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Muscat grapes (*Vitis vinifera* L.) -An insight into phytonutrient potential and an aromatized functional food

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Abstract

Vitis vinifera L., commonly known as Muscat grapes is an aromatic food product which has a beneficial effect on the human body due to its phytonutrient potential. Studies have reported that grapes have antiobesity, antidiabetic, antiinflammatory, anticarcinogenic, cardioprotective, vasorelaxant, phytoestrogenic, and neuroprotective properties. This study aimed to identify the phytonutrients such as alkaloids, flavonoids, phenol, steroid, tannin, and terpenoids of the coloured seeded grapes. Whole grapes were collected during the vegetation period and divided into three groups as whole fruit (W), fruit skin (P) and seed (S). All three samples were freeze-dried and soaked with the aqueous solution. Ultrasonic homogenized material was used for extraction. Further analysis is done in FTIR for qualitative measurement of the main functional group and antioxidant activity was determined through the DPPH method. The findings revealed that Muscat grapes may be considered as a natural rich source of phenolic amalgam with a notable amount of antioxidant capacity.

Keywords: Muscat grapes, functional food, FT-IR, phytonutrient, DPPH, antioxidant activity, phenolic amalgam.

1. INTRODUCTION

Vitis vinifera L. is the most widely consumable grape variety, commonly known as the Muscat grape. This coloured and seeded grape has a multifactorial beneficial effect on human health. Consumption of whole fruit as well as the skin, and seed separately gives the same effect on consumer health. This plant-based food is a very important source of macronutrients namely carbohydrates, proteins, lipids as well as secondary metabolites. Historically, an epidemiological observation of French people confirmed that Coronary Heart Disease is low in rate while their diet was rich in saturated fats, this scientific study is known as the “French Paradox”[3]. The explanation of this study was the consumption of red wine which is made from an aromatic fruit, the Muscat grape. The presence of a volatile phenolic group is the reason behind the aroma profile where esters and terpenes contribute to the fruity character that can be sensed and tasted promptly[21].

This phenolic amalgam reacts with proteins involved in gene expression and cell signalling, the anti-inflammatory transcription factors are suppressed by it, resulting in antioxidant activity against a variety of chronic inflammation-related diseases [10].

Through enzyme inhibitory activity [6] *Vitis vinifera* can treat non-communicable diseases like obesity, diabetes, cardiovascular disease, cancer, hypertension, reproductive problem, and neurological problem. Phytonutrients such as alkaloids, flavonoids, phenol, steroid, tannin, and terpenoids suppress obesity by inhibiting the pancreatic lipase, lipoprotein lipase glycerophosphate dehydrogenase [17,11,22]. Catechin, epicatechin, and quercetin are present in the grape seed and skin which treat diabetes with the help of the α-amylase

enzyme. Cancer can be nursed by cysteamine through inhibiting histone deacetylase [5]. Grape polyphenols stop the tyrosinase synthesis that has a positive effect on the pigmentary disorder as well as premature skin ageing [6,18]. The therapeutic aspect of Muscat grapes ranges from the supplementation industry to the cosmeceutical market paving the way for numerous research efforts focussing on the grape component.

2. MATERIALS AND METHODS

2.1 Sample Collection

Whole fresh grapes are collected as material from a home-grown grape plant at Ramalingam Colony as well as from Madhampatty grape farm, Coimbatore. Furthermore, samples were authenticated at Botanical Survey of India, Tamil Nadu Agricultural University (TNAU) Coimbatore, India (BSI/SRC/5/23/2022/Tech/491) and identified as *Vitis Vinifera* L. – VITACEAE, commonly known as Muscat grapes.

2.2 Sample preparation

Whole fresh grapes were washed thoroughly and categorized into three different forms of samples- whole fruit (W), fruit skin (P) and seed (S) and each group was divided into 50 raw materials. The skin and the seeds were peeled from the whole fruit to prepare the fruit skin (P) and seed (S) variety of samples. All three samples are pre-frozen at -20^{o}C for 24 h. For further process, samples were lyophilized using a freeze dryer. The frozen samples were freeze-dried under -45^{o}C temperature and a vacuum pressure of 0.010-0.012 for 7 days. Whole fruit took the longest time to dry than the other two samples as whole fruit had more water content than seed and skin. After lyophilisation, powdered samples were collected and stored in the glass vial, wrapped in an aluminium foil. This type of drying method guaranteed the retention of phytonutrients as a functional food [19].

2.3 Ultrasonic Homogenizer and FT-IR

To analyze the antioxidant activity of different forms of Muscat grapes, 5gm of each freeze-dried sample were diluted in aqueous form with 20 ml distilled water and were subjected to Ultrasonic Homogenizer (40 kHz) for 15 mins[20]. Obtained extracts were filtered through analytical filter paper.

