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# Encapsulation of Grape Seed Extract and Evaluation of its Potency against Gram-Negative Bacteria and Cervical Cancer

Pradeepa Subramanian[1], Chithra Shanmugasundaram[1], Jesteena Johney[2], Ragunathan R[2], Sam Ebenezer Rajadas[1#]
[1]Department of Biotechnology, V.S.B. Engineering College, Karur, Tamilnadu, India –639111
[2]Center of Bioscience and Nanoscience Research, Coimbatore, Tamilnadu, India - 641021

[#]Corresponding author: Dr. R. Sam Ebenezer
Associate Professor, Department of Biotechnology, VSB Engineering College

**Abstract:- One of the main cancers that kill women globally is cervical cancer, which has limited infectiousness and behaves epidemiologically like a vaginal illness. In a concept of multifactorial, stepwise carcinogenesis at the cervix uteri, smoking, and human papillomavirus (HPV) 16/18 are currently significant problems. Hence there is a need for therapeutics with lesser or no side effects. Grapes (*Vitis vinifera*) have recently gained scientific attention. Its rich high polyphenol content has been shown to improve human health, particularly in the prevention of cancer and cardiovascular disease. Resveratrol, quercetin, catechin/epicatechin, and other polyphenol bioactive compounds are present in significant amounts in grapes. Preservation of these contents is challenging to achieve significant shelf-life. In this study, we employed microencapsulation techniques to design a carrier medium that could preserve the Using the agar diffusion method, the encapsulated aqueous grape seed extract's antibacterial activity was assessed against Gram-negative bacteria, and anticancer activity was analyzed using Hela-cell lines.The outcome of the study, clearly indicates that the encapsulation does not alter the activity of the lead molecules present in the grape seed extract.**

***Keywords:-*** *Vitis Vinifera, Cervical cancer, Antioxidants, Polyphenol, Antibacterial and anti-cancer activity.*

## I. INTRODUCTION

Cervical cancer is the fourth most common cancer among women. Cervical cancer screening is acknowledged as an effective intervention for preventing advanced disease and death from cervical cancer (Arbyn *et al.,* 2020). Globally, an estimated number of 604,000 new cases and 342,000 deaths are reported in 2020 (Guo et al., 2021). In worldwide low and middle-income countries about 90% of the new cases and deaths in 2020 occurred (WHO 2020) (Hyuna Sung, *et.al.,* 2020). Even though 80% of cervical cancer cases occur in underdeveloped nations, cervical screening has significantly decreased the incidence of invasive cervical cancer in many industrialized countries. However, these countries have not received enough of the enormous advantages. This warrants the need for novel remedies from various sources preferably from nature. Grape seed has more content of phenolic acids and flavonoids. The majority of grapesbelong to the *Vitis vinifera*sub-sp. However, *Vinifera* hybrids of other *Vitis* species, as well as the related *genus Muscadinia* are also grown, Grapes are cultivated depending on regions where climate and disease pressure preclude the cultivation of *V. vinifera.*A little more than 80% of the harvest is used to make wine (Rani & Kekuda, 2014). Grapes are the most commonly consumed fruits and consist of a rich source of polyphenols (60-70%) (Markowitz et al., 2002).Only grape seed contained lipids and resins whereas grape seed and skin extracts were devoid of glycosides and phytosterols. Phytochemicals in plants are generally accepted as the health positive effects. Gallic acid decreased cancer cell viability via apoptosis induction. Antioxidant activity prevents free radical accumulation thatisknown tooccur during chronic diseases like cancer(Nasser et al., 2020).Grape seed extracts are known to induce significant inhibition of cell proliferation of cancer cell lines. These effects occur at both low and high concentrations.Grape seed flavanols inhibit alcohol-induced lipid peroxidation (lipofuscin production) and thereby protect the brain from the harmful effects of alcohol.It is critical to developing efficient systems for the targeted delivery of drugs and contrasts agents to tumour growth areas, as well as the stimulus-sensitive release of both drugs and agents; thus, it is at the forefront of the development of theranostic agents for the combined diagnosis and treatment of tumours (Chang D*et al.,* 2020).One strategy for producing new pharmacological forms of pharmaceuticals with minimal or no side effects is the encapsulation of drugs that have a substantial harmful effect on the body. This strategy has been beneficial for several commercially available medications. Encapsulation of hazardous medications in cancer therapy is becoming a viable prospect for effective treatment (De Cock *et al*., 2020).

