Nutritional composition, sensory quality and bioactivity of a dietary supplement based on the *Origanum compactum* Benth and *Foeniculum vulgare* Mill aerial part

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Summary

The present study aimed to develop capsules based on the powdered leaves of *Origanum compactum* Benth and *Foeniculum vulgare* Mill. For this, mixture of 500 g of powdered oregano leaves and 500 g of fennel powder was used and some primary components of the powder were characterized. In addition, total phenolics and flavonoids contents were determined. Also, quenching of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethyl-benzothiazoline-6-sulfhonic acid) (ABTS) free radicals was used to determinate the antioxidant activity. Several pharmaco-technical parameters of the powder were evaluated such as approximate solubility, conductivity, pH, dry matter and relative humidity. At the phytochemical level, total phenolics content of 0.29 ± 0.05 g·kg^{-1} (expressed as gallic acid equivalents) and 0.16 ± 0.02 g·kg^{-1} (expressed as rutin equivalents) for flavonoids were determined. The powder had a significant antioxidant activity, with a half-maximal inhibitory concentration (IC_{50}) of 0.34 ± 0.03 mg·ml^{-1} and 0.36 ± 0.02 mg·ml^{-1} in DPPH and ABTS assays, respectively. In addition, the powder was rich in carbohydrates (0.13 ± 0.29 g·l^{-1}), lipids (1.25 ± 0.29 g·kg^{-1}) and proteins (13.23 ± 0.85 g·kg^{-1}, expressed as bovine serum albumin equivalents). The present study has made it possible to provide a new approach to valorization of medicinal plants in food while producing improved drugs based on traditional medicine.

Keywords

antioxidant; capsule; *Foeniculum vulgare*; *Origanum compactum*

Agriculture introduced the first great changes in human nutrition. In a few thousand years, the population adopted a settled lifestyle, new foods and new habits appeared. A second great metamorphosis occurred during the industrial revolution [1]. This led us to consume increasingly refined foods, more and more present on our plates at the expense of "traditional" products.

Nowadays, the use of herbal treatments as a form of treatment, particularly for metabolic illnesses, is becoming increasingly popular [2–4]. According to Vantage Market Research [5], the market for plant-based foods was worth 40.21 × 10^9 USD in 2021 and is estimated to grow at a Compound Annual Growth Rate (CAGR) of 11.9 % to reach 161.9 × 10^9 USD in 2030 [6]. Similarly, the market for plant-based beverages, which was worth 26 × 10^9 USD in 2021, is expected to reach 67 × 10^9 USD by 2028 [7].

Along with their ability to produce proteins, lipids, carbohydrates and nucleic acids, plants also contain an array of secondary metabolites that are indirectly necessary to the plant's survival. They are involved in adaptation and defense processes [8]. A major group of aromatic and medicinal plants is the Lamiaceae family (also known as Labiatae), which is widely distributed throughout the world [9].

Oregano (*Origanum compactum* Benth.) is an endemic species of Morocco. It belongs to Lamiaceae family and is used as a condiment in the treatment of digestive disorders, as an analgesic, antibacterial and especially as a powerful antioxidant [10]. Fennel (*Foeniculum vulgare* Mill) is a medicinal plant of Apiaceae family, known for its carminative, digestive, galactogenic and diuretic effects. It is also indicated in the treatment of gastrointestinal and respiratory disorders. It is

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widely used in traditional medicine to treat abdominal pain, to calm colitis in children and infants, as well as in cases of constipation, conjunctivitis and stomach ache [11].

Both species occupy an important place in the therapeutic arsenal of humans, they are considered effective, both for the treatment of diseases and in traditional medicine, being major sources of antioxidants and bioactive metabolites. Their energetic nutritional value (fennel seeds), their endemism (oregano), and their richness in fibre facilitate intestinal transit with reduction of flatulence and bloating.

