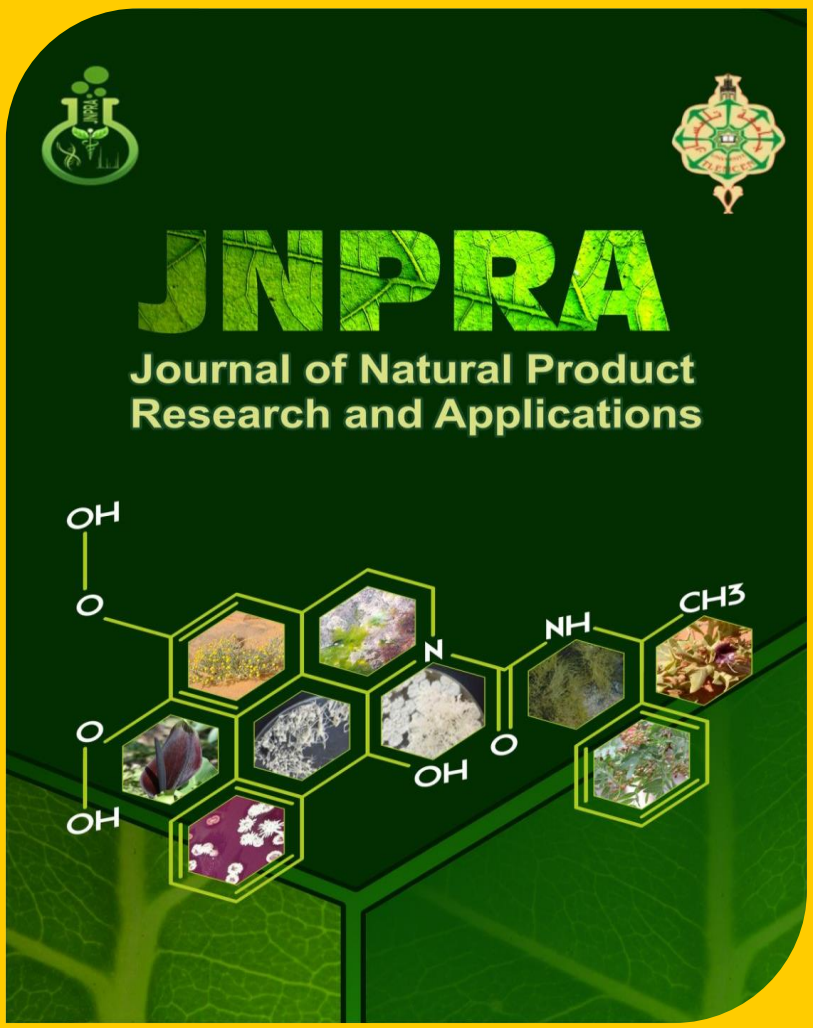
Antioxidant Activity and Hemolytic Effect of Hydro-Methanolic Extract and its Phenolic Enriched Fractions from Leaves and Stems of *Salvia officinalis* L. from Algeria

Souad MAHDI

Rachid AZZI

Farid Boucif LAHFA



Journal of Natural Product Research and Applications
Volume 1, Issue 2

Journal of Natural Product Research and Applications (JNPRA)

Home page : https://journals.univ-tlemcen.dz/JNPRA/index.php/JNPRA

Research Article

OPEN ACCESS

Antioxidant Activity and Hemolytic Effect of Hydro-methanolic Extract and its Phenolic Enriched Fractions From Leaves and Stems of *Salvia officinalis* L. from Algeria

Souad MAHDI, Rachid AZZI [*], Farid Boucif LAHFA

Laboratory Antibiotic, Antifungal, Physico- Chemistry, Synthesis and Biological Activity, Department of Biology, Faculty of Natural and Life Sciences, Earth and Universe Sciences, University of Tlemcen, Algeria
*Corresponding author : rachid.azzi@univ-tlemcen.dz; rachidbio@yahoo.fr.

Highlights

- Extract and fractions of *Salvia officinalis* exhibited potent antioxidant activity.
- This is the first report about *S. officinalis in vitro* hemolytic effect.
- All extracts, mainly ethyl acetate fraction, showed low hemolytic effect.

Graphical Abstract

Abstract

This study aimed to assess an *in vitro* antioxidant and hemolytic activities of crude hydro-methanolic extract, ethyl acetate and n-butanol fractions from leaves and stems of *Salvia officinalis L.* Extract and fractions prepared by maceration from *S. officinalis* were determined for their phytochemical composition and their contents in total phenolic and flavonoid. Antioxidant activity was evaluated by the methods of DPPH radical scavenging, ferric reducing antioxidant power and total antioxidant capacity. Hemolysis assay was carried out to evaluate the toxicity of the studied samples.

