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STUDY OF THE INFLUENCE OF SOLVENTS ON THE POLYPHENOLS CONTENT OF *SALVIA OFFICINALIS* L EXTRACTS AND THE EFFECT ON ANTIOXIDANT ACTIVITY

S. Tidjani, C. Adaika, S. Djaber

Department of Chemistry, Faculty of Exact Sciences, University of El Oued, 39000, Algeria

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ABSTRACT

Algerian *Salvia Officinalis* L aerial parts were extracted with pure solvents in increasing polarity (hexane, acetone, methanol and 80% aqueous methanol. All extracts were examined by assessing their content in polyphenols and the antioxidant levels were evaluated by two in vitro tests using spectroscopic and the voltametric methods. The quantitative assessment of total phenols and flavonoids by the colorimetric method make it clear that the extracts are very rich of these compounds. The hydro-methanol and methanol extracts gave the highest DPPH and $O_2^{\cdot-}$ radicals scavenging activities (12.15 µg/ml) and (0.704 mg/ml) respectively.

Keywords: *Salvia Officinalis* L, Antioxidant activity, DPPH, Radical superoxide $O_2^{\cdot-}$, Solvent extraction, Polyphenols, Flavonoids.

Author Correspondence, e-mail: soukaina-tidjani@univ-eloued.dz

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1. INTRODUCTION

The genus Salvia includes about 1000 species in the Lamiaceae family [1]; the studied plant is one of them. It's an aromatic plant, that has varying degrees of antibacterial, antioxidant,

antifungal, antiviral, cytotoxic, antiinflammatory properties, It also has antispasmodic, feverish, antiseptic, hepato-stimulant, choleretic and cholagogue and other biological activities [2,3]. The infusions of this plant are applied for the treatment of several diseases of blood circulation, digestive disorders and nervous system problems [4]. This genus *is* represented in Algeria by sixteen species, among which *S. Officinalis* L. its vernacular name in Algeria is: « souekennebi» [5,6]. Today, herbal remedies are gaining strength, because the effectiveness of certain antioxidant polyphenols and flavonoids compounds that can help to delay the progress of many chronic diseases as well [7]. A few studies have examined the impact of solvents on the polyphenol content [8].

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant material

The plant was collected in the North - East of the Algerian Sahara in July 2018, El-Oued, Algeria and then was identified by Dr. Slimani Noureddine, Biology department - El Oued University.

2.1.2. Chemicals

1,1-diphenyl-2-picrylhydrazyl, Aluminium chloride, sodium carbonate, Ferric chloride, Quercetin, Ascorbic acid, N,N-dimethylformamide, Gallic acid, tetrabutylammonium hexafluorophosphate.

2.2 Preparation of extracts

Preparation of extracts required an amount of 100 g of the dry powder of *S. Officinalis* L that was extracted in increasing polarity with successively n-hexane, acetone, methanol and 80 % aqueous methanol. The procedure of this study was described by Soares et al [9] with minor modifications inorder to provide the effect of solvent on the content of phenolic compounds. The macerate of the plant was filtered and concentrated to dryness, then kept in the fridge for further experiments.

2.2.1. Calculation of total polyphenols content

Analysis of the polyphenols content is based on the spectrometric method using the

Folin-Ciocalteu reagent, following the procedure adopted by Singleton [10]. A solution of 200 µl of extract was prepared, then transferred into a test tube and mixed with 1 ml of Folin-Ciocalteu reagent, already diluted 10-fold with water. After 5 min, 0.8 ml of Na_2CO_3 (7.5g /L) was added, the mixture was left for 30 minutes in the dark, the absorbance were then measured at 760 nm. Gallic acid was used as a reference to determine the polyphenols content and the results are expressed in Gallic acid equivalent GAE/g) in dry weight, corresponding to the graphic equation: Absorbance = 11.12 mg Gallic acid + 0.049 (r^2 = 0.991).

