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PHYTOCHEMICAL SCREENING AND PHYSICOCHEMICAL CHARACTERIZATION OF VEGETABLE OIL FROM THE *SIDERITIS INCANA*

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ABSTRACT

Natural substances are experiencing growing interest in their applications in various consumer products due to their importance. Plants represent an inexhaustible and renewable source of active ingredients for exploitation in a large field of application in the future. The soxhlet extraction method of oil (extracted from diethyl ether) gave an efficiency of 11.46%. The evaluation of the oil physicochemical parameters according to the Food Codex standards (1983) provides results such as the refractive index 1.4605, the potential hydrogen ((PH= 7), ultraviolet absorbance between 0.394 and 0.265, acid number 3.1997 mg of NaOH/g of oil, acidity 2.256%. The preliminary evaluation of the phytochemical composition of *Sideritis incana* confirms the presence of some chemical families such as saponins, volatile oils, alkaloids, flavonoids, cardenolides, tannins, anthocyanins, leucoanthocyanins.

Keywords: *Sideritis incana*, phytochemical screening, physicochemical parameters, vegetable oil.

Introduction

Sideritis incana L is a species that belongs to the endemic family of the Lamiaceae. The genus Sideritis includes more than 150 perennial and annual plant species widely distributed in the Mediterranean region (Kloukina *et al.,* 2020). In traditional medicine, Sideritis species have been used as teas, and flavoring agents (González-Burgos *et al.,* 2011).In addition, it is used as an antioxidant and antibacterial agent (Khelassi-Sefaoui *et al.,* 2021), antiulcer, vulnerative, antispasmodic, anticonvulsant, analgesic, anti-inflammatory, analgesic, and carminative are very rich in natural antioxidants, including flavonoids. Many studies confirm that almost all organs of aromatic plants contain essential oils.

Because of their fatty acid composition, vegetable and oily macerate are recognized for their specific virtues by the medical profession (Tomás-Lorente *et al.,* 1989). Moreover, antioxidants (tocotrienol, phytosterol,) and vitamins are recognized for their beneficial effects on health and beauty (Siham and Nesrine, 2021).

Vegetable oils are lipids and fats obtained from the leaves, fruits, or nuts of oleaginous plants, by various extraction processes, which remain liquid at room temperature. These oils are mainly made up of triglycerides known as triacylglycerol (TAG) consisting of a glycerol molecule attached to three unsaturated or saturated fatty acids combined as esters (Schuchardt *et al.,* 1998).

Vegetable oils are generally richer in unsaturated fatty acids. Furthermore, they contain a small proportion of other lipophilic substances such as sterols, free fatty acids, tocopherols, and other compounds. These oils and their various lipid components are widely used in the food, cosmetic, pharmaceutical, and oleochemical, industries (Rabasco Álvarez and González Rodríguez, 2000). The characteristics of an oil quality are defined by neutral taste, good stability, high resistance to oxidation, and a light color.

The objective of this study focuses on the characterization of some physicochemical parameters such as the refractive index, the potential hydrogen (pH), absorbance in the ultraviolet, acid index, acidity, and peroxide index of vegetable oil from the *Sideritis incana* plant.

Material and Methods

Plant Material

Collection of plant material

The *Sideritis incana* (SI) plant was harvested from the Ouenza province located in the Tebessa region (Algeria) in October 2019.

Drying plant material

The freshly harvested plant was left to dry in the shade and ventilated place at room temperature. Then, the process has been completed in the vacuum drying oven for 24 hours at T=35°C.

Grinding

Once dried, the plant material is finely ground and powdered using a mortar. To protect the material from humidity and light, it is stored in closed glass bottles covered with aluminum.

Phytochemical study

Phytochemistry is a study that gives the general composition of the selected plant.

Phytochemical screening

To highlight the presence or absence of certain compounds belonging to the chemical families of secondary metabolites, we carried out specific phytochemical tests based on color, turbidity, or precipitation reactions, using the methods described in the literature (Chaouche, T.-2011).

Preliminary tests:

Characterization tests are partly based on qualitative analysis, either on the formation of insoluble complexes using precipitation reactions or on the formation of colored complexes using color reactions (Badiaga, M.-2011).

Preparation of the infused at 10%

A mass of 2 g of the dry matter is covered with 20 ml of boiling water. After 3 to 6 min the mixture is stirred lightly and strained.

