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PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF VARIETY GRAPES FROM KURDISTAN IRAQ

Dalaram Sulaiman Ismael

ABSTRACT

This study was carried out to determine the amount of total phenols, total flavonoid and antioxidant activity of 7 grape cultivars grown in Kurdistan rejoin of Iraq. The results showed that total phenols, flavonoid and antioxidant activity in the berries varied among the investigated cultivars. Total phenolic content, total flavonoid content ranged from 112.77 to 249.19 mg GAE/100g FW, 584.23 to 288.55 mg of rutin equivalents/100 g of (fresh sample) respectly and antioxidant capacity value ranged from 41.79 to 92.30%. Tahlik cultivar had the highest value of antioxidant capacity, flavonoid and total phenolic content. The lowest total phenolic, flavonoid content and the lowest value of antioxidant capacity were found in Abhar cultivar. Present results showed statistically significant correlations with the free radical scavenging activity. There was a very strong positive correlation (R^2 =1, p <0.05) between the antioxidant activity and total phenolic content and a strong positive correlation (R^2 =0.9735, p <0.05) between the antioxidant activity and total flavonoid content.

Keywords: grape; phenolic; antioxidant activity; flavonoid

INTRODUCTION

Grape is a fruit grown in different parts of the world and widely cultivated because of its economic and importance in making juice, wine and raisins. South America has significant role in production and export of grapes **(Ruiz, 2011)**. Table grape used for eaten and other kind grapes are used mainly to produce wine. Italy is the main wine producer and France is the second wine producer in the World **(FAO, 2010)**. Grapes are rich sourse of flavonoids, anthocyanins and phenolic compounds, which have significant role for health benefits **(Yang et al., 2009)**. The anthocyanins participate in several reactions that responsible for changes the color of grape products, due to formation of polymeric pigments and through co pigmentation **(Wrolstad, et al., 2005)**. Grape has antioxidant properties it can prevent the oxidative damage of cells **(Park et al., 2003)**. The consumption of fresh grape has the health benefits which have associated with broadly known and connected to the extravagance of phenolic compounds, for example, anthocyanins, Gallic corrosive, catechin and a wide assortment of procyanidins. These mixes have been set up to have an extensive variety of biochemical and pharmacological impacts, for example, antiatherogenic, anticarcinogenic, cell reinforcement exercises and calming **(Dell Agli et al., 2004; Darra et al., 2012)**. Right now phenolic compounds are accepting much consideration because of their helpful wellbeing impacts identified with their capacity to ensure against oxidative cell harm if happened master oxidant and cancer prevention agent awkward nature. Truth is told responsive species play both a poisonous and gainful part and the harmony between them must be looked after **(Forman et al., 2010)**. Phenolic compounds have a wide spectrum of health benefits such as anti-mutagenic, anti-bacterial, anti-inflammatory, and antioxidant activity and minimize oxidative stress **(Celep and Rastmanesh, 2013)**. Polyphenolic compounds product the free radicals in the body and reduce damage to DNA **(Bub et al., 2003)**. Antioxidant properties of grapes are attributed at least partly to their phenolic content. Consumption of fruits rich in antioxidant substances such as phenolic nalyses proved that frequent adequate intake of fruits could help to prevent cardiovascular diseases **(Rautiainen et al. 2012)**. diabetes **(Hegde et al., 2013)** and cancer **(Wang et al., 2014)**. The majority of agro industrial residues of grape are mostly solid by products including stalks and the liquid filtrate; residues are composed of carbohydrates, vitamins, minerals, lipids, water, and compounds with important biological properties such as phenolic compounds and fiber, depending on the types of wastcompounds and vitamin C is inversely associated with risks of non communicable diseases. Epidemiological studies and meta-ae, climatic and cultivar **(Ahmad and Ali Siahsar, 2011)**.

Scientific hypothesis

The main object of this study was to evaluate the total phenolictotal content (TPC), total flavonoid content (TFC) and antioxidant activity using the spectrophotometrical determination of different varieties grape from different location in Kurdistan Rejoin of Iraq Republic. We assume there are different concentrations of flavonoids in different varieties of grapes.

