



Impact of Environmental Factors on Secondary Metabolite Diversity and Free Radical Scavenging Activity of *V.vinefera* (Jumbo Seedless) From Nashik Valley

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ABSTRACT

An environmental factor has great influence on biosynthesis of secondary metabolites in plants. Nashik valley is located at highest elevated point of the Maharashtra state of India. Thereby, vineyards of these areas are always subjected to various stressful environmental conditions. Present work aimed to study the effect of environmental factors on secondary metabolite diversity and free radical scavenging activity of different aerial parts of *V.Vinefera* from Nashik valley. Organic extracts are prepared by soaking the dry powder (10%) of different aerial parts viz. leaf lamina, stem and petiole of Jumbo seedless cultivar into 90% Methanol. Presence of different secondary metabolites was confirmed by phytochemical analysis. Detection of spots of secondary metabolites was observed by Thin layer chromatography (TLC) using the solvent system, N-Butanol: Acetic Acid: Water (4:1:5). Folin-Ciocalteu method was used to determine total phenolic content whereas, total flavonoid content was estimated by aluminum chloride colorimetric assay. Free radical scavenging activity of extract was carried out by DPPH assay. Findings of study showed that almost all the aerial parts of *V.vinefera* are rich source of secondary metabolites with medicinal values. Results of TLC showed presence of different spots of secondary metabolites. Rf value of flavonoid – quercetin was found to be 0.85. Total phenolic content of petiole was found to be highest (0.92 ± 0.10)

whereas leaf lamina showed lowest amount viz. 0.5 ± 0.07 mg GAE/g The total flavonoid content of petiole was found to be 0.28 ± 0.12 mg/g quercetin equivalent which was highest in comparison to stem (0.13 ± 0.04 quercetin equivalent) and leaf lamina (0.11 ± 0.01) mg/g quercetin equivalent. Satisfactory antioxidant activity (Radical scavenging activity) of different aerial parts were found in the order as, Stem (70.01%) > Petiole (67.00%) > leaf lamina (52.26%) respectively. The results showed positive linear correlation between total phenolic content and total flavonoid content of different aerial parts of black cultivar of *V.vinefera*. Therefore, our study emphasizes that environmental conditions of Nashik valley is significantly suitable for biosynthesis of various bioactive secondary metabolites in *V.vinefera*.

Keywords— *Climate change, environmental factors, Vitis vinefera, Secondary metabolites, Antioxidant*

I. INTRODUCTION

Secondary metabolites are known for their multiple functionality and bioactivity due to presence of more than one functional groups. [1]. Ability of any plant to compete and survive is profoundly affected by the ecological functions of their secondary metabolites. There is influence of external environmental factors

on the contents of secondary metabolites through their impact on growth rates, development and qualitative variation in secondary metabolites of plants.[2-3].It is reported that plants have ability to produce limitless secondary metabolites in response to the external attacks like biotic and abiotic stresses which include microbial and herbivore attack, temperature, altitudinal variation and light intensity etc. These defense mechanisms are generated by plants via triggering several simple to complex biochemical processes [4].

Temperature stress is one of important environmental factor known to stimulate the production of free radical scavenging enzymes. There are several reports regarding effect of temperature on composition or contents of phenolics or phenolic derivatives It is observed that both high and low temperatures has different effect on plants metabolites [5-6]. Solar radiation is also responsible factor for the plant growth as plants has ability to sense the variable light spectra and UV radiation .It shows significant variation in contents of secondary metabolites of plants including terpenoids, phenolic compounds and alkaloids etc. Altitude has proved to be important environmental factor showing significant effect on the contents of secondary metabolites in higher plants. It is reported that there is noteworthy variation in the phenolic content of plant due to effect of altitudinal variation. Genetically uniform plants growing at higher altitude showed higher antioxidant activity compare to plants at lower altitude [7-8].High altitudes and effect of enhanced UV-B radiation on contents of secondary metabolites has been discussed by Korner,1999 [9].The correlation of low temperature with increase of antioxidant secondary metabolite are described by .Ncube,2011.Enhanced productivity of phenolic compounds absorbing, UV-B with antioxidant is reported in response to enhanced UV-B radiation where, enhanced production is a protective response against damage from excessive UV-B radiation due to their UV-shielding properties [10-11].Soil nutrients and soil water availability has direct interlink with biosynthesis of secondary metabolites .Their abundance or limited availability has effect on contents of secondary metabolites in plants .Biosynthesis of secondary metabolites is also dependent of photosynthetic rate which is affected by duration and intensities of stress factors and limits the carbon and energy distribution for the biosynthesis of secondary metabolites and therefore quantitatively affect their levels in plants .In addition to climatic

factors, biotic stress factor is one of the important factors in which plants are exposed in environment of multiple herbivore and pathogenic attacks. [4-12]

