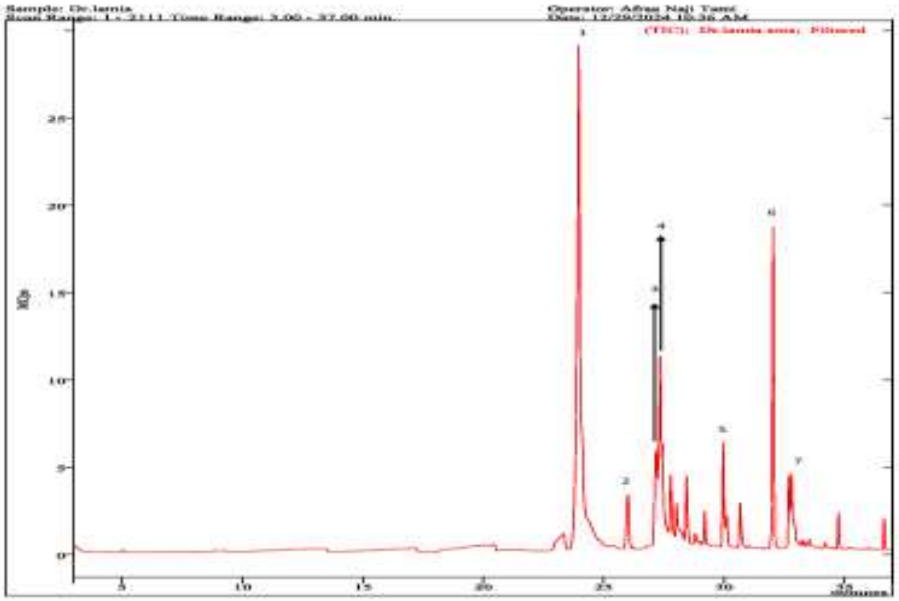


Figure1: Gas chromatography (GC) chromatogram of the petroleum ether extract of *Vitis vinifera* leaves, showing the detected phytochemical components within the retention time range of 3–37 minutes. Peaks are numbered according to the identified compounds.

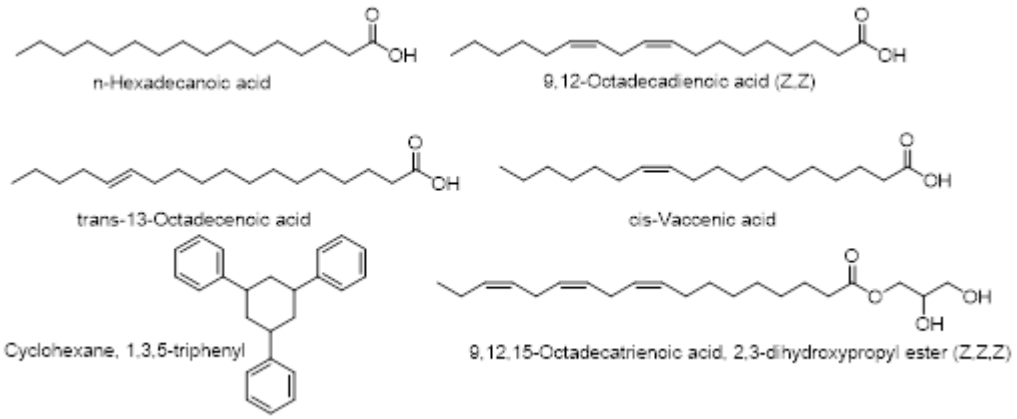


Figure2: Chemical structures of the major compounds identified in the petroleum ether extract of *Vitis vinifera* leaves, including fatty acids and related constituents

Table2: Results of GC MS Analysis Obtained from Petroleum Ether Extract of Vitis Vinifera Leaves

