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Research Article

SCREENING OF *Vitis vinifera* FOR FLAVONOID CONTENT AND FREE RADICAL SCAVENGING POTENTIAL

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ABSTRACT

V. vinifera is one of the important fruit crop, characterized by substantial number of soluble flavonoids including quercetin, the clinically proved therapeutic agent. Present study has been focused on analysis of flavonoids together with quercetin in aerial parts of *V.vinifera* and their correlation with radical scavenging activity (RSA). Two varieties of black cultivars viz. Sharad and Sonaka seedless were selected. Presence of flavonoid was confirmed by phytochemical analysis. Bands of flavonoids were detected by Preparative thin layer chromatography (PTLC). Total flavonoid content (TFC) and radical scavenging activity (RSA) was estimated by $AlCl_3$ colorimetric assay and DPPH assay respectively. Quantification of quercetin was done by HPLC. Findings of analysis showed high abundance of flavonoids in almost all parts of both cultivars. Rf value for quercetin was found to be 0.85. Aerial parts of both cultivars showed significant amount of TFC. Petiole of Sonaka seedless showed highest content of flavonoid (0.28 ± 0.06 mg/g QE). Whereas, stem of Sharad seedless showed less content (0.10 ± 0.02 mg/g QE). Concentration of quercetin in berry skin of Sharad seedless was found to be 2133.4 ppm. Order of RSA for both the cultivars was Petiole>Stem>Leaf lamina. In Sharad seedless less significant correlation was found between TFC and RSA. Whereas positive correlation was shown by Sonaka seedless Therefore, it can be concluded that in addition to skin, almost all the aerial parts of *V.vinifera* are rich source of flavonoids with noteworthy RSA i.e antioxidant potential. Thus, significant amount of flavonoids can be efficiently extracted from these aerial parts of *V.vinifera* which can be used for healing of various diseases and disorders

Keywords: Flavonoid, Quercetin, *V.vinifera*, Aerial parts, Antioxidant, Preparative TLC

INTRODUCTION

Grapevine (*V. vinifera*) is one of the important fruit crop, characterized by various bioactive compounds comprising large amount of soluble flavonoids, a group of natural polyphenols which plays important role in determination of coloration to flowers and fruits, attract pollinators as well as act as tissue protectors in case of pathogen attack or oxidative damage[1]. These polyphenols are produced by the phenyl propanoid pathway which has been distinctively described in different plant species[2,3]. In the berries and seeds of red and white cultivars of *V.vinifera* , the gene expression included in synthesis of flavonoids like anthocyanin is very well distinguished [4-7]. It was noticed that the genes, particularly responsible for anthocyanin synthesis were distinctively expressed in respect of cultivars and their parts. It was found that red and white cultivars show different temporal expression pattern of UDP-glucose: flavonoid 3-O-glucosyl transferase genes. Whereas, remaining genes from anthocyanin biosynthesis pathway are expressed in all the cultivars irrespective to berry colour [8]

It is clinically proved that flavonoids possess a variety of biological activities, including antioxidant, anti-allergic, anti-inflammatory, antiviral, anti-proliferative, and anti-carcinogenic activities, in addition to positive impact on mammalian metabolism[9]. As a dietary component also, they show health-promoting properties due high antioxidant capacity for both in vivo and in vitro systems [10, 11]. Among the various flavonoids, Quercetin is considered as one of the unique bioflavonoid that has been extensively studied and promoted for prevention as well as treating the conditions of the heart and blood vessels including atherosclerosis, high cholesterol, heart disease, and circulation problems. It is also used for diabetes, cataracts, hay fever, peptic ulcer, schizophrenia, inflammation, asthma, gout, viral infections, chronic fatigue syndrome (CFS), neurodegenerative diseases, preventing cancer, and for treating chronic infections of the prostate, etc.