MATERIAL AND METHODOLOGY

Chemicals and reagents

Folin Ciocalteu reagent (FCR), Gallic acid (GA), [DPPH=2, 2-diphenyl-1-picrylhydrazyl], rutin, [Na_2CO_3 = Sodium carbonate], Methanol (CH_3OH), Sodium nitrite, [$AlCl_3$ = Aluminum chloride], Sodium hydroxide were acquired from Sigma Chemical Co. in Bratislava, Slovakia Republic). The reagents and chemicals utilizes as a part of this investigation were of explanatory review (99%).

Material

Grape samples harvested at fully ripened and matured, Seven grape varieties including four of varieties black colors (Mula Hassan, Awilka. Mamarik, Tahlik) and three red colors (Sadani, Abhar, Kamali) were obtained from different location in (three Cities) in Kurdistan rejoin of Iraq Republic. The seven grape varieties are described in Table 1.

Extraction

For extraction of bioactive mixes from new grapes utilizing separated by the altered strategy depicted by **Yang et al. (2009)**. 200 g of grapes were mixed for 3 min in 200 g of 80% CH_3COCH_3) by utilizing Warring blender with low speed for expelling seeds. Included another sum (200 g of 80% CH_3COCH_3) after evacuation of the seeds, at that point the grapes were mixed for 2 min by a similar blender with fast, at that point the blend was homogenized by homogenizer for 5 min and sifted with vacuum under an ice shower. To expel (CH_3COCH_3) in the filtrate utilized a rotating evaporator at 40 °C until the point that the heaviness of the vanished filtrate break even with 20% of original filtrate weight. The concentrates were put away at -40°C until utilize.

Estimation of total phenolic content

Add up to phenolics decided utilizing Folin-Ciocalteu reagent (FCR) by the strategy portrayed **Lachman et al. (2003)**. Test removed (0.05 g to 1 mL of 80% methanol then 2.5 mL of (FCR) and 4 mL of distilled water added to a 50 mL carafe. Following 5 minutes 7.5 mL of [Sodium Carbonate=Na_2CO_3 (25%)] added to the carafe then the volume moved toward becoming 50 mL with distilled water. The blend was (permitted) brooded for (2.5 hours) at research temperature. At that point the absorbance was measured at 765 nm against a blank (80% methanol) by a spectrophotometer (Shimadzu710 Japan). The result was calculated as (mg GAE/100fw) the average content polyphenol compounds in samples were obtained from ±SD six replicates.

Estimation of total flavonoid content

For determination flavonoid content of grapes extract used the modification method according to **(Jia et al., 1999)**. 0.20 mL of 1:20 weakened grape separates was blended with 1.10 mL of refined water and with 0.070 mL of 4 % sodium nitrite arrangement at that point permitted to respond for 7 min. From that point forward, a 0.12 mL of 10% aluminum chloride was included and permitted extra respond for 7 min after that 0.60 mL of 1 M NaOH was included. The last volume make to 3 mL by including refined water. Absorbance of the blend was measured using spectrophotometer at a wavelength 510 nm against arranged clear from rutin standered. For assurance flavonoid content utilizing rutin standard bend and communicated as the mean ±SD for six replications of the sample (mg rutenE/100g Fw).

Determination of antioxidant activity

For the analysis of free radical scavenging activity 2, 2-diphenyl-1picryl hydrazyl (DPPH) was utilized by a changed technique for **Brand-Williams et al. (2005)**. To get a stock arrangement: 0.020 g DPPH was diuted to 100 mL methanol at that point kept in a cool and dim place. Prior to the examination, 1:10 dilution of the stock was made with methanol, at that point 3.9 cm^3 of the arranged DPPH was added to a cuvette and the absorbance at 515.6 nm was measured by spectrophotometer (UV/ VIS 710) at time At0 was written. After that 0.1 cm^3 of the solution (sample) was added and mixed then the absorbance was measured at 10 minutes (At10). The absorbance of time 0 and 10 minutes at 515.6 nm was measured. The dependence A = f (t) was measured.

The percentage of inhibition in given time (%) = (At0 – At10 / At0) x 100

Statisic analysis

The results were evaluated statistically using the Analysis of Variance. Procedure compares the data in seven varieties. The results assays were expressed as mean ±SD of six repeated samples. The data was utilized the F-test in the restricted investigation of change (ANOVA) by the F-test, if the *p*-value is less than 0.05, there is a statistically significant difference between the means at the 95% level; the Multiple Range Tests will indicate which implies are essentially unique in relation to others. This technique was utilized to separate between the methods for Fisher's minimum huge contrast (LSD) methodology. Examination was utilizing SAS programming 9.4. Connections among different parameters were likewise utilized and decided at $p < 0.05$, of six rehashed tests.

