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# Biological activities of phenolic extracts of a medicinal plant, endemic to the Algerian Sahara: *Salvia chudaei* Batt. & Trab

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## Abstract

The flora of the Algerian Sahara is rich in aromatic plants used in traditional medicine and many of them are endemic species that can be valued as a source of bioactive products. Phenolic compounds of plant material, find jobs in various sectors. This study was carried out to determine the total phenolic content and flavonoid, in addition to the evaluation of the biological activities of the phenolic compounds extracted from the aerial and underground parts of the medicinal plant endemic to the Algerian Sahara: *Salvia chudaei* Batt. & Trab. (Lamiaceae). This plant was subjected to two types of extractions (Soxhlet and maceration). The aerial part extract obtained by Soxhlet had a higher total phenolic content and flavonoid. The antioxidant activity of these extracts was evaluated using the DPPH test. Extracts from the aerial and underground parts obtained by maceration showed strong antioxidant activity with $EC_{50}$ values of 14 and 15 µg/mL respectively, whereas the $EC_{50}$ of the reference (ascorbic acid) was 22 µg/mL. The results of the anti-hemolytic activity carried out by the $H_2O_2$ test revealed that the extract of the underground part obtained by maceration has a good anti-hemolytic activity ($EC_{50}$ 55 µg/mL).

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## Introduction

Medicinal plants can be considered as reservoirs of bioactive molecules not yet explored, among these biomolecules, phenolic compounds (phenolic acids, flavonoids, condensed tannins etc.) (Karmakar *et al.,* 2011).The genus *Salvia*, the largest genus of the Lamiaceae family, includes nearly 900 species distributed throughout the world. Algeria has 23 species of the genus *Salvia* (Quezel and Santa, 1963). A large number of *Salvia* spp. are used as infusions and food flavorings, in cosmetics, perfumery and in the pharmaceutical industry. *Salvia* species have been described as species with a wide range of biological activities, including anti-cholinesterase, antibacterial, antimalarial, anticancer and antioxidant (Perry *et al.,* 2003; Tepe *et al.,* 2005; Kamatou *et al.,* 2008; Kotan *et al.,* 2008).

The aim of this study is to evaluate the antioxidant and anti hemolytic activities of the phenolic compounds extracted from the aerial and underground parts of the endemic species in the Algerian Sahara: *Salvia chudaei* Batt. & Trab.

## Materials and methods

*Plant material*

The plant material consists of the aerial and underground parts of *S. chudaei* harvested in October 2015 in the Tamanrasset region of southern Algeria, N 23 ° 81 '756 "East: E005 ° 93' 888".The plant was identified by the botanists of the National Institute of Forest Research -INRF- Research Station for the protection of arid zones-Tamanrasset (Algeria). A specimen of the plant was deposited in the herbarium (number PM/03) laboratory biogeochemistry desert environments, Ouargla University (Algeria).

*Extraction of phenolic compound*

The extraction of the phenolic compounds of *S. chudaei* was carried out by Soxhlet with a mixture of ethanol/water (80%), after three extraction cycles and duration of 100 min for the aerial part and 20 min for the underground part. Extraction by maceration was carried out by the same binary for 60 min, at 30°C for the aerial part and at 15°C for the underground part.

These conditions were chosen after an optimization of the extraction conditions carried out in the laboratory.

*Determination of total phenolic content (TPC)*

The total phenol concentration of the extracts of the different parts of the studied plant was determined by the method of Singleton and Ross (1965), using the Folin-Ciocalteu reagent. From a prepared stock solution of 10 mmol/L gallic acid, daughter solutions are prepared with concentrations ranging from 0.2 mmol/L to 2.4 mmol/L. 200 µl of each solution or extract are introduced into test tubes to which 1 mL of the Folin-Ciocalteu reagent (10%) is added. After 2 minutes, 4 mL of 20% (w/v) sodium carbonate ($Na_2CO_3$) is added. The solutions are kept in the dark for 2 hours at room temperature. The absorbance of each solution is read using a LNICAM-DISCPD2000-1 UV-Vis spectrophotometer at a wavelength of 765 nm against a blank (same solution without the gallic acid solution).For the phenolic extracts, the measurements were carried out by the same protocol. The TPC is expressed in milligrams of gallic acid equivalent per milligram of extract (mg EAG/mg extract).