## II. MATERIALS AND METHODS

### *A. Sample Collection*

Black grape seeds were collected from the Karur market. 1 kg of fresh samples are collected and the berries were finger pressed to remove pulp and juice. After the seeds were dried under sunlight. Then using a hammer to make seeds in fine powder.

*B. Preparation of grape seed extract*

Water has the more polar solvent and is used in the extraction of a wide range of polar compounds, which is used to dissolve a wide range of substances. The powdered seed was dissolved in distilled water (0.5% of extract) - 0.5g in 20 ml.

*C. Microencapsulation of Grape Seed Extracts:*

Sodium alginate (2.5% w/v in distilled water) and Calcium Chloride (2% w/v in distilled water) were prepared separately and kept for sterilization in an autoclave at $121^0$C for 15minutes. After sterilization this was cooled to room temperature and an equal amount of the seed extract was added and in a magnetic stirrer at $40^0$C for 2hrs. Small droplets of sodium alginate along with the plant extract pipetted out in calcium chloride solution for microencapsulation. This was incubated for 2hrs at room temperature, Finally, the capsules were filtered from the calcium chloride solution and dried until it turns into small dark beads (Jesteena Johney, *et.al.*, 2018). Encapsulation is the process where the mixture of coated with a polymer that protects exit against real negative influences (Grgic, *et.al.*, 2020).

## III. PHYTOCHEMICAL ANALYSIS

*A. Total phenol content*

The Folin-Coicalteu assay methodis used to measure the total phenolic content. The 1ml of the extract was mixed with 0.5ml of 10% Folin-Ciocalteu reagent and 1ml of the 20% $Na_2CO_3$ solution, the mixture was allowed to mix and incubated at $45^0$C for 15minutes in a shaking incubator. After incubation, the OD value wasmeasured at 765nm under a spectrophotometer. Gallic acid was used as a standard to calculate the mg/g of phenol content (Jaya Prakash, *et.al*,2019). The secondary compounds of phenolic compounds are widely produced through the shikimic acid pathway in plants (Naczk,*et.al.*, 2006). Phenolic compounds exist in plants in different forms, in a non-glycosylated form linked to other complexmolecules (Kammerer,*et al.*,2014).

*B. Total Alkaloid Content*

Alkaloids were determined with the dry weight of the sample after the experiment. 0.25gm of the sample was taken in a pre-weighed vessel and added 50 ml of the10% acetic acid, this was mixed and incubated at 37◦C for 3 hrs. To the solution, one-quarter of the water was added and the concentrated ammonium hydroxide was added drop by drop until get precipitation.The precipitation was separated from the vessel and washed with an ammonium hydroxide solution. This was filtered by filter paper and dried until getting constant weight to finalize the alkaloids content of the sample (Herrin Sheebu Gracelin, *et al.*, 2013).

$$\text{Percentage of alkaloids} = \frac{\text{Final weight of the sample}}{\text{Initial weight of the sample}} \text{X } 100$$

*C. Total Tannin Content*

Take 100µl of the extract are add 0.5 ml of Folin phenol reagent along with add 1ml of 35% of Sodium carbonate. After adding 1 ml of distilled waterit was incubated for 1 hour, and OD was measured at 765 nm using UV visible spectroscopy.

*D. Total Flavonoid Content*

Total flavonoid content (TFC) was measured using an aluminium chloride assay.1 ml of the extract was dissolved with 0.1 ml of 10% aluminium chloride solution and 0.1 ml of sodium potassium tartrate followed by 2.8 ml of distilled water. After adding the reagentsinto the tubes, tubes were incubated at room temperature for 30 minutes and OD wasmeasured at415 nm using a spectrophotometer (ELICO SL 159). Blank was maintained without adding the sample and standard quercetin was used to calculate the mg/g of the flavonoid content (Akbay *et al.*, 2003).

*E. DPPH Assay*

Stable 1,1- diphenyl-2-picrylhydrazyl radical was used for identifying the free radical scavenging activity of the extracts usingthe DPPH assay. DPPH solution (0.004%, w/v) was prepared in methanol(Grace Nirmala & Narendhirakannan, 2011). Stock solution (1 mg/ml) and standard ascorbic acid (0.05 g/ml) were prepared using methanol.500µlof the sample solution and 1ml of DPPH solution were added. Mixed the solution and incubate for 5 minutes. Along with 0.4 ml of 50mM tris HCL buffer, the tube was incubated in the dark for 30 minutes and the reading was measured at 517nm using a spectrophotometer(LT 291 labtronics microprocessor). Methanol was used as a blank, and the mg/g of the DPPH was calculated by using the ascorbic acid as a standard.

