



PRELIMINARY DETERMINATION OF WATER SOLUBLE VITAMINS, PHYTOCHEMICALS AND ANTIOXIDANT POTENTIAL OF DIFFERENT WINE SAMPLES

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ABSTRACT

Since ancient times, wine has been closely linked to the evolution of medicine. Wine is an alcoholic drink typically made from fermented grape juice. Due to modification of polyphenolic compounds during fermentation various studies on phytochemical composition and antioxidant activity in wines have emerged. In the present preliminary/pilot *in vitro* study, five different wine samples were subjected to estimation of Vitamin content, Anthocyanin content, Phenolic content, Flavonoid content, Ethanolic content and Antioxidant potential using various methods. The highest Vitamin B2 content was observed in Red Wine-1 followed by Red Wine-2 > Rose Wine > White Wine-2 > White Wine-1, whereas, Vitamin C content was noted in the order of White Wine-1 > White Wine-2 > Red Wine-1 > Rose Wine > Red Wine-2. The highest Anthocyanin content was revealed in Red Wine-1 followed by Red Wine-2 and Rose Wine, however, both the White Wine samples did not show Anthocyanin content. The highest Phenolic content was found in Red Wine-1 followed by Red Wine-2 > White Wine-2 > Rose Wine > White Wine-1. Similar pattern was observed for Flavonoid content. All the 5 wine samples revealed good antioxidant activity, which was detected using DPPH assay. Antioxidant potential and the components found in wine samples possess cosmetological-related properties. Hence, Wines may possibly find application in cosmetic industry.

KEYWORDS: Wines, Antioxidant, Flavonoids, Phenolic Content, Vitamins.

INTRODUCTION

Wine is one of the oldest traditional alcoholic beverages consumed worldwide. Wines can be classified as red, white, orange, or rose wine.^[1] Wine is an alcoholic drink typically made from fermented grape juice. Yeast consumes the sugar in the grapes and converts it to ethanol, carbon dioxide and heat. Different varieties of

grapes and strains of yeasts produce different styles of wine.^[2] White Wine can be made by the alcoholic aging of the non-shaded mash of green or gold hued grapes or from chosen juice of red grapes. Red Wine is formed from red grapes, which are literally closer to black in colour. Red Wine is considered to be the most classic in the kingdom of wines, mixing the delicious red grapes

with a wide range of aroma. Pink wine having a light pink tone are made up of a mix of “black” and “white” grapes, using the technology of manufacturing white wines. The wine industry in Maharashtra is rapidly developing since last 10 years in terms of area, production and marketing of wines.^[3]

Total of 80% of the grapes grown are grapes for wine production and they contain various polyphenolic compounds, which are extracted in the wines during winemaking. Grapes contain various secondary metabolites particularly flavonoids, phenolic acids, anthocyanins, amino acids and vitamins. Due to modification of polyphenolic compounds during fermentation various studies on phytochemical composition and antioxidant activity in wines have emerged.^[4] The present preliminary research work aims to determine the Vitamin content, Anthocyanin content, Phenolic content, Flavonoid content, Ethanol content and Antioxidant potential of five different wine samples.

MATERIALS AND METHODS

1) Procurement of Wine Samples

Total 5 wine samples, viz., two red wine, two white wine and one rose wine samples were included in the present *in vitro* study which were procured from local market in Mumbai.

2) Estimation of Vitamin B2

The Vitamin B2 content of different Wine Samples was determined according to the method of Dambature WL *et al.*^[5] with slight modification. For this, 1ml of Wine sample was dissolved in 9ml of 10% Methanol. The absorbance of this solution was measured on UV-Visible Spectrophotometer at 445nm. Riboflavin at various concentrations (1-10 ppm) in 10% Methanol was included as a standard. Vitamin B2 content was calculated from the equation ($y = 0.0368x - 0.004$, $R^2 = 0.9992$) obtained from the Riboflavin standard curve. All the determinations were done in triplicate The Vitamin B2 content was expressed in ppm. Experimental Results were expressed as Mean for analysis performed in triplicate.