*Dosage of flavonoids*

Quantification of flavonoids was carried out with aluminum trichloride ($AlCl_3$) (Bahorum *et al.,* 1996). Aluminum trichloride forms a yellow complex with flavonoids. The complex formed absorbs in the visible at 415 nm. The flavonoid used as a reference in this method is rutin. From the rutin solution 1 mmol/L, the concentrations solutions ranging from 0.01 to0.05 mmol/L are prepared. 1 mL of each solution of the rutin or extract is added to 1 mL of 20% (w/v) $AlCl_3$. After incubation in the dark for 40 minutes at room temperature, the assay is carried out by UV/Visible spectrophotometry at 415 nm. The flavonoid content (TF) is expressed in milligrams of rutin equivalent per milligram of extract (mg ER/mg extract).

*Antioxidant activity*

The DPPH (diphenylpicryl-hydrayl) method is based on the reduction of an alcoholic solution of the stable radical species DPPH• in the presence of a hydrogen-

donor antioxidant (AH), which results in the formation of a Non-radical form, DPPH-H (diphenyl picryl hydrazine) (Blois, 1958).

A volume of 100 µL of reference extract or antioxidant (ascorbic acid) is added to 3 mL of the DPPH solution ($5\times10^{-6}$ mol/L) freshly prepared in methanol. After a 30 min rest in the dark, the absorbance is measured at 517 nm against the white. The anti-radical activity of the extracts is expressed as a percentage inhibition of the DPPH radical according to the equation:

$$\% \text{ Inhibition} = ((Abs_{control} - Abs_{sample}) / Abs_{control})x100$$

The antioxidant efficiency of the extracts is determined by the calculation of the $EC_{50}$ parameter, which represents the inhibitory concentration necessary to decrease the free radical content in the reaction medium by 50%.The antioxidant capacity of a compound is higher the lower it's $EC_{50}$(Brand-Williams *et al.,* 1995).

*Anti-hemolytic activity*

The anti-hemolytic activity is carried out *in vitro* according to the protocol described by Manna *et al.* (2002), with some modifications. This activity is based on the power of phenolic extracts of *S. chudaei* to prevent the destruction of red blood cells.

The human blood used in this test is obtained by venous sampling from a non-smoker healthy volunteer. The blood collected in a heparin tube is centrifuged at 1500 g/5 min, after removal of the plasma; the pellet is washed three times with volumes of phosphate buffer (PBS without potassium, pH 7.4 and 0.1 M).

During each wash, the suspension is homogenized by simple turning of the tube, the supernatant and the interface layer are removed immediately after centrifugation. At the end of the last centrifugation, the cell pellet obtained is diluted with the same phosphate buffer to obtain a hematocrit of 4%. Two milliliters of the erythrocyte suspension are mixed with 5 mL of PBS solution.

Radical attack was induced by the addition of 0.5 mL of $H_2O_2$ to the pre-incubated erythrocyte suspension (15 min) with 1 mL of each daughter solution of ascorbic acid or extract at different concentrations. After two hours incubation with $H_2O_2$ at 37°C., the reaction mixture is centrifuged at 1500 g/10 min and hemolytic (hemoglobin release) is evaluated by measuring the absorbance of the supernatant at 540 nm.

The concentration of $H_2O_2$ in the reaction mixture was adjusted to cause 90% hemolytic of the red blood cells after 2 h of incubation.

The resistance of the blood to free radical attack is expressed by the percentage inhibition:

$$\% \text{ Hemolysis inhibition} = ((Abs_{control} - Abs_{sample}) / Abs_{control})x100$$

% hemolytic inhibition: percentage inhibition of hemolytic; $Abs_{Control}$: absorbance of the control; $Abs_{Sample}$: absorbance of the sample.

The anti-hemolytic activity of the extracts is determined by the calculation of the parameter $EC_{50}$ which represents the inhibitory concentration necessary to reduce 50% hemolytic of the red blood cells.

*Statistical analysis*

The data were analyzed using R (Windows GUI front-end) and MatLab (Version 16) softwares. All data were expressed as means ± standard deviations of triplicate 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 have been established between the different variables.

**Results and discussion**

*Quantitative estimation of total phenols and flavonoids*

The results of quantitative estimation of total phenolic, flavonoid content and the yield of phenolic extracts from the aerial and underground parts of *Salvia chudaei* using two types of extractions (Soxhlet and maceration) were mentioned in the table 1.

