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Biochemical Composition, Antioxidant Power and Anti-inflammatory of Dehulled *Sesamum indicum* Seeds and Its Coat Fraction

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Abstract

Sesamum indicum is one of the most important ancient oil crops in the world. In fact, this plant, in particular its seeds, is used for food, medicinal and industrial purposes. This article provides an evaluation of the biochemical composition, antioxidant and anti-inflammatory activities of dehulled sesame seeds *Sesamum indicum* and its coat fraction. Results analysis of our investigation showed that the level of total fatty oil, total protein and total sugars were present with 47.27%, 18.84% and 2.18% respectively. However; these compounds were significantly decreased in the coat fraction. On the contrary, the mineral content can be five times higher in the coat fraction, especially for calcium with a value of 831.11mg/100g, compared to dehulled seeds. Quantity of total phenolic and flavonoids were highest in the hydro-methanolic extract of seeds coat with 3.05±0.08mg GAE/g and 0.99±0.02 mg QE/g respectively. The same extract exhibited ant-inflammatory activity and antioxidant power, demonstrated by significantly decreasing in inflammation induced by carrageen in rats (85.56%), significant antioxidant activity (DPPH) (IC50: 2.12 mg/ml) and highest total antioxidant activity (95.5µg/ml). This study suggests that seeds coat of *sesamum indicum* contain interesting phytochemical potential and significant biological activities.

Keywords: *Sesamum indicum*; biochemical composition; mineral composition; phytochemical analysis; antioxidant activity; anti-inflammatory activity.

1. Introduction

Sesame (*Sesamum* genus) belongs to the family of Pedaliaceae that holds around 36 species (Bedigian *et al.*, 1986). Commonly called as "Simsim" in the Arabic world, it represents one of the most economically important and ancient crops. In terms of traditional and popular uses, the seeds are largely used in the cuisine preparation. Nutritionally, they are rich in fatty oil (44 - 58%), protein (18 - 25%), and carbohydrates (13.5%) (Bedigian *et al.*, 1986). The seed contains a high level of unsaturated fatty acids (Were *et al.*, 2006). Its protein fraction is rich in arginine, leucine and methionine amino acids (Namiki, 1995). Recently, studies have shed light on the interesting biological activities of sesame, especially the analgesic effect (Wu *et al.*, 2006), the antioxidant power (Cooney *et al.*, 2001) and the ability to reduce the plasma cholesterol (Moazzami *et al.*, 2006).

It is known that oxygen is the source of life of aerobic organisms; however, it can also represent a source of degradation for organisms (stress oxidative). In fact, it is the ultimate electron acceptor in the electron flow system. Therefore, most biological issues appear during the transfer of unpaired single electrons due to endogenous and exogenous bio processes, which lead to the development of free radicals (Davies, 2004), such as hydrogen peroxide H_2O_2, alkyl peroxides ROOH, superoxide O_2, hydroxyl radicals OH and peroxyl ROO (Gülçin, 2012).ROS (Reactive Oxygen Species) residues are the result of several biological and biochemical impairments that affect nucleic acids, lipids, proteins and carbohydrates (Halliwell, 1990; Elmastaş *et al.*, 2006). An antioxidant is defined as a substance that has the ability to significantly delay or inhibit the oxidation process, in low concentrations compared to the oxidizable substrate (Halliwell, 1990). The source of antioxidants could be endogenous (ex: superoxide dismutases), or exogenous (ex:

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flavonoids) (Arora *et al.,* 2002). Recently, a growing body of studies has focused on plant origin antioxidants as a safe substance (Namiki *et al.,* 1995). Phenolic compounds represent the major group of natural substances that act as primary antioxidants (Fawole, 2009). On the other hand, inflammation is one of the common manifestations of many diseases (Amabeoku, 2012). It is a biological response of exogenous and/or endogenous aggression such as infections caused by micro-organisms and damaged cells (Ferrero-Miliani *et al.,* 2007).The organism reacts by eliminating possible pathogens in order to revert the damaged tissue to its normal state (Lawrence *et al.,* 2007; Nathan, 2002). However, when inadequately controlled, inflammation can cause severe tissue damages (Alessandri *et al.,* 2013; Coussens *et al.,* 2002).Thus, the anti-inflammatory effect of steroidal and non-steroidal drugs has been widely studied (Barnes *et al.,* 1998;Landolfi *et al.,* 1994).