Fourier-transformation infrared spectroscopy (FT-IR) analysis was made with the extracted sample in the area 3600nm and mid-infrared spectral in the area1650-1600nm using Bharat Ratna Prof . C.N.R. Rao Research Centre laboratory. Obtained data from this area acknowledge the presence of the functional groups [2].

2.4 Antioxidant activity using DPPH radical (2,2-diphenyl -1-picryl-hydrazyl-hydrate)

All three samples were tested for free radical scavenging activity using the DPPH assay [14, 16]. Based on ascorbic acid as a standard, aqueous extracts [1] were assessed for antioxidant activity. The absorbance of the combination and blank was measured at 517nm at ambient temperature. The DPPH radical's scavenging effect was shown as a percentage inhibition against the concentration in the sample [13, 8].

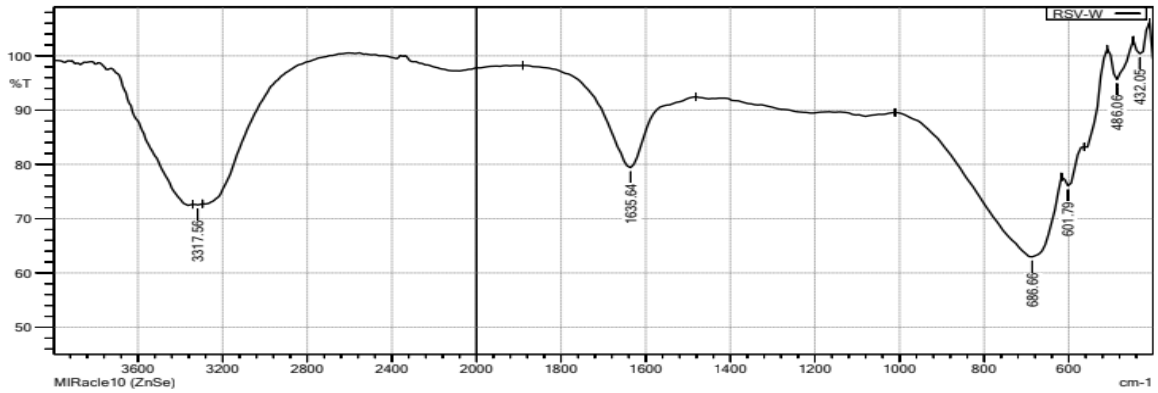
2.5 Statistical analysis

Each experiment value was calculated at t-Test and compared at correlation test in IBM SPSS statistics version 28.

3. RESULTS

3.1. FT-IR for functional group

The functional groups that may confirm the existence of polyphenolic substances can be identified using the FT-IR spectra in the homogenized extract: OH $_{alcoholic/phenolic}$(3400-3200 cm-1), C=C$_{aromatic}$(1650-1600cm-1), C-H$_{aromatic}$(700-420cm-1).



The wavenumbers (cm-1) of peaks identified in the sample extract-

Fig: 1 Lyofilized and homogenised Whole fruit(W) sample shows major peak specifically at 3317.56 cm-1 regions as the presence of the phenolic-OH group, at 1635.64 region shows another major peak which denotes the presence of C=O strength and lastly from 686.66cm-1 to 432.05 cm-1 region shows multiple peaks which considered as secondary structure composition of the molecule.

