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## PROXIMATE ANALYSIS OF FLAVONOIDS AND ANTIOXIDANTS IN *PHYLLANTHUS EMBLICA* AND *VITIS VINIFERA* PULP

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### ABSTRACT

Plants and their secondary metabolites have stimulated the development of innovative medications as well as a return to the use of herbal therapies for a variety of diseases. In the present study, an attempt has been made to identify the active ingredients present in *Phyllanthus emblica* pulp and *Vitis vinifera* pulp. The pulp of these plants was extracted by different solvents and subjected to phytochemical screening to assess the best extraction medium. Both the extracts were analyzed qualitatively and quantitatively for the presence of phenolics and flavonoids. The antioxidants in the fresh pulp of these plants were analyzed to determine the total antioxidant capacity of these plants. Finally, spectral studies were carried out to find the presence and structure of flavonoids in the *Phyllanthus emblica* and *Vitis vinifera* pulp juices. The antioxidant analysis of *Phyllanthus emblica* and *Vitis vinifera* pulp show near equal levels of antioxidant activity when compared with standard ascorbic acid. Quercetin, one of the flavonoids present in the extracts was identified by HPLC and the same has been confirmed by spectral studies. To summarize, the selected fruit pulps showed very notable biochemical flavonoids that are both beneficial and economical.

**Keywords: Flavonoids, Quercetin, *Phyllanthus emblica*, *Vitis vinifera*, HPLC, Spectral Studies**

## INTRODUCTION

Over millennia ago, natural products of some particular plants were utilized to cure various illnesses. Ancient India, China and Egypt all employed terrestrial plants as medicines and a staggering number of contemporary medications have been derived from them. There is a belief in the development of novel drugs that might be suitable for treating infections and their related pathological problems based on scientific analysis of important medicinal plant components utilized in traditional medicine [1, 2].

Plants generate a vast array of organic compounds, the majority of which don't appear to be directly related to growth and development. These chemicals, which are commonly referred to as secondary metabolites, are frequently distributed among specific taxonomic groups in the kingdom of plants. Terpenoids, phenolics, alkaloids, and flavonoids are some of the classes into which the secondary metabolites are divided [3].

Flavonoids, a significant family of natural products are secondary metabolites of plants with a polyphenolic structure that are commonly present in fruits and vegetables. Many flavonoids have evolved as bioactive molecules that interfere with nucleic acids or proteins and have antibacterial, insecticidal, and pharmacological activities. Flavonoids

perform a variety of defensive activities in the human body [4].

### *Phyllanthus emblica*

*Phyllanthus emblica*, sometimes referred to as Indian gooseberry or amla, is a fruit with six vertical stripes that are almost spherical, pale greenish-yellow in color, smooth and hard to the touch. Indian amla has a tart, bitter and astringent flavor. It also has high fiber content.

### Scientific Classification of *Phyllanthus emblica*

Kingdom - Plantae

Clade - Angiosperms

Order - Malpighiales

Family - Phyllanthaceae

Genus - *Phyllanthus*

Species - *P.emblica*



Figure 1: *Phyllanthus emblica* fruits

*Phyllanthus emblica* is a key ingredient in several Ayurvedic treatments, including Chyawanprash, a general tonic for people of all ages that improves entire physical and mental well-being, and Triphala. Ayurvedic medicine has historically utilized *Phyllanthus emblica* to treat fever, diarrhea, wounds, inflammation, and skin ulcers.

Antidiabetic, hypolipidemic, antibacterial, antioxidant, antiulcerogenic, hepatoprotective, gastroprotective, and chemopreventive activities are found in many plant sections. Some of the anticancer action of *Phyllanthus emblica* can be attributed to its antioxidant effect [5].

All parts of this plant are utilized medicinally, particularly the fruit, which has been used in Ayurveda as a strong rasayana and in traditional medicine to treat diarrhea, jaundice, and inflammation. Every 100 g of *P. emblica* fruit contains a remarkable amount of vitamin C ranging from 600 to 1,300 mg. The most abundant amino acids are glutamic acid (29.6%), proline (14.6%), aspartate (8.1%), alanine (5.4%), and lysine (5.3%) [6].

### *Vitis vinifera*

The common grape, *Vitis vinifera*, is a species of *Vitis*, one of the world's greatest fruit crops, with an estimated annual yield of 58 million metric tons. The grape is one such fruit that is consumed globally and can be eaten raw or in processed goods such as wine, juice, and sauce. Grapes are a good natural source of antioxidants since they contain several phytochemicals such as anthocyanin, catechin, epicatechin, resveratrol and proanthocyanidin, which have great antioxidant activity. Furthermore, these polyphenols have anticarcinogenic, anti-inflammatory, and antiproliferative effects [7].

