ISSN 2449-8955 European Journal of Biological Research *Research Article*

DOI: http://dx.doi.org/10.5281/zenodo.5129324

Phytochemical characterization, antioxidant and antibacterial activity of *Salvia officinalis* (L.) extracts from the Tiaret region

Rachida Bouteldja [1,2,*], Radhouane Doucene [3], Hebib Aggad [1], Fatima Zohra Abdi [4], Hamza Belkhodja [4], Mustapha Abdali [1], Khaled Zidane [3], Siham Abaid [5]

[1] Laboratory of Hygiene and Animal Pathology, Institute of Veterinary Sciences, University of Tiaret, Algeria

[2] Faculty of Natural and Life Sciences, Ibn Khaldoun University of Tiaret, Algeria

[3] Laboratory Reproduction of Farm Animals, Institute of Veterinary Sciences, University of Tiaret, Algeria

[4] Laboratory of Bioconversion, Microbiology Engineering and Health Safety, University of Mustapha Stambouli, Mascara, Algeria

[5] Laboratory of Eco-development of Spaces, Department of Environmental Sciences, Faculty of Natural and Life Sciences, University of Djilali Liabes, Sidi Bel Abbes, Algeria

* Corresponding author: E-mail: bouteldjarachida.92@gmail.com

Received: 29 April 2021; **Revised submission:** 25 June 2021; **Accepted:** 23 July 2021

https://jbrodka.com/index.php/ejbr

ABSTRACT: This work aims the valorization of a medicinal plant known by its traditional use, *Salvia officinalis* L. (Lamiaceae), by phytochemical characterization and evaluation of the antioxidant and antibacterial activity of their extracts. The antioxidant activity was assessed by the DPPH method and the antibacterial potential was determined by the diffusion method. The quantitative determination revealed that the ethanolic extract has a content of 8.04% for polyphenolic content and 17.4 % for flavonoids. The DPPH radical scavenging activity of *S. officinalis* showed that the ethanolic extract of *S. officinalis* presented the higher antiradical effect manifested with IC_{50} of 0.106±0.001 mg/ml. In addition, the antibacterial activity showed the strong capacity of *S. officinalis* methanolic extract to inhibit *B. subtilis*, *M. luteus*, *E. coli* and *S. aureus* with a diameter inhibition zone of 27.06±1.49; 15.43±2.23; 11.6±0.52 and 11.5±2.17 mm respectively. While the activity of the ethanolic extract was 26.62±2.97 mm against *B. subtilis*, 16.51±2.36 mm against *M. luteus*, 13.62±0.55 mm for *S. aureus*, *P. aeruginosa* (12.30±1.59 mm). The macrodilution method (MIC) showed a range of 625 to >5000 µg/ml. The study of the antioxidant and antibacterial activity of extracts of *S. officinalis* suggested that this plant represented a natural source of bioactive molecules with very important biological activities.

Keywords: *Salvia officinalis* L.; Polyphenols; Flavonoids; Antioxidant; Antibacterial.

Abbreviations: MIC: Minimal Inhibitory Concentration, DPPH: 2,2-diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$), IC_{50}: Median inhibitory concentration, *S. officinalis*: *Salvia officinalis*, AK: Antibiotic Amikacin.

1. INTRODUCTION

Medicinal plants make a big part of modern medicine by their richness in bioactive compounds of different parts of the plant. These molecules of natural origin participate in various fields such as cosmetic, food technology and pharmaceutical [1, 2]. Bioactive molecules are extra-nutritional constituents participating in physiological or cellular activities in humans and animals by providing biological activities; antioxidant, anti-inflammatory, anti-carcinogenic protect against metabolic disorders [3, 4].

The treatment of complex diseases requires the development of synthetic drugs. These products can cure various pathologies and cause adverse effects on human health with a more or less severe intensity [5]. To determine this growing threat, research is focusing on medicinal plants as the main resource for the therapeutic bioactive compound in addition to their use in health care by approximately 80% of the world's population [6]. In fact, they are sources of biologically active compounds; phenolic compounds with antioxidant properties and antimicrobial potentials [7].

Oxidative stress is an imbalance between the production of reactive oxygen species or free radicals and their removal by antioxidants. This phenomenon results from multiple external factors such as exposure to X-rays, ozone, smoking, air pollutants, nutritional deficiencies and industrial chemicals. It stimulates molecular attacks on biological membranes and tissues and causes or leads to several neurodegenerative diseases such as Parkinson's and Alzheimer's [8, 9].

Nowadays, with the appearance of side effects of synthetic drugs as well as the increase of resistance to conventional antibiotics, research is focusing on medicinal plants as the main resource for therapeutic agents to overcome this growing threat.

