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Study of the Antibacterial and Antioxidant Capacities of Fixed Oil of *Moringa oleifera* L. Cultivated in the Southwest of Algeria

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ABSTRACT

Background: Since time immemorial, humans have always been interested in lipids for various uses, namely food, cosmetology, medicine, *etc.* Many seeds, sources of oils, are increasingly studied for their nutritional and therapeutic properties.

Methods: The present study aims to valorize the vegetable oil obtained from the seeds of the *Moringa oleifera* plant widely cultivated in the southwest of Algeria, using the solid-liquid extraction technique. In addition, the fatty acid analysis of this oil, using the gas chromatography technique.

Result: An oil yield of 30.43% was obtained, revealed that it contains ten different compounds. The characterization of the oil obtained (physico-chemical properties such as organoleptic properties, density, viscosity, refractive index) proved to comply with international standards and AFNOR standards. In addition, it turned out that *Moringa oleifera* oil has a very interesting antioxidant power. However, this oil did not show any antibacterial activity on the strains tested.

Key words: Antioxidant, Antibacterial, Extraction, *Moringa oleifera* L., Vegetable oil.

INTRODUCTION

With the changes of lifestyle and living environment, numerous diseases and infections threaten humanity, including inflammatory disorder, diabetes and cancer and many microbial infection. Facing these health disorders, humanity use medicinal plant. Since long-time medicinal plants known for theirs beneficial to humanity. According to the report of the World Health Organization (WHO) about 80% of the world's populations rely mainly on traditional therapies, which involve the use of plant extracts or their active substances (Gupta and Sharma, 2014). Medicinal plants, which generally include several substances, are used for their proven active ingredients. These plants can contain volatile compounds (terpene compounds), flavonoids, tannins, saponosides, vitamins, proteins, amino acids, phospholipids, carotenoids, phytohormones, phenolic compounds and fatty acids (Rossignol, 2008; Legrand, 2007). Several medicinal plants have been reported for theirs precious source of phytochemicals. Among them Moringa or *Moringa oleifera* L., is one of the most widely distributed plants of a monogenic family Moringaceae, belongs to a unique genus that is the Moringaceae family which includes fourteen species (Morton, 1991). Moringa is classified as a local medicinal Indian herb that has turn out to be familiar in the tropical and subtropical countries (Razis *et al.*, 2014). In Algeria *Moringa oleifera* L. is the most popular plants encountered in southern of Algeria (Boulal *et al.*, 2020). *Moringa oleifera* L. has largely known for his phytotherapeutic value as preventive and treatment agent of numerous health dysfunction including, inflammation (Araújo *et al.*, 2013, Cui *et al.*, 2019), cardiovascular (Panda *et al.*, 2013), diabetic (Mehta *et al.*, 2009), haematological and hepatorenal disorders (Pisarello *et al.*, 2018), cancer (Ghosh et Capell, 2016). Numerous scientifics papers reported the antioxidant and antimicrobial proprieties (Ratshilivha *et al.,* 2014, Salem *et al.*, 2014, Xu *et al.*, 2019). Several compounds have been isolated from Moringa plant part. From *Moringa oleifera* leaves niazirin, niazirinin, 4-[4'-O-acetyl-α-L-rhamnosyloxy benzyl] isothiocyanate, niaziminin A and B (Ndhlal *et al.*, 2014). Other investigation revealed that Moringa leaves contain many essential oil (Chuang *et al.*, 2007). On another hand *Moringa oleifera* seeds have a high oil content of up to 40%. This oil, of oleic type with up to 76% oleic acid (Anwar and Bhanger *et al.*, 2003). In Algeria Moringa largely used due to their cosmetic-medicinal properties of vegetable oil. Thus, it would be interesting to enhance the Algerian biodiversity in terms of this substance that is extracted from plant seeds.

The main purpose of this work is the biological valorization of the fixed oils from *Moringa oleifera* L. seeds. We got particularly interested in this plant *Moringa oleifera* L., for its numerous advantages and virtues including its availability in the region, the richness of its seeds in fat (between 38

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and 40%), its adaptation to several types of soil and climatic conditions and its remarkable rusticity and very rapid initial growth with flowering from the first year after planting.

