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EXPERIMENTAL PAPER

Comparative analysis on bioactive compounds and antioxidant activity of Algerian fenugreek (*Trigonella foenum-graecum* L.) and Syrian cumin (*Cuminum cyminum* L.) seeds

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Summary

Introduction: Natural products represent a gold mine for scientists looking for compounds for the treatment of health problems and diseases with their different biological and pharmacological activities. However, recent research is focused on finding natural sources of antioxidants.

Objective: The objective of current research was to determine the phytochemical profile of Algerian fenugreek (*Trigonella foenum-graecum* L.), and Syrian cumin (*Cuminum cyminum* L.) seeds in order to characterize their phenolic compounds and to determine their antioxidant activities.

Methods: Total phenolic, flavonoids, condensed and hydrolysable tannins contents were quantified using Folin-Ciocalteu, aluminium chloride, vanillin and ferric chloride methods, respectively. Phenolic compounds were identified by HPLC method and antioxidant activity was measured using DPPH assay.

Results: The higher amounts of total phenolic compounds, flavonoids, condensed and hydrolysable tannins were given by fenugreek. Results of HPLC analysis of our plants showed that eight phytochemical compounds were found in cumin extract, and seven molecules in fenugreek extract. Moreover, fenugreek possessed higher antioxidant activity.

Conclusion: This study confirmed that our plants are a good source of phenolic contents and possess a high antioxidant activity.

Key words: *Trigonella foenum-graecum* L., *Cuminum cyminum* L., phytochemistry, HPLC, DPPH

Słowa kluczowe: *Trigonella foenum-graecum* L., *Cuminum cyminum* L., fitochemia, HPLC, DPPH

INTRODUCTION

The aetiology of a variety of diseases, including some types of cancer, atherosclerosis, cardiovascular diseases, neurodegenerative diseases, infections, chronic inflammatory diseases, diabetes and autoimmune diseases, has been highly affected by increased levels of free radicals and subsequent oxidative stress [1]. In order to treat problems of oxidative stress, researchers interest in plants which are important source of natural antioxidants (phenolic compounds, flavonoids and vitamins) [2]. Most of plants have ethnomedical practises known to present numerous medicinal properties in their extract [3], as well as antioxidant, antimicrobial, hypolipidemic, anti-inflammatory, anticancer, central nervous system, cardiovascular, analgesic, antipyretic, immunological, and many other pharmacological activities [4].

Cumin (*Cuminum cyminum* L.) is an essential seed spice used by humankind and one of the oldest known minor spices. It originates from Syria, Egypt, Turkey and Eastern Mediterranean area [5]. In several studies antidiabetic [6], antifungal [7], antibacterial [8], antioxidant [9], bronchodilatory [10], hepatoprotective and renoprotective [11], chemopreventive [12], antiepileptic [13], galactagogue [14], hypolipidemic [6], male antifertility [15], memoryenhancing and antistress [16] effect of *C. cyminum* seed extracts have been reported. Cumin contains numerous phytochemical effects

that have several biological activities, such as antimicrobial, insecticidal and antioxidant [17].

Fenugreek (*Trigonella foenum-graecum* L.) is one of the oldest *Fabaceae* family of medicinal plants native to central Asia, with exceptional medicinal and nutritional profile [18]. Fenugreek seeds possess wide range of properties, particularly bitter taste, aromatic smell, carminative characteristics, antibacterial and antioxidant effects [19]. Therefore, they are used as a common remedy in Indian and Chinese medicines for the treatment of diabetes and hypercholesterolaemia [20]. It has been stated that it has restorative and nutritional properties and stimulates digestive processes which are useful in healing various digestive tract ulcers [21]. Pharmacological characteristics such as antitumour, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant have been also recorded for fenugreek [22]. Phenolic acids, such as vanillic acid, coumaric acid, ferulic acid and gallic acid, are present in fenugreek seeds. There is a stronger antioxidant potential for these acids [23, 24].

