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Physicochemical Characterization and in Vitro Antioxidant Capacity of 35 Cultivars of Sesame (*Sesamum indicum.L*) from Different Areas in Morocco

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Abstract: ***Sesame (sesamum indicum.L) is one of the most important oilseed crops, having seeds that are highly valued as a traditional health food and recently, natural antioxidants have gained increased interest because natural food ingredients are safer than synthetic ones. Physicochemical analyses and bioactive molecular tests were carried out on 35 cultivars of sesame seeds; the antioxidant activity was also investigated. This activity was found to be high ranging between 60 and 63% for the samples considering other plants and the synthetic antioxidants like BHA and BHT, this result can be related to the large amount of phenolic and flavonoid contents with the values 3.8-3.9 mg/g and 0.13-0.14mg/g respectively. The flavonols content was ranging between 0.40 and 0.41mg/g. those results strongly suggest that phenolics compounds are a good natural antioxidant. Due to its all favorable properties; sesame seeds could be used in either food or cosmetic and pharmaceutical products.***

Keywords: Sesame (*sesamum indicum*), Antioxidant activity, Phenolic, Flavonoid.

1. Introduction

Free radicals and related species have attracted a great attention in recent years. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves, to donate different forms such as superoxide anion radicals, hydroxyl radicals, peroxyl, alkoxyl radicals, nitric oxide radical and peroxynitrite [1]. Those various derived are generated in our body by various endogenous systems, lead to extreme physicochemical and pathophysiological states [2]-[3]**.** Also, excessive generation of ROS, induced by various stimuli and which exceed the antioxidant capacity of the organism, leads to a variety of illness such as inflammation, diabetes, genotoxicity, the free radicals can adversely alter lipids, proteins, DNA and have been implicated in aging and a number of human diseases **[**4].In nutrition, in order to overcome the stability problems of food, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) have been use as food additives in many countries. But, it have been revealed that these compounds may be implicated in many health risks, including cancer and carcinogenesis [5]. In the recent years, there has been a growing interest in natural antioxidants of the plants and their use is gaining importance as nutraceuticals and phytoceuticals as they have significant impact on human health and preventing disease [6].

Natural antioxidant such as flavonoids, flavonols and phenolic compounds are found in various plant products and are known to protect easily oxidizable constituents of food from oxidation [7], it was found that antioxidants obtained from star fruit residues slowed the rancidity process of oil to a greater extent that did BHT. They stressed the high potential of this residue for preventing oil rancidity. The antioxidant compounds from seeds and oil may not only increase the stability of foods, by preventing lipid peroxidation and protect biomolecules and supramolecular structures from oxidative damage [8]. Phenolic compounds specifically are widely distributed in plants. They are known as important antioxidants because of their ability to donate hydrogen atom or an electron in order to form stable radical intermediate. They prevent the oxidation of various biological molecules. In fact, several oilseeds and their byproducts have been investigated for phenolic compounds for safe sources of natural antioxidant [9]-[7].

Sesame seed *(sesamum indicum.L)* is one of the world's most important and oldest oilseed crops with a high level content of antioxidant known to human health [10]. Sesame seed provides highly stable oil and nutritious protein and meals, used in sweetmeats and confectionery foods, and have varieties of medicinal properties. The seeds are used as a demulcent in respiratory affections, infantile cholera, diarrhea, dysentery and other bowel affections and bladder diseases. The seeds powder is useful in amenorrhea, dysmenorrheal, ulcers and bleeding piles [11]. Many studies on phenolic compounds, flavonoids and flavonols of sesame seeds, are reported to be one of the most potent free radical scavengers [12]-[13]. It is also reported that the sesame is a fairly high-value food crop, being harvested either for the whole seed usage or for cooking-oil extraction and consumed for its medicinal qualities **[**14**].**
The sesame seeds *(sesamum indicum.L)* contain approximately 20-25 g/100g protein, 50g/ 100g fat, 14g/100g carbohydrate, and 1-11g/100g fiber **[**15**].** It is

well- known that sesame has many functions for maintaining good health and it has been known for many years that sesame oil is highly resistant to oxidative deterioration, this remarkable stability is due to presence of a large quantity of endogenous antioxidants **[**16]-[17**]**. The sesame has received increasing interest as a source of good quality vegetable oil with antioxidative constituents; phenolics contents and flavonoids …..In fact, many studies indicated that antioxidant activity of many plant foods are highly correlated to their total phenolics [18]-[19].