3) Estimation of Vitamin C

The Vitamin C content of different Wine Samples was determined as stated earlier.^[6] For this, 40ml of Wine sample was taken in a beaker. The sample was decolourised by adding 1-2 g of activated charcoal in the same beaker. The solution was then filtered and colourless filtrate was obtained. To 20ml of this filtrate 150ml of Distilled Water was added and titrated against 0.005M Iodine Solution using starch solution as indicator. Titration was performed till blue-black endpoint was obtained. Ascorbic acid at various concentrations (10-100ppm) was included as a standard. All the determinations were done in triplicate. Mean values of triplicate determinations were used to plot the graph. Vitamin C content was calculated from the equation ($y = 0.1704x + 0.4667$, $R^2 = 0.9985$) obtained

from the Ascorbic acid standard curve. The Vitamin C content was expressed in ppm. Experimental Results were expressed as Mean for analysis performed in triplicate.

4) Estimation of Anthocyanin

The Anthocyanin content of different Wine Samples was determined according to the method of Lee *et al.*^[7] For this, two 1ml aliquots of Wine Samples were placed each in 10ml volumetric flask. The volume was completed with two buffer solutions, viz., potassium chloride buffer 0.025M (pH 1.0) and sodium acetate buffer 0.4M (pH 4.5) respectively. Absorbance was then measured simultaneously at 520nm and 700nm within 20-50min of preparation. All the determinations were done in triplicate. Mean values of triplicate determinations were used to plot the graph. Anthocyanin content was calculated from the equation:

Anthocyanin pigment (cyaniding-3-glucoside, equivalent mg/L =

$$\frac{A \times MW \times DF \times 1000}{\epsilon \times l}$$

$A = (A_{520nm} - A_{700nm})_{pH 1.0} - (A_{520nm} - A_{700nm})_{pH 4.5}$

MW (Molecular Weight) = 449.2 g/mol

DF (Dilution Factor) = $\frac{\text{Final Volume}}{\text{Initial Volume}}$

$\epsilon = 26900$ molar extinction coefficient for cyd-3-glu
1000 = factor for conversion from g to mg

5) Estimation of Ethanol Content

The Ethanol content of different Wine Samples was determined according to the Relative Density Method on Alcoalyzer. For this 50ml sample was distilled. The distillate was then analysed on Alcoalyzer (Anton-Paar). All the determinations were done in triplicate. Experimental Results were expressed as Mean for analysis performed in triplicate. Ethanol content was expressed as Alcohol Content (% v/v).

6) Phenolic Content Estimation

The total phenolic content of different Wine samples was determined using Folin-Ciocalteu reagent according to the method of Pandima Devi *et al.*^[8] with slight modification. Gallic acid at various concentrations (1 to 3.5 mg/ml) was included as a standard. All the determinations were done in triplicate in a microplate. Mean values of triplicate determinations were used to plot the graph. Total phenolic content was calculated from the equation ($y = 1.3829x - 1.2774$, $R^2 = 0.9715$) obtained from the Gallic acid standard curve. The total phenolic content was expressed as Gallic acid equivalent (GAE) in mg/ml. The colour controls were also included in the study. The OD values obtained for colour controls were subtracted from Test Values and then the Test Values were used for further calculation. Experimental results were expressed as Mean for analysis performed in triplicate.