The beneficial health effects of these two plants prompted us to investigate their secondary metabolites in order to provide important information on their chemical content and their pharmacological effects. Although, the goal of this paper was to determine and quantify the phenolic contents of fenugreek (*Trigonella foenum-graecum* L.), and cumin (*Cuminum cyminum* L.) seeds by HPLC method, and to investigate their antioxidant activity.

Our study is the first report on polyphenolic composition of methanolic extract of Algerian fenugreek and Syrian cumin.

MATERIAL AND METHODS

Sample preparation

According to Bouhenni *et al.* [25], Algerian variety of fenugreek (*Trigonella foenum-graecum* L.) and Syrian cumin (*Cuminum cyminum* L.) were the best ones, as compared to other varieties with a highest weight and a better germination rate. In this concept, these varieties were chosen for present study. Fenugreek seeds were grown in the region of Mostaganem, Algeria, and cumin seeds were grown in the region of Aleppo, Syria.

Samples of fenugreek and cumin were taken from a local market in Algeria in March 2017 in order to avoid the dust, the loss of aroma and colour which could occur as a result of their exposure to the direct sun light. They were sorted, washed, and dried at a room temperature then ground and screened at 200 μ m using electrical grinder. Resultant powders were analysed for their chemical and biological aspects.

Phytochemical analysis of fenugreek and cumin samples

Preparation of extracts

The extracts were prepared using maceration method, all the extraction procedures and conditions were performed as described by Gezici *et al.* [26]. 100 ml of 70% methanol was mixed with 10 g of each sample, the solutions were shaken at a room temperature for 24 h, then the mixtures were filtered using Whatman paper N°01 and evaporated using rotary evaporator. Dried extract was conserved in the refrigerator at 4°C for further analyses. Each extract was dissolved at 1mg/ml. Finally, yield extraction (w/w%) was determined.

Phytochemical tests

- *Determination of total phenolic content*

Total phenolic content was estimated according to the procedure described by Singleton *et al.* [27]

using Folin-Ciocalteu reagent. 0.5 ml of different concentrations of each extract and 2.5 ml of Folin-Ciocalteu were mixed with 1 ml of sodium carbonate (20%). This mixture was incubated for 30 min. in the dark at a room temperature. The absorption of the solution using the UV-Vis spectrophotometer was measured at 765 nm. A calibration curve was established using gallic acid as standard. The results were expressed as milligram of gallic acid equivalent (GAE) per 100 g of dry matter.

- *Determination of total flavonoids content*

Total flavonoids content of both extracts was determined using aluminium chloride method [28]. 1.5 ml of different concentrations of all extracts was mixed with 75 μ l of aluminium chloride solution and 0.5 ml of sodium acetate solution. The mixture was completed with distilled water until it reached a volume of 2.5 ml. Using a UV-Vis spectrophotometer, the absorption of the solution was measured at 415 nm after the incubation time of 30 min. at a room temperature in the dark. The results were expressed as milligram of quercetin equivalent (QE) per 100 g of dry matter through a calibration curve.

- *Determination of condensed tannins content*

Condensed tannins content was estimated using the method of Price [29]. 1 ml of each extract was added to 2.5 ml of 4% methanol vanillin solution and 2.5 ml of H₂SO₄. The absorbance was measured at 500 nm after 15 min. The results were expressed as milligram of catechin equivalent (CE) per 100 g of dry matter via a calibration curve.

- *Determination of hydrolysable tannins content*

Hydrolysable tannins content was estimated according to the method of Mole [30]. 500 μ l of the extract was mixed with 3.5 ml of the ferric chloride solution. After 15 s, the absorbance was measured at 660 nm. The results were expressed as milligram of tannic acid equivalent (TAE) per 100 g of dry matter via a calibration curve.

Phytochemical screening

Qualitative tests were realized in order to identify the presence of some phytochemical compounds in plants extracts according to Trease and Evans [31] and Sofowora [32], as shown in table 1.