MATERIALS AND METHODS

Plant material

Moringa oleifera L. seeds used in our study were harvested between mid-May and early June, 2018 in the Adrar region, located in the central part of the Sahara, southwest of Algeria (Fig 1).

Preparation of raw material

The different stages of oil preparation are given in the following diagram (Fig 2).

Extraction of *Morinaga oleifera* L. oil using the soxhlet method (Boulal *et al.*, 2019)

The extraction by specific nonpolar organic solvent (n-hexane) for determining the fat content was carried out in a Soxhlet type apparatus. This technique ensures a hot extraction of the fat contained in Moringa seeds powder that is placed in a cellulose cartridge and continuously soaked with the vapors of a solvent that was selected according to the polarity of the lipid active ingredients to be extracted. About 10 g of the crushed *Moringa oleifera* seeds weighed in the cellulose cartridge closed with carded cotton and introduced into a three-station Soxhlet fat extractor; each station has a 250 ml capacity. The extraction was carried out with n-hexane (300 ml) brought to reflux at 70°C for 6 hours. Once the extraction was completed, the hexane was removed through evaporation using a rotary steamer at 65°C.

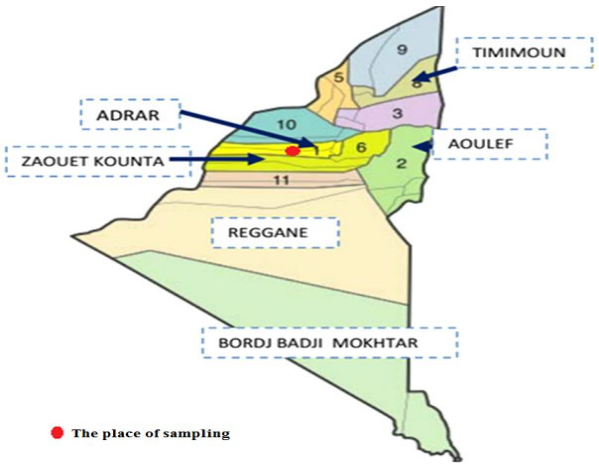

Fig 1: Presentation of the Moringa seed collection site.

Characterization of oil and seeds of *Moringa oleifera* L.

This characterization aimed to determine the fat content (ISO 659, 1988), weight of 1000 seeds (NA 730. 1991. ISO 520), moisture content (AFNOR, 1985), density (AFNT 60 214), refractive index (AFNT 60 212), acid index (AFNOR T60 204) and viscosity (NF EN 12092, August 2002).

Other qualitative and quantitative analyses

Gas chromatography analysis of fatty acids

The composition of the fatty acid methyl ester (FAME) of *Moringa oleifera* was determined by gas chromatography (GC) using a Clarus 680 GC from Perkin Elmer, Inc.

For this method we used a capillary column of type BPX70: 60.0 m × 0.25µm × 0.25 mm ID

The carrier gas: used is Hydrogen with flow rate: of 1.00 mL / min. The injection temperature and detector temperature: was fixed at 250°C and 240°C respectively. The oven temperature: is fixed at 200°C. The peak area is given by an integrator with paper speed equal to 0.5 cm / min.

Thin layer chromatography analysis

In order to draw conclusions about the product to be deposited on the TLC plate. Thin-layer, or plate, chromatography

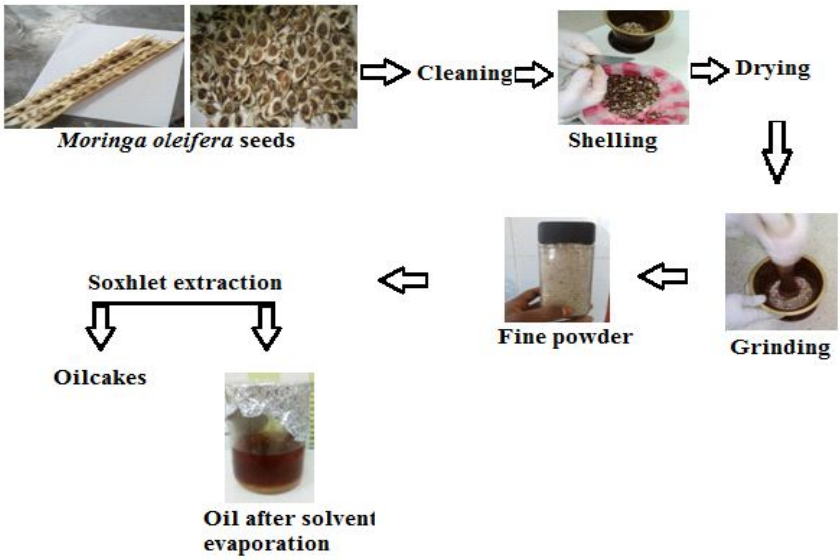

Fig 2: Diagram of oil extraction from *Moringa oleifera* L. seeds.