7) Flavonoid Content Estimation

The method of Oyedemi *et al.*^[9] with slight modification was used to estimate total flavonoids content of the Wine Samples. The method is based on formation of a complex flavonoids-aluminums. Briefly, a volume of 0.1 ml of 2% AlCl₃ ethanol solution was added to 0.1 ml of each test solution in a microplate. After one hour of incubation at room temperature, the absorbance was measured at 420nm using Multimode Reader (Synergy HT, BioTek). Yellow colour indicated presence of flavonoids. Quercetin at various concentrations (10 to 100 µg/ml) was included as a standard. All the determinations were done in triplicate. Mean values of triplicate determinations were used to plot the graph. Total flavonoid content was calculated from the equation ($y = 0.0205x + 0.1285$, $R^2 = 0.9949$) obtained from the Quercetin standard curve. The colour controls were also included in the study. The OD values obtained for colour controls were subtracted from Test Values and then the Test Values were used for further calculation. Total flavonoids content was expressed as quercetin equivalent in mg/ml. Experimental results were expressed as Mean for analysis performed in triplicate.

8) DPPH radical-scavenging assay

The free radical scavenging activities of Wine samples were measured by 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay with slight modification.^[10] For this, 0.1 ml of DPPH solution (0.1mM) in methanol was added to 0.1 ml of different Wine samples in a microplate. After incubating for 30 minutes in dark, the absorbance was measured at 517nm using Multimode Reader (Synergy HT, BioTek). Ascorbic acid at various concentrations (0.1 to 2 µg/ml) was included as a standard that showed IC₅₀ value of 1.65 µg/ml. Negative control without Wine samples was set up in parallel. The colour controls were also included in the study. The OD values obtained for colour controls were subtracted from Test Values and then the Test Values were used for further calculation. The percent DPPH scavenging activity was calculated as, DPPH scavenged (%) = $\frac{A_{\text{Negative control}} - A_{\text{Test}}}{A_{\text{Negative control}}} \times 100$. Where, A is absorbance. Experimental results were expressed as Mean for analysis performed in triplicate.

RESULTS

Table 1: Estimation of Vitamin B2.

No.	Wine Type	Vitamin B2 Content (ppm)
1	Red Wine-1	16.22
2	Red Wine-2	14.65
3	White Wine-1	0.43
4	White Wine-2	1.25
5	Rose Wine	4.5

Note: Mean of triplicate determinations

Table 2: Estimation of Vitamin C.

No.	Wine Type	Vitamin C Content (ppm)
1	Red Wine-1	5.49
2	Red Wine-2	4.71
3	White Wine-1	5.88
4	White Wine-2	5.50
5	Rose Wine	5.10

Note: Mean of triplicate determinations

Table 3: Estimation of Anthocyanin.

No.	Wine Type	Anthocyanin Content (mg/L)
1	Red Wine-1	16.12
2	Red Wine-2	13.02
3	White Wine-1	Nil
4	White Wine-2	Nil
5	Rose Wine	3.75

Note: Mean of triplicate determinations

Table 4: Ethanol Content Estimation.

No.	Wine Type	Ethanol Content (% v/v)
1	Red Wine-1	12.79
2	Red Wine-2	12.72
3	White Wine-1	12.34
4	White Wine-2	10.65
5	Rose Wine	12.99

Note: Mean of triplicate determinations

Table 5: Phenolic Content Estimation.

No.	Wine Type	Gallic Acid Equivalent (mg/ml)
1	Red Wine-1	237.2
2	Red Wine-2	209.5
3	White Wine-1	96.49
4	White Wine-2	109.2
5	Rose Wine	103.8

Note: Mean of triplicate determinations

Table 6: Flavonoid Content Estimation.

No.	Wine Type	Quercetin Equivalent (mg/ml)
1	Red Wine-1	0.617
2	Red Wine-2	0.392
3	White Wine-1	0.059
4	White Wine-2	0.242
5	Rose Wine	0.146

Note: Mean of triplicate determinations

Table 7: DPPH radical-scavenging Activity.