The effectiveness and success of a remedy depend strongly on the dose used and the frequency of use, while overdosing is dangerous even for non-toxic plants. According to ethnobotanicals surveys, traditional medicine practitioners recommend random and unspecific doses of "pinch, spoon or a handle" that can be used for varying lengths of time [12, 13], therefore, the risk of intoxication and/or compromise to human health is likely. However, it ensures consumer health protection by minimizing batch-to-batch variation; assures efficacy, safety, quality and acceptability of the polyherbal formulations.

The present study aimed to develop capsules based on *Origanum compactum* and *Foeniculum vulgare* aerial parts, for antioxidant and dietary supplement purposes. Capsules can be served also for antispasmodic effects and against aerophagia. For this, we proceeded to (i) characterize the primary metabolites of the species (proteins, lipids, carbohydrates), (ii) screen phytochemicals of powder extracts and (iii) evaluated the antioxidant activity of powder extracts.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethyl-benzothiazoline-6-sulfhonic acid) (ABTS), rutin and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemical reagents and solvents used in experiments were of analytical grade. Aqueous solutions were prepared using distilled water.

Plant materials

Fennel seeds were collected in the Er-Rachidia region in South-East Morocco (N 6° 7' 20", W 0° 14' 36", altitude 1 089 m) in April 2022. For oregano, the harvest was done in the Ksar El-Kbir region (N 34° 06' 03", W 3° 56' 36", altitude 134 m), North of Morocco. The samples were carefully placed in coolers and directly taken to the laboratory. Subsequently, the botanical identification was confirmed in the Bioactives, Health, and Environment Laboratory, Moulay Ismail University of Meknes, Morocco, and the reference specimen, carefully prepared and labelled, was deposited under the numbers F.V 01-2022 for *F. vulgare* and O.C 01-2022 for *O. compactum*. Fresh plants were left to dry in a dark and ventilated place for two weeks at room temperature of 24 ± 2 °C. Then, the parts to be used were stored in clean bags away from light and moisture.

Powder mixture preparation

The powder was obtained by grinding dry leaves using a laboratory grinder (R5 Plus, Robot Coupe, Heerhugowaard, the Netherlands) at room temperature (24 ± 2 °C) and mixed in equal quantities for analysis and capsule filling (500 g of powdered oregano leaves was mixed with 500 g of fennel powder).

Capsule preparation

Capsules were filled using a manual 100-unit capsule filler (K100 Premium; Kapselwelt, Hude, Germany). Fig. 1 shows the general flow diagram of the study.

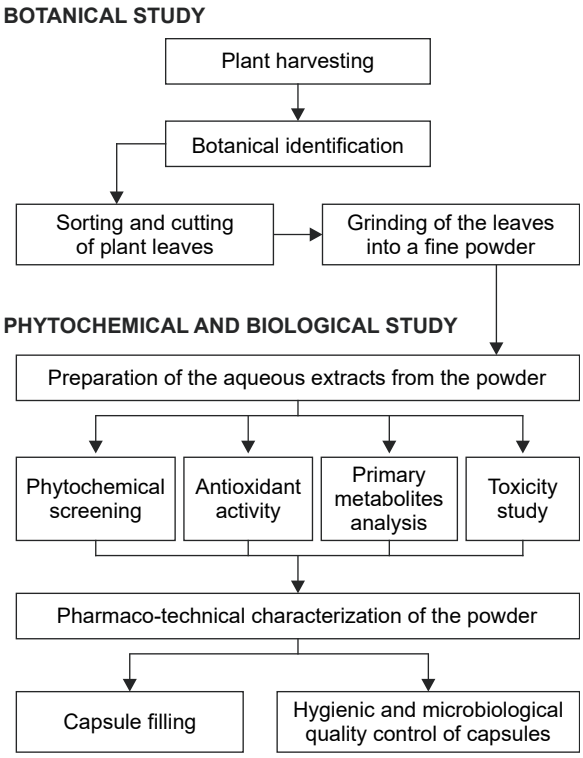

Fig. 1. Flow diagram of the study.

