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Determination of antioxidant activity, phenolic quantification of four varieties of fenugreek *Trigonella foenum graecum* L. seed extract cultured in west Algeria

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- ✓ Flavonoids
- ✓ Condensed tannins
- ✓ Western Algeria.

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Abstract

The differences between the metabolic content cultivars and their wild relatives are not only consequences for breeding and quality of food, but also for the study area increasingly growing wild introgression. Trigonella foenum graecum L. is a medicinal plant that is widely used in Algerian folk medicine. This work is a phytochemical study determining and comparing the amount of phenolic compounds and antioxidant activity of four different cultivars of fenugreek seeds grown at the National Institute for Agricultural Research (INRA) in Lamtar (Sidi Bel Abbes, North West Algeria). The total phenolic content in the extracts was determined using the Folin-Ciocalteu reagent and it ranged between 1,613 and 2,083 gallic acid equivalents mg/g of extract. The quantification of flavonoids was performed by a method with aluminum trichloride and sodium hydroxide, it varied between 1,847 to 3,778 catechin equivalents mg/g. The condensed tannins were determined by the method with vanillin under acidic conditions where contents expressed as catechin equivalent were about 0,730 to 1,051 mg/g. The antioxidant activity was analyzed in vitro using the DPPH reagent; among the varieties studied, the 2087 variety showed the highest antioxidant activity.

1. Introduction

Traditional medicine is widely practiced in all countries, and Algeria do not excluded. Today, 90% of the African population uses plants as a source of drugs (1).

The most important bioactive constituents of medicinal plants are the alkaloids, tannins, flavonoids, and phenolic compounds (10). These show considerable interest in the search for natural antioxidants and broad biological effects, including antibacterial, anti-inflammatory, anti-allergic, hepatoprotective, antithrombotic, antiviral, anticancer and vasodilatory action (16).

Trigonella foenum-graecum L. is an annual herbaceous aromatic plant (Fabaceae) (2), widely cultivated in Mediterranean and Asia countries, as it is a popular food (home remedies) consumed in various ways (6). It is rich source of calcium, iron, α-carotene and other vitamins (22). The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarins, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects, may inhibit cholesterol absorption and thought to help lower sugar levels (6).

Seeds and leaves of this plant have been used for centuries not only as food but also as an ingredient in traditional medicine (17). This plant is known for its very important medicinal therapeutic and nutritional properties seen traditional uses and pharmacological activities of the phytochemicals present in the extracts made from the seeds of this plant such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity, (8). In the Maghreb is used in the treatment of wounds, diarrhea, acne, dehydration, anemia, bronchitis, rheumatism, upset stomach, high blood pressure, constipation or as decoctions (14).

There are different varieties of fenugreek plan to cultivate based on soil and climatic conditions. In our work we studied four local varieties cultivated in West Algeria: 0057, 1063, 1138 and 2087.

The objective is to determine different seed extract of fenugreek by measuring total phenolics content, flavonoids and condensed tannins and their possible relation with antioxidant activity.

2. Materials and methods

2.1. Sampling

Four varieties of fenugreek were considered, it is: 0057, 1063, 1138 and 2087. The seeds grown in the same agro-ecological conditions in the National Institute of Agronomic Research (INRA) of Lamtar (Sidi Bel Abbes, Northwest Algeria) and collected in June 2013.

2.2. Methanolic extracts

Each 2 g of dry seeds were grinding by mortar in 10 minutes then was placed in an Erlenmeyer with 20 ml of methanol (96 %) during 24 hours. After filtration (using paper watt man 1ml), the methanolic solutions were evaporated under a reduced pressure in a rotary evaporator standard Buchi r-200 at 40°C. The weighed dry residues were taken again by 3 ml of methanol (15).

2.3. Quantification of polyphenols

2.3.1. Total phenols assay

The proportioning of total polyphenols by Folin-Ciocalteu reagent was described by (26). The reagent consisted of a mixture of phosphotungstic acid (h3pW12o40) and phosphomolybdic acid (h3pmo12o40). It was reduced, during the oxidation of phenols, in a mixture of blue molybdenum and tungsten oxides (21). The maximum concentration of color varied between 725 and 750 nm and it was proportional to the amount of polyphenols present in the vegetable extracts (7).

