Scientific Works. Series C. Veterinary Medicine. Vol. LXIX (2), 2023
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

IS ANTIOXIDANT CAPACITY CONNECTED TO BIOLOGICAL EFFECTS OF *SALVIA GLUTINOSA* L.?

Oana POP (COZMA)[1], Carmen Dana ȘANDRU[1, 2], Diana OLAH[1], Gheorghiță DUCA[2], Mariana RUSU[2], Aurel VASIU[1], Emoke PALL[1, 2], Cristian VĂLEANU[1], Marina SPÎNU[1, 2]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, 3-5 Mănăștur Street, 400372, Cluj-Napoca, Romania
[2]Institute of Research and Development for Montanology Cristian, 147 Street XIII, Cristian, Sibiu, Romania

Corresponding author email: marina.spinu@gmail.com

Abstract

The active principles extracted from plants prove to be useful in preventing or treating various diseases through their influence at molecular level. This potential of plants could reduce the negative effects of existing therapies due to lesser side effects and the results of bioavailability studies are encouraging. Hydro-alcoholic Salvia glutinosa L. extracts were examined for their antioxidant potential, anti-bacterial activity and in vitro immune stimulating effects. The dry aerial part of the plant (herba) was used for these experiments, after minced and solubilized in ethanol and also in aqueous solution. The antioxidant capacity was investigated by free radical scavenging effect over 1,1-diphenyl l-2-picrylhydrazyl (DPPH) radical, the antimicrobial effect by the Kirby Bauer radial diffusion test and the immune stimulating effect by tests on whole blood cultures. The results indicated a stronger antioxidant capacity (RSA% 88.89), antibacterial effect (G - E. coli, P. aeruginosa, x = 12 mm < G+, S. aureus, ATCC/clinical strains, x = 23 mm) and immune stimulation of the ethanol extract compared to the aqueous one. These results indicated the plant as a potential complex source to be implemented in alternative therapy.

Key words*: Salvia glutinosa* L.*, extract, antioxidant, antimicrobial, immune stimulating, therapy.*

INTRODUCTION

In many parts of the world medicinal plants have been used as components of the alternative therapy for numerous diseases (Palombo, 2011, Petrovska, 2012). Phytochemicals present in medicinal plants have discovered to be beneficial for the prevention and treatment of various diseases due to its capacity to act in multiple biological mechanisms (Zhang et al., 2011; Pereira et al., 2019). Other findings also suggested that some of the plants used in traditional medicine could provide antioxidant components useful for both therapy and preventive purposes.

One of the largest genera of *Lamiaceae* comprising approximately 980 species is genus *Salvia* (Hu et al., 2018). Originally from Europe and West Asia, *Salvia glutinosa* L. is a perennial herbaceous plant with wide distribution especially in the mountainous regions, being naturalized in several areas due to the appearance, smell and medicinal qualities it offers. The height (50-100 cm) at which it can reach is different depending on the pedo-climatic conditions. The morphological characteristics of this plant are: high and sticky stem, lanceolate leaves and provided with sticky glandular brushes, corolla consisting of yellow petals, with brown punctures, also provided with glandular brushes (Yin et al., 2023).

In Europe, *Salvia glutinosa* is widespread in mountain areas, mainly in deciduous forests, but other preferential areas are also reported, such as mixed coniferous and deciduous forests, wetlands at the edge of forests or on alpine pastures. There are numerous studies that describe the composition in active principles of *Salvia glutinosa*, which differs depending on the development conditions of the plant (microclimate, soil, geographical area), but also depending on the solvent used for extraction (Grdiša et al., 2015). Among the biologically active substances in the composition of *Salvia* are flavonoids, which are secondary metabolites of plants (Kennedy and Wightman, 2011).

The pharmacological effects of *Salvia* essential oils are based on the presence of more than 100 active compounds, which can be categorized

into monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, diterpenes, nonisoprenoid compounds and oxygenated sesquiterpenes (Fu et al., 2013; Szentmihályi et al., 2004). The most abundant components are 1,8-cineole, camphor, and a wide variety of thujenes (Russo et al., 2013).

The purpose of this study was to determine the effect of *Salvia glutinosa* extracts on bacteria and leukocytes, in connection to the antioxidant activity of the plant, to clarify the link between the *in vivo* effects of sage decoction used in traditional medicine on skin wounds and the true mechanisms underlying the repair processes in these cases.