Salvia officinalis L. (Sage) is a very popular plant in the traditional pharmacopeia of the Tiaret region (Algeria) for the treatment of various pathologies. Sage is certainly the queen of aromatic herbs and one of the oldest cultivated plants. It is an evergreen plant of the Lamiaceae family and belongs to the genus Salvia grouping more than 800 species. This group includes herbaceous plants (annuals and perennials) and semi-shrubs [10, 11]. The different parts of *Salvia officinalis* due to their richness in bioactive compounds such as flavonoids, alkaloids, tannins, glycosides can be essential elements for the inhibition of pathogenic bacteria and the reduction of different pathologies [11].

This research aims to evaluate the biological activity of the ethanolic (80%), methanolic (80%) and aqueous extracts of *Salvia officinalis* L. by the determination of polyphenolic and flavonoids contents followed by the study of the in vitro antioxidant and antibacterial activity against ATCC strains.

2. MATERIALS AND METHODS

2.1. Plant material and extraction

The aerial parts (leaves and stems) of *Salvia officinalis* were collected in April 2017 in the region of Tiaret (Algeria) (35° 23′ 17″ North, 1° 19′ 22″ East). The plant was washed and dried in the open air and then grinded to obtain a powder. The identification of the species was carried out by the botanical laboratory of the National Superior School of Agronomy (Algiers).

The extracts were prepared by maceration with a ratio of 5 g of powder mixed with 50 ml of the solvent (methanol 80%, ethanol 80% form BIOCHEM Chemopharma and distilled water) under continuous stirring with a shaker. After 24 hours, the mixture was filtered through filter paper and the filtrate was evaporated and dried at 40°C. The resulting residue was stored at a temperature of -20°C [12].

2.2. Quantitative analysis

2.2.1. Total Phenolic Content

The determination of total phenolic content was carried out by the Folin-Ciocalteu method [13]. A volume of 0.2 ml of different dilutions (1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 mg/ml) of ethanolic, methanolic, and aqueous extracts of S. officinalis was mixed with 1 ml of the freshly prepared Folin-Ciocalteu reagent (BIOCHEM Chemopharma) at 10% and 0.8 ml of sodium carbonate (Na_2CO_3) at 7.5%.

The mixture is incubated for 30 minutes at room temperature and then read by a spectrophotometer (Biochrom Libra S6) against a blank at a wavelength of 765 nm. Results were expressed in mg equivalent of gallic acid/g dry plant [14]. The tests were repeated in triplicate (Means ± SEM with n=3).

2.2.2. Total Flavonoid Content

The determination of flavonoids is done by the aluminum chloride method [15]. A volume of 0.5 ml of each dilution was mixed with the various extracts and added to 1.5 ml of methanol (95%), 100 µl of $AlCl_3$ prepared at 10% (w/v) plus 100 µl of sodium acetate (1 M) and 2.8 ml of distilled water. The mixture was stirred and incubated in the dark at room temperature for 30 min. The blank was made by replacing the extract with 95% methanol and the absorbance was determined by spectrophotometer (Biochrom Libra S6) at 415 nm. The results were expressed in mg quercetin equivalent/g dry plant [16]. The tests were repeated in triplicate (Means ± SEM with n=3).

2.3. Antioxidant activity

The antioxidant activity was performed by the DPPH (2,2-diphenyl-1-picrylhydrazyl, $C_{18}H_{12}N_5O_6$) radical scavenging assay. The protocol followed was the one described by Braca et al. with some modifications. An equal volume of the different dilutions (0.009 to 5 mg/ml) of the methanolic, ethanolic and aqueous extracts of S. officinalis was mixed with a volume of 0.004 % (w/v) methanolic solution of DPPH (Sigma Aldrich). Incubation was carried out for 30 min at room temperature. The absorbance was read against a control prepared for each concentration at 517 nm after 30 minutes of incubation in the dark and at room temperature with a spectrophotometer (Biochrom Libra S6) [17]. The tests were repeated in triplicate (Means ± SEM with n=3). The percentage inhibition was calculated by the following equation:

% Inhibition = [(Ac -Ae)/Ac] × 100.

With: Ac: Absorbance of the control solution which contains an equal volume of methanolic solution of DPPH and methanol. Ae: Absorbance of extract.

2.4. Antibacterial activity

The antibacterial potential of S. officinalis extracts was evaluated against seven referenced bacterial strains: *Micrococcus luteus* ATCC 14452, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027 and *Enterococcus faecalis* ATCC 29212, from the laboratory of Animal Hygiene and Pathology of the Veterinary Institute of Ibn Khaldoun University "Tiaret".

