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Comparative Evaluation of the Phytochemical Constituents and the Antioxidant Activities of Five Moroccan Pepper Varieties (*Capsicum Annuum L.)*

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Abstract: The aim of this study was to characterize the bioactive constituents and to evaluate the antioxidant activity of five pepper (*Capsicum annuum L.)* cultivars. Phenols, flavonoids, flavonols and ascorbic acid were quantified in the pericarp of the peppers studied. The antioxidant activity of their extracts was evaluated by the method of radical scavenging of DPPH•. The solvents used for the extraction of these metabolites are water, methanol and ethanol at 75%. Preliminary results have shown that the composition of the peppers in bioactive elements varies depending on the type pepper and the solvent extract. The hot and sweet red pepper showed high vitamin C content (respectively 34.49 and 33.4 mg / 100 g FW) followed by hot green pepper, sweet green pepper and yellow pepper. It was also found that capsaicin vary significantly ($P<0.005$) among peppers studied. Capsaicin contents ranged from 6, 78 to 32, 50 mg/100 FW. The maximum capsaicin content was noticed in hot green and hot red peppers. The highest contents of phenolic compounds, flavonols and flavonoids were in sweet and hot red peppers. The highest radical scavenging activity (IC50) was observed in red and yellow peppers for ethanolic and methanolic extracts.

Keywords: Phytochemical, Antioxidant activity, *Capsicum annuum* L., Pepper.

1. INTRODUCTION

The genus Capsicum comprises a large and diverse group of plants producing fresh fruits varying from sweet to hot. Originating from Latin American tropical regions, spreading from Chile to the southeastern United States, the Capsicum species are cultivated and appreciated around the world. Due to the unique flavor, spice uses, and presence of hot taste of the fruits, they are consumed fresh and in different forms of processed products[1].

Capsicum cultivars have been identified as potential solanaceous crop with high antioxidant activity[2]. They are important both economically and nutritionally, because they are excellent sources of natural colors and antioxidant compounds including flavonoids, phenolic acids and carotenoids[3, 4]. Intake of these compounds in food is an important health-protecting factor. They are also helpful in prevention of widespread diseases. There are growing evidences suggesting that antioxidants may maintain health and prevent many chronic diseases, such as certain cancers, cardiovascular diseases and other aging-related diseases[5].

Sweet pepper comprises numerous other chemicals including steam-volatile oil, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fiber, and mineral elements[6]. Many sweet pepper constituents have importance for nutritional value, flavour, aroma, texture, and colour. Many of these compounds are antioxidants that exert their biological effects through free radical scavenging, protein binding and interaction with human signal transduction pathways[7].

Phenolic are secondary metabolites in plants composed of phenolic acids and polyphenols (includes flavonoids). A number of studies have demonstrated phenolic and flavonoids to possess numerous biological, antioxidants, pharmacological, and medicinal properties, including antimutagenic, anticarcinogenic, anti-inflammation and anti-allergy properties, as well as having the ability to modify gene expression[3].

Peppers are also a good source of carotenoids, which can vary in composition and concentration owing to differences in genetics and maturation[8, 9]. Color of ripe pepper fruits originates from carotenoids, and two red carotenoids, capsanthin and capsorubin can naturally be found only in Capsicum sp.

Pepper (*Capsicum annuum L.*) is cultivated worldwide mainly as a fresh vegetable or a food additive. Presently, two cultivation types of pepper are largely distinguished based on the presence of pungency, that is, chili (hot) pepper and sweet pepper (paprika), which account for both consumption types. Chili pepper has a strong pungency and is used as a food additive (or a spice) to flavor food. Pungency, a major factor for consumption of the Capsicum fruits, is caused by capsaicinoids, which are unique alkaloids restricted to the genus Capsicum. The concentration of capsaicin varies according to the variety of pepper, the geographical origin and the climatic conditions. Capsaicin causes inflammation of the mucous membranes[10]. Green and red peppers are valued for their sensory attributes of color, pungency and aroma and capsaicin content of red pepper is one of the main parameter that determines its commercial quality[11]. Conversely, sweet pepper is non-pungent with larger fruits and is used as a vegetable. Sweet peppers are known to have resulted from the domestication of wild chili peppers, which are usually hot[12].

Valued for its attractive color and strong flavor, pepper is consumed widely throughout the world. In Morocco, as in many other countries, it is one of the most important vegetables in terms of both volume of production and commercial value. Capsicum spp. exhibit great genetic diversity in terms of color, size, shape, and chemical composition. Researchers have recently recognized that Capsicum fruit also vary greatly in their content of antioxidant vitamins and phytochemical. The objective of this study was to quantify the contents of phenolic acids, flavonoids, flavonols, capsaicin and ascorbic acid present in

the pericarp of five Moroccan peppers (*Capsicum annuum* L.) cultivars and to evaluate their antioxidant activity.

2. MATERIEL AND METHODS

2.1. Sample collection*:* Fresh hot and sweet green, Sweet yellow, sweet and hot red peppers *(Capsicum annum L.)* were obtained from a local supermarket in Béni Mellal, Morocco **(Fig.1)**. The fruit peppers were kept in cold storage at 10°C for 2 days prior to being processed. All fruit peppers were washed, trimmed, the cores discarded and the seeds and interlobular material removed. After being halved, the peppers were diced with a knife and were drained in a sieve to remove any excess fluid. Chemical analyses were conducted on fresh peppers.