RESULTS AND DISCUSSION

Total polyphenolic content

The total phenol content (TPC) for seven grape varieties was determined using the Folin-Ciocalteau method. The outcomes found that the levels of phenolic compound in the grape tests essentially vary ($p < 0.05$) among all examples utilized from the distinctive grape assortments. In the present investigation as appeared in (Table.1), it was distinguished, that aggregate polyphenols content in tests ran from (112.77 ±0.34 to 249.19 ±0. 29 mg GAE/100FW) mg of Gallic corrosive reciprocals per100 g fresh weight.

Table 1 The total phenolic, total flavonoid, contents and antioxidant capacity of 7 grape varieties.

Varieties	Locality	Color	**Total Phenolics (TPC)** ($mg.100g^{-1}$)	**Total Flavonoid (TFC)** ($mg.100g^{-1}$)	Antioxid **antcapacity (TAC)** (%)
MULA HASSAN	Erbil	Black	212.91 ±0.59a	498.96 ±0.48a	78.89 ±0.27a
AWILKA	Erbil	Black	124.36 ±0.37b	291.83 ±0.22b	46.41±0.40b
MAMARIK	Dohuk	Black	173.83 ±0.53c	380.91 ±0.30c	64.30 ±0.44c
TAHLIK	Dohuk	Black	249.19 ±0.29d	584.23 ±0.07d	92.30 ±0.56d
SADANI	Erbil	Red	128.31 ±0.4e	294.49 ±0.31e	47.67 ±0.29e
ABHAR	Sulaimanya	Red	112.77 ±0.34f	288.55 ±0.29f	41.79 ±0.25f
KAMALI	Dohuk	Red	236.14 ±0.41g	503.37 ±0.25g	87.22 ±0.64g

