OPTIMIZATION OF EXTRACTION CONDITIONS FOR PHENOLIC COMPOUNDS FROM *SALVIA CHUDAEI*

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ABSTRACT

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This study focused on optimization of the extraction conditions of phenolic compounds from the aerial and the underground parts of a medicinal plant, endemic in the Algerian Sahara: Salvia chudaei Batt. & Trab. (Lamiaceae). This plant has been subjected to two extraction methods (soxhlet and maceration), with optimization of extraction conditions (solvent, concentration, time and temperature). The best extracts based on the antioxidant capacity using two tests (FRAP and DPPH) were obtained by ethanol 80% as solvent and extraction period of 100 min for the aerial part and 20 min for the underground part by soxhlet method, whereas, 60 min at 30°C for the aerial part and 15°C for the underground part were appropriate for extraction by maceration.

Keywords: *Salvia chudaei*, optimization, extraction, phenolic compounds, antioxidant activity.

INTRODUCTION

For a long time, medicinal plants have played a key role in the conservation of human health and the survival of humanity (Iserin, 2001; Machiex et al., 2005). According to the World Health Organization (WHO), there are more than 4,000 medicinal plants, which constitute 90% of the African traditional medicine (OMS, 2003). Algeria has a great diversity of flora for therapeutic uses (Hamzaa et al., 2010).

Currently, industries are developing more and more processes using extracts and active ingredients of plant origin, such as polyphenols. These compounds have been particularly studied for their use in the pharmaceutical, cosmetic and food for their beneficial health effects (Hirasa and Takemasa, 1998), and their different biological properties such as anticarcinogenic, anti-thrombotic, and vasodilatory cardioprotective were reported by several workers (Middleton et al., 2000; Ksouri et al., 2007; Nijveldt et al., 2001).

The genus *Salvia*, the largest genus in the family Lamiaceae, includes around 900 species distributed worldwide. Many *Salvia* spp. are used as herbal tea and food flavorings, and in cosmetics, perfumes and pharmaceuticals. *Salvia* species have been reported to have a wide range of biological activities, including cholinesterase inhibitors, antibacterial, antimalarial, anti-cancer and antioxidant properties (Perry et al., 2003; Tepe et al., 2005; Kamatou et al., 2005; Kotan et al., 2008). Most of these reported beneficial characteristics were attributed to the presence of phenolic compounds.

However, to the best of our knowledge, optimization of extraction of phenolic antioxidants from *Salvia chudaei* has not been reported yet. Therefore, the objective of this study is to optimize the extraction conditions of phenolic compounds (solvent, solvent concentration, extraction time and temperature), from aerial and the underground parts of *Salvia chudaei* from the region of Tamanrasset (Algerian Sahara) by two extraction methods, maceration and Soxhlet.

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MATERIALS AND METHODS

Plant material

The plant material consists of aerial and underground parts of *Salvia chudaei* collected in October 2015 from Tamanrasset (Algeria Sahara; N 23 ° 81 '756 "East: E005 ° 93' 888"). The plant was identified by botanists from the National Institute of Forestry Research (INRF), Research Station for the Protection of Arid-Zones, Tamanrasset, Algeria. Representative specimens have been deposited at the Herbarium (PM/03) of the Laboratory of Biogeochemistry of Desert Environments, Ouargla University, Algeria. The samples are dried away from light and moisture, at room temperature. The grinding was carried out in a hammer mill and cutter (type: MFC) with 0.5 mm pore size (Catier & Roux, 2007).

Optimization of extraction conditions of phenolic compounds

To determine the best extraction conditions of the phenolic compounds contained in the aerial and underground parts of *S. chudaei*, two extraction methods, maceration and Soxhlet (VELP Scientifica Ser 148 Solvent Extractor) were used. Extractions series were performed to determine the type of solvent, concentration (%), time (min) and temperature (°C) suitable for extraction of the phenolic compounds with maximum antioxidant activity.

Effect of solvent type on extraction of phenolic compounds

Polyphenols were extracted in different organic solvents (acetone, ethanol and methanol) (Escribanobailon and Santos-Buelga, 2003; Naczk and Shahidi, 2004; Makhlouf *et al*., 2013; Vanessa *et al*., 2014; Shuangqin *et al*., 2015; Duong *et al*., 2015), using two extraction methods (maceration and Soxhlet). 5g of the aerial or underground parts of plant was extracted with 50 ml of solvent. The extraction was done for 30 min at room temperature and repeated three times for each solvent (Cujic et al., 2016). After evaporation of solvent with a rotary evaporator, the extracts were weighed to calculate the yield using a precision balance (PGW series with capacity 150 g and sensitivity of 0.01 mg). The most appropriate solvent was selected based on the highest antioxidant activity.

Effect of solvent concentration on extraction of phenolic compounds

Solvent concentration was assessed using ethanol/water mixtures at 20%, 40%, 60%, 80% and 100% (Chirinos et al., 2007; Kim et al.2007; Spigno et al., 2007). The best solvent concentration was chosen according to the highest value of antioxidant activity.

Effect of extraction time on extraction of phenolic compounds

The impact of different extraction periods (20, 30, 40, 60, 80 and 100 min) on the phenolic content was evaluated (Mehmet *et al.,* 2015; Shuangqin *et al*., 2015). Extraction was accomplished by applying the best solvent concentration, at ambient temperature. Extraction procedures were repeated as described above. The best extraction time was chosen based on the highest value of the antioxidant activity.

