Journal of Mines, Metals and Fuels, *71(12D): 235-242; 2023. DOI: 10.18311/jmmf/2023/47321*

Print ISSN : 0022-2755

Journal of Mines, Metals and Fuels

Contents available at: *www.informaticsjournals.com/index.php/jmmf*

Enhanced Antioxidant Activity of *Vitis vinifera* Extract Encapsulated in Nano Chitosan

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Abstract

Metals and minerals play an important role in maintaining a healthy human body. Various metals and minerals absorption in body are affected by the stress created due to reactive oxygen. Hence, it is essential for humans to supplement with a greater number of antioxidants. Polyphenols present in vegetables and fruits provide the antioxidants required for human diet. In recent years, biopolymers have gained tremendous interest in our daily life due to their enhanced pharmacological activity. The biopolymer like chitosan displays wide range of applications. On the other hand, natural plant extracts also gained considerable attention for their potential to treat various diseases. The plant extract encapsulation with chitosan may enhance the enzymatic activity and present study focuses on extraction of phytochemicals from leaf of Vitis vinifera and encapsulation of these phytochemicals in nano chitosan. Tender grape leaf (Vitis vinifera) are rich sources of antioxidant properties and can be extracted using various solvents like methanol, water in the ratio of 1:1, 1:2. Phytochemicals present in the Vitis vinifera extract act as reducing and capping agents while the nano-chitosan act as stabilizers. Leaf extract was carried out in Soxhlet apparatus using methanol as solvent. Fourier-transform infrared spectroscopy FT-IR was used to confirm the functional groups presence, Field Emission Scanning Electron Microscopy (FESEM) and Energy-Dispersive X-ray spectroscopy (EDX) to study the surface morphology of the prepared material. Free radical scavenging activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method using UV-visible Spectrometer were carried out for the prepared materials. The enhanced antioxidant property was achieved using a combination of Vitis vinifera extract and chitosan.

Keywords: *DPPH Method, Encapsulation, Nano Chitosan, Vitis vinifera Extract*

1.0 Introduction

Metals play a crucial role in biological systems, ranging from structural support to enzymatic activity regulation[1]. For instance, metals like iron, zinc, copper, and magnesium are essential for various cellular functions

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such as maintaining blood brain barrier, fluid level, and most important mineral absorption[2]. Iron is a key component of hemoglobin, enabling oxygen transport in blood, while zinc serves as a cofactor for numerous enzymes involved in metabolism and gene regulation[3]. Copper participates in electron transfer reactions, and magnesium is essential for stabilizing nucleic acids and ATP molecules. Similarly, minerals also play a vital role in maintaining overall health. Numerous factors influence mineral absorption, including the chemical form of the mineral, the presence of other dietary components, the wellbeing of gastrointestinal tract, and most importantly number of free radicals[4]. If the absorption of minerals and metals are abrupted due to nay physiological process then, these metals and minerals will interact to liberate Reactive Oxygen Species (ROS) in the body[5]. ROS, such as superoxide radicals (O2•−) and hydroxyl radicals (•OH), are highly reactive molecules that can damage cellular components such as DNA, proteins[6]. Hence, maintaining a balance of metals and antioxidants in the body is essential for preventing excessive ROS production and minimizing oxidative stress[6]. Antioxidants, found in various foods such as fruits, vegetables, and nuts, help neutralize ROS and protect cells from oxidative damage[7].

Traditionally grapes are used (*Vitis vinifera*) for abundance nutrients and regarded as wellness food in terms high Vitamin K. Presence of bioactive (poly) phenols including flavanols, stilbenes, flavones, hydroxycinnamic acid and anthocyanins make the fruit as multi nutrient food for humans. Grapes are used extensively inthebrewery industries. Himachal Pradesh's wild grape harvest was used to make regional wine. Currently, 90% of the total land is devoted to grape farming in peninsular India, which is flanked by the Arabian Sea, the Bay of Bengal, and the Indian Ocean[9-10]. The most abundant fruit in agriculture is the grapes globally cultivated across more than 750,000 hectares, out of which 77.44 million metric tons of grapes are used for wine production on an average annually.