Saponins (Foam test):

A mass of 1 g of the dry matter is weighed into a beaker in which 10 ml of distilled water is added and boiled for 5 min. The mixture is filtered and a volume of 2.5 ml of the filtrate is added to 10 ml of distilled water in a test tube. The test tube is shaken vigorously for about 30s. Then, the solution was left to rest for half an hour. The presence of saponins was revealed by an alveolar foam (N'guessan *et al.,* 2009).

Volatile oils

Macerate 1g of the dry matter in 4 ml of distilled water with constant agitation for 30 min. The extract is filtered and a volume of 2 ml of the filtrate is shaken with 0.1 ml of dilute NaOH and a small quantity of dilute HCl (Daira, N.E.H. *et al.,*-2016). A white precipitate is formed with the volatile oils.

Flavonoids

Macerate 1g of the dry matter in 15 ml of HCl diluted at 1% for 24 hours. Take 5 ml of the filtrate, and make it basic by adding NaOH (Kadri and Yahia, 2015). The appearance of a light yellow color in the upper part of the test tube indicates the presence of flavonoids.

Alkaloids

Macerate 1 g of MS in 10 ml of 1% HCl. After the filtration, a few drops of Mayer's reagent were added (Himour *et al.,* 2016). A white precipitate indicates the presence of the alkaloids.

Leuco-Anthocyanins

A volume of 5ml of the 10% infused is heated with 4ml (3/4 pure ethanol/HCl) in a water bath at 50°C for a few minutes. A cherry red color indicates the presence of leuco-anthocyanins.

Tannins

A portion of the 10% infused is diluted with distilled water in a ratio of 3 drops of 10% ferric chloride $FeCl_3$ (Hayat *et al.,* 2020). A blue or green color indicates the presence of tannins.

Cardenolides

Macerate 1g of MS in 20 ml of distilled water and filter the solution. Take 10 ml of the filtrate and extract it with a mixture of chloroform $CHCl_3$, and ethanol C_2H_5OH.

Evaporate the organic phase and dissolve the precipitate in 3 ml of glacial acetic acid, add a few drops of $FeCl_3$ followed by 1 ml of concentrated H_2SO on the walls of the test tube. The appearance of a green-blue color in the acid phrase indicates the presence of the cardenolides.

Anthocyanins

Based on infused (10%) color change with PH variation. A few drops of concentrated HCl are added to the infused at 10%. Then, a few drops of NH_4OH are added to the solution, which causes the change of the color indicating the presence of anthocyanins.

Extraction process:

Extraction by Soxhlet:

Soxhlet extraction is a simple and convenient method allowing the extraction cycle to be repeated infinitely with fresh solvent until the complete depletion of the solute in the raw material. (Ivanov, P. P.-2010).The fixed oils are extracted by Soxhlet according to the method described by Hassani. (1999) (Figure 1). The crushed plant material is placed in a cartridge, which will be exposed to the extraction solvent (150 ml Diethyl ether) carried out at an evaporation temperature of 40°C. After approximately 3 hours of extraction cycles, the cartridge is removed and the solvent loaded with plant extract is recovered to be concentrated to dryness under vacuum using a brand rotary evaporator (Heidolph HB digital Laborota 4000 efficient).

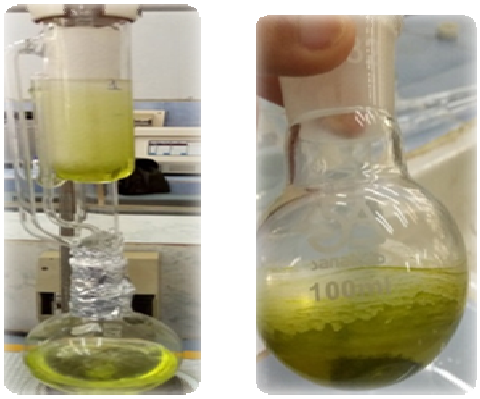

Fig. 1 : Fixed oil extraction.

Extraction efficiency:

The efficiency means the mass of the extract obtained after evaporation of the solvent compared to the initial mass of the plant subjected to the extraction. The rate of fixed oil was calculated by the following formula (Carré, 1953).

$$Rd\% = \frac{M}{M0} \times 100$$

R (%): efficiency expressed in %.

M: Mass in (g) of the resulting dry extract.

M0: Mass in (g) of the material used.