MATERIALS AND METHODS

The aqueous and ethanolic extracts of *Salvia glutinosa* were evaluated by a three step procedure, envisaging the antioxidant activity (a), the antibacterial effect (b) and the *in vitro* immune activity on whole blood cultures (c), as follows:

a. The total polyphenol content in aqueous solutions and in ethanolic extracts was estimated using the technique described by Blainski et al. (2013). The absorbance was read at 750 nm using a Shimadzu UV-VIS 1700 spectrophotometer. The standard curve was maintained by using different concentrations of gallic acid: 0, 0.25, 0.50, 0.75 and 1 mg/ml. The content in total polyphenols was expressed in gallic acid equivalents, mg GAE/100 ml sample.

To express the antioxidant activity of the plant extracts, the free radical scavenging effect was assessed over 1,1-diphenyl l-2-picrylhydrazyl (DPPH) radical, method proposed by Odriozola-Serrano et al. (2013) 100 µl of each sample was mixed with 3.9 ml of DPPH (0.025 g/l) maintained properly in the dark for 30 min. The absorbance of the samples was measured at 515 nm (Shimadzu 1700 UV-VIS) against a methanol/water blank (percent over standard DPPH absorbance).

b. The *in vitro* antimicrobial potential of watery and ethanolic extracts of *Salvia glutinosa* was evaluated by well-diffusion method against the following reference strains: *Staphylococcus aureus* ATCC 6538P, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 10145. For each bacterium, an inoculum was prepared by suspending 24 h pure culture in Mueller Hinton (MH) broth in order to dilute approximately to 10^{-6} colony forming units (CFU)/mL according to McFarland scale. The bacterial suspensions were "flood-inoculated" onto the surface of MH agar and dried. The extracts (60 µL) were placed into wells (three wells of six-millimeter diameter for each extract) made into the MH agar using a sterile matrix. The assay included 70% ethanol as the negative control and gentamicin as standard antibiotic. All tests were performed in triplicate. The diameters of the growth inhibition zones were measured after 24 h of incubation at 37°C. The data were statistically analysed for significance of the treatment effect by using Microsoft Excel software.

c. For monitoring the in vitro effect of the *Salvia glutinosa* extracts on leukocytes, blood samples were collected from adult bovine, by puncturing the jugular vein, during the official testing for transmissible disease surveillance. The samples were diluted with RPMI 1640 (Sigma-Aldrich, USA) medium (1:4) with 5% FCS (Gibco) and penicillin and streptomycin (Sigma-Aldrich). The diluted samples were added 96-well plate, 100 µl/well, 6 variants: (1) untreated control culture, (2) phytohaemagglutinin-M (PHA-M) (1 µl/well) (3) Alcohol (1.5 µl/ well), (4) concanavalin A (Con A) (1 µl/ well), (5) alcoholic extracts of *Salvia officinalis* (1.5 µl /well), (6) alcoholic extracts of *Vaccinium myrtillus* (1.5 µl/well). The cultures were incubated for 48 h at 37°C and 5% CO_2. Glucose concentrations were measured in the initial medium and in all variants, using orto-toluidine colorimetric test. 12.5 µl of the cultural medium were transferred to 0.5 ml of orto-toluidine reagent, heated for 8 minutes, added in cold water and evaluate using a spectrophotometer at 610 nm wavelength (Sumal PE2, Karl Zeiss, Germany), using the reagent as a blank. For transformation index (TI) the following formula was used: TI %=[(MG–SG)/MG] ´100, where TI, blast transformation index, MG, glucose concentration in the initial culture medium and SG, glucose concentration in the sample after incubation.

The data were statistically analysed for significance of the treatment effect by using Microsoft Excel software.

RESULTS AND DISCUSSIONS

Investigated by the lipide peroxidation system, the antioxidant activity of aqueous methanolic extracts of various sage species (*S. candelabrum*, *S. ringens*, *S. tomentosa*, *S. nemorosa*, and *S. glutinosa*) were dependant on concentration and similar to those observed for *S. officinalis* (Miura et al., 2002; Zupkó et al., 2001). The results of the Folin-Ciocâlteu method are presented in Figure 1 and reveal an increased amount of polyphenols in the ethanolic extracts, compared to the aqueous extracts, respectively the decoction and infusion of *Salvia glutinosa*. The differences between the two types of preparations lie, on the one hand, in the greater solubilizing capacity of alcohol compared to polyphenols and, on the other hand, in the temperature of obtaining the decoction and the infusion. The water used to prepare the teas was pre-heated to 100°C, which led to the denaturation of an important fraction of polyphenols, considering the fact that the optimal temperature for their recovery is 20-50°C (Brglez Mojzer et al., 2016).