Effect of extraction temperature on extraction of phenolic compounds

The extraction by maceration was carried out using the best solvent, concentration, extraction time, under different temperatures (15, 25, 30, 45 and 60°C) (Yap et al., 2009; Hismath et al., 2011; Bandar et al., 2013). The best extraction temperature was chosen according to the highest value of the antioxidant activity.

Evaluation of the antioxidant activity of the extracts

To evaluate the *in vitro* antioxidant activity of natural extracts, different methods were investigated. These methods involve the mixing of oxidizing species, such as free radicals, or oxidized metal complexes, with a sample which contains antioxidants capable of inhibiting the generation of radicals. These antioxidants can act according to two major mechanisms: either by transfer of hydrogen atoms or by electron transfer (Michel, 2011).

Free Radical Scavenging Activity: the diphenyl-picrylhydrazyl (DPPH) Test

The free radical-scavenging activity was determined by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay described by Blois (1958). Briefly, 6×10^{-6} $mol.L^{-1}$ solution of DPPH in methanol was prepared and 3 ml of this solution

was added to 100 µl of sample extract solution. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as *A* (blank). All measurements were performed in triplicate. The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\% \text{ inhibition} = ((A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}) \times 100.$$

Test FRAP (Ferric Reducing Antioxidant Power)

The FRAP method developed by Benzie & Strain (1996) corresponds to the reduction of a ferric complex tripyridyltriazine [(Fe (III) -TPTZ)$_2$] complex in a ferrous tripyridyltriazine [(Fe (II) -TPTZ)$_2$] blue color, with an antioxidant (AH), at pH 3.6 for maintaining iron solubility, which allows quantification by spectrophotometry. FRAP reagent is prepared by the following mixtures: 25 ml of acetate buffer (pH 3.6), 2.5 mL TPTZ (10 mmol) and 2.5 mL of iron chloride ($FeCl_3$- $3H_2O$) 20 mmol.L^{-1}. 150 µL of extract followed by 2850 µL of FRAP solution were added to the test tubes. The absorbance reading of the reaction medium was carried out after 30 minutes at 593 nm against the blank. The antioxidant activity of the extract was measured from the change in absorbance of the complex. The ascorbic acid calibration curve using different concentrations (10-100 mmol.L^{-1}) was used to calculate the antioxidant potential.

Statistical analysis

The experimental results were analyzed using R (WindowsGUIfront-end) and MatLab (Version 16) softwares. All data were expressed as means ± standard deviations of three replicate measurements. One-way analysis of variance (ANOVA) with Tukey's test was used to determine the significant differences ($p<0.05$) between the means. Similarly, Person's test correlations were established between the different variables.

RESULTS AND DISCUSSION

Selection of extraction solvent

The two parts of *S. chudaei* were subjected to the extraction of phenolic compounds by two methods: maceration and Soxhlet using the following three different solvents: acetone, ethanol and methanol.

Effect of solvent type on the phenolic extracts

The extraction yields of the phenolic compounds are illustrated in Table 1.

Table 1. Effect of solvent type, ethanol concentration, extraction time, and extraction temperature on the yield of phenolic compounds.

		Extracts			
Extraction parameters		**Ma**	**Ms**	**Sa**	**Ss**
Solvent type	**EA**	4.5 ± 0.0015	2.4 ± 0.0016	7.5 ± 0.0082	3.3 ± 0.0022
	EE	6 ± 0.0013	4.1 ± 0.0007	12.3 ± 0.0064	4.8 ± 0.0114
	EM	6.9 ± 0.0021	4.9 ± 0.0096	16 ± 0.017	2.5 ± 0.0196
Ethanol concentration	**20%**	9.7 ± 0.0441	5.6 ± 0.0004	14.5 ± 0.0123	23.2 ± 0.0045
	40%	22.3 ± 0.0010	4.2 ± 0.0091	15.6 ± 0.0072	10.7 ± 0.0045
	60%	16.86 ± 0.0017	18 ± 0.0028	14.3 ± 0.0028	7.3 ± 0.034
	80%	10.1 ± 0.0058	4.28 ± 0.0020	17.2 ± 0.0041	10.9 ± 0.023
	100%	6 ± 0.0036	3.1 ± 0.0037	15.4 ± 0.0066	12.7 ± 0.036
Extraction time	**20 min**	12.2 ± 0.0039	5 ± 0.0030	18.3 ± 0.0022	11.1 ± 0.3380
	30 min	12.4 ± 0.0005	6.3 ± 0.0001	22 ± 0.0010	11.2 ± 0.0035
	40 min	12.4 ± 0.0050	5.1 ± 0.0011	17.4 ± 0.0100	12.7 ± 0.0036

	60 min	12.3 ± 0.0023	5.7 ± 0.0046	38.5 ± 0.0086	13 ± 0.0087
	80 min	7.9 ± 0.0005	5.6 ± 0.0003	19.2 ± 0.0278	12.1 ± 0.0067
	100 min	11 ± 0.0152	8.5 ± 0.0167	84.84 ± 0.0225	12.8 ± 0.0099
Extraction temperature	**15°C**	9.6 ± 0.0150	2 ± 0.5522	ND	ND
	25°C	9.9 ± 0.0055	5.9 ± 0.0021	ND	ND
	30°C	11.7 ± 0.0085	6.3 ± 0.0004	ND	ND
	45°C	12.5 ± 0.0026	6.6 ± 0.0011	ND	ND
	60°C	13.3 ± 0.0034	7.2 ± 0.0029	ND	ND

