Bulletin UASVM Animal Science and Biotechnologies 70(1)/2013, 86-94
Print ISSN 1843-5262; Electronic ISSN 1843-536X

Comparative Antibacterial Activity of Different Plant Extracts in Relation to their Bioactive Molecules, as Determined by LC-MS Analysis

Cosmin POP, Dan VODNAR, Floricuța RANGA, Carmen SOCACIU

University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Science and Technology, 3-5 Mănăştur Street, Cluj-Napoca, Romania; carmen.socaciu@usamvcluj.ro

Abstract. The research of plant responses against antibiotics and chemical disinfectants is increasingly motivated by natural antimicrobial agents. We investigated hydrophilic extracts of leaves of Persian walnut (*Juglans regia*) and European Mistletoe (*Viscum album*) and White willow bark (*Salix alba*) which were characterized by their content in phenolic derivatives, using a high-throughput technique, HPLC-DAD. The extracts were obtained using 10% dry plant and hot acidulated water (around 85^{0}C) and kept for 48 hours then diluted successively (from 2 to 9 times). The antimicrobial activities were tested semi-quantitative ly on agar plates inoculated with *E. Coli, Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus* and *Salmonella enteritis*. The inhibition zones were measured after 24 hours of incubation. Using a liquid nutrient broth growth medium, a quantitative evaluation of antimicrobial effect was done. There were noticed to be significant the growth rates in the first 10 hours, the best inhibitory results being given by *Salix alba* extract which inhibited all bacteria. Meanwhile, walnut and mistletoe extracts showed different inhibition rates, depending on the bacteria and the extract dilution. The extracts' composition as determined by HPLC was compared with the antimicrobial activity, suggesting that willow extract is the richest in phenolics, especially in phenolic acids (salicylic acid derivatives). This study suggest that *Salix alba* bark and *Juglans regia* are available and efficient sources of natural antimicrobials and suggests the possibility of using their hydrophilic extracts as bio-disinfectants in food industry.

Keywords: aromatic plants, Persian walnut (*Juglans regia*), European Mistletoe (*Viscum album*) and White willow bark (*Salix alba*), antimicrobial activity, UV-VIS spectrometry, LC-MS analysis

INTRODUCTION

The healing potential of plants was related to their antioxidant and antimicrobial properties (Chaieb *et al.*, 2013; Kavak *et al*., 2010) due to their complex composition, rich in secondary metabolites (more than 10.000 known and characterized) especially phenolic derivatives, known yet as physiological, defense molecules against abiotic stress and pathogens. The large variety of phenolic derivatives can show antagonistic, additive or synergistic effects, as it was suggested by experimental studies.

Resistance of pathogenic organisms to antibiotics has become a worldwide problem with serious consequences on the treatment of infectious diseases. The increased and non-controlled use of antibiotics in human medicine, veterinary and agriculture increases the antibiotic resistance of bacteria that cause either community infections or hospital -acquired infections. Of particular interest are the multidrug resistant pathogens, such as *E.coli, Klebsiella pneumoniae, Acinetobacter baumanii, Staphylococcus aureus, Streptococcus pneumonia*, etc. which can be inhibited not only by antibiotics but also by natural extracts (WHO report, 2001; Alekshun *et al.*, 2007).

Bacterial cells develop their internal strategies to inhibit the effects of antibiotics and their resistance mechanisms may help in the discovery and design of new antimicrobial agents. European studies on antibiotics' consumption and resistance showed significant variations, most of them related to the difference between drug consumption and infection control in hospitals or food industry. In Romania, as it is reported, the antimicrobial resistance show levels above 25%, and an increasing trend in the last years, that is quite common in Southern and Eastern Europe. Also, some microorganisms are starting to exhibit resistance to chemical disinfection products in food industry (Oancea and Stoica, 2010; Zakavi *et al.*, 2013).

