

Lipids, proteins, phenolic composition, antioxidant and antibacterial activities of seeds of peanuts (*Arachis hypogaea* L) cultivated in Tunisia

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ABSTRACT

Fatty acid composition of peanut seed oil in four varieties cultivated in Tunisia showed that linoleic (C18:2), oleic (C18:1) and palmitic (C16) acids account for more than 84% for Chounfakhi and Massriya and for more than 85% of the total fatty acids of Trabilisia and Sinya seed oil respectively. Seed oil contents were significantly different ($P \leq 0.05$) and did not exceed 48%. The study of total phenolics revealed that Chounfakhi contained more total phenolics (2.1 mg GAE/g DW), followed by the Massriya and Sinya cultivars (1.35 mg GAE/g DW for each); Trabilisia presented the lowest total phenolic content with 1 mg GAE/g DW. Considerable antiradical ability was found, especially in the Trabilisia peanut seed cultivar (IC₅₀ = 1550 µg/ml), the Massriya and Sinya cultivars had, respectively, 720 and 820 mg/ml IC₅₀. In the Massriya variety the sterol fraction showed antibacterial activity against *Listeria ivanovii*, *Listeria innocua*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus hirae* and *Bacillus cereus*.

Keywords: *Arachis hypogaea*; oil; sterols; phenols; antioxidant activity; antibacterial activity.

INTRODUCTION

Peanuts (*Arachis hypogaea* L.) are grown in almost all tropical and sub-tropical countries; the most important producers are India, China, USA and West Africa (Krishnappa et al., 1999 ; Bhatti et al., 2010). Peanuts, known in Tunisia as “cacawya”, are mainly cultivated in the Cap Bon region (NE Tunisia) as well as in Nefza (NW) and Kairouan (Center) (Bornat, 2006). Compared to other oleaginous seeds, peanut seeds have a low amount of phospholipids (0.65 to 1.35%), 0.8% unsaponifiable (Yoshida et al., 2005), and 44 to 56% oil with a fatty acid composition mainly represented by oleic (37.94 to 41.90%), linoleic (34.59 to 37.51%), palmitic (12.22 to 13.30%), stearic (3.17 to 3.67%) and arachidic (1.63 to 1.85%) acids (Berry, 1982; Kaya et al., 2009). The total fatty acid composition was 10.44% and 33.51% for saturated and unsaturated fatty acids, respectively; the most abundant fatty acids of groundnut seed oil were oleic (C18:1), linoleic (C18:2) and palmitic (C 16:0), which together composed about 88.35% of the total fatty acids (Ingale and Shrivastava, 2011). This food is made up 22-30% protein, thus being an excellent source of energy (564 kcal/100 g); defatted peanut flour is a byproduct of peanut processing, which contains 47-55% proteins with high nutritional value (Yu et al., 2007). The main proteins are two globulins, arachine and conarchine, that represent at least 95% of the total seed proteins (Yoshida et al., 1995; Andersen et al., 1998; Han et al., 2009). Phytosterols and triterpenes make up the greatest proportion of the unsaponifiable components of vegetable oils (Azadmard-Damirchi et al., 2005); they are considered to have anti-inflammatory, anti-bacterial, anti-atherosclerotic, antioxidative (Lagarda et al., 2006), anti-ulcerative and antitumor properties in humans (Moreau et al., 2002), as well as contributing to the oxidative and thermal stability and shelf-life of vegetable oils (Beveridge et al., 2002;

Przybylski and Eskin, 2006, Alvarenga and Esteban, 2005). Peanut seeds have high nutritional and commercial value due to their protein, fatty acid, carbohydrate and fiber content, as well as vitamins, calcium, and phosphorous. Peanut oil is used in cooking and is also employed in the manufacture of margarines, cosmetics, pharmaceuticals and surfactants (Plessis and Steiman, 2004; Schirack et al., 2006; Nakai et al., 2008; Rodriguez et al., 2011).

The aim of this study was to analyze antioxidant activity and lipid, fatty acid, protein and phenolic composition in four groundnut varieties cultivated in Tunisia, as well as to test these compounds for antibacterial activity against reference strains.

2. MATERIAL AND METHODS

2.1. Plant material

The four groundnut varieties used in this study were Massriya, Sinya, Chounfakhi and Trabilisia. Their seeds were provided by the CRDA of Nabeul (NE Tunisia).