Ma= extract of the aerial part by maceration; **Ms**= extract the underground part by maceration; **Sa**= extract of the aerial part by Soxhlet; **Ss**= extract the underground part by Soxhlet; **EA=** acetone extract; **EE=** ethanol extract; **EM=** methanol extract; **ND=** not determined**.**

The yield of the aerial part was higher than the underground part and the methanolic extract gave the highest yield for the two extraction methods. However, for the underground part, the ethanolic extract using the Soxhlet method gave highest yield.

From the results obtained, we notice that yields vary an extraction method to another and a part of the plant to another. The variation in extraction yield following different extraction methods can be explained by the difference in solvent diffusion into the powder of the plants in the maceration step and possibly the nature of the solvents used for extraction (Naczk & Shahidi, 2004). Earlier results obtained by Mahmoudi et al. (2013) showed that acetone was the best extraction solvent with a yield of 19.29%, followed by water and methanol with 16.75% and 14% yield, respectively. Senol et al., (2010), who used other solvents for the extraction of phenolic compounds from different Salvia species (*S.adenocaulon, S. adenophylla, S. divaricata, S. spinosa, S. virgata S. staminea, S. potentillifolia*). The solvents used were dichloromethane, ethyl acetate and methanol. Highest yield was obtained from methanol, followed by dichloromethane and least from ethyl acetate.

Effect of solvent type on the antioxidant activity (FRAP and DPPH)

FRAP value for each sample was calculated from the calibration curve of ascorbic acid, expressed in milligrams of ascorbic acid equivalent per mg of extract (mg EAA/mg of extract). Determining the percent inhibition was determined for the method of DPPH. The results obtained are presented in Table 2.

Table 2. Effect of solvent type on antioxidant activity (FRAP and DPPH).

Solvent type		**EA**	**EE**	**EM**
DPPH (% inhibition)	**Ma**	73.88 ± 0.0010	91.29 ± 0.0007	70.64 ± 0.0006
	Ms	44.58 ± 0.0026	64.8 ± 0.0119	58.33 ± 0.7540
	Sa	72.95 ± 0.0004	75.74 ± 0.0020	46.64 ± 0.0010
	Ss	52.92 ± 0.0025	79.85 ± 0.0001	54.78 ± 0.0032
FRAP (mg EAA/mg of extract)	**Ma**	486.33 ± 0.011	533.66 ± 0.7638	473.77 ± 0.0038
	Ms	206.55 ± 0.9179	218.5 ± 0.0080	169.22 ± 0.7515
	Sa	224.66 ± 0.0040	498.33 ± 0.0032	414.66 ± 0.0081
	Ss	203.16 ± 0.0155	226.55 ± 0.0410	179.27 ± 0.0126

Ma= extract of the aerial part by maceration; **Ms**= extract the underground part by maceration; **Sa**= extract of the aerial part by Soxhlet; **Ss**= extract the underground part by Soxhlet; **EA=** acetone extract; **EE=** ethanol extract; **EM=** methanol extract.

Results indicated that ethanol was the best solvent followed by acetone and methanol for both tests, the two plant parts and for the two extraction methods. Statistically, the difference between the solvents was very highly significant ($p < 0.001$) for DPPH, whereas for the FRAP test, the difference was significant ($0.01 < P < 0.05$), and still ethanol solvent was the best.

According to Duong *et al.* (2015), the antioxidant activity by the DPPH test for soybean phenolics obtained by acetone, methanol and ethanol, showed that the highest inhibition rate was observed for the acetone extract (76.4%). Several studies have demonstrated that acetone was the best solvent for the extraction of proanthocyanidins and tannins (Chirinos *et al*., 2007; Tabart *et al*., 2007). On the other hand, Athamena et al., (2010) showed that ethanol was the best extraction solvent for polyphenols, because of their polarity and their good solubility in this compound, whereas ST-Pierre (2012) was in favor of ethanol, as a universal solvent, because it has a large polarity which enables it to extract as much polar molecules such as polyphenols, and non-polar molecules such as triterpenes or phytosterols.

The statistical study in our work showed that the variation of the concentration of total polyphenols and total flavonoids based on the part of the plant they were extracted from was very highly significant ($p < 0.001$). DPPH indicated that there was a highly significant difference ($0.001 < P < 0.01$), and for the FRAP, the difference was highly significant ($p < 0.001$) between the two plant parts, with best results obtained from the aerial part. This may be due to the influence of the distribution of the phenolic compounds in the plant (Naczk and Shahidi, 2004).

Non-significant difference ($P > 0.05$) between the values of FRAP of the two extraction methods was observed, whereas DPPH had a significant difference ($0.01 < P < 0.05$) between the two extraction methods. However, maceration seemed to be the best method of extraction of polyphenols. The correlation between the FRAP and DPPH was 59.6%. Maisuthisakul *et al*., (2008) found that the total content of flavonoids in ethanolic extracts of plants is related to the total phenolic compounds content.