In this study, we have evaluated the sesame seeds as a source of natural antioxidant on 35 cultivars of sesame seeds from the area Tadla-Azilal from Morocco. The content of total phenols, flavonoids and flavonols were determined, and the assessment of the antioxidant activity with the aim of classification and identification of the similarity and differences between the cultivars for their compound bioactive.

2. Materials and Methods

2.1 Plant Material

The accessions studied represented material collected from locations where sesame grows in the area Tadla-Azilal, Morocco (Fig1). The accessions of the sesame *(sesamum indicum.L)* were grown during the rainy season in the year 2012-2013, and their physicochemicals properties were evaluated.

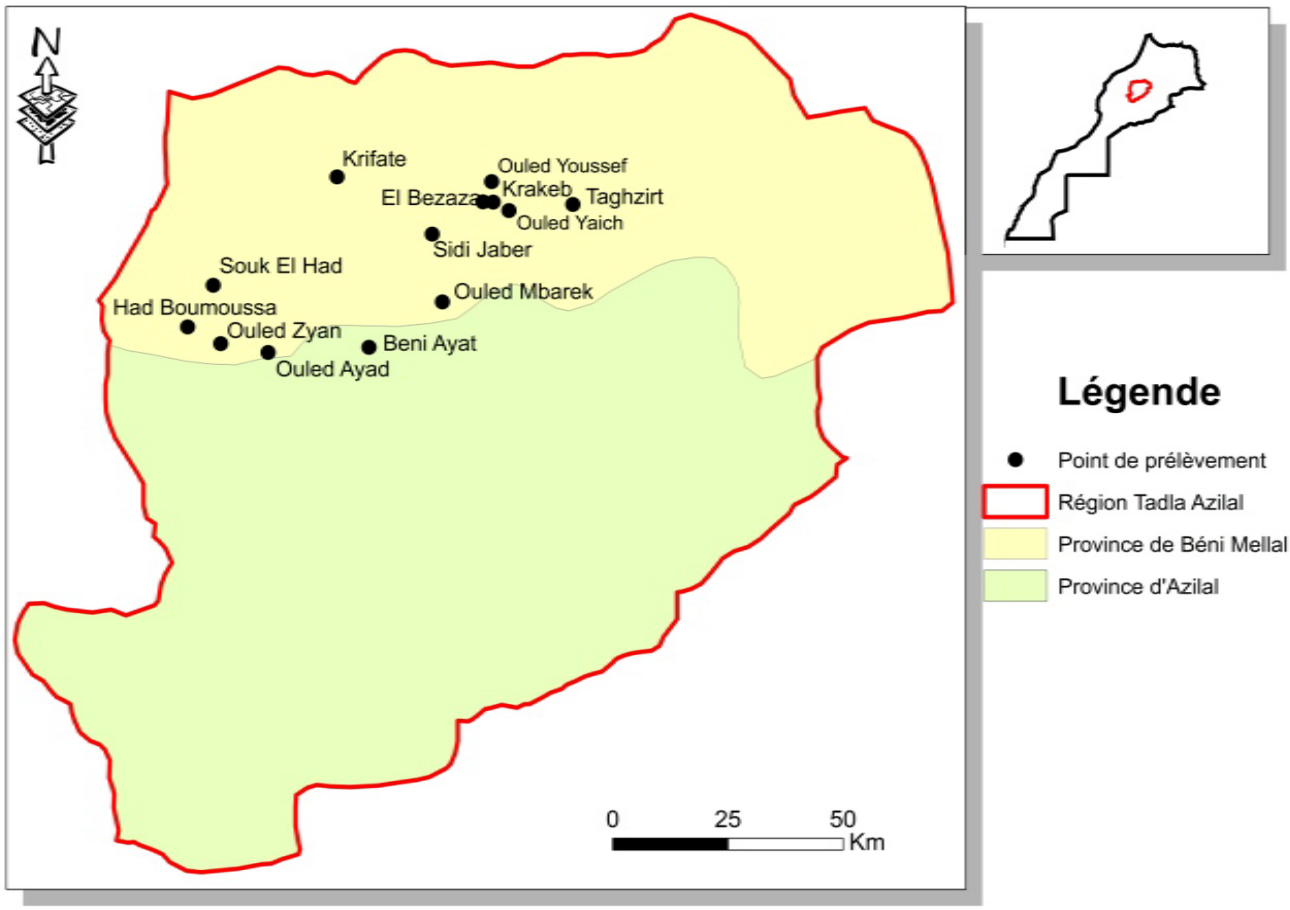

Figure 1: map shows the areas of sampling in the region Tadla-Azilal.

2.2 Chemicals and reagents

The solvents and the chemicals used were of analytical grade, ethanol and distilled water were used as solvent for extraction of antioxidants compounds. DPPH, Na_2CO_3, Folin-Ciocalteu, gallic acid, aluminium trichlorid, quercetin, BHT, BHA were stored at prescribed conditions in the laboratory.

2.3 Assessment of Bioactive Activity

2.3.1. Preparation of Seed Extracts

The seeds of each cultivar were ground in the mixer separately. 10g of the powder was weighed and suspended in 100ml of 90% ethanol and kept for shaking for 2 hours. After filtration, the samples were subjected for vacuum evaporation. The extract was redissolved in a 2 ml of 90% ethanol and assayed for its antioxidant activity, phenolic content and flavonoids [1].

2.3.2. DPPH Radical Scavenging Activity

For determination of the antioxidant activity of sesame extracts, the stable, 1 diphenyl-2-picryl hydrazyl (DPPH) radical was used [20]. An aliquot 0.5ml of DPPH solution was diluted in 4.5 ml of methanol, and 30µl of ethanolic solution sesame extract was added. A control without extract was also maintained. The mixture was shaken vigorously and allowed to stand for 45 minutes in the dark and the