Extract preparation

Qualitative phytochemical screening of the powder extracts was performed to identify the main groups of chemical constituents. For this, 10 samples of 0.5 g of the fine powder were macerated individually for 20 min by magnetic stirring in beakers containing 10 ml of distilled water. Then, the volume of the supernatant from each sample was taken and used for phytochemicals screening.

Total phenolics

The total phenolics content was determined using the colorimetric assay with the Folin-Ciocalteu reagent [14]. Briefly, 0.2 ml of diluted supernatnt was placed in a glass hemolysis tube with 1 ml of the 10-fold diluted Folin-Ciocalteu reagent. After vigorous shaking and standing of the mixture at 22 °C for 5 min, 0.4 ml of 75 $g \cdot l^{-1}$ Na_2CO_3 was added. After 90 min of incubation in the dark, the absorbance was measured at 765 nm. A standard curve was constructed under the same conditions using gallic acid. The results were expressed as gallic acid equivalents (GAE). The test was performed in triplicate ($n = 3$) and the results were expressed as mean ± standard deviation (SD).

Total flavonoids

Total flavonoids content was estimated by the aluminum chloride colorimetric assay, as described by Miliauskas et al. [15] with some modifications. For this, 0.5 ml of the newly prepared supernatant was added to 0.1 ml of 100 $g \cdot l^{-1}$ $AlCl_3$ and 0.1 ml of 1 $mol \cdot l^{-1}$ sodium acetate. Then, 1.5 ml of methanol and 2.8 ml of distilled water were added. After 30 min of incubation at room temperature (24 ± 2 °C), absorbance was measured at 415 nm. A standard curve was constructed under the same conditions using rutin. The flavonoids content was expressed as rutin equivalents (RE). The test was performed in triplicate ($n = 3$) and the results were expressed as mean ± SD.

DPPH radical-scavenging activity

The antioxidant activity was evaluated using the DPPH method [16]. For this, 0.5 ml of various concentrations of extract (0 $mg \cdot ml^{-1}$ to 1 $mg \cdot ml^{-1}$) was added to 3 ml of DPPH methanolic solution (15.77 $mol \cdot l^{-1}$). After 20 min of incubation at room temperature (24 ± 2 °C), the result of the decolorization process was measured at 517 nm with a UV spectrophotometer UV-2005 (J. P. Selecta, Abrera, Spain). A calibration curve was obtained using EDTA as standard. The antioxidant activity was expressed as a half-maximal inhibitory concentration (IC_{50}) value. The test was performed in triplicate ($n = 3$) and the radical-scavenging activity was expressed as percentage of inhibition (I) according to Eq. 1:

$$I = \frac{Ac - As}{Ac} \times 100 \quad (1)$$

where A_c is absorbance of the control (DPPH methanolic solution) and A_s is the absorbance of samples (DPPH with extracts).

ABTS radical-scavenging activity

The radical-scavenging activity using ABTS free radical was determined according to Re et al. [17]. The $ABTS^{\bullet+}$ stock solution was prepared by mixing 7 $mmol \cdot l^{-1}$ ABTS with 2.45 $mmol \cdot l^{-1}$ of potassium persulfate and allowed to react at room temperature (24 ± 2 °C) in the dark for 12–16 h. The stock solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 0.02 ml of extracts were allowed to react with 1.980 ml of $ABTS^{\bullet+}$ for 7 min and the absorbance was measured at 734 nm. The percentage of inhibition was calculated. A calibration curve was obtained using EDTA as standard. The antioxidant activity was expressed as IC_{50}.

Carbohydrates

The carbohydrates contents was determined by a phenol-sulfuric acid method according to Dubois et al. [18] with measuring absorbance 492 nm. The calibration curve was constructed using glucose as standard. The test was performed in triplicate ($n = 3$) and the results were expressed as mean ± SD.

