

ORIGINAL ARTICLE

Phytochemical Screening of Anti-Alzheimer's Compounds in the
Ethanolic Extract of Red Grape Seeds (*Vitis vinifera*.L)

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ABSTRACT

As per the available literature, Phytochemicals are organic chemical elements that exist naturally in plants. Red grape (*Vitis vinifera*.L) Seed Extract, by virtue of having several such qualities confers protection to animals including human beings. After screening, it was evident that the Ethanolic extract of Red Grape Seed showed the presence of polyphenolic compounds such as Flavonoids, isoflavonoids, Anthocyanidins, Phytoestrogens, Terpenoids, Carotenoids, Limonoids, Phytosterols, Glucosinolates Alkaloids, Glycosides. These phytochemicals, synthesized through a variety of metabolic processes in plants play a key role in not only defending the plants against environmental dangers including stress, drought, pathogenic attack etc. but also constitute an important source of alternative medical system to treat a number of diseases and disorders which were not cured by allopathic medicines.

Keywords: (RGSE), *Phytochemicals, *Polyphenols, *Flavonoids, *Human Health

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INTRODUCTION

With the aim of defending themselves from illnesses, plants produce phytochemicals, which have antibacterial properties. These anti-oxidants help protect cells from free radical damage and also promote health. They are used as a component of traditional medicine to treat various metabolic, immune and neurological diseases in people. Red Grape Seed Extract (RGSE), being rich in polyphenols acts as a potential antioxidant compound available than Vitamin C, Vitamin E and Beta-Carotene. As its name implies, Red Grape Seed Extract (RGSE) is derived from the small seeds of Red Grapes - the same kind that are pressed to make wine, extensively in Europe, which are believed to prevent and control numerous ailments by safeguarding cells against the ravages of unstable oxygen molecules called "Free Radicals". The most valuable flavonoids in Red Grape Seed Extract (RGSE) are "Procyanidolic Oligomers" (also known as "Proanthocyanidins"), commonly called "OPCs". Beyond their antioxidant powers, OPCs are thought to improve blood circulation and help strengthen blood vessels. These actions benefit people with heart disease and cancer. Experts compare its health benefits to those of Pycnogenol and in fact, many research studies examining the therapeutic effects of OPCs have relied on the use of Red Grape Seed Extract (RGSE). According to the experts at "American Institute for Cancer Research", it was well established that people who consume mostly plant-based diets had much reduced incidence of heart disease and cancers thus providing ample evidence on the advantages of phytochemicals [1]. Since previous reports have shown that Grapes have the majority of the above mentioned bioactive phytochemical constituents of pharmaceutical value, it was selected for my present research study work. The Therapeutic potential of these compounds were well described in the results section, supported by the available supporting evidences in discussion part of this manuscript.

Table 1: Phytochemicals and their potential benefits (Source: Roswellpark.org)

Phytochemical	Foods	Potential benefits
Carotenoids (beta-carotene, lycopene)	Cooked tomatoes, Orange squash, Carrots, Sweet potatoes and green plants such as Broccoli	May inhibit cancer cell growth, reduce risk of cardio vascular disease and boost immunity
Flavonoids	Berries, Apples, Citrus fruits, Soybeans, Coffee, Tea, Walnuts, Whole grains	May fight inflammation, decrease damage to DNA and reduce tumor growth
Anthocyanins	Berries	May help lower blood pressure
Isothiocyanates	Cruciferous vegetables, such as Broccoli, Cauliflower and kale	May protect against cancer and cardiovascular disease
Lutein and zeaxanthin	Dark, leafy greens, such as Spinach and Chard	May promote eye health

Authenticated data shows that Phytochemicals may have the ability to:

Assist in the regulation of hormones

- Reduce the inflammatory response
- Aid the immune system's function
- Defend DNA and cells from damage that may lead to cancer
- Some cancer cells' growth rate is slowed.

