Modeling of antiproliferative effects of *Salvia officinalis* L. essential oil optimized using Box–Behnken design

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**Modeling of antiproliferative effects of *Salvia officinalis* L. essential oil optimized
using Box–Behnken design**

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Abstract

Background

Salvia officinalis L. essential oil (SoEO) was mostly traditionally used to medicate various diseases as cancer. Then, the present work aims were: (1) to model the cytotoxicity effects of *Salvia officinalis* L. essential oil (SoEO) related to the human cancer cell lines kind (MCF-7 and HeLa) ; (2) to optimize the hydro-distillation extraction conditions of SoEO; and, (3) to determine the *in vitro* scavenging capacity of the free radicals DPPH[•], NO[•], ABTS⁺, and the ability to reduce Fe³⁺.

Methods

The cytotoxicity and anti-proliferative abilities were evaluated by measuring cell viability and then modeled. Two human cell lines: MCF-7 and HeLa were used. The optimization of SoEO extraction by hydro-distillation was carried out with Response Surface Methodology (RSM) using the Box–Behnken design

Results

The cytotoxicity activity against both tumor cell lines MCF-7 and HeLa was considerably important with IC₅₀ = 3.125 and 8.920 µg/mL, respectively. All treated cell lines showed a significant reducing in cell viability in response to the increasing oil concentration. The relative behaviors of both cell lines under SoEO treatment were modeled. The obtained optimal extraction yield was Y = 1.85 g/100 g d.b. The main identified fractions were camphene (23.7%), α-thujone (19.62%), 1,8-cineole (10.6 %), viridiflorol (5.9%), borneol (5.72%); β-thujone (5.4%); caryophyllene (3,83%). Also, SoEO was mostly able to scavenge DPPH[•] free radical, ABTS⁺ radical and hydrogen peroxide in an amount dependent manner (IC₅₀ = 0.97, 0.279 and 0.05 mg/mL, respectively).

Conclusion

The present work provides a preliminary platform for further investigation of the possible mechanism of *S. officinalis* essential oils and their individual compounds in cytotoxic and antitumor activity.

Keywords. *Salvia officinalis* L. ; essential oil ; response-surface methodology ; antioxidant activity ; cytotoxicity modelling ; MCF-7 ; HeLa.

1. Background

Salvia officinalis L. is a perennial shrubby herb belonging to the *Lamiaceae* family, originally found in the East Mediterranean region [1]. In Tunisia, some local regions consider it as a sacred plant named “tree of meriam” and use it in auto-medicinal applications to increase women’s fertility and/or against tumor status. It is also widely used as food condiment. Some studies explored the biological activities of this plant. Most of them reported antibacterial, antifungal and/or antioxidant activities of *S. officinalis* essential oil (SoEO) [2-5], and more recently, as an anti-insecticidal [5]. However, up to our bibliography, no works have been reported about the cytotoxic activity and potential anti-proliferative of the SoEO from Tunisia.

Many studies have described the diaphoretic and diuretic effects of SoEO [6], and their potentials as anti-diabetic [7], hypoglycemic [8], anti-inflammatory [9], antimicrobial [10], antioxidant [11], to prevent neurodegenerative disease [12] and as anti-tumoral [13-15].

Essential oils are the secondary metabolites of medicinal plants and have indicated lots of biological effects, and qualitatively they play a vital role as cytotoxic agents [16]. In fact, the predominant medicinally valuable metabolites identified were monoterpenes (e.g. β -thujone, 1,8-cineole, and camphor), which were mostly present in the distilled oil and used externally to cure inflammations, infections such as stomatitis, gingivitis, and pharyngitis, and as

antimicrobial agents. Also, diterpenes (e.g. carnosic acid) and triterpenes (oleanolic and ursolic acids) have shown potent HIV-1 RT inhibitory function [17-18].