Lipids

Lipids were extracted using differential solubility in Soxhlet as described by Milaiti et al. [19]. For this, 20 g of plant powder was extracted by refluxing with 200 ml of *n*-hexane at 70 °C for 6 h. Then, the solvent was removed by a rotary evaporator and the residue of initial mass was recovered. After 30 min, the flask was transferred to an oven at 120 °C, the residue was dried, cooled in a desiccator and weighed. The proportion of lipids (MG) was calculated using Eq. 2 and expressed in percent.

$$MG = \frac{Mb - IM}{MS} \times 100 \quad (2)$$

where Mb is the mass after 30 min of incubation in the oven at 120 °C. IM is the initial mass and MS is the mass of the sample.

Proteins

Three categories of proteins were extracted

and assayed including albumin, glutinin, and globulin. For this, a protocol of fractionation described by NASRI and TRIKI was used [20]. After extraction, the estimation of each protein category was carried out according to the BRADFORD method [21]. A standard curve was performed under the same conditions using bovine serum albumin as a reference. The results were expressed as bovine serum albumin equivalent (BSAE).

Sensory evaluation of the powder

The determination of organoleptic characteristics consisted of observing the powder, touching it, smelling and tasting it.

pH

An aqueous dispersion of the powder (5 g·l^{-1}) contained in capsules was prepared and filtered through filter paper (Macherey-Nagel, Dűren, Germany). The pH value of the filtrate was measured using a pH-meter HI 2211-PH/ORP meter (Hanna Instruments, Padua, Italy) at room temperature (23 ± 2 °C). A calibration step was conducted using HCl and NaOH solutions before the measurement process. The test was performed in triplicate ($n = 3$) and the results were expressed as mean ± *SD*.

Conductivity

Another aqueous dispersion of the powder was prepared and filtered for conductivity measurement. The conductivity value was determined by a conductivity meter pHenomenal -CO 3100L (VWR International, Radnor, Pennsylvania, USA) at room temperature (23 ± 2 °C). The test was performed in triplicate ($n = 3$) and the results were expressed as mean ± *SD*.

Approximate solubility

Solubility was determined by adding increasing volumes of water to 100 mg of the powder at room temperature (24 ± 2 °C), in a beaker containing 10 ml of bidistilled water. Continuous stirring of the mixture was carried out using a magnetic stirrer for 10 min after each addition of water. The solubility of the powder was checked visually. If the powder was not soluble, the test was continued in a 100 ml beaker for 24 h to 96 h (Tab. 1) [22].

Dry matter and relative humidity

An amount of 20 g of powder was wrapped in domestic aluminum foil and dried in an oven at 60 °C. After one week of drying, the sample was weighed. Dry matter (*DM*) and relative humidity (*RH*) were determined according to Eq. 3 and Eq. 4 and expressed in percent [23].

$$DM = \frac{Mf}{Mi} \times 100 \quad (3)$$

$$HR = \frac{Mi - Mf}{Mi} \times 100 \quad (4)$$

where *Mi* is the initial mass and *Mf* is the mass of samples after one week of drying.

Mass uniformity

The test was performed according to the European Pharmacopoeia [23] as follows: 20 units taken at random were weighed individually and the average mass was determines. The requirements state that individual mass of not more than 2 of 20 units may deviate from the average mass by a greater percentage than that indicated in Tab. 2, but the mass of no unit may deviate by more than twice that percentage.

Toxicity

To evaluate the potential toxicity of powder, the median lethal concentration (LD_{50}) was determined by brine shrimp (*Artemia salina* L.) lethality assay [24]. The extract was dissolved and diluted in artificial seawater (32 g of NaCl dissolved in 1 l of distilled water) and tested at final concentrations of 0.01, 0.1, 0.5 and 1.0 mg·ml^{-1}. Ten brine shrimp larvae were transferred to each test tube and artificial seawater was added to the final volume of 5 ml. After 24 h at 25–28 °C, surviving larvae were counted. The experiment was performed in triplicate, and LD_{50} values were determined by the

Tab. 1. Solubility of the the oregano-fennel powder as determined by OECD method [22].

Volume of water dissolving 0.1 g [ml]	Approximate solubility [g·l^{-1}]
0.1	> 1 000
0.5	200–1 000
1	100–200
2	50–100
10	10–50
100	1–10
> 100	< 1

Tab. 2. Mass uniformity of the prepared oregano-fennel capsules.

