

Bioactive Constituents and Antioxidant Activity of Moroccan garlic (*Allium sativum* L).

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Abstract

Garlic (*Allium sativum*) is a good source of total polyphenols content. These compounds reveal activity effectiveness not less than synthetics. The aim of current research is to study and compare an antiradical activity, phenolic, flavonoid and flavonol contents of garlic of five areas in Morocco. Those contents were determined using spectrophotometric method. Antioxidant activities were studied using two methods: DPPH and ABTS radical scavenging activity. On the basis of findings it turned out that total polyphenolic compounds and antioxidant activities varied from one area to another. Moreover, ABTS radical scavenging activity demonstrated better results compared to DPPH method and the results showed, in general, a good correlation could be found between antioxidant activity and polyphenolic compounds.

Keywords: Garlic, *Allium sativum*, polyphenol compounds, antioxidant activity.

1. Introduction

Garlic is a perennial plant of the Alliaceae related to onions, chives, shallots, and leeks. It is mainly used as a food flavoring agent and condiment in various foods and spices such as kimchi, mayonnaise, salad dressing, spaghetti, pickles, etc (Sun-Neo Lee et al., 2003). It is one of the world's oldest medicines and has been used not only for flavoring but also as a medicinal herb for its prophylactic and therapeutic properties. Garlic and garlic supplements are consumed in many cultures on account of their beneficial effects (Eleni Anifantaki et al., 2010). It has also been known as a medicinal plant applied as a medication for lowering blood pressure, reduction of serum cholesterol and triglycerides and inhibition of platelet formation (Reyhaneh sariri et al., 2002). The bioactive components of garlic are mainly responsible for the healing properties (S.G. Santhosha et al., 2013). Main pharmacological effects of garlic are attributed to its organosulphur compounds (Mohsen Arzanlou et al., 2010). It also contains many other sulfur containing compounds such as allin, ajoene, diallylsulfide, dithin, S-allylcysteine, and enzymes, vitamin B, proteins, minerals, saponins, flavonoids, and maillard reaction product, which are non-sulfur containing compounds (Muhammad Gulfraz, 2014). However, Sulphur and polyphenols present in garlic respond to the antibacterial, antifungal and antioxidant activity was carefully studied in previous reports (Yara S. Queiroz et al. 2009). Polyphenolic compounds are commonly bound in both edible and inedible plants and they have been reported to have multiple biological functions such as antioxidant, anti-inflammatory, anti-cancer and anti-microbial activities (Hilaire Macaire Womeni et al, 2013). These compounds reveal activity effectiveness not less than synthetics (Beata Drużyńska, Magdalena Wojda, 2007). Therefore, the objective of the present study is to measure and compare the content of polyphenolic compounds and the antioxidant activity of garlic.

2. Materials and methods

2.1. Plant Material

Garlic bulbs (*Allium sativum* L.) were collected from five locations in Morocco: Fez (Ain Cheggag), Meknes (Agourai), Ben Ahmed, Tetouan (Beni Hassane), and Marrakesh (Ait Ourir) (Fig.1). These areas are best known