Despite the wide therapeutic use of fruits of *V.vinifera*, there is still lacks of scientific data in the literature demonstrating, the existence of flavonoid contents in other aerial parts of *V.vinifera.* Therefore, the present study has been focused on qualitative and quantitative analysis of total flavonoids as well as quercetin content in different aerial parts and grape skin of black cultivars of *V.vinifera* and their antioxidant potential.

MATERIALS AND METHODS

Sample Collection

Fresh and healthy aerial plant parts (leaf lamina, stem and petiole, skin) of Black cultivars of *V.vinifera* viz. *Sharad seedless* and *Sonaka seedless* were randomly collected from vineyards of Nashik valley, Maharashtra India during June 2016.

Extraction

Collected plant material was carefully separated, cleaned, shade dried for fifteen days at room temperature and mechanically grinded to fine powder. The powder (10%) was subjected to solvent extraction with 90% methanol. It was allowed to

incubate at room temperature with gentle shaking for 72 hours. Supernatants were further evaporated to dryness .Hygroscopic yield of extract was noted with respect to dried plant material powder. The extracts thus obtained were dissolved in methanol at the concentration of 1 mg/1 ml and used for further analysis.

Qualitative Analysis
Phytochemical Screening

The preliminary phytochemical screening tests were carried out for detection of bioactive flavonoids in the plant extracts as well as to facilitate the qualitative separation and quantitative estimation of different flavonoids. Extracts were tested by using various reported methods with few modifications. The tests were identified by visual observation of colour change or by precipitate formation on addition of specific reagents to the test solution. Quercetin (HiMedia) was used as flavonoid standard.

Tests For Flavonoids

Lead Acetate Test[12]
In the 1ml extract, 4-5 drops of lead acetate solution (10%) were added. Appearance of yellow precipitate was an indication for the presence of flavonoids.

Sodium Hydroxide Test[13]
1ml of extract was dissolved in water and filtered. Add 1.5 ml aqueous sodium hydroxide (10%) to produce a yellow colour. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.

Shinoda Test[14]
Magnesium metal (0.5 gm) was added in the 1.5 ml extract followed by reaction with 1-2 drops of concentrated hydrochloric acid. Development of reddish pink or magenta color along with effervescence was an indication for the presence of flavonoids.

Aluminium Chloride Test[15]
1 ml extract was shaken with 0.25 ml of 1% aluminum chloride solution and observed for light yellow coloration. A yellow precipitate was an indication for the presence of flavonoids.

Sulphuric Acid Test For Anthocyanin[16].
In 1.5 ml of extract few drops of sulphuric acid were added. Development of yellowish orange color was an indication for the presence of anthocyanins

Preparative Thin layer Chromatography (PTLC)

Preparative Thin layer chromatography (PTLC) was carried out on precoated silica gel plates purchased from MERK(Germany). Methanolic extract of different aerial parts were loaded and air dried. Different solvent systems were optimized to obtain best resolution of loaded samples. N-Butanol: Acetic Acid: Water (4:1:5) was selected as best system for PTLC. The plates were developed at room temperature in pre saturated chamber and different bands were observed under UV at 254 nm and the plates were further developed with 5% $AlCl_3$.. Rf value for flavonoid in the selected solvent system was calculated by using Quercetin as a flavonoid standard.

Quantitative Analysis
Determination of Total Flavonoid Content [17]

Total flavonoids content was measured by $AlCl_3$ colorimetric assay with few modification in the method of D. Devi, 2013.Briefly, 60 µL sodium nitrate (5%) solution was added to mixture of 1000 µL of extract and 480 µL of distilled water and allowed to incubate for 5 min. Further, 60 µL $AlCl_3$ (10%) was added to the mixture and again incubated for 5 min. Afterwards, 400 µL of sodium hydroxide (1 mM) was added to it and the mixture was vortexed for few seconds followed by 30 min. incubation in dark at room temperature. Absorbance was measured at 510 nm against the blank. Formation of yellow coloration indicated presence of flavonoids. Quercetin was used as reference standard (0.1-1 mg/ml) and results were expressed in mg of quercetin equivalent (QE/g) of dry weight of extract. All the determinations were performed in triplicates.

