

EVALUATION OF THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *SAMBUCUS EBULUS* EXTRACT

STELIANA RODINO¹, ALINA BUTU^{2*}, PETRUȚA PETRACHE^{1,3}, MARIAN BUTU², CRISTINA-ELENA DINU-PÎRVU⁴, CĂLINA PETRUȚA CORNEA¹

¹University of Agronomic Sciences and Veterinary Medicine, Bucharest, Bd Marasti 59, 011464, Romania

²National Institute of Research and Development for Biological Sciences, Splaiul Independentei 296, 060031, Bucharest, Romania

³EUROCIMPIA SRL, 117490, Mirosi, Arges

⁴“Carol Davila” University of Medicine and Pharmacy, Faculty of Pharmacy, Physical and Colloidal Chemistry Department, Traian Vuia 6, 020956, Bucharest, Romania

*corresponding author: alina_butu@yahoo.com

Manuscript received: December 2014

Abstract

This study investigated the inhibitory activity of *Sambucus ebulus* (danewort) fruit extract on the growth of several gram positive and gram negative bacteria, *Bacillus subtilis*, *Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Escherichia coli* and on the mycelial growth of some fungal pathogens, namely, *Botrytis cinerea*, *Rhizoctonia solani* and *Phytophthora infestans*. The contents of phenols, flavonoids and the antioxidant capacity were determined using spectrophotometric methods. The extract showed antimicrobial activity against most of the tested strains. The best results regarding antibacterial activity were obtained against *P. fluorescens* and *E. faecalis*.

Rezumat

În această lucrare sunt prezentate rezultate privind activitatea inhibitoare a extractului din fructe de *Sambucus ebulus* (boz) atât asupra creșterii unor bacterii Gram-pozitive și Gram-negative, și anume, *Bacillus subtilis*, *Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Escherichia coli*, cât și asupra creșterii miceliene a unor fungi fitopatogeni *Botrytis cinerea*, *Rhizoctonia solani* și *Phytophthora infestans*. De asemenea, utilizând metoda spectrofotometrică a fost determinat conținutul de fenoli și flavonoide și activitatea antioxidantă a extractului obținut din fructe de boz. Extractul studiat a prezentat activitate antimicrobiană împotriva majorității tulpinilor testate. Rezultate bune privind activitatea antibacteriană au fost obținute pentru *P. fluorescens* și *E. faecalis*.

Keywords: *Sambucus ebulus*, antimicrobial activity, antioxidant capacity, polyphenols

Introduction

Plant extracts have a long history in human and veterinary medicine. The plant secondary metabolites represent a topic of major interest, being studied as effective sources for human and animal disease controlling agents [1, 6, 8, 9, 19].

In scientific literature, there are studies that demonstrate the fact that the alcoholic and aqueous extracts of *Sambucus* species possess important biological properties. Extracts prepared from roots or aerial parts of *S. ebulus* are used for the treatment of inflammatory diseases such as inflammatory joint diseases, rheumatic pain and sore throat [10]. The leaves of *Sambucus ebulus* L. are used in folk medicine against high fever, rheumatic pains, snake and insect bites, urticaria, oedema and open wounds. Previous studies indicated the cytotoxic, antiinflammatory, antibacterial, antiviral, antinociceptive, antiulcer and inhibition of *Helicobacter pylori* effects of

extracts obtained from different plant parts of *S. ebulus* [3, 4, 7, 11, 18]. *S. ebulus* is mentioned in Romanian folk medicine as a remedy in the treatment of rheumatic pains or colds [14].

The aim of the present work was to investigate the capability of *S. ebulus* extract to inhibit the *in vitro* growth of some gram positive and gram negative bacteria, *Bacillus subtilis*, *Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Escherichia coli* and of fungal strains, namely, *Botrytis cinerea*, *Rhizoctonia solani* and *Phytophthora infestans*, causing plant diseases. The ethanol extract was also studied for its antioxidant activity and polyphenol content.

Materials and Methods

The plant material and extraction

The fruits of *S. ebulus* were collected from fields located in Southern Romania. The plant material was identified by researchers from the

Biotechnologies Department of NIRDBS and authenticated voucher specimens were deposited in the Herbarium of the department.

The hydroalcoholic extract (ethanol 70 %) was obtained by sonication (30 minutes, 35°C, 34 Watts) followed by maceration (72 h, room temperature). The concentration of the final extract was 102 mg of dry weight / mL.