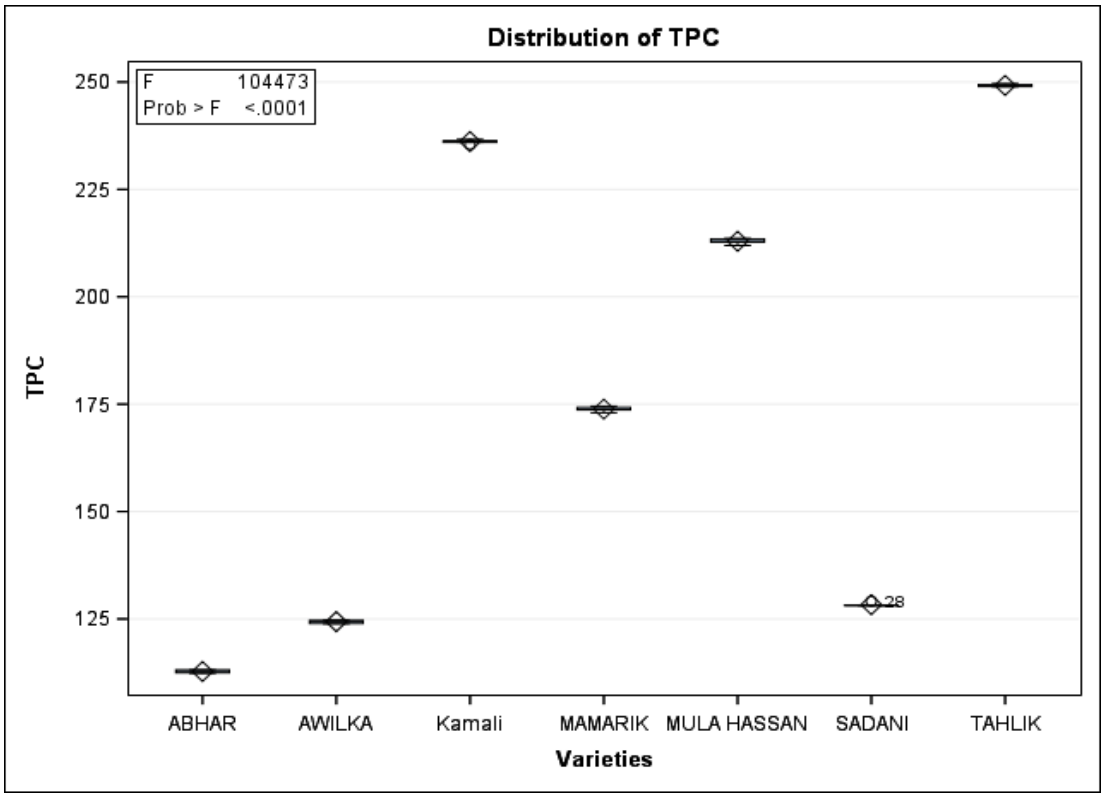

Figure 1 Distribution of total polyphenolic content.

As per picked up comes about, the polyphenols content (TPC) in all tried were fundamentally differenced relying upon grape cultivar and assortment.

Statistically significant highest value of total polyphenols was found in Tahlik black color and the lowest TPC content was found in Abhar red variety. According to the average contents of total polyphenols in fresh matter of grape, there is the following line showed in Table 1, Figure 1: Tahlik black color with (249.19 ±0.29 mg of Gallic acid equivalents/100g) > Kamaly red color (236.14 ±0.41) > Mula Hassan black color (212.91 ±0.59) > Mamarik black color (173.83 ±0.53) > Sadani red color (128.31 ±0.4) >Awilka black color (124.36 ±0.37) >Abhar red color (112.77 ±0.34) mg of Gallic acid equivalents /100g FW. The phenolic compounds composition of fruits depended on genotypes, environmental factors and postharvest processing conditions **(Benvenuti *et al.*, 2004; Kadir *et al.*, 2009)**. Many researchers have also been observed differences in the phenolic content among grape varieties **(Frankel et al., 1995; Simonetti et al., 1997; Burns et al., 2000)**. The composition and the quantity of phenolic compounds vary due to difference in varieties, species and maturity of the grapes, the place and location where the grapes are grown **(Burin et al., 2010)**.

Total flavonoid

The results as shown in Table 1, Figure 2 Tahlik variety obtained the highest total flavonoid content (584.23 ±0.07d mg rutin E/100 g FW) milligram rutin equivalents per 100 gram fresh weight grape followed by Kamali, Mula Hassan, Mamarik, Sadani, Awilka and Abhar. The difference might be due to variety grapes or the different factor: climate, cultivar, cultivation site and ecological factors, cultural practices or genetic factors influenced on phenolic and flavonoid content.

