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Review Article

Polyphenolic profile of Grape Seed Extract (gse) and evaluation of anti-oxidant/anti-aging properties

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Abstract

This paper aims at studying and analyzing polyphenolic content found in grape seed extract (GSE) through different conventional & modern extraction techniques. It also evaluates antioxidant and anti-aging properties of polyphenols extracted from grape seed. Owing to its anti-oxidant properties, polyphenols may help in slowing down skin aging process and may also be used in cosmetic formulations that can retard or slow down the process of aging by scavenging free radicals produced in the skin due to environmental degradation. Antioxidant potential/capacity of GSE has also been evaluated through in vitro and in vivo evaluation techniques.

Keywords: grape seed extract (GSE), Polyphenols, and antioxidants, *In vitro* & *In vivo* Evaluation

Introduction

Grape (vitis vinifera L) (family vitaceae) is not only the most abundant fruit but also a key horticulture crop in the world (Aurand, 2017). Majority of harvested grape is used in wine making. It is considered as one of the biggest sources of natural anti-oxidants having a long shelf life. Grape extract has been used as a key source of natural compounds in food and pharmaceutical industries. A grape berry consists of three distinct tissues (skin, pulp, and seed) as shown in figure 1. These tissues contain different groups of compounds like sugars, organic acids, minerals, amino acids, aroma compounds. Moreover, phenols like anthocyanins, flavonols, flavan-3ols, stilbenes, hydroxycinnamic and hydroxybenzoic acids are also found in grape tissues and seed (Harborne, 1989).

Many environmental factors like light intensity, temperature variation, altitude, soil types, water profile, wounding, plant growth regulators and canopy management, also affect the content and composition of grape phenolics (Downey *et al.*, 2006). The grape seed may also be obtained as residue from wine industries. GSE shows many health benefits related to cardiovascular protection, anti-aging, inflammation and skin care (Xia *et al.*, 2010).

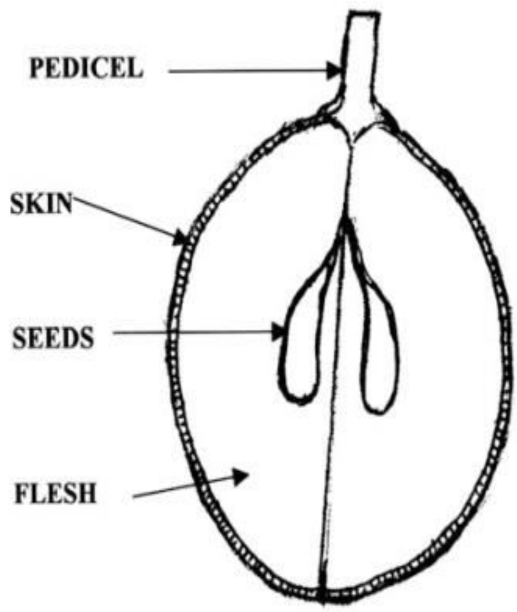

Figure 1: Mature grape berry.

Grape also contains many secondary metabolites which are rich in polyphenolic contents (Ivana petra *et al.*, 2017). These metabolites are also rich in antioxidant properties. Polyphenols are the major compound found in the seeds & skins of grape (Davidov-Pardo *et al.*, 2011). Figure 2 given below shows the different types of phenolic compounds detected in grapes.