Microbial Inoculum

The microbial strains belong to the Collection of the Biotechnologies Department of NIRDBS and Faculty of Biotechnologies from the UASVM, Bucharest.

Antimicrobial activity testing

The antibacterial activity was carried out by the disc diffusion method and the well diffusion method against *Bacillus subtilis*, *Enterococcus faecalis*, *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, and *Escherichia coli*.

Antifungal activity was achieved by the poisoned food method as described by Prashith et al. [13], against *Phytophthora infestans*, *Botrytis cinerea*, *Rhizoctonia solani*. According to the bibliographic study, there were used two different concentration of plant extract incorporated in the growth medium, 4% and 2%, respectively [15].

Plant extracts characterization

The total phenol content was determined by the Folin-Ciocalteu method [16] and was expressed as mg of gallic acid equivalents (GAE)/g of dry weight (DW). The total flavonoid content was determined by the aluminium chloride method as described by Erel et al. [6] and was expressed as mg of rutin equivalents (RE)/g of dry weight (DW). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay of the hydroalcoholic extracts was determined spectrophotometrically [17]. The antioxidant properties were expressed as DPPH %. The parameter EC₅₀ (µg/mL) was obtained by extrapolation from linear regression analysis.

Results and Discussion

Antimicrobial activity

For using the disk method, the most resistant strain was *B. cereus*, with an inhibition halo radius of only 1 mm. *B. subtilis*, *E. faecalis*, *S. aureus*, *P. fluorescens* were most susceptible to danewort extract, resulting an inhibition zone of 9 mm (Figure 1). *E. coli* strain proved to be resistant to *S. ebulus* extract and no antibacterial effect was observed.

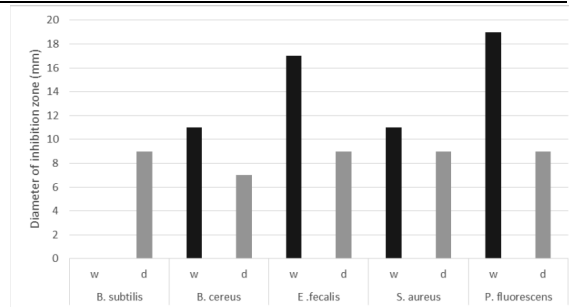


Figure 1.

Antibacterial activity of *S. ebulus* extract (w - well diffusion method; d - disc diffusion method)

Better results were obtained when employing the well diffusion method. The most susceptible strain was found to be *P. fluorescens* (19 mm), and the most resistant were *B. subtilis* and *E. coli* strains which did not develop any inhibition area.

In the case of fungal phytopathogens, *P. infestans* was the most susceptible, resulting in an inhibition of growth of 85%, when using a concentration of 4 % plant extract in the growing medium (Figure 2). At the same concentration, *R. solani* inhibition of growth was 78% and *B. cinerea* 63%. For the 2% concentration the distribution of the antifungal activity dynamics followed the same trend as before (Figure 2). It was observed that the growth inhibition was dose dependent decreasing with the concentration of each extract in the growth media.

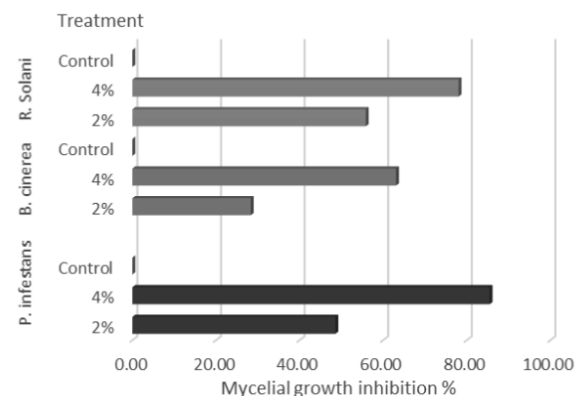


Figure 2.

The antifungal activity of *S. ebulus* extract

On the control Petri plate, the mycelium of *P. infestans*, began to grow on the fifth day after inoculation, while on the treated plates the growth was observed two days later. On the day 10, the control reached the margins of the plate, and on the 4% concentration in the growth medium, the colony was only beginning to grow (10 mm diameter) (Figure 3).