Table 1.

Phytochemical screening tests

Metabolites	Added reagent	Expected result
Flavonoids	KOH (50%)	yellow color
Tannins	FeCl ₃ (1%)	blue coloration
Alkaloids	HCl 2%+ Wagner reagent	brown precipitate
Sterols and triterpenes	acetic anhydride + H ₂ SO ₄ (98%)	red colour (surface) + greenish fluorescence
Terpenoids	chloroform + H ₂ SO ₄ (98%)	reddish brown coloration
Saponins	distilled water	formation of foam
Anthocyanins	chlorhydric alcohol+ isoamyl alcohol	reddish brown coloration
Cardiac glycosides	glacial acetic acid + FeCl ₃ (5%)+ H ₂ SO ₄ (98%)	brown ring
Reducing compounds	Fehlings (A+B)	brownish-red precipitate

Chromatographic analysis (HPLC)

Phytochemical compounds were identified using high-performance liquid chromatography (HPLC). According to their polarity in the solvents, the model of HPLC used was Shimadzu Nexera-I HPLC with autosampler and quaternary pump. Each extract was dissolved in methanol in a ratio of 1 part of extract to 5 parts of solvent. The extracts were analysed as such by injection into HPLC. The operating conditions are as follows: column: silica gel-C18 type Fortis C18, 150 x 2.1 mm x 3 µm, eluent: A – water, B – 0.1% formic acid, aqueous solution with pH=2.5, and C – acetonitrile, flow rate: 1 ml/min., injected volume: 5 µl, detector: DAD, spectrophotometric 220–400 nm, with chromatograms recorded at 254, 326 and 360 nm. The evaluation was based on a comparison of retention times and absorption maxima in the UV-Vis spectra.

In comparison with standards (standard pure phytochemical molecules), the resulting chromatographic profile is injected under the same operating conditions as that of the sample. The integrator determines the retention time (Rt) of each component by giving a peak on the chromatogram [33].

Antioxidant activity

The antioxidant power of plants extracts was evaluated by Shimada *et al.* [34] using the DPPH method. The DPPH solution (0.1 mM) was obtained by dissolving 4 mg DPPH in 100 ml methanol.

Serial dilution was prepared in order to obtain all increasing concentration required (0.1, 0.2, 0.3, 0.4, 0.5 mg/ml). The solutions were then incubated at room temperature in the dark for 30 min., and the absorbance was measured at 570 nm. The antioxidant activity was determined using the following formula:

$$\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100,$$

where

A_{control} – absorbance of DPPH solution without extract,

A_{sample} – absorbance of sample with DPPH solution.

The half-maximal inhibitory concentration (IC₅₀) was defined as the amount of antioxidant needed to decrease the initial DPPH concentration by 50%.

Statistical analysis

Statistical software program (SPSS version 20) was used to analyse our obtained results. The differences between the means were considered significant for *p* values lower than 0.05. Results were expressed as mean values and standard deviation with three repetitions.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS

Phytochemical analysis

The results of yield extraction, total phenolic, total flavonoid, condensed and hydrolysable tannins content in fenugreek and cumin extracts are presented in table 2.

Table 2.

Results of yield extraction, total phenolic, total flavonoid, condensed and hydrolysable tannins content of fenugreek and cumin

Analysis	Fenugreek extract	Cumin extract
Yield extract (%)	17.66±0.033	14.29±0.002
TPC (mg GAE/100 g DM)	115.3±0.01	91.6 ±0.001
TFC (mg QE/100 g DM)	80.98±0.066	66.04± 0.15
CTC (mg CE/100 g DM)	2.2±0.01	1.8±0.033
HTC (mg TAE/100 g DM)	1±0.045	0.205±0.001

TPC – total phenolic content; TFC – total flavonoids content; CTC – condensed tannins content; HTC – hydrolysable tannins content; DM – dry matter

The obtained results showed that the yield extract of fenugreek using maceration method and 70% methanol as solvent was higher (17.66±0.033%) than that of cumin (14.29±0.002%).