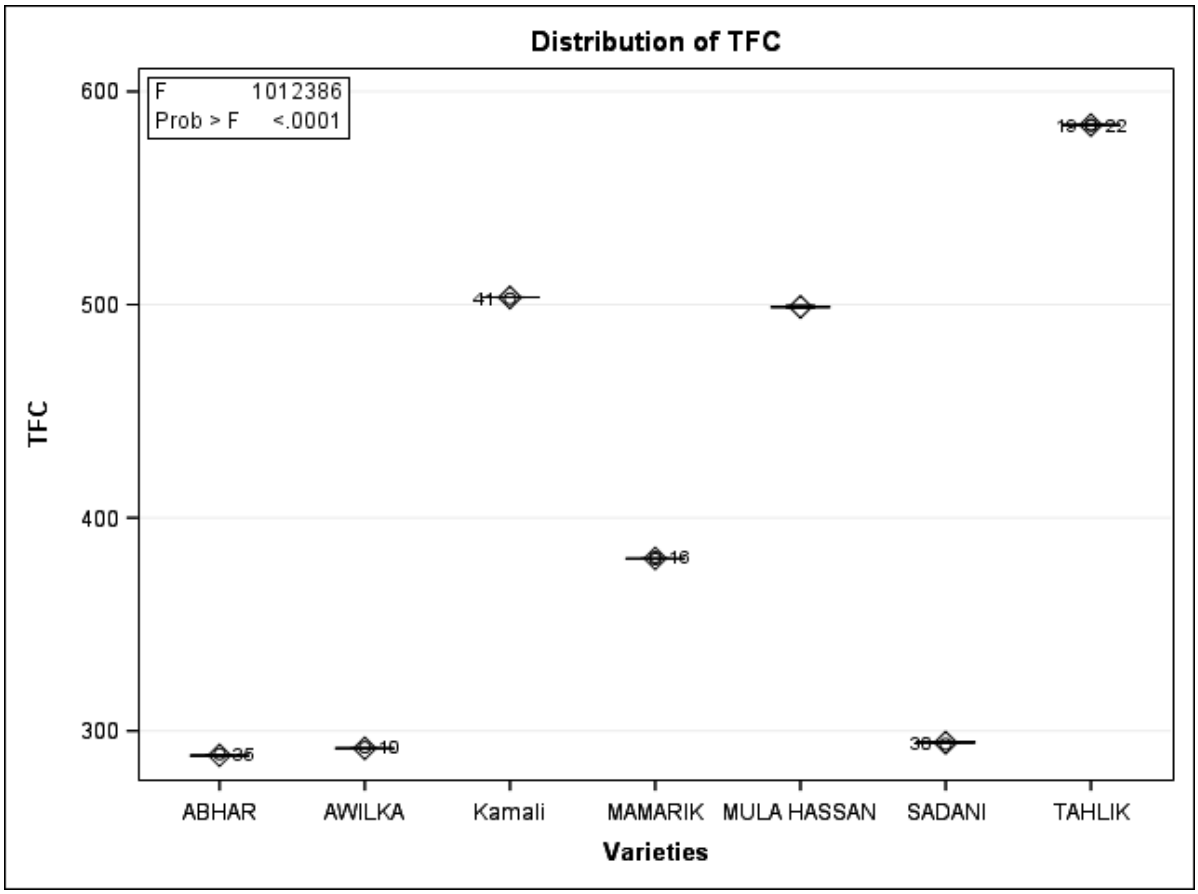

Figure 2 Distribution of total flavonoid content.