A volume of 200 µl of the extract was mixed with 1 ml of Folin-Ciocalteu reagent diluted 10 times with water and 0.8 ml of a 7.5 % sodium carbonate solution in a test tube. After stirring and 30 min later, the absorbance was measured at 765 nm by using a Jenway 6405 UV-vis spectrophotometer. Gallic acid was used as a standard for the calibration curve.

Total phenol compounds are reported as Gallic acid equivalents by reference to a standard curve (figure1). The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry matter or fresh matter and milligrams of gallic acid equivalents per g of methanolic extract.

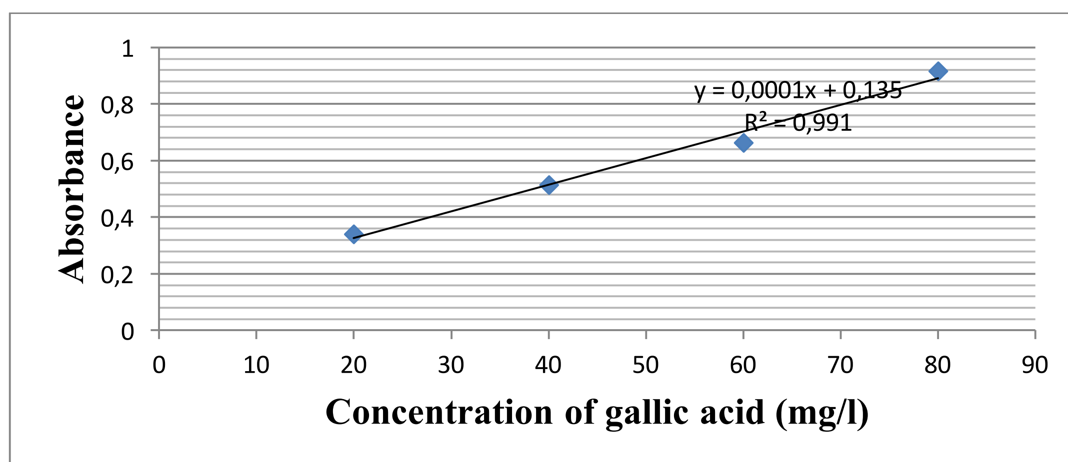

Figure 1: Calibration curve of gallic acid

2.3.2. Total Flavonoids assay

Quantification of flavonoids was performed following method (28) with aluminum trichloride and sodium hydroxide. Aluminum trichloride as a yellow complex with flavonoids and sodium hydroxide formed a complex pink that is absorbed in a visible 510 nm.

At t_0, 0.3 ml 5 % sodium nitrite was added to the flask. After 5 min, 0.3 ml of 10 % aluminum chloride was added. At 6 min, 2 ml of 1 m sodium hydroxide was added to the mix. At one time, the mixture was diluted to volume with the addition of 2.4 ml distilled water and thoroughly mixed. The absorbance of the mixture, pink in color, was determined at 510 nm versus; a blank contains all reagents except samples of extracts or fractions. Catechin was used as a standard for the calibration curve. The total flavonoid content of the extracts and fractions were expressed by reference to a standard curve (figure 2) as catechine mg equivalents per gram of dry or fresh matter and per gram of methanolic extract.

2.3.3. Condensed tannin assay

Condensed tannins were determined by vanillin under acidic conditions (18). This method is based on the ability of reacting with the vanillin units of condensed tannins in the presence of acid to produce a colored complex measured at 500 nm. The reactivity of vanillin with tannins involved only the first unit of polymer.