2-2. Vitamin C: The vitamin C content of the aqueous extract was determined using the method demonstrated by Benderitter et al. [13]. Briefly, 75 µL DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg CuSO4.5H2O in 100mL of 5M H2SO4) was added to 500 µL reaction mixture of 300 µL appropriate dilution of hydrophilic extract with 100 µL of 13.3% trichloroacetic acid and distilled water. The reaction mixture subsequently incubated for 3 hours at 370C, then 0.5mL of 65% H2SO4 (v/v) was added to the medium, and the absorbance was measured at 520 nm, and the vitamin C content of the sample was then subsequently calculated from the calibration curve prepared with ascorbic acid as standard.

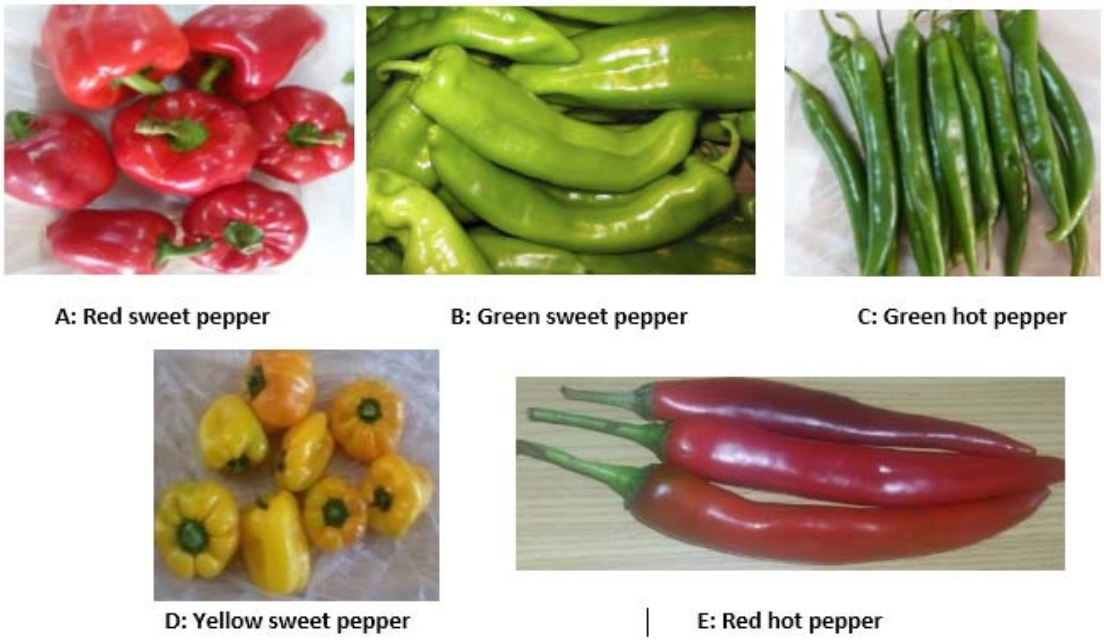

Figure 1: Samples of bell peppers used in the analysis.

2.3. Capsaicin: Capsaicin content in the samples was estimated by spectrophotometric measurement of the blue coloured component formed as a result of reduction of phosphomolybdic acid to lower acids of molybdenum[14]. Two grams of fresh sample was extracted with 10 ml of dry acetone using pestle and mortar. The extract was centrifuged at 10,000 rpm for 10 min and 1ml of supernatant was pipetted into a test tube and evaporated to dryness in a hot water-bath. The residue was then dissolved in 0.4 ml of NaOH solution and 3 ml of 3% phosphomolybdic acid. The contents were shaken and allowed to stand for 1 h. The solution was filtered to remove any floating debris and centrifuged at 5000 rpm for 15 min.

Absorbance was measured for the clear blue solution, thus obtained, at 650 nm using reagent blank (5 ml of 0.4% NaOH+3ml of 3% phosphomolybdic acid). Capsaicin content calculated from the standard curve was expressed as mg 100 g^{-1} on FW.

2.4. Preparation of extracts: A fresh fruit sample without seeds was sliced and stored at -20 °C for 16 h, before grinding it in a stainless steel blender for 30 s. The solvents used are deionized water, organic solvents (methanol and ethanol (25:75 v/v), in a ratio of 1:10, in an ice bath with a stirring mechanism for 15 min. The homogenate was centrifuged at 3640 rpm [15]. The resultant extracts were used to determine total phenolic, flavonoid and flavonol contents and their antioxidant activity. The extracts were conserved at 4°C until analysis.

2.5. ***Total phenolic:*** The content of phenolic compounds in the crude extract was determined considering the colorimetric method of Folin-Denis. In order to measure the phenolic content, 0.1 mL of the supernatant it was mixed with 2.8 mL of deionized water, 2.0 mL of 2% sodium carbonate (Na2CO3), and 0.1 mL of Folin Ciocalteau reagents[15]. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 750 nm and compared with the absorbance of the control of deionized water. Gallic acid was chosen as a standard. The calculation of total phenol content was based on the calibration curve of the gallic acid standard and the data was expressed as milligram gallic acid equivalents (GAE) 100 g-1 fresh weight.