The antioxidant activity measured by the DPPH test was similar to that described in literature for Romanian *Salvia glutinosa* (Mocan et al., 2020) that is X = 79.57±0.71 mg TE/g of extract for the tea and X = 88.21±0.63 mg TE/g of alcoholic extract. The radical scavanging activity varied in intensity based on plant sampling season (Figure 2).

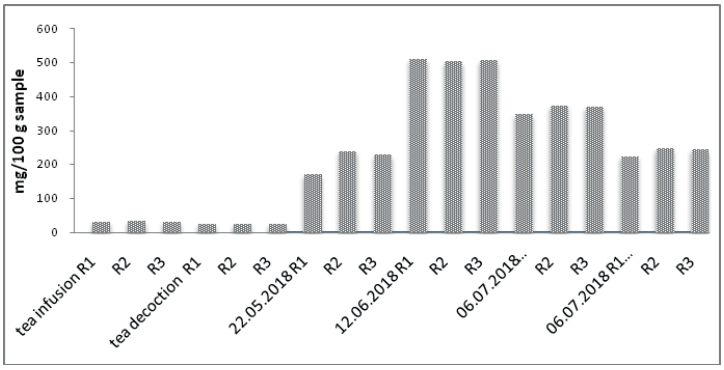

Figure 1. Total polyphenol content of different extracts of *Salvia glutinosa*. The R1, 2, 3 indicate the number of the replica for each sampling date ($p<0.05$ for the month of June versus May, and alcoholic extracts versus tea or decoction)

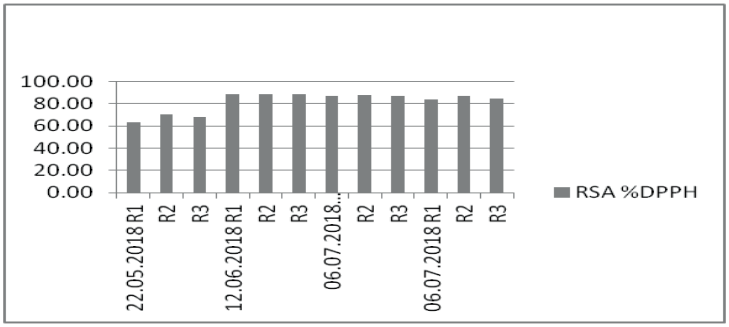

Figure 2. The radical scavenging activity (RSA%) depended on the sampling period of the plants, with higher values in June-July

The antimicrobial efficacy of the ethanolic extract and aqueous preparations of *Salvia glutinosa* showed different activity against different ATCC and field isolates of bacterial strains (Figure 3). As it is indicated by the inhibition diameters, the alcoholic extract was more active than the tea against all bacteria except *E. coli*, where surprisingly, the tea was

more active. These data are similar to those in literature, citing the efficacy of *Salvia glutinosa* alcoholic extract against numerous bacteria, including *B. cereus* (Mocanu et al., 2020). Nevertheless, the efficacy of the tea against *E. coli* was not mentioned, although the same effect is recognised for *S. officinalis* tea (Vogl et al., 2013). The statistically significant differences ($p<0.05$ - $p<0.001$) were recorded only when comparing the activity of antibiotics with that of the plant extracts.

In the blast transformation test of the leukocytes, the alcoholic extract induced stimulation of the mononuclear cells, when compared to the tea or decoction (Figure 4).

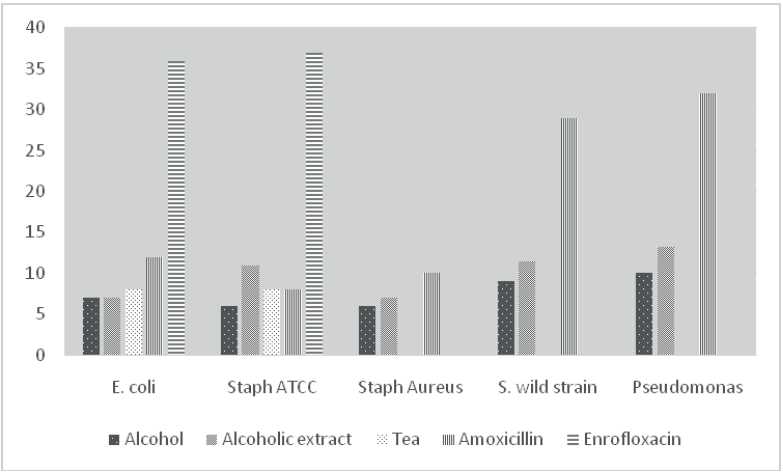

Figure 3. Antimicrobial efficacy of different *Salvia glutinosa* extracts when compared to ethanol and antibiotics