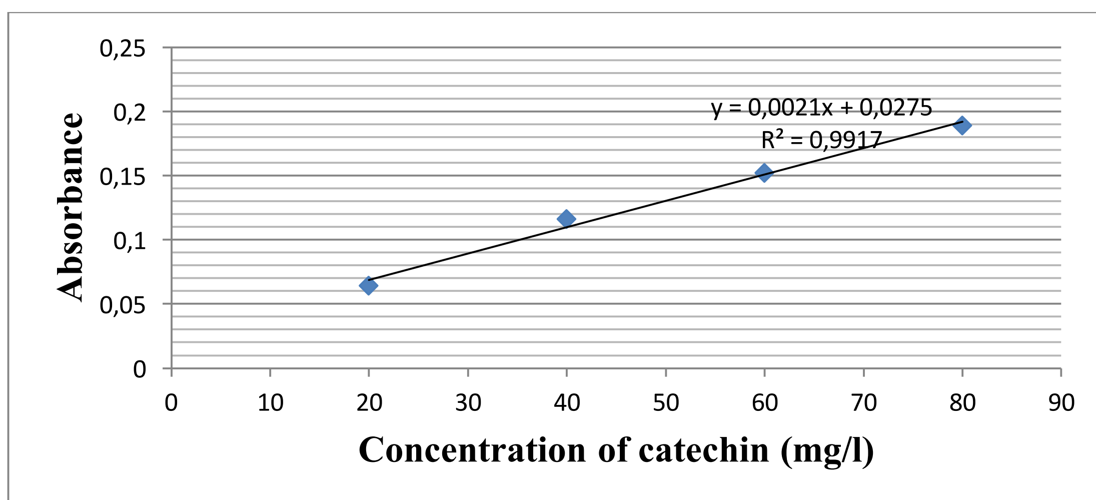

Figure 2: Calibration curve of catechin

The amounts of tannins were estimated and described by (11): counted 0,1ml sample condensation packed into a wrapped reaction tube, then enhanced by 3 ml vanilin 4% (b/v) then merged using a vortex mixer, immediately enhanced by 1,5 condensed HCL meal and mixed. Absorbance of the sample was read at λ 500 nm after incubation during 20 minutes at room temperature. The condensed tannin was expressed as catechin equivalent in mg/g of dry or fresh matter and catechin equivalent in mg/g of methanolic extract matter. Curve calibrates were drawn up by catechin equivalents by references to a standard curve (figure 3) as standard.

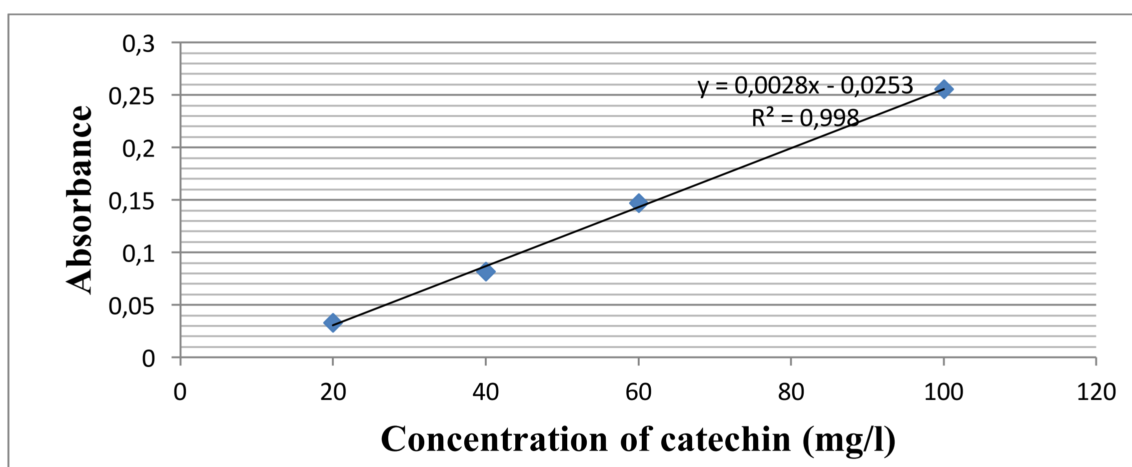

Figure 3: Calibration curve of catechin

2.4. DPPH scavenging assay

The hydrogen atoms donation ability of chemical compounds in the leaves and stems was measured on the basis to scavenge the 2,2-diphenyl-1-picrylhydrazil free radical. (23).