India is the leading country known for its highest tonnage per hector grape yield due to the favorable microclimatic condition in the world. Nashik, the Indian wine capital is located in the Western Ghats of Shyadri Vally near the Kalsubai Mountain which lies on Deccan Plateau. Nashik valley is the highest elevated point in Maharashtra state of India. The elevation ensures cooler growing conditions of *V.vinefera* .Since, microbial infections are more common in cooler climates, and vineyards of this region are frequently prone to various pathogenic attacks. Sunlight intensity is also higher in this region resulting in increased photosynthesis and increased production of secondary metabolites. Therefore, the present work aimed to study the effect of environmental factors on Secondary Metabolite diversity including bioactive phenolic compounds and free radical scavenging activity of different aerial parts of *V.Vinefera* from Nashik valley.

II. MATERIALS AND METHODS

Collection of Plant Material

Fresh and healthy aerial plant parts viz. leaf -lamina , Stem and Petiole of *Vitis vinefera* (Jumbo seedless) were randomly collected from the vineyard of Nashik valley, Maharashtra India during June 2016.

Preparation of Extracts

Collected aerial parts were cleaned, cut into small pieces and shade dried at room temperature for fifteen days and grounded to fine powder to store in air tight bags. Organic extracts are prepared by soaking the dry powder (10%) of each part into 90% Methanol and were incubated at room temperature with gentle shaking for 72 h. The supernatants obtained were used for further analysis.

Phytochemical Screening for Secondary Metabolites

Extracts were tested for the presence of different secondary metabolites like flavonoids -lead acetate test [13],cardiac glycosides -Salkowskii test [14], coumarins -NaOH Test [15],tannins -K₂Cr₂O₇ test[13],phenols -ferric chloride test [16], alkaloids-Mayer's reagent test [17], phytosterols-Salkowski

test [14], diterpenes-copper acetate test [18], ellagic acid test [19], and saponin-emulsion test [20] by using various reported methods. The tests were identified by visual observation of colour change or by precipitate formation on addition of specific reagents to the test solution.

Thin Layer Chromatography (TLC)

Different spots of secondary metabolites were detected by thin layer chromatography. Flavonoid (Quercetin) detection was carried out using pre coated silica gel plates purchased from MERK (Germany). Solvent system, N-Butanol: Acetic Acid: Water (4:1:5) was used to obtain best resolution of spots as well as bands. Band visualization was carried out Under UV (254nm) and thereafter by using 5%FeCl₃ as a visualization agent.

Determination of Total Phenolic Content

Total phenolic content was determined by Folin-Ciocalteu method of Zheng and Wang. [21]. Absorbance of the test samples were measured at 725 nm and content of phenolic in extracts were calculated using a Gallic acid (0.1-1.0 mg mL⁻¹) standard curve, and the results were expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

Determination of Total Flavonoids

The total flavonoid content was estimated using Aluminum chloride assay [22]. Absorbance of the test sample was measured at 510 nm and total flavonoid content was calculated as quercetin equivalents from a calibration curve of quercetin. The calibration curve was prepared in the same manner using 0.1-1 mg/mL of quercetin solutions in methanol.

Free Radical Scavenging Activity

The antioxidant potential of plant extracts (leaf lamina, stem and petiole) were estimated by the free radical scavenging ability or donation of hydrogen ions with reference to the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity by the spectrophotometric method reported by Santanu Sannigrahi. with few modifications [23]. 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 and percentage of DPPH scavenging activity or % inhibition was calculated by following equation,

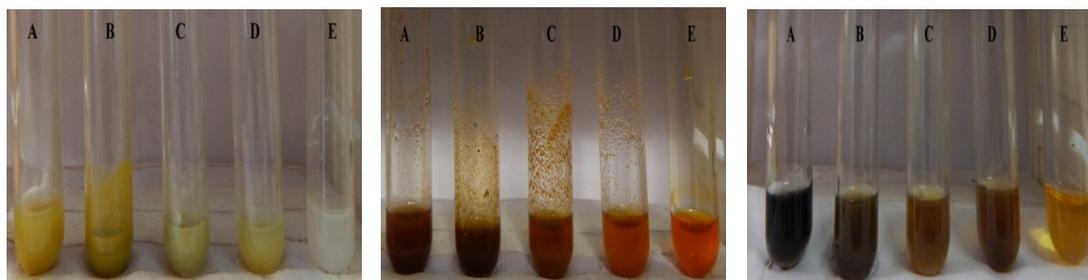
$$\text{Percentage DPPH scavenging activity} = (A_0 - A_1) / A_0 \times 100,$$

(Where, A₀=Absorbance of control, A₁=Absorbance of sample)