The natural medicines and plant extracts used as food supplements or bio-disinfectants, respectively serve the health needs for about 80% of the population, knowing that more than 65% of the world's population uses medicinal plants as a primary healing way (WHO Report, 2001; Barrett, 2006). In recent years, many possible sources of natural antibiotics have been in use for several infectious diseases, mostly bacterial and fungal, many of these natural "phytocides" belonging to phenolics' family (Sulaiman *et al.*, 2013; Poblocka-Olech *et al.*, 2010; Fox, 2006).

Our studies aimed to characterize hydrophilic extracts with antimicrobial activity made from leaves of Persian walnut *(Juglans regia*) (Ebrahizadeh *et al.*, 2013; Fernández-Agulló *et al.*, 2013) and European Mistletoe *(Viscum album)* (Paun *et al.*, 2011) as well from White willow bark *(Salix alba*) (Agbenin, 2013; Du *et al.*, 2007; Gupta *et al.*, 2012). The extracts where characterized by their content in phenolic derivatives by HPLC-DAD advanced technique and in parallel, evaluated, semi-quantitative by and quantitatively for their antimicrobial effects against *Staphylococcus aureus*, *Listeria monocytogenes, Bacillus cereus, E. Coli and Salmonella enteritis.* The possible correlations between the phenolics' extract composition and their antimicrobial effect may have good impact in deciding which extract can be used as potential bio-disinfectant in food industry (Harbourne *et al.*, 2009)

MATERIALS AND METHODS

Sample preparation. Hydrophilic extracts from leaves of Persian walnut *(Juglans regia*), European Mistletoe *(Viscum album)* and White willow *(Salix alba* shell) were made using 10% dry plant mixed with hot, acidulated water containing 1% HCl. The extract was made using 15 g dry (10–12% water) plants (15% plant), which were mixed with 85ml acidulated water solution (distil water + HCL 1%).

The plant and water mix was homogenized and left for maceration for 48 hours. The extract was filtered and kept in a freezer (2–4^{o}C) until used.

UV-Vis spectrometry and HPLC-DAD analysis. The phenolic concentration in extracts was determined by UV-Vis spectroscopy (Folin-Ciocâlteu method). The pH of each extract was measured and buffered to 7 before to be used in specific bacterial media. The LC-MS analysis was performed on an Agilent 1200 HPLC device coupled with diode array detection (DAD) and a gradient of 2 solvent mixtures, A and B, as follows: methanol/ acetic acid/water (10/2/88) (solvent A) and methanol/acetic acid/ water (90/3/7) (solvent B). The eluent flow was 1ml/min and detection set at 280 nm.

The solvent gradient was as follows: A from 100% to 85% (min 0-10), A from 85% to 50% (min 10-30), A from 50% to 15% (min 30-45), A from 15% to 100% (min 45-55).

Microbiological investigations. There were used the following microorganisms: *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *E. coli* and *Salmonella enteritis* all being provided by the Microbiology department at USAMV Cluj-Napoca.

For a preliminary screening, the semi-quantitative inhibition test was made on agar nutrient plates and, after 24 hrs the area of bacterial growth inhibition induced by each extract.

For quantitative analysis, each plant extract was diluted in water and mixed with liquid media (nutrient broth SIFIN made of potato extract 4 g/l, dextrose 20 g/l, agar 15 g/l, pH 5.6±0.2) inoculated with each type of bacteria.

