



## TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT CAPACITY OF COWPEA EFFECT OF VARIET AND LOCALITY

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### ABSTRACT

Leguminous seeds belong to plant foods which are generally rich in phenolic compounds. Cowpea seeds are a major source of plant proteins and vitamins for man, feed for animals. Polyphenolic compounds are secondary metabolites of amino acids present in many plant species, including legume. Their content depends on various factors, such as cultivar, pedoclimatic and cultivation conditions. The influence of cultivar, locality on the total polyphenols (TPC) and antioxidant activity (TAC) of Cowpea seeds was studied. Cowpea cultivars were cultivated under different climatic conditions in Iraq Republic. The main objective of the present work was to consider the changes of total polyphenols content in dependence on variety and to evaluate an antioxidant potential of three Cowpea varieties (white, light brown and red color) in different localities of Erbil City in Kurdistan Region Iraq and to evaluate the content of bioactive compounds (polyphenolics) in legumes commonly utilized in the human diet in Iraq, to compare their antioxidant capacity and to evaluate the influence of grown locality on observed parameters. Total polyphenols were determined by the Lachman's method and expressed as mg of Gallic acid equivalent per kg dry matter. Total antioxidant capacity was measured by the Brand-Williams method using a compound DPPH (2,2-diphenyl-1-picrylhydrazyl). Analysis of variance indicated significant differences ( $p < 0.05$ ) among locality and color for phenolic contents and antioxidant capacity. The various varieties of white color cowpea had significant influence on TPC and TAC and affected by locality too. From tested seeds the highest polyphenol content was measured in red color ( $802.323 \pm 15.937 - 825.700 \pm 8.494 \text{ mg.kg}^{-1} \text{ GAE}$ ). The lowest value was in white color ( $480.195 \pm 15.286 - 721.952 \pm 25.004 \text{ mg.kg}^{-1} \text{ GAE}$ ). The similar trend was observed at values of TAC. The highest TAC value was determined in red color ( $28.709 \pm 15.937 - 34.777 \pm 8.494\% \text{ DPPH}$ ). The lowest value was in white color ( $6.065 \pm 0.836\% - 9.578 \pm 0.884\% \text{ DPPH}$ ). The various varieties had significant influence on TPC and TAC according to used statistical analyses. Correlation between the phenolic contents and antioxidant activity was significantly positive ( $r = 0.783645$ ). Our results confirmed that legumes can be a good source of bioactive compounds in the human nutrition.

**Keywords:** Cowpea; total polyphenols; antioxidant capacity; locality

### INTRODUCTION

Cowpeas (*Vigna unguiculata* L. Walp), are an important part of the staple diet in many developing countries since the earliest practice of agriculture. The increasing agricultural production became an urgent issue since projections suggest that the global population will reach 9 billion people by the middle of this century (Godfray et al., 2010). Cowpea is a major staple food crop in sub Saharan Africa, especially in the dry savanna regions of West Africa. Cowpea has found utilization in various ways in traditional and modern food processing in the world. Traditionally in Africa, cowpeas are consumed as boiled vegetables using fresh and rehydrated seeds or processed into flour to make other food products (Odedeji and Oyeleke, 2011) noted that flour produced from whole seeds presents better functional properties compared to the dehulled seed flour which is common practice in processing of cowpea. Cowpeas provide a rich source of proteins and calories, as well as minerals and vitamins. A cowpea seed can consist of 25% protein and is low in anti-nutritional factors (Angel et al., 2003). This diet