2.2.2. Calculation of total flavonoids content

The analysis of flavonoids content was carried out using the spectrometric method with aluminium chloride reagent [11]. The absorbance was measured at 430 nm, quercetin was used as a reference to determine the flavonoids content and the results are expressed in Quercetin QE/g in dry weight, corresponding to the graphic equation: Absorbance = 49.27 mg Quercetin - 0.315 (r^2 = 0.978).

2.3. Determination of antioxidant activity

2.3.1. DPPH˙ radical scavenging assay

The aim of the current study was to determine the antioxidant power of the obtained extracts by evaluation of the scavenging ability of compounds towards the stable free radical 1,1-diphenyl-2-picrylhydrhazyl (DPPH) reduced to the corresponding colorless hydrazine upon reaction with hydrogen donors as described by Blois and Özturk with slight modification [12,13]. This method is based on the dissolution of the dry extracts n-hexane, methanol, hydromethanol and acetone of *salvia officinalis* L in methanol so as to obtain a concentration of 10.5 mg/ml (mother solution) for each extract. These serial dilutions of extracts dissolved in methanol were mixed with 200 µl of methanol 0.004% DPPH and left for 30 minutes in the dark, the absorbances were measured at 517 nm, using methanol as control. To determine the IC_{50} (concentration of plant compound which inhibits 50% of DPPH).

The capability to scavenge the DPPH radical of an antioxidant was calculated using the following equation:

$$(\%)\ DPPH\ Scavenging\ effect\ = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test extract or standard), and A_{sample} is the absorbance of the extract or standard. Ascorbic acid was used as a reference and the results are expressed in ascorbic acid equivalent Aa-E/g in dry weight.

2.3.2. Quenching of electrochemically generated superoxide anion ($O_2^{\cdot-}$)

One well-known early study that is often cited in research on superoxide anion radical scavening activities is that of Bourvellec and al in 2008 [14]. Samples of extracts rich in polyphenols were analysed using the same method to evaluate the antioxidant activity. The procedure is based on a cyclic voltammetry experiments using voltlab 40 model PGZ 301 potentiostat/galvanostat (Radiometer analytical SAS) driven by personel computer with voltamaster 4 software. All analyses have been conducted using three-electrode, a glassy carbon disk working electrod (diameter 3 mm), a platinium wire counter electrod and a reference electrode (SCE): saturated calomel electrode. The superoxide anion radical $O_2^{\cdot-}$ was generated by reduction of the commercial molecular oxygen (O_2). First we saturate the electrochemical cell with oxygène for 15 minutes, the cell alredy contain dimethylformamide as an organic solvent and tetrabutyl ammonium hexafluorophosphate salt (TBFP) at the concentration 0.1 M as an electrolyte, the mixture is stirred for 5 minutes in order to obtain a homogeneous solution, and then the extract of the plant was injected into the cell. The scan potential speed is 100 mv/s and the potentiel window was from -1,6 to 0,0 V versus (SCE) at room temperature 28 °C. Samples of plant extracts and the standard Quercetin (5 mg/ml) were successively added to the 10 ml of oxygen solution to get concentration in the range of 40 - 500 µg/ml.

The capacity of tests sample to reduce superoxide anion radicals $O_2^{\cdot-}$ was determited using the following equation:

$$(\%)\ O_2^{\cdot-}\ Scavenging\ effect\ = \frac{I_{P0} - I_{Px}}{I_{P0}} \times 100$$

Where I_{P0} and I_{Px} are successively the anodic current densities of superoxide anion radical $O_2^{\cdot-}$ in the absence and in presense of the plant extracts.