In the present study, the results showed that cumin extract expresses the higher phenolic content (115.3±0.01 mg GAE/100 g DM) than fenugreek extract (91.6±0.001 mg GAE/100 g DM). Also for total flavonoid, the highest content was given by fenugreek extract (80.98±0.066 mg QE/100 g DM), as compared to cumin extract (66.04±0.15 mg QE/100 g DM). Also fenugreek showed the higher value of condensed tannins (2.2±0.01 mg CE/100 g DM) in comparison with cumin (1.8±0.033 mg CE/100 g DM).

The results of qualitative assay of samples were shown in table 3. The results revealed the presence of flavonoids, tannins, terpenoids, anthocyanins and cardiac glycosides both in fenugreek and cumin extracts, with moderate difference. On the other hand, reducing compounds, alkaloids,

sterols, triterpenes and saponosides were absent in the tested extracts.

Table 3.

Results of phytochemical screening of fenugreek and cumin

Analysis	Fenugreek extract	Cumin extract
Flavonoids	++	+++
Tannins	++	+++
Alkaloids	–	–
Sterols and triterpenes	–	–
Terpenoids	++	+++
Saponosids	–	–
Anthocyanins	+	++
Cardiac glycosides	++	+++
Reducing compounds	–	–

(–): absent; (+): low presence; (++): medium presence; (+++): high presence

Chromatographic analysis HPLC

The molecular separation of fenugreek and cumin methanolic extracts was achieved by HPLC at three wavelengths: 254 nm, 326 nm and 360 nm. The findings obtained are visible in the peaks and retention time of chromatograms of each molecule. The results obtained are shown in the chromatograms with peaks and retention time of each molecule (fig. 1–6).

The results of HPLC showed the presence of fifteen compounds in *C. cyminum* extract (fig. 1–3) and eight compounds in *T. foenum-graecum* (fig. 4–6), which were identified by comparison between their retention times (Rt) with that of pure standards under the same experimental conditions.

Molecules identified in the methanolic extracts of *C. cyminum* and *T. foenum-graecum* are mentioned in table 4.

Eight phytochemical compounds could be identified in *C. cyminum* extract, namely: caffeic acid, isoquercetin, vanillic acid, myricetin 3-0, rutin, syringaresinol, citrusine, rosmarinic acid, *p*-coumaric acid. Seven compounds of *T. foenum-graecum* extract are: gallic acid, sinapic acid, caffeic acid, astrogenic acid, pyrogallol, hyperoside and ferulic acid. Other molecules could not be identified.

Figure 2.

HPLC chromatogram of *Cuminum cyminum* dry extract at 326 nm

Figure 5.

HPLC chromatogram of *Trigonella foenum-graecum* dry extract at 326 nm

Figure 6.

HPLC chromatogram of *Trigonella foenum-graecum* dry extract at 360 nm

Table 4.

Polyphenolic compounds of fenugreek and cumin analyzed by HPLC

Extract	Compounds	Retention time [min]
<i>Cuminum cyminum</i>	/	4.684
	/	7.881
	/	10.812
	caffeic acid	12.280
	/	14.322
	isoquercetin	15.165
	/	15.857
	vanillic acid	15.835
	/	18.179
	syringaresinol	19.007
	/	19.487
	myricetin 3-0 pentoside	20.030
	citrusine	20.602
	rosmarinic acid	21.197
<i>Trigonella foenum-graecum</i>	<i>p</i> -coumaric acid	25.719
	gallic acid	3.115
	sinapic acid	7.577
	caffeic acid	9.838
	asterogenic acid	10.146
	/	10.657
	pyrogallol	11.957
	hyperoside	16.480
	ferulic acid	17.647

Table 5.