### Scientific Classification of *Vitis vinifera*

Kingdom-Plantae

Clade- Angiosperms

Order - Vitales

Family-Vitaceae

Genus - *Vitis*

Species - *V. vinifera*



Figure

#### 2: *Vitis vinifera* fruits

The skin, pulp and seeds of *Vitis vinifera* contain the majority of the phenolic antioxidants. Grape skins and pulp include resveratrol, quercetin, anthocyanin and catechins, while the seeds are high in procyranidins. Notably, grape seeds contain roughly 20 times more proanthocyanidins than grape skins. The purple hues of grapes and red wines are ascribed to anthocyanins, which appear to be strongly linked to antioxidant capabilities [8].

In the present study, an attempt has been made to identify the active ingredients present in the *Phyllanthus emblica* pulp and *Vitis vinifera* pulp. The pulp of these plants was extracted by different solvents and subjected to phytochemical screening to assess the best extraction medium. The antioxidants in the fresh pulp of these plants

were analyzed to determine the total antioxidant capacity of these plants. Spectral studies were carried out to analyze the presence and structure of flavonoids in the *Phyllanthus emblica* and *Vitis vinifera* pulp juice.

## MATERIALS AND METHODS

### Plant source

The fruits of *Phyllanthus emblica* and *Vitis vinifera* were collected from the local market, Kanchipuram. The pulp of the fruits was separated and dried for 10-12 days in the shadow. Then the dried pulp was ground to powder using an electrical grinder and it was kept in an airtight container for further use.

### Preparation of solvent extracts

5gms of pulp powder was taken and packed between folds of filter paper and placed in a Soxhlet apparatus, run between 60-80°C using ethanol and chloroform as solvent.

### Qualitative Analysis of Phytochemicals

The qualitative analysis of phytochemicals was carried out on the pulp extracts using standard procedures to identify the constituents as described by Harborne (1973) [9].

### Quantitative Analysis

100 mg of the pulp tissue was weighed, uniformly homogenized with 1.0 ml of 0.5 M phosphate buffer of pH 6.9, and the homogenate was used for the following biochemical assays.

To determine the antioxidant potential and the respective principle compounds, the level of ascorbic acid [10], Tocopherol [11], Catalase [12], Superoxide Dismutase [13], Flavonoids [14], Total Phenol Compounds [14] and DPPH assay [15] were performed as per the standard proven methods.

### HPLC analysis of *Phyllanthus emblica* and *Vitis vinifera*

Chromatographic analysis by HPLC was carried out by Kromasil 100 C18v reversed-phase column (250 x 4.6mm) packed with 5µm diameter particles. The mobile phase was methanol: water: acetic acid (7:3:0.1 v/v/v). The mobile phase was filtered through a 0.45 µm membrane filter. Then deuterated ultrasonically before use. Flow rate and injection volume were 1 ml /min and 10 µl. The Chromatographic peaks of the analytes were confirmed by comparing their retention time and UV Spectra with those of the Quercetin. All chromatographic operations were carried out at ambient temperature.

About 5 mg of the given Quercetin sample was accurately weighed and taken into 10 ml volumetric flasks and dissolved in 10 ml mobile phase. To prepare 0.5 mg/ml sample solution, the volumetric flask contains standard solution kept for sonication for 10 mins. The standard solution was filtered with 0.45 µm membrane filter paper with a sample filter and analyzed by HPLC column.

### UV Spectrophotometry Analysis

The *Phyllanthus emblica* and *Vitis vinifera* fresh pulp juice were extracted and analyzed by UV spectrophotometer at the wavelength range between 200 and 500nm. The standard quercetin was also analyzed by UV spectrophotometer at the wavelength range between 200 and 500nm

### FT- IR Analysis

The *Phyllanthus emblica* and *Vitis vinifera* pulp were oven-dried at 60°C and ground into a fine powder using a mortar and pestle. Two milligrams of the sample were mixed with 100mg KBr (FT-IR grade) and then compressed to prepare a salt disc (3mm diameter). The disc was immediately kept in the sample holder and FT-IR spectra were recorded in the wavenumber range between 500 and 3500cm<sup>-1</sup>.