Average mass [mg]	Limit deviation [%]	Tolerated deviation for two capsules [%]
≤ 80	± 10.0	± 20
> 80 to < 250	± 7.5	± 15
≥ 250	± 5.0	± 10

LITCHFIELD and WILCOXON method [25]. Extracts were considered non-toxic if LD_{50} was greater than 1 mg·ml^{-1}.

Microbiological quality control of capsules

The microbiological quality control of capsules involved quantification of the bacterial load of finished products by germ enumeration techniques according to standardized procedures [26]. The enumeration concerned the total aerobic mesophilic flora (TAMF) and coliforms. For this purpose, plate count agar (PCA, Biokar Diagnostics, Allonne, France) and bromocresol purple broth with lactose (BCPL, Biokar Diagnostics) with deep seeding were used.

Statistical analysis

The data used in statistical analysis were obtained from three independent experiments (n = 3). Then, the results were expressed as mean ± SD. The analysis process was performed by GraphPad-8 Prism software (GraphPad Software, La Jolla, California, USA) and the Statistical Package for the Social Sciences version Statistics-20 (SPSS, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Pharmaco-technical parameters

The results of pharmaco-technical characterization tests of the powder are presented in Tab. 3. After grinding and sieving, The powder obtained was greyish-green in colour with a strong odour. In addition, the powder was classified as a "fine powder" with a homogenous particle size distribution and easy flow.

Mass uniformity of the capsules

High-quality capsule products should be developed and manufactured with a high degree of uniformity in the weight and content of the generated tablets, to ensure that patients will take an exact and appropriate dose of the medication. In this study, the individual mass of the 20 capsules tested ranged from 446 mg to 450 mg and the average mass was 448.7 mg. None of the capsules tested deviated from the average mass by ± 5 % (Tab. 2). It is therefore concluded, concerning the pharmacopeial standards [23], that the capsules conformed to the mass uniformity requirements (Fig. 2).

Tab. 3. Pharmaco-technical characteristics of oregano-fennel powder.

Parameter	Value
pH	7.32 ± 0.09
Conductivity [mS·cm^{-1}]	1.48
Approximate solubility [g·l^{-1}]	50–10
Relative humidity [%]	3.7 ± 0.2
Dry matter [%]	96.3 ± 0.1

Values represent mean ± standard deviation (n = 3).

Phytochemical characteristics of the powder

The phytochemical screening was conducted using the colorimetric and precipitation tests that reveal presence or absence of chemical compounds. The results obtained revealed the presence of flavonoids, condensed tannins, catechin tannins, triterpenes and steroids. Total phenolics content of 0.29 ± 0.05 g·kg^{-1} (expressed as gallic acid equivalents) and flavonoids content of 0.16 ± 0.02 g·kg^{-1} (expressed as rutin equivalents) were determined (data not shown).

Antioxidant activity

The antioxidant activity of the powder extracts

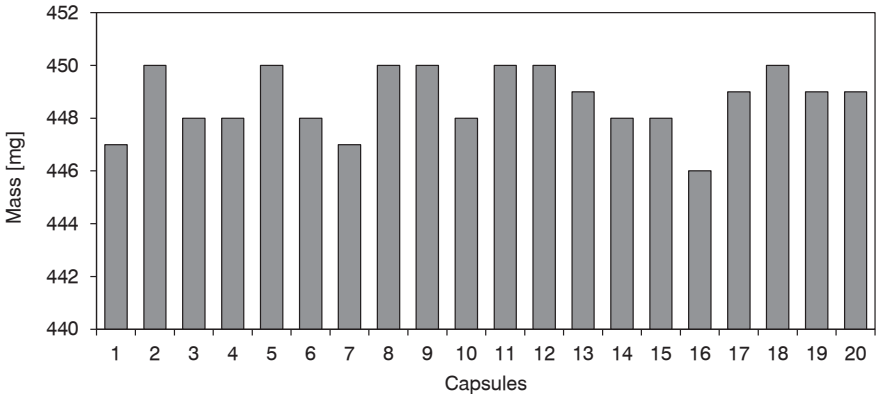

Fig. 2. Mass distribution of 20 oregano-fennel capsules according to the mass uniformity test.