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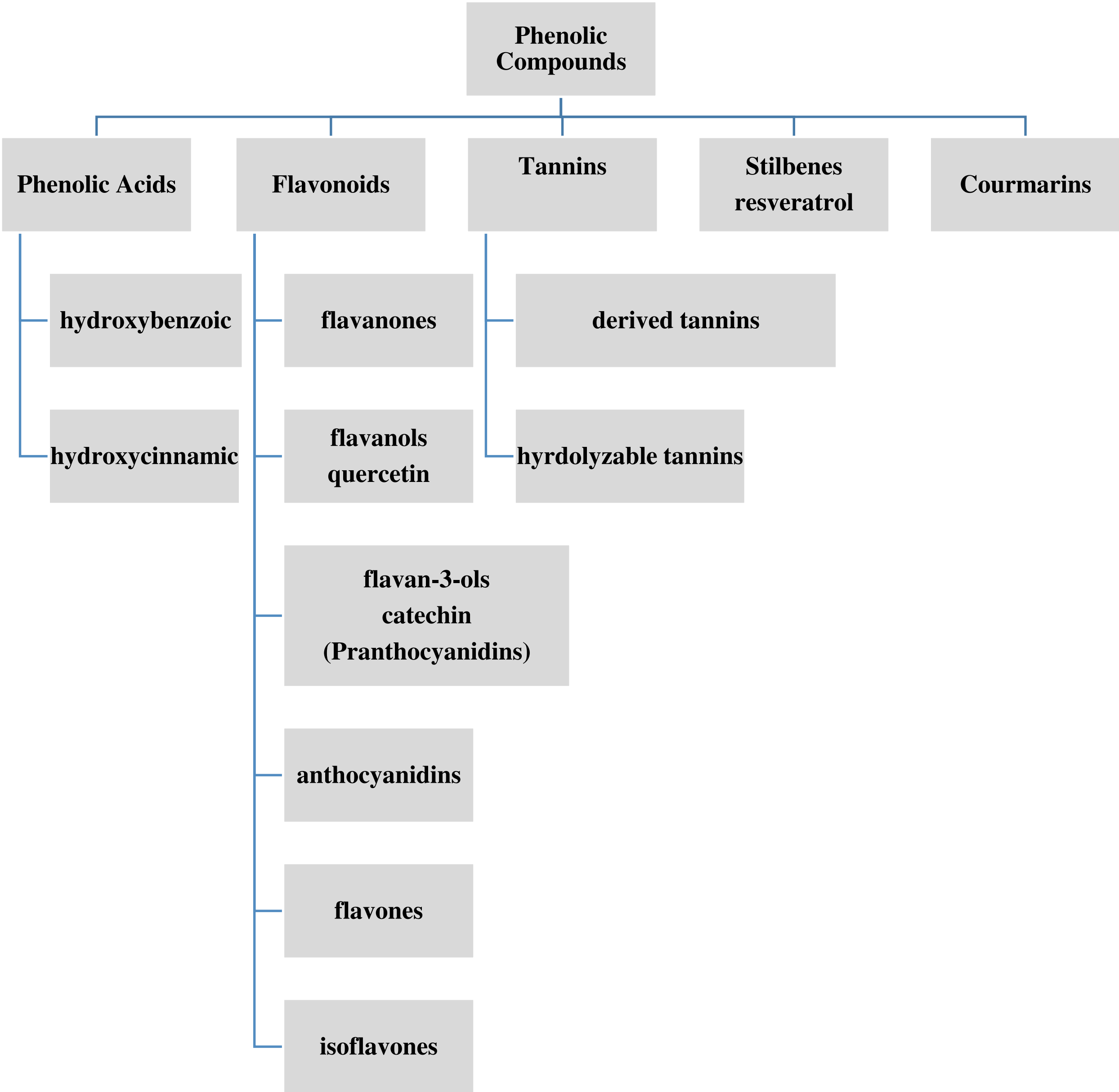

Figure 2: Different types of polyphenols detected in grape.

Grapes are recognized by their high phenolic contents. Two major categories of polyphenolic compounds found in the grapes can be classified as flavonoids (located in stems, skin, and seeds) & non-flavonoids (significantly found in pulp) as shown in figure 3. Polyphenolic compounds are substances having one or more than one hydroxyl groups located on the aromatic skeleton (Fernandes *et al.*, 2016). They can be classified as 'simple', 'highly polymerized', soluble or insoluble phenolic compounds.

