

Estimation of Total Phenols and Flavonoids in Extracts of *Chenopodium Quinoa*

Maroua Mesai Ahmed¹, Mounira Kadri¹

¹University Elchahid Hamma lakhder. El-oued, Algeria, marouamesai@gmail.com

¹University Elchahid Hamma lakhder. El-oued, Algeria and Laboratory of Biology, Environment and Health Laboratory, faculty of Nature Sciences and Life, Eloued, Algeria. mounira-kadri@univ-eloued.dz

Abstract: This study aims to know the phytochemical composition of *Chenopodium quinoa* Willd grains grown in Oum Touyour El-M' Ghair province. The primary chemical screening of some secondary metabolites in the extracts of quinoa grains, as the detection showed that the yellow quinoa grains contain all of the saponins, flavonoids, and the trace compounds in significant proportions, while the average proportions of tannins and alkaloids, and weak proportions of sterols and triterpenes were recorded. The number of polyphenols in the aqueous extract of quinoa was estimated at 37.94 µg AG E/mg Ex, while the tannins were at 60.63 µg CA E/mg Ex, and the flavonoid content gave 18,40 µg QeE/mgEx

Keywords—component; *Chenopodium quinoa* Willd, Secondary metabolite, Polyphenols, Flavonoid, Tannins.

1. INTRODUCTION

Nearly half of Peru's population lived in poverty in South America, despite the presence of quinoa crops, which originated in the Andean area and are high-nutrition crops, but they were not essential and were replaced by low-cost foods such as rice and pasta. Due to the low income and harsh nature of marginal and mountainous areas, farmers in these areas have adopted other methods of nutrition, such as relying on crops that are easy to grow and germinate and adapted to all climatic conditions, and quinoa was the most stable crop of the Chenopodiaceae family, which has a very high nutritional value compared to other grain crops. (Repo-Carrasco et al., 2003)

Recent Studies Have Focused On Chemical Ingredients and The Therapeutic Properties of quinoa Recognition as Functional and Cautious Food (FAO) Launched the International Year of Quinoa in 2013 To Enhance the Production, Preservation of this Crop as well as agriculture, Countries Take The Initiative To Employe Breeding Programs To Develop Varieties Commensurate with their Own needs based on climatic conditions, Some countries have been involved in a new idea to share and share a genetic plasma with other countries to initiate crop experiments and field assessments with FAO's technical support, these cooperative partnerships continue to enhance the expansion of the production of quinoa.

The Preservation of The Broad Diversity of This Crop Is Necessary For Its Stability (Rojas, 2003), Because It Is Important To Provide a Balanced Source For All Basic Amino Acids, and Range Of Other Nutrients, Such as Fatty Acids, As Well as a Source Rich In Phosphorus, Magnesium, and Iron (Van et al., 2010), And wide range of minerals, vitamins, fat and high-quality protein, these benefits have been able to introduce quinoa to the latest areas outside their original area, especially in the semi-tropical areas of the world (Bhargava, 2007).

This Broad Geographical Distribution is the Great Potential For This Type Frequent, Frost, Wind, Wind, Wind, Salinity,

and Also for Various Diseases, Parasites and Pests That Attack Crops (Jacobsen et al., 2015).

In addition to being a source of essential nutrients, vitamins, and minerals, it also possesses an important content of secondary metabolic compounds represented by polyphenols, flavonoids, anthocyanides, saponins, and tannins. These compounds give quinoa an important biological activity, which contributes to various physiological properties such as It has antimicrobial activity, antioxidants, anti-inflammatory, anti-tumor, and anti-cancer effects (Repo-Carrasco-Valencia et al, 2010). It is also considered a good food for people who are sensitive to gluten because it is free of gluten.

Global awareness of the need to grow quinoa seeds has increased in recent years thanks to activities under the name of the International Year of Quinoa, as 27 countries outside the Andean region participated, to enhance food security, during the period 2013-2015. Algeria was among these countries its cultivation witnessed great success in several areas of the home land, including the state of El-M'Ghair, as it has adapted to the dry climate of this area.