HPLC Analysis

HPLC Analysis was performed on Analytical Technologies Ltd.-HPLC 3000 series gradient binary systems. Column configurations were Grace, RP-18,250mm× 4.6 mm (ID), Particle size 5 µm held at room temperature using the solvent system, Methanol: Water (90:10)(v/v).Flavonoid-quercetin (50 ppm) was used as reference. Diluted Sample was detected at 270nmwith 20*ul* of sample injection at 0.8 ml/min (Isocratically)

Antioxidant Evaluation
DPPH Radical-Scavenging Activity[18]

The antioxidant potential of plant extract (leaf lamina, stem and petiole) was estimated by the free radical scavenging ability with reference to the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) as per spectrophotometric method reported by S. Sannigrahi,2010 with few modifications. Briefly, plant extract (100µl) was added into 100 µL methanolic solution of DPPH (0.1mM) final volume was made up to 2 mL with methanol. Whole mixture was shaken vigorously and incubated (30 min) in dark at room temperature. Absorbance was measured at 517 nm. Radical-Scavenging Activity toward DPPH was estimated from the equation as:

$$\% \text{ Inhibition} = [A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}] \times 100$$

RESULTS AND DISCUSSION
Qualitative Analysis of Flavonoids
Phytochemical Analysis

Methanolic extracts of leaf lamina, stem, petiole and skin were tested for the presence of flavonoid the tests were identified by visual observation of colour change or by precipitate or effervescence formation on addition of specific reagents to the test solution. Phytochemical screening showed that almost all the aerial parts including grape skin are rich in flavonoid content (Figure 1).

Preparative Thin layer Chromatography (P-TLC) [19]
Various solvent systems were tried to achieve a good resolution. Finally, solvent system, N-Butanol: Acetic Acid: Water (4:1:5) was used to obtain best resolution of different flavonoid bands. Rf value was compared to respective flavonoid standard (quercetin) which was found to be 0.85. (Figure 2)

Quantitative Analysis of Flavonoids
Estimation of Total Flavonoid Content

It was revealed that almost all the aerial parts of both varieties showed significant amount of flavonoid content. Petiole of Sonaka seedless showed highest flavonoid content (0.28 ± 0.06 mg/g QE) whereas, stem of Sharad seedless showed lowest flavonoid content (0.10 ± 0.02 mg/g QE). The total flavonoid content in Leaf lamina of Sonaka seedless and Sharad seedless

was almost found to be similar i.e.0.18 ±0.03 and 0.18 ±0.01 mg/g QE respectively. It was observed that stem of both the cultivars showed comparatively less amount of flavonoids, Sonaka seedless (0.15 ± 0.06 mg/g QE) and Sharad seedless (0.10 ± 0.02 mg/g QE) (Table 1)

HPLC Analysis

As per the chromatogram of standard quercetin, quantification of quercetin in the sample extract was carried out by comparing the retention time of sample with standard (Rt =2.8.). Concentration of quercetin in the skin of Sharad seedless grape berry was found to be 2133.4 ppm. (Figure 3)

Antioxidant Activity by DPPH Radical-Scavenging Activity

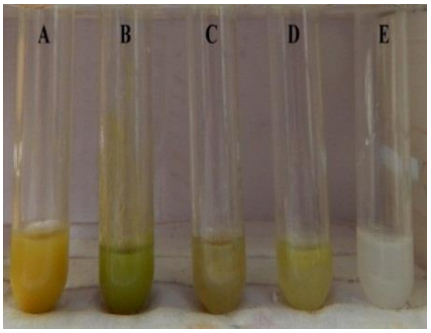
The DPPH radical scavenging activity of different aerial parts of both varieties of *V.vinifera* was carried out in triplicates. Ascorbic acid was used as reference compound. It was observed that petiole of Sonaka seedless, showed highest radical scavenging activity or % inhibition (84.54 %) in comparison with stem (74.90%) and leaf lamina (45.28%) Similarly, petiole of Sharad seedless showed highest radical scavenging activity (82.22 %) as compared to stem and leaf which was 70.23 % and 46.34 % respectively. Both the varieties showed order of radical scavenging activity as, Petiole>Stem>Leaf lamina. (Table 1)