Peak number	Retention time(R_t) (min)	Area %	%Total	Prob%	M.wt	Name of compound
Peak 1	23.969	3.266e+8	39.247	88.5	256	n-Hexadecanoic acid
Peak 2	27.173	4.144e+7	4.980	26.6	280	9,12-Octadecadienoic acid (Z,Z)
Peak 3	27.340	7.195e+7	8.646	19.1	282	trans-13-Octadecenoic acid
Peak 4	27.432	4.317e+7	5.188	19.4	282	cis-Vaccenic acid
Peak 5	29.962	2.670e+7	3.209	78.7	312	Glycidyl palmitate
Peak6	32.042	9.271e+7	11.141	81.3	312	Cyclohexane
Peak 7	32.687	1.660e+7	1.995	7.24	352	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester

3.3. Antioxidant Activity

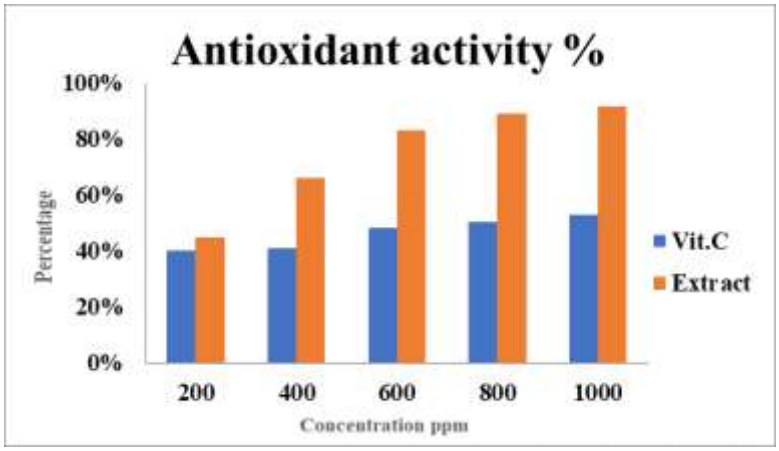
The extract's significant phenol and total flavonoid concentration has prompted investigations into its antioxidant properties, beginning with its capacity to scavenge free radicals in vitro. The methanolic aqueous extract of grape leaves was used to assess chemical free radicals, including DPPH, as well as other free radicals such as O2•−, OH•, and NO•, which are chiefly responsible for cellular oxidative stress. Given that grape leaf extract contains a wide range of bioactive compounds, several tests had been done to evaluate its antioxidant activity. The UAE method has demonstrated the process's effectiveness, producing a potent antioxidant extract, a free radical scavenging strategy, and IC50 measurement. The antioxidant activity of the methanolic: aqueous *vitis vinifera* leaves extract was examined against the DPPH radical scavenging method at the wave length 516 nm. The results in (table.3) revealed a clear concentration-dependent inhibitory effect at the highest concentration (1000 ppm), the compound exhibited a strong radical scavenging activity of 91.55%, while the lowest concentration (200 ppm) showed 44.69% activity. The calculated IC_{50}

value was estimated to be 249.55 ppm, indicating that the extract possesses remarkable antioxidant potency, while the ascorbic acid calculated value is 788.12 ppm as shown below in Table3, Fig.3A-B.

Table3: The Antioxidant Potency % Of The Meth:Aq. *Vitis Vinifera* Leaves Extract

Concentration	**Scavenging potency %**	
ppm	**L. Extract**	**vit.C**
200	45%	40%
400	66%	41%
600	83%	48%
800	89%	50%
1000	92%	53%
IC_{50} *(ppm)*	249.55	788.12