The root, seed, skin, cane, leaf, fruit, pomace, and stems contain a variety of phytochemicals compounds. Grapes contain a portion of bioactive compounds, nutritional constituents and ingredients used in various physiological processes, such as carbohydrates, fibers, proteins, vitamin C, minerals and sugar. Grapes are also considered to be used in therapeutical applications such as influenza, anemia, allergies, venerology and bronchitis, anti-platelet, anti-bacterial, anti-diabetic, protective effect, anti-cancer, anti-oxidant, anti-acne, anti-inflammatory, anti-hypertensive, anti-asthma, anti-viral, anti-fungal, anti-obesity, anti-cholinergic, anti-aging, anti-sunburn, anti-hyperpigmentation, anti-cataract and wound-healing activities[11-14].

2.0 Methods and Methodology

2.1 Materials

Grape leaf was purchased from local farms near Kolar. These leaves were ground into fine powder using an electric grinding machine (Model WX 511N National).

2.2 Preparation of Leaf Extract

About 4 gm of *Vitis vinifera* leaf was taken in a thimble. The extraction was carried out using different solvents (ethanol water and methanol)[7]. Methanol solvent (100%) was taken in 500 ml RB flask. Soxhlet was connected and extracted. Extraction was done for about 5 cycles around 6-8 h. Solvent was removed using Rota evaporator (SUPERFIT, Model: PBV-7D). And the extracts were stored in vials for further studies.

3.0 Preliminary Phytochemical Screening

The phytochemical analysis of the leaf extract was carried out to study the presence of chemical constituents using the standard procedures[15-18].

3.1 Qualitative Phytochemical Analysis

3.1.1 Test for Alkaloids (Mayer's test)

1ml of the extract was mixed with 3 drops of Mayer's reagent; the presence of alkaloids were identified white color precipitate.

3.1.2 Test for Flavonoids (Lead Acetate Test)

A yellow precipitate can be seen after 2 min of adding a small amount of the extract to the lead acetate.

3.1.3 Test for Tannins ($FeCl_3$ Test)

2ml of the extract was added to 0.3 ml of $FeCl_3$ solution appearance of bluish black or greenish black indicates the presence of tannins.

3.1.4 Test for Terpenoids (Salkowski test)

5 ml extract was mixed with 2 ml chloroform and 2 ml of concentrated sulphuric acid. A reddish brown color indicates that terpenoids are present.

3.1.5 Test for Carbohydrate (Benedict's test)

1ml of the extracts and 2 ml of Benedict's reagent were mixed and shaken slightly. If brick red precipitate seen it indicates the occurrence of carbohydrates.

3.1.6 Test for Protein (Ninhydrin test)

A small amount of extract was taken in a test tube and ninhydrin reagent was added, boiled for 2 mins. Appearance of a violet colour directs the presence of protein.

3.1.7 Test for phlobatannins

1-5 ml of extract was taken in a test tube dissolved in 1% of HCl and heated for 20 min. A red precipitate was observed indicating the presence of phlobatannins.

4.0 Preparation of Nano Chitosan and Encapsulation of Leaf Extract of *Vitis vinifera* in Nano Chitosan by Ionotropic Gelation Method

Nano chitosan was prepared by ionic gelation method as reported by [19-22] Chitosan (1%w/v) of molecular weight 12000 kDa (based on viscosity) was taken in a 250 ml beaker and dissolved in 1% acetic acid. Tween-80 (0.25% w/w) was added to the chitosan solution and it was maintained at pH 7 by adding 1 M NaOH and further stirred at 1200 rpm for 24 h at room temperature. After 24 h 0.25 ml of Tripolyphosphate (0.03-0.04% w/v [8], was added and stirred. Filter the obtained product withice cold water using Buchner funnel and dry the product. And the yields were stored in viols for further studies.