Fifty microliter of various extracts concentrations in methanol were added to 1,950 ml of a 0.025 g/l methanol solution DPPH.

After about a 30 min the incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was estimated utilizing the following recipe:

DPPH scavenging activity (%) = (A blank- A sample/A blank) X 100

Where a blank is the absorbance of the control reaction (containing all reagents except the test compound), a sample is the absorbance of the test compound. Extract concentration providing 50% inhibition (EC50) was estimated from the graph plotted of inhibition percentage against extract concentrations. The ascorbic acid methanol solution was applied as positive control (figure 4) (24).

2.5. Statistical analysis

All experiments were done in triple and results are expressed as means ± standard derivation (S.D.). Statistical analysis of results were done using StatSoft Statistica version 10. The data was subjected to one way Analysis of variance (ANOVA 1) using Microsoft Excel 2007 to compare the content of total phenols, flavonoids and condensed tannins of the 4 fenugreek varieties. Differences were considered significant when the p-values were less than 0.05.

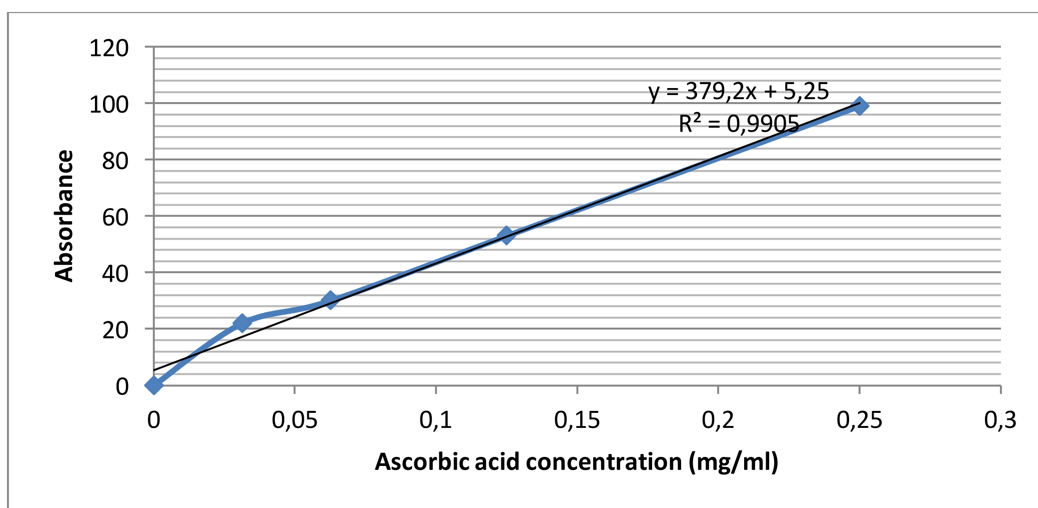

Figure 4: Calibration curve of Ascorbic acid

3. Results and discussion

3.1. The Quantification of polyphenols of the extracts of the Fenugreek seeds

Total phenolic content was expressed as gallic acid equivalent, flavonoids and condensed tannins contents were expressed as catechin equivalent. The results for the levels of phenolic compounds (Table 1 and Figure 5) show a heterogeneity of content between four varieties of fenugreek where the highest total phenolic, flavonoids and condensed tannins contents were observed in methanolic extract of the variety 2087. This heterogeneity was confirmed by testing the variance at the 5%. The results of the content of total phenols, flavonoids and condensed tannins confirmed those obtained by (3), (20) and (25) on fenugreek respectively.

Table 1: Total phenolic, flavonoids and condensed tannins contents of methanolic extracts of the seeds of *Trigonella foenum graecum* L.