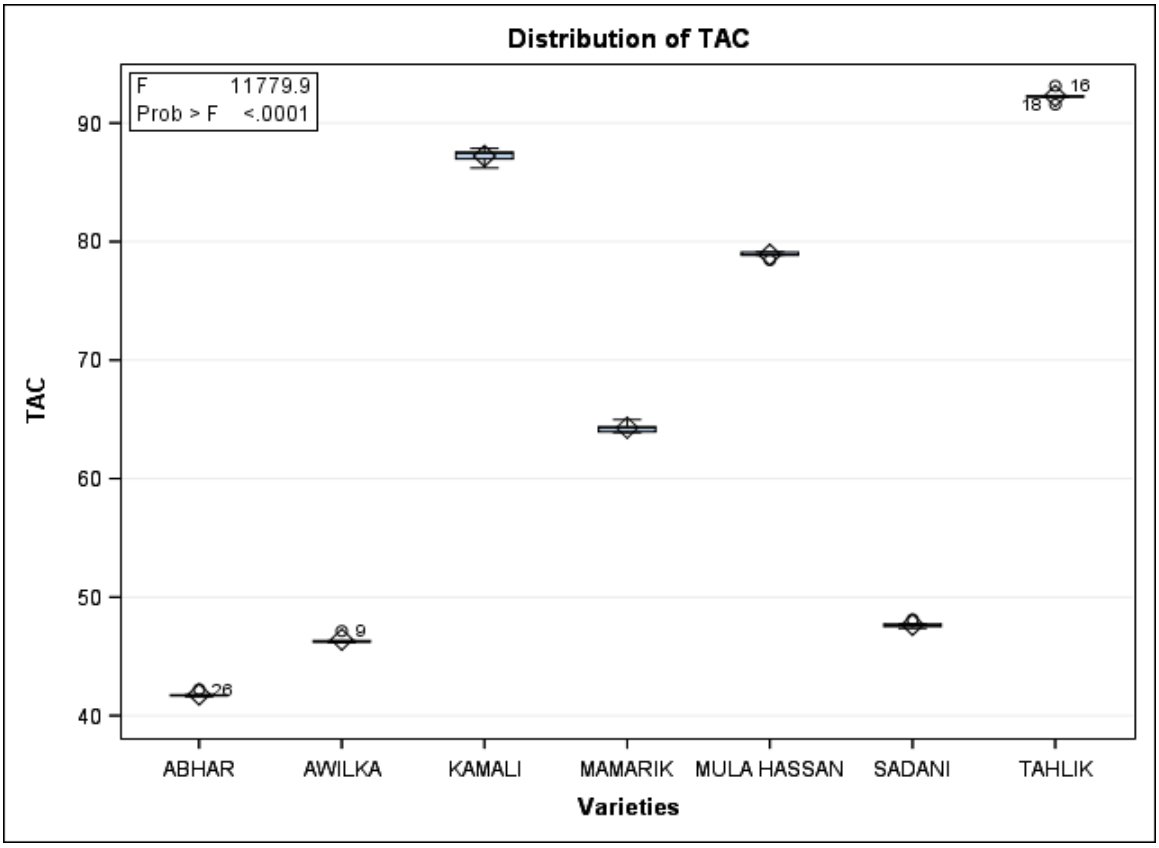

Figure 3 Distribution of antioxidant capacity.

DPPH ASSAY

The free radical rummaging exercises of concentrates rely upon the capacity of cancer prevention agent mixes (sample) to lose hydrogen atom and the basic compliance of parts **(Shimada et al., 1992)**. DPPH radical's has ability to bind hydrogen atom which has a radical scavenging property. The solution of DPPH prepared in methanol (CH_3OH) is converted into DPPH-H molecule in the presence of an antioxidant agent, as shown by the equation. Discoloration occurs due to the decreasing quantity of DPPH radicals in surroundings. The staining of DPPH in this manner mirrors the radical searching action of the investigated separate **(Guo et al., 2007)**. The strategy depends on the lessening of alcoholic DPPH• arrangements within the sight of a hydrogen giving cancer prevention agent (AH) to the non-radical frame DPPH-H.

DPPH• +A-H→DPPH-H + A•

The antioxidant capacity of the grapes samples as shown in Table 1. Figure 3.The antioxidant capacity values for seven varieties are significantly different and decreased by the order 92.30% Tahlik > 87.22 % Kamali > 78.89% Mula Hssan > 64.30 % Mamarik > 47.67 % Sadani > 46.41% Awilka > 41.7 9% Abhar. In present study the antioxidant activity of grape is influenced by their phenolic composition content **(Dávalos et al., 2005)**. The antioxidant capacity values ranged from 41.79 ±0.25 to 92.30 ±0.56 of the 7 grape samples, Tahlik black color from Duhok showed a higher value of antioxidant capacity and Abhar red color from Sulaimanya had a lowest antioxidant capacity.

Correlation between Antioxidant Capacity value and Total Polyphenols

Analysis of variance (ANOVA) was used for linear correlation coefficients to appraise (evaluate) the relationships between TAC with TPC, TFC. Our result optioned statically very strongly positive correlations (R =1; p <0.05) was found (Figure 4) between add up to cancer prevention agent limit esteems and aggregate polyphenol content and statically solid positive correlation

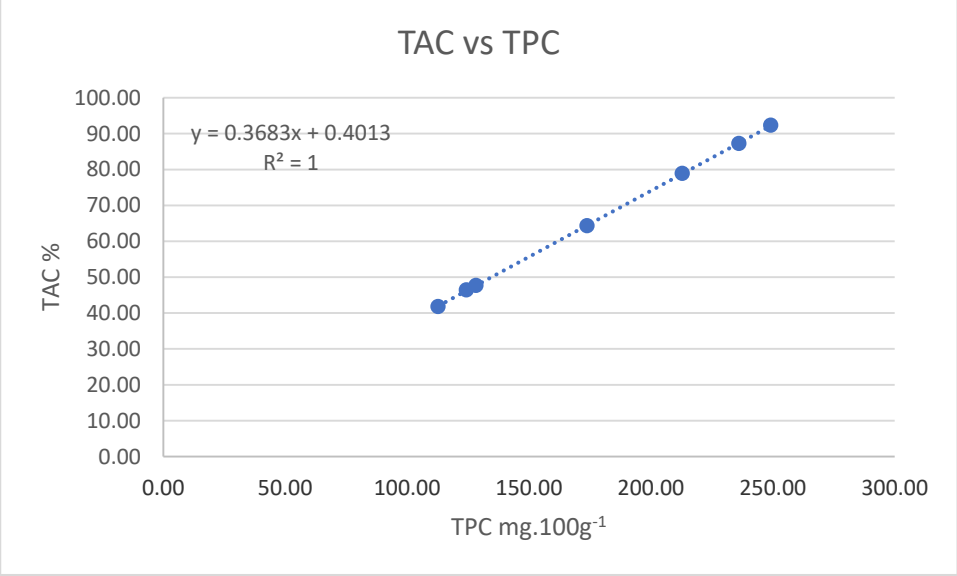

Figure 4 Correlation between Antioxidant Capacity value and Total polyphenolic content.