2.2. Determination of oil content

Oil content was determined by extracting dry material of peanut with petroleum ether using a Soxhlet apparatus (4h/42 °C) (Harwood, 1984). The extract was dried in a rotary evaporator and the oil content was determined as the difference in weight between a dried peanut sample before and after extraction (AOCS, 1989).

2.3. Saponification and TLC analysis

The unsaponifiable fraction of lipids was determined by saponifying 5 g of oil mixed with both 200 µl α -cholestanol

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and an ethanolic KOH 12% solution; the mixture was heated at 60 °C for 1.30 h. The unsaponifiable matter was extracted, washed, dried over anhydrous Na₂SO₄ and evaporated to dryness using N₂. The unsaponifiable matter was separated into subfractions on preparative silica gel thin-layer plates (silica gel 60G F254) using one-dimensional TLC with hexane–diethyl ether (6:4, v/v) as the developing solvent. The unsaponifiable fraction diluted in chloroform was applied on the silica gel plates. After developing, the plate was sprayed with 2,7-dichlorofluorescein and viewed under UV light.

2.4. GC–FID Fatty acids methyl ester analysis

Fatty acids were methylated using the method of Metcalfe et al. (1966) modified by Lechevallier (1966). Methyl esters were analyzed with a gas chromatography-flame ionization detector (GC-FID) using an HP 4890 chromatograph equipped with a flame ionization detector (FID) on a capillary column coated with supelcowaxTM 10 (30 m length, 0.25 id, 0.2 mm film thickness). Temperatures of the column, detector and injector were 200, 250 and 260 °C, respectively.

2.5. SDS-PAGE of proteins

Defatted peanut seeds (100 mg) were successively extracted with 1 ml distilled water, 1 ml 5.0 M NaCl, 1 ml absolute ethanol, and 1 ml 0.2 M phosphate buffer (pH 8.0) for the extraction of the albumin, globulin, prolamin and glutelin, respectively. Each extraction was shaken for 20 min in an Eppendorf tube and centrifuged at 10,000 g for 6 min. Protein fraction assays were performed following Bradford's method (1976). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a discontinuous buffered system according to the method of Laemmli (1970), using 12% separating gel and 3% stacking gel.

2.6. RP-HPLC phenol analysis

Colorimetric quantification of total phenolics was determined as described by Dewanto et al., (2002). All samples were analyzed in three replicates.

Phenolic compound analysis was carried out using a liquid chromatography RP-HPLC coupled with a UV-vis multiwavelength Waters 996 Photoperiod Array Detector: 190–400 nm. The separation was carried out in a 250×4.6-mm, LD 5-μm symmetry shield C18 reversed phase column. The mobile phase consisted of acetonitrile (solvent A) and water (80:20 v/v) with 1% formic acid (solvent B). The flow rate was kept at 1 ml/min. The injection volume was 20 μl and peaks were monitored at 275 nm. Peaks were identified by congruent retention times compared to standards. Analyses were performed in triplicate.

2.7. DPPH radical-scavenging activity

The DPPH-quenching ability of plant extracts was measured according to Hanato et al. (1988). The ability to scavenge the DPPH radical was calculated using the following equation: (1) DPPH-scavenging effect (%) = [(A₀ – A₁)/A₀]×100; where A₀ is the absorbance of the control at 30 min and A₁ is the absorbance of the sample at 30 min. All samples were analyzed in three replicates.

2.8. Antibacterial activity detection

The fractions of lipids and proteins were individually tested against a large panel of microorganisms including *Staphylococcus aureus* (ATCC 6539), *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 25922), *Enterococcus hirae* ATCC10541, *Listeria ivanovii* and *L. innocua* (RBL 30 and RBL 29 respectively), *Bacillus subtilis* and *B. cereus* (168 and ATCC11778 respectively). All strains were obtained from the Institut Pasteur de Tunis. The bacteriological agar was from Biokar Diagnostics (Beauvais, France). Nutrient broth (NB) was from Difco (Becton Dickinson, Le Pont de Claix, France). All the other media used in this study were manufactured by Biorad (Marnes-La Coquette, France) and Merck. Antibacterial activity is revealed by growth inhibition in the test strain, observed in solid medium. In the present study we used the well diffusion method described by Perez et al. (1990).