2.4.1. Agar diffusion method

The agar diffusion method was used to evaluate the antibacterial activity of the extracts of S. officinalis [18, 19]. Petri dishes are filled with Mueller-Hinton medium (BIOCHEM Chemopharma) in a liquid state and allowed to solidify, then the bacterial suspension is adjusted to 0.5 McFarland and seeded

with a sterile swab. Using sterile forceps, impregnate a Whatman paper disc (6 mm diameter) in 10 µl of each extract (ethanolic, methanolic and aqueous at a concentration of 20 mg/ml of the plant), and another for the solvent used as a negative control and another one an antibiotic (Amikacin 30 µg) represents the positive control. The disk is placed with slight pressure on the seeded agar. After diffusion of the extract for 2 hours at 4°C, the plates are maintained at 37°C for 24 hours. Antimicrobial activity is determined using a caliper by measuring the diameter of the zone of inhibition (mm) [18]. The tests were repeated in triplicate (Means ± SEM with n=3).

2.4.2. Macrodilution method

The minimum inhibitory concentration was performed by the macrodilution method. For this purpose, a series of 9 dilutions were prepared in Mueller-Hinton broth (BIOCHEM Chemopharma) (78 µg/ml to 10000 µg/ml) of the three extracts (methanolic, ethanolic and aqueous) of S. officinalis. The bacterial suspensions used were standardized to 0.5 McFarland (10^8 CFU) and then diluted 1:100 (10^6 CFU). Finally, 1 ml of the suspension was added to a tube containing 1 ml of the different dilutions prepared previously. A tube containing an antibiotic (Amikacin 30 µg) considered as a positive control and a tube with 1 ml of Mueller-Hinton broth considered as a negative control. The tubes are incubated at 37°C for 24 hours, then 100 µl of the tubes without bacterial growth are spread by a rake in plates containing solidified Mueller-Hinton agar and incubated at 37°C for 24 hours. After incubation, the MIC is the concentration of the plates with no growth [19, 20]. The tests were repeated in triplicate (Means ± SEM with n=3).

2.5. Statistical analysis

The statistical analysis of the data was performed using the Statistica software (version 8.0.725.0) via Anova with a single factor followed by the LSD test.

3. RESULTS AND DISCUSSION

3.1. Quantitative analysis

3.1.1. Total Phenolic Content

The results of polyphenol content are presented in the Table 1. The polyphenol content of S. officinalis extracts revealed a highly significant ($p \leq 0.001$) methanolic against aqueous extract with a ratio of 5.72 vs. 4.51%, respectively. In addition, we have noted a highly significant increase ($p \leq 0.001$) in ethanolic extract of 8.04% with the methanolic extract. Our results show the richness of the ethanolic extract of S. officinalis in polyphenols compared to the methanolic and aqueous extract. Pop et al. showed that the ethanolic extract of *S. officinalis* has a high level of polyphenols compared to methanolic extract [33]. Uygun et al. presented that the ethanolic extract of *S. amplexicaulis* was richer than methanolic and the aqueous extract [34]. Other comparative studies between the aqueous and the ethanolic extract at 50% and 96% showed that the 50% was the richer on phenolic content followed by the concentration 96% and finally the aqueous extract [21]. According to the results of the determination of polyphenols from *S. officinalis* a specific rate for each extract is presented. We can explain that the diversification of polyphenol levels can be translated into several parameters, including climate change, which has been a very important factor in their richness of phenolic compounds and similarly in their biological activity [35]. Or to the experimental conditions; temperature, time and method of extraction, the nature of the solvent and their percentage [36, 37]. Naczk and Shahidi, explained that phenolic compounds can guide the formation of insoluble complexes by their association with

plant components, as in the case of proteins and carbohydrates. Their solubility is based on their chemical nature in the plant, from simple to very highly polymerized, which can affect by the polarity of the solvents used [38].

3.1.2. Total Flavonoids Content

Flavonoid content was always related to polyphenols and richness in bioactive compounds. The flavonoid content in *S. officinalis* presented in Table 1. It showed a highly significant rate ($p \leq 0.001$) of ethanolic extract versus methanolic and aqueous extract (17.4±0.2 vs 14.33±0.98 and 14.46±0.3%).

Table 1. Polyphenols and flavonoids content of methanolic, ethanolic and aqueous extracts of *S. officinalis*.

Plant extracts	Polyphenols content %	Flavonoids content %
Me Ex	5.72***	14.33±0.98
Et Ex	8.04###	17.4±0.2•••
Aq Ex	4.51	14.46±0.3

*** Highly significant difference of methanolic vs. aqueous extract. ###Highly significant difference of ethanolic vs. methanolic extract. ••• Highly significant difference in ethanolic vs methanolic and aqueous extract.

This study reveals the high content of flavonoids in the ethanolic extract of *S. officinalis* versus the methanolic and aqueous extracts. These results were corroborated with those obtained by Alimpić Ana et al. where the ethanolic extract of *S. amplexicaulis* has a high flavonoid content than methanolic extract [22]. According to Duletić-Laušević et al. The ethanolic extract at 50% of *S. officinalis* of different varieties presented a high level of flavonoids compared to extract at 96% and aqueous [21].