Results of antioxidant activity of fenugreek and cumin

Extract	Extract concentration [µg/ml]	[%] Inhibition	IC ₅₀ [µg/ml]
Methanolic extract of fenugreek	1000	82.57	343.75
	500	80.59	
	250	39.20	
	125	35.84	
Methanolic extract of cumin	1000	68.51	588.55
	500	55.04	
	250	24.15	
	125	22.17	

Antioxidant activity

The results of the antioxidant power of plants extracts carried out by DPPH method were presented

in table 5. They showed that methanolic extract of fenugreek had the strongest radical-scavenging effect, as compared to cumin methanolic extract.

DISCUSSION

The result of percentage yield of fenugreek extract was approximately similar to the result of Abdouli *et al.* [35] (17.09±1.43%) and higher in comparison with the result of Sakhira *et al.* [36] which was 4.95%. Although, the percentage yield of cumin extract was higher than that of the earlier research of Megha [37] with an amount of 15.93%, and extremely higher than the result of Elghorab *et al.* [38] which was 4.08±0.17%. Thippeswamy and Naidu [39] observed that high yield extract was given with methanol solvent. Significant differences were observed among yields extraction between fenugreek and cumin ($p=0.000$).

The chemical composition of phytochemicals, the extraction method used, the particle size of the sample, the solvent used, as well as the presence of interfering substances affect the extraction yield [40].

This latter depends on the solvent with different polarities, extraction time, pH, temperature, and composition of the sample solvent as well as on the composition of sample. These are known as the most significant parameters under the same extraction time and temperature [41].

Similarly, this difference may be due to a greater solubility in methanol than in other solvents of extractable bioactive components such as carbohydrates and proteins [42].

The difference in extracted yields may be due to the difference in solvent polarities used, which also plays a key role in the increasing phytochemical compound solubility [42, 43].

Variations in the structure of phytochemical molecules also determine their solubility in solvents with different polarities [44].

Total phenolic contents of fenugreek were significantly lower than those found by Kaviarasan *et al.* [36, 45-48] with values of 480 mg GAE/100 g, 589±0.02 mg GAE/100 g, 1260 mg GAE/100 g, 2300 mg GAE/100 g and 5430 mg GAE/100 g, respectively. However, result of Benziane *et al.* [49] study on aqueous extract of fenugreek was extremely lower than present result: 18.9 mg GAE/100 g. While many studies have been carried out to estimate the amount of total phenolic contents in cumin, they gave a higher results than ours: Shan *et al.* [50], [17, 38, 39, 51-55] with

an amount of 230 mg GAE/100 g, 333–431 mg GAE/100 g, 685 mg GAE/100 g, 900 mg GAE/100 g, 1832 ± 0.23 mg GAE/100 g, 1920 mg GAE/100 g, 2466 mg GAE/100 g, 2950 ± 0.58 mg GAE/100 g and 3530 mg GAE/100 g, respectively.

The difference in phenolic composition between fenugreek and cumin ($p=0.000$) could be due to extraction method, plant organ, type of cultivar, harvest time, storage conditions, and genetic or geographical origin [56].

The season and sunlight duration are also known to affect the plant metabolism, since some compounds may be accumulated at a particular time to respond to environmental changes [57].

Several studies have also shown that solvent polarity contributes to substantially different phenolic compound extraction capacities in plants [58, 59].

The higher phenolic acid levels in methanolic extracts could be due to extraction of both non polar and semi polar soluble phenolic acids [39].