3. RESULTS AND DISCUSSION

3.1 extract yield, total phenolics and flavonoids content

The yields of the extraction with hexane and methanol as shown in Table 1 were higher compared to other solvants. Obtained results of total phenolic content (TPC) and total flavonoid content (TFC) are presented in the Table 1. The content obtained with acetone extract was the highest TPC (87.62 mg GAE/g DW), where as the total phenolic content of methanolic extract was much smaller (28.57 mg GAE/g DW). Besides that the content obtained with acetone extract from S. officinalis L was the highest content TFC (43.25 mg QE/g DW). Sonja Duletić-Laušević et al [15] found that alcohols extract of *Salvia officinalis* L plants originated from Belgrade have revealed to be more efficient in extracting phenolics constituents. Abdeslam Et-Touys et al [16] studies give that The TPC of MeOH, n-hexane and EtOH extracts from Salvia officinalis L of Morocco present the highest content. *Salvia* species are usually dispersed throughout the papers and details of the bibliography are often difficult to compare for the reason that of practical differences, type of extraction solvent affects the extraction of phenols as indicated before [17]. Many researchers showed that the difference between the yield of total phenolics and flavonoids content and the total extractable compounds were probably due to the plant part used for extraction, time of sampling, extraction techniques, environmental and genetic factors (location, temperature, climate, diseases, pests) [18-21].

Table 1: Extraction yield, total phenolics and flavonoids content

Tested extracts	Extraction yield %	TPC mg (GAE)/ g	TFC mg (QE)/g
Hexane	27.27	49.55	22.19
MeOH	10.14	28,57	19.66
MeOH/H_2O (80%)	2.26	48.95	15.94
Acetone	4.08	87,620	43.25

3.2. Antioxidant activity

There are many methods for measurement of antioxidant potential. Thus, in the second part of our study we assayed two in vitro antioxidant activity methods on extracts of the plant *Salvia officinalis* L: spectroscopic and voltametric methods.

3.2.1. DPPH radical scavenging activity

In DPPH assay, the hydro-methanolic extract of *S. officinalis* showed the best scavenging activity with an IC_{50} of (12.15µg/ml), followed by methanol extract (43.78 µg/ml) better than standard ascorbic acid (62.24 µg/ml). In general, *S. officinalis* extracts demonstrated strong antioxidant effect. Such properties have been previously reported from this plant [22, 23]. This effect is mainly due to antioxidant compounds presented in *S. officinalis* such as rosmarinic acid, carnosic acid, salvianolic acid and its derivatives carnosol, rosmanol, epirosmanol, rosmadial and methyl carnosate [24-26]. The methanolic extracts of the various parts of the *salvia Officinalis*, also show an efficiency in trapping the DPPH radical with IC_{50} values between (18.4 and 81.7 µg / mL) [27], whereas acetone extract demonstrated a lower activity with IC_{50} between (61.8 and> 5000 µg / mL), in conformity with the present work. The results of Albano and Miguel [28] recorded even higher IC_{50} than the other studies with an IC_{50} of 2.8 µg / ml, using different solvents in increasing polarity (diethylether, ethylacetate and ethanol) to finish with an extraction whith an ethanol / water solution (70:30, v/v). According to our IC_{50} values, it appears that the antioxidant capacity is higher for extracts of Salvia Officinalis with the following sequence: Aqueous methanol extract> methanol extract> hexane extract> acetone extract. This efficiency may be due to the fractionation procedure and the structure of molecules contained in the various extracts.

3.2.2. Free superoxide anion radical (O_2^{-}) radical-scavenging ability

This recent method based on the quenching of the (O_2^{-}) radical, was applied to *S. Officinalis* extracts for the first time. The measurement of the antioxidant capacity was estimated by

antioxidant index values AI_{50}, defined as extract concentrations necessary to consume 50% of the electrogenerated radical [29], calculated from the linear equation (y: the percentage antioxidant activity and x: extract concentration (tableau 2). The obtained results (figure 1) show that the addition of *S.Officinalis* extracts causes a proportional decrease of $(O_2^{\cdot-})$ anodic pic. It suggests that extracts reacts irreversibly with $(O_2^{\cdot-})$. With this characterization, the lower AI_{50} value, the more the sample has strong reactivity towards superoxide. It can be seen that AI_{50} of Methanol extract showed the lowest value (0.704 mg/ml) it corresponds to the highest antioxidant activity little different from that of the standard quercetin (0.232 mg/ml) followed by hydro- methanol extract (0.880 mg/ml). While the highest value was detected in acetone extract (1.946 mg/ml). It is assumed that the extracts with less antioxidant activity are less rich in flavonoids endowed with trapping activity free radicals whose activity is strictly related to the structure of the flavonic compound itself, of which numerous studies have established the relationship between the structure and the antiradical activity of the flavonoids [30]. The (Table 2) summed the results of antioxidant activity of all studied extracts.