The evolution of flavonoid levels was influenced by several genetic and environmental parameters, including climate, area, temperature, fertility, parasites and diseases; similarly, extraction efficiency depended on the diffusion rate and solubility of the solvent [23, 39]. This was demonstrated by Do et al., where the ethanolic extract presented a ratio 4 times higher than the aqueous extract according to polarity [40].

3.2. Antioxidant activity

The results obtained DPPH radical scavenging assay as a function of the evolution of antioxidant activity are presented in Table 2, showed that the ethanolic extract of *S. officinalis* presented a highly significant antioxidant capacity $p \leq 0{,}001$ versus the methanolic and aqueous extracts with an IC_{50} of 0.106±0.001; 2.82±0.05 and 1.74±0.005 mg/ml respectively compared to IC_{50} of ascorbic acid (0.13 mg/ml).

Table 2. IC_{50} of the methanolic, ethanolic, aqueous extracts of *S. officinalis* and ascorbic acid.

	Methanolic extract	Ethanolic extract	Aqueous extract	Ascorbic acid
IC_{50} (mg/ml)	2.82±0.05	0.106±0.001***	1.74±0.005	0.13

*** Highly significant difference in ethanolic vs methanolic and aqueous extract.

Antioxidants are chemical or biological agents capable of neutralizing the potentially damaging action of free radicals. To respond to free radical attacks, some types of antioxidant defenses have been developed. Their function is to maintain the balance of the cells [24]. Rasmy et al. revealed that the ethanolic extract (80%) of *S. officinalis* has better free radical scavenging potential than the aqueous extract [41]. Duletić-Laušević et al. proved the strong capacity of radical inhibition by the ethanolic extract of *S. officinalis*

followed by the dichloromethane, acetate and chloroform [23]. Mekhaldi et al. showed the high capacity of methanolic extract of S. officinalis compared to the essential oil [42]. According to the values obtained, a difference in inhibition capacity depending on the solvent used was observed. The ethanolic extract provided a high free radical scavenging capacity. The latter can be translated by the best extraction power of antioxidants and their richness in bioactive compounds. The latter represent a diverse range of molecules that are not necessary for cell life but play a major role in the interaction between cells and the environment [25]. Zhou and Yu, reported significant effects between the solvents applied in the extraction of antioxidants and the power of inhibition of free radicals of DPPH [43]. Tosun et al. showed a positive correlation between phenolic compounds and antioxidant activity. In addition, the extraction quality was linked to several parameters, including the polarity of the solvents applied in the extraction, the separation technique where organic solvents were more efficient than water because of their capacity to extract polyphenols [44]. The latter were known for their major participation in antioxidant activity [45, 46]. Several works proved that the ethanolic extract 80% was more effective compared to methanolic (80%) and aqueous because of its polarity. Their polarity provided a strong extraction capability of phenolic compounds and flavonoids [47-49].

3.3. Antibacterial activity

3.3.1. Agar diffusion method

Antibacterial activity against Gram-negative and Gram-positive bacteria of ethanolic, methanolic and aqueous extract of *S. officinalis* using the disc diffusion method by determination of zone of inhibition (Table 3).

Table 3. Results of antibacterial activity of *S. officinalis* extracts using the agar diffusion method by determination of inhibition zones (mm).

	Inhibition Zone (mm)			
Microorganisms	**Me Ex**	**Et Ex**	**Aq Ex**	**AK**
S. aureus	11.5 ± 2.17	13.63 ± 0.55	0	19.83 ± 0.28
E. coli	11.6 ± 0.52	13.8 ± 1.53	0	23 ± 1
M. luteus	15.43 ± 2.23	16.51 ± 2.36	9.6 ± 5.54	20 ± 1
B. subtilis	27.06 ± 1.49	26.62 ± 2.97	2.55 ± 4.40	21
B. cereus	7.34 ± 0.64	10.66 ± 0.28	9.02 ± 0.95	18.66 ± 0.57
E. faecalis	9.14 ±1.82	10.83 ± 0.28	6.45 ± 5.60	9.21 ± 0.4
P. aeruginosa	10.34 ± 1.22	12.30 ±1.59	9.11 ± 0.83	8.78 ± 0.91

Me Ex: Methanolic extract, Et Ex: Ethanolic extract, Aq Ex: Aqueous extract, AK : Antibiotic amikacin (30 µg).

