Functional properties of grape (*Vitis vinifera*) seed extract and possible extraction techniques - A review

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Received: 26-11-2014 Accepted: 21-09-2015 DOI: 10.18805/ag.v36i4.6668

**ABSTRACT**

Grape (*Vitis vinifera*) member of family *vitaceae*, is an excellent source of phenolics, particular in the skin and seeds. Phenolic compounds have attracted much interest recently, because they are potent antioxidant and exhibit various other physiological activities including anticarcinogenic, antimicrobial, anti-inflammatory and antihypertensive activities associated with reduced risk of chronic diseases like cardiovascular diseases. The method of processing and extraction play a vital role in the quality of nutraceuticals extracted. The traditional extraction techniques are time-consuming and require relatively large quantities of solvents. In view of increased awareness for health related compounds from plant materials, there is an increasing demand for better extraction techniques which are nontoxic, environmentally safe and inexpensive. This review paper discusses briefly on grape phenolics, important nutraceuticals extracted from grape seeds and its positive health effects. The possibility of application of novel extraction methods like sonication assisted extraction, microwave assisted extraction techniques and supercritical fluid extractions for efficient extraction of plant are discussed.

**Key word:** Antioxidants, Extraction techniques, Functional properties, Grape seed extract, Phenolics.

Grape (*Vitis vinifera*) member of family *vitaceae*, is one of the widely cultivated and most important fruit crops in the world (Satisha *et al*. 2008). Grape cultivation is believed to have originated in Armenia near the Caspian Sea in Russia, from where it spread westward to Europe and eastward to Iran and Afghanistan. It was introduced in India in 1300 AD by Moghul invaders from Iran and Afghanistan. It is a temperate crop which got adapted to sub-tropical climate of peninsular India. However, in India, grapes are cultivated because of the prevalence of congenial agro climatic conditions and is one of the most remunerative farming enterprises in India (Shikhamany 2001). In India, grapes are cultivated in an area of 111.4 thousand ha with a total production 1,234.9 thousand tons and productivity of 11.1 tons/ha.

*Vitis vinifera* is an excellent source of phenolics, particular in the skin, and the seeds. Total phenolic compounds in the red grape is contributed by skin 33%, seed 62%, juice 4% and pulp 1% (Anonymous, 2001). In addition they are also important source of phytochemicals such as gallic acid, catechin and epicatechin known for their antioxidantative capacity, (Table 1, Xia *et al*. 2010). The various other physiological effects of grape phytochemicals are anticarcinogenic (Agarwal *et al*. 2007), antimicrobial, anti-inflammatory (Xia *et al*. 2010) and antihypertensive activities (Peng *et al*. 2005). The antioxidative activity of phenolic compounds is mainly attributed to their free radical scavenging and metal chelating properties. In addition to that, they exhibit an effect on cell signaling pathways and on gene expression (Soobrattee *et al*. 2005; Dell Agli *et al*. 2005).

Grape is grown in India for table, raisin, wine and juice purpose (Somkuwar and Ramteke 2008). India currently has approximately 150,000 acres of table grapes and only 7,000 – 12,000 acres (this number is disputable) of wine grapes in production. Its chemical composition is profoundly influenced by enological techniques, the grape cultivar from which it originates, and climatic factors. Tannins and anthocyanins in grapes are responsible for the sensory properties of wine such as color and astringency (Yilmaz and Toledo 2004).

Seeds alone make up around 15% of the solid waste produced in wine industries. They are generally burnt and sometimes used for cattle feed, despite of they are the source of excellent oil for human consumption (Luque-Rodriguez *et al*. 2005). Grape skins and seeds produced in large quantities by the winemaking industry are increasingly used to obtain functional food ingredients. In addition it is a better source of antioxidative constituents than skins of grape. Functional ingredients of grape seed include several

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flavonoids such as monomeric flavanols, dimeric, trimeric and polymeric procyanidins, and phenolic acids. It is a complex matrix containing approximately 40% fiber, 16% oil, 11% proteins, and 7% complex phenols including tannins, in addition to sugars, and mineral salts, etc. The seed contains higher amounts of monomeric, oligomeric, and polymeric flavan-3-ols than the different parts of the grape. Polymeric proanthocyanidins represented the largest proportion of the total flavan-3-ol content in the grape seeds (Li et al. 2008).