complements the mainly cereal diet in countries that grow cowpeas as a major food crop. The seeds are a major source of plant proteins and vitamins for man, feed for animals, and also a source of cash income. The young leaves and immature pods are eaten as vegetables. Cowpea can be grown under rainfed conditions as well as by using irrigation or residual moisture along river or lake flood plains during the dry season, provided that the range of minimum and maximum temperatures is between 28 and 30°C (night and day) during the growing season. Cowpea performs well in agroecological zones where the rainfall range is between 500 and 1200 mm/year. Cowpea and horse gram are low in fat and are excellent sources of protein, dietary fibre, a variety of micronutrients and phytochemicals with potential health benefits (Kadam and Salunkhe, 1985; Siddhuraju and Becker, 2007). The nutritional and functional properties of their flours are comparable to chickpea flour (Sreerama et al., 2012). Due to their favourable flour functionality and their phytochemical-associated health benefits, these flours offer an enormous potential for the production of legume

composite flours. Pulses have shown numerous health benefits, e.g. lower glycemic index for people with diabetes, increased satiation and cancer prevention as well as protection against cardiovascular diseases due to their dietary fiber content (Chillo et al., 2008). There is a dearth of information on the specific health beneficial components in these lesser known legumes with specific reference to their phenolic compounds on the regulation of oxidative stress and their influence on enzyme activities associated with hyperglycemia and hypertension. These insights may help to exploit the use of under-utilized legumes as ingredients in composite legume flours and functional foods to promote their use in disease risk reduction and overall health. The health-promoting effects of dehulled cowpea flours derived from phenolic compounds and other antioxidants make this legume a potential source of functional food ingredients. Phenolic compounds (tannins, flavonoids and phenolic acids) are secondary metabolites in plants and as such are present in some plant foods (Manach et al., 2004; Wu et al., 2006). Several studies have reported on antioxidant and antiradical activity of tannins (Amarowicz, 2007). Cowpea is known also as containing a low amount of fat and high level of fiber which can prevent heart disease by reducing the low-density lipoprotein (Phillips et al., 2003). In addition, cowpea consumption increases glucose blood more slowly because of the slowly digestibility of the legume starch promoting its usage for diabetics (Phillips et al., 2003). Knowledge of the genetic basis and heritability of these health beneficiary phytochemical profiles is essential for efficient development of new cultivars for food processing industries and breeders. Phenolic extracts have been reported to retard lipid oxidation in oils and fatty foods (Rodriguez et al., 2007) vascular disorders including diabetes and hypertension (Van der Zwan et al., 2010). Therefore, it is important to control both blood glucose level and cellular redox status for managing these diabetic complications. Alpha-Amylase and alpha-glucosidase are key enzymes involved in starch break down and intestinal glucose absorption (McDougall and Stewart, 2005). Phenolic compounds constitute one of the most numerous and ubiquitously distributed group of plant secondary metabolites and are responsible for various beneficial effects in a multitude of diseases (Soobrattee et al., 2005). These are believed to work synergistically to promote human health through their antioxidant properties and their ability to modulate the activity of various enzymes. These phenolics are also potent inhibitors of  $\alpha$ -amylase and  $\alpha$ -glucosidase, the two important enzymes involved in the regulation of glucose homeostasis (McDougall and Stewart, 2005).

## MATERIAL AND METHODOLOGY

**Material.** Cowpeas (*Vigna unguiculata* L. Walp). Samples at full maturity were obtained from different localities in Erbil city, four varieties (C1, C2, C3, C4) were white color, two varieties (C5, C6) light brown and two varieties (C7, C8) red color were collected in Iraq.

**Extraction.** For 12 hours extraction, dry material (5 g) was used and continuously extracted by a Twisselmann extractor with methanol (80%, v/v).

**Total polyphenols determination.** Total polyphenols were determined by the method of Lachman et al. (2003) and expressed in mg eq. Gallic acid per kg dry matter. The total polyphenol content was estimated using Folin-Ciocalteu assay on the spectrophotometer Shimadzu 710 (Japan).

**Total antioxidant capacity determination.** The free radical scavenging activity of the extracts was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method of Brand-Williams et al., (1995).