was expressed as IC_{50} value. The results obtained showed IC_{50} of 0.34 ± 0.03 mg·ml^{-1} for DPPH assay and 0.36 ± 0.02 mg·ml^{-1} for ABTS assay. Also, the standard antioxidant EDTA had IC_{50} of 0.06 ± 0.20 mg·ml^{-1} (Fig. 3). Radical-scavenging activity is essential because of the damaging effect that free radicals have on food and biological systems [27]. Free radicals are produced as a consequence of biological processes [28]. ACIKGOZ and KARA [29] showed that *F. vulgare* seeds express the highest antioxidant power compared to other parts of the plant (leaves, flowers and bulbs), with the lowest IC_{50} value. Another study conducted by BEYAZEN et al. [30] on the aqueous extracts of *F. vulgare* showed a low antiradical activity compared to our results, with IC_{50} higher than 1 mg·ml^{-1}. According to KADRI et al. [31] and PUKALSKAS et al. [32], antioxidant activity depends on several factors, such as the concentration of extracts, the method of evaluation and the sensitivity of antioxidants to the test temperature. Indeed, a study conducted by OKTAY et al. [33] revealed a radical-scavenging effect of water and ethanol extracts of fennel seeds on the DPPH radical of 47.5 % and 36.5 %, respectively, at 0.25 mg·ml^{-1}.

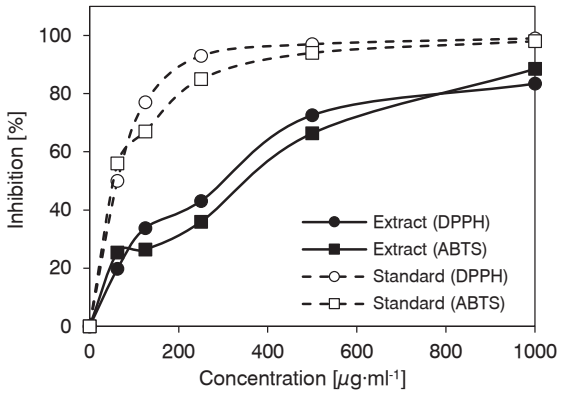

Fig. 3. Inhibitory effect of the powder oregano-fennel extracts on free radicals.

EDTA was used as standard.
DPPH – inhibitory effect determined by DPPH assay, ABTS – inhibitory effect determined by ABTS assay.

Tab. 4. Content of proteins in oregano-fennel powder.

Protein category	Content [g·kg^{-1}]
Albumins	4.52 ± 0.29
Globulins	4.79 ± 0.28
Glutelins	3.91 ± 0.27
Total	13.23 ± 0.85

Values represent mean ± standard deviation (n = 3).
The content of proteins is expressed as grams of bovine serum albumin equivalents.

Tab. 5. Content of primary metabolites in oregano-fennel powder.

Primary metabolite	Average
Carbohydrates [g·l^{-1}]	0.13 ± 0.29
Lipids [g·kg^{-1}]	1.25 ± 0.29
Proteins [g·kg^{-1}]	13.23 ± 0.85

Values represent mean ± standard deviation (n = 3).
The content of proteins is expressed as grams of bovine serum albumin equivalents.

Primary metabolites content and energy value

The results obtained from primary metabolites analysis are presented in Tab. 4 and Tab. 5. At present, no studies have been carried out on the dietary side of the species studied. The nutritional value and its considerable content of primary metabolites (carbohydrates, lipids, and proteins) may constitute a major contribution to the body's energy input. The energy value obtained for our capsules was 25 456 kJ·kg^{-1}. The present study is therefore a contribution to the valorization of primary metabolites of plant origin as a new source that can be exploited on an industrial scale for animal and human nutrition.

