Contribution to Optimising the Extraction of Active Ingredients from Common Sage (*Salvia Officinalis*.L).

Fatiha Ferhoum [1,2*]; Khadidja Mahdi [1,2,] *Nassima* Bourfis [1,2];Hamouche Aksas[2]; Fatima Benaisa [3,4];, Souhila Bensmail [2,5]; Djamila Djouahra-Fahem [2,5], Koussiala Tigrine[1] , Salim Lamine

[1] Department of Agricultural Sciences, Faculty of Nature and Life Sciences and Earth Sciences, University of Bouira, 10000, Algeria

[2] Food Technology Research Laboratory, Boumerdes University, Boumerdes 35000, Algeria

[3]Institute Desbrest of Epidemiology and Public Health (IDESP), University of Montpellier and INSERM,
Montpellier Department of Pneumology, Allergology and Thoracic Oncology, Monpelier University Hospital,
34090 Montpellier, France

[4] Common Core Department of Natural and Life Sciences, Targa Ouzemour, University of Bejaia, Bejaia 06000, Algeria

[5]Department of Biology, Faculty of Nature and Life Sciences and Earth Sciences, Bouira, 10000, Algeria

[6]Higher School of Saharan Agriculture, National Road N.06, Adrar, Algeria

Corresponding author: f.ferhoum@univ-bouira.dz

Received :22-05-2023 **Accepted:** 02-12-2023 **Published:** 25-12-2023

Abstract

Salvia officinalis. L, or common sage, is considered to be one of the most useful plants in the world because it is rich in bioactive substances and can be used in many fields: food, pharmaceutical and cosmetic. The present study consists of optimising the extraction of the main active ingredients from the aerial part of sage officinale. Firstly, we carried out a comparative study between the fresh plant and the dried plant (powder) to determine the extraction yield, polyphenol and flavonoid content and antioxidant activity. At the end of this section, we chose the dried plant on the basis of yield (17%) and antioxidant capacity (1.12E-04g/ml). Secondly, a mixing plan was applied to optimise the best solvent. At the end of this section we chose mixture No. 09 (16% distilled water, 16% acetone and 66% ethanol) as the extraction solvent for the rest of our experiments, as it has the highest antioxidant activity (1.36E-05 g/ml). The third part is devoted to a comparative study between the three extraction methods (maceration, ultrasound and soxhlet) where we chose the maceration method because it has a high antioxidant activity (1.36E-04 g/ml). Finally, we optimised the best method by applying a complete two-level factorial design of three parameters (temperature, quantity of plant material and extraction time) to determine the optimum extraction conditions (temperature of 50°C, quantity of 2g and time of 4h).

Key words: *Salvia Officinalis*., extraction, optimisation, yield, polyphenols, flavonoids, IC50

Tob Regul Sci. ™ 2023 ;9(2): 2724-2741
DOI : doi.org/10.18001/TRS.9.2.179

Introduction

Plants have played an essential role in human daily life for thousands of years. Their use in food, medicine and religious rituals dates back to ancient times. Ancient civilisations such as the Egyptians, Romans and Greeks were already aware of the beneficial properties of plants. They used plant extracts for their perfumes, medicinal properties and antimicrobial properties **(Fellah *et al.,* 2006; Laplante, 2009 and Ambe *et al* 2015).** However, it was not until the beginning of the 20th century that scientists began to take a more in-depth interest in plants. Scientific and technological advances at the time led to a better understanding of the chemical composition of plants and the mechanisms of action of their active components **(Fellah *et al.,* 2006)**. Today, research continues in the field of plant phytochemistry and pharmacology. Scientists explore plants to find new therapeutic compounds, study their effects on human health and discover potential medical applications. **(Koane, *et al.* 2011)**. Indeed, populations around the world, including in Africa, are facing an increase in non- communicable diseases such as cardiovascular disease, cancer and diabetes **(Aseervatham et al., 2013).** Faced with this situation, research is increasingly focusing on discovering new sources of biomolecules in medicinal plants. Plants have a long history of use in traditional medicine to treat various ailments and provide natural remedies. They contain a wide variety of chemical compounds, such as alkaloids, flavonoids, terpenoids, polyphenols, which may have potential therapeutic properties **(Soro *et al.,* 2012; Liu *et al.,* 2017).** It is interesting tonote that medicinal plants continue to be used in therapy, according to the World Health Organisation (WHO), around 80% of the population in developing countries. *Salvia officinalis. Salvia* officinalis, commonly known as Common Sage, is a plant widely distributed in the Mediterranean basin and appreciated for its many therapeutic virtues. It belongs to the Lamiaceae family and has a number of distinctive characteristics. It has long been known for its medicinal and aromatic properties **(Fleurentin, 2008).** Phenolic compounds are secondary metabolites present in many plants. They belong to a class of chemical compounds distinguished by their structure containing one or more phenolic groups. They are known for their remarkable antioxidant activity. They act by neutralising free radicals, which are reactive chemical species that can damage cells and contribute to oxidative stress. Oxidative stress is associated with various diseases, such as cardiovascular disease, cancer and ageing **(Cai *et al.,* 2004).** Our work consists of optimising the extraction of the main active ingredients from *salvia officinalis. L.*

2. Materials and methods

The working methodology followed in the course of this study is summarized in the diagram below.