**Statistical analysis.** Processing of the results was carried out by software STATGRAPHICS. Procedure compares the data in 8 varieties. The F-test in the ANOVA test whether there are any significant differences amongst the means. If the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means at the 95% confidence level; the Multiple Range Tests will tell which means are significantly different from which others. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure.

## RESULTS AND DISCUSSION

On the base of reached results there were estimated changes in the total polyphenols content and also changes in total antioxidant capacity values in dependence on Cowpea varieties in different localities.

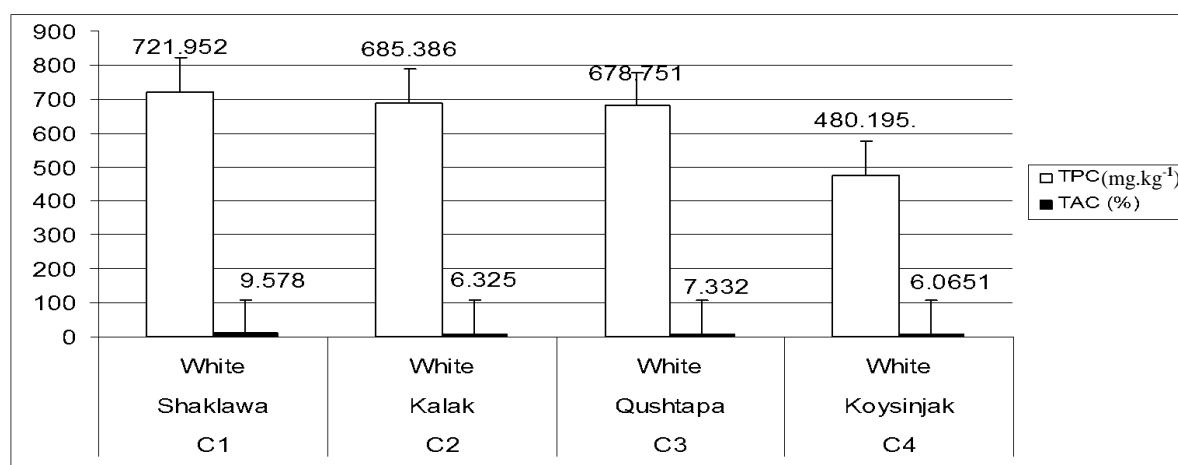
### 1 Evaluation of total polyphenol content and values of antioxidant capacity in white color Cowpea.

Following the total polyphenol content parameter in Cowpea white color (Figure 1, Table 1). According to the obtained results, the polyphenols content (TPC) in the tested was significantly different and was influenced by locality the highest value was reached in C1 (Shaqlawia) variety with  $721.952 \pm 25.004 \text{ mg.kg}^{-1}$ . When comparing this variety from total antioxidant capacity point of view, there was estimated value  $9.578 \pm 0.884\%$ . This is on the highest level of observed antioxidant capacity interval white color. For growing cowpeas are the best warm and dry areas. Therefore the C2 and C3 seeds have shown significantly lower TP content (these cultivars were grown in area suitable for growing cowpea). While the C1 variety, grown in mountain areas with higher rainfall, recorded higher amount of polyphenols. Lachman et al., (2006) investigated the effect of weather conditions on the TPC contents of potatoes and their results showed that an upland cooler site with higher rainfall provide the tubers with a higher content of TPC. C2 and C3 varieties were grown in a similar area condition, contain similar values of (TPC)  $685.386 \pm 40.956$ ,  $678.751 \pm 40.348 \text{ mg.kg}^{-1}$  respectively and the lowest value Of (TPC) was found in C4 variety Koysinjak with  $480.195 \pm 15.286 \text{ mg.kg}^{-1}$ . Manach et al., (2004) noted that environmental and genetic factors have a major effect on polyphenols content. Our results suggest that severe climatic conditions have caused a slight increase in the total content of polyphenolic substances in white color by locality.

**Table 1** Total phenolic content and antioxidant capacity (average and average deviation values) In chosen white Cowpea varieties after harvest in dray mass (mg.kg<sup>-1</sup> DM).