*F. FTIR Analysis*

Fourier transform infrared (FT-IR) spectra were done for the analysis of functional groups using an FT-IR spectrometer (Shimadzu). 400cm-1to 4000 cm-1 was used for the analysis to obtain an infrared spectrum of absorption of the sample. Fig 3.2. The present study proved that *Vitis vinifera* grape seeds especially have more alcoholic extracts, which play an important role in the inhibition of bacterial activity and growth (Abdul, *et al.*, 2016).

## IV. ANTIBACTERIAL ACTIVITY

The antibacterial activity of the sample was identified by using the well-diffusion method. Mueller-Hinton agar (39gm of media was dissolved in 1000ml of distiller water and sterilized under autoclave at $121^0$C for 15 minutes) was prepared, and poured onto a Petri plate for solidification, after solidification,100µl of the pathogenic culture (*Escherichia coli,Salmonella typhi, Klebsiella pneumoniae,*) were swabbed using a cotton swab and well.50µl of GSE was to the wells. Antibiotic disc (Cefazolin-CZ) was placed as a positive control, the plate was incubated at $37^0$C for 24 hrs. After incubation anti-bacterial activity of the sample was measured based on the zone of inhibition in mm (Jesteena Johney, *et.al.*,2018).

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## V. ANTICANCER ACTIVITY (MTT ASSAY)

HeLa Cell Line was used to study the anticancer activity of the GSE using MTT assay. The cell line was purchased from National Center for Cell Science, Pune, India and has been maintained further in the Center for Bioscience and Nano Science Research Laboratory Eachanari, Coimbatore, Tamil Nadu, India. After obtaining, the Cell Line was sub-cultured in DMEM medium with the addition of sodium carbonate, glucose and fetal bovine serum (10%).The cells were incubated ina $CO_2$ incubator with a pH of 7 to 7.5, temperature $37^0$C, and humidity 70-80% for 24-72 hrs. After incubation, the growth of the cell line was confirmed by viewing it under an inverted phase-contrast microscope.For the MTT assay, cells were again seeded in 96-well plates and allowed to adhere for 24 hours at $37^O$C in 5% $CO_2$ and 70-80% of humidity. The cell line withthe GSEs at different concentrations along with blank (DMSO), and control (CellLine), was incubated for 24 hrs. After incubation, the cells were washed with DMSO and trypsin. 20µl of MTT dye was added to each well, after slight mixing the plates were incubated for 24hrs at 37°C in a $Co_2$ incubator. The reaction mixture was then carefully taken out and formazan crystals were solubilized by adding 100µl of DMSO to each well and mixed thoroughly. After 24hrs, the absorbance of the purple colour was read at 570 nm using a 96-well plate ELISA reader (Robonik, India). After taking the % of cell viability was calculated by following the formula (Jesteena Johney*et.al.*, 2018).

## VI. RESULTS

*A. Sample collection and preparation of extracts*

Fresh Black grapes were bought in the Karur, Tamil Nadu, neighbourhood fruit market. These have been washed in water, the berries have been finger-pressed to extract the juice and pulp, and the seeds have been separated. after the seeds had been sun-dried. then smashing seeds into a fine powder using a hammer. For later usage, it was kept in a freezer. 20 ml of distilled water and 15 ml of methanol were combined with 0.5 g of powdered dry grape seed. The extract was centrifuged at 4500 rpm at 5°C for 15 minutes after the mixture was vortexed for 2 minutes and shaken with a washing bath at 70 pm for an hour at room temperature. A 0.45 m membrane filter was used to filter the mixture.