The first aim of this study was to optimize production extraction yield of essential oil and its chemical composition. The second was to investigate the antioxidant activity and cytotoxic effect against two tumor cell lines: a human breast cancer (MCF-7), and a human cervix adenocarcinoma (HeLa).

2. Materials and Methods

2.1 Samples

Salvia officinalis L. samples were collected in mature period: stage green color of leaves with flowers in April-May 2019 from south east of Tunisia (Zarzis, 33°36'12.4"N 11°01'59.8"E). Aerial parts of plant were collected and some of them dried at 40°C for 3 days until constant mass. The fresh and dried samples were subjected to hydro-distillation for 3h using a Clevenger-type apparatus. Indeed, the hydro-distillation gives a whitish distillate, presenting the emulsion. To acquire the operative essential oil, the method must be completed by a specific treatment for this emulsion. In fact, the salting out, using the sodium chloride (NaCl), is applied to separate the essential oil from the reaction mixture, and then a few washings by an organic solvent (cyclohexane) must be applied. The recuperated oils were measured and stored in dark glasses at 4°C for further studies. The extraction yield is obtained by equation (1):

$$Y(\text{g}/100 \text{ g d.b.}) = \frac{\text{mass of essential oil}}{\text{mass of dry matter}} \times 100 \quad (1)$$

2.2 Optimization of extraction of essential oil

A Box-Behnken design was chosen to optimize the essential oils extraction yield. Three factors were tested: drying (x_1), number of washings (x_2) and salt concentration (x_3). Table 1 presents the low, central and high levels for each factor.

Fourteen experimental conditions were defined by Box-Behnken design (Table 2). The obtained experimental results defined as the extraction yield are also presented in the same Table 2. The proposed mathematical model presenting the dependence of the response to the different factors, is presented as a second-degree polynomial (equation 2):

$$\hat{Y} = \beta_0 + \sum_{i=1}^n \beta_i \cdot x_i + \sum_{i=1}^{n-1} \sum_{j>i}^n \beta_{ij} \cdot x_i \cdot x_j + \sum_{i=1}^n \beta_{ii} \cdot x_i^2 \quad (2)$$

where β_0 , β_i , β_{ij} and β_{ii} are the regression coefficients, x_i is the centered reduced level of i^{th} factor (value is equal to -1, 0 or +1) and n is the number of factors.

The determination of model coefficients was carried out using least squares method.

2.3 GC/MS analyses

The *Salvia officinalis* L. essential oil was analyzed using an Agilent-Technologies 6890 N Network GC system using the protocol described by [19]. A sample of 1.0 μL was injected, using split mode (split ratio, 1:100). The composition was reported as a relative percentage of the total peak area. The identification and authentication of the SoEO compounds was realized using a comparison of their retention times to n-alkanes, and their mass spectra compared to the published data and spectra of authentic compounds (Wiley and NIST Library).

2.4 Antioxidant capacity assays

2.4.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay

The essential oil antioxidant activity was evaluated by its ability in quenching the stable free radical DPPH[•]. Basing on the Osawa and Namiki method [20], the radical scavenging activity against DPPH[•] free radicals was determined, and all experiment details were previously described in work of [21]. Then, The DPPH[•] radical scavenging activity was carried out according to the following equation (3):

$$I(\%) = \left[\frac{A_{517} \text{ of blank} - A_{517} \text{ of sample}}{A_{517} \text{ of blank}} \right] \times 100 \quad (3)$$

2.4.2 Acid 2,2'-azino-bis(3-éthylbenzothiazoline-6-sulphonique (ABTS⁺) free radical scavenging activity assay

The capacity of the extracts to reduce radical cation 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS⁺) was resolute referring to a published method [22]. ABTS⁺ radical scavenging ability was planned as percentage of inhibition (equation 4):

$$I(\%) = \left[\frac{A_{734} \text{ of blank} - A_{734} \text{ of sample}}{A_{734} \text{ of blank}} \right] \times 100 \quad (4)$$

The blank solution was prepared with ABTS⁺ and ethanol for essential oil. The results are expressed in IC₅₀. TROLOX (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as positive control.