Crude extract and fractions from *S. officinalis* contain different secondary metabolites and considerable contents of phenolic and flavonoids. They exhibit high antioxidant activity and low hemolytic effect. However, ethyl acetate fraction is characterized by the highest total phenolic (362.75 ± 0.07 µg GAE/mg DE) and flavonoid (263.27 ± 0.1 µg CE/mg DE) contents. Furthermore, this fraction shows the best antioxidant activity tested by DPPH, FRAP and TAC assays. The antiradical activity of ethyl acetate fraction against DPPH (IC_{50} = 208.51 ± 5.77 µg/mL) is close to that of ascorbic acid (IC_{50} = 206.43 ± 4.16 µg/mL). It exhibits a powerful activity to reduce iron (EC_{50} = 250 ± 0.001 µg/mL) and the best total antioxidant capacity (817.33 ± 0.06 µg/mL). It reveals the lowest hemolysis rate of 3.77 ± 0.02% with 200 mg/mL of fraction, after one hour of incubation.

The obtained results suggest that crude extract, ethyl acetate and n-butanol fractions from *Salvia officinalis* L. are considered efficient natural antioxidants and safe for human erythrocytes.

Keywords: Antioxidant; Hemolysis; Phenolic compounds; *Salvia officinalis.*

Abbreviations

GAE: gallic acid equivalents
CE: catechin equivalents
DE: dry extract
DPPH: 1-diphenyl-2- picryl hydrazyl
FRAP: ferric reducing antioxidant power
IC_{50}: inhibitory concentration of 50% of DPPH
EC_{50}: effective concentration to reduce 50% of iron
TAC: total antioxidant capacity
AAE: ascorbic acid equivalents
WM: water-methanol extract
EA: ethyl acetate fraction
n-B: n-butanol fraction
AA: ascorbic acid

1. Introduction

Oxidative stress and reactive oxygen species are considered as primary causes and central factors for the emergence of serious health problems and chronic diseases including cancer, cardiovascular disease, anemia, hypertension, diabetes mellitus and other disorders (Martins et al., 2020). They are characterized by the oxidation of cell constituents including polyunsaturated lipids leading to the damage of tissues (Gessner et al., 2017).

Consequently, natural substances with antioxidant properties are recommended. Phenolic compounds are widely distributed in plants with significant antioxidant capacity (Cheng et al., 2019). Therefore, the interest in phenolic-rich plant extracts increases in scientific researchers.

Salvia officinalis belongs to the most popular family of *Lamiacea*. It is highlighted in literature for its effective content in phenolic compounds and its therapeutic properties. Since earliest times, it is used as flavor, food condiment and mainly as herbal medicine to release pains (Rguez et al., 2019). Many authors reported the health benefits of this plant. It is used as antioxidant, diuretic (Korkmaz et al., 2016), spasmolytic (Jasicka-Mislak et al., 2018), antidiabetic (Farzaneh and Carvalho, 2015), anti-inflammatory (Giacometti et al., 2018), antidiarrhea (Elkhoudri et al., 2016), antiviral (Farzaneh and Carvalho, 2015) and anti-carcinogenic (Kaliora et al., 2014).

Membranes of erythrocytes are susceptible to oxidize. This is mainly due by their richness in polyunsaturated lipids (Phuse and Khan, 2018). Plant extracts can have either anti-hemolytic effect in protecting erythrocytes or hemolytic induction anemia. Therefore, test of hemolysis has a positive interest as a direct indication of toxicity for plant extracts (Burci et al., 2019).

The main objective of this work was to determine antioxidant activity of crude hydro-methanolic extract and fractions from *S. officinalis*. This activity was evaluated with DPPH radical scavenging, FRAP and TAC assays. *In vitro* assay of hemolysis was assessed to identify extract and fractions toxicity towards human red blood cells. Besides, all extracts were investigated for their phytochemical composition and their total phenolic and flavonoid contents.

2. Materials and Methods

2.1. Plant material and extraction

Leaves and stems from *Salvia officinalis* L. were collected from the city of Tlemcen − Algeria. Plant material was identified by the Laboratory of Botany, Ecology Department, Tlemcen University (Algeria).

After drying in the shade, plant material was submitted to water-methanol (30:70 v/v) maceration at room temperature for 72 hours. Then, it was filtered and rotarily evaporated at 50 °C till the evaporation of methanol. The remaining was submitted to liquid-liquid partition with ethyl acetate and n-butanol solvents. After the evaporation of the solvent in rotavapor at 50 °C, crude extract and fractions were collected.

2.2. Chemicals

All chemical reagents used in this study were purchased from Sigma-Aldrich Chemical.

2.3. Phytochemical analysis

2.3.1. Phytochemical composition

S. officinalis hydro-methanolic extract and fractions of ethyl acetate and n-butanol were screened for their phytochemical constituents. This investigation was carried out according to the methods described by Harbone (1998) and Bruneton (1999).

2.3.2. Determination of total phenolic content

For the determination of total phenolic content in *S. officinalis* extract and fractions, Folin–Ciocalteu assay was used (Li et al., 2007). We mixed 100 µL of each sample dilution extract (1 mg/mL) with 100 µL of Folin-Ciocalteu and 2 mL of Na_2CO_3 solution. After incubating the mixture in darkness for 30 min, we measured the absorbance at 700 nm. The calibration curve of gallic acid (50 - 400 µg/mL) was prepared as a standard. Results were expressed in gallic acid micrograms equivalent per milligram of dry extract (µg GAE/mg DE).