Oil content of grape seeds strongly depends on grape variety, though the usual range is 10–16% of dry weight. Grape seed contains typically 8 - 15% (w/w) of oil with recognized quality due to its high level of unsaturated fatty acids, namely oleic and linoleic acid. It consists mainly of triglycerides and the fatty acids 0–0.2% myristic acid (C14:0), 7–13% palmitic acid (C16:0), 3–6% stearic acid (C18:0), 0–0.9% palmitoleic acid (C16:1), 14–25% oleic acid (C18:1), 61–73% linoleic acid (C18:2), 0–0.6% linolenic acid (C18:3). The high content in unsaturated fatty acids (around 85–90%) are considered to be responsible for its health promoting effects like prevention of thrombosis and cardiovascular diseases, reduction of cholesterol in serum, dilation of blood vessels and regulation of autonomic nerves (Lui et al. 2001).

**Grape seed extract:** Grapes are rich in polyphenols and about 60-70% of grape polyphenols exist in grape seeds. The grape seed polyphenols are flavan-3-ol derivatives and are colourless in the pure state. When grapes are processed into wine, it is inevitable that some of the polyphenols will be leached into the liquid phase. However, an important portion will remain with the pomace, making it a valuable source of polyphenols that may have many applications as food and nutritional additives (Boussetta et al. 2009). Fig. 1 shows the basic chemical structures of proanthocyanidin of the grape extracts.

Proanthocyanidins extract of grape seed is a natural extract from the seeds of *Vitis vinifera* (Castillo et al. 2000), and contained 90% proanthocyanidins that are polymers of catechin. Proanthocyanidins are safe (Wren et al. 2002) to consume and known to possess a broad spectrum of pharmacological, medicinal, and therapeutic properties (Ray et al. 2001). The biologically active constituent of grape seed extract (GSE) - proanthocyanidins have a strong antioxidative effect in aqueous systems. In addition, GSE has been shown to possess antibacterial, antiviral, antifungal and antiparasite properties too (Heggers et al. 2002; Reagor et al. 2002). Because of their wide spectrum of pharmacological action, plant extracts containing proanthocyanidins are increasingly attracting the attention of both pharmacists and physicians.

In Japan, it is used as an anti oxidant in confectionery or processed fishery foods at a concentration of 0.01% to 1.0% (Yumiko et al. 2003). Several experimental and clinical studies have shown that GSE also has cholesterol-lowering effect (Preuss et al. 2002), cytotoxic effect on human cancer cells (Ye et al. 1999), cardioprotective properties (Sato et al. 2001), enhance bone formation with endochondral ossification (Wang et al. 2002) and has a stimulating action on angiogenesis in dermal wound healing (Khana et al. 2001). Further, the compound does not seem to induce any significant toxic effect (Wren et al. 2002).

<table>
<thead>
<tr>
<th>Source</th>
<th>Phenolic compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeds</td>
<td>gallic acid, (+)-catechin, epicatechin, dimeric procyanidin, proanthocyanidins.</td>
</tr>
<tr>
<td>Skin</td>
<td>Proanthocyanidins, ellagic acid, myricetin, quercetin, kaempferol, trans-resveratrol.</td>
</tr>
<tr>
<td>Lea</td>
<td>myricetin, ellagic acid, kaempferol, quercetin, gallic acid.</td>
</tr>
<tr>
<td>Stem</td>
<td>rutin, quercetin 3-O-glucuronide, transresveratrol, astilbin.</td>
</tr>
<tr>
<td>Raisin</td>
<td>hydroxycinnamic acid, hydroxymethylfurfural.</td>
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</tbody>
</table>
Bioactive properties of grape seed extract: Grape and red wine are consumed worldwide and have been reported to be associated with reduced risk of cancer. GSE has been linked to cancer prevention and therapy. Studies in carcinogen-induced and genetically engineered cancer models (Raina et al. 2007) have revealed a chemopreventive role of proanthocyanidins in GSE. GSE was also shown to inhibit the growth of a number of cancer cells in vitro and tumor growth in mice (Singh et al. 2004; Kaur et al. 2006). Recently, GSE was also reported to inhibit endothelial cell proliferation and tube formation on Matrigel and reduce vessel density in human prostate tumor (Veluri et al. 2006 and Agarwal et al. 2007).