Varieties	Locality	Color	TPC (mg.kg <sup>-1</sup> )	TAC (%)
C1	Shaklawa	White	721.952 ±25.004	9.578 ±0.884
C2	Kalak	White	685.386 ±40.956	6.325 ±0.429
C3	Qushtapa	White	678.751 ±40.348	7.332 ±0.404
C4	Koysinjak	White	480.195 ±15.286	6.065 ±0.836

Data expressed as means of six replications ± standard deviation. Values in the same column with the different letters present significant differences  $p < 0.05$  using F-test for independent samples.



**Figure 1** Average content of total polyphenols TPC (mg.kg<sup>-1</sup>) and Average content of total antioxidant capacity TAC (%) in chosen white cowpea varieties arranged by locality.

When comparing legumes from the antioxidant capacity (TAC%) point of view (Figure 1 and 3; Table 3).  $p$ -value  $< 0.05$  there is a statistically significant difference between the means of the 4 variables at the 95.0% confidence level. The highest value was reached in case of C1 (shaqlawa) variety with the value  $9.578 \pm 0.884\%$  c. The lowest value was found in case of C4 (koysinjak) variety with the value  $6.065 \pm 0.836\%$  a, C2 and C3 varieties with the value  $6.325 \pm 0.429\%$  a,  $7.332 \pm 0.404\%$  b respectively. Our results suggest that antioxidant capacity was affected by location too.

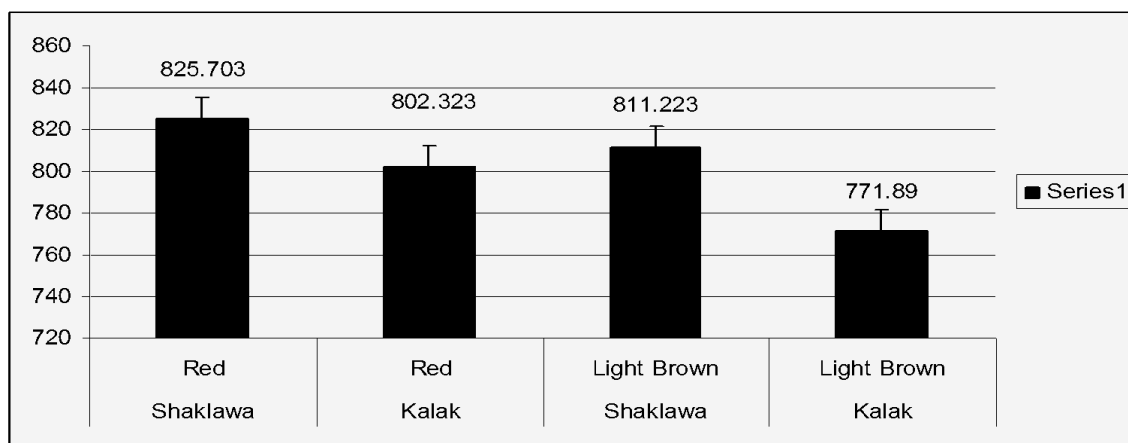
**2.1 Statistical evaluation of total polyphenol (TPC) content differences significance within of chosen varieties.**

When comparing all cowpea varieties, following in Table 2, there were significant differences according to used statistical methods on the all three types (white, light brown, red) observed confidence levels between almost the all observed varieties. Effect of varieties analysis (Table 2, figure 2) for the phenolic compounds contents (TPC) of seeds flours of tested showed the presence of significant variety differences ( $p < 0.05$ ).

**Table 2** Total phenolic content (average and average deviation values) in chosen Cowpea varieties after harvest in dray mass (mg.kg<sup>-1</sup>DM).