The results of antibacterial activity by agar diffusion method show the antibacterial effect of methanolic and ethanolic extracts of I against the tested strains with a zone of inhibition between 7.34-26.62 mm. On the other hand, the aqueous extract shows no effect against *S. aureus* and *E. coli* with low activity against most strains. Ghezelbash et al. showed that the ethanolic extract of *S. officinalis* stems were capable of inhibiting *E. coli*, *S. aureus*, *B. cereus* [12]. Abd-Elmageed and Hussein, demonstrated that ethanolic and aqueous extracts of *S. officinalis* have the potential to inhibit *S. aureus*, *P. aeruginosa*, *E. coli*, *B. cereus*, *M. luteus* and *B. subtilis* [50]. The ethanolic extract of *S. officinalis* can eliminate multi-resistant bacteria such as *S. aureus* and *E. coli* [51]. The activity of ethanolic and methanolic extracts inhibited the growth of *P. aeruginosa*, *B. subtilis*, *E. coli* and *S. aureus* [52]. Similarly, essential oils of *S. officinalis* reacted against *B.*

subtilis, *B. cereus*, *S. aureus*, *M. luteus* and *E. coli* [53]. In addition, other studies have shown that plant extracts belonging to the Lamiaceae family, such as sage, have biological activity against bacteria and yeasts. This inhibition can be explained by the richness of *S. officinalis* extracts in bioactive compounds such as phenolic compounds, alkaloids and terpenoids [11].

Several works have revealed that alkaloids, flavonoids, saponins, tannins, cardiac glycosides and terpenoids play a major role in the antibacterial activity through their ability to inhibit the growth of bacteria such as *E. coli*, *P. aeruginosa* and *S. aureus* [26-28, 54, 55]. Phenolic compounds cause disruptions in the cell wall leading to changes in permeability, leading to leakage of cell continuum or interfering with membrane proteins by a change in structure because of the strong correlation between toxicity and hydrophobicity of different phenolic compounds. In addition, bioactive compounds cause inhibitions of cell wall construction, microbial genetic material replication, biofilm formation, attachment motility and cell communication [29-31].

3.3.2. Minimal Inhibitory Concentration (MIC)

Our results of the minimal inhibitory concentration reveal a diversity of the bacterial inhibition capacity according to the extract used and the tested germ. These results show a low antimicrobial efficacy of methanolic extract of *S. officinalis* for 71.42% of tested bacteria (*S. aureus*, *E. coli*, *M. luteus*, *B. cereus* and *P. aeruginosa*) with a MIC equal to 5000 µg/ml, inactive (MIC >5000 µg/ml) for *B. subtilis* and high sensitivity of MIC = 625 µg/ml against *E. faecalis*. On the other hand, the ethanolic extract is inactive against *M. luteus*, *B. subtilis*, *B. cereus* (MIC >5000 µg/ml), *S. aureus*, *P. aeruginosa*, *E. coli* (MIC = 2500-5000 µg/ml) and *E. faecalis* (MIC = 625 µg/ml). The MIC of the aqueous extract is >5000 µg/ml for *E. coli*, *E. faecalis*, *S. aureus* and 1250-2500 µg/ml on *B. subtilis* and *M. luteus*, respectively and 625 µg/ml against *B. cereus* and *P. aeruginosa*.

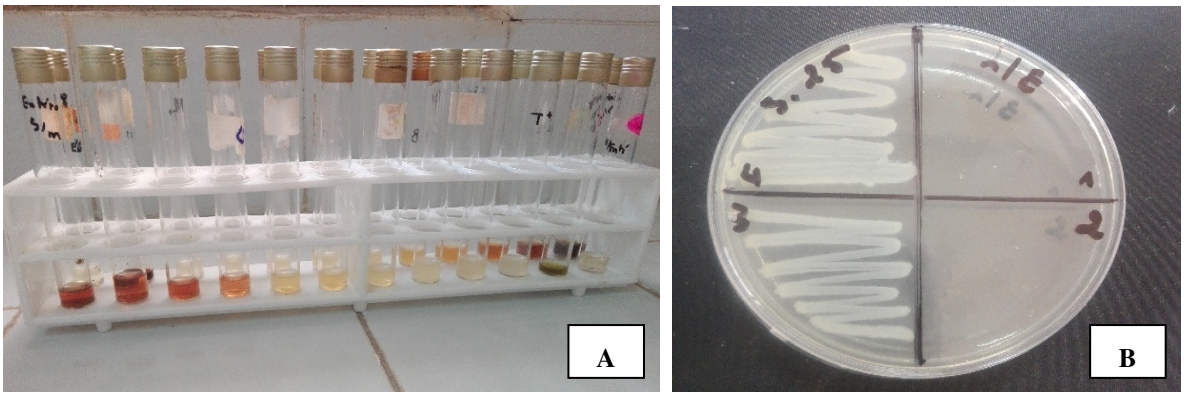

Figure 1. (A): MIC by macrodilution method, (B): inoculation of the tubes with no turbidity.