The antioxidant activity of grape seed polyphenols is more potent than vitamin C and vitamin E (Aldini et al. 2003). Many evidences indicate that regulation of reactive oxygen species play an important role in some forms of cardiovascular disease, including hypertension (Patrick 2006). Polyphenols present in red wine lower blood pressure and enhance nitric oxide bioactivity without up-regulating eNOS (López et al. 2008). Whereas polyphenolics derived from grapes increase the antioxidant levels and improve the endothelial function (Leifert and Abeywardena, 2008). The metabolic syndrome is an important risk factor for the development of both coronary artery disease (Malik et al. 2004) and type 2 diabetes mellitus (Laaksonen and others 2002). One manifestation of this state is the increase in Ox-LDL in plasma that is a “precursor” to the development of atherosclerosis. According to the observation made by Sivaprakasapillai and others (2009), GSE at a dose of 300 mg/d reduced Ox-LDL, particularly when the Ox-LDL concentrations were high. It is suggested that Phenolic compounds in the grape seed extract are absorbed and that its antioxidant properties could reduce the concentration of Ox-LDL in plasma.

Grape seed oil extraction techniques: Grape seed contains a broad range of nutraceutical compounds as mentioned in Table 2. The traditional methods for extracting grape seed oil consist of pressing the whole seeds in discontinuous-hydraulic press or the seeds are milled and heated in screw press (Flanzy 2000). At present, they have been replaced almost totally by solvent (hexane, generally) extraction after crushing seeds in roller mills and heating. Then, the crude oil is neutralized, bleached with activated carbon and clay and finally deodorized under vacuum. These extraction techniques which have been used so far are very time-consuming and require relatively large quantities of solvents. There is an increasing demand for new extraction techniques with shortened extraction time, better extraction efficacy, reduced organic solvent consumption, and less environmental pollution. Novel extraction methods including sonication assisted extraction, microwave-assisted extraction and supercritical fluid extraction are fast and efficient for extracting nutraceuticals from solid plant matrixes. These techniques offer the possibility of working at elevated temperatures and/or pressures, greatly decreasing the time of extraction.

**TABLE: 2  Bioactivities of some phenolic compounds from grapes (Xia et al.2010).**

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>Bioactivity</th>
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<tbody>
<tr>
<td>Resveratrol</td>
<td>Free radical scavenging</td>
</tr>
<tr>
<td></td>
<td>Antiproliferation</td>
</tr>
<tr>
<td></td>
<td>Enhancing plasma NO level</td>
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<tr>
<td></td>
<td>Regulating lipid metabolism</td>
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<td></td>
<td>Protection against membrane oxidation</td>
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<tr>
<td>Quercetin</td>
<td>Antibacterial</td>
</tr>
<tr>
<td></td>
<td>Enhancing plasma NO level</td>
</tr>
<tr>
<td>Catechin</td>
<td>Anticancer</td>
</tr>
<tr>
<td></td>
<td>Free radical scavenging</td>
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<tr>
<td></td>
<td>Antibacterial</td>
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<tr>
<td></td>
<td>Anti-inflammation</td>
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<tr>
<td></td>
<td>Protection against membrane oxidation</td>
</tr>
<tr>
<td>Flavone</td>
<td>Antiproliferation</td>
</tr>
<tr>
<td>Flavonol</td>
<td>Free radical scavenging</td>
</tr>
<tr>
<td>Procyanidin</td>
<td>Anticancer</td>
</tr>
<tr>
<td></td>
<td>Free radical scavenging</td>
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<tr>
<td></td>
<td>Anti-inflammation</td>
</tr>
<tr>
<td></td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>Vasorelaxation</td>
</tr>
<tr>
<td></td>
<td>Free radical scavenger</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>Free radical scavenger</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>Antibacterial</td>
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</tbody>
</table>