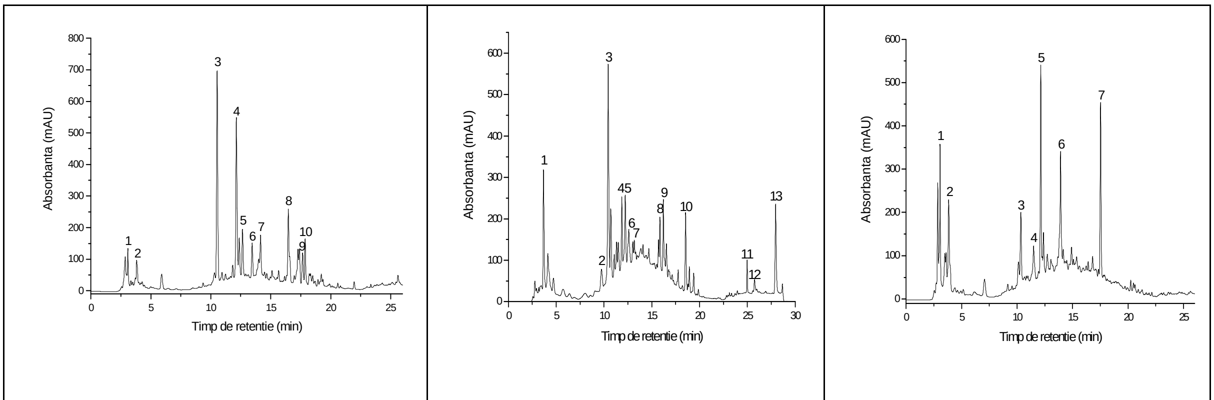
Three different dilutions were used: 5 ml extract + 5 ml broth+1 ml broth containing microorganism, 2 ml extract+ 8 ml extract +1 ml broth whit microorganism, 1 ml extract + 9 ml broth + 1 ml broth inoculated with each microorganism, abbreviated as E81, E72, E45 respectively). All determinations were compared with the control (broth inoculated with each microorganism). Their antimicrobial effect was monitored quantitatively after the inoculation with *E. coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus* and *Salmonella enteritis*. The growth was monitored by spectrometric measurement at 600 nm (Nano Drop spectrometer). The samples were measured at the inoculation time and every 2 hours, up to 24 hrs, incubated at 37^{o}C. The growth rate of different microorganisms was represented and compared with the control.

RESULTS AND DISCUSSIONS

UV-Vis and HPLC-DAD characterization of extracts. The initial pH of leaf and bark extracts of *Juglans regia*, *Salix alba*, *Viscum album* were 3, 1.73 and 3, respectively. To evaluate their antimicrobial effect the pH values were buffered with alkaline solution up to the neutral value of 7.

The *Salix alba* extract brought to the neutral pH, formed a precipitate which was difficult to be measured by optical methods. This extract contained, as demonstrated by HPLC-DAD, a high concentration of eriodictiol, isorhamnetin and Na-salicilate.

The total phenolic concentration of these extracts was expressed in gallic acid equivalents (GAE) per ml extract (data reported by Pop, 2013, unpublished) and the chromatographic fingerprint is presented in *Figure* 1.



Peak identified: 1- Cafeoylquinic acid; 2- Cumaroylquinic acid; 3-Hydrojuglone glucoside; 4-quercetin 3-rhamnoside; 5-kaemferol 3-glucoside; 6-kaemferol 7-glucoside; 7-juglone; 8-quercetin 3-glucoside; 9-quercetin 3-arabinoside; 10-theaflavin 3-gallate	**Peak identified:** 1- Dehydro-ascorbic acid; 2- p-coumaroyl glucoside; 3-eriodictiol 7-glucoside; 4,5-naringenin 7- and 5-glucoside; 6-catechin; 7-catechingallate; 8-naringin; 9-quercetin 3-glucoside; 10-naringenin dimer; 11-salicilate; 12-taxifolin; 13-isorhamnetin	**Peak identified:** 1-cafeoylquinic acid; 2- Betulinic acid; 3-resveratrol glucoside; 4-apigenin 7-glucuronide; 5-quercetin 3-acetyl-rhamnoside; 6-quercetin 3-rhamnoside; 7-theaflavin 3-gallate

Fig.1. Comparative HPLC-DAD fingerprints of phenolic derivatives found in *Juglans regia* (left), *Salix alba* (middle), *Viscum album* (right) extracts, as registerd at 280 nm.