These results are backed by those obtained in the work of Daoud et al., which show that the ethanolic extract of *S. officinalis* provides a MIC >5000 µg/ml against *S. aureus* [56]. Ali and Aboud, carried out a study the antibacterial activity of *S. officinalis* extracts on *E. coli*, *E. faecalis* and *S. aureus* and found a variable MIC of 2500-5000 µg/ml for the methanolic extract and 2500-10000 µg/ml for the aqueous extract [57].

4. CONCLUSION

According to the results obtained, it can be concluded that the ethanolic extract (80%) of *Salvia officinalis* L. can play a biological role as an antioxidant product by scavenging free radicals and antibacterial activity. The quantitative evaluation of polyphenols and flavonoids for the extracts of *S. officinalis* exposes that the ethanolic extract is the richest in polyphenols and flavonoids in comparison with the methanolic extract and the aqueous.

On the other side, the results of antioxidant activity by the DPPH test and the antibacterial activity revealed that the ethanolic extract of S. officinalis provides a better free radical scavenging power and antibacterial activity compared to the other extracts. All the results obtained from this study indicate the possibility of using ethanolic extract of S. officinalis in the therapeutic field as an antioxidant and antibacterial.

Authors' Contributions: RB, RD, HA designed and carried out the research. RB, RD, AM carried out the experiments. RB, RD, FZA, HB, KZ,SA analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interest: The authors have no conflict of interest to declare.

Acknowledgements: We would like to thank the Engineers working in the Laboratory of Animal Reproduction Farm and Laboratory of Animal Hygiene and Pathology of the Institute of Veterinary Sciences University Ibn khaldoun Tiaret. We are also grateful to the Directorate General of Scientific Research and Technological Development "DGRSDT".

REFERENCES

1. Sharma S, Kulkarni Sh K, Chopra K. Curcumin, the active principle of turmeric (*Curcuma longa*), ameliorates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol. 2006; 33: 940-945.
2. Wu S, Chappell J. Metabolic engineering of natural products in plants; tools of the trade and challenges for the future. Curr Opin Biotechnol. 2008; 19: 145-152.
3. Kris-Etherton PM, Kari DH, Andrea B, Stacie MC, Amy EB, Kirsten FH, et al. Bioactive Compounds in Foods: Their Role in the Prevention of Cardiovascular Disease and Cancer. Am J Med. 2002; 113: 71S-88S.
4. Walia A, Gupta AK, Sharma V. Role of Bioactive Compounds in Human Health. Acta Scient Med Sci. 2019; 3(9): 25-33.
5. Nisar B, Sultan A, Rbab SL. Comparison of Medicinally Important Natural Products versus Synthetic Drugs-A Short Commentary. Nat Prod Chem Res. 2017; 6: 308.
6. Oliveira GO, Braga CP, Fernandes Ana AH. Improvement of biochemical parameters in type 1 diabetic rats after the roots aqueous extract of yacon [*Smallanthus sonchifolius* (Poepp.& Endl.)] treatment. FCT. 2013; 59: 256-260.
7. Kaneria M, Kanari B, Chanda S. Assessment of effect of hydroalcoholic and decoction methods on extraction of antioxidants from selected Indian medicinal plants. Asian Pac J Trop Biomed. 2012; 2(3): 195-202.
8. Favier A. Stress oxydant et pathologies humaines. Ann Pharm Fr. 2006 ; 64: 390-396.
9. Islam TMM, Shekhar HU. Impact of Oxidative Stress on Human Health. In: Rani V, Yadav U, eds. Free Radicals in Human Health and Disease. New Delhi, Springer, 2015: 59-73.
10. Noémie V. Remèdes et recettes à la sauge: Les bonnes plantes de nos grands-mères. Brittany, Fleurus, 2013.
11. Ghorbani A, Esmaeilizadeh M. Pharmacological properties of *Salvia officinalis* and its components. J Tradit Complement Med. 2017; 1-8.