Sonication-assisted extraction: Ultrasonic-assisted extraction is one of the important techniques for extracting valuable compounds from vegetative natural sources. Resveratrol yield from grape by ultrasonication was found to increase by 24–30% compared with the conventional solvent extraction (Yong et al. 2006). Ultrasound is regarded as a promising technology for the extraction of phenolic compounds from solid materials due to its beneficial properties, involving high extraction efficiency, high reproducibility, low solvent consumption, easy-operating, low cost and low pollution to environment. Compared with other novel extraction techniques the ultrasound apparatus is cheaper and its operation is easier. Furthermore, the
ultrasound-assisted extraction like Soxhlet extraction can be used with any solvent for extracting a wide variety of natural compounds (Xia et al. 2006; Kiani et al. 2013).

Sound waves, which have frequencies higher than 20 kHz, act like mechanical vibrations in a solid, liquid, and gas. Unlike electromagnetic waves, sound waves when traveling in a medium involve expansion and compression cycles in the medium. Expansion pulls molecules apart, and compression pushes them together. The expansion can create bubbles in a liquid and produce negative pressure. The bubbles form, grow, and finally collapse producing high-speed jets of liquid at the solid boundary causing strong impact on the solid surface (Kiani et al. 2006).

It is necessary to take into account plant characteristics such as moisture content and particle size, and solvent used for the extraction in order to obtain an efficient and effective ultrasound-assisted extraction. Furthermore, many factors govern the action of ultrasound including frequency, pressure, temperature, and sonication time. A small change in frequency can increase the yield of extract (Zhao et al. 2007). The ultrasonic wave distribution inside an extractor is also a key parameter in the design of an ultrasonic extractor. In order to avoid standing waves or the formation of solid free regions for the preferential passage of the ultrasonic waves, additional agitation or shaking is usually used (Jian et al. 2006). Since ultrasound generates heat, it is important to accurately control the extraction temperature. The sonication time should also be considered carefully as excess of sonication can damage the quality of extracts (Xiaohua et al. 2006).

**Microwave-assisted extraction:** Microwave-assisted extraction offers a rapid delivery of energy to a total volume of solvent and solid plant matrix with subsequent heating of the solvent and solid matrix, efficiently and homogeneously. As water within the plant matrix absorbs microwave energy, cell disruption is promoted by internal superheating, which facilitates desorption of chemicals from the matrix, improving the recovery of nutraceuticals. The effect of microwave energy is thus strongly dependent on the dielectric susceptibility of both the solvent and the solid plant matrix (Tatke and Jaiswal 2011; Winny and Valérie 2012). There are two types of commercially available MAE systems viz., closed extraction vessels under controlled pressure and temperature, and focused microwave ovens at atmospheric pressure (Ravindra Naik and others 2012). The closed MAE system is generally used for extraction under drastic conditions such as high extraction temperature. The pressure in the vessel essentially depends on the volume and the boiling point of the solvent. The focused MAE system can be operated at a maximum temperature determined by the boiling point of the solvents at atmospheric pressure.

Moistening of samples with water that have relatively high dielectric constant was found to provide better recoveries. If a dry biomaterial is re-hydrated before extraction, the matrix itself can thus interact with microwaves and hence facilitate the heating process. The microwave heating leads to the expansion and ruptures of cell walls and is followed by the liberation of chemicals into the solvent (He and Xia 2011; Fanny et al. 2012). In this case, the surrounding solvent can have a low dielectric constant and thus remains cold during extraction. This method can be used to extract thermo-sensitive compounds like pigments (Satpathy et al. 2011).

Plant particle size and size distribution usually have a significant influence on the efficiency of MAE. The particle sizes of the extracted materials are usually in the range of 100 μm–2 mm. The plant material in fine powder form can enhance the extraction because the larger surface area of a fine powder provides better contact between the plant matrix and the solvent (Wilkowska and Biziuk 2010). Solvents such as ethanol, methanol, and water are sufficiently polar to be heated by microwave energy. Non-polar solvents with low dielectric constants such as hexane and toluene are not potential solvents for MAE. The extracting selectivity and the ability of the solvent to interact with microwaves can be modulated by using mixtures of solvents. One of the most commonly used mixtures is hexane-acetone (Hongyan et al. 2012; Rohit et al. 2012). A small amount of water can also be incorporated in non-polar solvents such as hexane, xylene, or toluene to improve the heating rate. During extraction, the solvent volume must be sufficient to ensure that the solid matrix is entirely immersed. Generally, a higher ratio of solvent volume to solid matrix mass in conventional extraction techniques can increase the recovery (Şeytaningsih et al. 2012).