To remove phenolic antioxidant compounds from various plant sources, acetone and ethanol are commonly used as extraction solvents (Spigno *et al*., 2007; Bazykina *et al*., 2002). They usually give high total yields, even if they are not very selective to phenols. As their chemical nature and the presence of one or more hydroxylated benzene rings in all phenolic compounds that are responsible for certain common properties, they are used to extract from the plant material, and characterize them chemically (Spigno et al., 2007). In light of these results, ethanol was found the most suitable solvent for the extraction of phenolic compounds from *S. chudaei*.

Effect of ethanol concentration on phenolic extracts

The extraction of phenolic compounds was made with different ethanol concentrations (20%, 40%, 60%, 80% and 100%). The results obtained are shown in Table 1.

The highest extract yield of the underground part obtained with 20% ethanol by Soxhlet method was 23.2%, followed by 17.2% yield from the aerial part with 80% ethanol. In the maceration method, the highest yield was obtained with 40% ethanol (22.3%) from the aerial part and 18% from the underground part with 60% solvent, whereas ile the 100% ethanol gives the least significant yields for both parts.

According to Yap et al., (2009), a high proportion of water in the solvent system promotes the extraction of total phenolics.

Effect of the ethanol concentration on antioxidant activity (FRAP and DPPH)

The results of the effect of the concentration of ethanol on anti-antioxidant activity made by both FRAP and DPPH methods are summarized in Table 3.

Table 3. Effect of extraction solvent concentration on antioxidant activity (FRAP and DPPH).

Ethanol concentration		20%	40%	60%	80%	100%
DPPH (% inhibition)	Ma	21.82 ± 0.0006	41.47 ± 0.0017	48.01 ± 0.0010	56.06 ± 0.0047	47.72 ± 0.0006
	Ms	23.24 ± 0.0015	26.56 ± 0.0020	48.05 ± 0.0042	49.62 ± 0.0146	33.14 ± 0.0015
	Sa	46.78 ± 0.0031	52.41 ± 0.0026	51.27 ± 0.0053	58.19 ± 0.0050	51.7 ± 0.0056
	Ss	8.19 ± 0.0032	47.86 ± 0.0046	55.25 ± 0.0069	56.06 ± 0.0002	49.57 ± 0.0165
FRAP (mg EAA/mg of extract)	Ma	115 ± 0.0173	240.66 ± 0.0136	327.33 ± 0.0070	445.66 ± 0.0015	364.33 ± 0.032
	Ms	39.38 ± 0.0040	48.16 ± 0.0053	136.27 ± 0.0268	178.33 ± 0.0274	108.11 ± 0.0117
	Sa	373.5 ± 0.0044	243.16 ± 0.0050	273.66 ± 0.0025	346.5 ± 0.0030	328.16 ± 0.0051
	Ss	3.61 ± 0.0962	125 ± 0.0046	146.88 ± 0.0015	154.88 ± 0.0110	147.77 ± 0.0150

Ma= extract of the aerial part by maceration, **Ms**= extract of the underground part by maceration, **Sa**= extract of the aerial part by Soxhlet, **Ss**= extract of the underground part by Soxhlet.

The antioxidant effects varied greatly between different extracts. These results show that extracts with ethanol (80%) have an important antioxidant power with both tests. In exception of aerial part extract by Soxhlet using ethanol (20%) presents the most important anti-radical power with FRAP test.

Variance analysis of two factors, concentration of solvent and the antioxidant activity, followed by the Tukey test showed a very highly significant difference ($P < 0.001$) by DPPH and a significant difference ($0.01 < P < 0.05$) by the FRAP assay. 80% ethanol was the best concentration used. However the difference in antioxidant activity by the FRAP test between the two plant parts was very highly significantly ($p < 0.001$) and was significant in the DPPH test, and it was higher in the aerial part. There was a significant difference ($0.01 < P < 0.05$) between the two extraction methods according to the DPPH test, and a non-significant difference ($P > 0.05$) by the FRAP test. The best method of extraction of phenolic compounds from *S. chudaei* was the Soxhlet method.

Duong et al. (2015) found that the inhibition rate of acetonic extracts from soybean (*Glycine max* L. Merrill) by DPPH test was 70%. Wissam et al. (2012) showed that 80% was the best ethanol concentration because the phenolic compounds in plants are polar compounds and are generally extracted with polar solvents. Combination of solvents such as methanol, ethanol, and acetone with water can improve the extraction of phenolic compounds (Naczk and Shahidi, 2004; Chirinos *et al.,* 2007; Kim *et al.,* 2007; Spigno *et al*., 2007; Tabart *et al*., 2007; Turkmen *et al*., 2007; Hismath *et al.,* 2011).

Ethanol-water mixtures, in particular, are more effective in the extraction of phenolic compounds (Yilmaz and Toledo, 2006; Pinelo *et al*., 2005; Penchev, 2010). Water plays an important role in the swelling of the plant material, while ethanol is liable to disrupt the bonding between the solute and the plant matrix and also allow better mass transfer of compounds. Therefore, the mixture of water and ethanol as solvent shows a synergistic effect which facilitates the phenol extraction. Furthermore, Wang and Weller (2006) reported that water and ethanol are the most commonly solvents used for extracting phenolic compounds with antioxidant power. Water and a low concentration of ethanol solvents may access the cells, but high ethanol concentration can cause protein denaturation, which prevents the dissolution of polyphenols during extraction (Yang et al., 2009). Accordingly, 80% ethanol concentration was chosen to determine the effect of time and temperature on the extraction of antioxidants.