2.6. ***Total flavonoids:*** Flavonoid content was determined by colorimetric assay [16]. In brief, 1ml of extract was mixed with 4ml of deionized distilled water and 0.3ml of 5% NaNO2. After 5min, 0.3ml of 10% AlCl3 was added, and after another minute, 2ml of 1M NaOH were added. The final volume was brought up to 10ml with deionized water, stirred, and lectures were taken at 415nm (UV-VIS spectrophotometer Cary 100, Varian Australia PTY LTD, Australia). Total flavonoids were expressed on a fresh weight (FW) basis as milligrams of quercetin equivalents per 100g.

2 .7. *Total flavonols:* Total flavonols content of extracts was determined by the method of Kumaran & Joel [17]. 2ml of extracts solution, 2ml of 20 g/l AlCl3 ethanolic solution and 3ml of 50g/l sodium acetate solution were prepared. The absorbance was read after 2.5 h at 20°C at 440nm. Total flavonols content were expressed on a fresh weight (FW) basis as milligrams of rutine equivalents per 100g.

2.8. Assay of DPPH radical scavenging activity: The free radical-scavenger activity was determined by the DPPH assay, as described previously by Campos *et al.*[18]. The antiradical activity of extracts was evaluated using a dilution series, in order to obtain a large spectrum of sample concentrations. This involved the mixing of 250µl of DPPH solution (6 mg of DPPH in methanol) with an appropriate amount of extract or compound, followed by homogenization. After incubation in the dark at room temperature for 30min, quantification of the remaining DPPH radicals was recorded by using absorption set at 517 nm. Antiradical efficiency was established using regression analysis at a 95% significance level ($P<0.05$). Results are presented in IC50 values, which represent the weight of sample, required to scavenge 50% of the DPPH radicals available.

2.8. Statistical analysais*:* The analysis was carried out in three replicates for all determinations. The mean and standard error of means were calculated. The data were analyzed by one way analysis of variance (ANOVA). Significance of the differences was defined as $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Vitamin C: Vitamin C (Ascorbic acid), a water soluble natural antioxidant, is present in peppers in considerable amounts when compared with other vegetables[19]. Vitamin C contents of the various

peppers ranged from 159.01 to 344, 9 mg/100g FW **(Fig. 2A).** These results confirm that the consumption of 100g of fresh peppers provides the recommended daily administ rat ion of ascorbic acid (100-200mg) [19]. Red hot and sweet pepper presented higher ascorbic acid content, whereas the lowest values were found in yellow sweet pepper, green hot and sweet pepper. These results exceed those obtained by Topuz and Özdemir[20] for peppers of Turkish origin (15.2 and 64.9 mg / 100g FW).

The vitamin C content found in this research was within reported ranges in other studies, for example, a variation ranged from 238.35 to 455.4 mg 100 g-1 FW was reported by Cruz-Pérez et al.[21]. Zhuang *et al.*[22] reported that the ascorbic acid content of peppers is mainly dependent on the cultivars. Peppers are considered an important source of ascorbic acid (170-280 mg/100 g FW); therefore, they have been attributed health benefits[23].

3.2. Capsaicin: Capsaicin is the compounds, which are responsible for the pungency of pepper fruits and their products [24]. Capsaicin and several related compounds are called Capsaicinoid[25]. Pure capsaicin is a volatile, hydrophobic, colorless, odourless, and crystalline to waxy compound. The pungency and flavour are fruit attributes of Capsicum[26] because Capsaicin and dihydrocapsaicin are an alkaloids that are responsible for 90 % of the intense organoleptic sensation of heat[27, 28]. These compounds are recognized for their therapeutic effects on gastric ulcers and rheumatoid arthritis[29]. Among the peppers capsaicin values averaged from 6,78 to 32, 5 mg/100g FW **(Fig. 2B).** Higher concentration was found in green hot pepper (32, 5 mg/100g FW) and red hot pepper (30, 36 mg/100g FW). The present finding agrees with those reported earlier showing that capsaicin content in green hot peppers was higher than the red ripe ones[30]. However, they are low compared to those obtained by Kumar *et al* [31] for bell pepper varieties (38-120 mg/100g). The content of capsaicinoids depends on several factors, including the genetic factor, cultivar, and stage of development of the fruit[32]. Furthermore, environmental conditions affect the accumulation of these alkaloids, mainly the temperature, light intensity[33], water stresses[34], and plant nutrition[35]. The importance of capsaicin active substance is gradually increasing due to its use in medicinal applications and the intensive areas of use of Capsicum sp. as a food complement.