The concentration of phenolic derivatives ranged from 1 to 2.6 mg/ml extract in the extracts (Pop *et al.*, 2013), with a highest concentration in willow extract. This extract proved to be rich eriodictiol, naringenin and quercetin glucosides, as well in catechins, salicilate and isorhamnetin, as determined by HPLC-DAD, all these bioactive molecules being responsible for its antibacterial activity.

The walnut extract was the second rich extract in phenolics, mainly represented by hydrojuglone derivatives (peaks 3 and 4), by quercetin derivatives (peaks 8-9) and theaflavin (peak 10). The mistletoe extract was mainly characterized by a higher content of quercetin and theaflavin.

Semi-quantitative evaluation of bacteria inhibition bz plant extracts. The inhibition zones of bacteria in agar plates were dependent on the extract type and the concentrations used. The best results were obtained by *Salix alba* extract which had the highest inhibition zones on agar plate against *Bacillus cereus* (2.72 mm), *Staphylococcus aureus* (1.10 mm), *Listeria monocytogenes* (3.08 mm) and *E. Coli* (0.85 mm). *Figures 2* and *3* show the inhibition zone of *Salix alba* extract against *Listeria monocytogenes* and *B.cereus.*

Fig. 2. The inhibition zone of Salix alba extract against Listeria monocytogenes

Fig. 3. The inhibition zone of Salix alba extract against Bacillus cereus

Table 1 shows the mean values of inhibition areas (expressed in mm) when the walnut and willow bark extracts, at three different dilutions were added to agar media inoculated with different microorganisms (*E. Coli*, *Staphylococcus aureus, Bacillus cereus, Salmonella enteritis*, *Listeria monocytogenes)*

Tab. 1

The mean values of inhibition area (expressed in mm) inoculated with different microorganisms, after addition of walnut and willow bark extracts

Microorganism tested	Walnut extract			Willow bark extract		
	E_{45}	E_{72}	E_{81}	E_{45}	E_{72}	E_{81}
E. coli	0.1	0	0	0.8	0	0
Staphylococcus aureus	0	0	0	1.1	0	0
Bacillus cereus	0	0	0	2.72	2.1	0.9
Salmonella enteritis	0.3	0	0	0	0	0
Listeria monocytogenes	0.8	0	0	3.08	2.6	0.5

In the inhibition test the willow extract had the best effect against *Listeria monocytogenes, Bacillus cereus* and no effect against *Salmonella.* The walnut extract inhibited *Listeria monocytogenes* and to some extent, *Salmonella enteritis* and *E. coli*. The extract from *Viscum album* showed no effect against these microorganisms. We correlated this weak effect with its low content of phenolics (almost 3 times lower than willow).

Quantitative evaluation of antimicrobial effects in liquid media. In all quantitative evaluations, the willow extract showed to have a 100% inhibitory effect during 24 hrs of incubation with all studies bacteria. The effects of the walnut and mistletoe extracts against *Staphylococcus aureus* were different and presented in *Figure 4*, left. After 24 hours of incubation it can be seen that the walnut extract inhibited the growth of this *Staphylococcus aureus* independently of the concentration (close values for E81, E72 and E45). The differentiation was not evident for mistletoe, where the effects were not dependent on the extract dilution and were close of the control (*Fig. 4*, right).

Meanwhile Braga *et al.* (2005) who used *Punica granatum* to inhibit the growth of *S. aureus* and also Zuo *et al*. (2008) showed a successful inhibition of *Staphylococcus* growth by other medicinal plants. In spite of its composition (but low in phenolic acids) *Viscum album* had almost no effect against *Staphylococcus aureus. Figure 5* shows the dynamics of *Listeria monocytogenes* growth in *vitro* using additions of the same extract dilutions (E81, E72 and E72) of *Juglans regia* and *Viscum album.* The walnut extract inhibited significantly the growth of *Listeria monocytogenes* when concentrated (E 81). Similar inhibition was also reported (Alvarez *et al*., 2006; Zuo *et al*., 2008).