## RESULTS AND DISCUSSION

The study of phytochemicals in medicinal plants is a highly helpful step that may be applied to the creation of new drugs. Previous research has demonstrated that the types of solvents employed during the extraction process are critical to the efficient extraction of phytochemical components. The phytochemical composition of the ethanol and chloroform extracts from *Phyllanthus emblica* and *Vitis vinifera* are depicted in **Tables 1 and 2**. Alkaloids, flavonoids, protein, carbohydrates, polyphenols, glycosides and terpenoids are among the phytochemical elements found in

the pulp of *Phyllanthus emblica* and *Vitis vinifera* as demonstrated by the ethanol and chloroform extracts.

Qualitative analysis of plant extract is very important to find out the presence of phytochemicals. Numerous types of bioactive compounds have been isolated from plant sources. The results of the phytochemical screening suggested that both extracts possess a very good store of polyphenols, flavonoids, alkaloids and saponins. Because of the existence of these phytochemicals, which have been shown to have significant therapeutic potential, the majority of plants are utilized to cure illnesses [16].

The amounts of flavonoids and phenolic compounds in *Phyllanthus emblica* and *Vitis vinifera* pulp are shown in **Table 3**. The results demonstrate that *Phyllanthus emblica* and *Vitis vinifera* pulp contain significant amounts of flavonoids and phenolic chemicals. Flavonoids are classified as flavones, flavanones, isoflavones, flavanols, chalcones, and anthocyanins based on their chemical structure, degree of unsaturation, and oxidation of the carbon ring. Each of these flavonoids is abundant in nature. Flavonoids are phytonutrients that belong to the polyphenol class and have long been employed in Ayurvedic treatment [17].

*Vitis vinifera* is one of the most widely planted fruit crops worldwide, and its

composition and qualities have been thoroughly studied, with multiple findings indicating the presence of high levels of phenolic compounds. These compounds have been linked to a wide range of health advantages in humans due to their bioactive qualities, including anti-inflammatory, anticancer, anti-aging, cardioprotective, neuroprotective, immunomodulatory, antidiabetic, antibacterial, antiparasitic, and antiviral effects [18].

Flavonoids belong to a class of low-molecular-weight phenolic compounds and are found in several parts of the plant. Flavonoids have been linked to a wide range of health-promoting properties and are an essential component in a wide range of nutraceutical, pharmacological, therapeutic and cosmetic applications [19]. This is due to their ability to control important cellular enzyme functions as well as their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic capabilities [20].

**Table 4** shows the levels and activities of antioxidants in *Phyllanthus emblica* and *Vitis vinifera* fruits. The results show significant levels and activities of antioxidants in *Phyllanthus emblica* and *Vitis vinifera* pulp tissue. Herbal extracts of amla and grapes exhibit a protective mechanism against oxidative stress by enhancing antioxidant enzyme activities. Numerous clinical disorders have been

related to the pathophysiology of oxidative stress caused by free radicals, with the majority of these illnesses stemming from a deficiency of natural antioxidant defenses. Non-enzymatic antioxidant defense mechanisms are the second line of protection against damage caused by free radicals [21].

Free radical scavenging potentials of *Phyllanthus emblica* and *Vitis vinifera* pulp extracts at different concentrations were tested by the DPPH method, and the results are shown in **Figures 3 and 4**. It is well understood that free radicals cause the autoxidation of unsaturated lipids. Conversely, antioxidants are believed to halt the free radical cycle of oxidation and transfer hydrogen from the phenolic hydroxyl groups, producing a stable end product that prevents the start of or spread of further lipid oxidation [22].

The findings demonstrate that *Phyllanthus emblica* and *Vitis vinifera* pulp extracts are primary antioxidants that react with free radicals and act as free radical scavengers. Results in a variety of lipid systems indicate that epicatechin, myricetin, quercetin, and catechin require dihydroxylation in both rings and the 3-position for antioxidant effect [23]. Antioxidants including mallonin, isomallotusin, isostrictinin and mallotusin have been reported to be present in *Phyllanthus emblica*, along with

phyllembilin, cinnamic acid, and chebulagic acid [24].

The HPLC chromatogram of quercetin, *Phyllanthus emblica* and *Vitis vinifera* at 254nm are given in **Figures 5, 6 and 7**. The results show that the characteristic retention time of the peaks in the HPLC chromatogram of quercetin include 1.522min, 2.02min and 2.33min. The characteristic retention time of the peaks in the HPLC chromatogram of *Phyllanthus emblica* at 2.026min and 2.383min reveal the presence of quercetin in this plant pulp. The characteristic retention time of the peaks in the HPLC chromatogram of *Vitis vinifera* at 1.520min, 2.062 and 2.312min reveal the presence of quercetin in this plant pulp. The other characteristic retention time of the peaks in the HPLC chromatogram of *Vitis vinifera* at 1.419min, 1.788min, 2.426min, and 2.753min are due to the presence of other flavonoids like naringin, hesperidin, sinensetin and resveratrol.