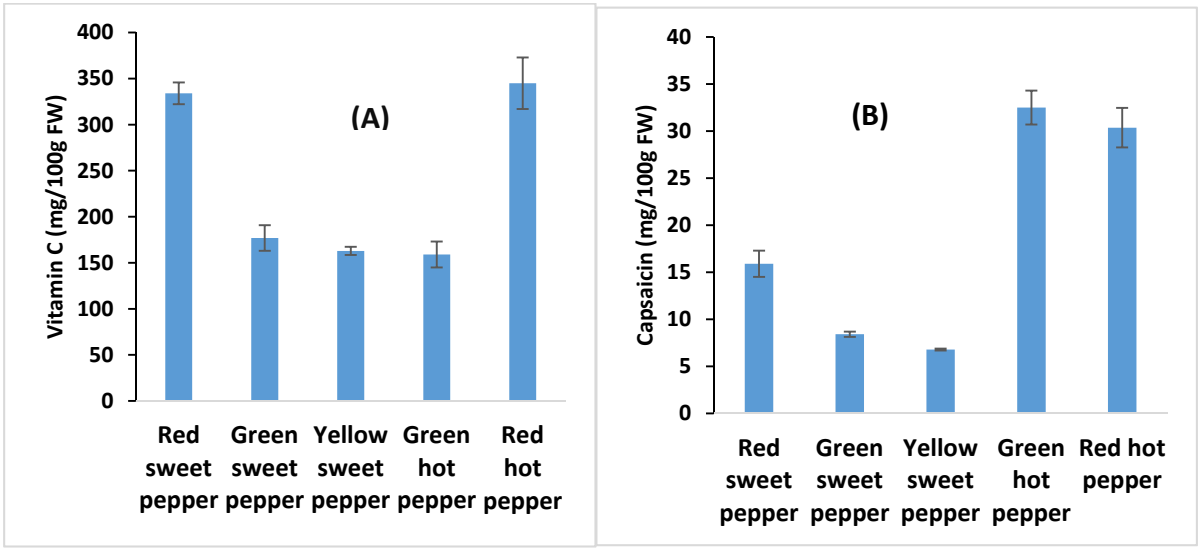

Figure 2: Vitamin C (A) Capsaicin content (B) in the five fresh peppers *(Capsicum annum L.).*

3.3. Total phenols: Peppers are an important source of total phenols, which are mainly localized in the peels[36].The results of total phenol contents of peppers samples are shown in **figure 3**. In general, high levels of total phenols were found in all the pepper extractions studied. The statistical analysis indicated a significant difference ($p \leq 0.05$) between peppers and solvent for extraction. Red peppers presented the highest value for aqueous extract (381, 02 mg GAE/100 g FW) and Green pepper the lowest (133, 12 mg GAE/100 g FW) for the ethanolic and methanolic extracts (**Fig. 3**). These results are in agreement with those reported by Kevers *et al.* [37]for red, yellow and green peppers (296, 284 and 215 mg/100g, respectively). The Yellow pepper presented a total polyphenol content of 329 mg GAE/100 g FW for ethanolic extract. Medina-Juarez *et al.*[38], Vinson *et al.*[39] and Sun *et al.*[40] reported lower levels of total phenols in Bell peppers than those found in the present study. Contrarily, Helmja *et al.*[41] reported a higher content of these compounds in pungent pepper (480 mg/100g FW).

The content in total phenols in the present study depended on the variety and color of the bell pepper fruits. Higher contents in total phenols were found in bell peppers with red color, followed by yellow, and then by Green. These results differ from the findings of Blanco-Ríos *et al*[42], who found that the variety Orion (green) had the highest concentration in this compound, while no differences were detected between the varieties Mazurca (red), Simpaty (orange), and Taranto (yellow). In another study, Sun *et al.* [40] found greater phenolic content in red peppers, followed yellow, and finally green, as in the present study.

It is well known that content of phytochemicals, including phenolic compounds present in vegetables, is affected by the specie and type of pepper, agronomic conditions, maturity[43], postharvest handling and pre and postharvest treatments applied to the fruit[44].

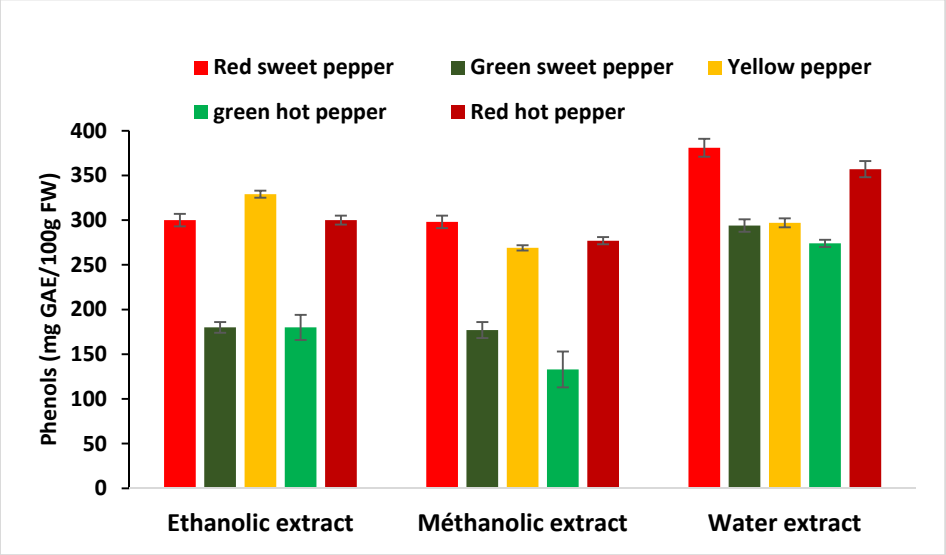

Figure 3: Total phenolic content in the five fresh peppers *(Capsicum annum L.).*