Importance of Phytochemical screening

Extraction, screening and identification of medicinally active chemicals present in plants is referred to as phytochemical screening. A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [2]. As a result, phytochemical screening is used to isolate diverse plant ingredients in order to evaluate their biological activity or therapeutic potential. In this context, it is appropriate to mention that since the introduction of Ayurvedic system in India, Red grape seeds have been known as potential antioxidants in nature in addition to other qualities such as being anti-diabetic, anti-cholesterol, anti-platelet and anti-chronic non-communicable illnesses [3,4]. Therefore, it is necessary to screen Phenolic compounds, Antioxidants, Tannins, Alkaloids, Carotenoids and Flavonoids in Red Grape Seeds which are conferred with multiple health-promoting qualities [5].

Significance of Phytochemical screening

Phytochemical screening is a quantitative and qualitative analysis method for identifying bioactive chemicals that are unknown in plant extracts [6]. Phytochemical screening in medicinal plants can also be used to find active molecules that cause toxins, in addition to identifying active compounds that are beneficial to the body's health (positive effects of herbal remedies) (negative effects of herbal medicines). As a result, screening medicinal plants for phytochemicals is necessary before proceeding with additional research [7,8]. It is a stage of a phytochemical investigation that seeks to give a general overview of the class of compounds found in the plants being studied. This knowledge will be useful in the development of complicated active components of chemical compounds found in medicinal plants. The discovery of crude pharmaceuticals relies heavily on phytochemical elements found in plants. It is critical for finding new sources of therapeutic and industrially relevant substances such as: Alkaloids, Flavonoids, Phenolic compounds, Saponins, Steroids, Tannins and Terpenoids.

MATERIAL AND METHODS

Collection of Grapes :

From the Red Grapes (identified as *Vitis vinifera (Linn)*), purchased from a local Grocery Market in Tirupati, A.P., India, Seeds were collected, dried in shade for one week and were milled to a particle size of less than 0.4 mm.

Preparation of Extraction:

The powder thus made was macerated in 75 percent 'Ethanol' for 72 hours at room temperature. The Ethanolic extract was evaporated to remove all traces of ethanol and the resulting 'Red Grape Seed Extract' (RGSE) was collected as a lyophilized powder [9]. The crude Red Grape Seed Extract was subjected to various standard screening techniques to isolate and identify the presence of different types of bioactive compounds.

Picture showing the preparation of Red Grape Seed Extract



Qualitative Analysis of Phytochemicals :

In the present study, about 9 important bioactive components viz.: Alkaloids, Flavonoids, Phenols, Anthocyanins, Tannins, Terpenoids, Saponins, Glycosides and Steroids were identified in the preliminary Phytochemical screening of Red Grape Seed Extract by adopting the standard methods as given below:

Table 2: Methods Employed for the Qualitative analysis of Phytochemicals in RGSE

S.No.	Name of the Phytochemical	Test Method used
1.	Flavonoids	Khandewal, 2008
2.	Phenols	Gibbs, 1974
3.	Alkaloids	Gibbs, 1974
4.	Anthocyanins	Farnsworth, 1966
5.	Tannins	Treare and Evans, 1985
6.	Terpenoids	Ayoola <i>et al.</i> , 2008
7.	Saponins	Kumar <i>et al.</i> , 2009
8.	Glycosides	Khandewal, 2008
9.	Steroids	Gibbs, 1974

RESULTS

In the present study, since 2 compounds are significant, the Quantitative analysis of the Red Grape Seed Extract was done only on these 2 constituents. The results have demonstrated that among these 2, the Total Phenolic compounds were highest (364 mg Gallic Acid Equivalent (GAE) /g of dry Seed extract) when compared with the Total Flavonoids Content (12.86 mg Quercetin Extract (QE)/g of dry seed extract). The significance of these 2 compounds is discussed in the light of available literature.