Effect of extraction time on yield and antioxidant activity

The extracts yields obtained following various extraction times are summarized in Table 1. The best yield was obtained with 100 min extraction from both parts of the plant extract by Soxhlet and maceration methods. However, the highest yield from the aerial part extracted by maceration was obtained following 30 and 40 min extraction. Some workers reported that extension of extraction time of phenolic compounds by solvents improved their performance (Chirinos *et al*., 2007; Drużyńska *et al*., 2007; Silva *et al*., 2009; Nicola *et al*., 2007). However, several researchers have drawn attention to the possibility of phenolic compounds oxidation if the extraction time was prolonged, which can lead to opposite results (low yield) (Naczk and Shahidi, 2004; Chirinos *et al*., 2007; Drużyńska *et al.,* 2007; Yap *et al.,* 2009). The kinetics of polyphenol extraction from plant material has been investigated in numerous studies (Chirinos *et al*., 2007; Silva *et al.,* 2009; Spigno *et al*., 2007). Similarly, antioxidant extraction kinetics from the leaves of *Melissa officinalis* has been slow. This process was divided into two phases (Herodez et al., 2003), a rapid phase explained by the fact that the solutes are present on surface sites of the plant material, and a slow phase corresponding to the molecular diffusion of the solute from internal sites through pores (Herodez *et al.,* 2003; Spigno *et al.,* 2007).
Results summarized in Table 4 indicated that the highest antioxidant activity was obtained following 60 minutes maceration of the two parts of the plant and by using both FRAP and DPPH tests (non-significant difference $p < 0.5$), however, the 80 min extraction from the aerial part showed by the FRAP test to have the highest antioxidant power.

Table 4. Effect of extraction time on the antioxidant activity (DPPH and FRAP).

Extraction time	FRAP (mg EAA/mg of extract)				DPPH (% inhibition)			
	Ma	Ms	Sa	Ss	Ma	Ms	Sa	Ss
20 min	426.33±0.0003	287.16 ± 0.0006	651.16 ± 0.0057	330.83 ± 0.0021	61.55 ± 0.0015	43.91 ± 0.0031	16.16 ± 0.0136	59.69 ± 0.0023
30 min	480.33 ± 0.0004	282 ± 0.0005	378 ± 0.0061	328.33 ± 0.0344	36.61 ± 0.0017	28.83 ± 0.0036	17.3 ± 0.0026	23.54 ± 0.0010
40 min	471.16 ± 0.0003	302.33 ± 0.0006	537.83 ± 0.0021	301 ± 0.0026	65.87 ± 0.0017	46.77 ± 0.0149	13.88 ± 0.0010	72.56 ± 0.0035
60 min	527.33 ± 0.0004	382 ± 0.0005	573.5 ± 0.0017	289.33 ± 0.0015	68.55 ± 0.0042	50.72 ± 0.0010	26.02 ± 0.0021	55.66 ± 0.0055
80 min	556.66 ± 0.0004	343.5 ± 0.0005	560.83 ± 0.0021	267.5 ± 0.0017	67.94 ± 0.0021	49.08 ± 0.0010	34.87 ± 0.0051	16.63 ± 0.0025
100 min	512.83 ± 0.0003	340.5 ± 0.0006	585.83 ± 0.0012	267.5 ± 0.0044	67.27 ± 0.0015	42.82 ± 0.0006	52.64 ± 0.0006	14.95 ± 0.0015

Ma=extract of the aerial part by maceration, **Ms**= extract the underground part by maceration, **Sa=** extract of the aerial part by Soxhlet, **Ss=** extract the underground part by Soxhlet.

The extracts of the aerial and underground parts, obtained by Soxhlet following 100 min and 40 min extraction provided the highest inhibition rate when using the DPPH test. However, with the FRAP test, the best value was noted 20 min extraction of both plant parts. In this study, the extraction time had a significant effect on antioxidant activity. Statistical analysis showed that there was no significant difference ($P > 0.05$) between the different durations of Soxhlet extraction of underground parts according to DPPH test. In addition, there was a very highly significant difference ($P < 0.001$) between the two plant parts of *S. chudaei* by FRAP test and a non-significant difference ($p < 0.5$) by DPPH test. However, the difference between the two extraction methods was very highly significant ($P < 0.001$) by DPPH test, and not significant ($P < 0.5$) by FRAP test, and maceration was the best method of extraction.

According to Galvan et al., (2012), two-phase extraction were observed, the first increase in the concentration of polyphenols in the beginning of the process followed by a slow extraction (after 60 min) characterized by low improving the polyphenol content with the evolution of the extraction.

Following these results, we find that 80% ethanol and a period of100 min for the extraction of polyphenols of the aerial part and duration of 20 minutes for the underground part are best extraction conditions by Soxhlet of phenolic compounds from the plant *S chudaei*. The phenolic extract obtained by maceration in 80% ethanol, and a period of 60 min., was chosen to study the effect of temperature.