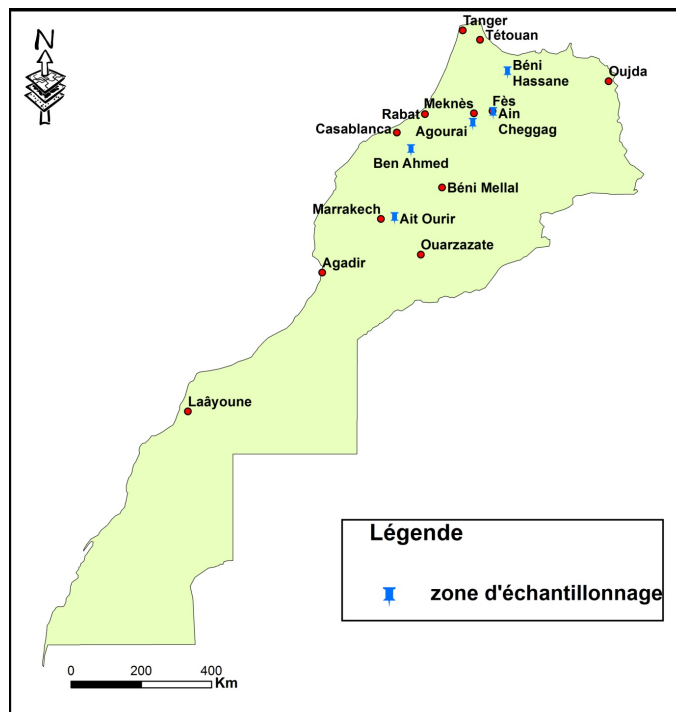


Figure 1. Map shows the areas of sampling garlic

2.2. Chemicals and reagents

The solvents and the chemicals used were of analytical grade, methanol was used as solvent for extraction of antioxidant compounds. DPPH, ABTS, potassium persulphate, sodium carbonate, Folin-Ciocalteu, gallic acid, aluminium trichlorid, sodium acetate were stored at prescribed conditions in the laboratory.

2.3. Extracts preparation

1 g of garlic bulbs were extracted using a method of maceration with 10 ml of methanol 80% (MeOH) for 76 h at room temperature. After the maceration, the extracts were collected, filtered through a filter system vacuum. The filtrate was evaporated under reduced pressure in a rotary evaporator at 35°C until the extracts became completely dry. After evaporation, the residues were dissolved in methanol and stored at 4°C until use. The extraction process was carried out in triplicate for each sample.

2.4. Determination of total phenol

The amount of total phenolic compounds in the extract was determined colorimetrically with the Folin–Ciocalteu (FC) reagent, using a slightly modified method of Biljana Bozin (2008). The reaction mixture contained 100 µl of extract, 0.5 ml of FC reagent and 2 ml of sodium carbonate solution and was kept in the dark under ambient conditions for 30 min to complete the reaction. The absorbance of the resulting solution was measured at 760 nm in a spectrophotometer. The concentration of total phenolic compounds was calculated as gallic acid (mg/g) equivalent (GAE) from the calibration curve using the equation: $Y = 0.0654 * x + 0.0309$, $R^2 = 0.9959$, where x is the absorbance and Y the gallic acid equivalent in mg/g. All measurements were carried out in three replicates.

2.5. Determination of total flavonoid

Measurement of total flavonoid content was determined spectrophotometrically according to Biljana Bozin (2008), using a method based on the formation of complex flavonoid-aluminium with the absorbivity maximum at 430 nm. 1 ml of the extract was separately mixed with 1 ml of 2% AlCl₃. After incubation in the dark under ambient conditions for 30 min, the absorbance of the reaction mixtures was measured at 430 nm. The flavonoid content was calculated as quercetin (mg/g) equivalent (QE) from the calibration curve using the equation: $Y = 0.0346 * x + 0.0023$, $R^2 = 0.9973$, where x is the absorbance and Y the quercetin equivalent in mg/g.

2.6. Total Flavonols

Total flavonols was determined by the method described by Otunola and Afolayan (2013). 2.0 ml of the extract was mixed with 2.0 ml of AlCl₃, and then 3.0 ml of sodium acetate solution (50 g/l) was added to the mixture.

This was incubated in the dark under ambient conditions for 2h30 min and the absorbance read at 440 nm. Total flavonol content was calculated as quercetin (mg/g) equivalent (QE) from the calibration curve using the equation: $Y = 0.0346x + 0.0023$, $R^2 = 0.9973$, where x is the absorbance and Y the quercetin equivalent in mg/g.

2.7. Determination of DPPH-radical scavenging capacity

The antioxidant activity was assessed on basis of the radical scavenging effect of the stable DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical and was determined by the method described by G.A. Otunola and A.J. Afolayan, (2013). Briefly, 0.5 ml of the extract was added to 2.5 ml of methanolic solution of DPPH, shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance of the reaction mixture at 517 nm was measured with a spectrophotometer. The percentage of free radical scavenging activity was calculated as follows:

$$I\% = [(Abs\ control - Abs\ sample) / Abs\ control] \times 100$$

Where Abs (control) is the absorbance of DPPH radical + methanol, and Abs (sample) is the absorbance of DPPH radical + sample extract or standard.