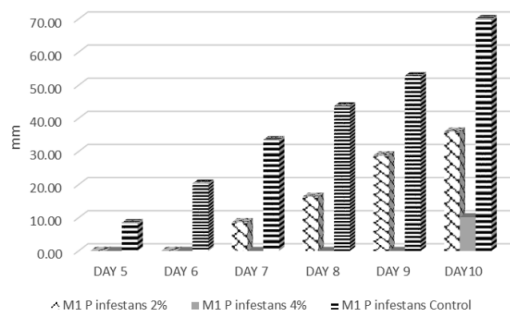


Figure 3.

The effect of *S. ebulus* extract on the mycelial growth of *P. infestans*

The most resistant fungal strain was *B. cinerea*. This is a plant pathogen attacking a wide range of horticultural species, causing pre and post-harvest grey mould of the fruits and vegetables. The complete growth of the control was observed on the fifth day after inoculation. At that moment, on the treated plates the mycelium was reaching 25 mm and 50 mm for 4% and respectively 2% plant extract in PDA (Figure 4.)

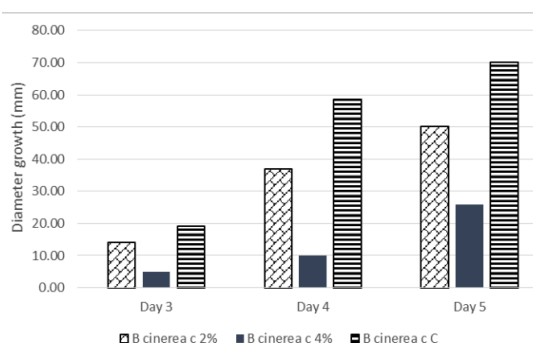


Figure 4.

The effect of *S. ebulus* extract on the mycelial growth of *B. cinerea*

R. solani mycelium presented the most rapid dynamic, the growth of the control being observed the second day after inoculation, reaching the maximum at four days after inoculation (Figure 5).

Due to economic, environmental and technical issues and to the more restrictive legal regulations in force, phytopathogens control is a difficult task to accomplish, and therefore complementary natural formulations could be a solution.

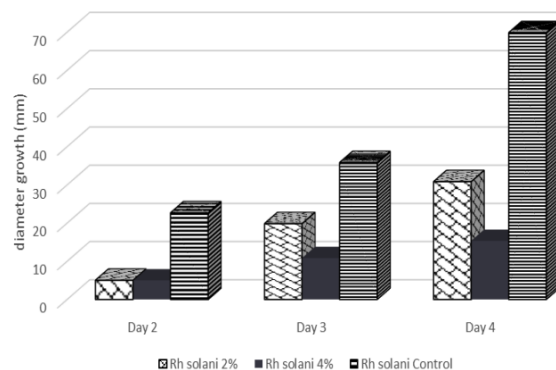


Figure 5.

The effect of *S. ebulus* extract on the mycelial growth of *R. solani*

Plant extract characterization

The total phenolic content (TPC) was determined based on the calibration curve achieved for gallic acid ($y = 0.00204x - 0.06775$, $R^2 = 0.9977$). The total flavonoid content (TFC) was determined based on the calibration curve for rutin ($y = 0.00348 + 0.000660x$, $R^2 = 0.9983$). The results provide a direct correlation between the values obtained for EC₅₀ factor and the for total phenolic and flavonoid contents (Table I).

Although in our country the therapeutic potential of danewort is less known, mentions on its medicinal properties were found back in 1973. Nevertheless other countries such as Turkey and Iran are paying special attention to this wild species [12]. Extracts of roots and leaves of *S. ebulus* have been used in traditional medicine to treat inflammatory joint diseases, rheumatic pain and sore throat [3] and there are several studies confirming the anti-inflammatory potential of danewort extracts [15].

Table I

TPC, TFC and antioxidant activity of *S. ebulus* extract

Total phenolic content (mg GAE / g DW)	Total flavonoid content (mg RE / g DW)	Antioxidant activity EC 50 (µg/mL)
126.502 ± 1.329	16.542 ± 0.471	68.45 ± 0.441

*Each value is the mean of three replicate determinations ± standard deviation

Conclusions

The extract obtained from the dried fruits of *S. ebulus* showed antimicrobial activity against most tested strains. The results indicated that this wild plant considered a weed has antibacterial and antifungal potential and therefore it may have practical applications in the development of an antimicrobial product based on natural compounds.

Acknowledgements

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.