Fenugreek Varieties	Total phenolic (mg GAE/gExt)	Total flavonoids (mg CE/gExt)	Condensed tannins (mg CE/gExt)
0057	1,613 ±0,32	2,342±0,45	0,821±0,009
1063	1,398±0,02	1,847±0,14	0,730±0,013
1138	2,047±0,03	2,086±0,08	1,040±0,003
2087	2,083±0,01	3,778±0,13	1,051±0,030
ANOVA I	+ (≠ S.N)	+ (≠ S.N)	+ (≠ S.N)

The data are displayed with means ± standard deviation of three replications.
(mg GAE/gExt=mg acid gallic equivalent/ g of extract, mg CE/gExt = mg catechin equivalent/g of extract)
+ (≠ S.N) = significant difference

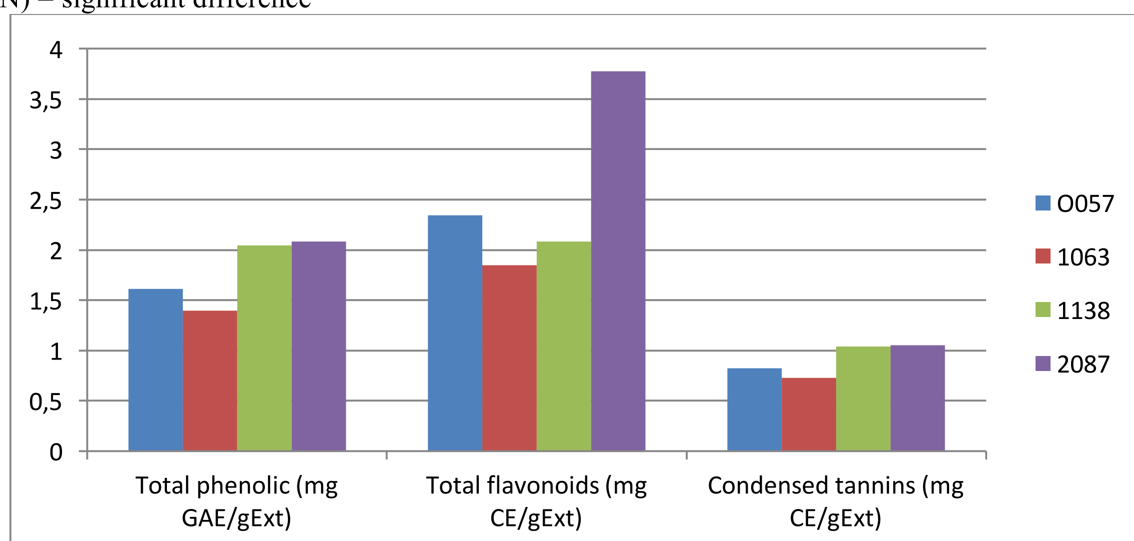

Figure 5: Total phenolic, flavonoids and condensed tannins contents of methanolic extracts of the fenugreek seeds.

3.2. The antioxidant activity of the extracts of the Fenugreek seeds

DPPH is a stable free radical having maximum absorption at 517 nm (12); that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (27).

Trigonella foenum graecum L. seeds extracts were tested for the DPPH free radical scavenging ability, the variety 2087 extract showed highest radical scavenging activity followed by 1063, 1138 and 0057 (figure 6). Table 2 shows the IC50 values (mg/ml) of methanolic extracts of the *Trigonella foenum graecum* extracts in the antioxidant activity evaluation assays. The lower value of IC50 indicates a higher antioxidant activity.

Table 2: IC50 values (mg/ml) of *Trigonella foenum graecum* L. methanolic extracts in the antioxidant activity evaluation assays

Fenugreek varieties	Concentration of methanolic extract (mg/ml)	DPPH IC50
0057	50,25	149 ,32
1063	61,00	99,77
1138	40,25	111,11
2087	40,75	80,98

IC50 (mg/ml): effective concentration at which 50% of DPPH radicals are scavenged.