Flavonoids, notably quercetin, have been demonstrated to reduce the beginning process of inflammation and improve the immune system [25]. To prevent excessive NO generation in macrophage cells, *Phyllanthus emblica* suppressed the synthesis of NO as well as key inflammatory enzymes, including COX-1, COX-2, and 5-LOX [26].

The UV spectra of quercetin, *Phyllanthus emblica* and *Vitis vinifera* are given in

**Figures 8, 9, and 10**. Strong absorption peaks are obtained between 200nm and 230nm, 252nm and 268nm, and 360nm, and 390nm for standard quercetin. Strong absorption peaks are obtained between 201nm and 322nm for *Phyllanthus emblica*. *Vitis vinifera* exhibits strong absorption peaks between 201.5 nm and 299 nm. The results reveal that the pulp juice of *Phyllanthus emblica* and *Vitis vinifera* contain flavonoids, including quercetin, as indicated by the prominent absorption peaks in their UV spectra.

The UV spectra of flavones and related glycosides show two strong absorption peaks commonly referred to as band I (300-380 nm) and band II (240-280 nm) [27]. It has also been reported that flavanones generally show a strong absorption peak in the range of 270-295 [28]. Flavonoids like quercetin, resveratrol and catechins have been considered as potential compounds for the treatment of atherosclerosis, a dangerous condition brought on by plaque accumulation in the arteries. Blocked blood flow in the arteries is a major risk factor for heart attacks and strokes, and it is less likely in those who eat a diet rich in flavonoids [29].

The FT-IR spectrum of *Phyllanthus emblica* and *Vitis vinifera* are given in **Figures 11 and 12**. The characteristic absorption bands corresponding to the vibration frequencies of  $1636\text{ cm}^{-1}$  and  $1638\text{ cm}^{-1}$  in the IR spectra

are due to the carbonyl groups of flavonoids. The characteristic absorption bands corresponding to the vibration frequencies of 2105 cm<sup>-1</sup> and 2110 cm<sup>-1</sup> in the IR spectra are due to the CH stretching of aromatic rings of flavonoids. The characteristic absorption bands corresponding to the vibration frequencies of 3320 cm<sup>-1</sup> and 3328 cm<sup>-1</sup> are due to the OH groups of flavonoids present in the *Phyllanthus emblica* and *Vitis vinifera* pulp.

The functional groups identified in the *Phyllanthus emblica* and *Vitis vinifera* are tabulated in **Tables 4 and 5**. The vibration frequencies obtained by FT-IR analysis corresponding to the –OH group, C=O group and C-H stretch indicate the presence of phenolic compounds, flavonoids and other phytochemicals in the extracts [30].

**Table 1: Qualitative Analysis of The Phytochemicals of Ethanol and Chloroform Extracts of *Phyllanthus Emblica***

S.no	Phytochemicals	Ethanol Extract	Chloroform Extract
1	Alkaloids	-	+
2	Flavonoids	+	+
3	Carbohydrates	+	-
4	Glycosides	-	-
5	Polyphenols	+	+
6	Tannins	+	-
7	Saponin	+	-
8	Terpenoids	+	-
9	Protein	+	-
10	Amino acids	+	+
11	Anthraquinone	-	+

**Table 2: Qualitative Analysis of The Phytochemicals of Ethanol and Chloroform Extracts of *Vitis Vinifera***

S.no	Phytochemicals	Ethanol Extract	Chloroform Extract
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Carbohydrates	+	+
4	Glycosides	-	+
5	Polyphenols	+	-
6	Tannins	-	+
7	Saponin	+	+
8	Terpenoids	+	-
9	Protein	+	-
10	Amino acids	+	+
11	Anthraquinone	+	-

**Table 3: The Levels of Flavonoid and Phenolic Compounds in The *Phyllanthus Emblica* and *Vitis Vinifera* Pulp Tissue**

S.NO	Plant Name	Flavonoid (µg/gm of tissue)	Phenolic Compounds (µg/gm of tissue)
1.	<i>Phyllanthus emblica</i>	79.4	92.7
2.	<i>Vitis venifera</i>	83.3	85

**Table 4: The Levels and Activities of Antioxidants in The *Phyllanthus Emblica* Pulp and *Vitis Vinifera* Pulp Tissue**