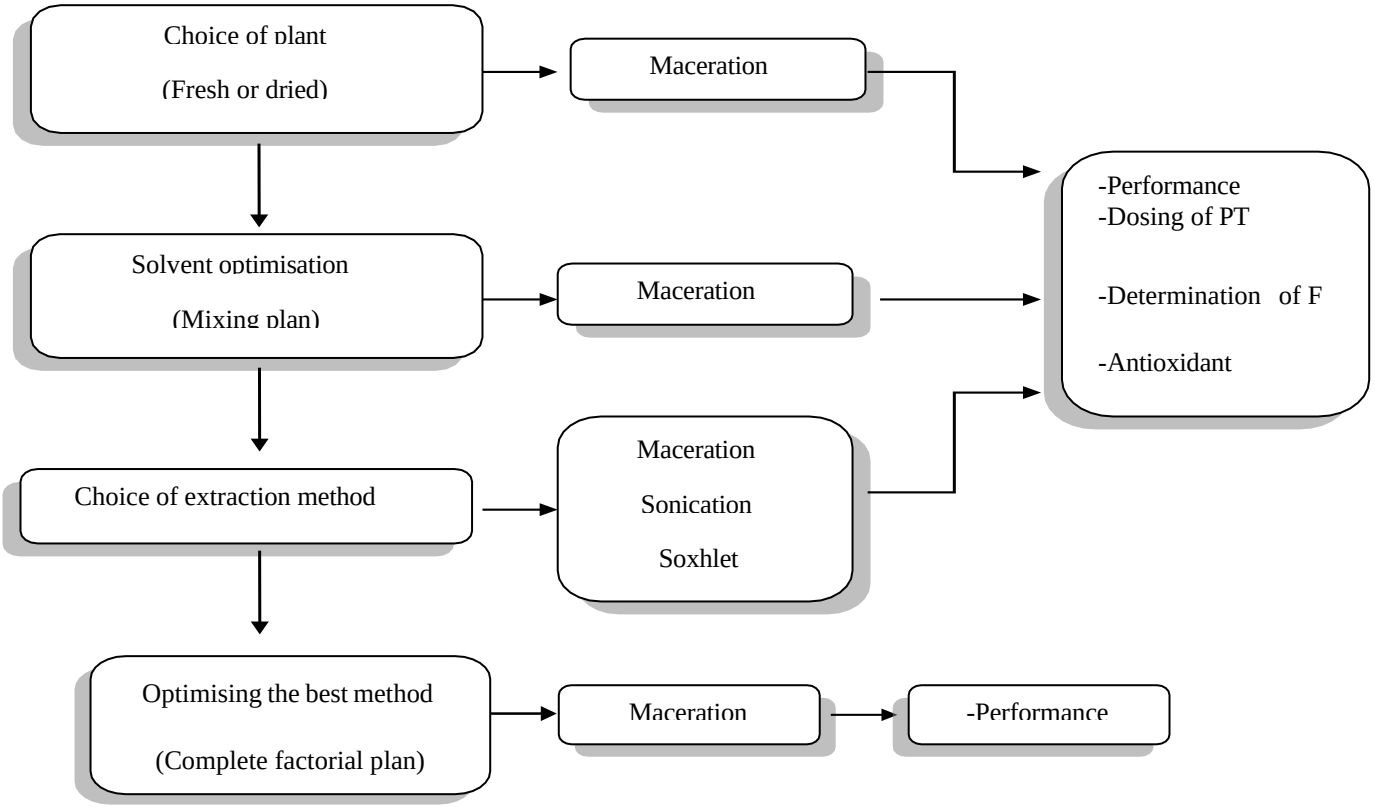

Figure 1 : Flow chart representing the methodology of our study

2.1. *Harvesting, drying and grinding*

The aerial part of Salvia officinalis. L, comprising the stems and leaves, was harvested before flowering. The harvest took place in March 2023 in the Aomar region, in the wilaya of Bouira, Algeria. The plant was dried naturally over a period of 15 days. The drying time may vary depending on various factors such as ambient humidity, plant density and other environmental conditions.

2.2 *Choosing the type of plant (fresh or dried)*

In this section we prepared extracts of the dried (powder) and fresh plant using the maceration method with ethanol as the solvent.

a) Extractions of the main active ingredients from Salvia Officinalis. L

Firstly, we used the maceration method to compare extraction from fresh and dried plants in order to optimise the yield of bioactive plant extracts.

Maceration is carried out according to the protocol described by **(Boryana *et al.*, 2006)**. Ethanolic extracts are prepared by maceration of 2 g of waste or fresh or dried plant in 20 ml of 70% ethanol. After stirring for 24 hours, the mixture is filtered and the filtrate is concentrated using a rotavapor. The yield is calculated using the formula described by **Harborne in 1998** :

$$(R\%) = (Me/ Mv) \times 100$$

R%: Represents the extraction yield as a percentage.

Me: Is the mass of extract obtained after evaporation of the solvent. It is measured in grams (g) or milligrams (mg).

Mv: Is the mass of the plant material used for extraction. It is also measured in grams (g) or milligrams

(mg).

b) Determination of total polyphenol content

Determination of total polyphenols in the ethanolic extract using the method described in the literature **(Kamazawa *et al.*, 2002; Singoleton, 1999)**

Add 5 ml of distilled water to 0.5 ml of sage extract and mix well; add 0.5 ml of folin- ciocalteus reagent and leave for 3 min. Add 0.5 ml sodium carbonate (10%), mix well and incubate for one hour at room temperature in the dark, then measure the absorbance at 760 nm.

c) Determination of flavonoid content

The total flavonoid content of sage ethanolic extracts was estimated using the method described in the literature (**Bahorun et al., 1996).** A volume of 1ml of extract of dried pumpkin waste or pulp is added to 1ml of aluminium chloride (AlCl3 at 2%), and the absorbance is measured at 430nm, after 1 hour of incubation. The quantity of flavonoids is calculated in mg quercetin equivalent per 1 g of sample (mg EQ/ g Ech), based on the standard curve prepared with quercetin.

d)Assessment of antioxidant activity

The antioxidant activity of ethanolic sage extracts is assessed by the DPPH radical free radical scavenging activity. According to **Molyneux (2004),** 2ml of ethanolic DPPH solution (0.1 mM) is added to 50 µl of extract solution and standard, then the tubes are incubated at room temperature in the dark for 30 minutes. Readings were taken at 517 nm. The results **are** expressed using the **following** formula

DPPH radical inhibition % (%) = [(AC-AE)/AC]×100

Hence : 1- **AC:** Absorbance of the control.

2-**AE:** Sample absorbance.

The IC50 value is calculated using linear regression with the concentration of the compounds tested on the abscissa and the percentage inhibition (I%) on the ordinate. The IC50 value represents the concentration of the tested sample required to reduce the activity of the DPPH radical by 50% **(Bouras and Houchi, 2013).**

2.3. Solvent optimisation (mixing plan)

Several extractions with a three-factor mixing plan using three types of solvent (ethanol, acetone and distilled water) were carried out on *salvia officinalis L* powder in order to compare yields, polyphenol and flavonoid content and antioxidant activity. The aim of these preliminary tests was to determine the best extraction solvent for this study.

2.4. *Optimisation of extraction methods*

After choosing the best extraction solvent for common sage, we began a comparative study between three extraction methods (maceration, already developed in part **2.1**, sonication and soxhlet) with the aim of optimising the best method.

To extract the active ingredients from la sauge officinale by ultrasound, we opted for the protocol described by(**Gargouri *et al.* 2019**) by making some modifications: Sonication of 1(g) of la sauge officinale in 50(ml) of 96% ethanol for 3 hours at 50°C, Filtration with Whatman number 1 filter paper, Concentration with a rotary rotavapor at 50°C under vacuum.