**Table 1.** Total phenolic, flavonoid content and the yield of phenolic extracts from *S. chudaei*.

| Extraction method | Plant part | TPC (mg EAG/mg extract) | TF (mg ER/mg extract) | Yield (%) |
|---|---|---|---|---|
| Maceration | Aerial | 0.016 ± 0.0004 | 0.062 ± 0.0005 | 11.7 ± 0.008 |
| | Underground | 0.004 ± 0.0001 | 0.03 ± 0.0003 | 2.07 ± 0.552 |
| Soxhlet | Aerial | 0.023 ± 0.0003 | 0.071 ± 0.0004 | 12.7 ± 0.003 |
| | Underground | 0.015 ± 0.0008 | 0.033 ± 0.0003 | 8.4 ± 0.337 |

TPC : Total Phenolic Content ; TF : Total flavonoïds.

The results of the assay reveal that the amount of the phenolic compounds in the crude extracts is greater by using the Soxhlet as the extraction method.

The largest amounts of total phenols are recorded in the aerial part, for both methods.

These quantities were (0.023 ± 0.0003 mg EAG/mg extract) for Soxhlet and (0.016 ± 0.0004 mg EAG/mg extract) for maceration. The amounts measured for the underground part were (0.015 ± 0.0008 mg EAG/mg extract) for Soxhlet and (0.004 ± 0.0001 mg EAG/mg extract) for maceration.

**Table 2.** Antioxidant activity of the phenolic extracts of *S. chudaei*.

| Extraction method | Plante part | $EC_{50}$ (µg/mL) |
|---|---|---|
| Maceration | Aerial | 15 ±0.38 |
| | Underground | 14 ±0.12 |
| Soxhlet | Aerial | 34 ±0.66 |
| | Underground | 34 ±0.32 |
| Ascorbic acid | | 22 ±0.27 |

The results of the estimation of the flavonoid content showed that the aerial part is the richest. The quantities ranged from (0.071 ± 0.0004mg ER/mg of extract) for the extract obtained by Soxhlet and (0.062 ± 0.0005mg ER/mg of extract) for those from the maceration, followed by extracts from the underground part, whose quantities ranged from (0.033 ± 0.0003mg ER/mg of extract) For Soxhlet and (0.03 ± 0.0003 mg ER/mg of extract) for maceration.

The results of the polyphenol and flavonoid assays revealed the presence of these compounds in both parts of *Salvia chudaei* with different amounts depending on the extraction method, the part of the plant and the type of the solvent. The aerial part was the richest ($p < 0.05$) in these compounds. There is a significant difference ($p < 0.05$) in the TPC and TF results.

The aerial part extract obtained with Soxhlet present the highest yield (12.7%), followed by the aerial part extract obtained with maceration (11.7%).

From the results obtained, we note that the yields vary from an extraction method to another and from a part of the plant to another.

This difference is explained by the difference in diffusion of the solvent in the plant powder in the maceration stage and probably by the nature of the solvents used for the extraction (Naczk and Shahidi, 2004).

In the literature, several plants of the genus *Salvia* were evaluated for their phenolic content (Asadi*et al.,* 2010).Indeed, numerous studies have shown that extrinsic factors (such as geographical and climatic factors), genetic factors but also the degree of maturation of the plant and the duration of storage have a strong influence on the polyphenol content (Aganga and Mosase, 2001; Hamia *et al.,* 2014).We can clearly see that the majority of these species, like the one targeted in this study, represent a promising source of phenolic compounds.

**Table 3.** Anti-hemolytic activity ($EC_{50}$)of the phenolic extracts of *S. chudaei.*

| Extraction method | Plant part | $EC_{50}$(µg/mL) |
|---|---|---|
| Maceration | Aerial | 87 |
| | underground | 55 |
| Soxhlet | Aerial | 120 |
| | underground | 22 |
| Ascorbic acid | | 16 |

*Biological activities of extracts of Salvia chudaei*

*Antioxidant activity*

The antioxidant capacities of the extracts of the plant studied were determined and compared with the activity of the standard, ascorbic acid. The results obtained for the DPPH test, expressed in terms of a 50% inhibitory concentration of the radicals ($EC_{50}$), are presented in table 2.

From these results, we note that the $EC_{50}$ values vary from an extraction method to another. Phenolic extracts obtained by aerial and underground maceration had nearly similar $EC_{50}$ values of 15 and 14 µg/mL, respectively. However, the Soxhlet extracts showed the same $EC_{50}$ value, which is 34 µg/mL and therefore less active than the first extracts obtained with maceration. Since these extracts have not shown any particular richness in total phenols or flavonoids, their important antioxidant activity can therefore be explained by the presence of other types of molecules endowed with this activity.