S. No.	PARAMETERS	PHYLLANTHUS EMBLICA PULP	VITIS VINIFERA PULP
1	Ascorbic acid (mg/gm of tissue)	124	74
2	Tocopherol (mg/ gm of tissue)	16	12
3	Superoxide Dismutase (µg of pyrogallol auto-oxidation inhibition/min)	0.54	0.33
4	Catalase (µg of hydrogen peroxide utilized / min)	102	136

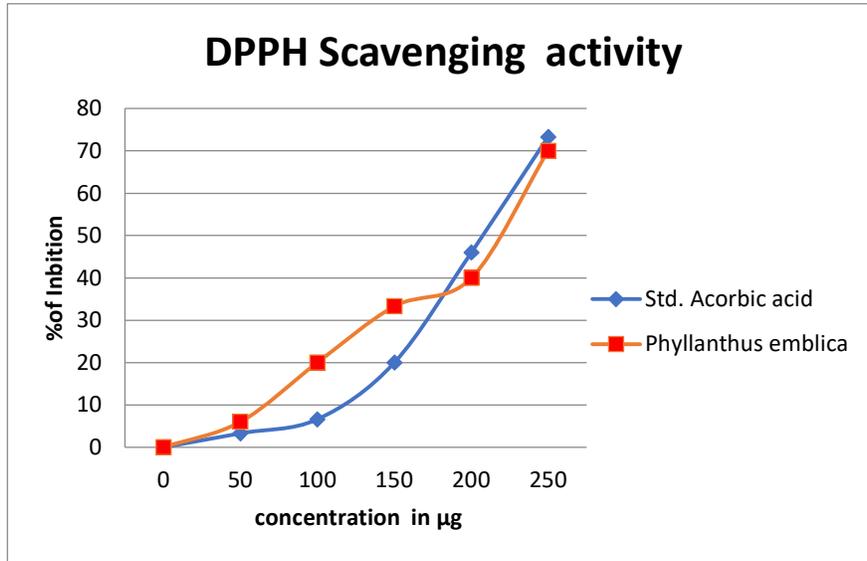


Figure 3: DPPH scavenging activity of *Phyllanthus emblica pulp*

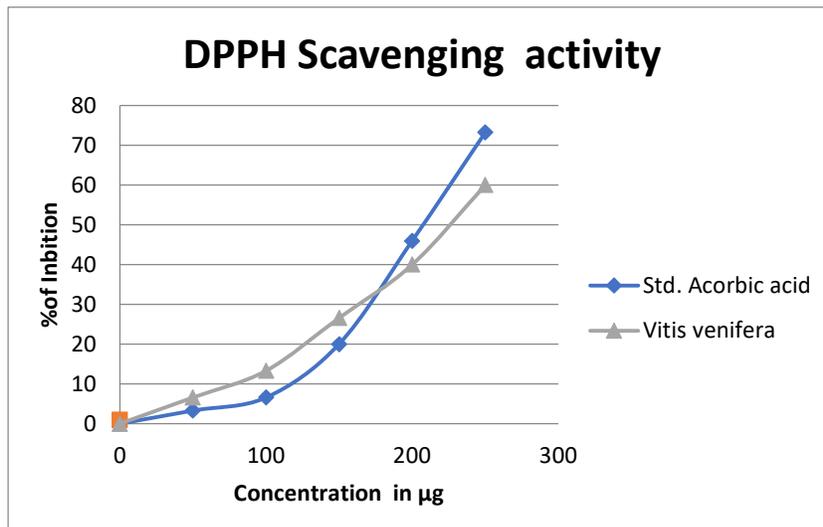


Figure 4: DPPH scavenging activity of *Vitis vinifera pulp*

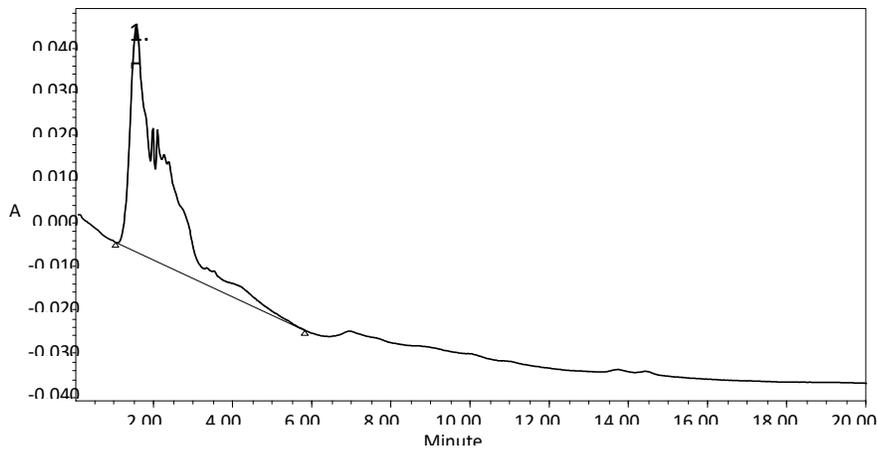


Figure 5: HPLC Chromatogram of Quercetin At 254nm

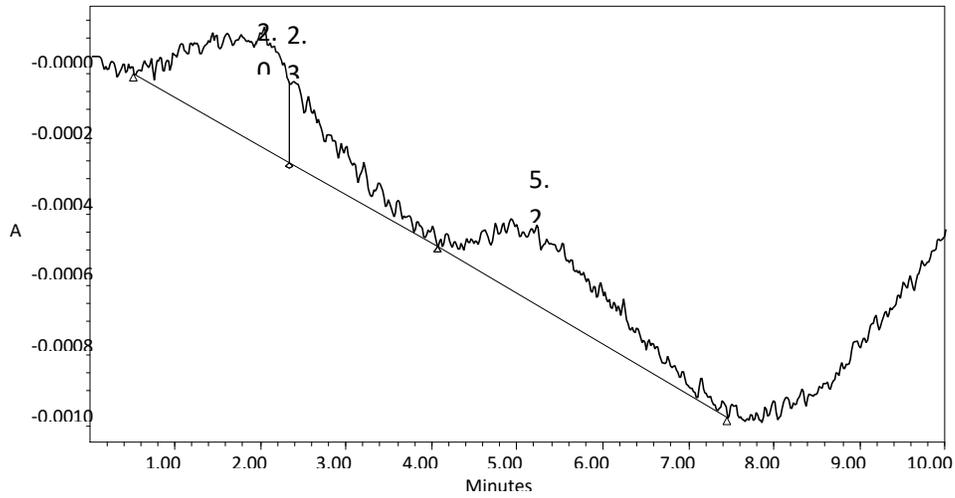


Figure 6: HPLC Chromatogram of *Phyllanthus Emblica* Pulp Juice at 254nm

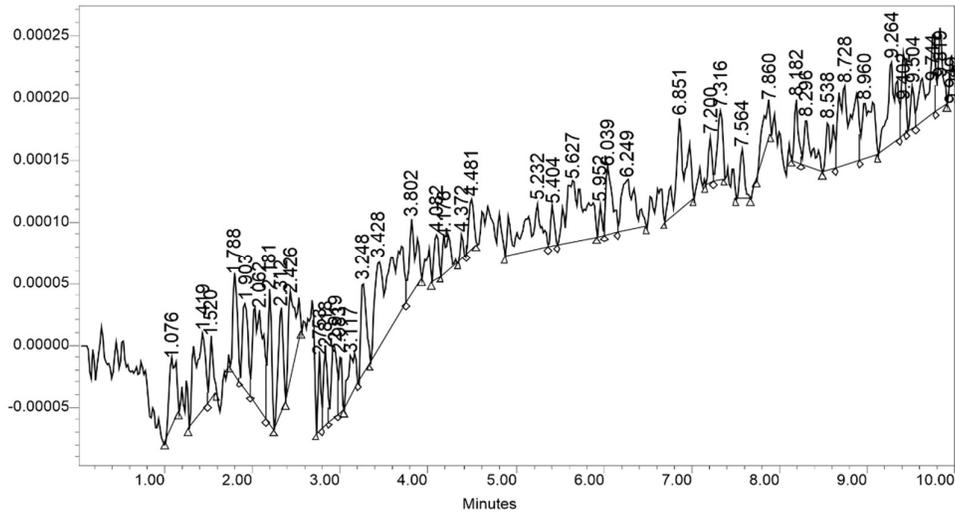


Figure 7: HPLC Chromatogram of *Vitis Vinifera* Pulp Juice at 254nm

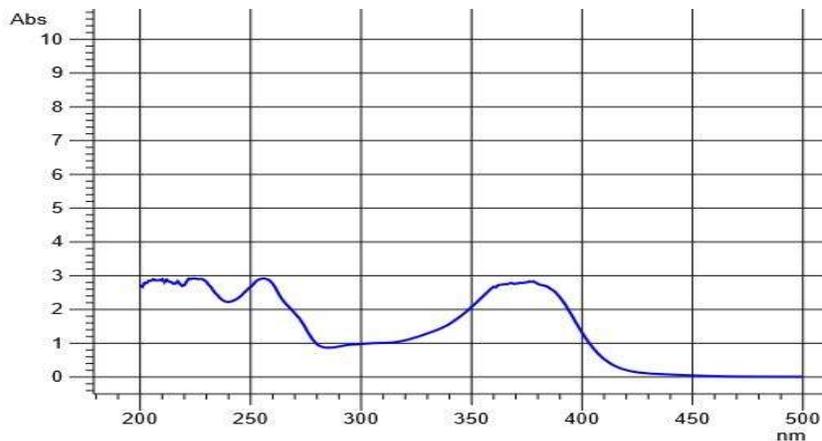


