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Original Research Article

Antioxidant and Antibacterial Activity of *Vitis Vinifera*

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Abstract

Grapes (*Vitis vinifera*) are usually known grape species that belong to the Vitis genus in the Vitaceae family and come from western Asia and southern Europe. This normally grows well in temperate countries. Many studies show that the consumption of grapes and various other parts of the fruit, especially the leaf, provides many health benefits. These are universally appreciated fruit for their delicacy, nutrition and are recognised as a functional food. The skin leaf and seeds of grapes are known to be rich sources of phenolic compounds, both flavonoids, and non-flavonoids. Grape leaf expressed high antioxidant and antimicrobial properties compared to grape skin extract which explained the medicinal activities of grape leaf extract. This review highlights the antioxidant and antibacterial activity of *Vitis vinifera* leaf. The various scientific databases and search engines such as PubMed, Elsevier, Springer, Frontiers, Google Scholar, Scopus, Science Direct, and MDPI provide various informations about the antioxidant and antibacterial properties of *Vitis vinifera* leaf, seed, stem. In some countries, grapes are utilised for traditional uses, such as drug therapy for blood- forming, anemia, allergies, wound care, colds and flu, carminative, bronchitis, diarrhoea, and anti- phlegm. The main phytochemical compounds in *Vitis vinifera* are phenolic compounds, aromatic acids, flavonoids, proanthocyanidins, and stilbenoids. *Vitis vinifera* has been highlighted by its many positive effects on human health, including antibacterial, anti-inflammatory, and antioxidant activity due to its rich phytochemical content. *Vitis vinifera* shows as a vital and renowned source of compounds with important biological activity. Wines and winery bioproducts, such as grape pomace, skins, leaf and seeds, are rich in bioactive compounds against a wide range of human pathogens, including bacteria, fungi, and viruses. However, little is known about the biological properties of vine leaves.

Keywords: Grapes, leaf (*Vitis vinifera*), Antioxidant Activity, Antimicrobial Activity.

Introduction:

The starting of human civilization, medicinal plants have always endured a component of human society to fight and treat different diseases. In particular, in the Indian systems of medicine Rigveda, Charak Samhita, and Sushruta Samhita such explanations have been documented. According to estimates of the World Health Organization, nearly 75% of the world's population now a day's uses herbs and other traditional medicines to treat diseases of different natures[1,2,3]. Nevertheless, currently medical as well as pharmaceutical research is being carried out towards evaluation and development of plant-derived natural components to treat different human diseases[4,5,6].

The grape (*Vitis vinifera*) has been well recognized worldwide for over 2,000 years as one of the edible sweet fruits and acknowledged for its wide spectrum of biological activities[3]. Its taxonomic position is as follows: Group Thalamifloreae, Order Rhamnales, Family Vitaceae, Genus Vitis, Species vinifera. It is a deciduous woody climber with coiled climbing tendrils and large leaves. It has small, pale, green flowers in the summer followed by bunches of berry fruits that range from green to purple-black. The grape has been utilised as folk medicine for its biological properties since ancient times. The leaves of the plant, which have astringent and haemostatic activities, are utilised in the treatment of diarrhoea, haemorrhage, varicose veins, haemorrhoids, an inflammatory disorder, pain, hepatitis, and free radical related diseases and externally for centuries in Anatolia to heal wounds and drain furuncles[2,4,7]. The juice of the leaves has been also advised as an antiseptic for eyewash[3,5]. Some of the above-cited diseases could be correlated to viral or bacterial agents. Moreover, in recent years, the leaves are utilised in the formulation of dietary antioxidant supplements [6,7].

Grape: a good source of nutrients

The grape belongs to the berry family as it is found attached to the stem. Many berries make up a cluster or bunch of grapes. The fruit of the grape is one of the most agreeably edible foods, having many confirmed nutritional and medicinal properties for consumers. The grape is a good source of water (*82%), carbohydrates (12–18%), proteins (0.5– 0.6%), and fat (0.3–0.4%). Additionally, the grape contains notable amounts of potassium (0.1–0.2%), vitamin C (0.01–0.02%), and vitamin A (0.001–0.0015%) and also has a small amount of calcium (0.01–0.02%) and phosphorus (0.08–0.01%). Grapes are also a vital source of other nutrients like boron, 4 a possible substance for bone health. The essential parts of the berry include skin, pulp, and seeds. Nutritional analysis per serving with grape halves gives *78 calories of energy, *0.3 g of protein, *19 g of carbohydrate, *0.23 g of fat (*3% calories from fat), *0.18 mg of sodium, *155 mg of potassium, *0.4 g of fiber, *20 ng of calcium, *30 ng of phosphorus, and *1mg of vitamin C[7,8].