A similar procedure was followed for the plant material encapsulation by adding 2ml of leaf extract of *Vitis vinifera* to the chitosan containing tween-80 and stirred for 30 mins at 1200 rpm followed by addition of sodium hydroxide of tri poly phosphate with continuous stirring for another two hours at 1200 rpm. Later washed repeatedly with distilled water and dried for 24 h. And the yields were stored in viols for further studies. The yield of encapsulation of flower extract in nano chitosan is calculated.

5.0 Determination of Total Antioxidant Activity

5.1 Antioxidant Studies of *Vitis vinifera* Leaf Extract and Polyphenols Extracted from *Vitis vinifera* Leaf in Nano Chitosan will be Carried out by DPPH Method

Antioxidant activity of leaf extract of *Vitis vinifera* encapsulated in nano chitosan by DPPH method: The scavenging effect of leaf extract of *Vitis vinifera* encapsulated in nano chitosan was done by using the 2, 2-diphenyl-1-picrylhydrazyl-hydrate method as per the standard methods. 1 gram of *Vitis vinifera* leaf extract encapsulated in nano chitosan was dissolved in 3ml

Table 1. Encapsulation of *Vitis vinifera* in nano chitosan in different concentrations.

S. No.	Beakers No.	Quantity (W/V)	Tween (%)	pH	RPM	TPP (1%) (W/V)	Plant extracts
1	EEONCS-E	0.5 gm	0.003	6.8	1200	10ml	2ml
2	EEONCS-F	0.5 gm	0.002	6.8	1200	10ml	2ml
3	EEONCS-G	0.5 gm	0.003	7.0	1200	10ml	2ml
4	NCS	0.5 gm	0.002	7.0	1200	10ml	-

of acetic acid diluted with 99% methanol. A series of concentrations ranging from 5 µg/l to 25 µg/l. After 30 mins the radical scavenging activity was recorded by measuring the absorbance of prepared concentration of *Vitis vinifera* extract encapsulated in nano chitosan in UV-Visible spectrometer at 517nm. DPPH was taken as standard and Ascorbic acid as reference. Even the leaf extract and nano chitosan antioxidant were recorded. Percentage inhibition of free radical of DPPH by the *Vitis vinifera* extract encapsulated in nano chitosan was calculated by using the following equation[23].

$$I\ \% = \frac{A_0 - A_1}{A_0} \times 100$$

A_O = Absorbance of the sample, A_1= Absorbance of Control

6.0 Result and Discussion

6.1 Phytochemicals Screening

The plant extract was screened for the presence of secondary metabolite, mainly Flavonoids, Carbohydrate, Saponin, Terpenoids and Glycosides, depending on general phytochemical methods. In some of these tests precipitation occurs after the addition of reagent and some test were based on observations that color changes occurred only after the addition of specific reagents. The phytochemical tests result of *Vitis vinifera* extracted with methanolic solvents are presented in Table 2. The present study demonstrated the presence and absence of extracted phytochemical compounds in the extract. The highest number of phytochemical was observed in the methanol extract of *Vitis vinifera*[24,25]. The results of the photochemical analysis of the *Vitis vinifera* leaf extract is as shown in given Table 2.

6.2 FT-IR Analysis

6.2.1 FT-IR Spectra of Vitis vinifera Leaf Extract

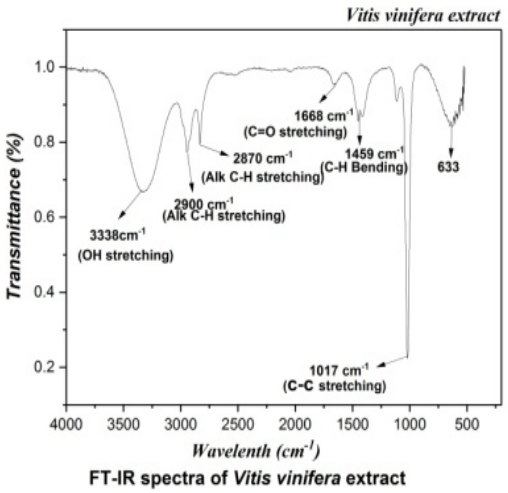


Figure 1. FT-IR spectra of *Vitis vinifera* leaf extract.