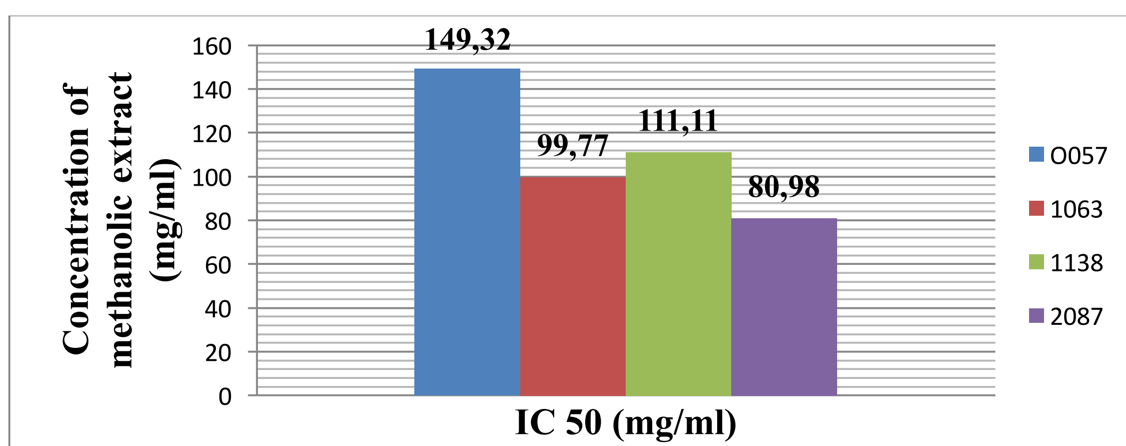

Figure 6: Antioxidant activity of methanolic extracts of *Trigonella foenum graecum* L. seeds using DPPH method determined at different concentrations.

There was a high positive correlation (R^2 = 0,989 in 0057, R^2 = 0,995 in 1063, R^2 = 0,978 in 1138, R^2 = 0,987 in 2087) between the total phenolic content, concentration of methanolic extract and antioxidant activity in the seeds extracts (Figure 7).

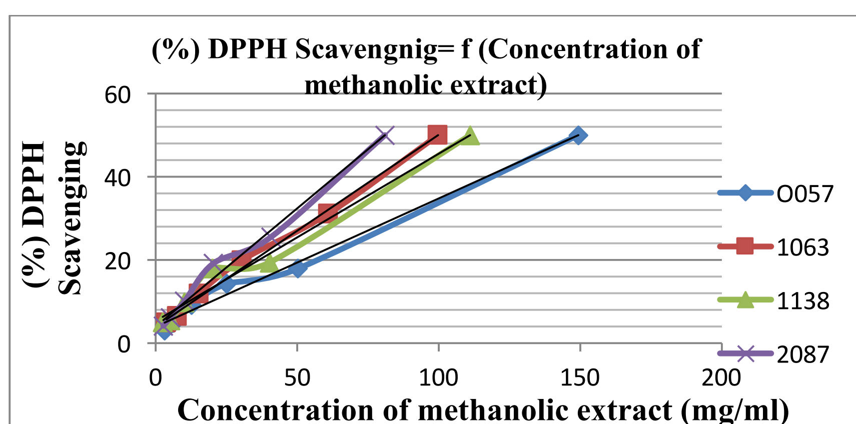

Figure 7: DPPH scavenging activity (%) of methanolic extracts of *Trigonella foenum graecum* L.

The data of this study have shown that high total phenol content and concentration of methanolic extract increases antioxidant activity. This result corroborates the results of (13) which show a strong positive correlation between the total phenolic content and antioxidant activity in the fenugreek extracts.

The natural antioxidants are the subject of much research and a new breath to the exploitation of secondary metabolites generally and particularly polyphenols. Hence the extract of *Trigonella foenum graecum* exhibited potent DPPH activity, (19). The high free radical scavenging activity of the fenugreek seed explained by (4) by the presence of polyphenols in this plant methanolic extract, also a polyphenol-rich extract from the seeds of fenugreek reduces the oxidative haemolysis and lipid peroxidation in normal and diabetic human erythrocytes (9).

Conclusion

The content value of phenolic compounds is related to the variety and the significant variations were observed in total phenolic, flavonoids, condensed tannins contents and antioxidant activity depending on the variety of fenugreek. The variety of 2087 and 1138 show high yields of phenolic compound, compared to the 0057 and 1063 varieties. The metanolic extract of fenugreek 2087 seeds shown highest antioxidant activity (scavenging activity of DPPH %). The antioxidant activity can be correlated with the polyphenolic components present in the extract. These preliminary results are interesting and we think further investigation on this seeds.

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