was primarily performed for the analysis of a mixture of products (Mendham, 1999).

Infrared spectroscopy analysis

Fourier transform infrared spectroscopy (FTIR) was used as a tool to confirm the chemical structure of our products. The FTIR absorption spectrum was recorded, within the range from 4400 to 400 cm^{-1}, on an IR CARY 660 FTIR ATR type device, by spreading the essential oil diluted in carbon tetrachloride (CCl4) on a KBr disk.

Biological activities

Determination of the antibacterial activity

a. Bacterial strains

In order to evaluate the antimicrobial activity of *Moringa oleifera* L. vegetable oil; twelve bacterial strains from Gram negative and Gram positive group are used including: *Klebsiella pneumoniae* (ATCC 700603), *Acinetobacter baumannii* (ATCC 1906), *Salmonella enterica* (ATCC 14028), *Serratia marcescens Bizio* (ATCC 14756), *Staphylococcus aureus* (ATCC 43300), *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecium* (ATCC 35667), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (ATCC 14579) and *Enterococcus faecalis* (ATCC 29212). These strains brought from the Microbiology Laboratory at Kasdi Merbah University in Ouargla (Algeria).

b. The antibacterial activity

The antibacterial activity tests were carried out by the direct contact technique (Agar diffusion method) (Luciana and Layara, 2017). The sensitivity of the different strains to the vegetable oil under study is classified according to the inhibition zone diameter including not sensitive (-) for Ø < 8 mm, sensitive (+) for Ø between 9 and 14 mm, very sensitive (++) for Ø between 15 and 19 mm, extremely sensitive (+++) for Ø > 20 mm (Leitão *et al.*, 2002; Ponce *et al.*, 2003).

Determination of antioxidant power by the DPPH method

To assess the antioxidant activity of vegetable oil, it was decided to use the DPPH (2, 2-diphenyl-1-picrylhydrazyl) method proposed by Arora *et al.* (2013) with certain modifications.

It is interesting to know that the DPPH° test allows measuring the anti-radical activity of plant extracts in a model system (organic solvent, room temperature). This test measures the capacity of an antioxidant to reduce the chemical radical DPPH° (2, 2-diphenyl-1-picrylhydrazyl) by transferring one of hydrogen. The DPPH°, which was initially purple, turned into DPPH-H, pale yellow (Fig 3).

According to Sanhita (2016), the inhibition of the DPPH free radical in percentage (I%) may be calculated as follows:

$$I\% = \frac{\text{Ablank- A sample}}{\text{Ablank}} \times 100 \qquad .....1$$

Where Ablank is the absorbance of the control sample (containing all reagents without the test product) and Asample is the absorbance of the test sample. The graph of the variation of inhibition percentage as a function of the vegetable oil concentration makes it possible to determine the IC_{50} which corresponds to 50% inhibition; this represents the antioxidant activity of vegetable oil. This value was compared to that found for the reference compound.

RESULTS AND DISCUSSION

Extraction yield

The oil content of *Moringa oleifera* seeds by Soxhlet extraction was found to be 30.437% of the weight of dry seeds. By comparing this figure with the results reported in previous works, it was noted that the yield obtained was slightly lower than the percentages 40%, 41%, 36.48% and 34.5% obtained by (Goja, 2013; Pereira *et al.,* 2016b; Domínguez *et al*., 2017; Boulal *et al*., 2019), respectively. These findings related to the region climate, soil type and the operating conditions. Therefore, we deduced that the fixed oil yield of the same species can vary depending on several parameters such as the plant species and the harvest season.