2.3.3. Determination of flavonoid content

Flavonoid content in extract and fractions from *S. officinalis* was estimated by the method of Zhishen et al. (1999). For 250 µL of sample dilution (1 mg/mL), 75 µL of sodium nitrite at 15% and 1 mL of distilled water were added. Then, we added to the mixture volumes of 75 µL of aluminum chloride at 10% and 1 mL of sodium hydroxide at 4%. After 15 min, the absorbance was measured at 510 nm. Catechin was prepared as the standard from concentrations 50 to 400 µg/mL. Flavonoid content was calculated from calibration curve ($y = 0.003\ x$; $R^2=0.997$). Results were expressed in microgram catechin equivalents per milligram dry extract (µg CE/mg DE).

2.4. Antioxidant activity

2.4.1. Antiradical activity (DPPH assay)

Antiradical activity of *S. officinalis* samples was assayed according to the method of Dandlen et al. (2010). 50 µL of each extract dilution (100 - 1000 µg/mL) was added to 1950 µL of methanolic solution of DPPH freshly prepared. For each concentration, a blank was prepared containing 50 µL of extract and 1950 µL of methanol. The control test was carried out by mixing 50 µL of methanol with 1950 µL of DPPH methanol solution. After incubation of 30 min in the dark, absorbance was measured at 517 nm using spectrophotometer. Ascorbic acid (80 - 350 µg/mL) was used as a positive control following the same procedures. Antiradical activity was calculated using the following equation: Antiradical activity % = [(A control - A sample) /A control] x 100 (A: absorbance). The IC_{50} values were determined as

concentrations of samples producing 50% inhibition of DPPH radical. These values were determined graphically from the DPPH inhibition percentage curve in terms of different concentrations of samples.

2.4.2. Ferric reducing antioxidant power assay

The FRAP assay was performed according to the protocol of Karagozler et al. (2008). 100 µL of each sample dilution (100 - 700 µg/mL) was mixed with 250 µL of phosphate buffer solution (0.2 M; pH 6.6) and 250 µL of potassium ferricyanide $K_3Fe(CN)_6$ at 1%. The mixture was incubated for 20 min. Then, 250 µL of trichloroacetic acid at 10% was added. After centrifugation, 500 µL of the supernatant was collected then diluted with 500 µL of distilled water. Then, 100 µL of $FeCl_3$ at 0.1% was added. Absorbance was measured at 700 nm against a blank. Ascorbic acid (25 - 400 µg/mL) was used as a reference antioxidant. EC_{50} values of *S. officinalis* samples were determined as efficient concentrations reducing 50% of iron.

2.4.3. Total antioxidant capacity (TAC) assay

Total antioxidant capacity (TAC) was evaluated by phosphomolybdenum method proposed by Prieto et al. (1999). 200 µL of sample dilution was added to 2 mL of molybdate reagent solution. The tubes were incubated at 95 °C for 90 min and the absorbance was measured at 695 nm. Standard calibration curve using ascorbic acid (50 - 500 µg/mL) was prepared in the same experimental conditions. Total antioxidant capacity values were expressed in microgram ascorbic acid equivalents per milligram of dry extract (µg AAE/mg DE).

2.5. Hemolytic assay

The method of Henneberg et al. (2013) was adopted for *in vitro* hemolytic assay. Phosphate buffered saline solution (10 mM; pH 7.4) was used to wash human erythrocytes. Rates of hemolysis were tested for concentrations of 25, 50, 100 and 200 mg/mL from *S. officinalis* extract and fractions. 20 µL of each sample dilution was incubated with 1980 µL of erythrocytes solution for one hour at 37 °C. Absorbance of supernatant was measured at 540 nm. Positive and negative controls were prepared in the same experimental procedures. Hemolysis rates were calculated according to the following equation: Hemolysis rate % = $[(A_{sample} - A_{\text{negative control}})/ A_{\text{positive control}}] \times 100$ (A: absorbance).

2.6. Statistical analysis

Experiments were conducted in triplicate and data were expressed as mean ± standard deviation. Graphs were plotted by Microsoft Office Excel 2007 software. Analysis of variance (one-way ANOVA) and Dunnett post-hoc test were performed to test the significance differences between IC_{50}, EC_{50} and control mean values. Values of $p < 0.05$ were considered statistically significant using Graph Pad Prism 5 software (version 5.03, 2009).

3. Results

3.1. Phytochemical composition, total phenolic and flavonoid contents

Phytochemical composition of *S. officinalis* hydro-methanolic macerated extract and fractions indicated the presence of various families of secondary metabolites. Flavonoid, saponins, free quinones, tannins, terpenes and coumarins were the constituents found in the crude extract. However, fractions were lacked from some of these compounds (Table 1).

Contents of total phenolic and flavonoid varied in *S. officinalis* samples ranging from 271.11 ± 0.02 to 362.75 ± 0.07 µg GAE/mg DE and 189.13 ± 0.01 to 263.27 ± 0.1 µg CE/mg DE, respectively. The highest contents of these compounds were exhibited by ethyl acetate fraction. Results were presented in Table 2.