Total flavonoids analysis expressed that the amount of total flavonoids contents in fenugreek was higher than the amounts found by Abdouli *et al.* [35, 46, 60] which were 0.77 mg QE/100 g, 31.8 mg QE/100 g and 20.8–65.3 mg QE/100 g, respectively. However, Sakhira *et al.* [36, 61, 62] revealed a raised amount: 136 to 274 mg QE/100 g, 145 mg QE/100 g, 377.8 mg QE/100 g and 4990 mg/100 g OE, respectively. Regarding cumin, significant differences in total flavonoids contents were remarked relatively to previous study of Rebey *et al.* [51] with a value of 56 mg QE/100 g for Tunisian cumin seeds and 88 mg QE/100 g for Indian cumin seeds. Our amount was significantly higher than that showed by Munuswamy and Ramachandiran [17] (15.1 mg QE/100 g). However, other researchers, Zhang *et al.* [52, 53, 63] found higher contents: 102 mg QE/100 g, 560 mg QE/100 g and 4656 mg QE/100 g, respectively.

The significant differences between plants ($p=0.000$) in the terms of flavonoids were explained by several factors such as genotypic and environmental differences within species [64], choice of parts tested [65], time of taking samples [66] and determination methods [67].

The presence of condensed tannins in fenugreek agrees with the results of Abdouli *et al.* [35, 46, 68] with significant differences: 2.3 mg CE/100 g, 0.29 mg CE/100 g, and 0.78 mg CE/100 g, respectively.

Rahmani *et al.* [61] found higher value (73 ± 0.013 – 105.1 ± 0.030 mg CE/100 mg), although, Benziane *et al.* [49] observed that fenugreek aqueous extract showed a highest amount of condensed tannins: 8.69 mg CE/100 g. The levels of present

results were lower than that reported in Iranian fenugreek seed genotype (380 mg CE/100 g DM) [69] and hugely lower than that reported in Yemen genotype (2000 mg CE/100 g) [62]. Levels measured in fenugreek seeds were far below threshold level (5000 mg CE/100 g DM) mentioned by Muller-Harvey [70]. Besides, a lower condensed tannins content was recorded in cumin, in comparison with fenugreek. The present result was lower than results of Rebey *et al.* [51, 54] (200 mg CE/100 g, 4228 mg CE/100 g), and extremely lower than that of Rebey *et al.* [53] with an amount of 6571 mg CE/100 g for Tunisian cumin seeds and 6137 mg CE/100 g for Indian cumin seeds. Cumin is known to contain large amount of tannins [71].

The difference in findings ($p=0.000$) may be due to extraction methods and solvents used [72], cultivar type, growing conditions, maturity stage at harvest, storage conditions and sample preparation method [47]. In contrary to hydrolysable tannins contents, there is no substantial difference between these two plants.

Results of the phytochemical screening of methanolic extracts of fenugreek did not exclusively agree with the report of Asmena *et al.* [73] which shows the absence of flavonoids, tannins and cardiac glycosides, even the presence of alkaloids steroids and carbohydrate. However, Sumayya *et al.* [3] assent with our study, and found many secondary metabolites in fenugreek extract like flavonoids, tannins, phenols, carbohydrate, glycosides, anthocyanin and terpenoids. Further Rodolfo *et al.* [74, 75] studies showed the presence of flavonoids, steroids, alkaloids, and saponins in fenugreek extract. Gorinstein *et al.* [76], also reported the presence of terpenoids, tannins and absence of anthocyanin.

A recent study of Megha *et al.* [37] concerning the qualitative analysis of cumin registered a moderate presence of alkaloids, flavonoids, steroids, carbohydrate, phenol and terpenoids, whereas tannins, saponins, protein, glycosides and cardiac glycosides were reported to be absent in this plant extract. Furthermore, Himanshu *et al.* [77] reported the richness of cumin extract with alkaloids, glycosides, flavonoids, tannins, terpenoids and phenolic compounds which are in the contrary to our results.

Qualitative and quantitative analysis of important individual phenolics in the spices may help reveal the structure-activity relationships of antioxidant phenolics and to explain the relationships between total antioxidant activity and total phenolic contents [50].

In the *C. cyminum* extract, the separation of flavonoids at retention times between 10 and 20 min.

is observed, which are not derived from quercetin, having absorption maxima between 330 and 345 nm. At 20.030 minute, a polyphenol appears, that seems to be of the tannin class, probably a complex or condensed tannin. Tannins also appear at 4.864 min. and 7.881 min., respectively. At retention times higher than 20 min., polyphenols appear; that seems to be of the coumarin class.