12. Ghezelbash GhR, Parishani MR, Fouani MH. Antimicrobial activity of *Salvia officinalis* acetone extract against pathogenic isolates. J. Herb Drug. 2015; 5(4): 215-218.
13. Lamuela-Raventós RM. Folin-Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. In: Measurement of Antioxidant Activity & Capacity: Recent Trends and Applications. John Wiley & Sons Ltd, 2018: 107-115.
14. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticult. 1965; 16: 144-153.
15. Bag GC, Grihanjali Devi P, Bhaigyabati Th. Assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three hedychium species of manipur valley. IJPSR. 2015; 30(1): 154-159.
16. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from Licaniae flora. J Ethnopharmacol. 2002; 79(3): 379-381.
17. Dehpour A, Ibrahimzadeh MA, Seyedfazelet N, Seyed M. Antioxydant activity of the methanol extract of *Ferula Assafoetida* and its essential oil composition. Grasas Aceites. 2009; 60(4): 405-412.
18. Mazari K, Bendinerad N, Benkhechi Ch, Fernandez. Chemical composition and antimicrobial activity of essential oil isolated from Algerian *Juniperus phoenicea* L and *Cupressus sempervirens.* J Med Plant Res. 2010; 4(10): 959-964.
19. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016; 6: 71-79.
20. Schwalbe R, Steele-Moore L, Goodwin AC. Macro- and Microdilution Methods of Antimicrobial Susceptibility Testing, In Antimicrobial Susceptibility Testing Protocols, 1st edn. CRC Press, New York, 2007; 75-78.
21. Duletić-Laušević S, Alimpić AA, Živković J, Gligorijević N, Šavikin K, Radulović S, et al. Evaluation of bioactivities and phenolic composition of extracts of *Salvia ofcinalis* L. (Lamiaceae) collected in Montenegro. Bot Serb. 2019; 43(1): 47-58.
22. Alimpić A, Oaldje M, Matevski V, Marin PD, Duletić-Laušević S. Antioxidant activity and total phenolic and flavonoid contents of *Salvia amplexicaulis* lam. extracts. Arch Biol Sci Belgrade. 2014; 66(1): 307-316.
23. Duletić-Laušević S, Alimpić A, Pavlović D, Marin PD, Lakušić D. *Salvia officinalis* of different origins Antioxidant activity, phenolic and flavonoid content of extracts. Agro Food Industry Hi Tech. 2016; 27(1): 52-55.
24. Vidyarthi N, Mazumdar A. Oxidative stress and antioxidant enzymes. Pharmacophore. 2014; 5(6): 889-894.
25. Verpoorte R, Alfermann AW. Secondary metabolism. In: Metabolic Engineering of Plant Secondary Metabolism. Springer Science & Business Media, Kluwer Academic Publishers, 2000: 1-23.
26. Doughari JH. Antimicrobial Activity of *Tamarindus indica* Linn. Trop J Pharm Res, 2006; 5(2): 597-603.
27. Iwalokun BA, Usen UA, Otunba AA, Olukoya DK. Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. Afr J Biotechnol. 2007; 6(15): 1732-1739.
28. Agbafor KN, Akubugwo El, Ogbashi ME, Ajah PM, Ukawandu CC. Chemical and Antimicrobial Properties of Leaf Extracts of *Zapoteca portoricensis*. J Med Plants Res. 2011; 5(5): 605-612.
29. Mickymaray S. Efficacy and Mechanism of Traditional Medicinal Plants and Bioactive Compounds against Clinically Important Pathogens. Antibiotics. 2019; 8(257): 1-57.
30. Öncül N, Karabıyıklı Ş. Mechanism of antibacterial effect of plant based antimicrobials. Ukr Food J. 2016; 5(3): 541-549.
31. Stefanović OD. Synergistic Activity of Antibiotics and Bioactive Plant Extracts: A Study Against Gram-Positive and Gram Negative Bacteria. In: Kırmusaoğlu S. Bacterial Pathogenesis and Antibacterial Control. Croatia, BoD – Books on Demand, 2018: 23-48.