Table 2. Photochemical qualitative analysis of *Vitis vinifera* leaf extract

S. No.	Phytochemicaltest	Observation	Result
1	Alkaloid	Creamy white precipitate	-
2	Flavonoids	Yellow colouration	+
3	Tannin	Blue black colouration	-
4	Terpenoids	No reddish brown colouration	+
5	Carbohydrate	Reddish colour precipitate	+
6	Protein	Violet or pink colour	-
7	Phlobatannin	Red precipitate	-
8	Saponin	Producing foam	+

+: present, -: absent

6.2.2 FT-IR Spectra of EVVNCS-E

The FT-IR spectra of *Vitis vinifera* leaf extract indicated broad band at 3338 cm^{-1} for O-H stretching indicating the presence of phenolic compounds. A strong C=O stretching, Alk C-H stretching and C-H bending was observed at 1668 cm^{-1}, 2870 cm^{-1}, and 1459 cm^{-1} respectively along with C-C at 1017 cm^{-1}.

The FT-IR spectra of EVVNCS-E (Figure 2) indicated broad band at 3336 cm^{-1} for O-H stretching present in phenolic compounds of leaf extract. C=O stretching, Alk C-H stretching and C-C stretching were also recorded at 1660 cm^{-1}, 2784 cm^{-1}, and 1021 cm^{-1}, respectively.

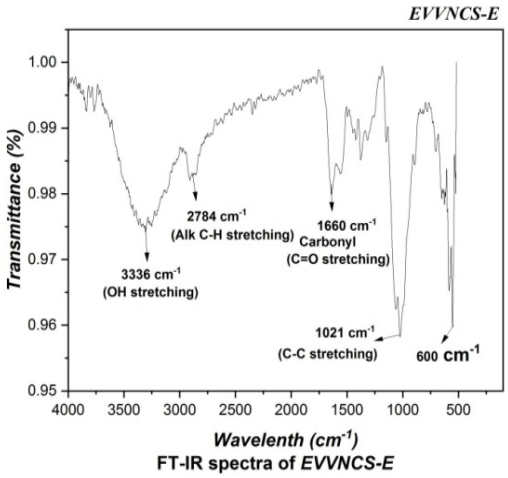


Figure 2. FT-IR spectra of EVVNCS-E.

6.3 SEM Analysis

6.3.1 SEM image of EVVNCS-E

The morphological shape and size of encapsulated *Vitis vinifera* leaf in nano chitosan were studied by scanning electron microscopy as shown in Figure 3. Which indicate the particles size of 140-170 nm. From the SEM image (Figure 3), it is observed that the encapsulated *Vitis vinifera* leaf in nano chitosan is irregular in shape.

6.3.1.1 Energy Dispersive X-Ray (EDX) Analysis of EVVNCS-E

From the EDX graph (Figure 4) of encapsulated *Vitis vinifera* leaf in nano chitosan, it was observed that

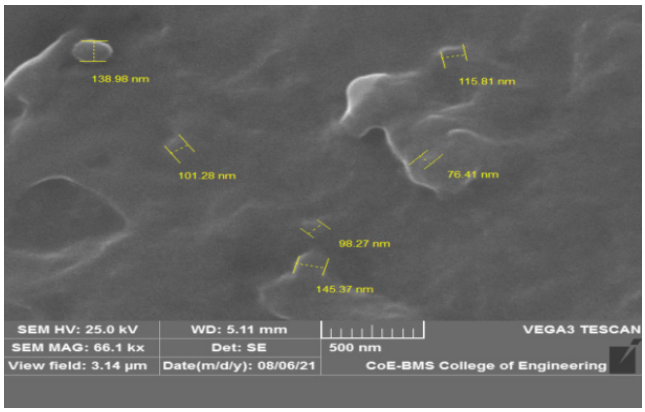


Figure 3. SEM image of EVVNCS-E.