Correlation Between Total Flavonoid Content and DPPH % Radical Scavenging Activity

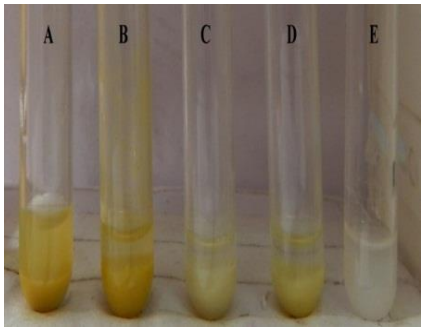
The anti-oxidant activity was correlated with the amount of total flavonoid content present in the respective extracts. In case of Sonaka seedless, highest % inhibition activity was shown by the petiole with comparatively high content of flavonoids. Likewise, leaf lamina with less flavonoid content showed lower percent of inhibition activity. It was noticed that Sonaka seedless has positive correlation between flavonoid content and antioxidant activity for all the aerial parts. It was observed that petiole of Sharad seedless with high flavonoid content showed high % inhibition. However, stem with comparatively less flavonoid content showed high % inhibition activity in comparison to leaf lamina. The possible reason may be presence of other bioactive phenolic compounds contributing to antioxidant activity of stem. (Table 1)

Statistical Analysis

All experiments were performed in triplicates. All values were expressed as mean ± standard deviation (SD) of three separate experiments. Linear correlation between total flavonoid content and DPPH % radical scavenging activity of aerial parts of both cultivar was calculated to establish a relationship between them. Results revealed that Sonaka seedless variety showed satisfactory linear correlation between both the parameters with R^2 value, 0.8035. Whereas, the linear correlation was not much significant in Sharad seedless cultivar, showing R^2 value, 0.6312. (Figure 4)



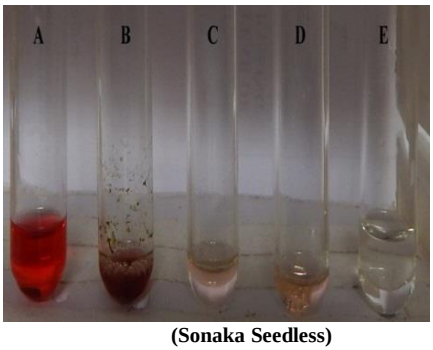
(Sonaka Seedless)



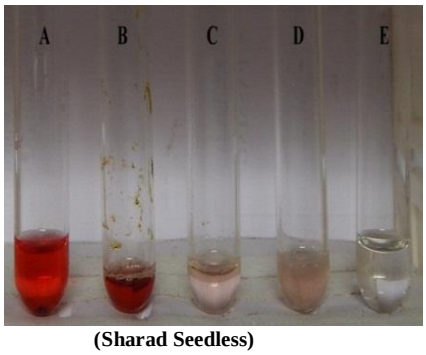
(Sharad Seedless)

(A.Positive Control B.Leaf lamina C.Stem D. Petiole E.Negative Control)

[1a. Lead Acetate Test]



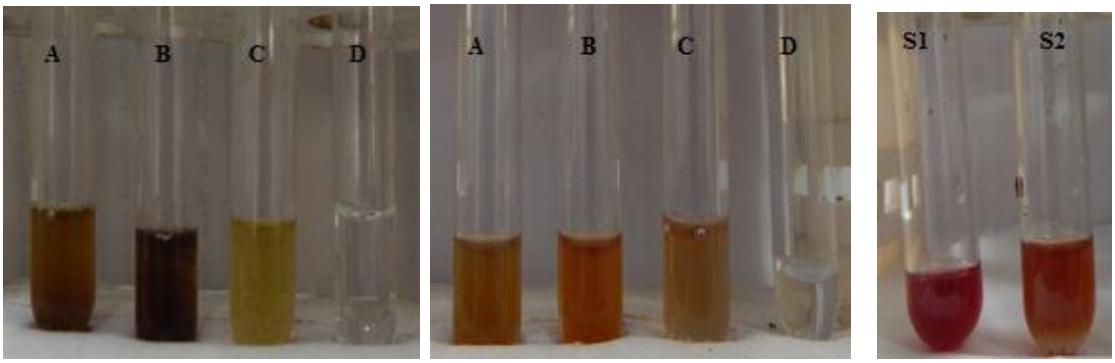
(Sonaka Seedless)