3.4. Flavonoids and flavonols: Phenolic compounds, especially flavonoids, possess different biological activities, but the most important are antioxidant activity, which is associated with a reduced risk of cancers and cardiovascular diseases[45, 46]. Peppers contain a very rich polyphenol pattern, which includes hydroxycinnamates, flavonols and flavones[36]. Flavonoids are a family of compounds with a C6–C3–C6 skeleton structure. Flavanols, flavonols and anthocyanins are included in this group

The total flavonoids content **(Fig. 4A)** was significantly higher in the red hot pepper (60, 9 mg QE/100g FW for methanolic extract and 50, 1 mg QE/100g FW) for ethanolic extract). The best yields of flavonoids for the peppers studied are obtained for methanolic extracts. However, the lowest yields are obtained for the aqueous extracts. Howard *et al.*[43] reported flavonoid contents from 17.17 to 85.49 mg QE/100g FW in similar pepper varieties (*Capsicum annuum* L.). Sun *et al.*[40] and Lee et al[3] reported total flavonoids from not detectable to 80mg QE/100mg FW in Bell peppers of different colors. They observed a content of total flavonoids similar to those found in this study. Marinova *et al* [47] found a range of flavonoids between 13.7 (red pepper) and 27.4 (green pepper) mg QE/100g FW. These values contrast with those reported by Materska and Perucka[48], who observed greater contents of flavonoids in red peppers than in green ones.

The flavonol contents vary depending on the type of solvent used for their extraction. The best yields of flavonols are obtained with the aqueous and methanolic extracts (**Fig.4B**). The red sweet pepper has the highest content of flavonol for aqueous extracts (17, 25 mg RE/100 g FW). Among peppers studied, the flavonol values averaged from 2, 27 to 9, 83 mg RE/100g FW in green peppers, and from 2, 68 to10, 78 RE/100g FW in yellow sweet pepper. These results are in agreement with those obtained by Hallmann & Rembiałkowska[49], where flavonol contents varied from 8 to 14 mg/100g FW. These variations in flavonoid, flavonol and phenol contents have been associated to pepper maturity, type of cultivar and growing conditions[43].

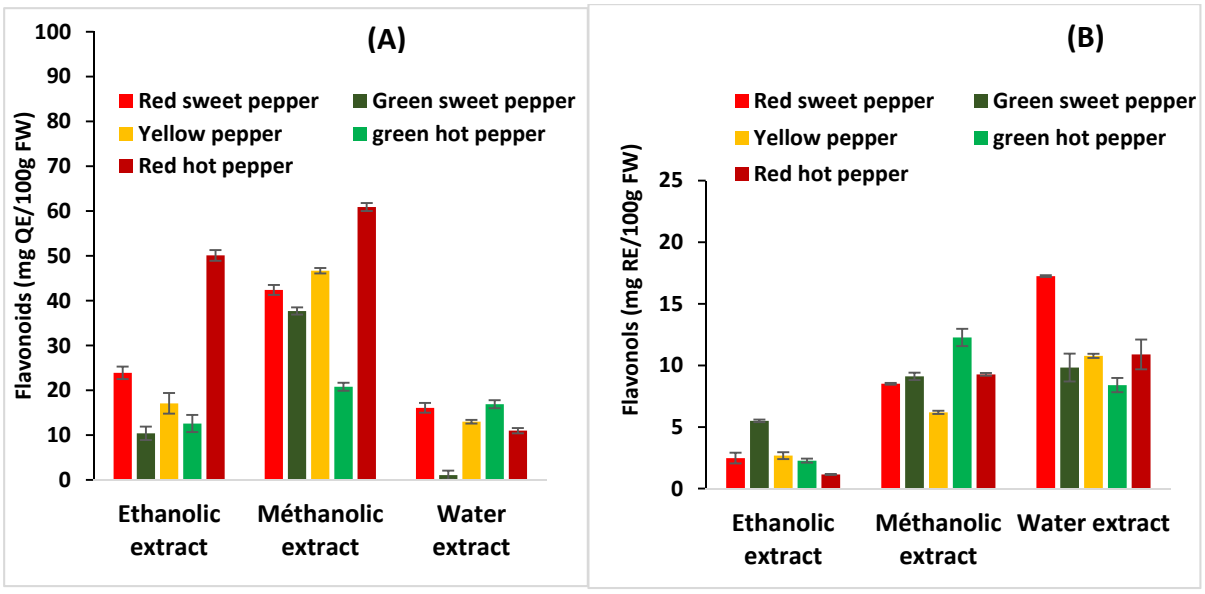

Figure 4: Flavonoids (A) Flavonols (B) content in the five fresh peppers *(Capsicum annum L.).*

3.5. Antioxidant activity: Among vegetables, bell peppers have been extremely popular for the abundance and types of antioxidants they contain. The phytochemical antioxidants that deserve special mention due to their strong capacity to scavenge free radicals are polyphenols, which are found in high quantities in bell peppers, whose levels vary strongly during growth and ripening[50]. Antioxidant activity is an important parameter to establish the health functionality of a food product.