The mistletoe extract (*Fig. 5*, right) had a weaker effect against *Listeria monocytogenes*, as compared with walnut, but a good relation dose-effect.

The investigations made by Hasmik *et al*. (2012) on *Punica granatum* extract confirmed the inhibition of the *Listeria*, unfortunately no literature data were found on walnut and mistletoe.

The walnut extract inhibited the growth of *Bacillus cereus* after incubation during 24 hours (*Fig. 6* left) significantly when it was concentrated (E81), similar to other authors (Hyejung *et al.*, 2013). The mistletoe extract (*Fig. 6* right) had also a strong effect after 6 hrs if the concentrated E81 extract was used. Similar results were obtained recently by Gutiérrez-Larraínzar *et al.* (2013).

The walnut extract inhibited the growth of *E. coli* after 24 hours of incubation (Fig. *7 left*) in a dose-dependent manner, as observed also by Wong and Kitts (2006). A similar effect can be seen for *Viscum album* (*Fig. 7 right*) extract.

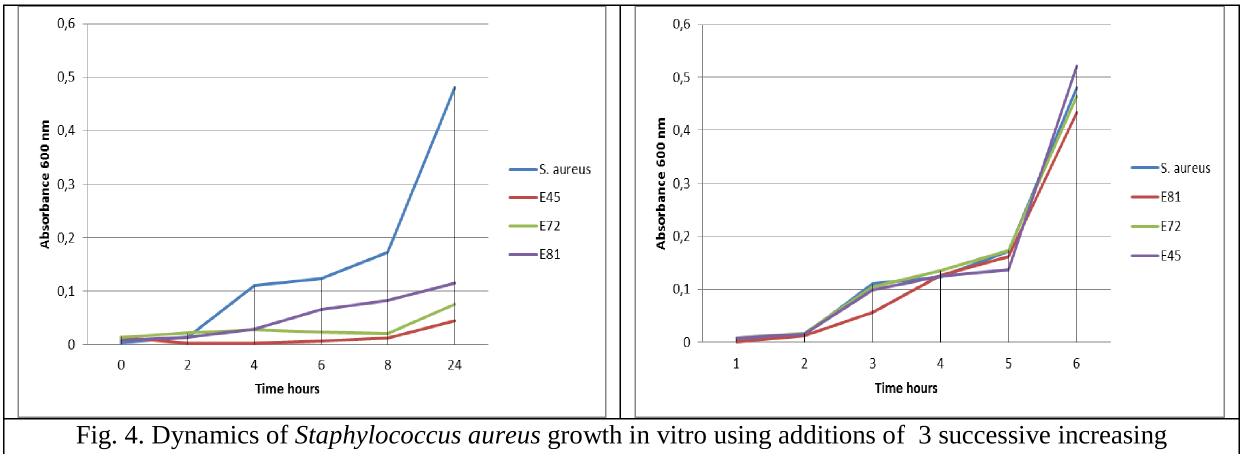


Fig. 4. Dynamics of *Staphylococcus aureus* growth in vitro using additions of 3 successive increasing concentrations (from E45 to E81) of *Juglans regia* and *Viscum album*