Table 2: IC_{50} (µg/ ml) and AI_{50} (mg /ml) values of *Salvia officinalis* L extracts and standards obtained using DPPH and $O_2^{\cdot-}$ radical scavenging activity.

Sample	Methods	Equation	r^2 values	$O_2^{\cdot-}$ AI_{50} (mg/ml)	DPPH IC_{50} (µg/ ml)
Hexane	DPPH	y = 0.875 x + 2.365	0.996	-	54.0.6
	$O_2^{\cdot-}$	y = 32.70 x + 16.15	0.928	1.035	-
Methanol	DPPH	y = 0.793 x +15.20	0.846	-	43.78
	$O_2^{\cdot-}$	y = 0.720 x -1.213	0.990	0.704	-
MeOH/H_2O	DPPH	y = 2.226 x + 22.93	0.940	-	12.15
	$O_2^{\cdot-}$	y = 47.53x + 8.167	0.992	0.880	-
Acetone	DPPH	y = 0.535 x + 18.28	0.979	-	59.16
	$O_2^{\cdot-}$	y = 24.91x + 1.519	0.846	1.946	-
Ascorbic acid	DPPH	y = 0.643x + 9.943	0.956	-	62.244
quercetin	$O_2^{\cdot-}$	y = 218.5x – 0.801	0.982	0.232	-

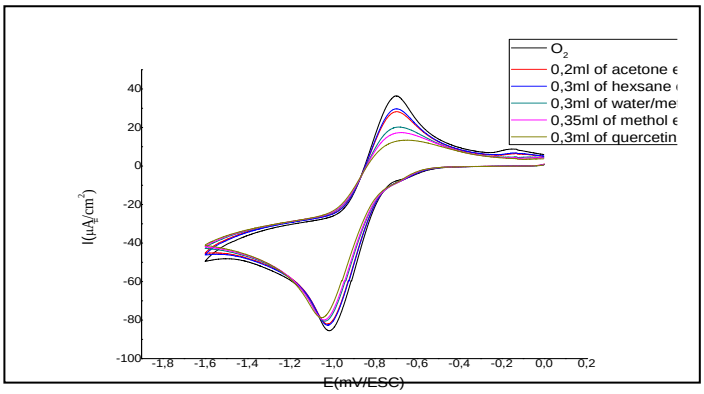

Fig.1. Cyclic voltammograms of the O_2 / $O_2^{\cdot-}$ redox couple in oxygen-saturated DMF/ 0.1 TBFP of tested extracts

4. CONCLUSION

The present study proposed to carry out the quantification of polyphenolic and flavonoids compounds, and the evaluation of the antioxidant activity of Algerian *Salvia Officinalis* L by different solvent and extraction method. According to our results, acetone and hexane extracts have high total phenolic and flavonoids content, but in parallel the results of their capacity as an antioxidant remains moderate. The richness of extracts of methanol and hydro-methanol in phenolic compounds, compared to the other extracts, is the main cause of the result obtained. It is assumed that the extracts with less antioxidant are less rich in flavonoids compounds that are endowed with a trapping activity of free radicals, whose activity is strictly related to the structure of the flavonic compound itself of which numerous studies have established the relation ship between the structure and the antiradical activity of the flavonoids. We concluded that, extraction method is very important in the selection of the most appropriate extract for further study remains to be discussed.

5. ACKNOWLEDGEMENTS

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