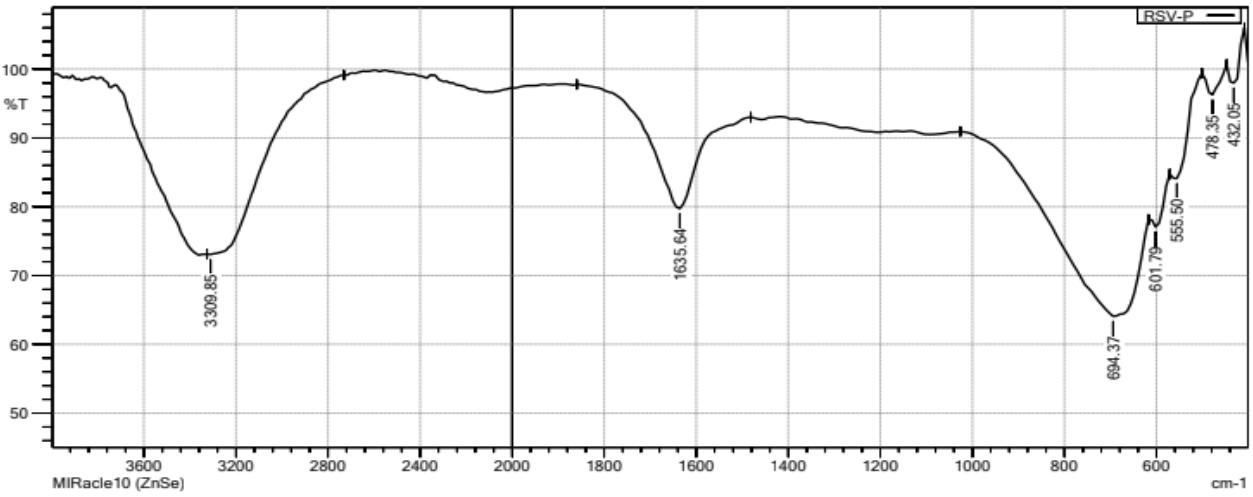


Fig: 2 Lyophilized and homogenised Fruit skin(P) sample shows major peak specifically at 3309.85cm-1 region as the presence of the phenolic-OH group, at 1635.64 region shows another major peak which denotes the presence of C=O strength and lastly from 694.37cm-1 to 432.05 cm-1 the region shows multiple peaks which were considered as secondary structure composition of the molecule.

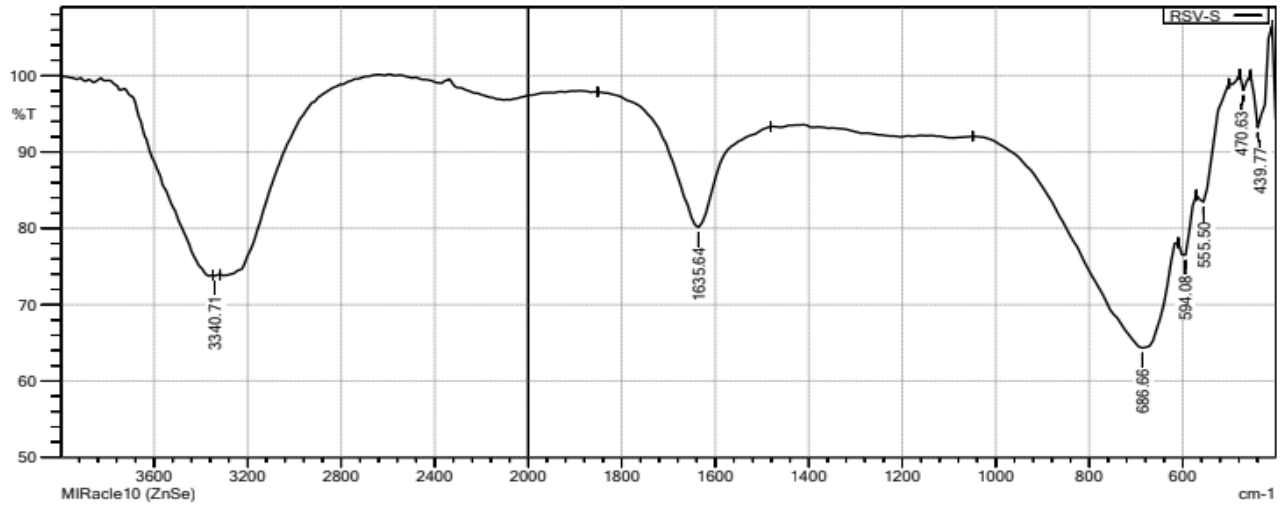


Fig: 3 Lyophilized and homogenised Seed(S) sample show major peak specifically at 3340.71cm-1 region as the presence of the phenolic-OH group, at 1635.64 region shows another major peak which denotes the presence of C=O strength and lastly from 686.66cm-1 to 439.77 cm-1 region shows multiple peaks which considered as secondary structure composition of the molecule.

Recorded data were interpreted as the presence of flavonoids and non-flavonoid content such as resveratrol, tannin acid, catechin, hydroxycinnamic acid, and gallic acid[9,15].

3.2 Antioxidant activity (DPPH method)

Aqueous extract of whole fruit (W), fruit skin (P) and seed (S) was plotted for antioxidant activity by the DPPH method against percentage inhibition. The percentage of extract scavenging activity is shown in Table 1.

Table 1: DPPH free radical scavenging activity of Whole fruit (W), Fruit skin (P) & Seed(S)

Serial no.	Concentration (µg/ml)	Whole fruit (W) (%)	Fruit skin (P) (%)	Seed(S) (%)
1.	10	61.74	76.52	95.65
2.	**50**	80.87	**96.52**	93.04
3.	150	89.57	85.22	88.70
4.	250	86.09	76.52	82.61
5.	350	80.87	65.22	80.00
6.	500	74.78	54.78	75.65
7.	**750**	65.22	**47.83**	65.22

In a concentration-dependent manner, the DPPH radicals were scavenged and showed 47.83% inhibition at the highest concentration of 750 µg/ml which is the lowest value and 96.52% inhibition at the concentration of 50 µg/ml which is the highest value Table 1 depicts that both the percentage values were from fruit skin (P) sample.