This study focus on the chemical content of the yellow quinoa plant grains in the area of El-M'Ghair with a view to valuation, where many problems were subtracted: The extent to contain yellow quinoa pills on food, effective and metal materials

The current study was first addressed to the preliminary detection tests represented by the chemical limitation of secondary metabolism and then discussed to estimate the nutritional value (carbohydrates-protein) for the yellow chino water disintegration, and various effective substances (Phenols - Flavonoids - Tanins) have been estimated at all of the water and methanolic extracts.

2. MATERIALS AND METHOD

2.1 Materials

Quinoa was obtained from Oum Thiour Wilaya M' Chair is located in the Algerian Sahara.

EL

ELM'Ghair region is located in the southeast of the Algerian country from the Sahara. Its lands extend between 5,9293 longitudes and 33,9530 latitudes, with an area of 8835 km².

EL M'Ghair enjoys the fertility of its soil until it is suitable for cultivation, It was famous for its vegetables, fruits, and palm cultivation, and it gained international fame for its production of dates.

The yellow quinoa was ground by an electric grinding machine and left for a short period to ventilate so as not to be exposed to moisture, and then kept in paper bags (Bourkhis et al., 2009).

2.METHODS

2.1 PREPARATION OF QUINOA EXTRACTS

Five plant extracts were prepared for the initial chemical inventory and determination of polyphenols, flavonoids, and tannins, represented by:

Preparing the aqueous and methanolic extract by boiling:

Putting 10g of powder seeds plant in 100ml of distilled water or methanol (80%), where it is extracted in a condensing device for 1 hour, followed by a filtration process (Azzi, 2013), the extracts are used to detect secondary metabolites.

•Preparation of the aqueous and methanolic extract by soaking:

Putting 10g of vegetable powder extracted with 100ml of distilled water or methanol (80% soaked for 24h at laboratory temperature, then filtered (with repeating the process 3 times). The extracts are dried using a rotary evaporator (Rotavapeur), to obtain the crude extract, which is kept at 4°C (Mannet al., 2008; Abalake et al., 2011). The extracts are used to detect secondary metabolites, and the crude extracts are used to determine Both polyphenols, flavonoids, and tannins.

•Prepare the acid extract:

Soak 10g of vegetable powder in 50ml of dilute sulfuric acid (1/10), for 24h after its expiration. The filtration is done, and the extract is used to detect alkaloids (Sandrine, 2005)

Extracts yield:

Calculates the yield of the extracts by the formula given by (Falleh et al., 2008)

$$Rd \% = [P/P'] \times 100$$

Rd = yield of extracts

P = weight of the extract

P' = weight of plant matter

2.2 Primary chemical screening

This chemical detection aims to identify the most important active substances present in the aqueous and methanolic extracts of the vegetative and root system of the quinoa plant, which are flavonoid saponins, alkaloids, tannins, sterols, triterpenes, and glycosides, following the method of (Harborne, 1998 ; Trease et Evans, 1989).

•Detection of flavonoids:

In a test tube, mix 5 ml of the extract with 1 ml of amyl alcohol (Alcool iso-Amylique), followed by 1 ml of HCl, and 0.5 g of magnesium.

•Detection of saponins:

To find out the richness or poverty of the plant from Saponins, we used the method of measuring the length of the foam, where we prepare a tube for each of the aqueous and methanolic extracts, we take 2 ml of each extract and then add 1 ml of distilled water, shake the tubes horizontally for a minute, then leave to cool for 3 minutes Then we measure the length of the foam in each tube.

•Detection of reducing compounds:

1 ml of the obtained filtrate was taken with 2 ml of distilled water and 20 drops of liqueur de Fehling's solution were added, followed by heating in a water bath.

The appearance of the red precipitate is evidence of the presence of returned compounds.

•Detection of tannins:

To detect the presence of tannins, we mix 1 ml of the extract with 1 ml of distilled water and add 1 to 5 drops of dilute FeCl₃ (1%) solution.

The appearance of a greenish-blue color indicates the presence of catechic tannins.

The appearance of the blue color indicates the presence of gallic tannins.

•Detection of alkaloids:

To 1ml of the extract is added 3–5 drops of alkaloid reagents Wagner's reagent, Dragendroff's reagent, and Mayer's reagent.

Wagner's reagent: The appearance of a brown precipitate indicates the presence of alkaloids.

•Dragendroff Reagent: The appearance of an orange precipitate indicates the presence of alkaloids.