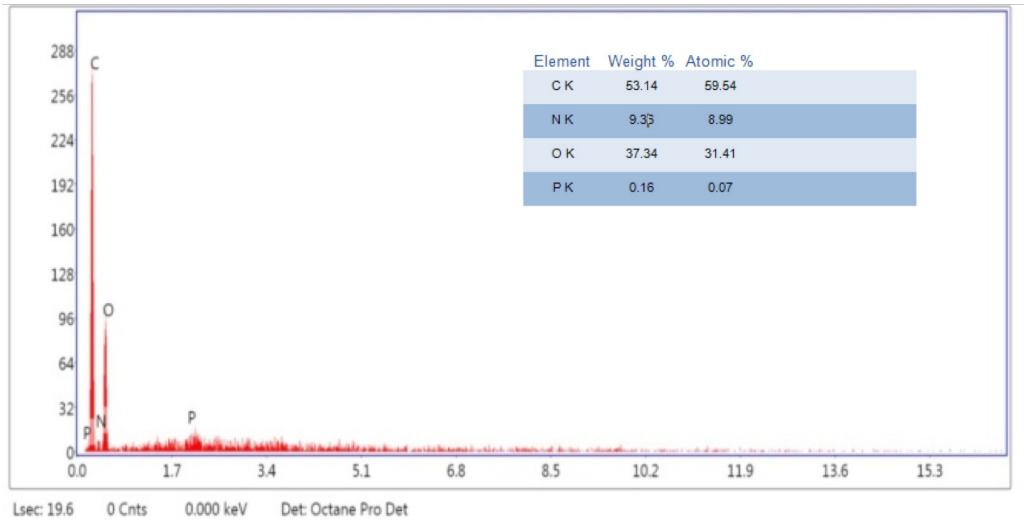


Figure 4. EDX graph of EVVNCS-E with elemental component.

carbon, oxygen, nitrogen, phosphorus were the elements present. In EVVNCS-E the percentage of carbon was 59.54%.

6.3.2 SEM Image of Nano Chitosan

The morphological shape and size of nanochitosan were studied by Scanning Electron Microsopy (SEM) as shown in Figure 5. which indicates the particles size of 80-120nm and surface appeared to be rough with cuboidal shape nanoparticles[25].

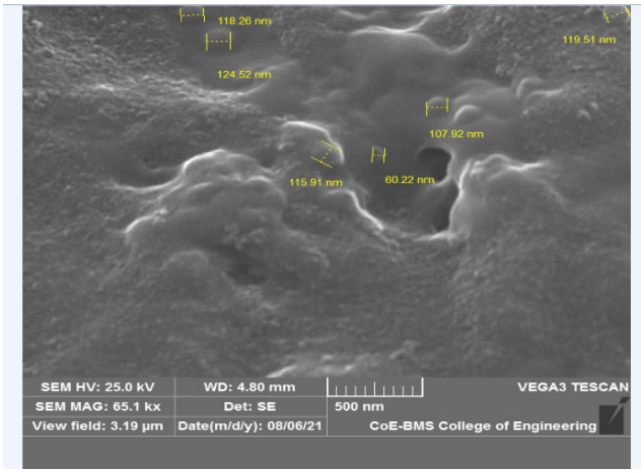


Figure 5. SEM image of nano chitosan.

6.3.2.1 Energy Dispersive X-Ray (EDX) Analysis of Nano Chitosan

From the EDX graph (Figure 6) of nano chitosan, it was observed that carbon, oxygen, calcium, nitrogen, phosphorus were the elements present.