Varieties	Locality	Color	TPC (mg.kg <sup>-1</sup> )		
			M ± SD	LSD I.	LSD II.
C1	Shaklawa	White	722.952 ±25.004	c	C
C2	Kalak	White	685.386 ±40.956	a	B
C3	Qushtapa	White	678.751 ±40.349	b	B
C4	Koysinjak	White	480.195 ±15.286	a	A
C5	Shaklawa	Red	825.703 ±8.493	c	E
C6	Kalak	Red	802.323 ±15.938	b	E
C7	Shaklawa	Light Brown	811.223 ±7.200	b	E
C8	Kalak	Light Brown	771.890 ±7.440	a	D

Notes: Data expressed as means of eight varieties ± standard deviation. Since the P-value of the F-test is less than 0,05, there is a statistically significant difference between the means of the 8 variables at the 95,0% confidence level. Values in the LSD I. column present significant differences using LSD tests among white varieties (3 homogenous groups) and among red and light brown varieties (3 homogenous groups). Values in the LSD II. column present significant differences using LSD tests among all varieties, 5 homogenous groups are identified.



**Figure 2** Average content of total polyphenols TPC (mg.kg<sup>-1</sup>) in red and light brown color.

The TPC varied from 480.195 mg.kg<sup>-1</sup> (white color Koysinjak) to 825.700 mg.kg<sup>-1</sup> (red color shaklawa), our result similar, according to the results of **Dalaram et al., (2013)** recorded a range of (555.12 ±4.13 – 969.30 ±6.39) mg.kg<sup>-1</sup> for TPC in chickpea seeds. As shown in (Table 2 and 3) cultivar significantly affected phenolic accumulation and antioxidant capacity of flours from cowpea seeds. The cultivar shaklawa with a red seed coat, possessed the highest AOA and TPC levels followed by light brown seeds then in white color seeds. As shown in (Table 2, 3) According to **Warington et al., (2002)**, **Siddhuraju and Becker (2007)**, pigmented cowpea varieties had favorable factors that enhance AOA of seeds. The total polyphenolic compounds contents (TPC) in red color with the range (802.323 ±15.938 to 825.703 ±8.493) mg.kg<sup>-1</sup> higher than content (TPC) in light brown color with the range (771.890 ±7.440 to 811.223 ±7.200) mg.kg<sup>-1</sup> and the lowest contents (TPC) was found in white color with the range (480.195 ±15.286 to 722.952 ±25.004) mg.kg<sup>-1</sup> **Papoulias et al., (2009)** pointed out that polyphenols accumulation in plants is affected by genetic factors, environmental and cultural conditions and also various stresses (**Kravic et al., 2009**), storage and cooking (**Manach et al., 2004**).

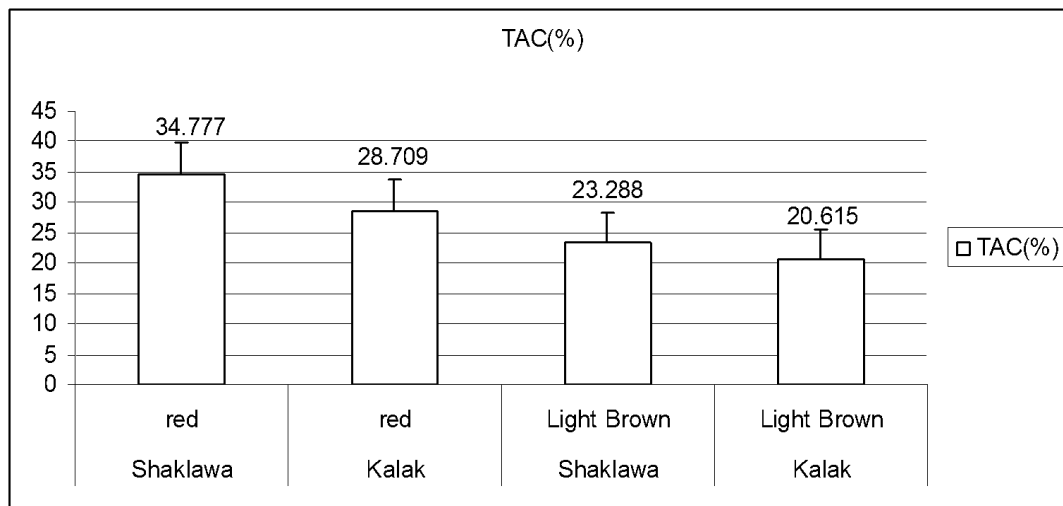
## 2.2 Statistical evaluation of antioxidant capacity (TAC) differences significance within the frame of chosen Varieties