Soxhlet extraction is based on a distillation process which is widely used in laboratories and food and non-food industries **(Cunha, *et al.* 2004).** 1g of sauge officinale is introduced into the Soxhlet cartridge. 150

ml of 96% Ethanol are poured into the flask and 25ml into the extractor, the extraction is carried out for 6 hours until the material is exhausted. Then Concentration in a rotary rotavapor at 50°C under vacuum.

2.5. *Optimisation of the best method (maceration)*

Once the best method has been chosen, many parameters can influence the extraction yield of officinal suage, such as time and temperature, and the nature of the solvent (**table 1**).

In this section we have chosen to study the effect of the following three factors: (extraction temperature, quantity of plant material and extraction time) on extraction yield using a full factorial design.

Table 1: Full factorial design invoices

	Factors	Low level (-1)	High level (+1)
X1	Temperature	20°C	50°c
X2	Quantity	1g	2g
X3	Time (duration)	2h	4h

3. Results and discussion

3.1 Results of biochemical characterisation of fresh and dried plants

The results of the biochemical characterisation are summarised in Table 2.

Table 2: biochemical characteristics of common sage

Parameters	Yield (%)	Total polyphenols (mg EQAG/gMV)	Flavonoids (mgEQQ/gMV)	EC50 (g/ml)
Fresh plant	3,5±0,3	131,23±4	68,25±1,8	1,8*E-4
Dried plant	17±038	77±2,5	74,962,3	1,12*E-4
Ascorbic acid	/	/	/	0,8*E-4

a. Extraction yield

The results obtained in this study show that the dried plant (powder) gave a high yield (17%), compared with the fresh plant with a low yield (3.5%).

The ethanol extraction yield for sage officinalis was 17%, close to the 18.24% reported in the study by **Roby *et al.* (2013).** On the other hand, the study by **Menaker *et al.* (2004)** reported a very low extraction yield (1.32%) for an ethanolic extract of *Salvia officinalis. L compared with* our results.

Extraction yields can vary depending on many factors, such as temperature, the type of solvent, the ratio between the mass of the powder and the volume of the solvent, as well as the extraction technique used, the quality and nature of the plant material used, the sample preparation method and the analytical techniques used to determine the extraction yield. **(Louli *et al.,* 2004; Naczk and Shahidi, 2004).**

b. Total polyphenols

From the results in Table 2, we can see that the fresh plant is the richest in polyphenols with 131.23 mg EAG/g extract compared to the dried plant with 77.55 mg EAG/g extract.

The study by **Miliauskas *et al* (2004)** revealed total polyphenol contents ranging from 9.7 to 24 mg EAG/g extract for different Salvia species. These variations can be attributed to genetic differences and

plant growth conditions.
The study conducted by **El Gabbas *et al.* in 2019** reported a high level of phenolic compounds, namely 120.30 ± 0.72 mg EAG/g extract.
The **Achat** study **in 2005** obtained a content of 54.77 mg catechin equivalent/g DM of total polyphenols in sage. The difference between these two studies can be attributed to the use of different measurement standards.
The content of phenolic compounds can be influenced by several factors: geographical factors, genetic factors, the extraction method and the quantification method can also influence the estimate of the total phenol content **(Aganga, 2001).**

c. **Flavonoid assay**

The data in Table 2 show that the dried plant has a slightly higher flavonoid value than the fresh plant, at 74.96 and 68.25 mg EQ/mg MV respectively.
The studies by **Dewanto *et al.* (2002) and Miliauskas *et al.* (2004)** reported flavonoid contents of different sage species ranging from 0.3 to 13.8 mg EQ/g extract, which differs from your results. Similarly, the study by **Et-Touys *et al.* (2016)** revealed a flavonoid compound content of 31.05±0.62 mg EQ/g in the methanolic extract of *Salvia officinalis L*, while **Hammoudi (2015)** found a flavonoid content of 148 mg EQ/g E in the ethanolic extract of Salvia chudaei, a higher value than in our study.
Flavonoid levels in plants are influenced by a combination of genetic and environmental factors. Adverse environmental conditions can stimulate flavonoid production as a plant defence mechanism (**Bouteldja *et al.*, 2021).**

d. **Anti-free radical activity**

According to the IC50 data obtained for samples of common sage (fresh and dried) and ascorbic acid, the results show that the extracts have low antioxidant activity compared with ascorbic acid.
We found that the dried plant exhibited more intense free radical scavenging activity with an IC50 of 1.12 * 10-4 g/ml, compared to the fresh plant with an IC50 of 1.8 * 10-4 g/ml. This suggests that the drying process may increase the antioxidant activity of the plant.
Studies by **Soobrattee *et al* (2005)** also showed significant antioxidant activity in sage extract, but less than that of ascorbic acid (AUC). These results are in line with our own.
The study by **Martin *et al.* (2015)** showed that all extracts of *Salvia officinalis. L* have good efficacy in scavenging the DPPH radical, with IC50s between 18.3 and 32.97 µg/mL for the hydro-methanolic extract. These results are significantly lower than those obtained in our study. The work of **Hamrouni-Sellami, *et al* 2013** showed that the methanolic extract of *Salvia officinalis.L* has an IC50 of 2.78E-04 g/ml, a higher value than that obtained in our study. This difference can be attributed to the nature of the solvents used in the extraction.

3.2. Optimisation of extraction solvent (mixing plan)

The results of the blending plan for yield, total polyphenols, flavonoids and CI values50 are shown in the table below:

Table 3: Mixing plan results

No. of Mix	*distilled water X1*	*% ethanol X2*	*% acetone X3*	*R %*	*Polyphenols (mgEAG/g)*	*Flavonoids (mgEQ/g)*	*IC 50 (g/ml)*
1	100	0	0	9,5	64,45	30,71	5,11E-04
2	0	100	0	17,5	87,43	80,15	1,61E-04
3	0	0	100	8,75	**229,18**	**184,78**	1,29E-04
4	50	50	0	19,5	53,65	45,6	2,79E-04
5	0	50	50	14	140,87	137,38	1,43E-04
6	50	0	50	14,5	54,72	45,59	1,41E-04
7	33	33	33	20	60	53,13	4,54E-04
8	66	16	16	22	31,51	29,23	2,33E-04
9	16	66	16	**24**	93,1	86,61	**1,36E-05**
10	16	16	66	20,5	78,45	72,31	1,61E-04

a) **Performance**

By analysing the results of table N°03, we notice that the yields of the various mixtures obtained by the mixing plan are variable, the highest yield is that of the extract of mixture N°9 (ethanol 66%, acetone 16%, distilled water 16%) with a percentage of the order of (24%), The yield percentages of most of the other mixtures are close, ranging from 14 to 22%, except for the aqueous extract (mixture No. 01) and the acetone extract (mixture No. 03), which are less important, with values of 9.5 and 8.75% respectively.
According to the work of **(Benabied *et al.*, 2022),** the percentage yield for the 100% ethanolic extract is around 35.4% and for the 50% ethanolic extract is 30.2%. These values are higher than those found in our study (17.5% and 19.5% respectively).
According to the study by **(Khenfer , 2016),** the acetone extract yield (100%) gave a percentage of 4.5%, a lower value than our results of 8.75%.