• Detection of sterol compounds and triterpenes:

We relied on the Liebermann Buchard reaction, where 10 ml of the extract is evaporated, the precipitate is dissolved in 0.5 ml of chloroform and 0.5 ml of anhydride acetic is added to it, followed by the addition of 1 ml of concentrated sulfuric acid (H₂SO₄).

3. 3.MATERIALS AND METHOD QUALIFICATION OF PHYTOCHEMICALS

3.1Quantitative of polyphenols

The determination of the total polyphenols of the aqueous extract of yellow quinoa seeds was carried out according to the method (Singleton et al., 1977; Singleton et al., 1999), where the Folin-ciocalteur reagent is used.

We put in a test tube 125 ul of plant extract, 500 ul of distilled water, 125 ul of Folin-ciocalteur, after 3 minutes, add 1250 ul of sodium carbonate (Na₂CO₃) (7%) and 1ml of distilled water, leave the mixture in the dark And at room temperature for 90 minutes, then read the absorbance in a

spectrophotomètre at a wavelength of 760 nm, the standard curve was prepared from gallic acid dissolved in distilled water with different concentrations (25ug/ml-600ug/ml), with the same previous treatment we were able to obtain the graph of the standard curve of gallic acid, the results are expressed as micrograms of gallic acid equivalent per milligram of extract (ug AGE/mg extract).

3.2 Quantification of flavonoids

The flavonoids were determined among the studied plant extracts according to the method (Turkoglu et al., 2007) by reacting them with aluminum trichloride (AlCl₃), to form a yellow-colored complex, as we mixed in a test tube 500 ul of plant extract with 1500 ul of methanol, 100 ul of acetate Sodium (Na₂COOK) 100ul of aluminum chloride (ALCl₃), left in the dark and at room temperature for 40 minutes, then read the absorbance intensity in the opticalspectrophotometerat the wavelength of 415 nm. The standard curve wasprepared from the flavonoid (Quercetine) dissolved in methanol at different concentrations (0ug/ml - 200ug/ml) and with the same previous treatment, The standard curve was obtained for quercetin, and the results are expressed as several micrograms equivalent to quercetin per milligram of extract(μg CAE/ mg extract).

3.3Quantification of tannins

The quantitative determination of tannins was carried according to the (Sun,1998) method using vanillin, by taking 500 of the extract and adding to it 3 ml of a solution (vanillin/methanol 4%) and 1.5 m of hydrochloric acid HCl, mixed well and then left for some time. Then the absorbance was measured at a wavelength of 500 mm by a spectrophotometer and the results are expressed in micrograms equivalent with catechin per mg of extract (μg CAE / mg extract)(Sun et al .,2008).

4.RESULTS AND DISCUSSION

After preparing the aqueous and methanolic extracts of quinoa grains by soaking, the extracts were dried using a rotary evaporation device, and from it, the crude vegetable extract was obtained, Then the yield was calculated

Table 1: Yield of aqueous and methanolic extracts

	Aqueous extract	Methanol extract
<i>Plant matter mass (g)</i>	50	50
<i>Extract mass (g)</i>	1.36	1.71
<i>yield %</i>	2.72	3.42

4.1 Chemical screening results

Table 2: Phytochemicals present in the aqueous and methanol extract of Chenopodium quinoa

photochemical	Chemical nature of the extract	
	Aqueous extract	methanolic extract
Flavonoids	-	+++
Tanins	++	++
Alkaloids	++	++
Saponin	+++	+++
Reducing compounds	+++	-
Sterol compounds and triterpenes	++	+-

+++ : Strong intensity reaction, ++ : Medium intensity
+ : Weak intensity reaction, - : Non detected
+- : trace

4.2 Determination of polyphenol content

The quantitative estimation of polyphenols was carried out according to the method (Singleton and Rossi, 1965), where the Folin-ciocalteu reagent is used, as it quantitatively expresses the total polyphenol content of the studied extracts based on the linear equation of the standard curve for gallic acid, The amount of polyphenols in the aqueous extract of yellow quinoa is estimated at 37.94±1.91 μg AGE/mg extract.