The 50% inhibition concentration (IC₅₀) was then obtained from a linear regression plot of percentage inhibition against concentration of the extract.

2.8. ABTS radical scavenging activity

The method described by Otunola and Afolayan (2013) was used to determine the ABTS scavenging activity. Two stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate v/v were mixed together, allowed to react for 12 h at room temperature in the dark and used as the working solution. This was further diluted by mixing in 1 ml of freshly prepared ABTS solution to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. The extract was allowed to react with 1 ml of the ABTS+ and the absorbance was read at 734 nm after 7 min. the percentage ABTS+ inhibition was calculated as follows:

$$\% \text{ ABTS+ scavenging activity} = (Abs\ (control) - Abs\ (sample) / Abs\ (control)) \times 100$$

Where Abs (control) is the absorbance of ABTS radical + methanol, and Abs (sample) is the absorbance of ABTS radical + sample extract or standard.

The 50% inhibition concentration (IC₅₀) was then obtained from a linear regression plot of percentage inhibition against concentration of the extract.

3. Results and discussion

The results of phenolic contents are shown in figure 2. The data shows that they varies widely from one area to another and ranges from 0.037 to 0.286 mg/g. Tetouan cultivar (Beni Hassane) records the highest value of phenolics contents (0.286 mg/g) followed by Fez (0.176 mg/g) and Marrakesh (0.087 mg/g). While for Meknes (Agourai) and Ben Ahmed, the concentration of phenols is 0.045 and 0.037 mg/g respectively. From a comparative view point, the values found in this analysis are generally smaller to than those reported by Hilaire Macaire et al. (2013) and Biljana Bozin et al. (2008) with respective mean values of 13.4 mg/g and 0.98 mg/g. While, the values obtained from Tetouan and Fez cultivars are finding slightly high to than those reported by Hala, M. Abdou (2011) and Muhammad et al., 2014 with respective mean values of 0.125 mg/g and 0.152 mg/g, and generally, all the values obtained in this analysis are found high to than those found by Yara S. Queiroz et al. (2009) (6.99 -8.32 µg/mg). As shown in the figure 3, flavonoids contents varies from 0.360 mg/g to 0.785 mg/g. Fez and Tetouan cultivars are also found to contain the highest levels 0.6 mg/g and 0.750 mg/g respectively, followed by Marrakesh with 0.49 mg/g. While a low level (0.36 mg/g) was found in Meknes and Ben Ahmed cultivars which have almost the same value of flavonoid contents. The levels of flavonoids are higher than those of phenols in all the regions studied. These results are similar to those found in the literature (Ji-Sang Kim et al., 2013, Biljana Bozin et al., 2008, and Muhammad G. et al., 2014). Furthermore, Flavonols contents are significantly highest compared to the phenols and flavonoids contents. This agrees with the results of the present study of Otunola and Afolayan (2013), and the values range from 0.1 mg/g to 2.5 mg/g (figure 4). Generally, both Tetouan and Fez cultivars record the highest values of polyphenolic compounds. This significant differences between results may be likely due to environmental differences (namely climate, location, temperature, fertility, diseases and pest exposure), planting conditions, harvesting, method of irrigation, soil type and storage conditions of samples.