C. cyminum extract appears to be the richest in polyphenols. Among the flavonoids, those from 14.322 min. and 12.280 min., respectively, are the majority, representing 30.5% and 20.8%, respectively, of total of the most important polyphenols. Others are under 11%.

In the case of *T. foenum-graecum* extract at 3.115 min. a tannin is separated, probably a gallic acid derivative, then at minutes 7.577, 10.146, 10.657 and 11.957 flavonoids with maximum absorption between 330 and 340 nm, and at over 15 minutes polyphenols that seems to be from coumarin class. At minute 9.838, a polyphenol with a spectrum specific to caffeic acid derivatives appears.

Among the majority of flavonoids, the predominant quantity is the one from minute 10.657, this being in proportion of 47.3%, and the component separated at 11.957 min. in proportion of 26.9%.

Previous study about the characterization of secondary metabolites in cumin observed the presence of coumaric acid, luteolin, syringic acid, cinnamic acid for Tunisian cumin seeds and trans-2-dihydrocinnamic acid as well as flavone for Indian cumin seeds [51]. Eventually, Rebey *et al.* [53] found that phytochemical characterization of cumin revealed the presence of eighteen phenolic compounds, including gallic acid, caffeic acid, dihydroxyphenolic acid, dihydroxybenzoic acid, chlorogenic acid, syringic acid, vanillic acid, coumaric acid, ferrulic acid, rosmarinic acid, cinnamic acid, flavonoid, leutolin, catechin, coumarin, apigenin, amentoflavone and flavone. Also, Shan *et al.* [50] demonstrated that bioactive compounds present in cumin were phenolic acids, flavonoids, coumarins, caffeic acid, kaempferol and others compounds which were not determined. Ani *et al.* [78] could identify some phenolic compounds in cumin seeds such as gallic, caffeic, ellagic protocatechuic, ferulic acids and also flavonols, such as quercetin and kaempferol.

Concerning phenolic profile of fenugreek, some major components such as catechin, epicatechin, gallic acid, caffeic acid, coumaric acid, cinnamic acid, vanillic acid were previously found in fenugreek ethanolic extract [36] and others: gallic acid, chlorogenic

acid, *p*-coumeric acid, ferulic acid, sinapic acid and quercetin in the methanolic extract [79].

Four phytochemical compounds were found in fenugreek aqueous maceration extract, namely: genistein, kaempferol, vanillin and myrecitin, while three compounds were identified in aqueous decoction extract, namely: kaempferol, rutin, and vanillin [49].

Also Swati *et al.* [80] exhibit that characterization of phenolic compounds present in fenugreek extract by HPLC could identify seven contents: vitexin, isovitexin, kaempferol dirhamnoside, kaempferol rhamnoside, quercetin, leuteolin and apigenin. In other research it was observed that flavonoid glycosides and kaempferol were two major phenolic compounds found in the aqueous extract of fenugreek [81].

The significant difference in the phenolic profile obtained by HPLC between fenugreek and cumin confirmed previous data of total phenolic and flavonoid contents. These differences could be related to many factors, such as genotype, stage of maturity, growing and climate conditions, harvest time and even post-harvest conditions [82]. Also, the results of chromatographic analysis using HPLC depend on the separating power of the column, the flow velocity and the composition of the mobile phase and column temperature [83].

Our study showed that free radical scavenging activity of cumin was lower than fenugreek. Regarding IC_{50} , the lowest value was observed by fenugreek ($343.75 \pm 0.01 \mu\text{g/ml}$), followed by cumin ($588.55 \pm 0.01 \mu\text{g/ml}$). While, the results establish that fenugreek has more ability to scavenge the free radicals as compared to cumin.