b) **Determination of polyphenols and flavonoids**

The results of our study show that total polyphenol contents vary between 31.71 and 229.18 mg EAG/g of plant matter in the different mixtures analysed. The acetone extract (blend No. 03) had the highest concentration of total polyphenols, at 229.18 mg GAE/g extract, as did flavonoids, at 184.78 mg QE/g extract.
The low solubility of total polyphenols in pure solvents is due to the inability of these solvents to break the strong hydrogen bonds formed between proteins and polyphenols; the addition of water makes these bonds more sensitive, and therefore the total polyphenols more extractable **(Sripad *et al.*, 1982).**
According to **Farhoosh (2009)**, acetone is a solvent that showed high content of total polyphenols and flavonoids due to its better selectivity and low viscosity. This is in line with the results of our study, where we obtained high values of total polyphenols and flavonoids with the acetone extract, namely 229.18 mg EAG/g extract and 184.78 mg EQ/g extract respectively.
According to **Duletić-Laušević *et al.* (2019),** the 50% ethanolic extract of *Salvia officinalis L* is richer in total polyphenols (TPP) with a content of 102.12 mg EAG/g, compared to the aqueous extract which has

a content of 77.44 mg EAG/g. However, in our study, we found that the aqueous extract was richer in TPP, with a content of 64.45 mg TPE/g, compared to the 50% ethanolic extract, which contained 53.65 mg TPE/g.
According to **Bouteldja (2021),** the flavonoid content of the ethanolic extract is 37.53 mg EQ/g, which is lower than our result. On the other hand, the flavonoid content of the aqueous extract is 31.03 mg EQ/g, which is similar to our results.

c) Anti-free radical activity

A lower concentration of IC50 (50% inhibitory concentration) indicates higher antioxidant activity. Analysis of the data shows that blend No. 09 has the lowest IC50 (**1.36E-05 g/ml), and** therefore a higher antioxidant power. A low AAO is marked by blend N° 01 (aqueous extract) with a very high IC value50 (5.11E-04 g/ml).
According to **Albno and Miguel (2011)**, the hydroethanol extract of *salvia officinalis. L* has an IC value50 of 2.8 µg/ml, i.e. a higher antioxidant activity than that found in mixture No. 4 (IC50 = 2.79E-04 g/ml). The results obtained by **(Bouteldja, 2021)** show that the ethanolic extract of *Salvia officinalis. L* and the aqueous extract *have* IC values50 of 0.106 and 1.74 mg/ml respectively. These two values are lower than those found in our study.
According to **Chirinos et al (2007)**, a mixture of acetone and distilled water is considered to be the most effective solvent for extracting polyphenols. Water combined with acetone creates a moderately polar medium that favours both the extraction of phenolic compounds and the preservation of their antioxidant activities.
According to **Spigno *et al* (2007),** varying the concentration of the solvent, using mixtures of different proportions with distilled water, can modify the extraction capacity and help improve the efficiency of the solvent to extract a greater number of compounds.
Note: Based on the results obtained previously, we chose the plant with the highest antioxidant activity and the best extraction solvent. We therefore adopted the dried plant (powder) **and** mixture No. 09 **as the solvent for the rest of our experiments.**

d) Statistical analysis

The aim of this part is to determine the best response in order to optimise the best solvent mixture. The results obtained were processed using JMP software.

Analysis of variance

The values of the coefficient of determination R^2 calculated during the analysis of the results of the responses studied (yield, total polyphenol content, flavonoid content and antioxidant activity) by the "*actual by Predicted Plot*" diagrams *(***Fig 2)** are of the order of: 0.84, 0.94, 0.95 and 0.93 respectively, while the *p-values are of* the order of 0.2231, 0.0609, 0.0474 and 0.0782 respectively. This means that the responses for yield, PPT and IC50 gave insignificant effects (their *p-values>0.*05) which means that the models are invalid, on the other hand the flavonoids response to a significant effect *(p-value<0.*05) which means that the model is valid.

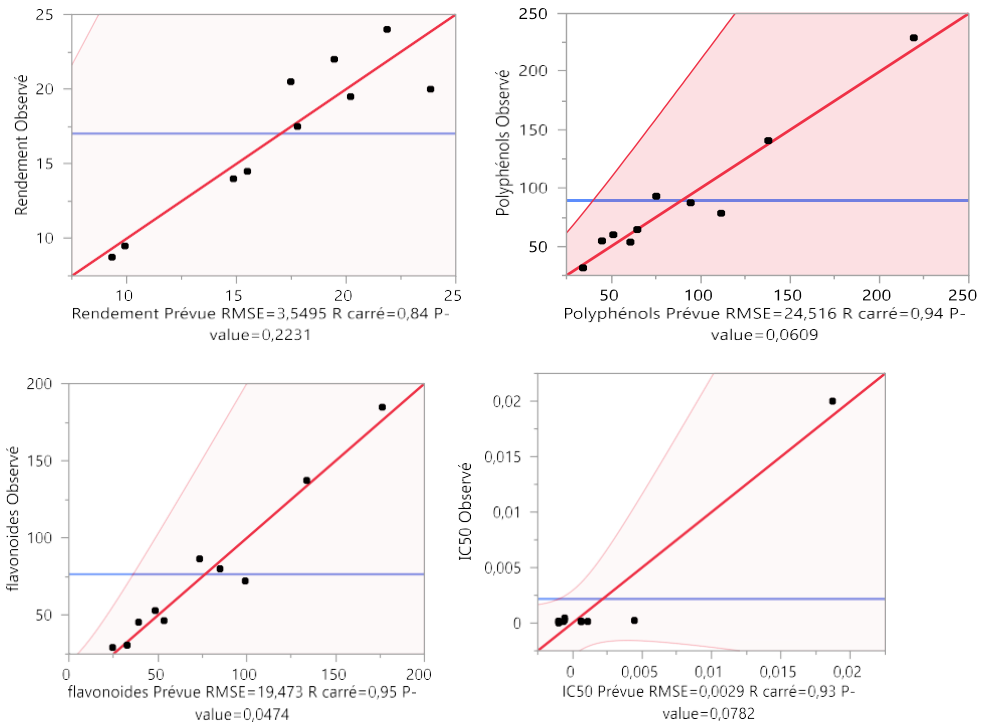

Figure 2: "*Actual by Predicted Plot*" diagram of the experimental results of the responses studied by the mixing design.