Sesame seeds are largely processed in many ways;"Tahini" is one of the food products obtained after industrial transformation of sesame seeds. Thus, dehulling is primarily used to obtain clear and white sesame seeds which are a main ingredient in the "Tahini" preparation. The coat fraction is undesirable (industrial residues) (Elleuch *et al.,* 2007). The aim of the current study is to demonstrate that the sesame by-products obtained by means of dehulling may have bio-medicinal properties. For this purpose, this research will shed light on the phytochemical composition, antioxidant effect and anti-inflammatory activity of the dehulled and seed coat of sesame.

2. Materials and Methods

2.1. Preparation of plant material

A Moroccan sesame sample was collected from the agricultural province of Taounat situated in the north of Morocco at 34° 33' North, 4° 39' West. To get fraction coat, raw seeds were soaked in water at room temperature to facilitate the peeling off the coat, the seeds were then dehulled manually. Seeds coat fraction (SC), which is very light in weight and brown in color, and dehulled seeds (DS) were separated and stored at 4 ºC.

2.2. Biochemical composition

2.2.1. Total Oil content

Total oil content of SC and DS was extracted using the Soxhlet system (Abaza *et al.,* 2002). In fact, 2g of dried samples was extracted using hexane solvent in a Soxhlet extractor. The oil was then recovered by evaporating the solvent using a rotary vacuum evaporator. Total oil content was calculated according to the following formula (1):

(1) Total oil content (%) = (Weight of extract)/ (Weight of sample) x 100

2.2.2. Total protein content

For determination of total nitrogen concentration, the Kjeldahl method was used as described by McKenzie and Wallace (McKenzie *et al.,* 1954). Then, the total nitrogen content was multiplied by 6.25 to determine the protein content of sesame seeds (Khalid *et al.,* 2003). Briefly, a sample of 1g was digested with 8 ml of concentrated H_2SO_4 into the Kjeldahl flask, in the presence of a catalyst (potassium sulfate, copper sulfate) until the color of the mixture changed to greenish. Then, to distill the sample, 15 ml of NaOH (30%) was added using a semi-automatic distillation system. 4% boric solution was used to collect the produced nitrogen NH_3. The titration was conducted with H_2SO_4 in the presence of mixed indicator solution (bromocresol green and methyl red). The following equation (2) was used to estimate the total Nitrogen concentration:

(2) Total protein content (%) =6.25× [V (H_2SO_4) ×N (H_2SO_4) ×0.014×SW]

Where: V (H_2SO_4): volume of H_2SO_4 used for titration, N (H_2SO_4): the normality of H_2SO_4 used for titration, SW: sample dry weight. 0.014: mili equivalent of nitrogen.

2.2.3. Total soluble sugars

100mg of ground seeds of SC and DS was extracted with 4 ml of ethanol (80%); the mixture was then placed in a water bath at 80°C for 30 min**.** After centrifugation (10 min at 4500 rpm), the supernatant was collected and the sugar content was analyzed with anthrone reagent (0.2% (w/v) anthrone in sulfuric acid). The absorbance was read at 625 nm by using a spectrophotometer and then converted into its glucose equivalent (mg/g) (Dubois *et al.,* 1956).

2.2.4. Mineral content

The mineral contents, including potassium, phosphorus, calcium, sodium, magnesium, were analyzed by inductive coupled plasma mass spectrometry (ICP-MS) (Zhao *et al.,* 1994). Briefly, 0.1 g of dried seeds of SC and DS was digested using concentrated HCl for 5 hours. After being cooled, the sample was then diluted with de-ionized water. All samples were analyzed using ICP-MS.