Biological activities of grape

Although a large number of compounds have been isolated from the grape, only some of them are having biological and medicinal properties[1]. The major medicinal qualities of grape and its constituents are antioxidant, anticarcinogenic, immunomodulatory, antidiabetes, anti-atherogenic, neuroprotective, anti-obesity, anti-aging, and anti-infection. In particular, antioxidant property oxidative stress is a hallmark of various health problems. Resveratrol (3,5,40-trans-trihydroxystilbene) is a natural phytoalexin profusely found in grapes and red wine, which has potent antioxidant

properties[2,3]. Over the years several analogs, i.e., 3,4-dihydroxy-trans-stilbene (3,4-DHS), 4,40- DHS, 4-hydroxy-trans-stilbene, and 3,5-DHS, of resveratrol have been synthesized and have been found to have a weakened effect on free radical-induced peroxidation. Thus, all these trans-stilbene derivatives are potent antioxidants against both 2,20-azobis (2-amidinopropane hydrochloride)- and iron-induced peroxidation. The most potent antioxidant activity was noticed with 3,4-DHS, followed by 4,40-DHS, resveratrol, 4-hydroxy-trans-stilbene, and 3,5-DHS respectively. Further, resveratrol is also described to show a strong inhibitory effect on 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced aryl hydrocarbon receptor DNA binding activity as well as on the expression of cytochrome P450 1A1 and 1B1, which are known to oxidize 17b-estradiol to produce catechol and estrogens. Thus, resveratrol protects the tissues from oxidative stress as well as catechol estrogen-induced damage. Some study shows that proanthocyanidin, a variant of resveratrol, isolated from grape seed extract also showed antioxidant protection in smokeless tobacco-induced cellular injury, and this activity has been assigned to be due to alteration in Bcl-2 and p53 expression in in vitro and in vivo systems. Moreover, the skin and seeds of grapes are better sources of phytochemicals like gallic acid, catechin, and epicatechin, which are suitable raw substrates for the production of antioxidative dietary supplements. Antioxidant thiol conjugates could also get from the white grape pomace. Analysis of Concord grape juice has shown that it is a rich source of flavonoids, having greater antioxidant efficacy, as seen in vitro, than α-tocopherol (vitamin E). Further, Concord grape juices have also been found to increase serum antioxidant capacity and thereby be more protective than α-tocopherol against low-density lipoprotein (LDL) oxidation in healthy adults. Based on these observations the consumption of the fruit of the grape and or its constituents may be part of therapeutic regimens to suppress oxidative stress-related threats. Another important group of components present in grapes is the anthocyanins, which belong to the flavonoid family. In recent years, several studies have shown that anthocyanins display a wide variety of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic effects. Antioxidant effects of anthocyanins have been mainly studied and assessed elsewhere [9,10,11,12].

Antioxidant activity of *Vitis vinifera*

Sample collection

To collect the samples, firstly grapes have to be gathered at their technological maturity, like from the Thondamoothur region, Coimbatore. Approximately 500 undamaged and disease-free berries are randomly fragmented from clusters. The grapes are dried with filter paper and weighed. The skins and seeds are correctly separated manually from pulp, dried with filter paper, weighed and made into powder, and stored until analyzed [8].

Extraction

The *Vitis vinifera* were shade dried and reduced to coarse powder in a mechanical grinder. The powdered plant material was extracted using ethanol. In this method dried *Vitis vinifera* powder was taken in a round bottom flask. And was extracted by Soxhlet extraction for 48 hours at 60 °C temperature. Cooled it and filtered it by vacuum filtration unit using Whatman filter paper no.1.

Then the filtrate was collected into flask and extract obtained was evaporated concentrated extract was dried. And dried plant extract was collected and stored in air tight container at room temperature [8,13,14].

Antioxidant activity

The free radical scavenging capacity is analyzed by DPPH assay which is a radical

generating substance of free radical scavenging abilities of various antioxidants [8].

Procedure

Usually, 4 ml of 0.1mm DPPH is taken at various concentrations (9mg, 17.5mg) are added vitamin A and vitamin C (9mg, 17.5mg) are also taken and to this 4ml of DPPH is added. All the tubes are incubated at room temperature in dark for 1 hour. After incubation to all the tubes 1ml of distilled water is added. The color changed from deep violet to pale yellow color. The reagent without the extract is used as the blank. The absorption is measured colorimetrically at a standard wavelength [8,15,16].

Antibacterial activity of *Vitis vinifera* leaf extract

Antibacterial assay

The antibacterial assay is done by Kirby Bauer testing. The test organisms grow in a smooth "Lown of confluent" on the Petri plate except in a clear zone around the antibiotic discs, which inhibited the growth of the organisms and indicate the susceptibility of the organisms[8,17,18].

Screening of antibacterial activity

For antibacterial activity commercial antibiotic tetracycline is used. The standards of the antibiotic disc are taken from the container using forceps and placed gently on the surface of the inoculated Petri plates. The extracts of various solvents are taken in separate containers. The sterile disc that is soaked in various solvent extracts is then placed in the inoculated plates. The Petri dishes are then kept at 37°c for 24hrs in an inverted position. The inhibition zone of microbes is observed[8,9,13,19].

Microorganisms and culture conditions

Gram-positive bacterium *Staphylococcus aureus*, and Gram-negative bacterium *Eischheria coli* are used in this study. Each bacterial strain is incubated in Luria Broth (LB) medium at 37°C for overnight. After incubation, the bacterial solution is centrifuged two times and washed by using PBS (phosphate buffer saline, pH 7.0), and the test bacterial solution is prepared with PBS to give a concentration of 107 CFUs/mL by using a haematometer[8].