Table 1. Phytochemical screening of hydro-methanolic extract and its fractions from *S. officinalis.*

Secondary Metabolites	Hydro-methanolic extract	Ethyl acetate fraction	n-butanol fraction
Flavonoids	+	+	-
Saponins	+	-	-
Free quinines	+	+	+
Anthraquinones	-	-	-
Tannins	+	+	+
Terpenes	+	-	-
Alkaloids	-	-	-
Coumarins	+	+	-
Reducing compounds	-	+	-

+:presence/ -: absence

Table 2. Total phenolic and flavonoid contents in hydro-methanolic extract and its fractions from *S. officinalis.*

Extract/Fractions	Total phenolic (µg GAE/mg DE ± SD; n=3)	flavonoid (µg CE/mg DE ± SD; n=3)
Hydro-methanolic extract	271.11 ± 0.02	230.36 ± 0.06
Ethyl acetate fraction	362.75 ± 0.07	263.27 ± 0.1
n-butanol fraction	295.75 ± 0.06	189.13 ± 0.01

Values were expressed as mean ± SEM (n = 3). GAE: gallic acid equivalents, CE: catechin equivalents, DE: dry extract.

3.2. Antioxidant activity

3.2.1. DPPH radical scavenging activity

The radical scavenging ability of hydro-methanolic extract, ethyl acetate and n-butanol fractions were calculated as inhibition percentage of DPPH in terms of different concentrations of samples (Figure1). This activity was concentration dependent. Ascorbic acid was used as the reference antioxidant. It exhibited the highest antiradical activity. However, ethyl acetate fraction showed approximately similar antiradical activity, compared to control.

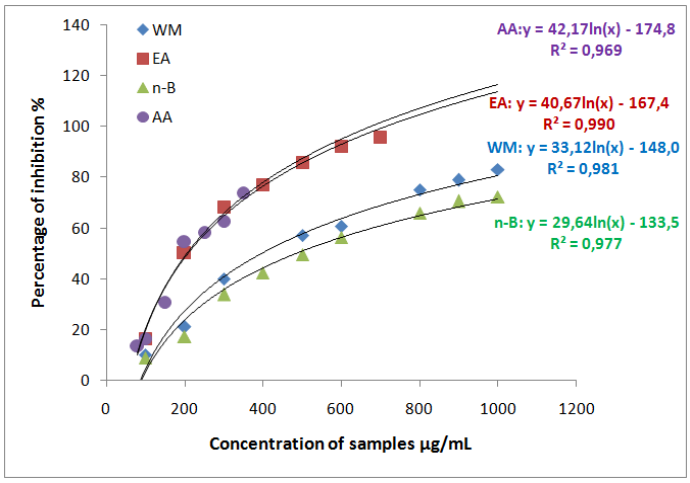


Figure1. Percentage inhibition of DPPH activity of hydro-methanolic extract, fractions and ascorbic acid. WM: hydro-methanolic extract; EA: ethyl acetate fraction; n-B: n-butanol fraction; AA: ascorbic acid

Results were expressed in IC_{50} values as the amount of sample needed to reduce 50% of the DPPH radical obtained by logarithmic regression from the percentage antiradical activity values (Figure 2). All samples were found to present antiradical activities with different IC_{50} values. Hence, the highest activity was recorded for ethyl acetate fraction (IC_{50} = 208.51 ± 5.77 µg/mL). Statistical analysis did not mention significant difference ($p > 0.05$) between the activity of this fraction and that of the ascorbic acid (IC_{50} = 206.43 ± 4.16 µg/mL).

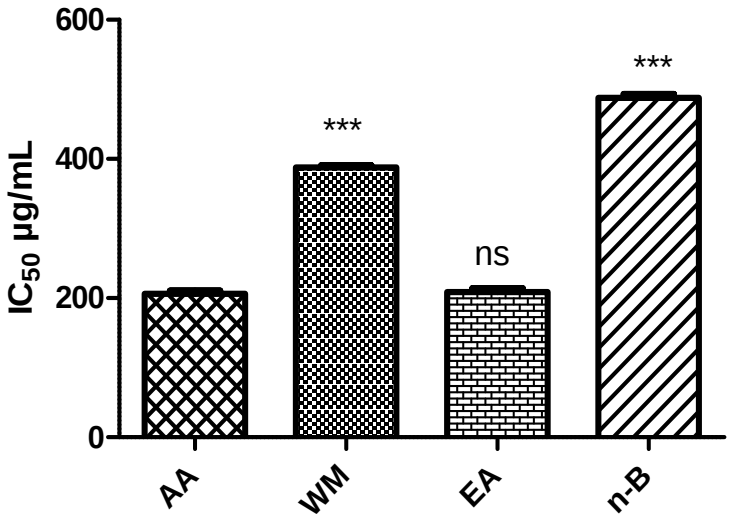


Figure 2. IC_{50} values of hydro-methanolic extract and fractions of ethyl acetate and n-butanol of *S.officinalis* obtained by DPPH assay in comparison with IC_{50} of ascorbic acid. WM: hydro-methanolic extract; EA: ethyl acetate fraction; n-B: n-Butanol fraction; AA: ascorbic acid; Values were represented as mean ± SEM (n = 3); ***: $p < 0.001$, ns: $p > 0.05$ versus control.

3.2.2. Ferric Reducing Antioxidant Power (FRAP)

The reductive capacity of *S. officinalis* hydro-methanolic extract and its fractions was presented by linear curves of absorbance at 700 nm according to different concentrations of samples (Figure 3). Results were expressed as concentration of samples corresponding to an absorbance of 0.5. Results of EC_{50} values were presented in Figure 4.