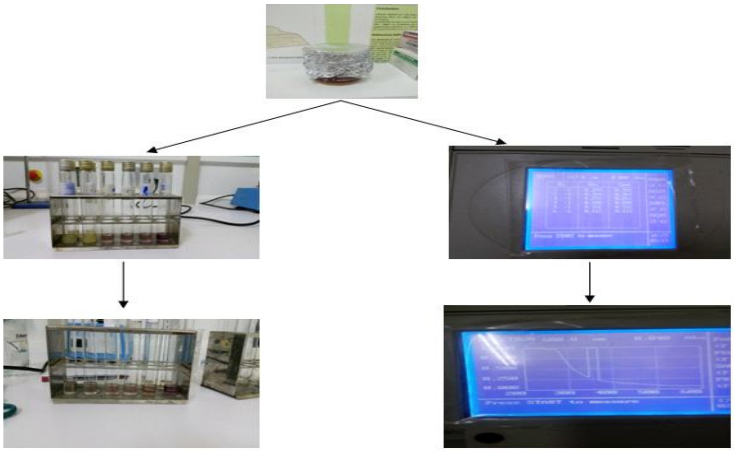

Fig 3: Determination of antioxidant power.

Table 1: Organoleptic characteristics of *Moringa oleifera* seed oil..

	Aspect	Color	Odor
AFNOR ISO	Liquid at room temperature	Light yellow to dark yellow	Characteristic
Our oil	Oily liquid	Dark yellow	Natural hazelnut

Organoleptic characteristics

The organoleptic parameters, such as appearance, color and odor, of our heavy oil summarized in the (Table 1). The organoleptic and physico-chemical properties make it possible to check and control the quality of the vegetable oil. Our tests carried out according to a precise protocol and obeying the ISO standards. For the *Moringa oleifera* vegetable oil, the ISO standard of AFNOR NF EN ISO 659 (1998) is in force.

Determination of the physico-chemical parameters

Density

Lipid density, one of the purity criteria allows detecting the presence of foreign bodies. The result obtained, *i.e.* D_{20} = 886.05 kg/m^3, is close to olive oil density; whose general standard given by the *Codex* Alimentarius is between 910 and 916 kg/m^3. This value is lower than the value 897.5 kg/m^3 obtained by Mofjur *et al.* 2014 and 896.7 kg/m^3 reported by Boulal *et al.*, 2019.

Refractive index

The refractive index provides interesting information on the purity and group of the oil under study. Its value was J_{20} = 1.46465 at 20°C. Our oil is classified as non-drying since its refractive index falls within the range [1.465 - 1.467]. The value found complies with the ISO standard and is close to the refractive index of olive oil; it varies between 1.467 and 1.470.

Acid index

The acid number calculated according to the relation previously mentioned. It was found that IA equal to 9.04 mg of NaOH per g of oil. This result is very similar to those reported in Mofjur *et al.*: 8.62 mg of KOH per g of oil (Mofjur *et al.*, 2014).

It is worth recalling that the acid number helps detecting the presence of *free fatty acids* (FFA) generated by a hydrolysis reaction or an oxidative reaction. The production and storage conditions of seeds, expiration date, processing time and handling tools favor the increase of this acid number (Pereira *et al.*, 2016a). The acid number generally defines the quality of the oil and characterizes its purity and stability at room temperature. *Moringa oleifera* oil has an acid number that is higher than that of soybean oil with a maximum of 3 mg of KOH/g oil. However, lower than that of olive oil which varies between 2 and 16 mg of KOH/g of oil.

According to the pervious experiments, nonpolar solvents, such as hexane, give oil with a relatively high acid number. Consequently, shorten the shelf life of the oil extracted from *Moringa oleifera* L. depends on the ripening and storage conditions of seeds.

Table 2: Physicochemical parameters of the oil from *Moringa oleifera L* seeds.

Parameter	Value	Reference
Yield (%)	30.437	40.
Density	0.88605	0.8990
Refraction index	1.4646	1.4600
Acid number (%)	09.04	1.940
Viscosity (kg/m^{-1}.s^{-1})	106.727	92.60

Table 3: Retarding ratios of the observed spots.