absorbance was measured at 515nm. The antioxidant activity of the extract was calculated using the formula,

% scavenging activity= ((Absorbance sample – Absorbance control) / Absorbance control) X 100.

The synthetic antioxidants (BHA and BHT) prepared in ethanol (0,1 ml of each) were allowed to react with 3.9ml of the DPPH solution and vortex and then the absorbance was measured at 515 nm after 45 min.

2.3.3. Total Phenolic Content

The amount of total phenolic compounds was measured using the method [21]; 15mg of extract was dissolved in 1ml of 90% ethanol. A 10µl aliquot of the resulting solution was added to 2ml of 2% Na2CO3 and after 2 minutes 100µl of Folin-ciocalteu reagent (diluted with water 1:1) was added. After a further 30 minutes, the absorbance was measured at 750nm. The concentration was calculated using gallic acid as standard, and the results were expressed as mg gallic acid equivalents per mg extract.

2.3.4. Total Flavonoid Content

The flavonoid content was determined using the method [22]; 1ml of the extract was added to 1ml of aluminium trichlorid $ALCL_3$ (2%).After 15 min of incubation. The absorbance was measured at 430 nm and the results were expressed an mg quercetin equivalents per mg extract.

2.3.5. Total Flavonols

Total flavonol content was determined by the method of [23]. To 2.0ml of extract solution, 2.0ml of 2% $AlCl_3$ ethanol and 3.0 ml (50g/l) sodium acetate solutions were added. The absorption at 44 nm was recorded after 2,5h at 20°C. Extract samples were evaluated at a final concentration of 0.1mg/ml. total flavonols content expressed as quercetin equivalent (QE).

2.4. Statistical Analysis

Statistical analyses were conducted using SPSS (Statistical Program for Social sciences) version 17.0 for window. All analyses were performed in triplicate and data reported as means ± standard deviation (SD).