**Supercritical fluid extraction:** Supercritical Fluid Extraction (SFE) is a potential alternative to conventional extraction methods using organic solvents for extracting biologically active components from plants (Kagliwal et al. 2012; Omar et al. 2013). Supercritical state is achieved when the temperature and the pressure of a substance are raised over its critical value. The supercritical fluid has characteristics of both gases and liquids. The dissolving power of a supercritical fluid solvent depends on its density, which is highly adjustable by changing the pressure or/and temperature. Also the fluid has a higher diffusion coefficient and lower viscosity and surface tension than a liquid solvent, leading to more favorable mass transfer (Ansari and
SFE offers unusual possibilities for selective extractions and fractionations because the solubility of a chemical in a supercritical fluid can be manipulated by changing the pressure and/or temperature of the fluid. Furthermore, supercritical fluids have a density of a liquid and can solubilize a solid like a liquid solvent. The solubility of a solid in a supercritical fluid increases with the density of the fluid, which can be achieved at high pressures (Zuknik et al. 2012; Mann et al. 2013).

Many nutraceuticals such as phenolics, alkaloids and glycosidic compounds are poorly soluble in carbon dioxide and hence not extractable. Addition of polar co-solvents (modifiers) to the supercritical CO₂ is known to significantly increase the solubility. Among all the modifiers including methanol, ethanol, acetonitrile, acetone, water, ethyl ether and dichloromethane, methanol is the most commonly used because it is an effective polar modifier and is up to 20% miscible with CO₂. A mixture of modifiers can also be used in SFE. The best modifier usually can be determined based on preliminary experiments. One disadvantage of using a modifier is that it can cause poor selectivity (Liu et al. 2009; Ruiz et al. 2012).

Preparation of plant materials is another critical factor for SFE of nutraceuticals. When fresh plant materials are extracted, the high moisture content can cause mechanical difficulties such as restrictor clogging due to ice formation. Although water is only about 0.3% soluble in supercritical CO₂, highly water-soluble solutes would prefer to partition into the aqueous phase, resulting in low efficiency of SFE. Some chemicals such as Na₂SO₄ and silica gel are thus mixed with to retain the moisture for SFE of fresh materials (Van et al. 2012a). Plant particle size is also important for a good SFE process. Large particles may result in a long extraction process because the process may be controlled by internal diffusion. (Van et al. 2012b). The extraction time has been proven to be another parameter that determines extract composition. Lower molecular weight and less polar compounds are more readily extracted during supercritical CO₂ extraction since the extraction mechanism is usually controlled by internal diffusion (Miguel et al. 2010; Girotra et al. 2013).

The dissolved nutraceutical compounds can be recovered from the fluid by the reduction of the density of the supercritical fluid, which can usually be reduced by decreasing pressure (Zarena et al. 2012; Pimentel et al. 2013). Therefore, SFE can eliminate the concentration process, which usually is time-consuming. Furthermore, the solutes can be separated from a supercritical solvent without a loss of volatiles due to the extreme volatility of the supercritical fluid. (Glisic et al. 2010; Domingues et al. 2013).

CONCLUSION

With wide spectrum of pharmacological action, grape seed extracts containing proanthocyanidins are increasingly attracting the attention of both pharmacists and physicians. Biofunctional properties of grape polyphenols have created a lot of interest recently. Type of extraction method used plays a significant role as the extraction method, temperature and time has impact on the yield and quality of the nutraceutical compound extracted. Efficient extraction technique could aid in complete utilization of the active compounds in the fruit. Traditional solid–liquid extraction methods require a large quantity of solvent and are time consuming. In addition, the large amount of solvent used has serious environmental problems. Novel extraction techniques have been developed as an alternative to conventional extraction methods, which need to be ventured for large scale production. These techniques have to be adopted to take advantage of extraction time, solvent consumption, quantity and quality of nutraceuticals extracted.

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