(Sharad Seedless)

(A. Positive Control B. Leaf lamina C. Stem D. Petiole E. Negative Control)

[1b.Shinoda Test]



(Sonaka Seedless) **(Sharad Seedless)** **(S1)** **(S2)**

(A. Leaf lamina , B.Stem , C. Petiole , D.Negative Control S1. Grape Skin -Sonaka Seedless S2. Grape Skin -Sharad Seedless)
[1c.Anthocyanin Test]

Figure 1: Phytochemical Tests for Flavonoids

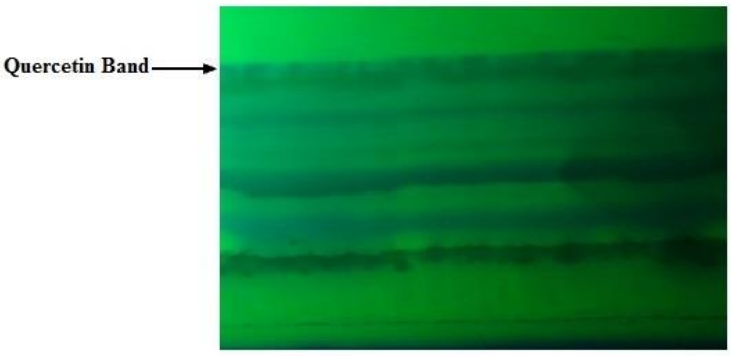


Fig.2. Preparative TLC of Petiole (Sonaka Seedless) Under UV -254nm

Figure 2: Preparative TLC of Petiole (Sonaka Seedless) Under UV -254nm

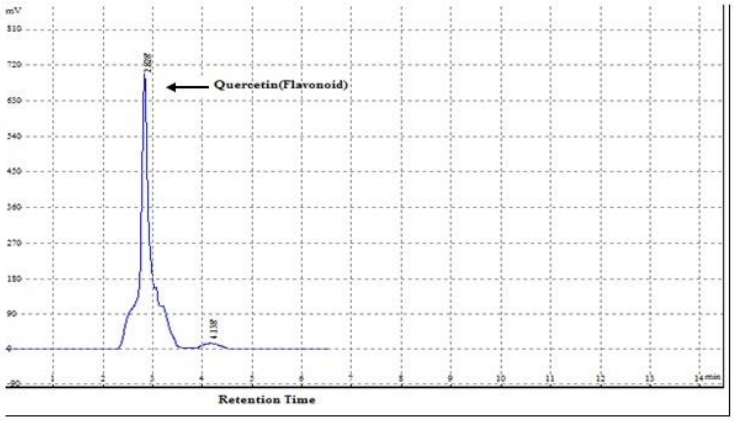
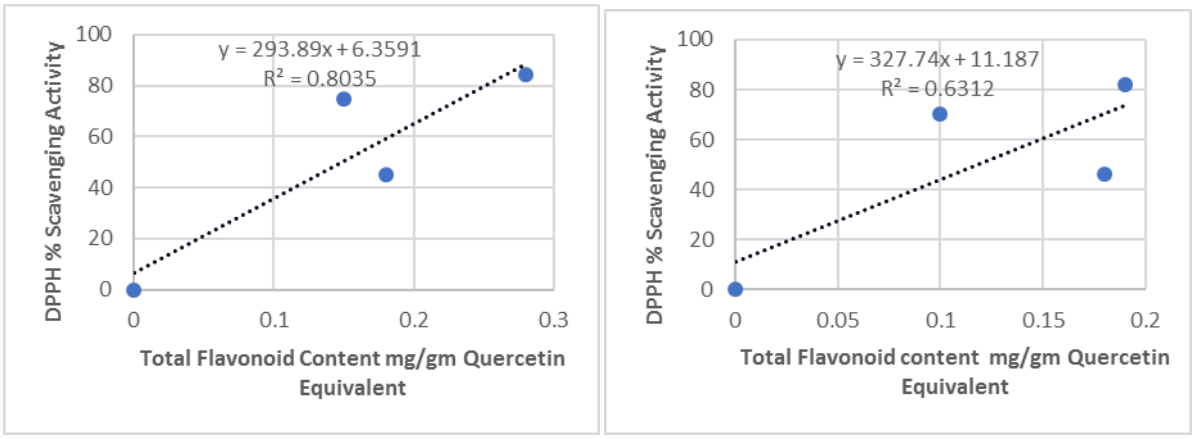