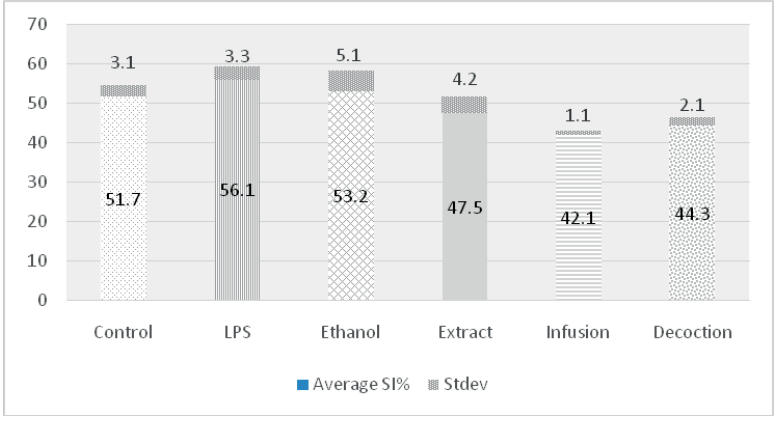

Figure 4. The blast transformation indices calculated for various *in vitro* treatments: the alcoholic extract induced an increased stimulation of the leukocytes when compared to aqueous preparations

The need to discover new methods in diminishing the antibiotic resistance phenomenon directed research towards the investigation of the bacteriostatic or bactericidal capacity of some plant extracts. Current studies in the field, which verify the bioavailability of *Salvia officinalis* and other *Salvia* species extracts' biological activity at cellular level are becoming more and more numerous, thus emphasizing the bactericidal and antioxidant properties of this genus.

The data in the literature supports the antimicrobial properties of *Salvia glutinosa*. Testing the ethanolic extract of *Salvia* on some clinical isolates and some reference strains of *Streptococcus pyogenes* reveals that a

concentration of 125 µg of Salvia extract is necessary to alter the structure of the bacterial wall and exert a bactericidal effect (MBC) (Wijesundara et al., 2019). The results of the present study followed the same dose-effect pattern, based on the type of bacterial isolate investigated. This locally resourced plant provides encouraging solutions to implement in antibacterial therapy, further enhanced by the radical scavanging activity and its immune stimulating effect.

CONCLUSIONS

The bioavailability of the active principles of *Salvia glutinosa* L. in different cell culture systems (bacteria and cells of the circulating immune system) depends on several factors, such as: the extraction method, the type of cells used, the harvesting period and the amount of polyphenols.

The antioxidant effect due to the increased amount of polyphenols can be exploited for protection against oxidative damage and for anti-inflammatory properties in veterinary medicine as well. The ethanolic extracts active against bacteria could represent an alternative to antibiotic therapy especially against infections involving *Staphylococcus* spp., but the range of dosages needs to be expanded. The immune stimulating efficacy of the extracts represents an asset for their combined positive biological effects.

The observations made on all three different cell types justify the use of the obtained extracts as adjuvant therapy in the therapy of skin wounds.

AUTHORS' CONTRIBUTION

All authors had equal contribution in the study design, sampling, sample processing, data processing and writing the paper.

REFERENCES

Blainski, A., Lopes, G., de Mello, J. (2013). Application and Analysis of the Folin-Ciocalteu Method for the Determination of the Total Phenolic Content from Limonium Brasiliense L. *Molecules*, *18*(6), 6852–6865.

Brglez Mojzer, E., Knez Hrnčič, M., Škerget, M., Knez, Ž., Bren, U. (2016). Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects. *Molecules (Basel, Switzerland)*, *21*(7), 901.

Fu Z. H., Wang H., Hu X., Sun Z., Han C. (2013). The pharmacological properties of *Salvia* essential oils; 3(7): 122–127.

Grdiša M, Jug-Dujaković M, Lončarić M, Carović-Stanko K, Ninčević T, Liber Z, Šatović Z. (2015). Dalmatian sage (*Salvia officinalis* L.): A review of biochemical contents, medical properties and genetic diversity. *Agric Conspec Sci*.; 80(2): 69–78.