Spot	H_s (cm)	R_f
Spot 1	2.2	0.44
Spot 2	3.2	0.64
Spot 3	4.4	0.88

Determination of viscosity

The viscosity found for *Moringa oleifera* L oil cultivated in South of Algeria was very higher (μ = 106.727 kg.m^{-1}.s^{-1}). The obtained results indicated that the oil extracted from *Moringa oleifera L.* from the Adrar region may be a fairly good lubricant for watch mechanisms and machines, and also as a fuel for oil lamps. It also revealed to have interesting antiseptic and anti-inflammatory properties.

The physico-chemical analysis results of the oil produced from the *Moringa oleifera* seeds at 20°C are summarized in the (Table 2).

The obtained results allow concluding that *Moringa oleifera* L oil cultivated in Adrar complies with AFNOR international standards (French Association for Standardization).

Quantitative and qualitative analyses

Thin layer chromatography

Moringa oleifera L. oil was placed on a TLC plate under a UV lamp at 254 nm; three spots with different retarding ratios (R_f) were then observed. As the retarding ratio is characteristic of a given substance with an eluting torque on a given support, it can be concluded that Moringa oil contains three different compounds (Table 3).

Gas chromatography analysis

Analysis of the fatty acid profile, using the CPG / FID technique, revealed that the oil was rich in monounsaturated fatty acids (77.1%) in which the oleic acid was predominant with an average content of 74.1%. In contrast, the saturated fatty acid content was only 18.6%.

On another hand, polyunsaturated fatty acids were present in small amounts (4.3%).

Table 4: Fatty acid composition (%) of *Moringa oleifera* seed oil.

Fatty acids	Formula	Systematic name	Structure	Result
Myristic	$C_{14}H_{28}O_2$	Tetradecanoic	14:00	0.1
Palmitic	$C_{16}H_{32}O_2$	Hexadecanoic	16:00	7.9
Palmitoleic	$C_{16}H_{30}O_2$	Hexadec-9-enoic	16:01	1.7
Stearic	$C_{18}H_{36}O_2$	Octadecanoic	18:00	5.5
Oleic	$C_{18}H_{34}O_2$	*Cis*-9-Octadecenoic	18:01	74.1
Linoleic	$C_{18}H_{32}O_2$	*Cis*-9-*cis*-12 Octadecadienoic	18:02	4.1
Linolenic	$C_{18}H_{30}O_2$	*Cis*-9-*cis*-12	18:03	0.2
Arachidic	$C_{20}H_{40}O_2$	Eicosanoic	20:00	2.3
Eicosanoic	$C_{20}H_{38}O_2$	*Cis*-11-eicosenoic	20:01	1.3
Behenic	$C_{22}H_{44}O_2$	Docosanoic	22:00	2.8
Saturated				18.6
Monounsaturated				77.1
Polyunsaturated				4.3
Total				100

Fig 4: The IR spectrum of *Moringa oleifera* seed oil.

According to (Vigneron *et al.*, 2006; Lecerf, 2011), vegetable oils can therefore be grouped based on the nature of their most abundant fatty acids, *i.e.* oleic, linoleic or α-linolenic.

Thus, *Moringa oleifera* L. oil is very rich in oleic-type monounsaturated fatty acids (MUFA C18:1, ω9) and its fatty acid composition is quite similar to that of olive oil which contains 75% oleic acid (Charrouf and Guillaume, 2010).

The saturated fatty acids found in *Moringa oléifera L.* oil are myristic (0.1%), palmitic (7.9%), stearic (5.5%), arachidic (2.3%) and behenic (2.8%) acids, as indicated in (Table 4).

IR spectroscopy analysis

Fig 4 shows the infrared spectrum of *Moringa oleifera* seed oil. An infrared spectroscopy data table is also provided.

The results of the IR spectroscopy analysis give the following values:

- Absorption bands at 2921.976 cm^{-1}: Symmetrical valence vibration of a saturated C-H alkyl group.
- Absorption bands at 2852.602 cm^{-1}: Asymmetric valence vibration of a saturated C-H alkyl group.
- Absorption bands at 1745.117 cm^{-1}: Valence vibration of a C = O carbonyl group.
- Absorption bands at 1161.160 cm^{-1}: Valence vibration of a C - O group.