The antioxydant capacity of fruits and vegetable has been tested using a wide variety of methods. In the present study, free radical (DPPH) scavenging assay were used to evaluate the antioxidant activity of fresh pepper. Data are reported in **Figure 5**. All extracts were able to reduce the stable free radical DPPH to the yellow-colored DPPH. The radical scavenging activity (IC_{50}), exerted by Moroccan

peppers extracts, ranged from 52, 21 to 323, 4 µg/ml. The best antioxidant activities are obtained for the red hot pepper (IC_{50} value: 61, 83 to 70, 14 µg/ml), yellow sweet pepper (IC_{50} value: 72, 57 to 82 µg/ml) for the methanolic and ethanolic extract and red sweet pepper (IC_{50} value: 78,1to 81, 8 µg/ml) for the various extracts. Green peppers presented modest radical scavenging activity with an IC50 value ranged from 220, 87 to 323, 4 µg/ml for methanolic and ethanolic extracts.

In the present study, the antioxidant activity was significantly affected by the cultivar. On average, the red bell peppers showed higher activity, followed by yellow and finally green. Chávez-Mendoza *et al.* [51] reported that the green fruits have a lower activity than the red ones. The results were similar to those of Blanco-Ríos *et al.*[52], who found higher antioxidant activity in the red peppers, but followed by green ones, then yellow.

Phenolic compounds play a major role in the antioxidant activity in the bell peppers [40]. In the present study, red and yellow peppers presented the highest concentration of total phenols in all extract, which coincides with a higher antioxidant activity.

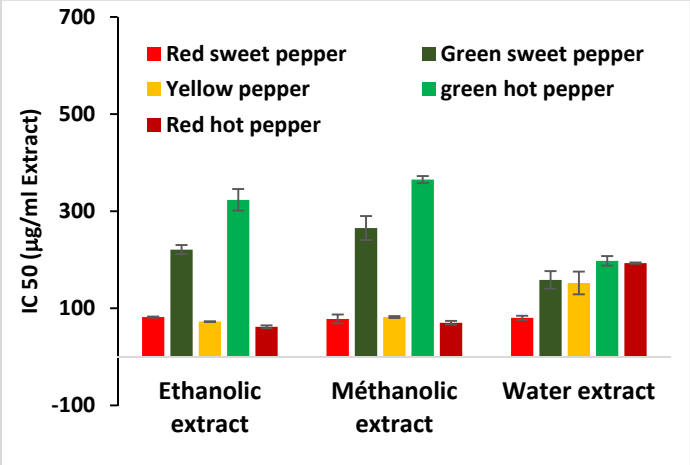

Figure 5: Antioxidant activity of the various extracts fresh peppers *expressed in IC_{50}.*

3.6. Correlations: The antioxidant activity of bell peppers can be attributed also to the content in vitamin C, carotenoids, and capsaicinoids, and therefore it is important to analyze the correlation between the antioxidant activity and the bioactive compounds of bell peppers due to the influence of other soluble compounds in addition to polyphenols, which could affect the total antioxidant capacity[53].

Linear regressions were performed with collected data in order to know which bioactive compounds are contributing to antioxidant activity (**Fig. 6, 7, 8).** Results revealed that the total phenolic contents were highly correlated to antioxidant activity. The correlation coefficients were r^2=0.96, r^2=0.98 and r^2=0.87 respectively for aqueous, methanolic and ethanolic extracts. Earlier, Lee *et al.*[3] reported that phenolic compounds correlated well (r^2=0.86) with antioxidant activity. Zhuang *et al.* [22] found a correlation between the antioxidant activity and the total phenols in bell pepper varieties. Similary, Medina-Juárez *et al.*[38] reported a close correlation between total phenols and antioxidant activity in bells peppers harvested in Zamora, Sonora, México (r = 0.91).