III. RESULTS AND DISCUSSION

Phytochemical Screening

Methanolic extracts of leaf lamina, stem and petiole were tested for the presence of different secondary metabolites. The tests were identified by visual observation of colour change or by precipitate formation on addition of specific reagents to the test solution. Phytochemical screening showed that almost all the aerial parts are rich in secondary metabolite which are medicinally important compounds. (Figure.1)

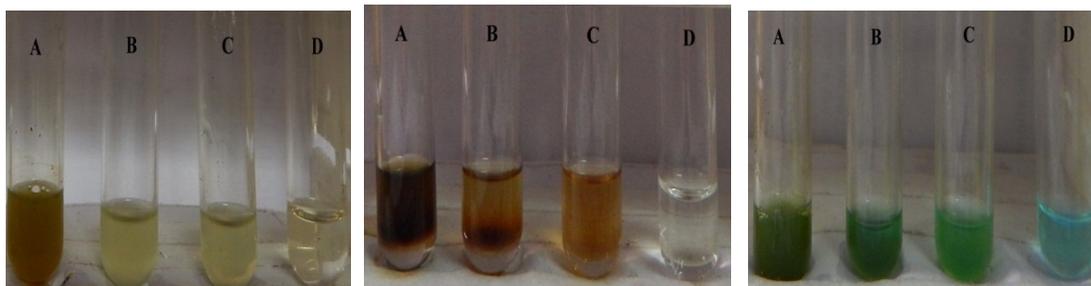


1. Test for Flavonoid

2. Test for Tannin

3. Test for Phenols

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

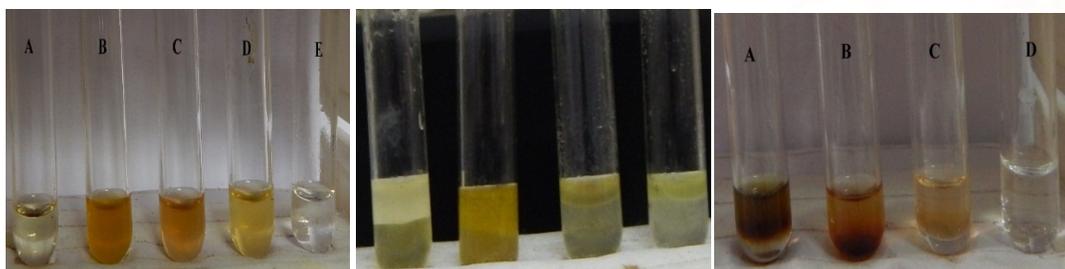


4. Test for Alkaloids

5. Test for Phytosterols

6. Test for Diterpens

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



6. Test for coumarins

7 Test for Saponin

8 Test for Cardiac Glycoside

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

Fig.1 - Phytochemical screening tests for detection of secondary metabolites

Thin Layer Chromatography (TLC)

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 spots as well as bands. The R_f value of flavonoid – quercetin was found to be 0.85.[24]. (Fig. 2)

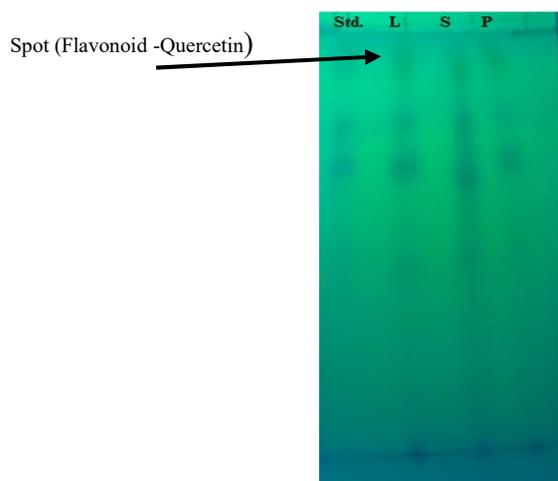


Fig 2: TLC of Jumbo seedless cultivar of *V.vinefera* under UV (254nm) (Std-Flavonoid Standard: Quercetin, L-Leaf lamina , S-Stem ,P- Petiole)

Determination of Total Phenolics

Total phenolic content was determined by Folin-Ciocalteu method and expressed as mg gallic acid equivalent(GAE) per gram of extract using a standard curve of gallic acid ($y = 0.5724x + 0.3721$, $r^2 = 0.968$). Results obtained showed that the total phenolic content of petiole was highest (0.92 ± 0.10) whereas leaf lamina showed lowest amount viz. 0.5 ± 0.07 mg GAE/g .Noteworthy amount of phenolic content of 0.64 ± 0.04 mg GAE/g was observed in stem. (Fig.3)

Determination of Total Flavonoid

The total flavonoid content was determined by aluminum chloride colorimetric method and expressed as quercetin equivalents in mg/g dry powder, and fractions using a standard curve of quercetin ($y = 0.3369x + 0.0149$, $r^2 = 0.9701$). The total flavonoid content of petiole was found to be 0.28 ± 0.12 mg/g quercetin equivalent which was highest in comparison to stem (0.13 ± 0.04 quercetin equivalent) and leaf lamina (0.11 ± 0.01) mg/g quercetin equivalent) (Fig.3)