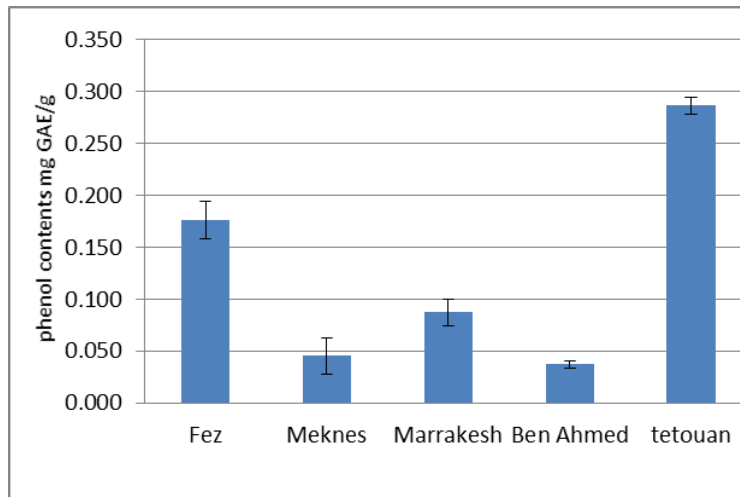


Figure 2. Phenolic content in five areas of Morocco

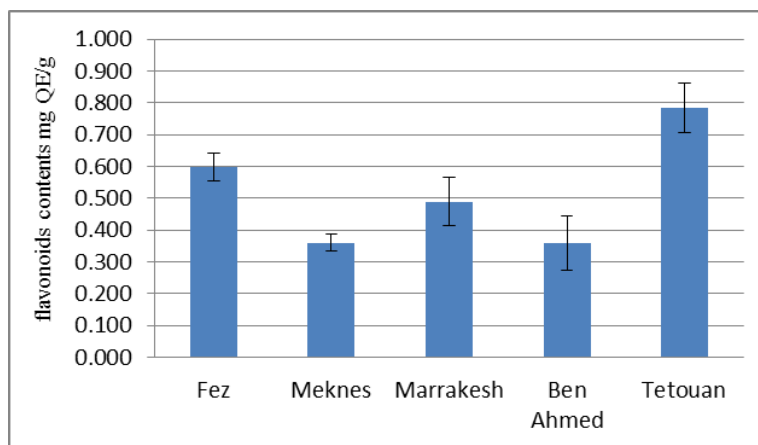


Figure 3. Flavonoids content in five areas of Morocco

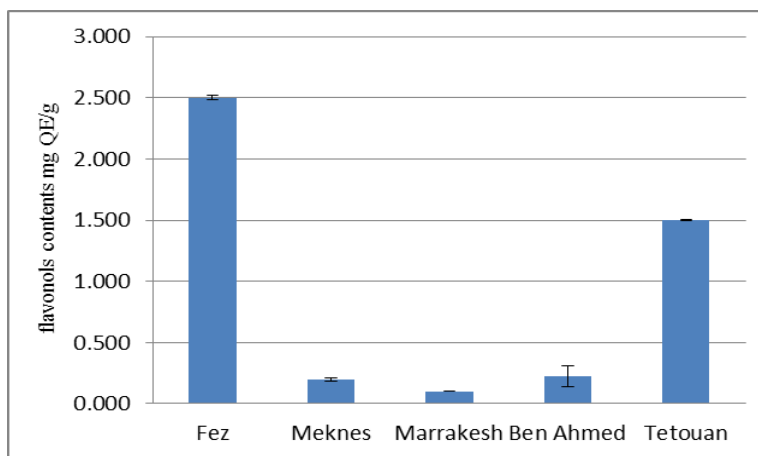


Figure 4. Flavonols content in five areas of Morocco

The antioxidant activity of plant extracts vary with assay methods Therefore a single assay may be in adequate (Yen, G.C., Duh, P.D., Su, H.J, 2005). For this reason we cross checked antioxidant activities of extracts with two antioxidant activity assays namely DPPH and ABTS radical scavenging activity. In the DPPH

assay, and as shown in figure 5, all the cultivar are found to possess the higher antioxidant activity, and the high value is revealed on Meknes cultivar (60.85 %) followed by Fez (59.79 %) while the low value (51 %) is shown on Ben Ahmed cultivar. These results are higher than those found by G.A. Otunola and A.J. Afolayan (2013) with mean value of 20 % and 35 %. The IC₅₀ (concentration of sample needed to scavenge 50% of DPPH) was calculated by linear regression of plots and it ranges from 1.94 mg/ml to 11.23 mg/ml. For the second method based on the ABTS assay, the IC₅₀ ranges from 1.3 mg/ml to 5.31 mg/ml. The activities of the extracts from all area against ABTS+ are significantly high to the DPPH assay and these trends are not far to those reported by Beata Drużyńska, Magdalena Wojda (2007). The high value is always recorded in Tetouan cultivar with 81 % followed by Fez cultivar with 80.59 % while the low value is revealed on Meknes cultivar with mean value of 49.37 % (Figure 6). Good antioxidant activity of garlic extracts towards ABTS may result from the fact that, according to literature, one of polyphenol compounds in garlic is quercetin treated as a very good scavenger of ABTS cation-radicals (Ananth Sekher Pannala et al., 2001 and Anna Maria Nuutila et al., 2003).