2.4.3 Hydrogen peroxide radical scavenging assay (H₂O₂)

Scavenging activity of hydrogen peroxide was resolute using the reference method [23]. Also, the all precision experiment details were described in our previous work [21]. The percentage of H₂O₂ scavenging was determined using equation (5):

$$I(\%) = \left[\frac{A_{230} \text{ of blank} - A_{230} \text{ of sample}}{A_{230} \text{ of blank}} \right] \times 100 \quad (5)$$

2.4.4 Nitric Oxide radical scavenging assay (NO[•])

Nitric oxide generated from aqueous sodium nitroprusside solution interacts with oxygen to produce nitrite ions at physiological pH, which may be quantified and determined according to Griess Illosvoy reaction [24]. A solution of sodium nitroprusside (20 mM) was prepared in phosphate buffer (0.5 M, pH 7.4). The reaction mixture is composed of 2 mL of sodium nitroprusside and 250 µL of each sample, and incubated at 25°C for 150 min. After incubation, a volume of 1 mL of each solution was taken and diluted with 1 mL of Griess reagent (1% sulfanilamide, 2% H₃PO and 0.1% N-1-anphthyl ethylenediamine). The mixture is incubated again for 30 min at room temperature (25°C), and then the absorbance is measured at 546 nm against the blank. Ascorbic acid was used as standard. The pink chromophore generated during diazotization of nitrite ions with sulfanilamide and subsequent coupling with α-naphtylethylenediamine were measured using a spectrophotometer at 546 nm. The percentage of NO[•] trapping of the extract was calculated according to the following formula (6):

$$I(\%) = \left[\frac{A_{546} \text{ of blank} - A_{546} \text{ of sample}}{A_{546} \text{ of blank}} \right] \times 100 \quad (6)$$

2.4.5 Ferric reducing-antioxidant power (FRAP)

This method is based on the capacity of the plant to reduce ferric iron (Fe³⁺) to ferrous iron (Fe²⁺). The mechanism is known to be a marker of electron donor activity [25]. To 1 mL of the sample at different concentrations (0.0625, 0.125, 0.25, 0.5 and 1 mg/mL), 2.5 mL of a buffer solution phosphate (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide

(K₃Fe(CN)₆) solution were added. The mixture is incubated at 50°C for 20 min and then cooled to room temperature. Then, 2.5 mL of 10% trichloroacetic acid (TCA) is added to stop the reaction, and then the tubes are centrifuged at 3000 rpm for 10 min. 2.5 mL of the supernatant are then added to 2.5 mL of distilled water and 500 µL of 0.1% solution of iron trichloride (FeCl₃, 6H₂O) [25]. Absorbance reading is done against a blank at 700 nm using a spectrophotometer. Ascorbic acid is used as a positive control. The increase in absorbency in the constituents indicates the raise in iron reduction. The percentage of iron reducing power is calculated by the following equation (7):

$$I(\%) = \left[\frac{A_{700} \text{ of blank} - A_{700} \text{ of sample}}{A_{700} \text{ of blank}} \right] \times 100 \quad (7)$$

2.5 Determination of the antiproliferative activity

2.5.1 Human Cell Line (MCF-7 and HeLa)

Two tested tumor cell lines were used in this study: MCF-7 and HeLa. The first tumor cell line, MCF-7, is a stable epithelioid cell line originally obtained from the pleural effusion of a female patient with metastatic breast cancer whose disease responded to hormone therapy [26]. The second one, HeLa, is a transformed line expressing the HPV18 virus (Human Papiloma Virus). This adherent line is obtained from tumor cells from cancer of the cervix of a 31-year-old woman [27]. These cells have substantial amounts of estrogen receptor [28].