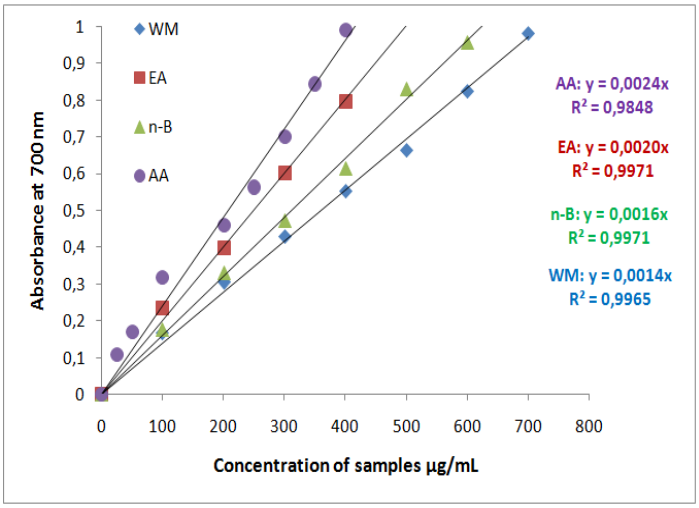


Figure 3. Reductive capacity of iron of ascorbic acid, hydro-methanolic extract, ethyl acetate and n-butanol fractions. WM: hydro-methanolic extract; EA: ethyl acetate fraction; n-B: n-Butanol fraction; AA: ascorbic acid.

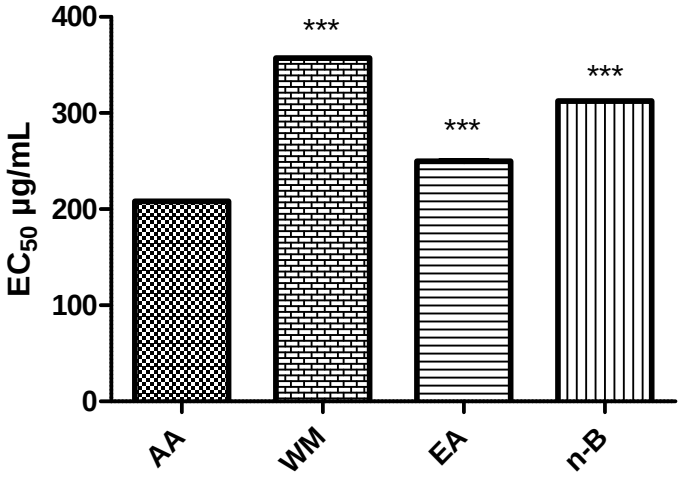


Figure 4. EC_{50} values of hydro-methanolic extract and fractions of ethyl acetate and n-butanol of *S.officinalis* obtained by FRAP assay in comparison with ascorbic acid. WM: hydro-methanolic extract; EA: ethyl acetate fraction; n-B: n-Butanol fraction; AA: ascorbic acid; Values were represented as mean ± SEM (n = 3); ***: $p < 0.001$ versus control.

According to results, all samples of *S. officinalis* possessed the capacity to reduce iron. However their reducing activity remained lower than that of ascorbic acid (EC_{50} = 208.33 ± 0.001 µg/mL). Ethyl acetate fraction revealed the most effective reducing activity (EC_{50} = 250 ± 0.001 µg/mL) as compared with that of hydro-methanolic extract (EC_{50} = 357.14 ± 0.02 µg/mL) and n-butanol fraction (EC_{50} = 312.5 ± 0.06 µg/mL).

3.2.3. Total Antioxidant Capacity (TAC)

Total antioxidant capacity of *S. officinalis* samples was evaluated by the reduction of molybdate method. The equation of calibration curve of ascorbic acid was used to calculate the values of total antioxidant capacity ($y = 0.0019x$; $R^2 = 0.9973$).

Results of this assay (Figure 5) showed that all studied samples of *S. officinalis* reduced molybdate. Indeed, ethyl acetate fraction had the highest total antioxidant capacity (817.83 ± 0.06 µg AAE/mg DE) whereas the crude extract showed the lowest one (238.1 ± 0.01 µg AAE/mg DE).

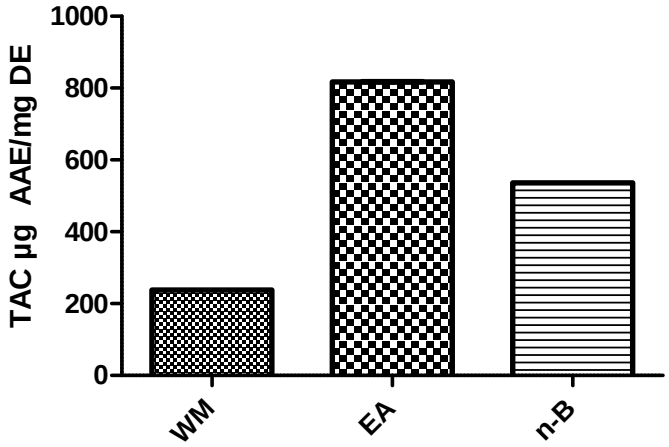


Figure 5. TAC values of hydro-methanolic extract and fractions of ethyl acetate and n-butanol of *S. officinalis.* WM: hydro-methanolic extract; EA: ethyl acetate fraction; n-B: n-Butanol fraction

3.3. Hemolytic effect

Hemolysis rates were assayed with four concentrations from *S. officinalis* samples: 25, 50, 100 and 200 mg/mL, after 60 min of incubation at 37 °C. Results were shown in Figure 6.