The conducted regression analysis shows the existence of a positive correlation between polyphenolic compounds and the antioxidant activity tested by ABTS and DPPH method at a significance level of $p=0.05$ in the 95% trust limits. A conclusion on the existence of a relation between the contents of polyphenols and antioxidant activity of extracts can also be based upon literature data. (Sain Cricq de Gaullejac et al., 1999, Hilaire Macaire Womeni et al., 2013, Safaa Y. Qusti et al., 2010, Biljana Bozin et al., 2008, Yara S. Queiroz et al., 2009).

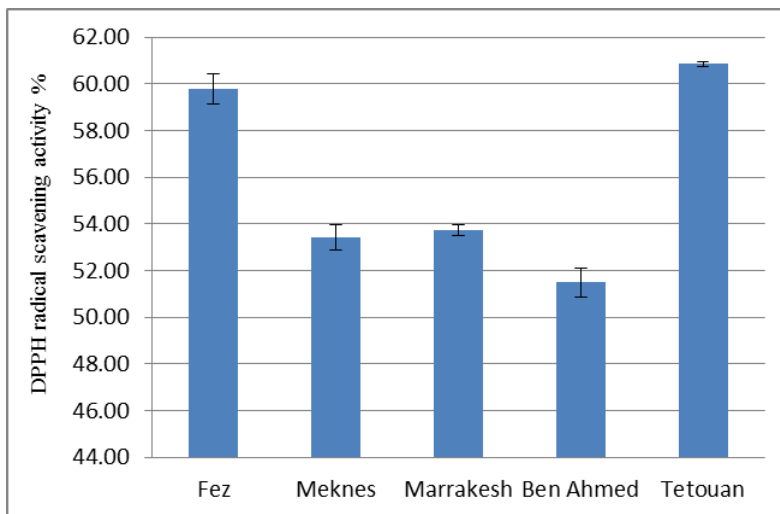


Figure 5. DPPH antiradical activity in five areas of

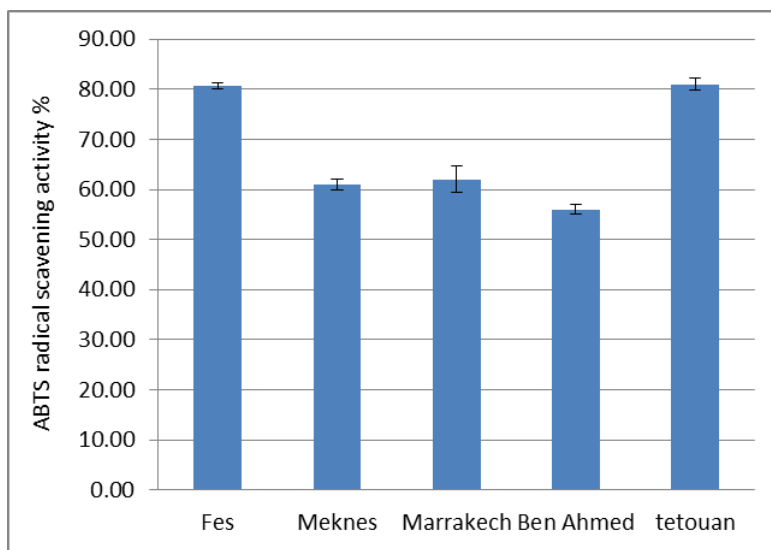


Figure 6. ABTS antiradical activity in five areas of

4. Conclusion

This study researched and compared the contents of polyphenolic compounds and the antioxidants activities of 5 cultivars from different areas in Morocco for their acclaimed health benefits. A large variability in these contents was observed among the cultivars which allow us to create a differentiation on the groups. The five cultivars represent a good source of natural antioxidants and they could be considered as useful sources of materials for human health. This antioxidant effectiveness was dependent on the action of polyphenolic compounds. However, further research is required to investigate the organosulphur compounds which are also responsible for the healing properties of garlic.

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