References

1. Cowan M.M., Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, 1999; 12: 564.

2. Ebadi A.G., Hisoriev H., Review on distribution of *Sambucus ebulus* L. in the North of Iran. *American-Eurasian J. Agric. & Environ. Sci.*, 2011; 10(3): 351.
3. Ebrahimzadeh M.A., Mahmoudi M., Pourmorad F., Saeidnia S., Salimi E., Anti-inflammatory and antinociceptive properties of fractionated extracts in different parts of *Sambucus ebulus*. *Mazandaran Uni Med Sci.*, 2006; 16(54): 35.
4. Ebrahimzadeh M.A., Mahmoudi M., Salimi E., Antiinflammatory activity of *Sambucus ebulus* hexane extracts. *Fitoterapia*, 2006; 77(2): 146.
5. Erel S.B., Reznicek G., Senol S.G., Yavasogulu N.U., Konyalioglu S., Zeybek A.U., Antimicrobial and antioxidant properties of *Artemisia L.* species from western Anatolia. *Turk. J. Biol.*, 2012; 36: 75.
6. González-Lamothe R., Mitchell G., Gattuso M., Diarra M.S., Malouin F., Bouarab K., Plant antimicrobial agents and their effects on plant and human pathogens. *Int. J. Mol. Sci.*, 2009; 10(8): 3400.
7. Ghesmati M., Survey of antibacterial activity of *Sambucus Ebulus* extracts against *Staphylococcus aureus* ATCC 1341 and *Pseudomonas aeruginosa* ATCC 2785. *Journal of Biology Science*, 2008; 1(3): 73.
8. Gyawalia R., Ibrahim S.A., Natural products as antimicrobial agents. *Food Control*, 2014; 46: 412.
9. Hadacek F., Secondary metabolites as plant traits: current assessment and future perspectives. *Crit. Rev. in Plant Sciences*, 2002; 21(4): 273.
10. Hiermann A., *Sambucus* in: Blaschek W., Ebel S., Hackenthal E., Holzgrabe U., Keller K., Reichling J., Schulz V., editors. 6th ed. 2007, vol. 14. Wissenschaftliche Verlagsgesellschaft mbH; Stuttgart, Germany: 173-189.
11. Mahboubi A., Kamalinejad M., Shalviri M., Karbasi Z., Jafariazar Z., Asgharian R., Evaluation of antibacterial activity of three Iranian medicinal plants. *African Journal of Microbiology Research*, 2012; 6(9): 2048.
12. Popescu R., Chirigiu L., Bubulica M.V., Averis L.M.E., Qualitative screening of secondary metabolites from *Sambucus ebulus* roots by multiple analytical techniques. *Rev. Chim.*, 2014; 65(2): 148.
13. Prashith Kekuda T.R., Kavya R., Shrugashree R.M., Suchitra S.V., Screening of selected single and polyherbal Ayurvedic medicines for antibacterial and antifungal activity. *Anc. Sci. Life.*, 2010; 29(3): 22.
14. Ranković B., Kosanić M., Stanojković T., *Stereocaulon paschale* lichen as antioxidant, antimicrobial and anticancer agent. *Farmacia*, 2014; 62(2): 306.
15. Rodino S., Butu A., Butu M., Cornea C.P., *In vitro* efficacy of some plant extracts against damping off disease of tomatoes. *Journal of International Scientific Publications: Agriculture and Food*, 2014; 2: 240-244.
16. Schwaiger S., Zeller I., Pölzelbauer P., Frotschnig S., Laufer G., Messner B., Pieri V., Stuppner H., Bernhard D., Identification and pharmacological characterization of the anti-inflammatory principal of the leaves of dwarf elder (*Sambucus ebulus* L.). *J. Ethnopharmacol.*, 2011; 133(2-4): 704.
17. Vulic J.J., Tumbas V.T., Savatovic S.M., Dilas S.M., Cetkovic G.S., Canadanovic-Brunet J.M., Polyphenolic content and antioxidant activity of the four berry fruits pomace extracts. *Acta Periodica Technologica*, 2011; 42: 271.
18. Yesilada E., Gurbuz I., Tokar G., Anti-ulcerogenic activity and isolation of the active principles from *Sambucus ebulus* L. leaves. *J. Ethnopharmacol.*, 2014; 153(2): 478.
19. Zia-Ul-Haq M., Ahmad S., Stankovic M., Sultan M.T., Imran I., Velter V., Badiu D., Halichidis S., Hangan T., Antimicrobial and antioxidant potential of *Ipomoea hederacea*. *Farmacia*, 2014; 62(6): 1181.