Estimation of model coefficients

Flavonoids response

The estimated flavonoid response coefficients are shown in Table 04 :

The results in **table 4** show that only the two factors X2 (ethanol) and X3 (acetone) are considered to have significant effects (their *p-values<0.*05); on the other hand, the factor X1 (distilled water) and all the interactions X X12 , X X13 , X X23 and X X X123 are considered to have non-significant effects (their p-values>0.05).

Table 4: Effects of the variables studied on flavonoids

Term	designation	Estimate	Error standard	t ratio	Prob. > |t|
Water(Mixture)	X1	32,766618	18,82437	1,74	0,1801

Term	designation	Estimate	Error standard	t ratio	Prob. > |t|
Ethanol(Blend)	X2	85,016618	18,82437	4,52	**0,0203***
Acetone(Mixture)	X3	176,32662	18,82437	9,37	**0,0026***
Water*Ethanol	X1X2	-21,47353	94,75795	-0,23	0,8353
Water*Acetone	X1X3	-261,4135	94,75795	-2,76	0,0702
Ethanol*Acetone	X2X3	12,486471	94,75795	0,13	0,9035
Water*Ethanol*Acetone	X1X2X3	-525,2188	624,7087	-0,84	0,4622

3.3 Optimisation of extraction methods

In Table 5, we compared three extraction methods: extraction by maceration (room temperature, 24h), extraction by ultrasound (40°C, 2h) and extraction by soxhlet (70°C, 6h).

Table 5: Results of the comparative study of extraction methods

Parameters	Yield (%)	Total polyphenols (mg EQAG/gMV)	Flavonoids (mgEQQ/gMV)	EC50 (g/ml)
Maceration	24 ± 1,5	93,1 ± 3,2	86,61±1,3	1,36*E-5
Ultrasound	18 ± 0,7	66,39 ±2,9	55,08 ± 2,3	3,29*E-4
soxhlet	30 ± 2,3	92,7 ± 1,4	84,64± 1,9	6,59*E-4

Extraction yield

According to the results obtained in Table 5, extraction by Soxhlet gave the highest yield (30%), followed by extraction by maceration (24%) and ultrasound (18%), in agreement with the work of (**Bucic-kojic *et al.,* 2006),** which emphasises the positive effect of temperature on the extraction of phenolic compounds using the Soxhlet method.

According to the results of **(Hammoudi, 2015)**, the yield of acetone and ethanolic extract of *S. chudaei* by ultrasound gave values in the order of 4.44%, 14.8% respectively, and by maceration the yield of ethanolic extract gave a value of 4.56%. These values are lower than those found in our study.

The presence of water-soluble substances, such as protein compounds or carbohydrates, can contribute to the increase in extraction yield when using the Soxhlet method. These substances can be extracted more efficiently with this method due to the use of a continuously refluxing solvent at a high temperature (**Békro *et al,* 2007**).

Quantification of polyphenols and flavonoids

The results in Table 5 show that the maceration method has the highest levels of total polyphenols and flavonoids in the *Salvia officinalis.L* extract, with values of 93.1 mg EAG/g E and 86 mg EQ/g E respectively. The soxhlet extraction method gave slightly lower contents, with 92.7 mg EAG/g E for total polyphenols and 84.6786 mg EQ/g E for flavonoids. Finally, ultrasonic extraction gave lower contents of

the order of 66.39 mg EAG/g E for total polyphenols and 55.08 mg EQ/g E for flavonoids.
According to the work carried out by **(Pop *et al.,* 2015)**, ethanol extraction of sage shows polyphenol contents by maceration (19.49mg EAG/g E)) followed by ultrasound (19.06mg EAG/g E) at the end by soxhlet (18.91mg EAG/g E). **Jakovljević, *et al.,* (2019)** on *salvia officinalis. L* found polyphenol contents for the 40% ethanolic extract (137.11 mg EAG/g E) and for the aqueous extract (42.199mgEAG/g E) by maceration. These values are lower than those found in our study.
The results obtained by **(Jakovljević, 2019)** reveal that the 40% ethanolic extract of *Salvia officinalis. L* by maceration contains 40.91 mg EQ/g E and the aqueous extract contains 20.62 mgEQ/g E of flavonoid compounds. These results are inferior to those of our study.
According to **(Bouteldja, 2021)**, the flavonoid content by maceration of ethanolic and aqueous extracts varies between 37.53 and 31.03 mg EQ/g E.

Anti-free radical activity

According to the results shown in Table 5, maceration has a high and effective antioxidant activity with an IC value50 of 1.36E-04 g/ml followed by soxhlet (6.59E-04 g/ml) and a low IC50 value for ultrasound (3.29E-04 g/ml).
According to the work carried out by **(Pop et al ,2015)**, the ethanolic extract of S. *officinalis* has an antioxidant activity varying between 70.83 and 78.43% for the three extraction methods (maceration, ultrasound and soxhlet), which are lower values than those obtained in our results.
According to studies carried out by **(Jakovljević, et al., 2019), the** 60% ethanolic extract and the aqueous extract of S. *officinalis* by maceration have IC50% values of 0.078 and 0.355 mg/ml, values close to our results. This difference may be due to the nature of the solvents.
The results obtained by **(Bouteldja, 2021)** show that the ethanolic extract of *Salvia officinalis. L* and the aqueous extract *have* IC values50 of 0.106 and 1.74 mg/ml.
The extraction method chosen must allow complete extraction of the compounds of interest while minimising their chemical modification. Water and aqueous mixtures of ethanol, methanol and acetone are commonly used in the extraction of phytochemicals. The solubility of phenolic compounds can be influenced by several factors, including their degree of polymerisation, their interaction with other constituents present in the plant and the type of solvent used **(Sun *et al.*, 2005).**

3.4. Optimisation of the best extraction method (full factorial design)

The results of the experiment matrix are summarised in Table 06 and the desirability test is illustrated in **Fig. 3**.