## VII. MICROENCAPSULATION OF GRAPE SEED EXTRACT

The ionic gelation procedure was used to create grape seed extract microcapsules. The active ingredient is dissolved in an aqueous solution of calcium chloride and sodium alginate for the anionic gelation process, which is extruded through a syringe needle or nozzle. The produced emulsion was then poured into a beaker glass to create microcapsules. The microcapsules were weighed at 2g in total as shown in Fig.1 and Fig.2


Fig. 1: Microencapsulated GSEs before drying (A)and after drying (B)

*A. Phytochemical Analysis of GSEs*

Phytochemical analysis of the encapsulated GSEs was carried out to look for the impact of the encapsulation process on the phytochemical characteristics of GSEs. Significant changes in the phytochemical constituents were noticed. The values are mentioned in Table 1.

Table 1: Phytochemical analysis of encapsulated GSEs

| **Phytochemical Constituent** | **Quantity** |
|---|---|
| Total Phenolic Content (TPC) | 82mg/ml |
| Total Tannin Content (TTC) | 11mg/ml |
| Antioxidants | 249mg/ml |

## VIII. FTIR ANALYSIS

FTIR analysis revealed that the C-I groups of the extract have a distinct and extremely intense bond (432/cm). The frequency asymmetrical patterns of the aromatic group C-Br are shown by the sharp bond (501/cm). The C-CI group's aliphatic bond's frequency patterns were represented by a different bond (756/cm). The 825/cm bond, which corresponds to the ester anhydrate group's (C=O) frequency, is a pattern. The frequency of pattern group C-F is represented by the bonds at 1010 cm/cm and 1064 cm/cm. The results of the current study clearly show the chemical makeup of grape (V. vinifera) seed extracts.

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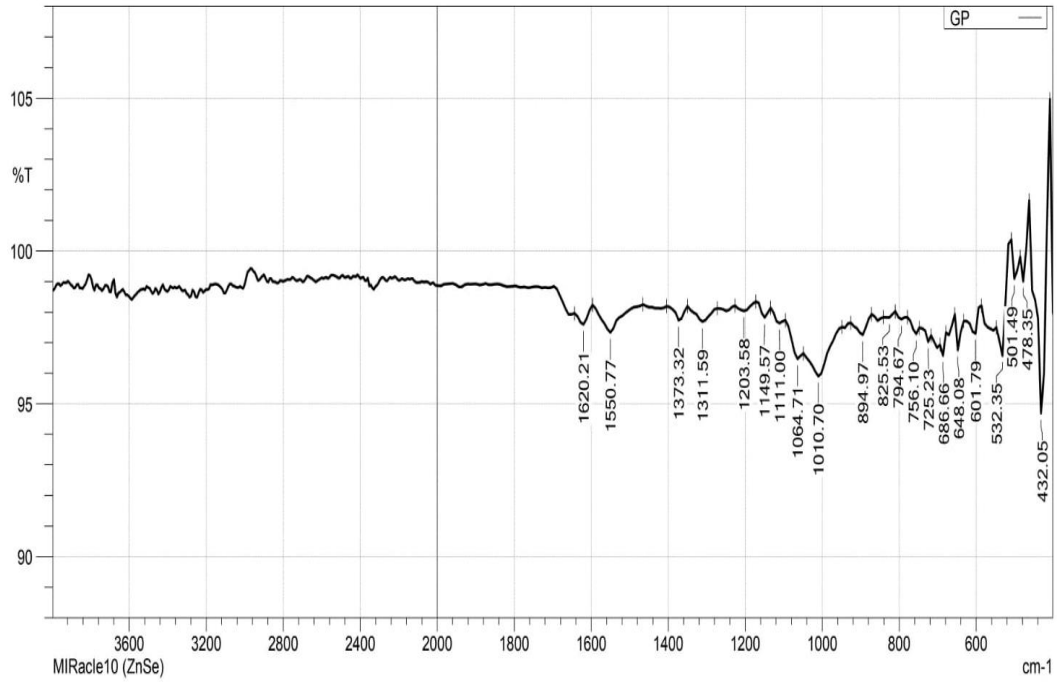


Fig. 2: FTIR analysis of the grape seed extracts

## IX. ANTIMICROBIAL ACTIVITY

The extracts of encapsulated GSEs showed significantly similar antimicrobial activity against selected standard isolates of *Klebsiella pneumoniae, Salmonella typhi,* and *Escherichia coli*(Fig.3 and Table 2)