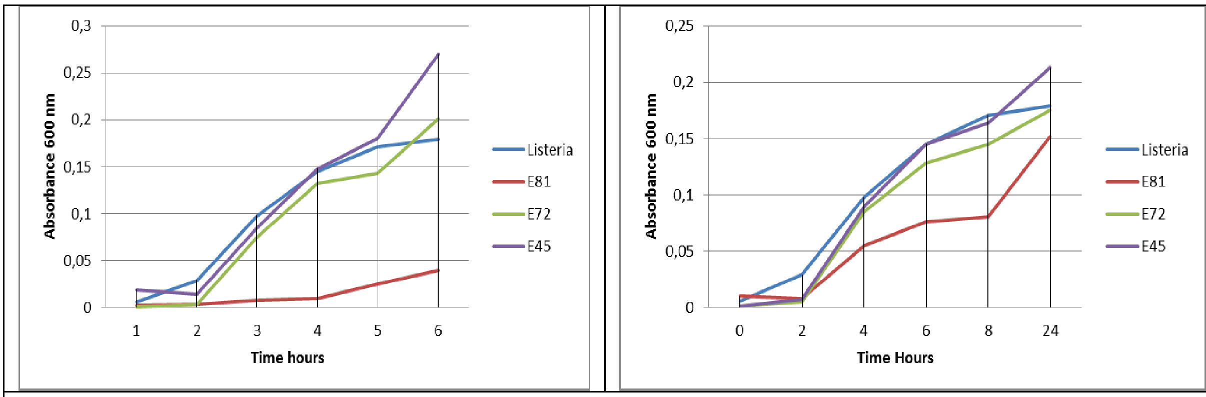


Fig. 5. Dynamics of *Listeria monocytogenes* growth in vitro using additions of 3 successive increasing concentrations (from E45 to E81) of *Juglans regia* and *Viscum album*

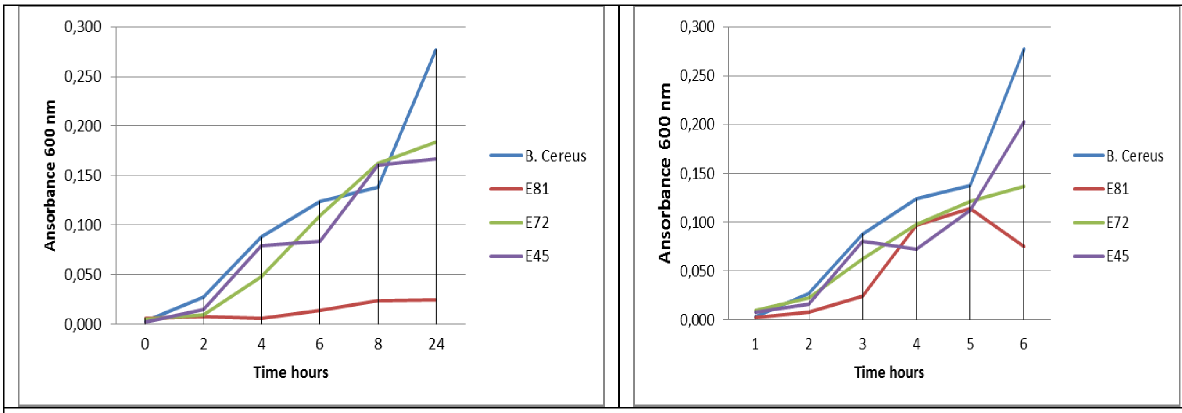


Fig. 6. Dynamics of *Bacillus cereus* growth in vitro using additions of 3 successive increasing concentrations (from E45 to E81) of *Juglans regia* and *Viscum album*