Table 3. Total Phenolic and Total Flavonoids in Grape Seed Extracts

Sample	Total phenolic compounds(mg/g) a	Total flavonoids(mg/g) b
Red Grape Seed (Ethanol extract)	364 ±17.04	12.86 ±0.874

a: mg Gallic Acid Equivalent (GAE) /g of dry seed extract; **b:** mg Quercetin Extract (QE)/g of dry seed extract. Each value is expressed as the mean ± SD (n=3).

Qualitative Phytochemical Analysis:

Among the 9 compounds, identified by qualitative analysis, the amount of Flavonoids and Tannins was highest while that of Anthocyanins, Alkaloids, Phenols, Saponins, Glycosides and Steroids was medium and Terpenoids was least as indicated by the **number of “+” symbols**.

Table.4: Qualitative Phytochemical Analysis of Red Grape Seeds Ethanol Extracts

PHYTOCHEMICAL CONSTITUENT	ETHANOLIC EXTRACT OF GRAPE SEED
Flavonoids	+++
Tannins	+++
Anthocyanins	++
Alkaloids	++
Phenols	++
Saponins	++
Glycosides	++
Steroids	++
Terpenoids	+

Phytochemical constituents of Red Grape Seed (RGSE). The strength of signs viz. (+++), (++) , (+) refer to high, moderate and low phytochemicals respectively.

DISCUSSION

It is very interesting to note that among several organic solvents used to extract the biochemical compounds from any of the natural source of either plant or animal origin, ethanol was established as the most safe and efficient extractor as evidenced by the previous observations [10,11], according to whom the Total Flavonoid's Content in different solvents decreased in the following order: Acetone extract (70%) > Ethanol extract (70%) > Methanol extract > Water. The results obtained have demonstrated that the Total Phenolic compounds in Red Grape Seed extract was: 364 mg Gallic Acid Equivalents (GAE) /g of dry seed extract which was relatively lower than as reported by [12], where in his studies the Total Polyphenols content was 506.25 mg Gallic Acid Equivalents (GAE)/100g. However, the Total Flavonoids content in Red Grape Seed Extract was 12.86 mg Quercetin Extract (QE)/g of dry seed extract which was relatively lower in comparison to the research reports of [10], where the Total Flavonoids in ethanolic extract was: 13.75 mg Quercetin Extract (QE)/g grape seed. These observations were supported by several previous reports where, after thorough screening of 26 samples of six white grape varieties and 44 samples of four red grape varieties from different areas of Castilla-La Mancha, Spain, it was reported that different varieties of Grapes, made up of a heterogeneous combination of proantho-cyanidin monomers (5–30%), oligomers (17–63%) and polymers (11–39%) contained several types of bioactive compounds such as: catechin, epicatechin, epicatechin gallate, procatechuic acid, procyanidin B1, procyanidin B2, procyanidin B3, and procyanidin B4 [13,14]. Another study found that (+) - catechin (11%) was the most abundant constituent in *V.vinifera* (Tinta del pais) grape seeds, followed by (+)-epicatechin (10%), (-)-epicatechin-3-O-gallate (9%), epicatechin 3-O-gallate-(48)-catechin (B1-3-O-gallate) (7%), Epicatechin-(48)-epicatechin (dimer B2) (6%). The main phenolic components found in muscadine grape seeds were: (+)-catechin, epicatechin, and gallic acid. According to [15], the concentration of epicatechin, (+)-catechin, gallic acid, total anthocyanin, and total phenolics in muscadine grape seeds were 1299 mg/100 g of fresh weight (FW), 558 mg/100 g of FW, 6.9 mg/100 g of FW, 4.3 mg/100 g of FW, and 2179 mg/g GAE, respectively.

CONCLUSION

Based on these results, supported by the earlier findings, it can be suggested that RGSE can be recommended as one of the potential therapeutic compound for health- promoting activities in general and to treat the most serious neurological diseases, including Alzheimer's disease in particular.

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