Fig.3 HPLC Chromatogram of Flavonoid (Quercetin) of Skin (Sharad Seedless Grape Berry), at 270nm and Rt= 2.8 min

Figure 3: HPLC Chromatogram of Flavonoid (Quercetin) of Skin (Sharad Seedless Grape Berry), at 270nm and Rt= 2.8 min



[I. Sonaka seedless] **[II. Sharad seedless]**

Figure 4: Linear Correlation Between Total Flavonoid Content and DPPH % Radical Scavenging Activity of Different Aerial Parts of

Table 1: Total Flavonoid Content (TFC) and % Inhibition of Aerial Parts of *V.vinifera*

Sample	TFC mg/g Quercetin Equivalent	% Radical Scavenging Activity
Sonaka Seedless		
Leaf Lamina	0.18 ± 0.03	45.28
Stem	0.15 ± 0.06	74.90
Petiole	0.28 ±0.06	84.54
Sharad Seedless		
Leaf Lamina	0.18 ± 0.01	46.34
Stem	0.10 ± 0.02	70.23
Petiole	0.19 ± 0.12	82.22

*All values were expressed as the mean of triplicates ± standard deviation (SD).

CONCLUSION

In present study, the phytochemical test for flavonoid detection showed high abundance of flavonoids in almost all the parts of both cultivars of *V.vinifera.* Among the identified flavonoids quercetin is clinically proved novel medicinal biomolecule with diverse therapeutic potential which shows antioxidant, anti-inflammatory, anti-cancer, antimicrobial, psychostimulant, cardio protective, and neuroprotective properties. In this regard, the further analysis and quantification was carried out with reference to quercetin. Preparative TLC with the solvent system, Butanol: Acetic Acid: Water (4:1:5) showed best resolution for different flavonoid bands of which, Rf value for quercetin was found to be 0.85. It was revealed that different parts of both cultivars showed significant amount of total flavonoid content. Petiole of Sonaka seedless showed highest content of flavonoid (0.28 ± 0.06 mg/g QE) whereas, stem of Sharad seedless showed less content (0.10 ± 0.02 mg/g QE). Concentration of quercetin in berry skin of Sharad seedless was found to be 2133.4 ppm by HPLC. It was noticed that the order of radical scavenging activity for both the cultivars was Petiole>Stem>Leaf lamina. It was found that Sharad seedless variety showed less significant correlation between total flavonoid content and antioxidant activity for all the aerial parts. Whereas satisfactory positive correlation was shown by cultivar of Sonaka seedless. Therefore, based on the outcomes of this research, it can be concluded that in addition to skin, almost all the aerial parts of *V.vinifera* are rich source of flavonoids with noteworthy radical scavenging as well antioxidant potential. Thus, significant amount of flavonoids can be efficiently extracted from these aerial parts of *V.vinifera* which can be used for healing of various diseases and disorders

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