3. Results and discussion:

Antioxidant activity of sesame extracts:

The free radical scavenging ability of sesame seeds (*sesamum indicum*) extracts were analysed by DPPH method. The DPPH radical is commonly used for the assessment of antioxidant activity in vitro and is foreign to biological systems; he is a very stable organic free radical with deep violet colour which gives absorption maxima within 515-528 nm range. Upon receiving proton from any hydrogen donors, mainly from phenolic, it loses it chromophore and became yellow, which mean that the antioxidants react with DPPH, reducing a number of DPPH molecules equal to the number of their available hydroxyl groups. The DPPH radical is considered to be a model of a stable lipophilic radical. The yield of ethanolic extracts of sesame was found to be ranging between 60% ±0.29 for one cultivar, 61%± 0,29 for 9 cultivars, 62%± 0,38 ±0,46 for 16 cultivars and 63% for the others 8 cultivars. The cultivars R, S and M' were found to possess the higher antioxidant activity, and the low value was shown on J' cultivar (Table 1); this result was similar to the result found by Vishwanath and *al*., with the value 61%±2.09 This difference between the cultivars may be due to the nature of the soil and the seeds, the colour of the cultivars or the method of the culture. This activity is believed to be mainly due to their redox properties, which play a great role in adsorbing or neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [24]. As the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases their DPPH radical scavenging activity also increases and can be defined as antioxidant activity [25].

DPPH radical-scavenging abilities of the different cultivars of sesame seeds along with the reference standards BHA and BHT are shown in Fig 2. The extracts demonstrated a concentration-dependent scavenging activity by quenching DPPH radicals: DPPH radical scavenging activities of different cultivars increased with increased content. The scavenging activities of the extracts were significantly higher ($p<0,05$) than that of BHT and BHA with the values 48% and 57% respectively. It has been proven that antioxidant activity of plant extracts is mainly related to the concentration of the phenolic compounds present in the plants [26].

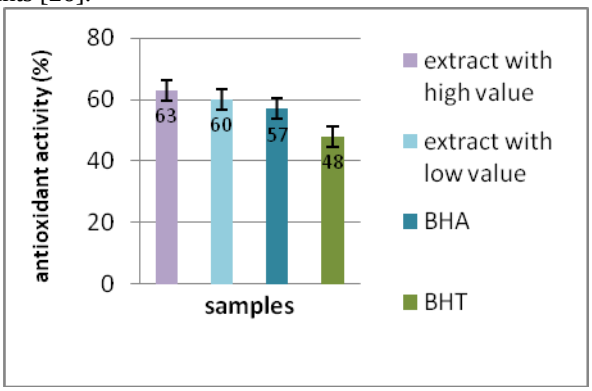

Figure2: Percentage of antioxidant activity of sesame extracts and standards antioxidants by DPPH radical scavenging method

The results of the DPPH free radical scavenging suggest that the different extracts from the different cultivars and area are capable of scavenging free radicals via electron- or hydrogen-donating mechanisms and thus should be able to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices. This result showed the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage.

Total phenol, flavonoids and flavonols contents:

Phenolic compounds are distributed in the plant. They are an important antioxidant because of their ability to donate a hydrogen atom or an electron in order to form stable radical intermediates. Hence, they prevent the oxidation of various biological molecules [27]. In fact, several oilseeds and their by-products have been investigated for phenolic compounds in search for safe sources of natural antioxidants [28].

The total phenolic compound was determined by the Folin-Ciocalteu phenol method, this method measures the reduction of the reagent by phenolic compounds with the formation of a blue complex that can be measured at 760 nm. Also the flavonoids possess a broad spectrum of chemical and biological activities including radical scavenging properties. Such properties are especially distinct for flavonols.

The values of phenolics content, flavonoids are shown in table 1, and were ranging between 3.75mg/g± 0. 05 and 3.92mg/g ±0. 03, 0.13±0.003and 0.14mg/g±0.006, those results were similar to those found by Vishwanath and *al.*, 2011. The flavonols compounds were between 0.40 and 0.41mg/g (table 1). in this study , the phenolic compound of sesame extracts was found to be higher than potato peels (2,91mg/g) [29] and banana (2.32mg/g) [30],carrot (1.52mg/g) [30].