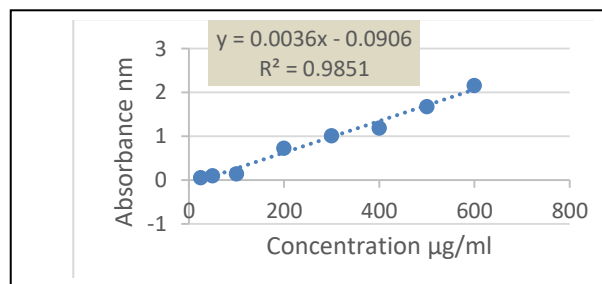


Fig. 1.: Standard curve for estimation of polyphenols

The values of phenolic compounds vary from generation to generation depending on environmental factors, including soil nutrients and precipitation. Some plant species have been reported to produce large amounts of phenolic compounds in highland and semi-arid areas (Penocuo et al., 2018).

The leaves of the quinoa plant contain high levels of phenols compared to the grains, as it was concluded that the distribution of phenols among the members of the plant depends on the cultivated variety (Miranda et al., 2013).

4.3 Determination of flavonoids

The quantification of flavonoids was carried out according to the (Dehpour, 2009) method, which reacts with aluminum chloride, $AlCl_3$, and we use quercetin to determine the equation of the linear curve.

The number of flavonoids in the methanolic extract of the yellow quinoa plant was estimated at $18.40 \pm 0.01 \mu gQE / mg$ extract.

The value of flavonoids appears to be significant in the current study compared to other studies as it was estimated at 36.2 mg/g (Carrasco et al., 2010).

In another study, it was concluded that there is a contradiction in flavonoid species in terms of their response to light under germination conditions, where the proportion of flavonoids Rutin in light increased by 2900 mg/kg, and the value of flavonoids isovitexin and flavonoid Vitexin 1455 mg/kg in the dark and this indicates that the proportions of flavonoids in Plant correlate of photovoltaic conditions (Paškoe et al., 2008). A study of the antioxidant activity of *Teucrium polium* stated that the content of flavonoids could differ even within the same study because of the standard curve where the concentration of flavonoids in terms of Rutin was twice their quantity in terms of Quercetin because the slope of the standard curve of quercetin is equal to twice the slope of the rutin curve.

4.4 Determination of tannins

The content of tannins in the aqueous and methanolic extracts of *Chenopodium quinoa* Willd quinoa was estimated at $58 \mu gECA / mg$ extract and $60.63 \mu gECA / mg$ extract, respectively. stated through his study of different varieties that the distribution of tannins is at the level of the peel and may be subjected to peeling or washing, which reduces the value of tannins, especially those treated (Chuane et al., 1992). In another study, the value of tannins in quinoa was higher compared to soybeans This is due to the applied temperature factor as a variable in the study (Valencia, 2004). Production of tannins just like the rest of the other phenolic substances that are resistant to various stresses, and also considering tannins among the most abundant compounds among plants.

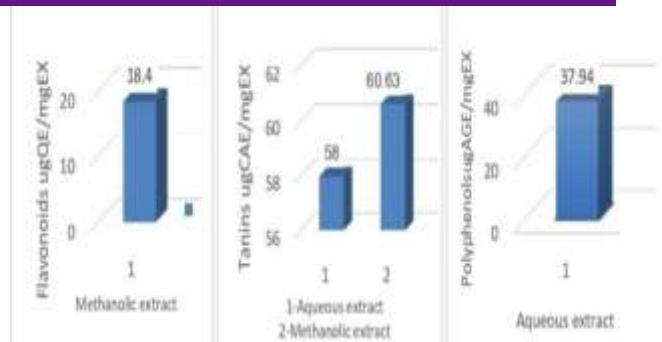


Fig. 2.: Total Phenolic and flavonoid content in quinoa seeds.

5. ACKNOWLEDGMENT

We extend our thanks and gratitude to the biology lab at the College of Natural and Life Sciences and to everyone who supported the study, El oued, Algeria, for their support during this work.

6. CONCLUSION

The growing global demand for nutritious and healthy foods and the need for the environmental and economic sustainability of agricultural production is boosting the cultivation of quinoa worldwide. However, agronomists face a challenge in promoting quinoa as a food crop, particularly in marginal environments where agricultural production is inefficient due to unfavorable climatic conditions, low soil fertility, and market restrictions. In the Middle East and North Africa, experiments are being conducted to test different types of quinoa under agricultural production systems to introduce and improve the production of quinoa.

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