The antioxidant activity of our extracts was compared to that of vitamin C, used as a reference antioxidant. The latter is often used as a synthetic antioxidant in the agro-food industries. We observed that extracts obtained by maceration have a very high antioxidant power compared to that of vitamin C ($EC_{50}$ = 22 µg/mL), whereas Soxhlet extracts have been shown to be much less active than vitamin C.

Statistically, the difference between the results of the different parts and the two extraction methods used is very highly significant ($p < 0.001$).

We did not find a linear correlation between the $EC_{50}$ values and the phenol or flavonoid amounts. This was expected since the values reported in tables 1 and 2shows an independence of the antioxidant activity of the total phenol and flavonoid amounts.

This observation of the antioxidant activity of the extracts of this plant depends on other chemical structures of antioxidant compounds contained in these extracts.

In the study of Krimat *et al.,* (2015), the aerial part of *Salvia chudaei*, the $EC_{50}$ (measured by DPPH) of the diethyl ether extract was the most active than the hydro-methanol extract and the chloroform extract. In addition, Matkowski *et al.* (2008) studied the antioxidant activity of phenolic extracts of the roots and leaves of the three plants of the genus *Salvia* (*S. miltiorrhiza, S. verticillata, S. przewalskii*). The reported $EC_{50}$ for root extracts of the first and second species was lower than that of leaf extracts, whereas for the third species the $EC_{50}$ of the leaves was lower than the roots.

Victor *et al.* (2007) tested dichloromethane, ethyl acetate, methanol and aqueous *Salvia pratensis* extracts. The latter did not exhibit an interesting antioxidant activity.

Numerous studies carried out on natural products have shown that phenolic compounds are particularly responsible for antioxidant activity (Apostolidis *et al.,* 2007, Nickavar *et al.,* 2008, Kamatou *et al.,* 2008).

*Anti-hemolytic activity*

The results of the anti-hemolytic activity of the various phenolic extracts shown in table 3 showed that vitamin C had a greater inhibitory capacity than those of the phenolic extracts of *Salvia chudaei.* We have observed that the extract of the underground part, obtained by Soxhlet, exhibits the highest activity ($EC_{50}$ value 22 µg/mL against the underground part extract obtained by maceration ($EC_{50}$55 µg/mL). At the same time, the $EC_{50}$ of the aerial part extract obtained by maceration was lower than that of the Soxhlet extract. They are 87 and120 µg/mL, respectively.

In the literature consulted, no studies on the anti hemolytic activity of *S. chudaei* Batt. & Trab. was discussed. However, studies of the antihemolytic activity of extracts of other plants have been carried out in particular by the work of Suboh *et al.* (2004), which have reported that the extract of *Nigella sativa* seeds protect the erythrocytes from damage Oxidative effects induced by hydrogen peroxide ($H_2O_2$).

Erythrocytes are a very suitable cell model for the study of oxidative stress. Because of their ease of isolation, their simplicity, the richness of their polyunsaturated fatty acid membranes and the high oxygen and hemoglobin cell concentration, these cells are extremely susceptible to oxidative damage (Wajeman *et al.,* 1992; Arbos *et al.,* 2008; Çimen, 2008).

This cellular system could be very useful for the study of the protective effect of the phenolic extracts of the aerial and underground parts of the studied plant against oxidative hemolysis induced by $H_2O_2$.

Under the conditions of this test, the free radicals are generated in the aqueous medium and react with the membrane lipids of the erythrocytes during hemolytic. Nevertheless, lipophilic antioxidants are the most effective, since hydrophilic antioxidants act significantly but they can not directly protect the red cell membrane. In addition, hydrophobic compounds which enter the lipid double layer or indirectly regenerate other antioxidants and/or enter into various enzymatic processes (Lesgards, 2000).

Since ascorbic acid is classified as a water-soluble compound, it can then act against free-radical propagation in the aqueous phase. However, our phenolic extracts show activities against free radicals that are less effective than that observed for ascorbic acid.

By establishing the relationship between total phenols, flavonoids and the total antioxidant capacity of the different extracts of the studied plant and their antihemolytic activity, Person's test correlations shows that there is a correlation between the total phenol contents (68.4%) And total antioxidant capacity but this relationship is low with flavonoids (19.1%).

On the other hand, there is no correlation between the antihemolytic activity and the total polyphenol and flavonoid contents and their antioxidant activity.

It would be interesting to isolate and then identify the active ingredients responsible for these activities and then evaluate them *in vivo*.

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