Fig 4: IC_{50} Value for *Vitis vinifera* L.

The standard value of IC_{50} was 5.68 and the values for whole fruit (W), fruit skin (P), and seed(S) IC_{50} was 4.8, 4.36, and 2.8114 respectively.

3.4 Statistical analysis

Peak wavenumbers of FT-IR value and IC_{50} value of antioxidant assay were compared using Pearson correlation and t-test. The P-value of correlation with antioxidant activity and the functional group is 0.289 and a comparison of functional group value evaluation reveals a significant result value is lower than 0.05.

4. DISCUSSION

4.1 FT-IR for functional group

In the infrared spectrum, X-axis denotes the intensity and peaks are called the absorbance band. Y-axis is noted as absorbance or frequency. The sum of infrared light absorbance or transmitted by the substance being analysed. The absorbance bands are grouped into two groups-group frequency and fingerprint frequency. Group frequency is the characteristics of minor groups of atoms or functional groups such as OH and C=C, CH_2. These are ordinarily seen above 1500 cm-1 in the infrared spectrum. They are conventionally unique to a specific functional group making them a reliable means of identifying the functional group in a molecule. Fingerprint frequency, these are highly characteristic of a molecule as a whole. These types of absorbance are typically sen below 1500 cm-1 in the infrared spectrum, some functional groups will result in this region of the spectrum being reliable for identification but the absence of a band is often more indicative than the presence of a band in this region.

Biochemical composition namely carbohydrates, lipid, especially secondary structure of protein is reflected under this wavenumber [12].

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical tool based on a high resolution that offers a speedy and non-destructive investigation of chemical constituents and describes the structural compound. Lyophilized and homogenized aqueous sample of Muscat grape exposes (Fig 1, Fig 2, Fig 3) the positive results of the presence of a cis and transform

of resveratrol and anthocyanins. maldivin, flavonoids like s kaempferol, myricetin, and quercetin as the peaks were from 3400-3200cm-1 region. Gallic acid, chlorogenic acid, terpenes, and terpenoids confirm their presence as the peaks shown in the 1600 cm-1 region and below.

4.2 Antioxidant activity (DPPH method)

The ability of hydrogen transfer to a radical determined the usage of DPPH radical solution in the experiment using an aqueous extract of *Vitis vinifera* sample [23,7]. A rapid change in colour, from pale yellow to colourless, proves the quality of scavenging capacity in the presence of aqueous extract. As Table 1 depicted whole fruit (W), Fruit skin (P) and seed(S) scavenged 61.74%, 76.52% and 95.65% inhibition at a concentration of 10 µg/ml that can be standardized with ascorbic acid (90.43%). It may be interpreted that seed extract exhibits the highest scavenging capacity among other examined extracts.

IC_{50} values constitute the efficacy to accomplish its 50% scavenging quality where IC_{50} value and antioxidant activity were inversely proportionate. According to Blois (1958) [4] and Fidrianny *et al*., (2015) [8], the lower the IC_{50} value, the higher the antioxidant activity. Hence, its exhibited that the presence of antioxidant properties in grape seed is maximum whereas whole fruit contains minimum phytonutrient properties (Fig 4).

Depending on the experimental values, it is confirmed that there is negative relationship between antioxidant activity and phyotnutrient presence. Phytonutrients are the organic components produced by the the plants and antioxidants are plant chemicals which protect the body from free radicals. Not all phytonutrients act like an antioxidant and it is not as essential as antioxidant, whereas antioxidants activity of a food item provide the electron-scavenging elements to body mechanism.

5. CONCLUSION

The present study concluded that the antioxidant activity ($p=0.289$) and significant amount ($p=<0.05$) of phytonutrients present in Muscat grapes but there is no relationship between phytonutrients and antioxidant activity as the sample extract is aqueous in nature and depending on the values of antioxidant activity it is advisable that small amount of grape seed consumption is good for health. Further pre-clinical and clinical studies need to be performed as the consumable exact amount of dose need to be confirmed through experiment. Individual extraction of the phenolic compound from *Vitis vinifera* exhibits good results in the pharmaceutical industry. The inclusion of this food into the daily diet is very much beneficial as it acts as a functional food as well as a fibre component. This aromatic food can entice a positive change in the sensory part of the body.

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