2.2.5. Total Phenolic Content

For extracts preparations, methanol: water (70:30 v/v) was added to ground and dried samples of SC and DS, with constant shaking for 8 hours. The extracts were then filtered using Whatman filter paper and concentrated under reduced pressure. Total phenolic content of each hydro-methanolic extract was determined according to the method of Folin and Ciocalteu (1927), with minor changes. Briefly, the appropriate dilution of each extract was mixed with 1ml of the diluted Folin–Ciocalteu reagent. Then, 2 ml of 5% sodium carbonate solution was added. After incubation for 2 hours at room temperature, the absorbance was measured at 750 nm. Results are expressed as Gallic Acid Equivalents (GAE).

2.2.6. Total Flavonoids Content

To evaluate the total flavonoids content, 0.1ml of hydro-methanolic extracts of SC and DS were mixed with aluminium chloride methanolic solution (10%) and 0.1ml of sodium acetate. After incubation, the absorbance was measured at 415nm. Results are expressed as Quercetine equivalents (Ordoněz *et al.,* 2006).

2.3. Evaluation of the Antioxidant Activity

2.3.1. DPPH scavenging activity

The effect of extracts on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging was estimated according to the method of Molyneux (Molyneux *et al.,* 2004) with minor modifications. Different concentrations of hydro-methanolic extracts were added to the DPPH

solution (0.5 mM), and then the mixture was incubated at room temperature for 30 min. The absorbance of the solution was measured at517 nm. Ascorbic acid was used as a positive control. The proportion of the DPPH radical scavenging is calculated using the following equation (3):

(3) % inhibition of DPPH radical = [(Ac – Ae)/Ac)]*100

With Ac: Absorbance of the control and Ae: Absorbance of the extract.

The inhibition % of DPPH radical was then used to calculate IC50, which is the anti-radical concentration required to cause 50% of inhibition.

2.3.2. Total Antioxidant Capacity (TAC)

Total antioxidant capacity was carried out using the phosphomolybdenum method according to Prieto *et al.* (1999). The tubes, containing a mixture of hydro-methanolic extract solutions of SC or DS, and reaction solution(0.6 M sulfuric acid, 28mM sodium, and 4 mM of ammonium molybdate), were incubated at 95°C for 90 min. After the cooling process, the solution absorbance was measured at 695 nm. The antioxidant activity was expressed as ascorbic acid equivalents.

2.4. Evaluation of the anti-inflammatory activity

The anti-inflammatory activity of SC and DS was evaluated by the carrageenan induced rat paw oedema assay previously reported by Winter *et al.* (1962). Rats were divided into five groups, each containing five adult animals. Group I served as a negative control group receiving normal saline, groups II–IV were given topical application of cream formulated in our laboratory by mixing the neutral cream with each extract at doses of 10% and 15%. Diclofenac gel was used for groupV, as a positive control group. Oedema was induced by subplantar injection of 0.1 ml carrageenan (1%, w/v) into the right hind paw of each rat. The paw size was measured just before the carrageenan injection, and then immediately at 3, 4, 5, and 6h after the injection of carrageenan. Percentage inhibition of oedema thickness in treated animals compared to the control group was calculated according to the following formula (4):

(4) % inhibition of oedema = [(Sc – St) / Sc]*100

Sc: the Mean increase in paw size of control group; St: the mean increase in paw size of the treated groups.

2.5. Statistical analysis

Statistical analysis was performed using SYSTAT 12. Data were subjected to one-way analysis of variance (ANOVA) in order to determine significant differences among the treatments. The results were considered significant at P<0.05.