2.5.2 Culture medium

The cell lines were grown in RPMI 1640 (Rosewell Park Memorial Institute) (Gibco) medium. The medium was added with 2 g/L sodium bicarbonate (HCO₃Na) and adjusting the pH to 7.2 with 1 N HCl, the mixture was filtered through a filter of 0.22 µm and then

supplemented with 10% fetal bovine serum (FBS) (Gibco), gentamycin 1%, and L-glutamine 2 mM. Cells were grown at 37°C in a humidified atmosphere of 5% CO₂.

2.5.3 MTT test

The proliferation rates of MCF-7 and HeLa tumor cells after treatment with essential oils were determined by the colorimetric 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The yellow compound MTT is reduced by mitochondrial dehydrogenases to the water-insoluble blue compound formazan, depending on the viability of cells. MCF-7 and HeLa cells (240×10^5 in each well) were incubated in 96-well plates for 48 hours in the presence or absence of essential oil. Twenty microliters of MTT solution (Sigma) (5 mg/mL in PBS) were added to each well. The plate was incubated for 4 h at 37°C in a CO₂-incubator. One hundred and eighty microliters of medium were removed and added the same volume with DMSO-methanol. This reaction can be monitored quantitatively by spectrophotometry. The DO at 570 nm reflects the activity of mitochondrial cytochromes. This activity can be considered as an index of cell proliferation [29].

2.6 Statistical analyses

All biological measurements were conducted on the basis of three independent experiments. Statistical significance difference was evaluated using independent Student test. The difference between individual means was deemed to be significant at $p < 0.05$.

About testing the significant effects of factors and their interactions in model (equation 2), the analysis of variance test (ANOVA) was applied ($p < 0.05$).

The experimental design and all statistical analysis were performed with STATISTICA 12.0 Software, StatSoft, Inc.

3. Results and discussion

3.1 Yield optimization of essential oil

The experimental results (Table 2) were used to determine the model coefficients (equation 8) and to identify the different influences of factors (*via* ANOVA test in Table 3) on the extraction yield of SoEO essential oil as response. Ferhat *et al.* [30] showed an oil yield of aerial parts ranging from 1.1 to 1.2% based on dry weight, which is higher than previously investigated samples, cultivated in Tunisia [3]. Therefore, all these yields values and those obtained in this work are included in the range of 0.4-2.2% of sage essential oil yields from a variety of European sources [31, 32].

$$\hat{Y} = 1.120 - 0.491 \cdot x_1 + 0.095 \cdot x_2 - 0.164 \cdot x_3 - 0.028 \cdot x_1 \cdot x_2 + 0.115 \cdot x_1 \cdot x_3 - 0.009 \cdot x_2 \cdot x_3 - 0.086 \cdot x_1^2 + 0.159 \cdot x_2^2 - 0.513 \cdot x_3^2 \quad (8)$$

Equation (8) presents all model coefficients. Drying in linear term (x_1) and concentration of saline solution in quadratic term (x_3^2) have the only significant effects on yield of essential oil extraction (equation 8 and Table 3; $p < 0.05$). Moreover, drying of the matter before extraction have a negative effect on the extraction yield, and this is the most important significant effect ($p < 0.01$). This result was observed previously for another matter's essential oil [33, 34]. Then, to produce a higher extraction yield of essential oil, it is important to establish it with fresh matter. There are no important interactions between factors (equation 8, Table 3 – $p > 0.05$).

The quality of the determined model was carried out principally with three coefficients: coefficient of determination $R^2 = 91\%$; adjusted coefficient of determination $R^2_A = 71\%$ and the root mean squared error $RMSE = 0.29$ g/100 g d.b. Moreover, the model presents a not significant lack-of-fit criterion ($p > 0.05$). Therefore, the model is considered as suitable for

all tested experimental data and it presents an important validity overall chosen experimental field. So, the established model can be adopted for this study work.