Sakhira *et al.* [36] observed that fenugreek extract presents a similar IC_{50} to our result with a concentration of $285.59 \pm 2.01 \mu\text{g/ml}$ with higher radical scavenging activity, however, other researchers, such as Mashkor *et al.* [47] and Rababah *et al.* [48], present lower radical scavenging activities: 65–68% and 10%, respectively. Regarding to cumin, previous study of Thippeswamy and Naidu [39] showed that radical scavenging activity of cumin was similar to our result: IC_{50} $520 \mu\text{g/ml}$, while others, such as Rebey *et al.* [51], present higher activities: $6.24 \mu\text{g/ml}$ for Tunisian cumin seeds and $15.14 \mu\text{g/ml}$ for Indian cumin seeds, Rebey *et al.* [53]: $20.17 \mu\text{g/ml}$ and Zhang *et al.* [52]: $102.42 \mu\text{g/ml}$. Aljuhaimi *et al.* studies [55, 84] gave a lower activities with IC_{50} : 825 – $1124 \mu\text{g/ml}$ and $2000 \mu\text{g/ml}$, respectively.

In addition, major differences in antioxidant activity between these two plants ($p=0.000$) are primarily due to the difference in the polarity of the solvents

used and therefore to the different effects of extractability on the antioxidant compounds [85, 86].

It is widely agreed that the antioxidant potential of phenolic compounds is often linked to the chemical composition of individual compounds, depending on a variety of factors, including geographical variation [87], harvest time [88], environmental and agronomic conditions [89], plant botanical components [90] and methods of extraction [91].

The literature presents ample evidence for the biological and biomedical activities of cumin, including its use as a treatment of a variety of diseases, such as chronic diarrhoea and dyspepsia, acute gastritis, diabetes, and cancer which have generally been ascribed to its bioactive constituents such as phenols and flavonoids [92]. Although, several animal studies and clinical data show that the use of fenugreek seeds can be useful in lowering cholesterol and blood glucose level. It has been found that compounds present in fenugreek extracts increase bile secretion and reduction in blood cholesterol, also administration of extracts from fenugreek seeds has a beneficial effect on blood glucose level, as it was confirmed by many studies performed in animals and in humans. It seems that such activity of compounds contained in fenugreek seeds is beneficial for people struggling with concomitant diseases in the metabolic syndrome [93]. In this context, the present study allowed to identify bioactive compounds which are economically important as drugs (pharmaceuticals) in medical field.

CONCLUSION

In our study, higher amounts of total phenolic compounds, flavonoids, condensed and hydrolysable tannins were found in fenugreek extract seeds.

Furthermore, the results of chromatographic analysis by HPLC revealed the presence of caffeic acid, isoquercetin, vanillic acid, myricetin 3-O-rutinoside, syringaresinol, citrusine, rosmarinic acid, and *p*-coumaric acid in cumin seeds and gallic acid, sinapic acid, caffeic acid, asterogenic acid, pyrogallol, hyperoside and ferulic acid in fenugreek seeds, this diversity could be explained by several factors such as genotype, stage of maturity, growing and climate conditions, harvest time and even post-harvest conditions.

It can be concluded that fenugreek extract possess a very strong antioxidant activity which showed an IC_{50} value of $343.75 \pm 0.01 \mu\text{g/ml}$ followed by cumin extract ($588.55 \pm 0.01 \mu\text{g/ml}$). This significant antioxidant potential may be due to polyphenolic

constituents and flavonoids present in these two plants.

Therefore, fenugreek and cumin extracts seem to be a valuable source of natural antioxidants and may be applied commercially in pharmaceutical industry as drugs in the future.

Further scientific assessment of tested plant amalgams should be performed, including fractionation and further characterization of phytochemicals to identify the active components responsible for the biological activities, to determine the antioxidant effect of isolated molecules with maximum activity and to establish that the overall antioxidant effect is a measure of all the components present together and working together or their individual capacities.

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