Table 6: Matrix of yield variables studied

No. Exp	*Configuration*	*X1*	*X2*	*X3*	*Rexp (%)*	*Rprédit (%)*
1	- - -	20	1	2	5	4,8
2	+ - -	50	1	2	8	10,8
3	- + -	20	2	2	7,5	7,3
4	+ + -	50	2	2	14	8,3
5	- - +	20	1	4	11	7,8

6	+ - +	50	1	4	**17**	16,8
7	- + +	20	2	4	8,5	13,8
8	+ + +	50	2	4	**18**	17,8
9	000	35	3	1,5	9,75	10,925
10	000	35	3	1,5	10,25	10,925

From the results in Table 10, we can see that experiment N°8 gives a high yield (R=18%) followed by experiment N°6(R=17%), while the lowest percentage is recorded by experiment N°1(R=5%).

The results of the desirability test are shown in Figure 3.

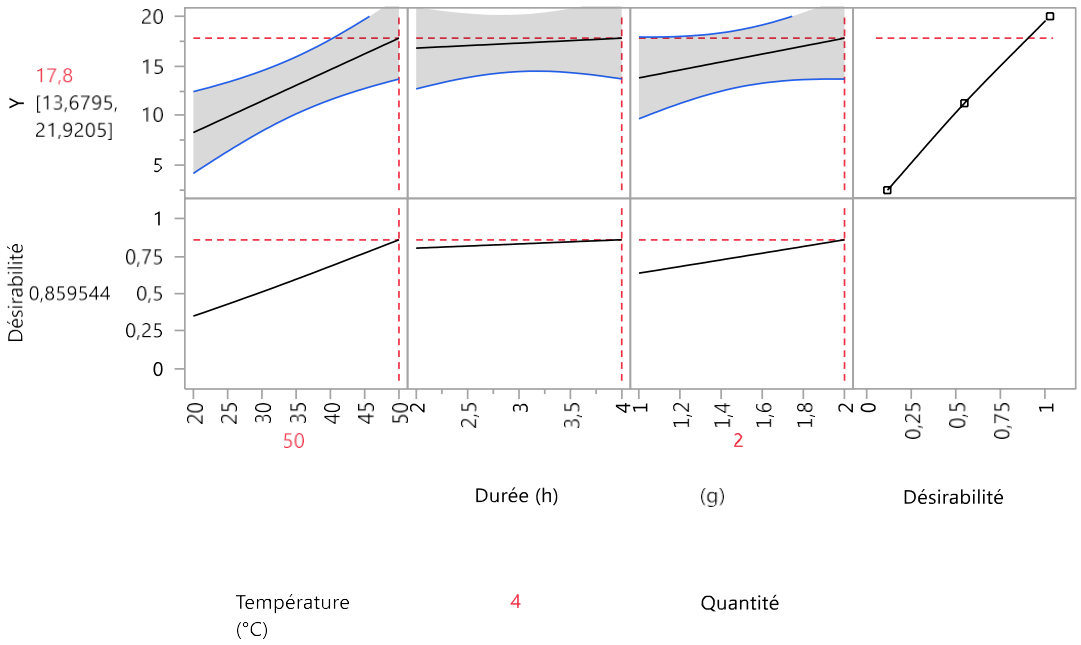

Figure3: Desirability test diagram (prediction profiler) for extraction yield.This figure shows the effects of three factors on the extraction yield response.

Experimental results indicate that extraction yields varied between 5 and 18%. An increase in temperature (X1) from 20 to 50°C led to an increase in extraction yield. This increase in temperature can increase the solubility of phenolic compounds in the solvent and improve their diffusion coefficient, making them easier to extract.

In addition, increasing the amount of plant material (X2) from 1g to 2g also has a proportional effect on the increase in extraction yield. This means that the greater quantity of plant material used in the extraction leads to a higher yield.

With regard to the extraction time (X3), the increase from 2 to 4 hours had only a slight effect on extraction yield. However, according to the study by **Spigno *et al* (2006)**, a longer contact time between the solvent and the plant material can improve the extraction rate.

The desirability test plot showed that the yield desirability value was close to 1, confirming the validity of the model used. This suggests that the experimental conditions used in the study were ptimized to obtain

a high extraction yield of the compounds of interest.

The influence of the factors studied on yield

According to the results in Table 07, we can see that temperature(X_1) and the quantity of plant material(X_2) with $p=0.0118$ and 0.0183 respectively, are factors with significant effects, whereas duration(X_3) does not have a significant effect on the response studied (extraction yield).It can be seen that temperature, quantity of plant material and duration have positive effects on the extraction yield of *salvia officinallis. L.* based on the sign of the coefficients of each variable.

Table 7: Effects of the variables studied and their interactions on the extraction yield of common sage established by the complete factorial design 2^3

Term	Sum of squares	Coefficient	Standard error	*t -value*	*P-value*
Constant		10,925	0,306696	35,62	**0,0008***
Temperature (°C)(20,50)	78,125000	3,125	0,342897	9,11	**0,0118***
Quantity (g)(1,2)	50,000000	2,5	0,342897	7,29	**0,**0183*
Duration (h)(2,4)	6,125000	0,875	0,342897	2,55	0,1253
Temperature (°C)*Duration (h)	6,125000	0,875	0,342897	2,55	0,1253
T (°C)*Quantity (g)	4,500000	0,75	0,342897	2,19	0,1602
Duration (h)*Quantity (g)	12,500000	-1,25	0,342897	-3,65	0,0677
T (°C)*Duration (h)*Quantity (g)	0,000000	0	0,342897	0,00	1,0000

Analysis of variance

The quality of the full factorial design applied is estimated from the results of the analysis of variance (**Table 8**), the model misfit and the experimental error (**Table 9**).

Table 8: Results of the analysis of variance of the full factorial design 2^3 for extraction yield.

Source	Degrees of freedom	Sum of squares	Medium square	F Report
Model	7	157,37500	22,4821	23,9013
Residues	2	1,88125	0,9406	**Prob. > F**

Corrected total 9	159,25625	**0,0407***

R^2 =0.98817/R^2 adjuste=0.946843

According to the R^2 obtained of 0.98817 and the adjusted R^2 of 0.946843 and the model p- value of 0.0407 for the response (extraction yield), we can estimate that the statistical model applied is not very significant (valid model).