32. Shovon LS, Prianka S, Nigarin S. In vitro evaluation of phytochemical components and antimicrobial activity of the methanolic extract of *Tridax procumbens* L. against pathogenic microorganisms. J Pharmacogn Phytochem. 2016; 5(5): 42-46.
33. Pop A, Muste S, Mureșan C, Pop C, Salanță L. Comparative study regarding the importance of sage (*Salvia officinalis* L.) In terms of antioxidant capacity and antimicrobial activities. Hop Med Plants. 2013; 1(2): 41-42.
34. Uygun M, Uygun DA, Aktaş K, Özdemir C, Özkan M, Karagözler AA. Antioxidant activity of *Salvia Tchihatcheffii* leaf extracts. Ege Uni J Faculty Sci. 2012; 36(1/2): 18-33.
35. Kumar S, Yadav A, Yadav M, Yadav JP. Effect of climate change on phytochemical diversity, total phenolic content and in vitro antioxidant activity of *Aloe vera* (L.). BMC Research Notes. 2017; 10(60): 1-12.
36. Lee KW, Kim YJ, Lee HJ, Lee ChY. Cocoa Has More Phenolic Phytochemicals and a Higher Antioxidant Capacity than Teas and Red Wine. J Agric Food Chem. 2003; 51: 7292-7295.
37. Hammoudi R, Khenfer S, Medjouel M, Tlili ML, Mohammed MH. Optimization of extraction conditions for phenolic compounds from *salvia chudaei*. LSJ. 2017; 18(2): 234-243.
38. Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. J Pharmaceut Biomed. 2006; 41: 1523-1542.
39. Abarca-Vargas R, Zamilpa A, Petricevich VL. Development and validation of conditions for extracting flavonoids content and evaluation of antioxidant and cytoprotective activities from *Bougainvillea* x *buttiana* Bracteas (var. rose). Antioxidants. 2019; 8(264): 1-16.
40. Do QD, Angkawijaya AE, Tran-Nguyen PhL, Huynh LH, Soetaredjo FE, Ismadji S, Yi-Hsu J. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatic. JFDA. 2014; 22: 296-302.
41. Rasmy NM, Hassan Amal A, Foda MI, El-Moghazy MM. Assessment of the Antioxidant Activity of Sage (*Salvia officinalis* L.) Extracts on the Shelf Life of Mayonnaise. WJDFS. 2012; 7(1): 28-40.
42. Mekhaldi A, Bouznad A, Djibaoui R, Hamoum H. Phytochemical Study and Biological Activity of Sage (*Salvia officinalis* L.). Int J Biol Vet Agricult Food Engin. 2014; 8(11): 1165-1169.
43. Zhou K, Yu L. Effects of extraction solvent on wheat bran antioxidant activity estimation. Lebensm-Wiss Technol. 2004; 37: 717-721.
44. Tosun M, Ercisli S, Sengul M, Ozer H, Polat T, Ozturk E. Antioxidant Properties and Total Phenolic Content of Eight Salvia Species from Turkey. Biol Res. 2009; 42: 175-181.
45. Lafka TI, Sinanoglou V, Lazos ES. On the extraction and antioxidant activity of phenolic compounds from winery wastes. Food Chem. 2007; 104: 1206-1214.
46. Sepahpour S, Selamat J, Manap Mohd YA, Khatib A, Razis AFA. Comparative Analysis of Chemical Composition, Antioxidant Activity and Quantitative Characterization of Some Phenolic Compounds in Selected Herbs and Spices in Different Solvent Extraction Systems. Molecules. 2018; 23(402): 1-17.
47. Koffi E, Sea T, Dodehe Y, Soro S. Effect of the type of solvent on the extraction of polyphenols from twenty-three Ivorian factories. JAPS. 2010; 5: 550-558.
48. Anwar F, Przybylski R. Effect of solvents extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.). Acta Sci Pol Technol Aliment. 2012; 11(3): 293-301.
49. Grujic N, Lepojevic Z, Srdjenovic B, Vladic J, Sudji J. Effects of Different Extraction Methods and Conditions on the Phenolic Composition of Mate Tea Extracts. Molecules. 2012; 17: 2518-2528.
50. Abd-Elmageed MAM, Hussein BA. Cytotoxicity and antimicrobial activity of *Salvia officinalis L.* flowers. SJMS. 2008; 3(2): 127-130.

51. Mosafa E, Yahyaabadi S, Doudi M. In-Vitro Antibacterial Properties of Sage (*Salvia officinalis*) Ethanol Extract against Multidrug Resistant *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Zahedan J Res Med Sci. 2014; 16(10): 42-46.
52. Veličković DT, Karabegović IT, Stojičević SS, Lazić ML, Marinković VD, Veljković VB. Comparison of antioxidant and antimicrobial activities of extracts obtained from *Salvia glutinosa* L. and *Salvia officinalis L.* Hemijska industrija. 2011; 65(5): 599-605.
53. Ben Khedher MR, Hammami M, Arch Jonathan RS, Hislop DC, Eze D, Edward TW. et al. Preventive effects of *Salvia officinalis* leaf extract on insulin resistance and inflammation in a model of high fat diet-induced obesity in mice that responds to rosiglitazone. PeerJ. 2018; 6: 1-22.
54. Mariita RM, Oguge N, Ogol CKPO, Okemo PO. Methanol Extract of Three Medicinal Plants from Samburu in Northern Kenya Show Significant Antimycobacterial, Antibacterial and Antifungal Properties. Res J Med Plant. 2011; 5(1): 54-64.
55. Abdulhamid A, Fakai IM, Sani I, Argungu AU, Bello F. Preliminary Phytochemical and Antibacterial Activity of Ethanolic and Aqueous Stem Bark Extracts of *Psidium guajava*. Am J Drug Discov Dev. 2014; 4: 85-89.
56. Daoud S, Alqahtani M, Alkhalifa DH. Biosynthesis of silver nanoparticles using *salvia officinalis*. Int J Curr Res. 2015; 7(10): 21548-21552.
57. Ali MR, Aboud AS. Antimicrobial activities of aqueous and methanolic extracts from *Salvia officinalis* and *Salix acmophylla* used in the treatment of wound infection isolates. Ibn Al Haitham J Pure Appl Sci. 2010; 23(3): 1-14.