2.9. Statistical analysis

The data (three replicates) were statistically evaluated using the JMP SAS version 12.6 software (Statistical Analysis System). (SAS, Institute INC, Box 8000, Cary, North Carolina 27511, USA).

3. RESULTS AND DISCUSSION

3.1. Physicochemical characteristics:

The average compositions of the four peanut seed varieties are shown in Table 1. It shows significantly different oil contents ($P \leq 0.05$) which were very high in Chounfakhi and Sinya (about 48%), whereas in Trabilisia and Massriya they were slightly lower (45 and 46%, respectively). These differences were probably due to the different origins of the four varieties, the environmental conditions they were subjected to and/or year effect. For 1000-seed weight Trabilisia exhibited the lowest mean, but this showed no effect on its oil content. However, differences between the four varieties were not statistically significant ($P > 0.05$). Similarly, no significant differences were found in moisture. The values of acid index were significantly different ($P \leq 0.05$) in these groundnut varieties. Trabilisia and Massriya showed the highest values, which shows that their oils contain a lot of free fatty acids and are therefore of low quality. This may be due to the fact that seeds used in this work were not freshly harvested or were not well stored. Chounfakhi and Sinya, however, showed acid indexes which conform to the international norms of *codex alimentarius*.

Total unsaponifiable content did not exceed 1% in the four groundnut varieties and varietal differences were not statistically ($P \leq 0.05$) significant (Table 1). In the literature it was reported that unsaponifiable contents did not exceed 2% in oil seeds such as those of rapeseed and linseed (Sebei, 2005; Sebei et al., 2007).

3.2. Biochemical composition:

Protein composition: The four groundnut varieties exhibited relatively low protein content (7–12%); the highest were found in Chounfakhi and Massriya (Table 1). In addition, differences between all varieties were statistically significant ($P \leq 0.05$).

In general, protein content in groundnut seeds is around 20-30% (Andersen et al., 1998). The local variety Chounfakhi can produce an appreciable quantity of protein, which encourages its cultivation in regions where it can adapt to climatic and edaphic conditions. Compared to other plant proteins, especially commercial soy protein isolate, the functional properties of peanut proteins were much poorer, which might greatly limit the application of peanut proteins in many food formulations (Liu et al., 2012). Using adequate buffers, we isolated all protein classes in seeds of the four varieties and found that they were almost totally composed of globulins (95%), albumins, prolamins, and glutelins being with negligible contents (Table 1). No significant ($P \leq 0.05$) difference was found in the content of each protein class between the four varieties.

SDS-PAGE of total proteins and their fractions: This qualitative study was performed in SDS-PAGE. Figure 1 illustrates the electrophoretic profiles of total proteins in the four groundnut varieties. It does not reveal marked varietal differences. The major proteins belong to type A, represented with bands with molecular weight (MW) varying between 100 and 50 KDa (high MW), type B (medium MW, from 35 to 25 KDa), types C and D (MW from 15 to 12 KDa), and type E (low MW, less than 10 KDa). According to Figure 2, which represents globulin electrophoretic profiles in the four groundnut varieties, there were four major bands: Band A (proteins with MW between 50 and 35 KDa), Band B (proteins with MW around 25 KDa), Band C (proteins with medium MW between 15 and 25 KDa), and Band D (proteins with low MW (around 10 KDa).

Fatty acid composition: Fatty acid composition of peanut seed oil is given in Table 2, which shows that linoleic (C18:2), oleic (C18:1) and palmitic (C16) acids accounted for more than 84% for Chounfakhi and Massriya and for more than 85% of the total fatty acids in Trabilisia and Sinya seed oil, respectively. This study shows that the oils of Tunisian peanut varieties contained oleic and linoleic acids at relatively high levels 32.63-39.65%, 27.16-41.38%, 30.31-41.85% and 32.12-