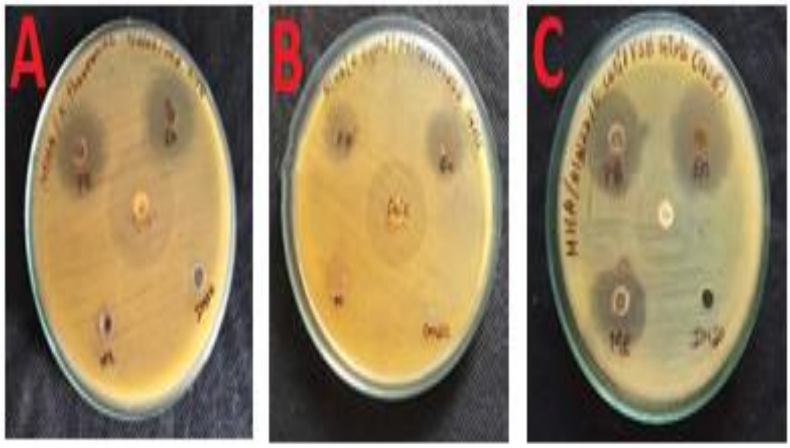


Fig. 3: Antibacterial activity of encapsulated GSEs against Klebsiella pneumoniae, Salmonella typhi, and Escherichia coli

Table 2: Zone of inhibition by free and encapsulated GSEs

| Micro-organism | Free GSE | Aqueous Encapsulated GSE | Methanolic Encapsulated GSE | 0.5 % DMSO |
|---|---|---|---|---|
| *Escherichia coli* | 9mm | 11mm | 10mm | - |
| *Klebsiella pneumoniae* | 11mm | 11mm | - | - |
| *Salmonella typhi* | 9mm | 9mm | - | - |

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## X. EFFECTS OF ENCAPSULATED EXTRACT *VITIS VINIFERA* ON CERVICAL CANCER CELL LINE(HeLa)

The encapsulated GSEs were tested for their anticancer activity against the cervical cancer cell line HeLa. 60% of cell death was observed in the wells exposed to 25 µg/ml of the encapsulated GSEs (Fig. 4 & 5).

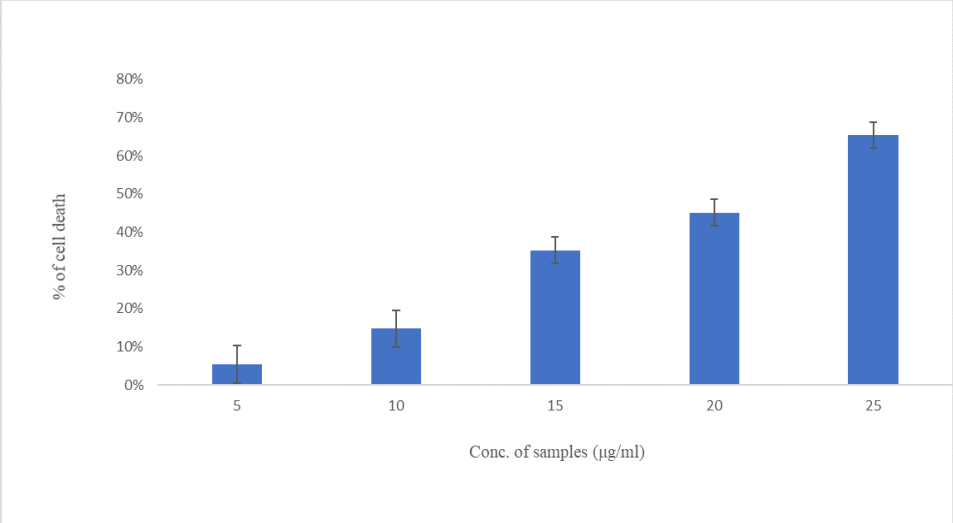


Fig. 4: Graphical representation of Anticancer activity of encapsulated GSEs on HeLa Cells