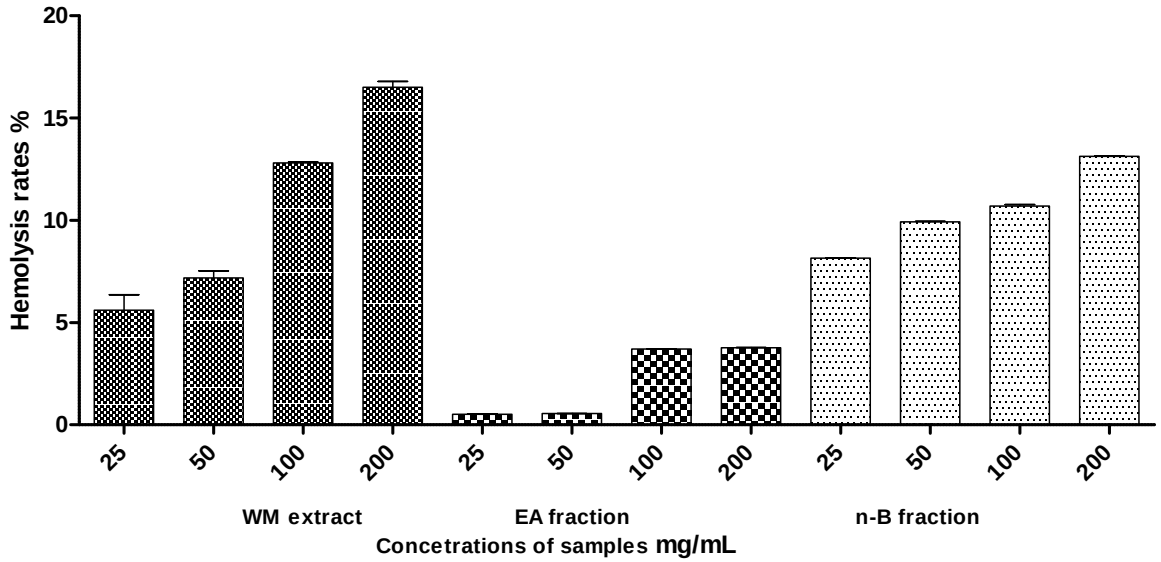


Figure 6. Hemolysis rates of hydro-methanolic extract and its fractions from *S. officinalis.* WM: hydro-methanolic extract; EA: ethyl acetate fraction; n-B: n-butanol fraction; Values were performed as means ± SEM (n = 3).

Results revealed that hemolysis rates were dose dependent. We noticed that these rates increased slightly with concentrations of ethyl acetate fraction: 0.51 ± 0.06% (25 mg/mL), 0.55 ± 0.01% (50 mg/mL), 3.7 ± 0.04% (100 mg/mL) and 3.77 ± 0.02% (200 mg/mL). Thus, ethyl acetate fraction exhibited the least hemolytic effect, compared to hydro-methanolic extract and n-butanol fractions.

4. Discussion

The chemical composition of *S. officinalis* confirmed its richness in pharmaceutical compounds. This composition depends on the plant geographic origin and the harvest season (Duletić-Laušević et al., 2019). The quantity and the quality of phenolic constituents in plants are affected by the climatic changes, the plant stage of development and the plant parts used in extraction (Kumar et al., 2017). Moreover, the solvent and the method used for the extraction of these compounds determine their quantity (Venkatesan et al., 2019).

In terms of quantity and quality, *S. officinalis* is characterized by the abundance of effective phenolic compounds (Duletić-Laušević et al., 2019). In previous studies, Jasicka-Misiak et al. (2018) obtained 93.8 ± 3.1 and 63.9 ± 2.9 mg GAE/g extract of total phenolic in two types of *S. officinalis* methanolic extracts. Garcia et al. (2016) found amounts of 1.88 ± 0.01 and 1.61 ± 0.95 mg GAE/mg of total phenolic in hydroalcoholic and aqueous extracts from Brazilian plants.

According to literature data, phenolic compounds of *S. officinalis* are excellent antioxidants. This plant is a source of phenolic acids and flavonoid which have potent antioxidant properties by the mechanism of donating hydrogen (Andrei et al., 2018). In their study on

different species of *Salvia* from Romania, Andrei et al. (2018) found that *S. officinalis* exhibited the highest antioxidant capacity.

In the current study, ethyl acetate was found to be the most efficient solvent in extracting total phenolics and flavonoid. Ethyl acetate fraction showed a powerful antioxidant activity revealed by DPPH, FRAP and TAC assays. Its antioxidant effect was suggested to be correlated to its high phenolic content. Saba et al. (2019) revealed the potential reducing power and chelating ability of *S. officinalis* leaves ethanolic extract which were correlated with its high content in phenolic compounds.