A



B

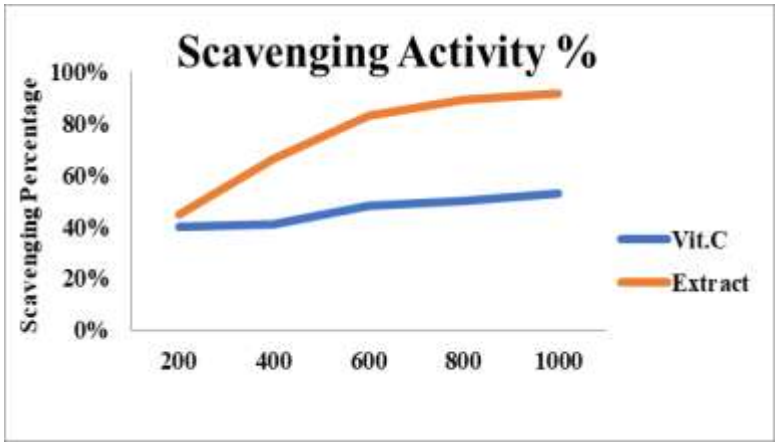


Figure3: Antioxidant and free radical scavenging activities of the petroleum ether extract of *Vitis vinifera* leaves compared with vitamin C (Vit. C) at different concentrations (200–1000 ppm). **(A)** Percentage antioxidant activity showing a concentration-dependent increase for the extract compared with Vit. C. **(B)** Percentage scavenging activity illustrating the superior radical scavenging potential of the extract relative to Vit. C across all tested concentrations.

4. Discussion

In the current study the ultrasound assisted extraction technique and two extraction solvent were used to extract the hydrophilic and hydrophobic compounds found in the dried *Vitis vinifera* leaves collected in Karbala/Iraq. The drying prosses was done at room temperature and not by oven in order to preserve the active compounds that may be destroyed by heat (Lamiaa *et.al*., 2024). The advancement of new technology has enhanced extraction procedures, making them more efficient and environmentally friendly, leading to products that are more affordable and freer from organic solvents (Panja, 2018). These procedures are occasionally termed "cold extraction techniques" due to the low temperature employed during the extraction of a molecule, which exerts negligible influence on the stability of the isolated molecules (Tiwari, 2015). The contents of fibre is high in Grapevine leaves with lower calories about (13 kcal), and the amounts of vitamins K and A also elevated. According to research, the concentration of antioxidants in grape leaves have ten times than grape juice or pulp contents (Gabler *et al*., 2003). Grape vine leaves are known to contain polyphenols and phenolic compounds, indicating their potential as a source of antioxidants. The metabolic profile of *Vitis vinifera* leaves revealed a variety of antioxidant compounds, indicating a significant potential for antioxidant activity. The antioxidant capacity of grape leaves is notable, measuring at 2402 198 mol TE per 100 g of fresh weight (Muresan *et al*., 2010). The application of organic solvents for the identification of non-polar components in *Vitis vinifera* leaves reveals that fatty acids are the most prevalent molecules. Another study indicates that alpha-linolenic acid is the most concentrated fatty acid in the leaves (Veskoukis *et al*., 2012). Given that polyphenols serve as food additives, it is vital to investigate the impact of heating on their antioxidant activity. The impact of boiling and steaming on carotenoids, polyphenols and their antioxidant qualities differed based on the type of leaf (Gunathilake *et al*., 2018). Consequently, the findings of the study provide valuable insights for selecting the optimal cooking procedure of leaves to preserve their antioxidant properties. In both humans and animals, a decline in motor and cognitive functions may be more vulnerable to the impacts of oxidative stress and inflammation (Zhangetal. *et al*, 2013). *Vitis vinifera* leaves contain a wide range of polyphenols, including anthocyanins and flavonoids, as well as organic acids including malic acid, oxalic acid, and tartaric acid. Polyphenols present in vine leaves interact with a wide range of pathways, including neuronal and glial signalling. Furthermore, vine leaves suppress ROS formation, neuronal damage from neurotoxins, and neuroinflammation, leading in better neurological health (Borai ,*et al*.,2017).

5. Conclusion

The excellent antioxidant potency results revealed from the methanolic aqueous extract of *vitis vinifera* L. tested against the DPPH with a noteworthy IC50 (249.55 µg/mL) as comparison with ascorbic acid (vit. C) , IC50 (788.12). It is good evidence the rich methanolic aq. Extract content of phenolic compounds mainly as flavonoids with high potency to scavenge free radicals likes OH, N and superoxide radicals then leads to

reduce ROS levels in cells which are a byproduct of oxidative stress and thus providing cells a great protection against oxidative stress. The pharmacological effected saponin, tannin and less of alkaloids also found in the methanolic aq. Extract. The non-polar component extracted with petroleum ether rich with terpenoids and fatty acids. Finally, as a result of study the *vitis venefera* L. collected from Iraqi garden are a bio residue part of the plant has a powerful role to treat diseases related with inflammation and oxidative stress.

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