40.06% respectively for the Chounfakhi, Trabilisia, Sinya and Massriya varieties. Saturated fatty acids (SFA) accounted for 19.93%, 26.04%, 22.03 and 20.67 of total fatty acids for Chounfakhi, Trabilisia, Sinya and Massriya, respectively. The main saturated normal chain fatty acids were myristic, palmitic, stearic, behenic and arachidic acid. The ratio of unsaturated to saturated fatty acids was 4.01 in Chounfakhi, 2.84% in Trabilisia, 3.53 in Sinya and 3.83 in Massriya. According to Ingale and Shrivastava (2011), the total fatty acid composition of peanut seeds was 10.44 and 33.51% for saturated and unsaturated fatty acids, respectively. The most abundant fatty acids of groundnut seed oil were oleic (C18:1), linoleic (C18:2) and palmitic (C16:0), which together composed about 88.35% of the total fatty acids. The most abundant saturated fatty acid in groundnut seed oil was palmitic acid (6.20 percent); the main unsaturated fatty acids were oleic acid (16.28%) and linoleic acid (16.35%). The source of variability may be genetic (plant cultivar, variety grown), seed quality (maturity, harvest-caused damage and handling/storage conditions), oil processing variables, or accuracy of detection, lipid extraction method and quantitative techniques (Rodrigues and et al., 2011).

This preliminary study shows that peanut seed oils contain high relative percentages of linoleic acid (C18:2 ω 6). Potential effects of linoleic acid on health include anticarcinogenic, antiatherogenic and antidiabetogenic modulating properties; it has also attracted interest in the scientific community because of its potential effects on body composition, reducing body fat mass and increasing lean mass (Hernandez-Diaz et al., 2010). To enhance peanut seed oil, it was investigated as an alternative source for the production of a biodiesel fuel (Kaya and et al., 2009). The maximum oil to ester conversion was 89% and the viscosity of biodiesel oil is nearer to that of petroleum diesel; the calorific value is about 6% less than that of diesel.

Phenol analysis and antioxidant activities: The total phenolics, expressed as mg GAE/g dry peanut seeds per cultivar, are presented in Table 3. Chounfakhi contained the most total

TABLE 1
Physiochemical characteristics of four Tunisian peanut seed cultivars (Mean values, n = 3)

	Chounfakhi	Trabilisia	Sinya	Massriya
Moisture (%)	8.48 ± 0.54	7.38 ± 0.42	7.74 ± 0.31	7.3 ± 0.4
WTS (g)	765 ± 10.2	334 ± 11,3	702 ± 12.4	885 ± 13.41
Oil content (g.100 g ⁻¹)	48.53 ± 1.9	45.32 ± 1.1	48.08 ± 1.7	46.38 ± 1.5
Index acid	1.68 ± 0.05	3.92 ± 0.12	2.24 ± 0.09	3.36 ± 0.1
Unsaponifiables (%)	1.1 ± 0.07	0.74 ± 0.02	0.55 ± 0.03	0.98 ± 0.04
Total Proteins (mg.g ⁻¹ FW)	120 ± 1.32	68 ± 0.81	105 ± 1.27	86 ± 1.09
Globulins (%)	95 %	94%	95%	95%
Albumins (%)	3 %	3%	2%	3%
Prolamines (%)	1,5 %	1,5 %	2%	1%
Glutelines (%)	0,5 %	1,5 %	1%	1%

WTS: Weight of one thousand seeds

phenolics (2.1 mg GAE/g DW), followed by the Massriya and Sinya cultivars (1.35 mg GAE/g DW for each), Trabilisia presented the least total phenolic content, with 1 mg GAE/g DW. As found for total phenolic content, antioxidant activity showed differences between peanut seed varieties.

The HPLC study of *Arachis hypogea* seed extract identified 6 phenolic compounds in the Massriya cultivar, with rutin trihydrate and p-coumaric acid as the major compounds

(6.79% and 6.35%, respectively), 7 phenolic compounds in the Sinya cultivar with p-coumaric acid as the major compound (4.03%), 8 phenolic compounds in the Chounfakhi cultivar with p-coumaric acid as the major compound (4.87%) and 5 phenolic compounds in Trabilisia cultivar with rutin trihydrate as the major compound (4.52%). The compounds identified were (Table 4): caffeic acid, dihydroxyphenylacetic acid, syringic acid, p-coumaric acid, rutin trihydrate,