In vitro hemolysis assay was used to indicate the effectiveness of *S. officinalis* samples as natural antioxidant free from adverse effects of toxicity. This assay is a very practical and useful method to test plant extract toxicity (Burci et al., 2019). Membranes of erythrocytes contain high level of polyunsaturated fatty acids, which explain their fragility towards oxidation (Phuse and Khan, 2018).

Plant extract content in saponins may explain its toxicity (Zheng et al., 2019). It was the case of hydro-methanolic extract which exhibited the highest hemolysis rate, compared to ethyl acetate and n-butanol fractions. However, the least hemolytic effect of ethyl acetate fraction might be justified by its highest content of phenolic compounds related to its potent antioxidant activity. *S. officinalis* infusions are considered safe except its excessive consumption that may lead to the appearance of some side effects (Yashaswini et al., 2019).

5. Conclusion

Quantitative analysis revealed the richness of *S. officinalis* hydro-methanolic extract, ethyl acetate and n-butanol fractions in total phenolics and flavonoid. The highest contents of these compounds were exhibited by ethyl acetate fraction. This fraction showed the most interesting results in antioxidant assays and hemolysis rates. Its antiradical activity against DPPH was comparative to that of ascorbic acid. It revealed the highest reduction of iron and potent total antioxidant capacity. Additionally, extract and fractions from *S. officinalis* exhibited low hemolytic activity. This study may lead to new antioxidants drugs formulations safe in consumption and prevent possible anemia. Further studies should be carried out to extract and purify *S. officinalis* phenolic compounds involved in the prevention from oxidative stress damages.

Acknowledgement

The research is financed by the Algerian Ministry of Higher Education and Scientific Research (MERS) and General direction of scientific research and technological development (DGRSDT).

Author Contribution Statement

Azzi Rachid supervised the findings of this work; Souad MAHDI: Carried out the survey; Farid Boucif LAHFA: discussed the results and contributed to the final manuscript.

Conflict of interest
No conflict of interest was reported by the authors.

ORCID
Rachid AZZI: 0000-0001-6979-7773

References

Andrei, M., Cadmiel, M., Simone, C., Marcello, L., Marina, S., & Gianina, C. (2018). Determination of phenolic compounds in different species of salvia from Romania (*Salvia transsylvanica, Salvia glutinosa, Salvia officinalis*) and their biological activities. *Acta Medica Marisiensis,* 64, 14.

Bruneton, J. (1999). Pharmacognosie-Phytochimie-Plantes médicinales, 3ème édition, Tec & Doc. Lavoisier: Paris.

Burci, L.M., da Silva, C.B., Rondon, J.N., da Silva, L.M., de Andrade, S.F., Miguel, O.G., de Fátima Gaspari Dias, J., & Miguel, M.D. (2019). Acute and subacute (28 days) toxicity, hemolytic and cytotoxic effect of Artocarpus heterophyllus seed extracts. *Toxicology Reports*, 6, 1304–1308. https://doi.org/10.1016/j.toxrep.2018.02.006

Cheng, K., Dong, W., Long, Y., Zhao, J., Hu, R., Zhang, Y., & Zhu, K. (2019). Evaluation of the impact of different drying methods on the phenolic compounds, antioxidant activity, and *in vitro* digestion of green coffee beans. *Food science and nutrition*, 7(3), 1084–1095. https://doi.org/10.1002/fsn3.948

Dandlen, A.S., Sofia Lima, A., Marta Mendes, D., Graça Miguel, M., Leonor Faleiro, M., João Sousa, M., Pedro Luis, G., Barroso José, G., & Cristina Figueiredo, A. (2010). Antioxidant activity of six Portuguese thyme species essential oils. *Flavour and fragrance journal,* 25(3), 150–155. https://doi.org/ 10.1002/ffj .1972.

Duletić-Laušević, S., Alimpić Aradski, A., Živković, J., Gligorijević, N., Šavikin, K., Radulović, S., Ćoćić, D., & 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. https://doi.org/10.2298/BOTSERB1901047D.

El khoudri, N., Baali, A., & Amor, H. (2016). Maternal morbidity and the use of medicinal herbs in the city of Marrakech, Morrocco. *Indian journal of traditional knowledge,* 15(1), 79–85.

Farzaneh, V., & Carvalho, I.S. (2015). A review of the health benefits potentials of herbal plant infusions and their mechanism of actions. *Industrial Crops and Products,* 65, 247–258. https://doi.org/ 10.1016/j.indcrop.2014.10.057

Garcia, C.S.C., Menti, C., Lambert, A.P.F., Barcellos, T., Moura, S., Calloni, C., Branco, C.S., Salvador, M., Roesch-Ely, M., & Henriques, J.A.P. (2016). Pharmacological perspectives from Brazilian *Salvia officinalis* (*Lamiaceae*): antioxidant, and antitumor in mammalian cells. *Anais da Academia Brasileira de Ciências*, 88(1), 281–292. https://doi.org/10.1590/0001-3765201520150344.

Gessner, D.K., Ringseis, R., Eder, K. (2017). Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. *Journal of Animal Physiology and Animal Nutrition*, 101(4), 605–628. https://doi.org/10.1111/jpn.12579.