3. Results

3.1. Proximate composition

The proximate chemical composition of SC and DS is illustrated in Table 1, total Oil, total protein and total soluble sugars were found to be higher in the whole fraction with 47.27%, 18.84 % and2.18% respectively than the coat fraction. Furthermore, we observed variations of mineral distribution between dehulled seeds and its coat fraction (Table 1). In fact, phosphorus was concentrated in the DS with 14.09±1.28 mg/100g. Unlike calcium, which is almost 5 times higher in the coat fraction (831.11±1.5 mg/100g) compared to dehulled seeds (166.28±1.32 mg/100g). Also, the value of sodium was found to be 43.9% higher in SC than DS. As for potassium and magnesium, they have the same quantity both in DS and SC with about 377± 4.56 and 258±3.2 mg/100g.

	Dehulled seeds	**Seeds coat**
Total oil%	47.27±0.6a	5.34±0.15b
Total Protein%	18.84±0.09a	7.57±0.1b
Total soluble sugars %	2.18±0.08a	0.75±0.03b
Mineral compounds		
Calcium(mg/100g)	166.28±1.5b	831.11±1.32a
Potassium(mg/100g)	414.6±4.56a	340.48±3.2a
Magnesium(mg/100g)	239.4±3.21a	278.58±2.5a
Sodium (mg/100g)	14.09±1.28a	25.12±2.67b
Phosphorus(mg/100g)	576.23±2.1a	76.41±1.81b

Table 1. Biochemical composition of dehulled seeds and seeds coat of sesame

Levels of all components measured in the DS and SC fractions were found significantly different (*P* >0.05%). Data is presented as Means ± Standard Error (SD).

The effect of dehulling on total phenolic content and total flavonoid content is shown in Table 2, indicating that SC was the richest source of poly-phenols. In fact, the value of total phenolic compounds and total flavonoids of SC was 37.4% and 67.54%higher than DS.

Extract type	**Total phenolic content (mg GAE /g)**	**Total flavonoids content (mg QE/g)**
Dehulled seeds	1.13±0.0.7*	0.1 ± 0.04**
Seeds coat	3.05 ± 0.08*	0.99 ± 0.02**

Table 2. Total phenolic and flavonoids content of dehulled and seeds coat (on dry weight basis)

Total phenolic (*) and flavonoids (**) content of the DS and SC fractions were found significantly different (*P*> 0.05). GAE: gallic acid equivalents; QE: Quercitin Equivalents. Data is presented as Means ± Standard Error (SD).

3.2. Evaluation of the antioxidant Activity

The extract of SC exceeded DSin regards to DPPH scavenging activity, in all doses used Fig.1. However, both extracts showed a low antioxidant activity when compared with the capacity of ascorbic acid to reduce DPPH. In fact, the inhibition percentage of DS was 23%, 51% and 59% for the concentrations of 5mg/ml, 10mg/ml and 15mg/ml respectively, compared to those of the ascorbic acid: 62%, 88% and 99% respectively within the same concentrations. In SC, the inhibition percentage of DPPH radical was 37%, 72% and 85% respectively within the same concentration. Based on Fig.1, the IC50 found for the extract of SC corresponded to2.39mg/l, compared to ascorbic acid which has an IC50=2.12 mg/ml.

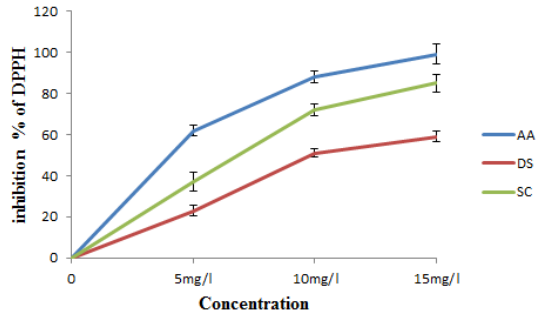

Figure 1. Antioxidant activities of the hydro-methanolic extracts of dehulled and seeds coat as assessed by the DPPH method compared to ascorbic acid. AA: ascorbic acid, DS: dehulled seeds, SC: seeds coat. Data is presented as Means ± Standard Error (SD).