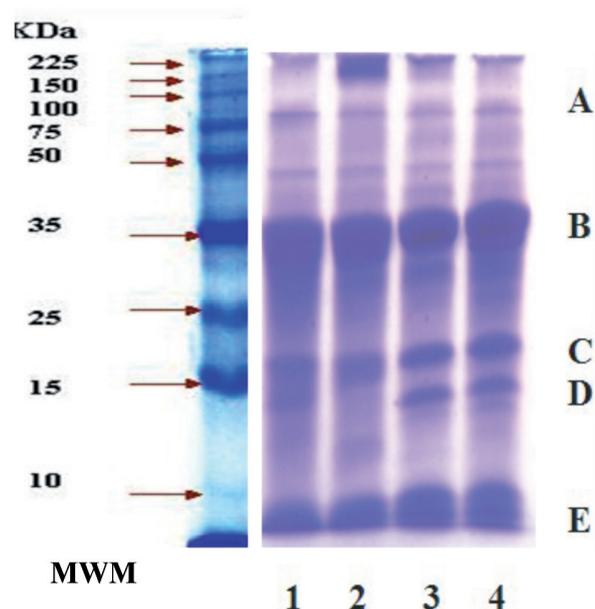


Figure 1: SDS-PAGE profile of total proteins extracted from different peanut cultivars. MWM: Molecular weight marker (kDa); 1: Chounfakhi; 2: Trabilisia; 3: Sinya; 4: Massriya

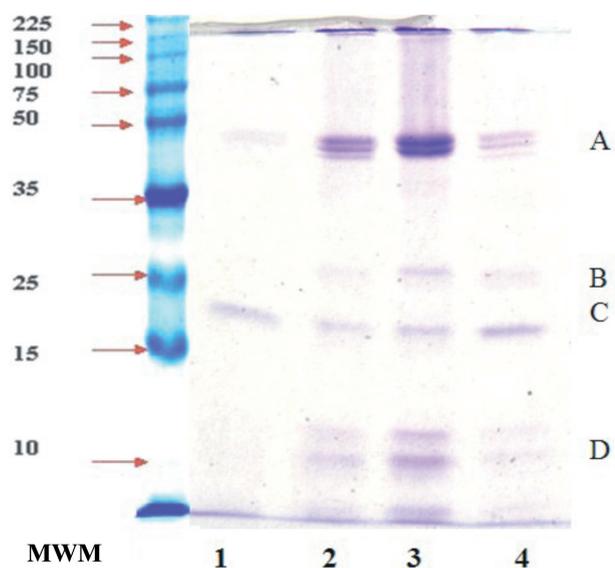


Figure 2: SDS-PAGE profile of globulins extracted from different peanut cultivars: MWM: Molecular weight marker (kDa); 1: Chounfakhi; 2: Trabilisia; 3: Sinya; 4: Massriya

TABLE 2

Fatty acid compositions (% of total fatty acid) of peanut cultivars

Fatty acids	Chounfakhi	Trabilisia	Sinya	Massriya
C14	1.19	0.77	0.79	1.56
C16	12.11	17.45	13.34	11.89
C16:1	3.43	1.93	2.15	3.17
C18	4.01	4.12	4.1	4.59
C18:1	32.63	27.16	30.31	32.12
C18:2	39.65	41.38	41.85	40.06
C18:3(w ₃)	1.63	1.27	1.31	1.41
C22	0.58	1.1	0.98	0.39
C22:1	2.73	2.22	2.35	2.57
C24	2.04	2.6	2.82	2.24
SFA	19.93	26.04	22.03	20.67
MUFA	38.79	31.31	34.81	37.86
PUFA	41.28	42.65	43.16	41.47

All values given are means of three determinations.

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

nephtoresorinol, trans-2-dihydroxycinnamic acid and dihydrate quercetin. Sales and Resurreccion (2010) reported that peanut skins contain flavonoids such as epigallocatechin, epicatechin, catechin gallate and epicatechin gallate; proanthocyanidins including procyanidin B, A-type and B-type procyanidin dimers, trimers and tetramers; phenolic acids such as chlorogenic, coumaric, caffeic and ferulic acids; and the stilbene trans-resveratrol. Peanut kernels contain caffeic, ferulic, and coumaric acids (Sales and Resurreccion, 2009) and the stilbenes trans-resveratrol and its glucoside, trans-piceid. Free p-coumaric acid, along with three tentatively identified esterified derivatives, was the predominant soluble polyphenolic that contributed to the antioxidant capacity of peanut kernels (Talcott et al., 2005 a).