No.	Wine Type	DPPH Scavenged (%)
1	Red Wine-1	61.43
2	Red Wine-2	87.44
3	White Wine-1	86.55
4	White Wine-2	84.30
5	Rose Wine	90.13

Note: Mean of triplicate determinations

DISCUSSION

Wine is a beverage from the alcoholic fermentation of ripe and fresh grapes or fresh grape juice. Historical records show that the medicinal use of wine by man has been a practice made for over 2000 years. Thus since ancient times, wine has been closely linked to the evolution of medicine.^[11] In the present preliminary *in vitro* study, vitamin content, anthocyanin content, ethanol content, phytochemical content and Antioxidant potential of 5 different wine samples were evaluated using various methods.

Thirteen vitamins are universally recognised at present and classified by their biological and chemical activities. Vitamins are classified as either water soluble or fat soluble. In humans there are 13 vitamins: 4 fat soluble (A,D,E,K) and 9 water soluble (8 B Vitamins and Vitamin C).^[5] Vitamins metabolic roles mostly concern coenzyme in diverse pathways, reduction-oxidation systems, antioxidant activities as well as membrane integrity, cellular signalling, cellular protection etc. B-group vitamins have been shown to display a catalytic function and act as coenzymes in diverse metabolisms.^[12] A critical role of vitamin C is the synthesis of connective tissue, particularly collagen.^[11] In addition, vitamin C is an important antioxidant which can remove and neutralise oxidants in the body.^[8] Along with its role in collagen synthesis, there is also evidence that vitamin C increases the proliferation of dermal fibroblasts, a function important for wound healing.^[13] In the present pilot study, the highest Vitamin B2 content was observed in Red Wine-1 followed by Red Wine-2 > Rose Wine > White Wine-2 > White Wine-1 [Table-1], whereas, Vitamin C content was noted in the order of White Wine-1 > White Wine-2 > Red Wine-1 > Rose Wine > Red Wine-2 [Table-2].

Besides, Anthocyanins are wide spread and biologically active water-soluble phenolic pigments responsible for a wide range of vivid colours that are present in fruits, vegetables and coloured grains. As per scientific literature Anthocyanins have a potential applications in different industrial fields, namely in the textile and food industries, as well as in the development of photosensitizers for dye-sensitized solar cells, as new photosensitizers in photodynamic therapy, pharmaceuticals, and in the cosmetic industry, mainly on the formulation of skin care formulations, sunscreen filters, nail colorants, skin and hair cleansing products, amongst others. Anthocyanin extracts from red wine were found to exhibit UV-filter capacity, attenuate the production of reactive oxygen species in human skin keratinocytes and fibroblasts and showed inhibitory activity of skin-degrading enzymes (Tyrosinase, elastase, hyaluronidase, and collagenase) in the absence of cytotoxic effects.^[14] In the present *in vitro* study, the highest Anthocyanin content was revealed in Red Wine-1 followed by Red Wine-2 and Rose Wine, however, both the White Wine samples did not show any Anthocyanin content [Table-3].

The Ethanolic content of the wine samples was determined by using alcolyzer and the content was noted in the range of 10.65% to 12.99% [Table-4] which was found to be within specified limits.

Moreover, Phenolics and Flavonoids are commonly known as the largest phytochemical molecules with antioxidant characteristics. Phenolics are naturally occurring compounds present in several foods such as fruits, cereals, vegetables and beverages. The prominent health benefits of phenolic compounds are antioxidant activity, antibacterial, antiviral, neuroprotective potential, anticancer activities, appropriate for skin health and suitable for wound healing. Moreover, flavonoids are considered as an important constituent in different pharmaceutical, medicinal, nutraceutical and cosmetic applications.^[15]

Phenolic compounds are diverse group of bioactive metabolites that include flavonoids, flavones, flavanols, quinones and tannins.^[16] Phenolic acids possess much higher *in vitro* antioxidant activity than well-known antioxidant vitamins. Radical scavenging mechanism is known for the antioxidant activity of phenolic acids.^[17] Further, phenolic compounds contribute to antioxidative action through various mechanisms, viz., scavenging free radicals, by stabilizing lipid peroxidation, through redox properties and by chelating metals.^[18,19]