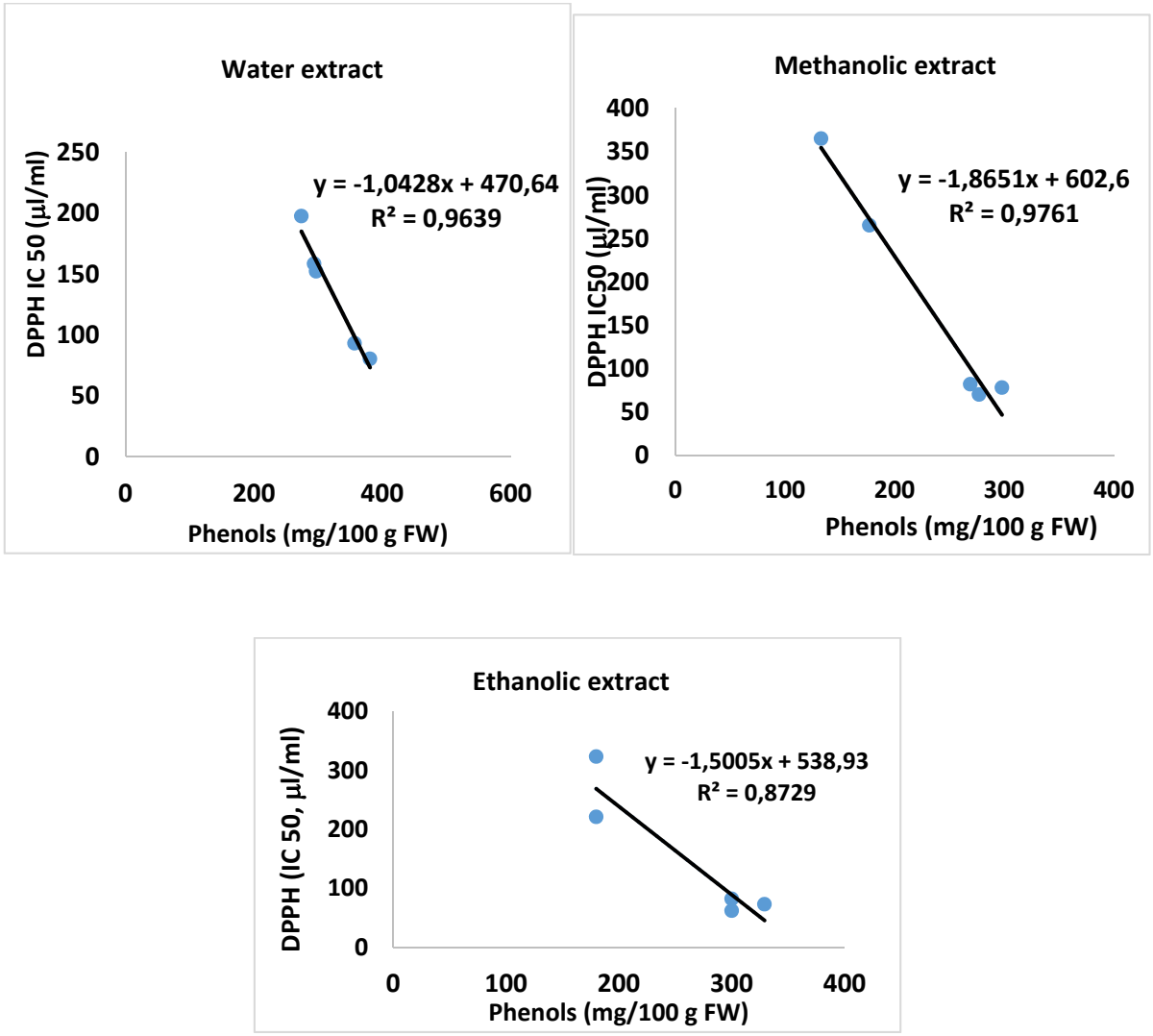

Fig 6: Correlation between antioxidant capacities (DPPH) and total phenolic**.**

While, Segura et al.54 did not found correlation between total phenolic compounds and antioxidant capacity in Chiltepin and Habanero peppers, which may indicate that antioxidant capacity could be affected by the presence of phytochemicals such as carotenoids.However, total content of flavonoids **(Fig. 7)** and flavonols do not correlate with the antioxidant power observed for extract peppers in this study, except methanolic extract who present a significant positive but weaker correlation between total flavonoids and antioxidant activity (r^2=0.76). Zimmer et al. (2012), Medina-Juárez *et al.* reported that the contents of flavonoids and total phenolic compounds correlated strongly with the antioxidant activities.

The strongest significant lineal correlations were found between vitamin C content with the free radical scavenging activity (DPPH•) for the aqueous extract (r^2=0.88) (**Fig. 8).** By contrast, for the methanolic and ethanolic extracts, the antioxidant activity and the content of vitamin C are weakly correlated. Hwang *et al.* [55] reported a positive correlation between ascorbic acid, total phenols and antioxidant activity of cooked peppers. Campos *et al.*[56] found high correlations between antioxidant activities and total phenolic contents in mashua and oca tubers. It is well known that phenolic compounds exert antioxidant activity in biological systems.