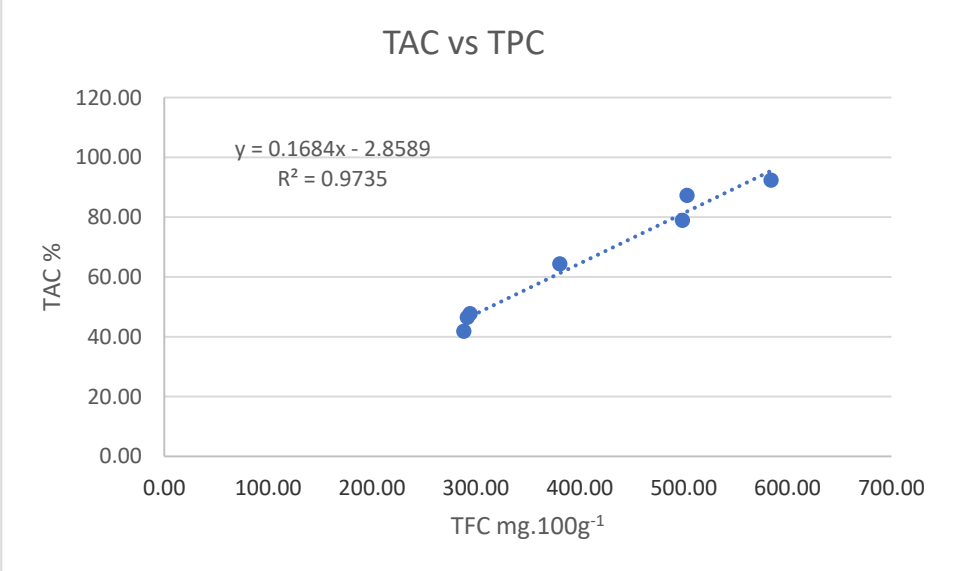

Figure 5 Correlation between Antioxidant Capacity value and Total Flavonoid content.

(R = 0.9735; p <0.05) between cell reinforcement limit esteem and aggregate flavonoid content was discovered (Figure 5). A positive relationship between's aggregate phenolic and cell reinforcement limit has likewise been discovered **(Hulya-Orak, 2007)**. In exhibit examine a high connection comes about among add up to phenolic substance and cancer prevention agent limit are in concurrence with the consequences of numerous specialists. (0.97 and 0.95, p <0.05) between cancer prevention agent limit and aggregate phenolic content (measured by the FRAP techniques). Numerous analysts found a high connection among cancer prevention agent limit, add up to phenols, flavonoid, anthocyanins content content **(Wang and Lin, 2000; Burns et al., 2000)**]. The outcomes exhibited here in gave significant information of cell reinforcement limit, phenolic substance and flavonoid content for seven grape assortments, the outcome demonstrated that there is a high relationship among expanded phenolic content and the expanded cancer prevention agent limit **(Susana et al., 2014)**.

CONCLUSION

The phenolic content, antioxidant activity, and the correlation between phenolic content and antioxidant capacity were studied in varieties grape. The comparison of seven grape varieties showed that Tahlik variety grape provided significantly highest total phenolic content (average value of 249.19 ±0.29 mg.100g^{-1} FW grape), total flavonoid content (584.23 ±0.07 mg of rutin equivalents/100gFw), as well as strong antioxidant capacity (92.30 ±0.56%). showing that there is a correlation between increased phenolic content and increased antioxidant activity. Strong correlations were found between total phenolic, total flavonoid contents and their antioxidant activities.

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Contact address:

Dalaram Sulaiman Ismael, Salahaddin University in Erbil, College of Agriculture, Department of Food Technology, Erbil Azady show street 45-123/62, Iraq Republic, E-mail: dlaram.dizayee@gmail.com