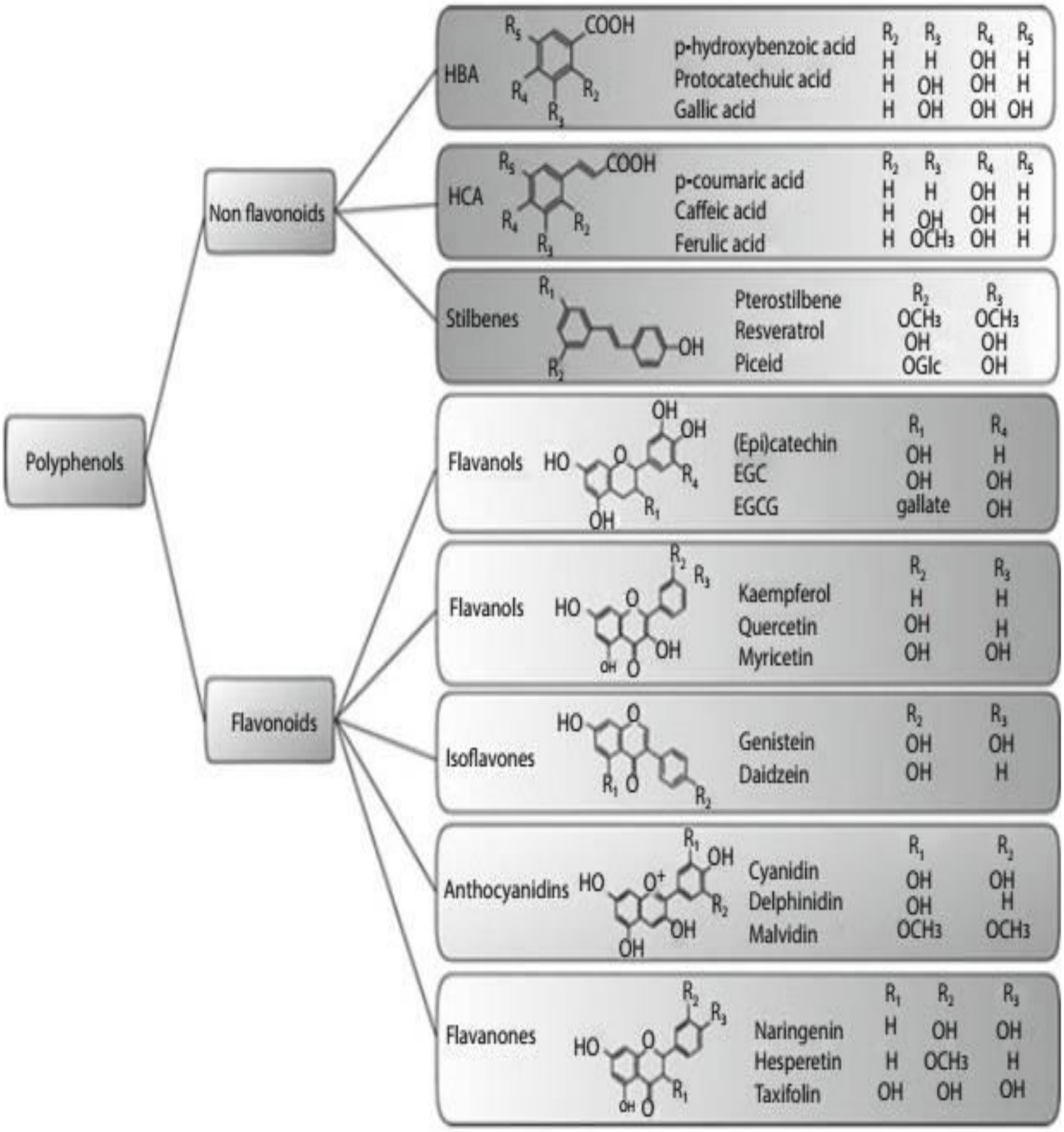

Figure 3 : Chemical composition of polyphenols.