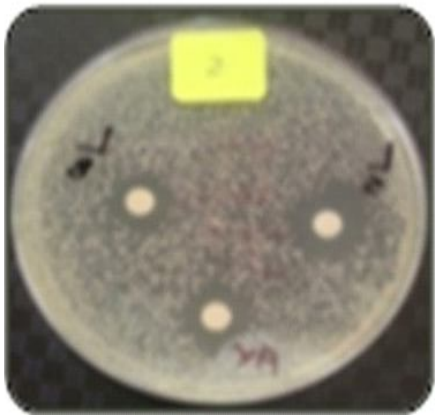

Fig 5: Inhibitory effect of our oil on the bacterial strains tested.

Biological activities

Antibacterial capacity

The antibacterial activity of *Moringa oleifera* oil was evaluated against 12 pathogenic bacterial strains via the disc diffusion method (Fig 5). During the experiment, it was noticed that our oil was not sufficiently effective against all the bacteria used.

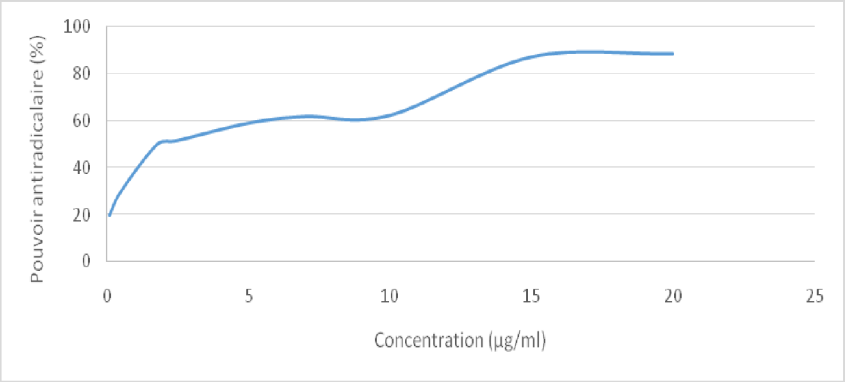

Fig 6: Curve for the determination of the free-radical scavenging activity as a function of the inhibitory mass concentration of HV of *Moringa oleifera* L. seeds.

Antioxidant capacity

The free radical scavenging method for DPPH (2, 2-diphenyl-I-picrylhydrazyl) was used in order to determine the antioxidant activity of the fractions obtained for the species under study. The test consisted in reducing the amount of DPPH dissolved in methanol. Note that the addition of an antioxidant to a DPPH solution leads to its discoloration which is directly proportional to the antioxidant capacity of the added product; this would induce a decrease in the absorbance measured at 517 nm. This addition therefore provides a practical means of measuring the antioxidant activity of our vegetable oils.

- The DPPH reduction test allows determining the oil concentration that causes the 50% reduction in DPPH (IC_{50}) as well.
- Once the inhibition percentages were calculated according to the vegetable oil concentrations and absorbances of the samples prepared, the results obtained were presented in the form of a curve representing the inhibitory power of *Moringa oleifera* seeds.

These results were used to plot the following curve (Fig 6).

The projection of the percentage of free-radical scavenging activity on the axis of the inhibitory mass concentration of HV allows noting that IC_{50} = 1.78318584 (µg/ml).

Therefore our oil has a very strong antioxidant activity, which confirms that this oil is capable of capturing free radicals.

CONCLUSION

This study aimed at valorizing the *Moringa oeifera* L. seed oil, in the region of Adrar, for its different virtues in the pharmaceutical, parapharmaceutical, medicinal, cosmetic, food industry fields and also as a source of biofuels. The vegetable oil from *Moringa oleifera* seeds was obtained by solid-liquid extraction using the soxhlet technique. For this, a quantity of 1 kg of dry seeds was used, with n-hexane as solvent. After 6 extraction cycles, a yield of 30.437% was obtained. Characterization of the oil obtained indicated that the physicochemical properties, such as organoleptic properties, GC, TLC, IR, density, viscosity and refractive index, complied with international standards and AFNOR standards. Furthermore, *Moringa oleifera* oil proved to have a very high antioxidant power; however, this oil did not exhibit any antibacterial activity on the strains tested. Moreover, the results obtained in this study are very encouraging and point to very promising prospects for cosmetic, medical, pharmaceutical and food applications in the future. From the economic and social standpoint, this plant turns out to be a significant source of raw materials and financial resources.

ACKNOWLEDGEMENT

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