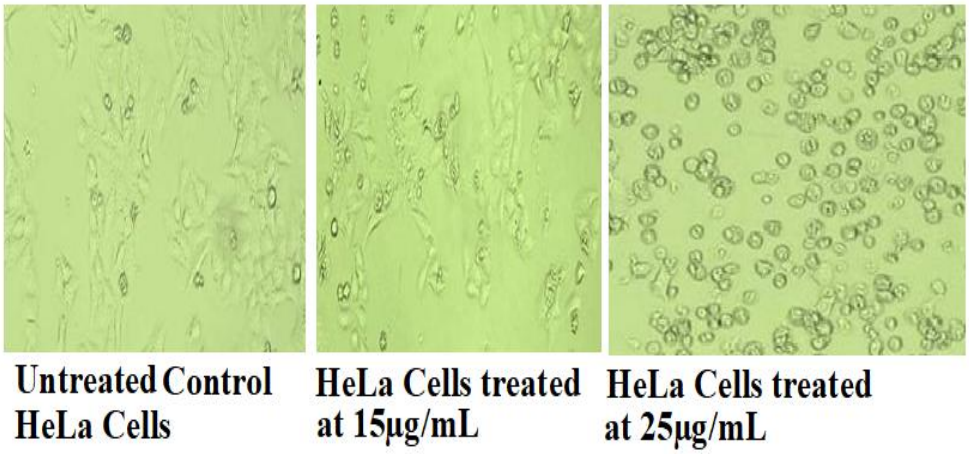


Fig. 5: Microscopical representative image of HeLa cell lines exposed to different concentrations with *V. vinifera* seeds

## XI. DISCUSSION

The findings of the current study show that GSE significantly lowers survival by causing apoptotic cell death in Hela cell lines. It also greatly inhibits growth and cell multiplication. An innovative and fruitful endeavour is the investigation of the antibacterial and antioxidant properties of natural extracts and their usage as substitutes for antimicrobials and food preservatives. Significant antibacterial efficacy against numerous foodborne pathogens has been demonstrated by grape seed extracts. GSEs can substitute synthetic chemicals that may have cytotoxic effects by acting as antibacterial food additives. The grape seed extract was extracted and subjected to UV-VIS and FTIR analysis in the current investigation. The resultant extracts have antibacterial and anticancer effects because they are high in polyphenolic chemicals. Investigating the presence of polyphenolic chemicals is crucial to elucidating the antibacterial and anticancer potentials of the extracts. The extracts' TPC was in the neighbourhood of 82 mg. This is consistent with the findings of other researchers (Martin et al., 2019), who found that the TPC ranged from 7.10 to 107.8 mg GSE/g DW in various grape seed extracts. The TPC in the GSEs of 10 grape varieties was measured in a study by (Lenka Sochorova et al., 2020), and the readings were in the range of 8.79-11.27 mg GAE/g. The extracts' TF fell between 40.05 to 52.01 mg. DPPH tests were used to determine the extracts' antioxidant capabilities. The DPPH assay evaluates an antioxidant's ability to reduce the DPPH radical. The strong scavenging action of GSEs is due to the -OH functional group and its location in the polyphenol structure. Results of the DPPH test, from 249 mg. Tannins are secondary metabolites present in several food products based on plant material. Plant tannins are a wide group of natural phenolic compounds with molecular weights ranging from 500 to 3000 Da. They are currently classified into three major subgroups: (1) hydrolysable tannins, (2) condensed tannins, and (3) phlorotannins. Hydrolyzable tannins are highly soluble in water. Tannins provide antimicrobial, antioxidant, and nutraceutical agents(Singh & Kumar, 2020).The typical strategy for preventing cancer cell proliferation is the induction of apoptosis, cell cycle arrest,

or a combination of these two modalities. The results of the current study showed that GSE enhanced the rate of apoptosis in HeLa cells, indicating that GSE inhibited the proliferation of HeLa human cervical cancer cells via initiating the apoptosis cell cycle. Several inferences may be made about the effect of the total polyphenolic content and composite profile of the investigated extracts on their antioxidant and antibacterial potentials based on the results. It is clear from comparing our findings to those of other writers that the values of TPC, TFC, and TTC are comparable and frequently much higher. According to the data, grape seed extracts have the strongest antibacterial and antioxidant properties. It is important to carry out more investigations in which the polyphenols are isolated and their actions against resistant bacteria were examined to properly understand which polyphenol has the highest inhibitory impact. Based on these data, it will be possible to obtain enriched extracts with higher antioxidant and antimicrobial activities in the future. These findings will make it possible to produce enhanced extracts in the future that have better antibacterial and antioxidant activity.

## XII. CONCLUSION

Grape and grape products are important and have rich in bioactive components. Grape seeds and pulp has fatty acids and tocopherols and minerals. The current study demonstrated conclusively that the chemical composition of grape seed extracts does not get altered by the encapsulation while exhibiting significantly similar biological activity against gram-negative bacteria and cervical cancer cell lines.

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