Table 9: Misfits and experimental error for extraction yield.

Source	Degre e s of freedom	Sum of squares	Medium square	F Report
Misadjustment	1	1,6000000	1,60000	5,6889
Pure error	1	0,2812500	0,28125	**Prob. > F**
Total error	2	1,8812500		0,2527

The two experiments at the centre point for the three factors (experiments 9 and 10 in Table 10) are used to estimate the pure (experimental) error. The results in Table 13 show that the pure error is insignificant for the response analysed, with a *p-value of* around 0.2527 greater than 0.05. This shows that the experimental results are very slightly different from those predicted by the model. This shows that the experimental results are very slightly different from those predicted by the model. The diagram in **Fig. 4** makes it easier to visualise these conclusions, where most of our experimental results presented by the black dots lie on the bisecting line of the diagram.

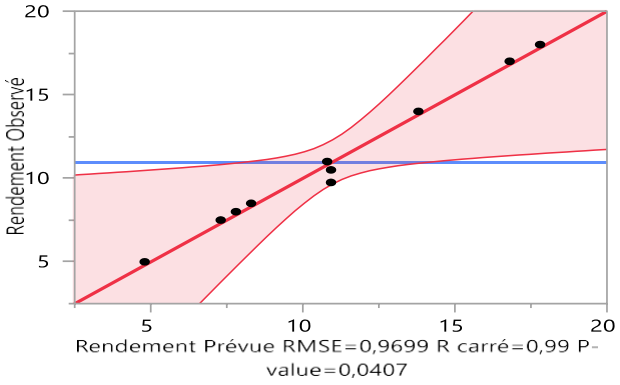

Figure 4: Diagram of experimental results for full factorial design

Conclusion

In the course of this study, we optimised the extraction of phenolic compounds from sage officinale, concluding that dry plants give the best results in terms of yield, polyphenols, flavonoids and anti-free radical activity.

Optimisation of the best extraction solvent by applying a mixing plan. This indicated that the mixture (16% distilled water, 16% acetone, 66% ethanol) gave satisfactory results. Statistical analysis of the solvent optimisation results showed that the response for flavonoids had a significant effect *(p-value<0.*05).

The results of the comparative study between the three extraction methods show that maceration is the best extraction method for common sage.

References

[1] **Achat S. (2005)**. Study of protein-polyphenol interactions. Case study of Salvia officinalis with bovine serum albumin protein. Magister dissertation, 67p.

[2] **Aganga, A. A., & Mosase, K. W. (2001).** Tannin content, nutritive value and dry matter digestibility of Lonchocarpus capassa, Zizyphus mucronata, Sclerocarya birrea, Kirkia acuminata and Rhus lancea seeds. *Animal Feed Science and Technology*, *91*(1-2), 107-113.

[3] **Albano, S. M., & Miguel, M. G. (2011).** Biological activities of extracts of plants grown in Portugal. *Industrial Crops and Products*, *33*(2), 338-343.

[4] **Ambe, A. S., Ouattara, D., Tiebre, M. S., Vroh, B. T. A., Zirihi, G. N., & N'guessan, K. E. (2015).** Diversity of medicinal plants used in the traditional treatment of diarrhoea in the markets of Abidjan (Côte d'Ivoire). *Journal of Animal & Plant Sciences*, *26*(2), 4081-4096.

[5] **Aseervatham, G. S. B., Sivasudha, T., Jeyadevi, R., & Arul Ananth, D. (2013).** Environmental factors and unhealthy lifestyle influence oxidative stress in humans-an overview. *Environmental Science and Pollution Research*, *20*, 4356-4369.

[6] **Bahorun, T., Gressier, B., Trotin, F., Brunet, C., Dine, T., Luyckx, M., & Pinkas, M. (1996).** Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. *Arzneimittel-forschung*,*46*(11),1086-108

[7] **Bekro, Y. A., Mamyrbekova, J. A., Boua, B. B., Bi, F. T., & Ehile, E. E. (2007).** Ethnobotanical study and phytochemical screening of Caesalpinia benthamiana (Baill.) Herend. et Zarucchi (Caesalpiniaceae). *Sciences & nature*, *4*(2), 217-225.

[8] **Bouteldja, R., Doucene, R., Aggad, H., Abdi, F. Z., Belkhodja, H., Abdali, M., ... & Abaid, S. (2021).** Phytochemical characterization, antioxidant and antibacterial activity of Salvia officinalis (L.) extracts from the Tiaret region. *European Journal of Biological Research*, *11*(3), 356-366.

[9] **Bucić-Kojić, A., Planinić, M., Tomas, S., Bilić, M., & Velić, D. (2007).** Study of solid-liquid extraction kinetics of total polyphenols from grape seeds. *Journal of food engineering*, *81*(1), 236-242.

[10] **Cai, L. (2004).** Metallothionein as an adaptive protein prevents diabetes and its toxicity. *Nonlinearity in biology, toxicology, medicine*, *2*(2), 15401420490464367.

[11] Chirinos, R., Rogez, H., Campos, D., Pedreschi, R., & Larondelle, Y. (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua (Tropaeolum tuberosum Ruíz & Pavón) tubers. *Separation and Purification Technology*, *55*(2), 217-225.

[12] Cunha, I., Sawaya, A. C., Caetano, F. M., Shimizu, M. T., Marcucci, M. C., Drezza, F. T., ... **& Carvalho, P. D. O. (2004).** Factors that influence the yield and composition of Brazilian propolis extracts. *Journal of the Brazilian Chemical Society*, *15*, 964-970.

[13] **Dewanto, V., Wu, X., & Liu, R. H. (2002).** Processed sweet corn has higher antioxidant activity. *Journal of Agricultural and food Chemistry*, *50*(17), 4959-4964.

[14] **Duletić-Laušević, S., Alimpić-Aradski, A., Živković, J., Gligorijević, N., Šavikin, K., Radulović, S., ... & Marin, P. D. (2019).** Evaluation of bioactivities and phenolic composition of extracts of Salvia officinalis L.(Lamiaceae) collected in Montenegro. *Botanica Serbica*, *43*(1), 47-58.

[15] **El Gabbas, Z., Bezza, K., Laadraoui, J., Ait Laaradia, M., Kebbou, A., Oufquir, S., ... & Chait, A. (2019).** Salvia officinalis, rosmarinic and caffeic acids attenuate neuropathic pain and improve function recovery after sciatic nerve chronic constriction in mice. *Evidence-Based Complementary and Alternative Medicine*, *2019*.