Figure 1 presents the extraction yield of essential oil's dependence to different factors: drying and washings (a), drying and concentration of saline solution (b) and washings and saline solution (c). The higher linear influence of the drying on the extraction yield is shown in figure 1.a and figure 1.b. Also, the quadratic saline solution concentration influence is shown in figure 1.b and figure 1.c. The presented graphic results confirm those obtained previously.

The maximum extraction yield was carried out ($Y = 1.85$ g/100 g d.b.) with a desirability of 90%. The corresponding extraction must be without drying; five washings must be applied to the distillate using a saline solution concentration with 238.3 g/L.

3.2 Composition

Composition of the obtained optimum essential oil of *S. officinalis* collected from “Zarzis” (south east of Tunisia) was determined by GC-MS (Table 4). 40 constituents, representing 99.41% of total SoEO have been identified. The main fractions were camphene (23.7%), α -thujone (19.62%), 1,8-cineole (10.6%), viridiflorol (5.9%), borneol (5.72%); β -thujone (5.4%); caryophyllene (3.83%) (with 2.03% *trans*-caryophyllene; 0.97% caryophyllene-oxide and 0.83% β -caryophyllene); α -Terpineol (2.41%) and α -humulene (2%) (Table 4). Our composition was so comparative to only one Tunisian study reported by [5] (Table 5), in which they show that SoEO collected from “Chott Mariem” – a region at central east of Tunisia was characterized by the presence of 49 components representing 97.97%; with camphor (25.14%), α -thujone (18.83%), 1,8-cineole (14.14%), viridiflorol (7.98%), β -thujone (4.46%), β -caryophyllene (3.30%); and α – humulene (2.48%) as the major components. Nevertheless, our composition differs from others Tunisian studies, mostly regarding to geographic origin of sample collected (Table 5). Therefore, Hayouni *et al.* [3] found that

essential oil contents of *S. officinalis* collected from north of Tunisia was mainly composed by 1,8-cineole (33.27%), β -thujone (18.40%), α -thujone (13.45%), borneol (7.39%), β -elemene (4.82%), camphor (3.31%) and α -pinene (2.74%). While Bouaziz *et al.* [4] identified 44 compounds representing 96.32% of the total of essential oil of *S. officinalis* L. collected from south of Tunisia (Table 5). Among them, 21 were monoterpenes hydrocarbons (20.77%), 14 were oxygenated monoterpenes (60.21%), six were sesquiterpenes hydrocarbons (10.28%), and two were oxygenated sesquiterpenes (5.06%). These compounds were commonly found in the essential oil of *S. officinalis* L. however, with different concentrations depending on plant geographic origin [35], environmental factors and on harvest season [36]. Moreover, the change in the essential oil compositions might arise from genetic differences [31, 37, 38], the developmental stage of the plants and extractions method [39].

3.3 Antioxidant activity of *S. officinalis* essential oil

Numerous methods have been used to evaluate antioxidant activity of the studied essential oil; and IC₅₀ values obtained for all test are shown in Table 6. Comparing the obtained results and their positive controls, *S. officinalis* essential oil exhibited a main antioxidant effect to scavenging DPPH[•] radical (Figure 2a), ABTS⁺ (Figure 2b), FRAP (Figure 3), and NO[•] (Figure 3), but relatively lower than the synthetic antioxidant BHT, TROLOX, vitamin C used as positive controls. Moreover, it shows a comparable effect as vitamin C as standard in H₂O₂ test (Figure 3).

At all tested concentrations, *Salvia officinalis* L. essential oil showed a considerable antioxidant activity. The inhibiting percentage of free radical DPPH increased with elevated concentration of essential oil, and as also with BHT (Figure 2a). In fact, the *Salvia officinalis* essential oil has a substantial anti-radicular effect attending 50%. Also, it is attending 85% compared to TROLOX as positive control with ABTS⁺ essay (Figure 2b). Using a t-test we

can validate a significant difference between mean of DPPH[•] and ABTS⁺ with essential oil and their positive control ($p < 0.05$). These antioxidant activity results were amply reported and linked to polyphenol rich's oil from sages' leaves, but with some difference of concentrations which may be related to origins, season of plant cultivate and extract manner [10, 40].