Figure 8: UV Spectra of Quercetin

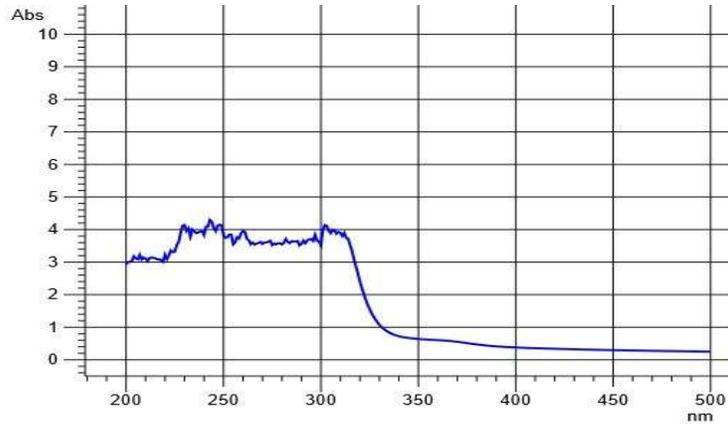


Figure 9: UV Spectra of *Phyllanthus Emblica* Pulp Juice

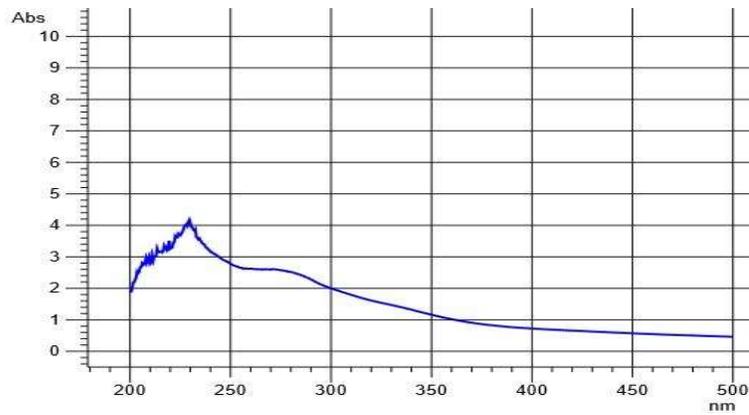


Figure 10: UV Spectra of *Vitis Vinifera* Pulp Juice

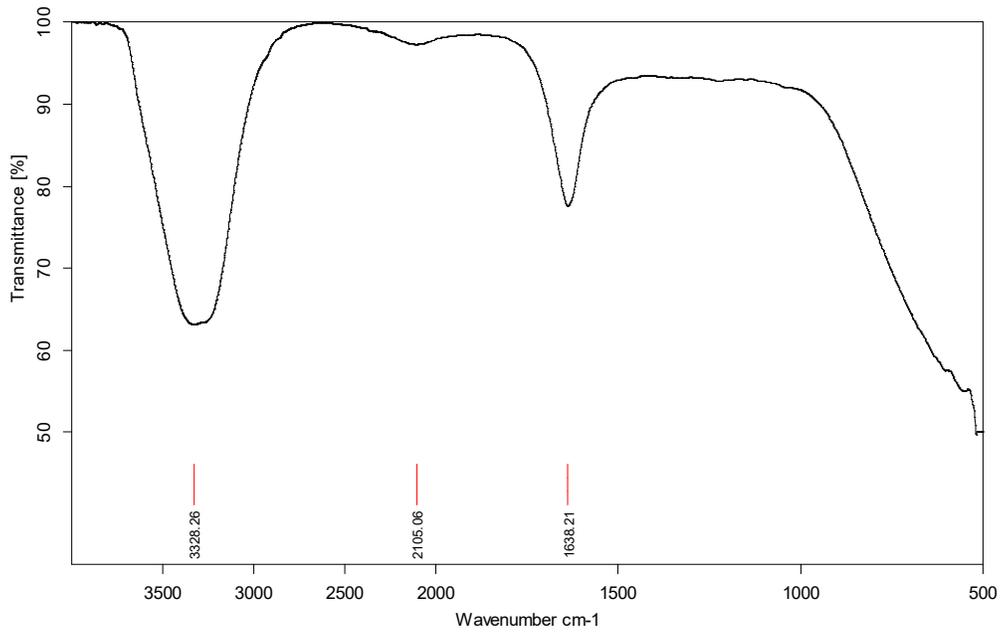


Figure 11: FT-IR Spectrum of *Phyllanthus Emblica* Pulp Juice

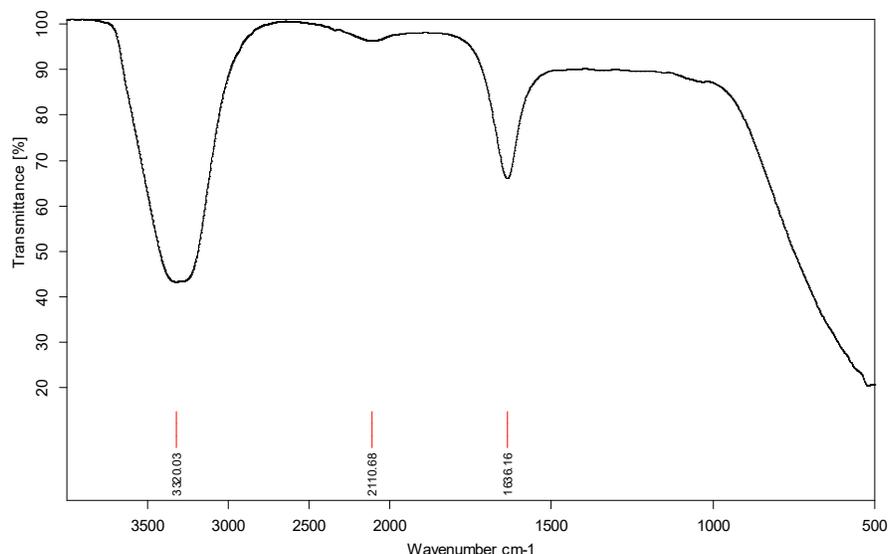


Figure 12: FT-IR Spectrum of *Vitis Vinifera* Pulp Juice

Table 4: FTIR Spectral Peak Values and Functional Groups of *Phyllanthus emblica*

Peak values	Functional groups
3328.26	OH group
2105.06	CH stretching
1638.21	C=O carbonyl group

Table 5: FTIR Spectral Peak Values and Functional Groups of *Vitis vinifera*

Peak values	Functional groups
3320.03	OH group
2110.68	CH stretching
1636.16	C=O carbonyl group