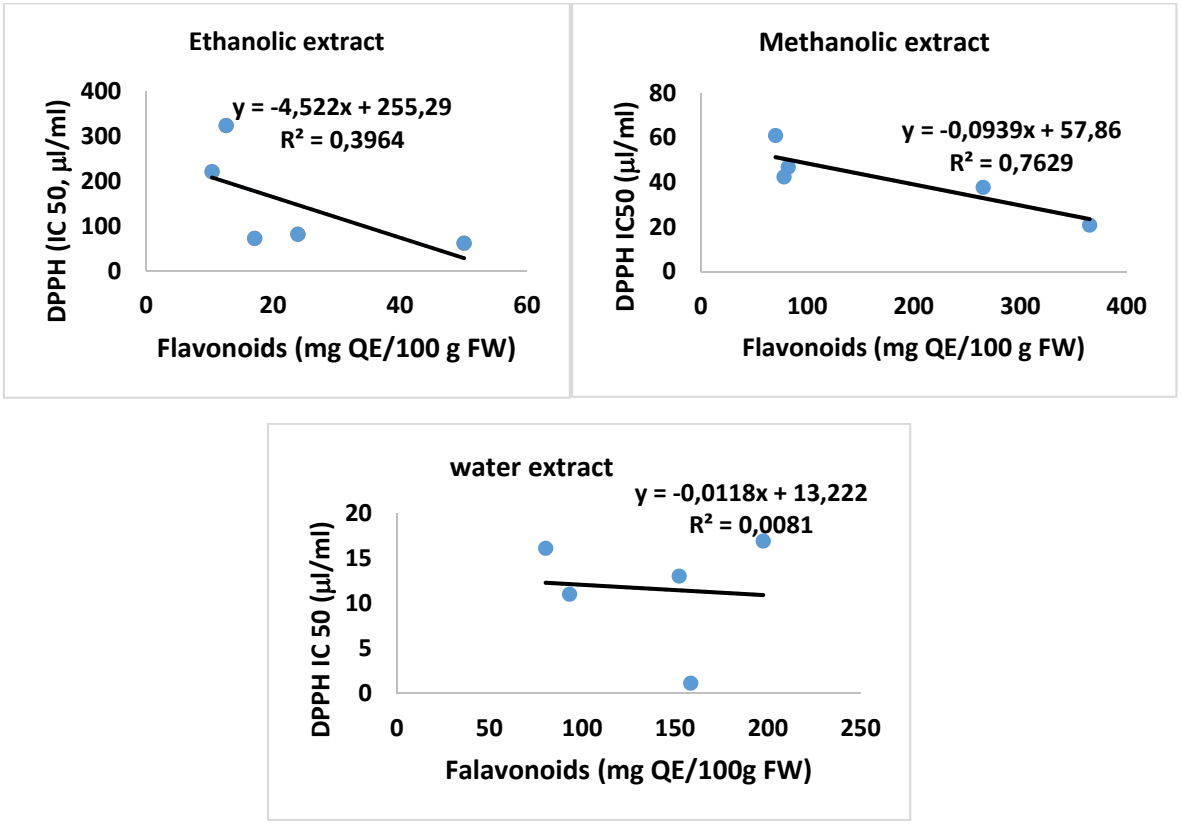

Figure 7: Correlation between antioxidant capacities (DPPH) and Flavonoids.

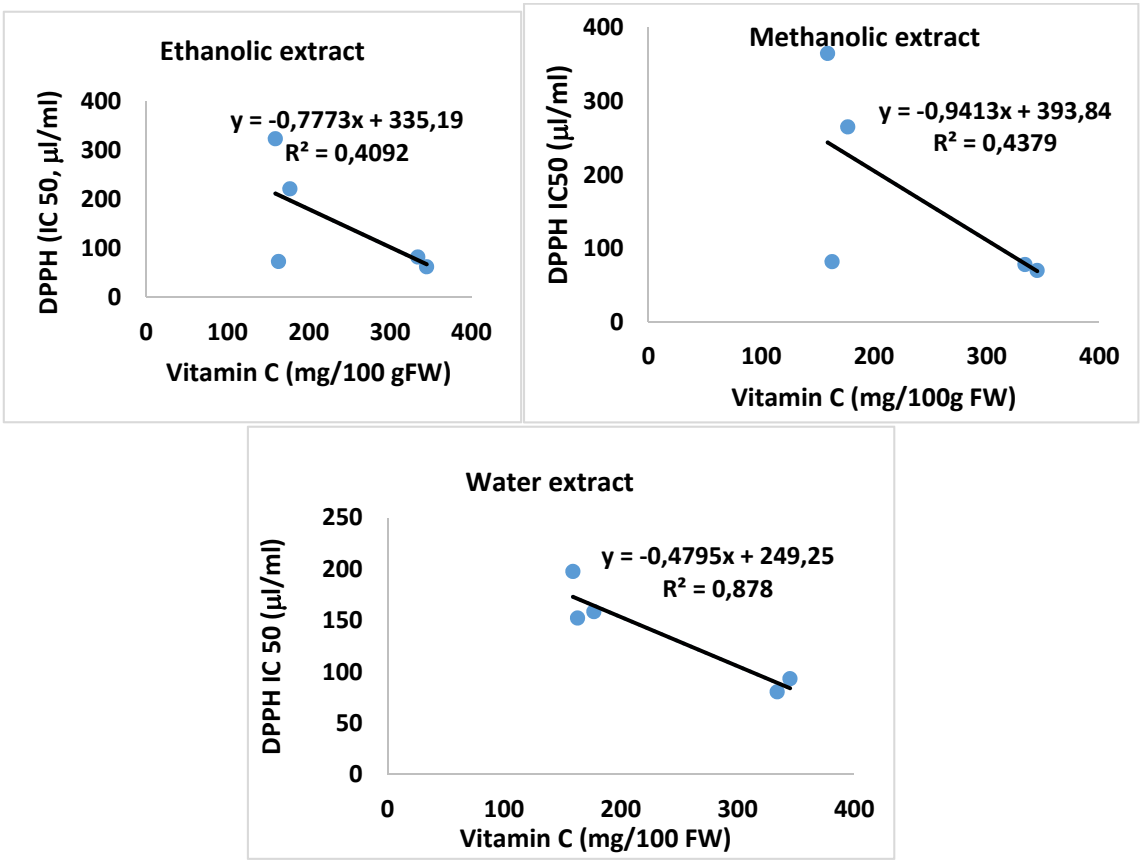

Figure 8: Correlation between antioxidant capacities (DPPH) and Vitamin C.

4. CONCLUSION

Extracting solvent significantly affected total polyphenol, flavonoids and flavonols contents and antioxidant activities of peppers extracts. According to the results obtained, the pepper cultivars studied have levels of phenolic constituents that contribute to a high antioxidant activity and may be considered as a good source of natural antioxidants. Red and yellow peppers had the highest antioxidant capacity, which correlated with the highest levels of total phenols. However, other compounds (ascorbic acid, flavonol and flavonoid) present in the peppers could contribute to the antioxidant activity and therefore should be considered in order to understand know the individual contribution of each group of phytochemicals to the total antioxidant activity. In conclusion, among the pepper cultivars studied, red and yellow peppers showed to have the best antioxidant properties and can be suggested as preferable for human consumption.

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