Concerning DPPH scavenging activity, a considerable antiradical ability was found, especially in the Trabilisia peanut cultivar (IC₅₀ = 1550 µg/ml); the Massriya and Sinya cultivars had, respectively, 720 and 820 mg/ml IC₅₀. The Chounfakhi cultivar presented the lowest value of IC₅₀ (550 µg/ml).

Nuts are a good source of a wide range of bioactive compounds with health benefits. Many of the bioactive compounds that occur in nuts are associated with the oil fraction. Nuts contain tocopherols, tocotrienols, phytosterols and many different flavonoids, including isoflavones and quercetin. There is scientific evidence that health benefits are mediated by the bioactive components in the oil fraction of the nuts (Jonnala et al., 2006).

3.3. Antibacterial activity

Groundnut seed oil of the four varieties showed no antibacterial activity against all studied strains. This was not surprising, since no data were found in literature mentioning an antibacterial activity in fixed oil. This is probably due to the fact that fixed oil is composed almost entirely of triacylglycerols (TAG) that are structurally composed of aliphatic carbon and hydrogen chains that induce no antibacterial activity. Phytosterols, minor compounds of plant oils, constitute the major fraction of their unsaponifiables. Groundnuts showed phytosterol content varying from 900 to 3000 ppm; the most represented ones are β-sitosterol (48%), campesterol (10%), and stigmasterol (5%) (Dewanto et al., 2002). We found that sterols from the Massriya oil exhibited antibacterial activities against *Listeria ivanovii*, *Listeria innocua*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus hirae* and *Bacillus cereus* (Table 5). For triterpenic alcohols and carbohydrates that can be considered as terpenes, antibacterial activity was not clear. This was probably due to their low concentrations. Indeed, only few strains were inhibited by these compounds.

4. CONCLUSION

In summary, this study revealed that Tunisian peanut seeds are a rich source of many important components that have good

TABLE 3

Total polyphenol and antioxidant activities (DPPH scavenging ability and reducing power) of Tunisian peanut cultivars (Mean values, n = 3)

	Total phenolic content (mg GAE/g DW)	DPPH scavenging activity (IC ₅₀ µg/ml)
Massriya	1.35 ± 0.08	720 ± 8.42
Sinya	1.35 ± 0.05	820 ± 5.38
Chounfakhi	2.1 ± 0.09	550 ± 7.23
Trabilisia	1 ± 0.06	1550 ± 21.47

TABLE 4

Identified phenolic compounds in peanut seed extracts analyzed by RP-HPLC

Peak Name	% Area Massriya	% Area Sinya	% Area Chounfakhi	% Area Trabilisia
Caffeic acid	4	1.22	1.78	1.48
Dihydroxyphenylacetic acid	5.43	2.5	1.67	ND
Syringic acid	1.4	0.93	1.86	1.98
p-Coumaric acid	6.35	4.03	4.87	2.18
Rutin trihydrate	6.79	3.35	2.29	4.52
Nephtoresorinol	2.04	2.14	2.06	3.91
Trans-2-dihydroxycinnamic acid	ND	2.55	0.8	ND
Dihydrate quercetin	ND	ND	5.4	ND

TABLE 5
Antibacterial activity of sterols

	Chounfakhi	Trabilsia	Sinya	Massriya
<i>Listeria ivanovii</i> (RBL30)	-	-	+	+
<i>Listeria innocua</i> (RBL29)	-	-	-	+
<i>Escherichia coli</i> (ATCC25922)	-	-	-	-
<i>Pseudomonas eruginosa</i> (ATCC15442)	-	-	-	+
<i>Staphylococcus aureus</i> (ATCC6533)	-	-	-	+
<i>Enterococcus hirae</i> (ATCC10541)	-	-	-	+
<i>Bacillus subtilis</i> (168)	-	-	+	-
<i>Bacillus cereus</i> (ATCC11778)	-	-	-	+

(-): Inactive; (+): Inhibition diameter between 8–10 mm.

nutritional attributes and appear to have a very positive effect on human health (polyunsaturated fatty acids (w_3 and w_6), phenolic components, antioxidant biomolecules, antibacterial activity...). The high levels of fatty acids and protein content make peanuts a healthy food for human and animal nutrition. Crude peanut seed oil has a good potential as alternative fuel, which we will use in Tunisia as a source of biodiesel as perspective for our work.

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