[16] **Et-Touys, A., Fellah, H., Mniouil, M., Bouyahya, A., Dakka, N., Abdennebi, H., ... & Bakri, Y. (2016).** Screening of antioxidant, antibacterial and antileishmanial activities of Salvia officinalis L. extracts from Morocco. *British Microbiology Research Journal*, *16*(5), 1-10.

[17] **Farhoosh, R., Khodaparast, M. H. H., & Sharif, A. (2009).** Bene hull oil as a highly stable and antioxidative vegetable oil. *European journal of lipid science and technology*, *111*(12), 1259-1265.

[18] **Fellah, S., Romdhane, M., & Abderraba, M.** (2006). Extraction and study of essential oils from Salvia officinalis. l harvested in two different regions of Tunisia. *Journal-Societe Algerienne De Chimie*, *16*(2), 193.

[19] **Fleurentin, J., & Hayon, J. C. (2008).** *Les plantes médicinales: traditions et thérapeutique*. Ed. Ouest-France.

[20] **Gargouri, W., Osés, S. M., Fernández-Muiño, M. A., Sancho, M. T., & Kechaou, N. (2019).** Evaluation of bioactive compounds and biological activities of Tunisian propolis. *Lwt*, *111*, 328-336.

[21] **Goupy, J., & Creighton, L. (2009).** *Introduction aux plans d'expériences: avec applications*. Dunod. 321 P.

[22] **HAMMOUDI, R. (2015).** *Biological activities of some secondary metabolites extracted from some medicinal plants of the Algerian Southern Sahara* (Doctoral dissertation).

[23] **Hamrouni-Sellami, I., Rahali, F. Z., Rebey, I. B., Bourgou, S., Limam, F., & Marzouk, B. (2013).** Total phenolics, flavonoids, and antioxidant activity of sage (Salvia officinalis L.) plants as affected by different drying methods. *Food and Bioprocess Technology*, *6*(3), 806-817.

[24] **Harborne, A. J. (1998).** *Phytochemical methods a guide to modern techniques of plant analysis*. springer science & business media.

[25] **Jakovljević, M., Jokić, S., Molnar, M., Jašić, M., Babić, J., Jukić, H., & Banjari, I. (2019).** Bioactive profile of various Salvia officinalis L. preparations. *Plants*, *8*(3), 55.

[26] **Kamazawa, S., Kigawa, J., Kanamori, Y., Itamochi, H., Sato, S., Iba, T., & Terakawa, N. (2002).** Multidrug resistance gene-1 is a useful predictor of Paclitaxel-based chemotherapy for patients with ovarian cancer. *Gynecologic oncology*, *86*(2), 171-176.

[27] **KASRANI. K., & MOUH.OUB I.. (2019).** *Biochemical characterization and evaluation of biological activities of phenolic extracts of salvia officinalis L species* (Doctoral dissertation, Abdelhafid Boussouf University centre-Mila).

[28] **Koane, J. N., Syssa-Magalé, J. L., & Ouamba, J. M. (2011).** P291-Phytochemical and pharmacological studies of some Central African medicinal plants with antidiabetic properties. *Diabetes & Metabolism*, *37*(1), A102-A103.

[29] **Laplante, J. (2009).** Medicinal plants, knowledge and society: the view of South African Rastafarians. *Drogues, santé et société*, *8*(1), 93-121.

[30] **Liu, S., Wang, X., Liu, M., & Zhu, J. (2017).** Towards better analysis of machine learning models: A visual analytics perspective. *Visual Informatics*, *1*(1), 48-56.

[31] **Louli, V., Ragoussis, N., & Magoulas, K. (2004).** Recovery of phenolic antioxidants from wine industry by- products. *Bioresource technology*, *92*(2), 201-208.

[32] **Martins, N., Barros, L., Santos-Buelga, C., Henriques, M., Silva, S., & Ferreira, I. C. (2015).** Evaluation of bioactive properties and phenolic compounds in different extracts prepared from Salvia officinalis L. *Food chemistry*, *170*, 378-385.

[33] **Menaker, A., Kravets, M., Koel, M., & Orav, A. (2004).** Identification and characterization of supercritical fluid extracts from herbs. *Comptes Rendus Chimie*, *7*(6-7), 629-633.

[34] **Miliauskas, G., Venskutonis, P. R., & Van Beek, T. A. (2004).** Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food chemistry*, *85*(2), 231-237.

[35] **Molyneux, P. (2004).** The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. sci. technol*, *26*(2), 211-219.

[36] **Naczk, M., & Shahidi, F. (2004).** Extraction and analysis of phenolics in food. *Journal of chromatography A*, *1054*(1-2), 95-111.

[37] **POP, A. V., TOFANĂ, M., Socaci, S. A., Nagy, M., BORŞ, M. D., SALANȚĂ, L., & Vlaic, R. (2015).** Studies on total polyphenols content and antioxidant activity of methanolic extracts from selected Salvia species. *Bulletin UASVM Food Science and Technology*, *72*, 1.

[38] **Roby, M. H. H., Sarhan, M. A., Selim, K. A. H., & Khalel, K. I. (2013).** Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (Thymus vulgaris L.), sage (Salvia officinalis L.), and marjoram (Origanum majorana L.) extracts. *Industrial Crops and Products*, *43*, 827-831.

[39] **Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999).** [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in enzymology* (Vol. 299, pp. 152-178). Academic press.

[40] Soobrattee, M. A., Neergheen, V. S., Luximon-Ramma, A., Aruoma, O. I., & Bahorun, T. (2005). Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutation Research/Fundamental and Molecular mechanisms of mutagenesis*, *579*(1-2), 200-213.

[41] **Soro, L. C., Atchibri, L. O., Kouadio, K. K., & Kouamé, C. (2012).** Evaluation of the nutritional composition of leafy vegetables. *J. Appl. Biosci*, *51*, 3567-3573.

[42] Spigno, G., Tramelli, L., & De Faveri, D. M. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of food engineering*, *81*(1), 200-208.

[43] **Sripad, G., Prakash, V., & Rao, M. N. (1982).** Extractability of polyphenols of sunflower seed in various solvents. *Journal of Biosciences*, *4*, 145-152.

[44] **Sun, Y., Chen, J., Li, A., Liu, F., & Zhang, Q. (2005).** Adsorption of resorcinol and catechol from aqueous solution by aminated hypercrosslinked polymers. *Reactive and Functional Polymers*, *64*(2), 63-73.