Determination of the physicochemical characteristics of the oil:

Physical characters:

Refractive index

It is the ratio between the sine of the angles of incidence and refraction of a light ray of a determined wavelength, passing from the air into the oil fixed at a constant temperature (Lion, P. H.-1969).

Depending on the refract meter used, either directly measure the angle of refraction or observe the limit of total reflection; the oil is maintained under the conditions of isotropism and transparency. The measurement was performed using an Abbe refractometer (Denis *et al.,* 1997).

Absorbance in the ultraviolet

The oxidation of fatty substances in particular those containing linoleic acid leads to the formation of linoleic hydroxy-peroxide a conjugated diene, which absorbs near 232 nm. If the oxidation continues, "secondary products" are formed in particular diketones and unsaturated diketones absorb around 270 nm. Absorbances are determined at wavelengths 270 nm and 232 nm.

Potential Hydrogen (pH)

The determination of the pH is carried out according to the AFNOR method (1986). This method is based on the potential difference existing between two electrodes immersed in the product (Afnor, N.-1986).

Chemical characters:

Acid value

The acid number is the number of mg of potassium hydroxide necessary for the neutralization of the free acids contained in one g of fatty substance (Lion.-1995). The acid index is the quantity of potash expressed in mg necessary to neutralize the free fatty acids contained in one g of oil. This Index provides valuable information on the quality of the oil conservation (Ullah and Bano, 2011).

Acidity

The acidity of fat is the number of mg of sodium hydroxide (NaOH) required to neutralize the free fatty acids (FFA) contained in 1g of fat. It measures the amount of GLA present in a fatty substance (Kandji, -2001).

The principle of the method consists of a titration of the free fatty acids present by an ethanolic solution of sodium hydroxide.

Peroxide index

The peroxide index is a measurement allowing the quantity of peroxides present in fat to be estimated (Ozkan *et al.,* 2007). Peroxides are characteristic constituents of the oxidation of unsaturated fatty acids. The determination is based on their property of releasing iodine from potassium iodide in acidic media. The iodine released is measured by the reaction with the thiosulphate. It is known that 1ml of 0.01 N thiosulphate corresponds to a quantity of 80 mg of oxygen fixed to the fatty acids (Lion, 1995).

Results and Discussion

Phytochemical screening

The results of the phytochemical tests carried out on the plant are summarized in table 1.

Table 1 : Phytochemical screening of the *Sideritis incana.*

Chemical component	Presence / absence
Saponins	+
Volatile oils	+
Flavonoids	+++
Alkaloids	+++
Leuco-Anthocyanins	+++
Tannins	+++
Cardenolides	+++
Anthocyanins	+

Definitely positive test: (+++), Moderately positive test: (+)

Phytochemical screening results are similar to those of Khelassi-Sefaoui. (2021) (Khelassi-Sefaoui *et al.,* 2021), which approves the presence of certain chemical families. However, other chemical families are not detected. This can be explained by a difference in geographical, physicochemical, or biological parameters such as the difference in the harvesting site including the environment of the plant, the light, the precipitation, the topography, and the season, the type of soils, harvest time, the extraction methods, and the part of the plant selected of the study or their phytochemicals.

Extraction processes

Solvent extraction (Soxhlet)

Extraction of the oil was carried out by Soxhlet using ethyletherlike as a solvent. After extraction and elimination of solvent, the efficiency obtained is 11.46%. According to Bojović *et al.* (2011), lipids (vegetable oil) are important bio compounds in terms of calories and fatty acid intake. Lipids are organic materials insoluble in water but soluble in organic solvents. The obtained results show that *Sideritis incana* is very rich in lipids.

Physico-chemical characteristics
Physical characters:
Refractive index :

The measurements are carried out with an Abbe refractometer with a temperature fixed at T=23°C. The table below summarizes the results of the analyzed oil refractive index.

Table 2 : Result of the refractive index.