Toxicity and microbiological quality of capsules

From the point of view of consumer protection, evaluation of toxicity and microbiological quality of capsules was carried out in the first instance. The results obtained using the brine shrimp test on *A. salina* larvae showed that extract of *F. vulgare* and *O. compactum* had no toxicity against shrimp larvae ($LC_{50} > 1000$ μg·ml^{-1}, data not shown). The main advantages of using *A. salina* in toxicity testing are speed, cost-effectiveness and ease of use. This test can be considered as an alternative to in vitro cell culture and in vivo tests [34]. A good correlation between the results of the acute oral toxicity test in the animal model and this one have been reported. Thus, it represents a useful tool for predicting the acute toxicity of plant extracts [35]. Also, LAHLOU [36] showed that the powder and aqueous extract of *O. compactum* did not exert any toxic effect on *Artemia* larvae. Thus, according to KISHORE et al. [37], mice receiving the highest dose (60 mg·ml^{-1}) of *F. vulgare* extract showed no toxicity or mortality. According to the bacterial enumeration results (Tab. 6), TAMF value was below 10^3 CFU·ml^{-1}. This microflora includes Enterobacteriaceae, bacilli, staphylococci, pseudomonads, lactic acid bacteria or other potentialy pathogenic microorganisms [27]. The

Tab. 6. Content of microorganisms in oregano-fennel dispersion, powder and capsules.

	In aqueous dispersion	In initial powder	Per capsule
	[CFU·ml^{-1}]	[CFU·kg^{-1}]	[CFU]
TAMF	1.4×10^2	1.1×10^5	48.7×10^2
Total coliforms	4.2×10^2	3.2×10^5	1.1×10^2
Fecal coliforms	1.4×10^2	1.1×10^5	0.3×10^2

Values represent mean ± standard deviation (n = 3).
TAMF – total aerobic mesophilic flora.

total microflora informs about the preparation conditions and the efficiency of raw material treatment from reception to manufacturing. According to the standards, the most contaminated samples are those which present a rate of total flora higher than 10^5 CFU·ml^{-1} and 10^7 CFU·kg^{-1}. They are considered unsatisfactory [38]. Coliforms were also present at less than 10^3 CFU·ml^{-1}. The results obtained showed the hygienic conformity of capsules ($<10^5$ CFU·ml^{-1} for TAMF, $<10^3$ CFU·ml^{-1} for total coliforms and $<10^3$ CFU·ml^{-1} for fecal coliforms (data not shown).

The bacteriological conformity of our capsules, combined with non-toxicity of the plants used, ensures overall product safety. Our results are promising regrading long-term conservation of the capsules, both in terms of hygienic conformity and because the galenic form of our product (capsules) is easier to conserve than other galenic forms (syrups, pills).

CONCLUSIONS

In the end, it emerges that phytotherapy remains a practice still widely used by the Moroccan population for the treatment of many diseases despite the socio-economic development and better medical care of the patients. The formulation of the food supplement in the form of capsules based on selected medicinal plants is part of the development of local virtues. The present study evaluated some physico-chemical and biological properties of these plant species. The biological activity of powder aqueous extracts from *F. vulgare* and *O. compactum* showed interesting results, in terms of antioxidant activity and phenolics content. Thus, the phytochemical screening showed the richness of the two species studied in secondary metabolites. In addition, the toxicity assay showed no toxic effects of plant extracts. The nutritional value of the manufactured capsules showed a considerable contribution of primary metabolites (carbohydrates, lipids and proteins). The results obtained contribute to providing a new approach to valorization of medicinal plants as nutritional supplements while delivering biologically active compounds based on traditional medicine. By valorizing medicinal plants into finished products such as capsules, the active ingredients are more available, with rapid absorption and the appearance of the expected therapeutic effect. In this way, capsules enable the expected therapeutic effects to be achieved with a minimum quantity of raw material, as opposed to food, which requires consumption of a higher quantity. In addition, access to a balanced, healthy diet is not always easy for many people. In this context, medicinal plants represent a major contribution in terms of food and medicine.

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