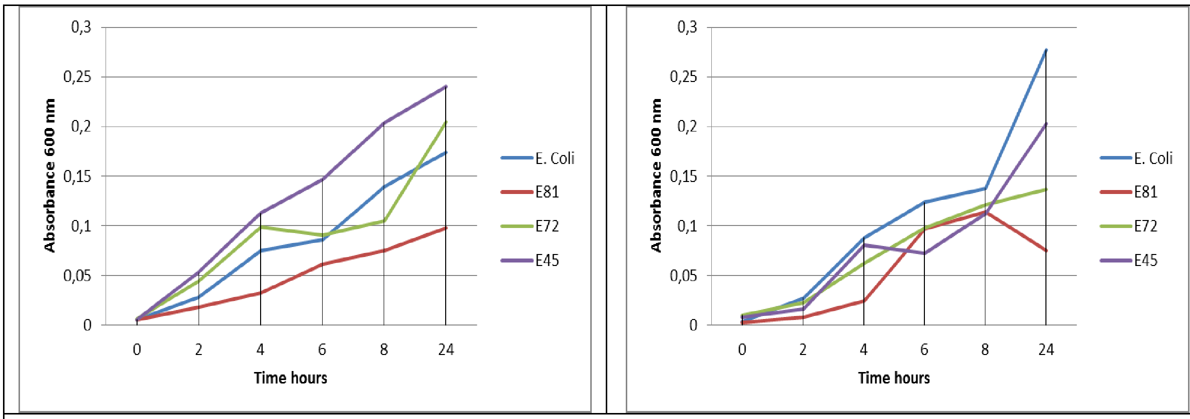
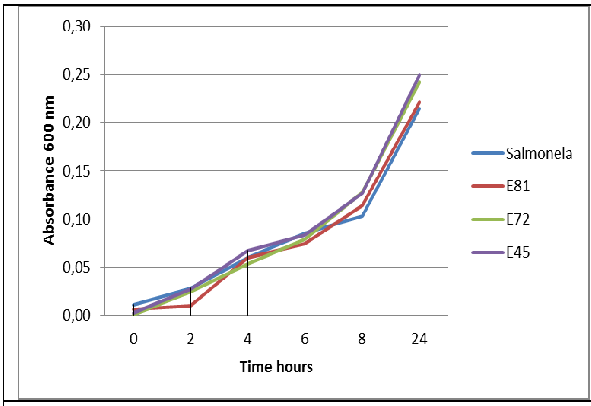


Fig. 7. Dynamics of *E coli* growth in *vitro* using additions of 3 successive increasing concentrations (from E45 to E81) of *Juglans regia* and *Viscum album*

After 24 hours of investigation in *Figure 8* it can be seen that the *Juglans regia* extract has inhibited the growth of *Salmonella enteritidis* at its highest concentration (1:1) and less for the other two concentrations. In a recent study, had demonstrated the growth inhibition potential of *Salmonella* using plants extract from *Vaccinium corymbosum* (Shen *et al.*, 2014). Meanwhile, the *Viscum album* extract has almost no effect against *Salmonella.* The inhibition test has revealed some interesting values for the three extracts. Extract from *Aloe secundiflora* showed an inhibition effect against salmonella (Waihenya *et al.*, 2002).

In liquid media, all *Salix alba* extracts, independently of their dilution, showed the best antimicrobial activity (100% inhibition), and especially against *Bacillus cereus* and *Staphylococcus aureus.*



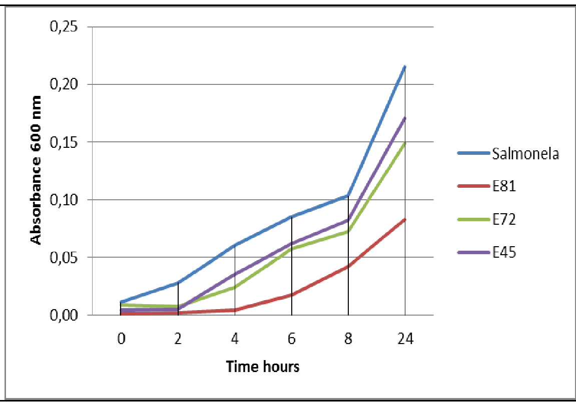


Fig. 8. Dynamics of Salmonella growth in vitro using additions of 3 successive increasing concentrations (from E45 to E81) of *Juglans regia* and *Viscum album*

CONCLUSION

This study confirms that dried leaf hydrophilic extracts of Persian walnut *(Juglans regia*), European mistletoe *(Viscum album)* and especially White willow bark *(Salix alba*) has good antibacterial effects. All these three extracts contain many phenolic derivatives, especially flavonoids derivatives and triterpenoids, as shown by HPLC-DAD analysis. By a semi-quantitative screening of their antimicrobial effect on agar media, willow bark extract showed to be the most active antibacterial agent, especially against *Listeria monocytogenes* and *Bacillus cereus.*

The quantitative evaluation of antimicrobial effect in liquid media, followed by UV spectrometric measurements, revealed that willow bark extract, the richest one in phenolic acid derivatives (salicylic acid) was the most efficient antimicrobial agent, while walnut and mistletoe had specific antibacterial activity, dependent on the extract dilution and type of bacteria.