Phenolic content is commonly estimated using the Folin-Ciocalteu (FC) method, which measures the reducing capacity of the sample. This method involves reacting the sample with the FC reagent, then adding a base (like sodium carbonate) and measuring the absorbance of the resulting blue-coloured solution at a specific wavelength. The absorbance is then correlated to a standard curve, usually using gallic acid as a reference, to quantify the total phenolic content.^[20] Hence, total phenolic content of Wine samples was determined by Folin-Ciocalteu method. The highest Phenolic content was displayed by Red Wine-1 followed by Red Wine-2 > White Wine-2 > Rose Wine > White Wine-1 [Table-5].

Besides, total flavonoid content was determined by Aluminium chloride method. Flavonoid content in plant extracts can be estimated using the aluminum chloride colorimetric assay, which involves forming a complex between the flavonoids and aluminum chloride, and measuring the absorbance of the resulting solution at a specific wavelength using a spectrophotometer.^[20] In the present study, the highest Flavonoid content was displayed by Red Wine-1 followed by Red Wine-2 > White Wine-2 > Rose Wine > White Wine-1 [Table-6]. Almost every group of flavonoids has a capacity to act as antioxidants. Flavonoids can prevent injury caused by free radicals in various ways and one of the way is the direct scavenging of free radicals. Flavonoids stabilize the reactive oxygen species by reacting with the reactive compounds of the radical.^[21] The antioxidant potential of

flavonoids is more robust than vitamin C and Vitamin E.^[22]

Antioxidants are becoming ever more interesting to scientists in food field and medicinal professionals due to their protective roles in food against oxidative deterioration and in the body against oxidative stress-mediated pathological processes.^[23] Antioxidants regulate various oxidative reactions naturally occurring in tissues. Furthermore, they terminate or retard the oxidation process by scavenging free radicals, chelating free catalytic metals and also by acting as electron donors. In general, *in vitro* antioxidant tests using free radical traps are relatively straightforward to perform. Among free radical scavenging methods, DPPH method is furthermore rapid, simple (not involved with many steps and reagents) and inexpensive in comparison to other test models. DPPH method is based on the reduction of DPPH a stable free radical. The free radical DPPH gives a maximum absorption at 517nm (purple colour). When antioxidants react with DPPH, the stable free radical becomes paired off in presence of a hydrogen donor and is reduced to DPPH-H and as a consequence the absorbance decreases from the DPPH radical to the DPPH-H form, resulting in decolorization (yellow colour). More the decolorization more is the reducing ability. The test has been the most accepted model for evaluating free radical scavenging activity of any new drug. Furthermore, the conventional cuvette assay can be replaced by 96-well plate assay.^[24] In the present *in vitro* study, the antioxidant potential of different Wine samples was detected using DPPH assay. The highest antioxidant activity was noted with Rose Wine followed by Red Wine-2 > White Wine-1 > White Wine-2 > Red Wine-1 [Table-7].

Thus, the present *in vitro* study has demonstrated presence of Vitamin B2, Vitamin C, Phenolic Compounds, Flavonoid compounds, Anthocyanin content and Antioxidant potential of wine samples. Literature review and current findings indicate that Wine samples contain compounds that possess cosmetological-related properties such as anti-aging, anti-inflammatory, skin-whitening and wound healing properties. Hence, wines can be possibly one of the useful sources for Cosmetic Industry.

CONCLUSION

In the present pilot study, Vitamin B2, Vitamin C, Anthocyanin, Ethanolic, Phenolic and Flavonoid Contents as well as Antioxidant potential of 5 different Wine samples were determined using various assays. The highest Vitamin B2 content, Anthocyanin, Phenolic and Flavonoid contents were observed in Red Wine-1, whereas, the highest Vitamin C content was noted in White Wine-1. Furthermore, all the 5 wine samples revealed strong antioxidant potential. The components found in Wine samples along with Antioxidant potential suggest that Wines might find application in cosmetic industry.

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