Concerning Total antioxidant capacity TAC, Fig.2 shows the total antioxidant capacity of SC and DS which increased with concentration of each extract. The value of TAC for SC hydro-methanolic extract showed 95.5µg/ml of ascorbic acid equivalent at 100 µg/ml concentration, this value decreased by 43% for DS ($p < 0.05$).

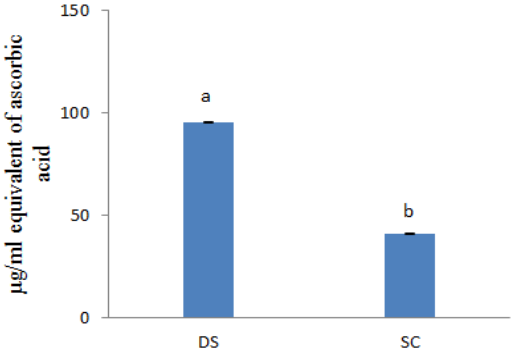

Figure 2. Total antioxidant capacity of the hydro-methanolic extracts of dehulled and seeds coat in ascorbic acid equivalent. DS: dehulled seeds; SC: seeds coat. Data is presented as Means ± Standard Error (SD).

3.3. Evaluation of the anti-inflammatory activity

As illustrated in Figure3, after 6 hours of carrageenan injection, both doses of 10% and 5% of SC extracts showed maximum inhibition of carrageenan (70% for 5% and 85.56% for 10%) in comparison to Diclofenac at 1% which produced an inhibition of 78.9%. Thus, inhibition of inflammation from DS has shown the lowest effect after 6hours, which reached 46% and 58.17% respectively for 5% and 10% respectively.

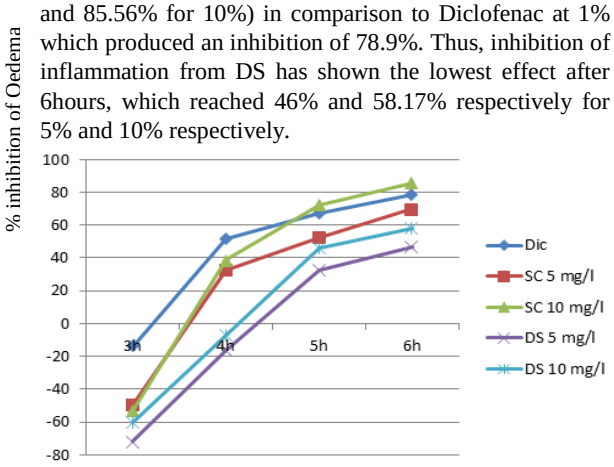

Figure 3. Anti-inflammatory effects of dehulled seeds and seeds coat in the carrageenan induced rat paw oedema test. Dic: Diclofenac 1%, SC: hydro-methanolic extract of seeds coat, DS: hydro-methanolic extract of dehulled seeds.

4. Discussion

In this study, phytochemical composition, antioxidant effect and anti-inflammatory activity of the dehulled and seeds coat of sesame were evaluated.

Concerning our results, the data revealed that the dehulling process increased protein, oil, and soluble sugar content in DS when compared to SC; this difference in concentration is likely due to the storage localization of those natural compounds in the endosperm layers of sesame seeds as has been shown by El-Adawy and Mansour (2000). On the other hand, it is known that that magnesium (intracellular cation) and potassium (extracellular cation) play a preventive role in blood pressure and cardiac issues (Swaminathan, 2003 and Weaver, 2013). In fact, it has been reported that a diet containing 20% sesame can slow aging and decrease lipid peroxidation (Yamashita *et al.*, 1990 ; Namiki, 2007). In addition, phosphorous is another important nutrient for maintaining bone formation. Our observation showed that phosphorus was highly concentrated in the DS, contrary to the sodium that was found to be higher in SC than DS. As to potassium and magnesium, they have the same quantity both in DS and SC. These results confirm the findings reported by Wang *et al.* (2008) for *Pisum sativu.* Calcium plays a crucial role not only in physiological reactions (vascular contraction, neutral transmission…) but also in preventing osteoporosis (Park *et al.*, 2011). Our study showed that the quantity of calcium was higher in coat fraction than dehulled seeds. Chang *et al.* (2002) reported that oxalate binds to calcium to form calcium oxalate, a type of crystal, which is found to be localized mainly in the seed coat. The nutritionally available calcium was estimated to be less than 25% of the total calcium (Namiki, 2007).