Effect of extraction temperature on yield and antioxidant activity

The impact of temperature (15°C - 60°C) on the extraction of phenolic compounds was evaluated and the results obtained are summarized in Table 1. The highest extraction yields were obtained at the highest temperature (60°C). This is not in agreement with an earlier report by Majhenic et al. (2007) who indicated that higher yields of solids were obtained at room temperature. The choice of the extraction temperature was an important step in a series of experiments conducted to optimize the extraction conditions, and the results obtained are illustrated in Table 5.

Table 5. Effect of extraction temperature on the antioxidant activity evaluated by the DPPH and FRAP tests.

Temperature (°C)		**15**	**25**	**30**	**45**	**60**
DPPH(% inhibition)	**Ma**	60.68 ± 0.0969	61.46 ± 0.0040	76.95 ± 0.0023	63.36 ± 0.0969	59.67 ± 0.0006
	Ms	41.16 ± 0.0006	29.19 ± 0.0050	35.85 ± 0.0021	35.51 ± 0.0006	40.26 ± 0.0046
FRAP (mg EAA/mg of extract)	**Ma**	445.33 ± 0.0031	471.16 ± 0.0051	493.83 ± 0.0065	465.83 ± 0.0050	468.5 ± 0.0017
	Ms	329.83 ± 0.0059	276.5 ± 0.0044	274.33 ± 0.0025	276.83 ± 0.0035	310.33 ± 0.0032

Ma= extract of the aerial part by maceration, **Ms=** extract the underground part by maceration.

The results of antioxidant activity revealed that it was highest at 30°C for extraction of the aerial part, but differences were not significant (P> 0.05). Extraction at 15°C from the underground part gave higher antioxidant activity with both tests. The difference between the activity of extracts from both parts of the plant was highly significant (P <0.001), with higher activity obtained from extracts of the aerial part. Previous studies showed that extrinsic factors (such as geographic and climatic factors), genetic factors, plant maturation stage and storage period have a strong influence on the polyphenols content (Aganga and Mosase, 2001; Hamia *et al*., 2014).

Generally, the temperature has a positive effect on the extraction of phenolic compounds from plant sources (Bucic-Kojic et al., 2007; Harbourne et al., 2009; Spigno et al., 2007). This effect could be explained by the greater solubility of polyphenols in the solvent. Higher temperature can imcrease diffusion of extracted molecules, reduce its viscosity and improve mass transfer. An increase of the extraction temperature may also affect the structure of the plant matrix by increasing the permeability of cell walls and weakening the interaction between phenolic compounds and macromolecules (proteins, polysaccharides) and therefore facilitates the extraction process (Prasad *et al*., 2009; Al-Farsi and Lee, 2007). However, excessive temperatures during extraction or drying affect the stability of some phenolic compounds due to reactions involving chemical and enzymatic degradation, or a thermal decomposition of some compounds (45-100°C) (Fischer *et al.,* 2013; Cisse *et al*., 2012).

Based on all the above, maceration at 30°C was considered the optimal temperature for the extraction of phenolic compounds from the aerial parts of *S. chudaei* and maceration at 15°C for maceration of underground parts. Furthermore, best conditions to remove phenolic compounds by maceration of the aerial and underground parts of *S. chudaei* was in ethanol at 80% as solvent, with three cycles lasting 60 minutes at temperature of 30°C for the aerial part and 15°C for the underground part. As for the Soxhlet extraction, best results were obtained with 80% ethanol as solvent, three cycles of extraction lasting 100 min for the aerial part and 20 min for the underground part. It would be interesting to identify the active compounds responsible for antioxidant activity in further studies and evaluate the pharmacological activities *in vivo* of these compounds.

REFERENCES

Aganga, A.A. and Mosase, K.W. 2001. Tannins content, nutritive value and dry matter digestibility of *Lonchocarous capussa*, *Ziziphus mucropata*, *Sclerocarya birrea*, *Kirkia acuminata* and *Rhuslancea* seeds. *Anim Feed Sci Tech*., 91: 107-113.

AL-Farsi, M.A. and Lee, C.Y. 2007. Optimization of phenolics and dietary fiber extraction from date seeds. *J Food Chem*., 108: 977-985.

Athamena, S., Chalghem, I., Kassah-Laouar, A., Laroui, S. and Khebri, S. 2010. Activité anti-oxydante et antimicrobienne d'extraits de *Cuminum cyminum* L. *Lebanese Science Journal*, 11: 1-13.

Bazykina, N.I., Nikolaevskii, A.N., Filippenko, T.A. and Kaloerova, V.G. 2002. Optimization of conditions for the extraction of natural antioxidants from raw plant materials. *Pharm Chem J.,* 36(2): 100-103.

Benzie, I.F.F. and Strain, J.J. 1996. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Anal Biochem*. 239(1): 70-76.

Blois, M.S. 1958. Antioxidant determination by the use of a stable free radical. *Nature*, 181: 1199-1200.

Bucic-Kojic, A., Planinic, M., Tomas, S., Bilic, M. and Velic, D. 2007. Study of solid–liquid extraction kinetics of total polyphenols from grape seeds. *J Food Eng*., 81:236-242.

Chirinos, R., Rogez, H., Campos, D., Pedreschi, R. and Larondelle, Y. 2007. Optimisation of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruíz, Pavón) tubers. *J Sep Purif Technol*., 55:217-225.