Phenolic compounds are generally divided into different categories and sub-cateogires depending upon their chemical composition and structures such as acetophenones, and phenylacetic acids (C6-C2), derivatives of cinnamic acid (C6-C3), courmarins, isocoumarins and chromones (C6-C3), chalcones, dihydrochalcones, aurones, flavans, flavons, flavanones, flavanols, leucoanthocyanidins, anthocyanidins, anthocyanins, biflavonyls, benzophenones, xanthones and stilbenes, quinines, liganans, neolignans, tannins and polymeric compounds such as lignin and phlobaphenes (Harborne and Simmonds, 1964). Phenolic acids, precursor of flavonoids contain one carboxylic acid functional group and divided into hydroxycinnamic acids and hydroxybenzoic acids (Fernandes *et al.*, 2016). The Figure 4 given below shows the chemical structures of major polyphenolic components detected in the grape seed.

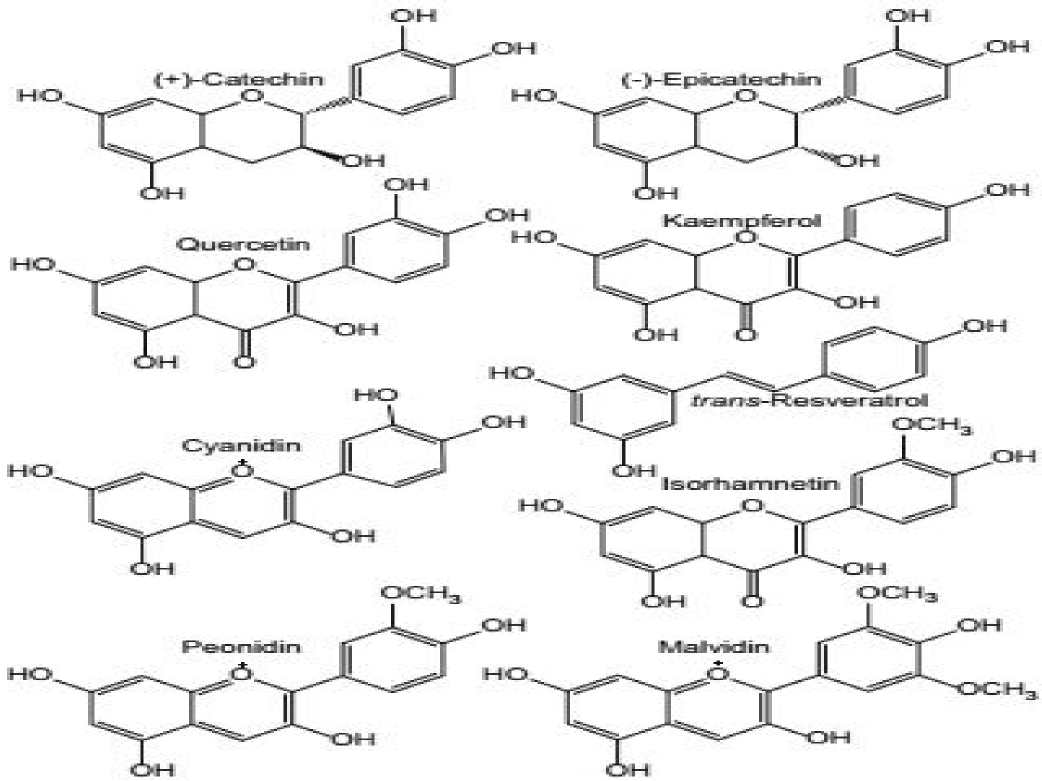

Figure 4: Chemical structures of major phenolic elements of grapes.

The total concentration of phenolic compounds in grape were 374.6, 23.8, 2178.8 and 351.6 mg/g GAE (Gallic acid equivalent) in skin, flesh, seed and leaf respectively (Content, 2003). However, average percentage of extractable polyphenols in grape tissues is: 60-70% in the seed, 10% or less in the pulp and 28-35% in the skin. The polyphenolic content of the seed may range from 5 to 8 wt % (Nawaz *et al.*, 2006).

Polyphenolic profile of grape seed extracts

Grapes are considered a rich source of phenolic compounds. The grape seed structure is usually divided into five key parts:

(i) cuticle and epidermis
(ii) outer integument or soft seed coat
(iii) medium integument or hard seed coat
(iv) inner epidermis
(v) endosperm and embryo

Polyphenols have been mainly found in the epidermis and in the outer integument (DOWNEY *et al.*, 2003). While grape ripens, different environmental factors and endogenous enzymes modify phenolic composition (Fernandes *et al.*, 2016).