The results suggest that phenolics compounds are important for this plant and others, the values obtained corresponded well with the antioxidant activity. In fact, many medicinal plants contain large amounts of antioxidants such as polyphenols. In different study, the sesame seed extracts as compared to the synthetic antioxidant was found to possess potential antioxidant activity [1].

Table 1: The antioxidant activities, the content of polyphenols, flavonoids and flavonols of Sesame extracts

Name	*Sources*	*Activité Antioxydante %*	*Polyphenol content mg EQ GA per G extract*	*Flavonoids content mg EQ Q per G extract*	*Flavonols Content mg per G*
A	bni ayat	61,17±0,29	3,82±0,03	0,13± 0,002	0,41±0,03
B	taghzirt2	62,33±0,58	3,82±0,03	0,14±0,006	0,4±0,04
C	krakeb1	62,33±0,58	3,82±0,03	0,14±0,005	0,41±0,02
D	krakeb2	63±0,01	3,82±0,03	0,13±0,005	0,4±0,03
E	ouled zian 2	63,17±0,29	3,82±0,03	0,13±0,005	0,4±0,04
F	ouled yaich1	63,17±0,29	3,8±0,02	0,13±0,003	0,4±0,04
G	charika d'ouled youssef	62,33±0,29	3,83±0,03	0,13±0,006	0,4±0,03
H	taghzirt1	62,42±0,38	3,82±0,03	0,13±0,007	0,41±0,03
I	ouled yaich2	61,17±0,29	3,88±0,03	0,13±0,006	0,41±0,03
J	had boumoussa1	61,17±0,29	3,85±0,05	0,13±0,004	0,4±0,03
K	had boumoussa2	61,71±0,29	3,75±0,05	0,13±0,003	0,4±0,03
L	ouled barkat1	61,5±0,5	3,78±0,03	0,13±0,002	0,4±0,04
M	taghzirt3	62±0,01	3,83±0,03	0,13±0,002	0,41±0,03
N	taghzirt4	62,92±0,14	3,9±0,04	0,14±0,008	0,4±0,03
O	souk el had	63,17±0,29	3,92±0,03	0,14±0,005	0,41±0,04
P	taghzirt8	62,83±0,29	3,92±0,03	0,14±0,006	0,41±0,03
Q	lbazaza1	63,17±0,29	3,92±0,03	0,14±0,003	0,4±0,02
R	ouled barkat2	63,33±0,29	3,92±0,03	0,14±0,004	0,4±0,05
S	krifat	63,33±0,29	3,92±0,03	0,14±0,004	0,4±0,06
T	lbazaza2	62,83±0,29	3,88±0,03	0,14±0,003	0,4±0,03
U	ouled zian1	62,83±0,29	3,88±0,03	0,14±0,005	0,4±0,05
V	taghzirt6	62,83±0,29	3,88±0,03	0,14±0,004	0,4±0,05
A'	sidijaber	62,83±0,29	3,88±0,03	0,14±0,006	0,4±0,03
B'	ouled ayad	62,83±0,29	3,88±0,03	0,14±0,003	0,4±0,05
C'	taghzirt7	62,83±0,29	3,87±0,06	0,14±0,003	0,4±0,06
D'	taghzirt5	62±0,02	3,83±0,03	0,13±0,006	0,4±0,06
E'	souk sebt ouled slimane1	62,17±0,29	3,92±0,03	0,14±0,003	0,4±0,07
F'	lbazaza3	62,17±0,29	3,82±0,03	0,14±0,007	0,4±0,06
G'	had boumoussa	61,17±0,29	3,82±0,03	0,13±0,006	0,4±0,07
H'	ouled mbark	61,83±0,29	3,88±0,03	0,13±0,006	0,4±0,03
I'	lbazaza4	61,17±0,29	3,9±0,05	0,13±0,003	0,41±0,05
J'	had boumoussa 4	60,17±0,29	3,83±0,03	0,13±0,007	0,41±0,05
K'	krakeb3	61,57±0,51	3,82±0,03	0,13±0,003	0,41±0,04
L'	krakeb4	62±0,03	3,82±0,03	0,13±0,006	0,41±0,04
M'	souk sebt ouled slimane2	63,33±0,29	3,82±0,03	0,14±0,002	0,4±0,06