Trial	White	Oil	C.A standard
1	1.33	1.462	1.463 – 1.478

*: C.A standard: Codex Alimetarius

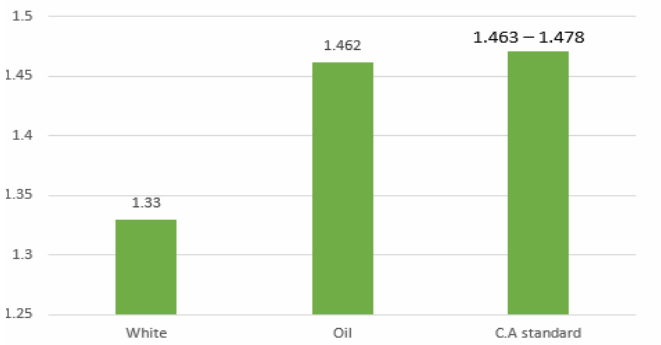

Fig.2: Result of the refractive index.

For oils, the refractive index is considered as purity criteria. It varies according to two important conditions, the wavelength of the incident light and the temperature at which the analysis is carried out. The value of the refractive index of Sideritis incana oil (1.4620) is within the range established by CODEX ALIMENTARIUS (1983). Storage conditions affect the oil quality in various ways mostly by hydrolysis or oxidation Ognyanov *et al.* (2021). In this case, the storage oil is not suitable for consumption.

The potential hydrogen (pH)

The pH meter is used to determine the pH of oils. The obtained results show that this oil has a neutral pH (pH= 7). This finding allows us to say that the acidity level is relative to the hydrogen potential (Dogan *et al.,* 2018).

Absorbance in the ultraviolet

We dissolved 0.5 g of oil in 50 ml of hexane. This implies that the concentration is 1g/100 ml. The R=E232 /E270 ratio is generally greater than 10 or 20 in virgin fatty substances and less than 2 in refined fatty substances. The findings are directly in line with previous findings of (Mariod *et al.,* 2005).

Table 3 : Result of the hydrogen potential of the analyzed oil.

Wavelength (λ nm)	**232**	**270**	**232 Dilution (1/2)**	**270 Dilution (1/2)**	**232 Dilution (1/4)**	**270 Dilution (1/4)**
Absorbance	3,860	3.013	1.671	0.560	0.394	0.265
Extinction K	3,860	3.013	1.671	0.560	0.394	0.265
Report E232/E270	1,280		2.980		1.480	
C.A standard for E232/E270 ratio	Max (2)					

For λ 232 nm dilution (1/4): E= 0.394
For λ 270 nm dilution (1/4): E= 0.265

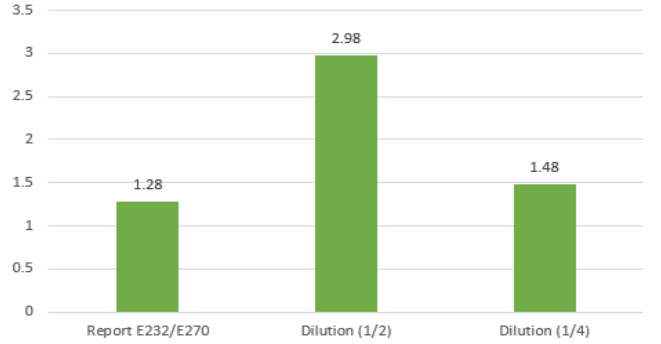

Fig.3: Result of the hydrogen potential of the analyzed oil.

The obtained results are conform to the standard established by the 1983 Alimentarius Codes. All fatty substances contain epoxides and hydroperoxides. Indeed, the conjugated dienes and the primary oxidation products of fatty acids are formed by the rearrangement of the double bonds of the alkyl radical of polyunsaturated fatty acids. When conjugated diene structures such as linoleic hydroperoxide are present, they absorb light at around 232 nm.

Conjugated trienes (in the case of the presence of fatty acids with three double bonds) and secondary oxidation products such as α-unsaturated aldehydes and ketones absorb the light around 270 nm. The determination of the absorbance near 232 and 270 nm makes it possible to detect and evaluate the quantities of the oxidation products: the greater the extinction at λ=232 nm the more it is peroxidized. Similarly, the stronger the extinction at λ=270nm the richer it is in secondary oxidation products and reflects a low aptitude for preservation (Wolff J.P.-1968).

Chemical characters

Determination of acid value:

The obtained value revealed a medium high acid number of 3.19976 mg NaOH/g oil. It is mean that there is a good number of free fatty acids in lipids, comparing with the CA standard (2.2–7.26 mg NaOH/g oil). These findings are consistent with the study of Sevindik (2019). The following table summarizes the results of the acid value of analyzed oil.

Table 4 : Result of the acid number of the analyzed oil.