The chemical constituents of grape seed expressed in %age of the total mass is : 25-45% water, 34-36% sugars, 13-20% oils, 4-6% tannins and phenolics, 4-6.5% nitrogenous compounds, 2-4% minerals and 1% fatty acids. Resveratrol is also an important non-flavonoid polyphenol in grape seeds. The trans-resveratrol content has been found to be 1.42 ± 0.18 mg per 100 g dry mass of white grape seeds (Kammerer *et al.*, 2004).

The composition of the endosperm is formed of a lipid fraction made of around 50% linoleic acid 30% oleic acid, 10% saturated fatty acids and 1% unsaponifiable residue. The minerals present in the seeds take up 4% to 5% of their total weight and the distribution of cations differs from that of the other parts of the grape (Chedea *et al.*, 2010). Calcium tends to be the most abundant followed by potassium, magnesium and sodium and then much lower level of iron, manganese, zinc and copper in that order. Outer layers of the seeds contain high amounts of tannins. It also depends upon crop type that the content is 22% and 56% of the total polyphenols of the grape. Procyanidins amounts for 67-86% and an important proportion of the total gallic acid and caffeic acid. The woody part is surrounded by a thin film that has also substantial concentrations of tannins (Ribéreau-Gayon *et al.*, 2006, Katalinić *et al.*, 2010).

Polyphenolic compounds found in grape seeds are flavonoids, flavan-3-ols, (catechin, epicatechin and epicatechin-3-O- gallate monomers) and their polymers. The most abundant flavan-3-ols monomers are (+)-catechin and (-)-epicatichen, and also (+)-gallocatechin, (-)-epigallocatechin and their 3-O-gallic acid aceters (Fernandes *et al.*, 2016). Other compounds present in the grape seeds are phenolic acid precursors (gallic acid) and stilbenes (Chedea *et al.*, 2012). It is also important to note that the degree of polymerization of the prcoyanidins may also determine the antioxidant activity. The higher the degree of polymerization, the greater is the antioxidant activity (Perumalla and Hettiarachchy, 2011). Standardized grape seed extracts contain 74-78% oligomeric proanthocyanidins and less than 6% of free flavanol monomers on a dry weight basis. The level of gallate in the seeds is higher than those found in the stem and skin (Pinelo *et al.*, 2006).

Flavonoids are primarily synthesized in the seed and skin of the grapes as they are mainly located in the seed. Flavonols and anthocyanins are primarily situated in the skin, and flavan-3-ols and their proanthocyanidin polymers are also get synthesized mainly in seed and stem (Jackson, 2008).

Extraction of polyphenols from gse

This section presents the traditional and new technologies to extract polyphenols from GSE. The quality and quantity of the polyphenolic contents obtained by various techniques mainly depend upon kind of seeds, grape varieties, maturity on harvest, the climate conditions of the production area, vinification process and other treatment done on grapes (Fernandes *et al.*, 2016). Moreover, other factors are also important as the solvent or combination of solvent chosen, extraction procedures and finally quality of material picked its storage conditions and pretreatment. The development of an extraction process of polyphenol compounds is a key step followed by performance of isolation protocols and compounds identification. The extraction process involves the following key mechanisms: (i) transport of solvent from the bulk solution to the external surface of the matrix (ii) solvent penetration and diffusion in the solid matrix (iii) solubilization of the components (iv) transport of the solute (s) through the solid matrix and (v) transport of the solutes from the external surface of the solid to bulk solution (Conde *et al.*, 2013).