The major components of the essential oils, as camphor, 1,8-cineol and α -pinene have been reported to have high antioxidative activity [41].

Also, the scavenging ability of sage essential oil on hydrogen peroxide is shown on Figure 3.

The essential oil of *S. officinalis* was capable of scavenging hydrogen peroxide in an amount dependent manner. 0.05mg of essential oil of exhibited 50% scavenging activity on hydrogen peroxide. Wang *et al.* [42] and more recently Tamil Selvi *et al.* [43] suppose that the antioxidant activity of sage oil due to presence of monoterpenes; and several sesquiterpenes compound; and it is assumed that the contribution of minor and major compounds exhibited this activity and not only one or few active molecules [42].

3.4 Modelling of cytotoxicity assay

To evaluate cytotoxicity of the essential oils against MCF-7 and HeLa, MTT-based cytotoxicity assay was performed. The results are expressed as percentage of cytotoxicity versus the concentrations of the essential oil. Our results showed a dissimilar cytotoxic effect of the essential oil of the *Salvia officinalis* L. against both tumor cell line MCF-7 and HeLa (Figure 4). It can be clearly noted that MCF-7 cells were the most affected cells followed by HeLa after 48 h. The proliferation of HeLa cells was considerably reduced by 50% exposure with 8.92 $\mu\text{g/mL}$, when IC_{50} was obtained with 3.12 $\mu\text{g/mL}$ against MCF-7 (Figure 5). Moreover, the effect was proportional to the applied concentration.

To model the observed cytotoxicity behavior of SoEO against two cells lines, a power model type (Cytotoxicity = $a \times \text{concentration}^n$) was chosen and a Matlab algorithm was carried out and applied to the corresponding cytotoxicity versus concentration evolution in order to identify the model parameters using the fitting procedure. The used version of Matlab was version 2017b; © 1984-2017 The MathWorks, Inc. The results of power model parameters for MCF-7 and HeLa cells lines are presented in equations (9) and (10), respectively:

$$\text{Cytotoxicity (\%)} = 43.15 \cdot \text{Essential oil concentration}^{0.180} \quad (9)$$

$$\text{Cytotoxicity (\%)} = 26.92 \cdot \text{Essential oil concentration}^{0.318} \quad (10)$$

All the statistical parameters show a very interesting quality of fitting: coefficient of determination ($73.44 \leq R^2 \leq 97.77\%$); adjusted coefficient of determination ($68.13 \leq R^2_{\text{Adj}} \leq 97.33$) and the root mean squared error ($1.84 \leq \text{RMSE} \leq 4.13\%$). The modelling results of the cytotoxicity effect of SoEO against the used cells lines are shown in Figure 5.

Bakkali *et al.* [16] suggest that cytotoxic activity of the essential oil on the tumor cell lines may be ascribed to its antioxidant activity, since antioxidants are believed to be anti-mutagenic and anticarcinogenic due to their radical scavenging properties.

For all tested cell lines, an increase of the cytotoxicity with the concentration of the essential oil was observed indicating a dose dependent effect. Examinations of the obtained results showed that cytotoxic effects *Salvia officinalis* L. essential oil were different between tumor line MCF-7 and HeLa. These differences could be attributed to the diversity of molecules of essential oil and affinity effect on one type of tumor more than others. In fact, this plant has been widely investigated for its chemical composition and pharmacological profile. Some diterpenoid quinines (royleanone- SAR 3, horminone- SAR 26, and acetyl horminone- SAR 43) isolated from the roots of *S. officinalis* are able to exert cytotoxic and DNA-damaging activity in human colon carcinoma Caco-2 cells and human hepatoma HepG2 cells cultured [44-46]. As well a difference effect regarding too complex various molecules composition

essential oils and several terpenoids compounds belonging mainly sesquiterpene and to monoterpene chemical groups may well associated to diverse functional groups dotted such as alcohols, phenols, ketones, etc. [16]. These compounds might operate separately or in a synergic way.