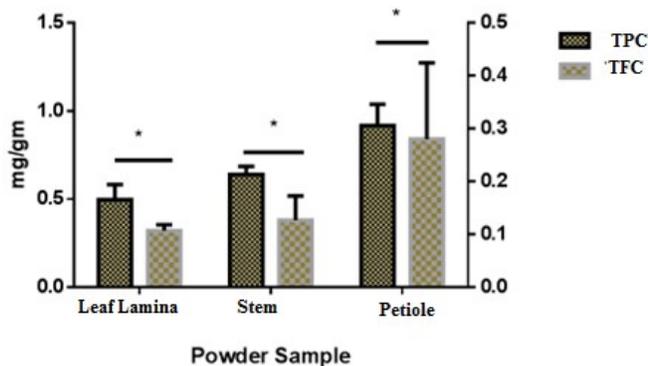


Fig.3. Total phenolic content and Total flavonoid content of Jumbo seedless cultivar

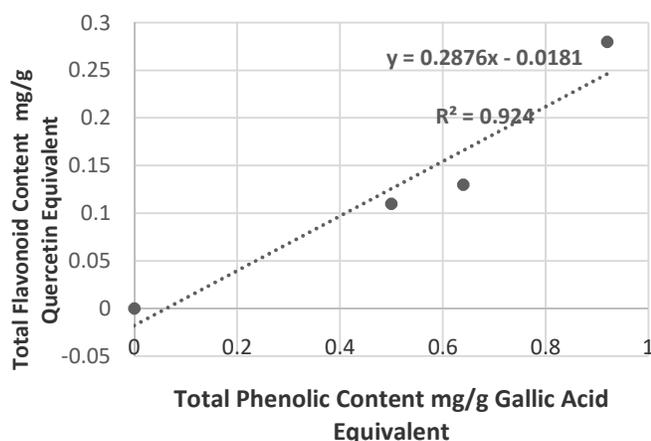


Fig 4: Linear correlation between total phenolic content and total flavonoid content of aerial parts of Jumbo seedless cultivar

Radical Scavenging Activity

The DPPH radical scavenging activity of the polyphenolic extract of different aerial parts of *V.vinefera* is carried out in triplicates. Ascorbic acid was used as reference compound. Order of radical scavenging activity of different aerial parts were found as , Stem(70.01%)>Petiole (67.00%)> leaf lamina(52.26%) respectively .The reduction of DPPH by the extract was either due to the transfer of hydrogen atom or transfer of an electron as most of the secondary metabolites are effective hydrogen donors, which makes them good antioxidants .

Statistical Analysis

All experiments were performed in triplicates. All values are expressed as mean ± standard deviation (SD) of three separate experiments. Linear correlation

between total phenolic content and total flavonoid content of aerial parts of Jumbo seedless cultivar was calculated to establish a relationship between them. Results revealed that there is a satisfactory linear correlation between both the parameters showing R² value, 0.9249. Pearson's Coefficient was found to be 0.9617. The results of statistical analysis indicates that there is strongly positive correlation between total phenolic content and total flavonoid content of studied aerial parts of Jumbo seedless cultivar of *V.vinefera* (Fig.4)

CONCLUSION

The present study showed that almost all the aerial parts of *V.vinefera* are rich source of secondary metabolites like flavonoids ,cardiac glycosides coumarins ,tannins ,phenols, alkaloids ,phytosterols , diterpenes, ellagic acid, and saponins with health potential. TLC revealed the presence of different spots of which R_f value of flavonoid –quercetin was found to be 0.85. Total phenolic content of petiole was found to be highest (0.92± 0.10) whereas leaf lamina showed lowest amount viz. 0.5± 0.07mg GAE/g The total flavonoid content of petiole was found to be 0.28±0.12mg/g quercetin equivalent which was highest in comparison to stem (0.13 ±0.04quercetin equivalent) and leaf lamina (0.11±0.01) mg/g quercetin equivalent. Satisfactory antioxidant activity (Radical scavenging activity) of different aerial parts were found in the order as, Stem (70.01%)>Petiole (67.00%)> leaf lamina (52.26%) respectively. The present work tries to establish a correlation between total phenolic content and total flavonoid content of different aerial parts of black cultivar of *V. vinefera*. The results also revealed the strong positive linear correlation between both the parameters. As Nashik is located at highest elevated point in Maharashtra, vineyards of these area are subjected to various stressful environmental conditions and thereby has positive impact on grapevine profile. Therefore, our study emphasizes that climatic conditions of Nashik region are very much suitable for biosynthesis of various bioactive secondary metabolites in *V.vinefera*.

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