Giacometti, J., Bursa´c Kova˘cevi´c, D., Putnik, P., Gabri´c, D., Biluši´c, T., & Kreši´c, G. (2018). Extraction of bioactive compounds and essential oils from mediterranean herbs by conventional and green innovative techniques. *Food Research International*, 113, 245–262.

Harborne, J. (1998). Phytochemical Methods. In: A Guide to Modern Techniques of Plant Analysis, 3rd ed, 203–234. *Chapman and Hall Thomson Science*, UK.

Henneberg, R., Otuki, M.F., Furman, A.E.F., Hermann, P., Nascimento, A.J., & Leonart, M.S.S. (2013). Protective effect of flavonoid against reactive oxygen species production in sickle cell anemia patients treated with hydroxy urea. *Revista Brasileira de Hematologia e Hemoterapia*, 35(1), 52–55. https://doi.org/10.5581/1516-8484.20130015.

Jasicka-Mislak, I., Poliwoda, A., Petecka, M., Buslovych, O., Shlyapnikov, V.A., & Wieczorek, P.P. (2018). Antioxidant phenolic compounds in *Salvia officinalis* L. and *Salvia sclarea* L. *Ecological Chemistry Engineering S.*, 25(1), 133–142. https://doi.org/10.1515/eces-2018-0009

Kaliora, A.C., Kogiannou, D.A.A., Kefalas, P., Papassideri, I.S., & Kalogeropoulos, N. (2014). Phenolic profiles and antioxidant and anticarcinogenic activities of Greek herbal infusions; Balancing delight and chemoprevention? *Food Chemestry*, 142, 233–241. https://doi.org/10.1016/j.foodchem.2013.07.056.

Karagözler, A.A., Erdag, B., Emec, Ç.Y., & Uygunet, D.A. (2008). Antioxidant activity and proline content of leaf extracts from Dorystoechas hastata. *Food chemestry*, 111, 400–407.

Korkmaz, M., Karakuş, S., Özçelik, H., & Selvi, S. (2016). An ethnobotanical study on medicinal plants in Erzincan, Turkey. *Indian journal of traditional knowledge*, 15(2), 192–202. http://hdl.handle.net/123456789/33967.

Kumar, S., Yadav, A., Yadav, M., & Yadav, J.P. (2017). Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of Aloe vera (L.). *Burm.f. BMC Research Notes*, 10(1), 60. https://doi.org/10.1186/s13104-017-2385-3

Li, H.B., Cheng, K.W., Wong, C.C., Fan, K.W., Chen, F., & Jiang, Y. (2007). Evaluation of antioxidant capacity and total phenolic content of different fraction of selected microalgae. *Food Chemistry*, 102(3), 771–776.

Martins, T.F., Palomino, O.M., Álvarez-Cilleros, D., Martín, M.A., Ramos, S., & Goya, L. (2020). Cocoa Flavanols Protect Human Endothelial Cells from Oxidative Stress. *Plant Foods for Human Nutrition*, 75(2), 161–168. https://doi.org/10.1007/s11130-020-00807-1.

Phuse, S.S., & Khan, Z.H. (2018). Assessment of hemolytic effect of Cassia flower extracts on human RBCs. *Journal of Drug Delivery & Therapeutics*, 8(6-s), 18–20. https://doi.org/10.22270/JDDT.V8I6-S.2169.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemestry*, 269, 337–341. https://doi.org/10.1006/ABIO.1999.4019.

Rguez, S., Msaada, K., Hammami, M., Daami-Remadi, M., Chayeb, I., Ksouri, R., & Hamrouni-Sellami, I. (2019). Diurnal variation of the chemical composition and its repercussion on the biological activity polyphenolics of *Salvia officinalis* aerial parts. *International Journal of Plant Science and Horticulture*, 1(1), 07–21. https://doi.org/10.36811/ijpsh.2019.110002.

Saba, A., Suhayla, M.K., & Raheem, Z.H. (2019). Study the Antioxidant Activity of Sage (*Salvia Officinalis*) Leaves Extract. *Indian Journal of Public Health Research & Development*, 10(3), 556. https://doi.org/10.5958/0976-5506.2019.00557.6.

Venkatesan, T., Choi, Y. W., & Kim, Y. K. (2019). Impact of Different Extraction Solvents on Phenolic Content and Antioxidant Potential of Pinus densiflora Bark Extract. *BioMed Research International*, 2019, 1–14. https://doi.org/10.1155/2019/3520675.

Yashaswini, S., Fagan, J., & Schaefer, J. (2019). Ethnobotany, phytochemistry, cultivation and medicinal properties of Garden sage (*Salvia officinalis L.)*. *Journal of Pharmacognosy and Phytochemistry*, 8(3), 3139–3148.

Zheng, S., Wang, Y., Liu, H., Chang, W., Xu, Y., & Lin, F. (2019). Prediction of Hemolytic Toxicity for Saponins by Machine-Learning Methods. *Chemical Research in Toxicology*, 32, 1014–1026. https://doi.org/10.1021/acs.chemrestox.8b00347.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555–559. https://doi.org/10.1016/S0308-8146 (98)00102-2.