The highest value was reached in case of red color (shaklawa red) varieties as it is (Figure 3, Table 3) in Table with the range (28.709 ±0.425 to 34.777 ±1.827 c) %. The lowest value was found in case of white color variety with the range (6.065 ±0.836 to 9.578 ±0.884) %, and for light brown color with the range (20.615 ±0.665 to 23.288 ±0.678) %. In comparison with results of **Kavalcova et al., (2014)**, where the interval of statistically significant highest value of antioxidant activity was recorded in onion from (20.22 ±0.53 to 25.76 ±0.53) % and statistically significant the lowest value of antioxidant activity was recorded in garlic (from 4.05 ±0.20 to 5.07 ±0.47) %, our results in cowpeas light brown color with the range (20.616 ±0.665 to 23.288 ±7.199) % are similar. In comparison with results in variety in onion from (20.22 ±0.53 to 25.76 ±0.53) % of **Kavalcova et al., (2014)**, our results in white color cowpeas with value range (6.065 ±0.836 to 9.578 ±0.884) % are higher than garlic of **Kavalcova et al., (2014)**.

**Table 3** Average content of total antioxidant capacity TAC (%) in chosen cowpea varieties (average and average deviation values).

Varieties	Locality	Color	TAC (%)		
			M ±SD	LSD I.	LSD II.
C1	Shaklawa	White	9.578 ±0.884	c	C
C2	Kalak	White	6.326 ±0.429	a	a, b
C3	Qushtapa	White	7.333 ±0.404	b	B
C4	Koysinjak	White	6.065 ±0.836	a	A
C5	Shaklawa	Red	34.778 ±1.828	c	F
C6	Kalak	Red	28.696 ±0.401	b	E
C7	Shaklawa	Light Brown	23.288 ±7.199	b	D
C8	Kalak	Light Brown	20.616 ±0.665	a	D

Notes: Data expressed as means of eight varieties ± standard deviation. Since the *p*-value of the F-test is less than 0,05, there is a statistically significant difference between the means of the 8 variables at the 95,0% confidence level. Values in the LSD I. column present significant differences using LSD tests among white varieties (3 homogenous groups) and among red and light brown varieties (3 homogenous groups). Values in the LSD II column present significant differences.