7.0 Antioxidant Activity

7.1 DPPH Radical Scavenging Test

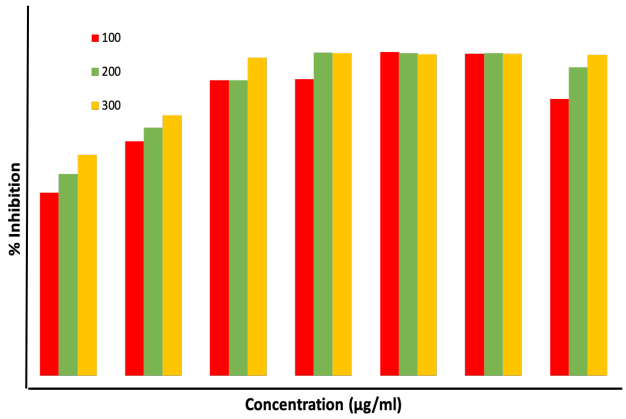


Figure 7. % inhibition of ascorbic acid, plant extract, encapsulated VV, Nano chitosan and chitosan.

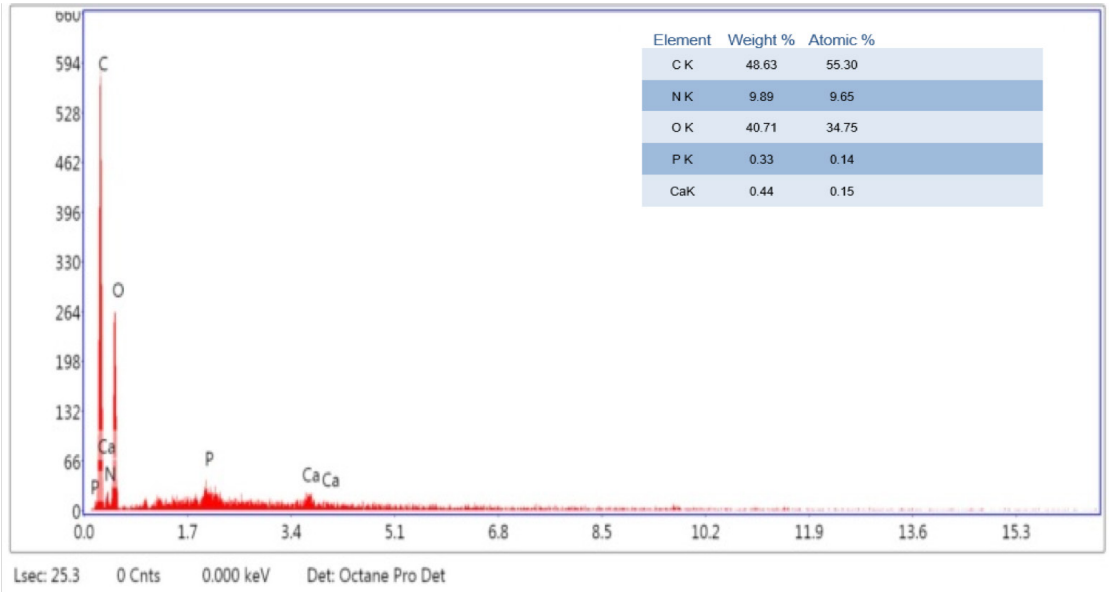


Figure 6. EDX graph of Nano chitosan with elemental component.

8.0 Conclusion

Vitis vinifera is a bioactive compound having several pharmacological activities such as anti-oxidative, anti-inflammatory and antimicrobial activities. The antioxidant activity result indicates that the DPPH antioxidant activity of the phytochemical encapsulation of *Vitis vinifera* leaf extract in nano chitosan was a good source of antioxidant and a promising biomaterial for further studies. According to the UV recorded data, the results indicate that leaf extract (Figure 7) had high inhibition % of free radical when refereed with nano chitosan[26]. However, the antioxidant activity of Vitis vinifera leaf extract encapsulated in nano chitosan showed 97% for 5µg/ml of leaf extract of *Vitis vinifera* in nano chitosan. Further, these prepared materials can enhance the absorption of metals and minerals in the human body.

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