Cisse, M., Bohuon, P., Sambe, F., Kane, C., Sakho, M. and Dornier, M. 2012. Aqueous extraction of anthocyanins from *Hibiscus sabdariffa*: experimental kinetics and modeling. *J Food Eng.,* 109: 16-21.

Cujic, N., Šavikin, K., Jankovic, T., Pljevljakušic, D. and Zdunic, G. 2016. Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique Svetlana Ibric. *Food Chem*., 194:135-142.

Drużyńska, B., Stepniewska, A. and Wolosiak, R. 2007. The influence of time and type of solvent on efficiency of the extraction of polyphenols from green tea and antioxidant properties obtained extracts. *Acta Sci. Pol. Technol. Aliment.*, 6:27-36.

Duong, T., Phan, T. and Ha, T. 2015. Effects of extraction process on phenolic content and antioxidant activity of soybean. *J Food Nutr Sci*., 3(1-2): 33-38.

Escribano-Bailon, M.T., Santos-Buelga, C. 2003. Polyphenols extraction from foods. In methods in polyphenol analysis. Royal Society of Chemistry. Thomas Graham House, Science.

Fischer, U.A., Carle, R. and Kammerer, D.R. 2013. Thermal stability of anthocyanins and colourless phenolics in pomegranate (*Punica granatum* L.) juices and model solutions. *Food Chem*., 138:1800-1809.

Galvan, D., Kriaa, K., Nikov, I. and Dimitrov, K. 2012. Ultrasound assisted extraction of polyphenols from black chokeberry. *Sep Purif Technol*., 93: 42-47.

Hamia, C., Guergab, A., Rennane, N.E., Birache, M., Haddad, M., Saidi, M. and Yousfi, M. 2014. Influence des solvants sur le contenu en composes phénoliques et l'activité antioxydante des extraits du *Rhanterium adpressium*. *Annales des Sciences et Technologie*, 47: 33-38.

Hamzaa, N., Berkea, B., Chezea, C., Aglib, A.N., Robinsona, P., Ginc, H. and Moorea, N. 2010. Prevention of type 2 diabetes induced by high fat diet in the C57BL/6J mouse by two medicinal plants used in traditional treatment of diabetes in the east of Algeria. *J Ethnopharmacol*., 128: 513-518.

Harbourne, N., Marete, E., Jacquier, J.C. and O'riordan, D. 2009. Effect of drying methods on the phenolic constituents of meadowsweet (*Filipendula ulmaria*) and willow (*Salix alba*). *LWT - Food Sci Technol.* 42: 1468-1473.

Hirasa, K., Takemasa, M. 1998. Spice science and technology. New York: Marcel Dekker.

Hismath, I., Wan, A.M. and Ho, C.W. 2011. Optimization of extraction conditions for phenolic compounds from neem (*Azadirachta indica*) leaves. *Int Food Res J.,* 18(3): 931-939.

Iserin. P. 2001. Encyclopédie des plantes médicinales, Larousse VUEF, 2^{éme} Ed. Vol. 14. Paris. 335 pp.

Kamatou, G.P.P., Viljoen, A.M., Gono-Bwalya, A.B., Van Zyl, R.L., Van Vuuren, S.F., Lourens, A.C.U., Başer, K.H.C., Demirci, B., Lindsey, K.L., Van Staden, J. and Steenkamp, P. 2005. The *in vitro* pharmacological activities and a chemical investigation of three South African *Salvia* species. *J Ethnopharmacol.,* 102: 382-390.

Kim, J.M., Chang, S.M., Kim, I.H., Kim, Y.E., Hwang, J.H., Kim, K.S. and Kim, W.S., 2007. Design of optimal solvent for extraction of bio-active ingredients from mulberry leaves. *Biochem Eng J.,* 37: 271-278.

Kotan, R., Kordali, S., Cakir, A., Kesdek, M., Kaya, Y. and Kilic, H. 2008. Antimicrobial and insecticidal activities of essential oil isolated from Turkish *Salvia* hydrangea DC. ex Benth. *Biochem Syst Ecol*., 36:360-368.

Ksouri, R., Megdiche, W., Debez, A., Falleh, H., Grignon, C. and Abdelly, C. 2007. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. *Plant Physiol. Biochem.,* 45: 244-249.

Machiex, J., Fleuriet, A. and Jay-Allemand, C. 2005. Composés phénoliques des végétaux. Presses polytechniques et universitaires Romandes. Pp 75-85.

Mahmoudi, S., Khali, M., Mahmoudi, N. 2013. Etude de l'extraction des composés phénoliques de différentes parties de la fleur d'artichaut (*Cynara scolymus* L.). *Nature et Technologie*, 2: 35-40.

Maisuthisakul, P., Pasuk, S. and Ritthiruangdej, P. 2008. Relationship between antioxidant properties and chemical composition of some Thai plants. *Food Compos Anal*., 21: 229-240.

Majhenic, L., Knez, Z. and Kerget, M.S. 2007. Antioxidant and antimicrobial activity of guarana seed extracts. *Food Chem.,* 104:1258-1268.

Makhlouf, C., Noureddine, T. and Hayette, L. 2013. Extraction des compounds phénoliques et activité antioxydante *in vitro* des graines de figue de barbarie. *Journal of economic development*, 67: 467-475.