4. Correlations

In this study, significant correlations existed between total phenolic contents and antioxidant activity in all the extracts studied ($p<0.05$) and ($p<0.01$), the R^2 (coefficient of correlation) of relations between total phenolic content, flavonoids and DPPH-scavenging activity was 0.8832 and 0.8504. This is due to the fact that the chemistry behind these methods is based on the same redox properties. Our results are similar to previous studies. It was been reported that correlations of total phenolic content and antioxidant activity of different extracts from seeds, sprouts and breads were linear [32].

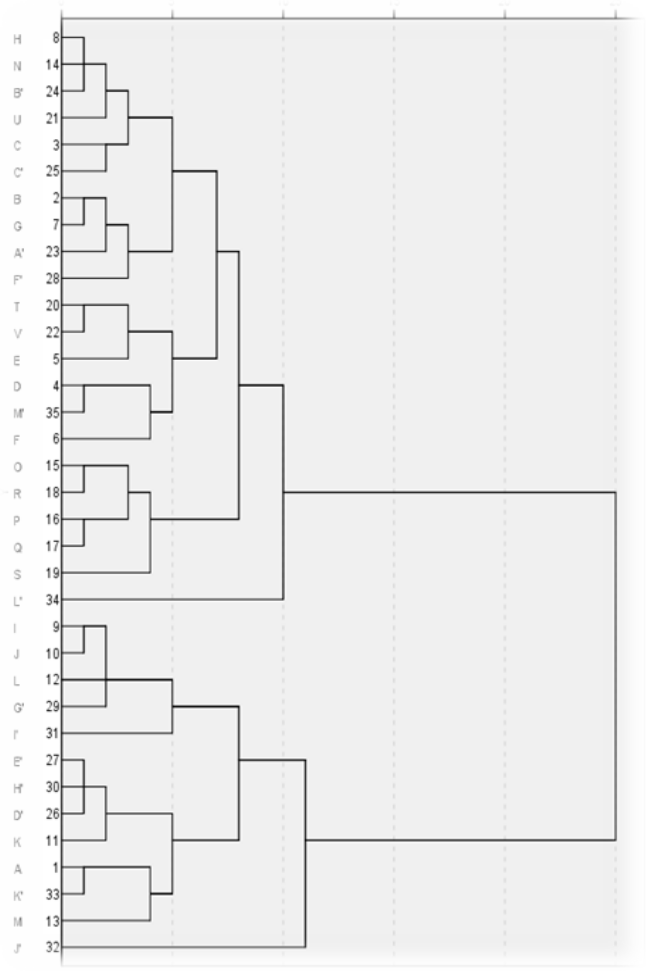

Figure 3: Dendrogram resulting classification of 35 cultivars of sesame from different regions.

The hierarchical clustering based on phenolic compounds, flavonoids, flavonols and the antioxidant activity was carried out (Fig3), the resulting dendrogram puts in evidence a clear separation between the 35 cultivars from different regions to specified groups which means a significant population distribution based on bioactive molecules, this difference may be due also to the effect of environment or genetic variability.

5. Conclusion

This study researched the contents of bioactive compounds and the antioxidants activities of 35 cultivars from different areas in Morocco. A large variability in these contents was observed among the cultivars which allow us to create a differentiation on the groups. Sesame extracts has stabilization efficiency comparable to commonly employed synthetic antioxidants BHT and BHA at their legal limit, they can be recommended as a potent source of antioxidants for stabilization of food systems, especially unsaturated vegetable oils. The phenolic compounds appear to be responsible for the antioxidant activity of sesame seeds, those seeds could be part of the nutritional for human, and may be used successfully as a key ingredient in Halva, Tahini, and in other colourful rice and noodle dishes for its aroma and flavour, although further studies are required to reveal whether they contain other antioxidative constituents.

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