Furthermore, some studies prove the cytotoxicity effect of *S. officinalis* essential oil, against A549 and NCI-H226 cells which are treated with various concentrations of SoEO and with a combination of two and three of its main constituents (1,8-cineole, α -thujone and camphor) [47]. So recently, Privitera *et al.* [48] mention again that the whole oil of *S. officinalis* and a mixture of α -thujone, 1,8-cineole and camphor induced a significant reduction of cell viability at doses of 100 μ g/mL and 200 μ g/mL, after a 48-hour incubation on breast (MCF-7), cervical (HeLa) and prostate (LNCaP) cancer cells. At the same concept, our Tunisian SoEO was reach of α -thujone (19.62%) and 1,8-cineole (10.6%), which could enhance the cytotoxicity of whole *Salvia officinalis* essential oil against MCF-7 and HeLa tumor cell line. In addition, Siveen and Kuttan [49] showed that α -thujone improved the cytotoxicity of colon cancer cells and inhibited lung metastasis of B16F-10 cells by inhibiting tumor cell proliferation, adhesion and invasion; while Biswas *et al.* [50] had a relevant an antiproliferative activity of α -thujone against melanoma. Also, Nikolić *et al.* [51] according the cytotoxic activity to the presence of camphor, 1,8-cineole and α -thujone.

Generally, the activity of an essential oil is accredited and influenced by the major compounds. All the same, the minor compounds might also be of crucial importance [52].

4. Conclusion

The results obtained show that optimum yield of *S. officinalis* L. essential oil from Tunisian south east with RSM was 1.85 g/100 g d.b. with a reach variation chemical compound.

The *in vitro* experimental study clearly reveals the considerable antioxidant activity and the cytotoxic effect of *S. officinalis* essential oils against tumor cell line; suggesting the possibility of using this essential oil as potential source of antioxidant ingredients for the food, cosmetic, drug and pharmaceutical industry. The modelling of the cytotoxicity effect display that the Tunisian *S. officinalis* could be considered as an important Mediterranean medicinal plant and one of highly promising natural agent as drug tumor therapy. Therefore, the present work provides a preliminary platform for further investigation of the possible mechanism of *S. officinalis* essential oils and their individual compounds in cytotoxic and antitumor activity. More studies are necessary to explore the molecular mechanisms of this anticancer potential.

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Author contribution statement

IK, AB and BH carried out the design of the study and the statistical analyses. IK, AE, NT, BG, EBM, and BH carried out the experimental study. IK, AB and BH carried out the analyses and discussion of the results. All authors conceived the study and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Availability of data and material

No data were used to support this study.

Code availability

Not applicable.

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Figures

Figure 1

Evolution of extraction yield of essential oil as function of (a) drying and washings (at saline solution of 262.5 g/L), (b) drying and saline solution (at washings of 4) and (c) washings and saline solution (at drying of 50%).

Figure 2

In vitro antioxidant activity, DPPH• (a) and ABTS+ (b) in *Salvia officinalis* L. essential oil

Figure 3

In vitro antioxidant activity, NO•, H2O2 and FRAP in *Salvia officinalis* L. essential oil

Figure 4

Anti-proliferative activity at different concentrations of the *Salvia officinalis* L. essential oil against MCF-7 and HeLa cell line by MTT assay. Results are expressed as means±standard deviation of three determinations.

Figure 5

Modeling results of cytotoxicity effect of SoEO against three cells lines: experimental data of MCF-7 (o) and HeLa (*); power model (continuous and discontinuous lines)