Mehmet, A., Akalina, K., Kubilay, T., Selhan, K. 2015. Sage oil extraction and optimization by response surface methodology Mehmet. *Ind Crop Prod*. 76: 829-835.

Michel, T. 2011. Nouvelles méthodologies d'extraction, de fractionnement et d'identification : Application aux molécules bioactives de l'argousier (*Hippophae rhamnoides*). Thèse de doctorat en chimie Analytique Phytochimie. Université d'Orléans.

Middleton, E., Kandaswami, C., Theoharides, T. 2000. The effects of plants flavonoids on mammalian cells: Implication for inflammation, heart disease, and cancer. *Phamacol rev.,* 52: 673-751.

Nazck, M. and Shahidi, F. 2004. Extraction and analysis of phenolics in food. *J Chromatogr A.* 1054: 95-111.

Nicola, E.D., Owen, J.C., Rosemary, F.W., Kevin, A.M., Yeap, F. and Nigel, BP. 2007. Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol–water mixtures. *Food Chem*., 101:1417-1424.

Nijveldt, J., Van Nood, E., Van Hoorn, D., Boelens, P., Van Norren, K. and Van Leeuwen, P. 2001. Flavonoids: a review of probable mechanisms of action and potential applications. *Am j clin nutr*. 74: 418-425.

OMS, 2003. (Organisation Mondiale de la Santé) Principes méthodologiques généraux pour la recherche et l'évaluation relatives à la médecine traditionnelle. Pp 31-35.

Perry, N.S., Bollen, C., Perry, E.K. and Ballard, C. 2003. *Salvia* for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. *Pharmacol Biochem Behav*., 75: 651-659.

Pinelo, M., Rubilar, M., Sineiro, J. and Nunez, M. J. 2005. Effect of solvent, temperature, and solvent-to-solid ration the total phenolic content and antiradical activity of extracts from different components from grape pomace. *J Agric Food Chem*., 53: 2111-2117.

Prasad, K.N., Yang, B., Zhao, M.M., Ruenroengklin, N., Jiang, Y.M. 2009. Application of ultrasonication or high-pressure extraction of flavonoids from Litchi fruit pericarp. *J Food Process Eng*. 32(6): 828-843.

Senol, F., Orhan, I., Celep, F., Kahraman, A., Dogan, M., Yilmaz, G. and Sener, B. 2010. Survey of 55 Turkish *Salvia* taxa for their acetylcholinesterase inhibitory and antioxidant activities. *Food Chem*., 120:34-43.

Shuangqin, M., Xijuan, T., Jiangtao, D., Peng, L., Wenchao, Y., Xiaoqing, M., Wenbin, C. and Zhenhong, W. 2015. Soxhlet-assisted matrix solid phase dispersion to extract flavonoids from rape (*Brassica campestris)* bee pollen. *J Chromatogr. B.,* 1005: 17-22

Silva, E.M., Rogez, H. and Pompeu, D.R. 2009. Optimisation of extraction of phenolics from fruits of *Euterpe oleracea* using Response Surface Methodology. *Bioresource Technol.* 100: 6076-6082.

Spigno, G., Tramelli, L. and De Faveri, D.M. 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *J Food Eng.,* 81: 200-208.

ST-Pierre, F. 2012.Caractérisation physico-chimique de bois et d'écorces de *Betula alleghaniensis* et *Acer saccharum* de différentes vigueurs. Doctorat s'sciences. Université Laval.

Tabart, J., Kevers, C., Sipel, A., Pincemail, J., Defraigne, J. and Dommes, J. 2007. Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and stability during storage. *J Food Chem.,* 105: 1268-1275.

Tepe, B., Daferera, D., Sokmen, A., Sokmen, M. and Polissiou, M. 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chem.*, 90: 333-340.

Turkmen, N., Velioglu, Y.S., Sari, F., Polat, G. 2007. Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. *Molecules.* 12(3): 484-496.

Vanessa, M., Renata, L., José, R., Souzab, C., Zequic, E., Leite, M., Gisely, C., Lopesa, M. 2014. Extraction of flavonoids from *Tagetes patula*: process optimization and screening for biological activity. *Rev Bras Farmacogn.* 24: 576-583.

Wang, L. and Weller, C.L. 2006. Recent advances in extraction of nutraceuticals from plants. *Trends Food Sci Technol*., 17(6): 300-312.

Wissam, Z., Bashour Ghada, B., Wassim, A. and Warid, K. 2012. Effective extraction of polyphenols and proanthocyanidins from pomegranate's peel. *Intl J Pharm Pharm Sci*. 4(3): 675-682.

Yang, B., Liu, X. and Gao,Y. 2009. Extraction optimization of bioactive compounds (crocin, geniposide and total phenolic compounds) from Gardenia (Gardenia jasminoides Ellis) fruits with response surface methodology. *Innov Food Sci Emerg*., 10: 610-615.

Yap, C.F., Ho, C.W., Wan Aida, W.M., Chan, S.W., Lee, C.Y. and Leong, Y.S. 2009. Optimization of extraction conditions of total phenolic compounds from star fruit (*Averrhoa carambola* L.) residues. *Sains Malaysiana*, 38(4): 511-520.

Yilmaz, Y. and Toledo, R.T. 2006. Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. *J Food Compos Anal*., 19: 41-48.