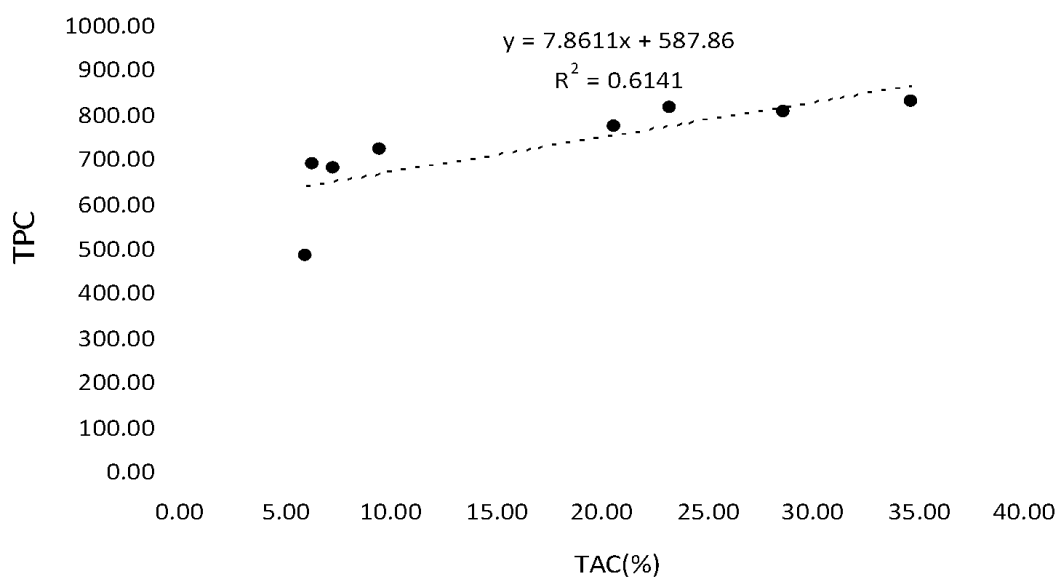
**Figure 3** Average content of total antioxidant capacity TAC (%) in red and light brown varieties using LSD tests among all varieties, 6 homogenous groups are identified.

Significant variation of the AOA among varieties was also pointed out for many grains (Adom and Liu, 2002), for wheat flour (Akond et al., 2010) and for common bean (Golam et al., 2011). For fababean (Dalaram et al., 2013), with the range  $16.62 \pm 0.81$  to  $24.54 \pm 0.64\%$ . Mokgope (2007) noted that in general, the efficacy of phenolic constituents as antioxidants depends on factors such as the number of hydroxyl groups bonded the aromatic ring, the site of bonding, mutual position of hydroxyls in aromatic ring and their ability to act as hydrogen or electron donating agents and free radical scavengers.

**2.3 Correlation between the total antioxidant activity values and total phenolics contents:**

ANOVA linear correlation coefficients were used to assess the relationships between TPC and TAC, Correlation: Our result confirmed a strong statically

correlations between total polyphenol content and total antioxidant capacity values. A statistically strongly significant correlation ( $R = 0.783645$ ;  $p < 0.05$ ) was found (Figure 5). Amarowicz et al., (2005) analyzed the extracts of fababean, broad bean, adzuki bean, red bean, pea, red lentil and green lentil seeds using 80% (v/v) acetone and confirmed a statistically significant correlation between the total antioxidant activity values and total phenolics ( $p = 0.01$ ). A strong correlation between total polyphenol content and antioxidant activity ( $R = 0.86$ ;  $p < 0.05$ ) was observed also by Akond et al., (2011) in common bean and a statistically strongly significant correlation ( $P\text{-value } 2.391E-06$ ;  $R = 0.802$ ) was found between total polyphenol content and total antioxidant capacity values by Dalaram et al., (2013) in lentil. According these authors this finding suggests that total polyphenol content is a good predictor of in vitro antioxidant activity.



**Figure 4** Correlation between TP and TAC.

## CONCLUSION

Cultivar significantly affected phenolic accumulation and antioxidant capacity of flours from cowpea seeds. The present study showed that there are also differences in these traits among red, brown and white cowpea cultivar. The Shaqlawa cultivar with red seed coat possessed the higher AOA and TPC levels than light brown color seed coat and white color seeds cowpea. The positive interrelationship between these two parameters demonstrates that the antioxidant activity depends mainly on polyphenols contents. Based on our results the followed order of total polyphenol content as well as total antioxidant capacity in investigated cowpea obtained from Iraq can be created: red color cowpea >light brown color cowpea >white color cowpea. The present study of cowpea is also affected by locality. Our results confirmed that legumes can be a good source of bioactive compounds in the human nutrition. The consumption of cowpea provides potential nutraceuticals for human health. Prevention of degenerative diseases associated with free radical damage, in addition to their traditional role of preventing protein malnutrition.

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**Acknowledgments:**

This work was supported by grant VEGA No. 12345.

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