It has been shown that the flavonoids like Quercetin 3-b-D-glucopyranoside, kaempferol 3-b-D-glucopyranoside, quercetin and kaempferol extracted from *P. emblica* have antioxidant properties. Flavonoids of the quercetin class (Quercetin and quercetin 3-b-D-glucopyranoside) had stronger antioxidant activity than kaempferol and its derivative (kaempferol 3-

b-D-glucopyranoside) [31]. Flavonoids, according to their structure, have a variety of preventive actions against cancer, diabetes, cardiovascular disease and neurological illnesses. By complexing with oxidizing species, hydroxyl groups in flavonoids give these chemicals the capacity to scavenge and stabilize free radicals, lowering oxidative

damage, which is a hallmark of various chronic illnesses [32].

## CONCLUSION

For the treatment of many diseases, natural products derived from medicinal plants have been used for long period. There are more than 270,000 higher plants in earth. So far phytochemical research has only been done on a tiny percentage of it. The structure and existence of significant flavonoids in *Phyllanthus emblica* and *Vitis vinifera* were uncovered by the current spectral analysis. The results of this investigation show that *Vitis vinifera* and *Phyllanthus emblica* have strong antioxidant properties that protect against free radical damage. Quercetin, gallic acid, ellagic acid, polyphenols and other flavonoids are responsible for the antioxidant properties of *Phyllanthus emblica* and *Vitis vinifera*. Over the past ten years, flavonoids and phenolic compounds have gained a lot of interest in the literature and numerous possible positive effects have been clarified. More research is necessary to improve the benefits of flavonoids in the diet for better human health.

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