Volume of NaOH (ml)	Acid number (AI) mg NaOH/g oil CA standard (mg NaOH/g oil)	CA standard (mg of NaOH/g of oil)
0.80	3.19976	2.2 – 7.26

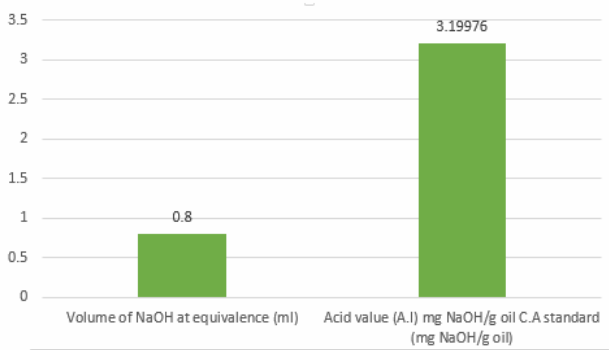

Fig.4: Result of the acid number of the analyzed oil.

The acid number evaluates the amount of free fatty acids. The acid index measures the quantity of free fatty acids resulting from the hydrolytic reactions of triglycerides. The obtained value of the acid index are conform to those of the Codex Alimentarius standard (1983). This explains that the oils are rancid without the transformation of triglycerides into fatty acids and glycerols. (Sevindik, 2019).

Determination of Acidity

Acidity is an oil quality criteria depends essentially on the storage conditions (light, humidity, temperature and oxygen)(WOLFF, J.P.-1991).

Table 5 : Result of the acidity of the analyzed oil.

Volume of NaOH at equivalence (ml)	Acidity %	C.A standard
0.8	2.256	Max 3.3%

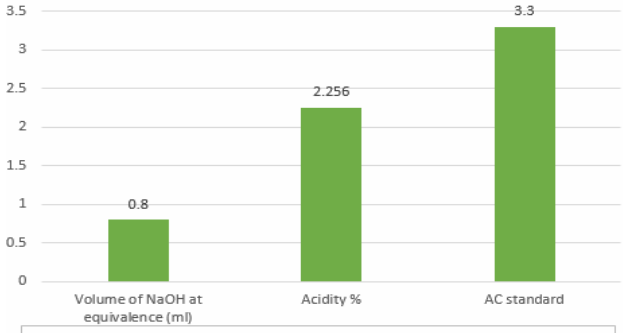

Fig.5: Result of the acidity of the analyzed oil

According to Gökbulut *et al.* (2017) a low acidity level contributes to giving the oil greater stability against air oxidation. It is recommended for vegetable oil to have a low acidity level (lower than the 3.3% standard imposed by codex alimentarius) to support long conservation without deterioration (Onyeike and Acheru, 2002). The results showed that the analyzed oil has an acidity adequate to that of the Codex Alimentarius standard (1983) (3.3% max).

Determination of the peroxide number:

This is a very important criterion for assessing the first stages of oxidative deterioration of the oil. The peroxides contained in the fresh oil can play a significant role as initiators or catalysts of the oxidation reactions during its storage.

Table 6 : Results of the peroxide index of the analyzed oil.

Volume of H_2SO_4 at V0 equivalence (ml) Blank	Volume of H_2SO_4 at equivalence V (ml) oil	Peroxide index (meq of O_2/Kg of oil)	CA standard (meq of O_2/ Kg of oil)	AOCS standard (meq of O_2/Kg of oil)
2.7	20	86	5.2 – 7.0	0 to 12

Because of its sensibility, the peroxide index is a very useful criteria for assessing the first stages of oxidative deterioration (Perrin, 1992). It indicates the amount of fatty rancid acids (Aïssi *et al.,* 2009). In this study, the obtained value is higher than that of the Codex Alimentarius and American Oil Chemist's society standard. This can be explained by errors in the titration process through the measurement and the test sample, which contains more of the solvent (Charami *et al.,* 2008).

Conclusion

This work contributes to the phytochemical study of the Algerian flora plant *Sideritis incana* (Lamiaceae family). The physicochemical characteristics of the oil from the powder were determined. These characteristics show that this oil has interesting physicochemical properties. The complete study of the phytochemical screening highlights the presence of other chemical compounds, which have interesting biological activities, in particular polyphenolic substances. The overall analysis of the different properties allows us to say that this oil is appropriate and suitable for use in the food industry and/or in phytomedicine.

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