Table 1: Comparative evaluation of different extraction techniques

Name of Technique	Key Characteristics
Conventional Solvent Extraction	(i) It is a time consuming technique extraction time ranges from few hours to several days that's why it is not considered as time efficient technique. (ii) The key variables in this technique include solvent type, relative solvents proportion, solvent/sample ratio, temperature, and particle size (Fernandes *et al.*, 2016). (iii) Methanol as a solvent is usually employed in the extraction of grape seed. However, its toxicity restricts analytical procedures (Fernandes *et al.*, 2016). (iv) It is observed that Long extraction time or higher temperature causes degradation of phenolic compounds in grape seeds (Fernandes *et al.*, 2016).
Ultrasound assisted Extractions (UAE)	(i) It is Simple, efficient and inexpensive technique(Anđelković *et al.*, 2014) (ii) It is used to extract Phenolic acid, tartaric acid and anthocyanins from grape seed and their by-products (Zhou *et al.*, 2014, Manco *et al.*, 2010, Ghoshal *et al.*, 2009) (iii) It is time-efficient technique (10-34 minutes) (Laugerette *et al.*, 2011). (v) It demands extra attention to avoid degradation of susceptible solutes (Fernandes *et al.*, 2016).
Microwave assisted Extractions (MAE)	(i) It is Simplest, fastest, and comparatively economic technique for extraction (Fernandes *et al.*, 2016). (ii) It requires less solvent and also provides higher yield and shorter extraction time. (iii) It heats material internally and externally without thermal degradation (Fernandes *et al.*, 2016) (iv) It uses microwave energy to effect molecular movements and rotation of polar solvents with permanent or induced diploes(Bäckhed *et al.*, 2007).
Enzyme assisted Extractions (EAE)	(i) It is enzymes dependent technique. (ii) The enzyme treatment hydrolyzes polyphenols released into low molecular weight phenolics that might increase bioactivity of these phenolics (Chamorro *et al.*, 2012, Xu *et al.*, 2014, Fernández *et al.*, 2015)
Accelerated solvent extraction (ASE)	(i) It is Pressurized fluid extraction (Fernandes *et al.*, 2016). (ii) It is efficient, innovative and environmentally clean technology (Fernandes *et al.*, 2016). (iii) It helps to extract anthocyanins and procyanidins from grape seed varieties.
Subcritical water Extraction (SWE)	(i) It is an environmental friendly technology (Fernandes *et al.*, 2016). (ii) It is safe technique. (iii) It is used for extracting both polar and non-polar compounds (Duba *et al.*, 2015). (iv) It is a pressure-regulated technique (Herrero *et al.*, 2012).
Super critical fluid Extraction (SFE)	(i) It is based upon the properties of the fluid under supercritical conditions to facilitate extractions and purification of natural compounds from solid samples (Fernandes *et al.*, 2016). (ii) It is re known for its rapid response Green technology (environment friendly), safe, high availability and purity (Fernandes *et al.*, 2016). (iii) In this technique two major steps are involved: a- Extracting soluble substance from the solid matrix by the super critical fluid solvents b- Separating extracted compounds from the supercritical solvents after the expansion.
Super critical anti-solvent Extractions (SAE)	(i) It is used to fractionate polar compounds from an organic solvent (Fernandes *et al.*, 2016). (ii) It is carried out under controlled Experimental conditions using pressure and temperature ranging from 8-15 MPa and from 35-60 Degree C (Fernandes *et al.*, 2016). (iii) When the operational variables chosen between 15Mpa and 40 Degree C, the extract was enriched the antioxidants by more than 150 % with respect to starting extract (Fernandes *et al.*, 2016).
Hot water extraction technique	(i) It is the most effective, rapid technique and its yield highest percentage of phenolic content from GSE (Yemis *et al.*, 2008).

The most widespread technique used is conventional extraction with solvents (CSE), but owing to its operational conditions like the use of organic solvents with their toxicology and environmental problems led to growth of new processes of extraction using different technologies (Fernandes *et al.*, 2016).

During the study in order to obtain polyphenol from GSE we examined different extraction techniques in detailed and also analyzed efficiency, cost effectiveness, rate and its percentage yield and suitable temperature parameters. The Table 1 given below shows the key characteristics of different extraction techniques to obtain GSE.