Determination of microbial growth inhibition

A 500 mL of mid-logarithmic phase bacterial cultures (107 CFUs/mL) is inoculated in 4.5 mL LB medium to make the final concentration of 106 CFUs/mL. A 2.0 mg/mL of GPP(Grape pomace polyphenols) stock solution is prepared and then serial two-fold diluted in a 1.0 and 0.5 mg/mL using LB medium and also control (without samples) are taken to measure the growth inhibition of *Eischheria coli* and *Staphylococcus aureus*. The GPP is diluted using 1% Dimethyl sulfoxide. The cultures are incubated in a rotary shaker at 37°C and growth inhibition is obtained by measuring the absorbance at OD 600 nm using a UV-VIS spectrophotometer. Absorbance readings are taken for 360 min, followed by 60, 120, 180, 240, 300, and 360 min intervals. A growth curve is plotted with the obtained absorbance readings. All the measurements are done in triplicate[8,11,13,18].

Determination of antimicrobial activities as killing effect

The GPP is added to 5.0 ml of PBS (phosphate buffer saline, pH 7.0) containing 500 mL of mid-logarithmic phase bacterial culture (107 CFUs/mL) to prepare the final concentration 10.0 mg/mL and 106 CFUs/mL. The tested bacterial cultures with samples are incubated at 37°C for 60, 120, 180, 240, 300, and 360 min intervals to decide the log survival ratio. The visible colony forming units (CFUs/mL) is measured with the above time intervals using MacConkey and Mannitol salt agar plate,

incubated at 37°C for 24 h to measure the log reduction of *Eischheria coli* and *Staphylococcus aureus*. In addition, the dose-dependent killing effect is also assessed using mid-logarithmic phase bacterial cultures (107 CFUs/mL) that are inoculated in a 500 mL PBS (phosphate buffer saline, pH 7.0) to make the final concentration of 106 CFUs/mL. In the 500 ml of PBS, the final concentration of GPP is 3.0, 6.0, 9.0, and 12.0 mg/mL to measure the killing effect of *Eischheria coli* and *Staphylococcus aureus* incubated at 37°C for 6 h. After incubation to measure the killing effect of bacteria a serial 10-fold dilution is prepared using PBS (pH 7.0) and plated onto MacConkey and Mannitol salt agar plates and incubated at 37°C for 24 h. And then log survivals are set out using visible colonies on agar plates, while the count detection limit is maintained between 5 and 50 CFUs.

The bacterial killing effect is calculated using the following formula: killing effect (Log CFUs/mL) log10nc−log10np, where nc and np are CFUs/mL of mock and treated cells. All the data represent the mean of triplicate tests[8,19,20,21].

Conclusions

The fruit of the grape is probably by consumers worldwide for its taste and is confounded with a bunch of nourishing properties. The health beneficial qualities of active ingredients are common in different parts (e.g., fruit, stems, leaf and seeds) of grapes. This further adds on benefits of this fruit, thereby appealing the consumer for its inclusion to the diet of humans to circumvent disease, infections, and relaxants. Keeping in mind all the reality explained here, it is valuable mentioning that the intake of grapes in the consistent diet of humans could help a lot in supporting good health. Similarly, the purified products acquired from the fruit could demonstrate to be probable agents in the prevention and treatment of several diseases. *Vitis vinifera*, with its various varieties, has phytochemical compounds similar to each other. The major phytochemical compounds are stilbenoid, phenolic compounds, aromatic acids (hydroxycinnamic and hydroxybenzoic acid), flavonoids, proanthocyanidin. Every part of *Vitis vinifera* is abundant in phytochemical compounds, which differ from one component to another. Every part and compound carried therein has benefits for humans, as designated by the many pharmacological activities established. The pharmacological activity depends on the part of the grapevines and the type of extract used. Therefore, *Vitis vinifera* can be favourable for humans in traditional use and research development. There is a deficiency of current studies on genotoxicity and toxicity. Much recent research is required concerning the genotoxicity and toxicology study of *Vitis vinifera.*

Discussion

Grape *Vitis vinifera* are suggested to have antimicrobial activity, antioxidant activity, and various other benefits to human beings. The anti-fungal and antibacterial effect of grape plant extract against common clinical isolates and drug-resistant pathogenic strains were assessed by many studies. The bactericidal effect of grape leaves extract is considered for by the presence of stigmasterol. Stigmasterol is a sterol molecule that causes degradation of bacterial components by surface interaction and pore formation in the bacterial cell wall. It might also be connected to the presence of tannins which has the capacity to inactive microbial adhesions, enzymes and cell envelope transport proteins, their complexity with polysaccharide and their capacity to improve the morphology of microorganisms. Therefore, this observation is expressive of the antibacterial effect of grape *Vitis vinifera plant* extract. The structure-activity correlation assays revealed that the hydroxyl group of the phenolic compound is found to be productive against *E.*

coli and the benzene ring is effective against *Staphylococcus aureus*. The grape extract could be a possible antibacterial agent and this effect can additionally be made obvious with upgraded methodologies.

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