Hu G.-X., Takano A., Drew B.T, Liu E.-D., Soltis D.E, Soltis P.S., Peng H., Xiang C.-L. (2018). Phylogeny and staminal evolution of *Salvia* (Lamiaceae, Nepetoideae) in East Asia, *Annals of Botany*, 122: 4, 649–668,

Kennedy D. O., Wightman E.L. (2011). Herbal Extracts and Phytochemicals: Plant Secondary Metabolites and the Enhancement of Human Brain Function, *Adv Nutr.*; 2(1): 32–50.

Miura, K., Kikuzaki, H., & Nakatani, N. (2002). Antioxidant activity of chemical components from sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) measured by the oil stability index method. *Journal of agricultural and food chemistry*, *50*(7), 1845–1851.

Odriozola-Serrano, I.; Aguilo-Aguayo, I.; Soliva-Fortuny, R.; Martin-Belloso, O. (2013). Pulsed electric fields processing effects on quality and health-related constituents of plant-based foods. *Trends Food Sci. Technol*., 29, 98–107.

Palombo EA, Traditional Medicinal Plant Extracts and Natural Products with Activity against Oral Bacteria: Potential Application in the Prevention and Treatment of Oral Diseases, Evidence-Based Complementary and Alternative Medicine Volume 2011, Article ID 680354

Pereira, A. S. P., den Haan, H., Peña-García, J., Moreno, M. M., Pérez-Sánchez, H. ve Apostolides, Z. (2019). Exploring African Medicinal Plants for Potential Anti-Diabetic Compounds with the DIA-DB Inverse Virtual Screening Web Server. *Molecules*, *24*(10), 2002.

Petrovska B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy reviews*, 6(11), 1–5.

Russo, A., Formisano, C., Rigano, D., Senatore, F., Delfine, S., Cardile, V., Rosselli, S., & Bruno, M. (2013). Chemical composition and anticancer activity of essential oils of Mediterranean sage (*Salvia officinalis* L.) grown in different environmental conditions. Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association, 55, 42–47.

Szentmihályi K., Csedo C., Then M. (2004). Comparative study on tannins, flavonoids, terpenes and mineral elements of some *Salvia* species. 629:463–470.

Yin D., Luo W., Yang Z., Shuai Qi, Chen K., Gou A. (2023). Morphological and biochemical studies of *Salvia guaranitica* St. Hil. under simulated deposition with different amounts of dust, Ecotoxicology and Environmental Safety, 249:114404.

Zhang, L., Ravipati, A. S., Koyyalamudi, S. R., Jeong, S. C., Reddy, N., Smith, P. T., Bartlett, J., Shanmugam, K., Münch, G., & Wu, M. J. (2011). Antioxidant and anti-inflammatory activities of selected medicinal plants containing phenolic and flavonoid compounds. *Journal of agricultural and food chemistry*, *59*(23), 12361–12367.

Zupkó I, Hohmann J, Rédei D, Falkay Gy, Janicsák G, Máthé I. (2001). Antioxidant activity of leaves of *Salvia* species in enzyme-dependent and enzyme-independent systems of lipid peroxidation and their phenolic constituents. *Planta Medica*,67, 366-368.

Mocan, A., Babota, M., Pop, A., Fizeșan, I., Diuzheva, A., Locatelli, M., Carradori, S., Campestre, C., Menghini, L., Sisea, C. R., Soković, M., Zengin, G., Păltinean, R., Badarau, S., C Vodnar, D. C., & Crișan, G. (2020). Chemical Constituents and Biologic Activities of Sage Species: A Comparison between *Salvia officinalis* L., *S. glutinosa* L. and *S. transsylvanica (Schur ex Griseb. & Schenk) Schur*. *Antioxidants (Basel, Switzerland)*, *9*(6), 480.

Vogl, S., Picker, P., Mihaly-Bison, J., Fakhrudin, N., Atanasov, A. G., Heiss, E. H., Wawrosch, C., Reznicek, G., Dirsch, V. M., Saukel, J., & Kopp, B. (2013). Ethnopharmacological *in vitro* studies on Austria's folk medicine--an unexplored lore in vitro anti-inflammatory activities of 71 Austrian traditional herbal drugs. *Journal of ethnopharmacology*, *149*(3), 750–771.

Wijesundara Niluni M., Rupasinghe H. P. Vasantha (2019). Bactericidal and Anti-Biofilm Activity of Ethanol Extracts Derived from Selected Medicinal Plants against Streptococcus pyogenes, *Molecules,* vol. 24, 1165