The identification of bioactive compounds and their activities from industrial residues has been the subject of many studies. The peeled parts of many fruits have been found to have a higher concentrate of phenolic compounds than the edible parts. According to our results, the SC extract showed higher phenolic and flavonoids content than the DS extract. Elleuch *et al.* (2007) had shown that the polyphenols are associated with dietary fibers in the sesame coat. The same observation has been registered for coats of numerous fruits like peanut (Yen *et al.*, 1994), pea (Watanabe *et al.*, 1997), wheat (Ohta *et al.*, 1994) and bean (RodrõÂguez *et al.*, 1994). Phenolic compounds are known for their antioxidant activities and are considered as the most predominant antioxidants (Abaza *et al.*, 2002).

Many studies highlighted the role of plants, whose extracts have a total antioxidant capacity, in prevention of oxidative stress (Abushouk *et al.*, 2017; Uddin *et al.*, 2018). The antioxidant power of the extracts of DS and SC were shown by two complementary spectrometric methods. The free scavenging activity efficiency is indicated by the neutralization of free radicals (DPPH), which arepurple-colored (Zhao *et al.*, 1994). The TAC is based on green phosphomolybdenum complex formation. In fact, at acidic pH, Mo (V) is reduced to Mo (VI). Based on our data, the DPPH scavenging activity of SC was found to be higher in DS. This difference can be explained by the ability of coat extract to act as hydrogen atom donors to free radicals. Our results also showed that the

value of TAC for SC hydro-methanolic extract is widely higher when compared with DS. This confirms previous findings of Chang *et al.* (2002), who showed that the seeds coat of soybeans had greater antioxidant activity than whole soybeans. Shahidi *et al.* (2006) found that black sesame coat has significant effects on oxidation of human low density lipoprotein (LDL) cholesterol and also on ferrous ion chelating capacity. Several studies demonstrated the strong and positive relationship between quantity of phenolic compounds and the antioxidant effect (Das *et al.*, 1990). Balasundram *et al.* (2006) showed that phenolic compounds play an important role in antioxidant activity by creation of phenoxyl radicals, which is possible due to giving up hydrogen atoms from their hydroxyl groups to radicals.

It has been clearly shown that significant anti-inflammatory activity was observed in seeds coat extracts. This result can confirm our findings regarding the interesting contents of total phenolic in the hydro-methanolic extract of seeds coat. Geronikaki and Gavalas (2006) had revealed the implication of antioxidant compounds in anti-inflammatory activity. Several previous studies have shown the positive correlation between phenolic compounds quantity and anti-inflammatory activity (Wiseman *et al.* (2001) and Kong *et al.* (2000). Thus, these results suggest that the seed coat of sesame can be valorized as potential resource of antioxidant and anti-inflammatory effects.

5. Conclusion

In this study, coat and dehulled seeds of sesame were evaluated for their basic composition and biological activities. Based on the presented results, the dehulled seeds could be considered as a good source of fixed oil and protein. However, coat is showed rich by phenolic and flavonoids content. Also, it had showed optimum antioxidant efficiency by two complementary test systems. Furthermore, the coat methanolic extract possesses a good anti-inflammatory activity. It is recommended to consider sesame seed coats for therapeutic consideration whereas seeds are rich with nutrients as traditional food.

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