Antioxidant properties of polypenols extracted from gse

Imbalance among antioxidants, Reactive Oxygen Species (ROS) and free radicals are in the origin of alteration in the cell molecules causing oxidative stress. Antioxidants may help to maintain an adequate antioxidant status in the body. Grapes are known to provide different antioxidants during the last 100 years (Leong *et al.*, 2016).

The study confirmed the antioxidant properties of GSE and its potential protection against lipid oxidation (Schevey and Brewer, 2015). Antioxidant properties largely depend upon chemical structure of phenolic compounds. Mainly, it depends upon ability to donate an electron and their ability to de-localize the unpaired electrons within the aromatic structure. ABTS, ORAC, FRAP, TBRAS assays may be used to gauge antioxidant properties of GSE (Maier *et al.*, 2009).

Grape seed has been found to exhibit the highest capacity to show antioxidant properties (García-Lomillo *et al.*, 2014). GSE also showed higher antioxidants efficacy than synthetic antioxidants, like α-tocopherol, ascorbic acid & gallic acid (Shafiee *et al.*, 2003). Both antioxidants and anti-bacterial capacities of the grape seed aqueous extracts were lower when extracted from seeds after wine making (Adámez *et al.*, 2012).The de-oiling method influence the ultrasound extraction of phenolics from grape seed.

Ethyl acetate extract of seeds from V. vinifera varieties cultivated in Grece, contained different low molecular weight compounds: Catechin was the most abundant Polyphenols accounts for 49 % of the total content followed by epicatechin, epicatechin gallate and Procyanidin, whereas epigallocatechin and gallic acid were in lower amounts. Procyanidin B1 is one of the most important radical scavengers in grape seed extract (Guendez *et al.*, 2005). The total proanthocyanidins quantity of the extract had the highest positive correlation with the antioxidant action and also phenolic content and color of extracts were found to be correlated (Bucić- Kojić et al., 2009).

Strong correlation among FRAP (Ferric Reducing Antioxidant Power) and total Phenolics, flavonoids and flavan-3-ols content was observed for the seed extract from red wine grapes (Doshi *et al.*, 2015). Hot water extracts from grape seeds showed the highest content of Polyphenols among different winery residues. The extract containing the highest Phenolic content also showed the highest antioxidant activity measured by DPPH test. Total Phenolic contents showed a key correlation with DPPH and ABTS values in the grape seed extract obtained from different varieties produced in Turkey, extracted with 70%

acetone and with total phenolic content ranged from 33 to 58 mg GAE/100g extract (Yemis *et al.*, 2008). The polymeric fraction of GSE showed the higher antioxidant capacity than the monomeric and oligomeric fractions according to ABTS, DPPH and FRAP (Ky *et al.*, 2014).

Grape seed Procyanidins present higher free radical scavenging activity than that of Vitamin C (Spranger *et al.*, 2008). As per molar concentration, polymeric procyanidins are the most potent antioxidants followed by oligomeric procyanidins.

Anti-ageing activity of the grape and its products is the combination of grape seed extract with co-enzyme Q10, Luteolin & Selenium showed remarkable improved skin elasticity (De Luca *et al.*, 2016). Grape phenolic compounds are also used as a good UV-B rays protector and human dermal fiberoblasts and markedly prevents fiberoblast collagen degradation by blocking the matrix metalloproteinase production (Bae *et al.*, 2009, . Bae *et al.*, 2010)

Conclusion

GSE are a natural source of polyphenols having strong anti-oxidants properties, confirmed both by in vitro assays and also by in vivo experiments. Different extraction techniques/technologies may be utilized for the proficient extraction and concentration of polyphenolic fraction including the extraction with conventional solvents, the use of pressurized solvents or the enzymes, microwave or ultrasound as technical tool.

Future prospects

Keeping in view of comparative characteristic of key extraction techniques, there is dire need to carry out further study in order to develop economic, cost effective, time-efficient and environment friendly technique for extraction of polyphenols from GSE. Moreover, further study may also be carried out to formulate anti-aging skin formulations of GSE and perform its non-invasive biophysical study on human skin.

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