In conclusion, our experimental data suggest possibilities to use the white willow bark and walnut leaf extracts as natural preservatives and bio-disinfectants against a large range of bacteria, which can contaminate the food production areas and food products. These types of extracts can be used also as ingredients for innovative formulas, based on natural, friendly solvents and possibly as atomized or lyophilized powders to obtain water-soluble bio-disinfectants for food industry.

REFERENCES

1. Acero-Ortega, O., L. Dorantes, H. Hernandez-Sanchez, M.S. Tapia, G. Gutierrez-Lopez, S. Alzamora, A. Lopez-Malo (2006). Response surface analysis of the effects of Capsicum extract, temperature and pH on the growth and inactivation of *Listeria monocytogenes*, J. Food Eng. 67: 247–252.

2. Agbenin, N.O. (2013). Effect of aqueous extract of some plants on soil micro-fauna and micro-flora *in vitro*. Arch. Phytopathol. and Plant Protection. 7: 299-301,

3. Agnolet, S., S. Wiese, R. Verpoorte, D. Staerk (2012). Comprehensive analysis of commercial willow bark extracts by new technology platform: Combined use of metabolomics, high-performance liquid chromatography-solid-phase extraction-nuclear magnetic resonance spectroscopy and high-resolution radical scavenging assay. J.Chromatogr. A. 1262: 130-137.

4. Alekshun, M.N. and S.B. Levy (2007). Molecular mechanisms of antibacterial multidrug resistance, Cell. 128: 1037-1050.

5. Amer B., O. J. Juvik, F. Dupont, G. W. Francis, T. Fossen (2012). Novel aminoalkaloids from European mistletoe (*Viscum album L.).* Phytochem.Lett. 5 (3): 677-681.

6. Barrett, J.F. (2005). Can biotech deliver new antibiotics? Curr. Opin. Microbiol. 8:498–503

7. Braga, L.C., J.W. Shupp, C. Cummings, M. Jett , J.A. Takahashi , L.S. Carmo, E. Chartone-Souza, A.M. Nascimento (2005). Pomegranate extract inhibits *Staphylococcus aureus* growth and subsequent enterotoxin production. J.Ethnopharmacol. 96: 335–339.

8. Chaieb, K., B. Kouidhi, R.B. Slama, K. Fdhila, T. Zmantar, A. Bakhrouf (2013). Cytotoxicity, antibacterial, antioxidant, and antibiofilm properties of tunisian juglans regia bark extract Journal of Herbs. Spices and Medicinal Plants. 19 (2): 168-179.

9. Du, Q., G. Jerz, L. Shen, L. Xiu, P. Winterhalter (2007). Isolation and structure determination of a lignan from the bark of *Salix alba*. Natural Product Research, 21 (5): 451-454.

10.Ebrahimzadeh, M.A., S.F. Nabavi, S.M. Nabavi (2013). Antihemolytic activity and mineral contents of *Juglans regia L.* flowers. Eur. Rev. Med. and Pharmacol. Sci. 17(14): 1881-1883.

11.Ferech, M., S. Coenen, K. Dvorakova, E Hendrickx, C. Suetens, H. Goossens (2006). On behalf of the ESAC Project Group. European Surveillance of Antimicrobial Consumption (ESAC): Outpatient antibiotic use in Europe, J. Antimicrob. Chemother. 58(2): 401-407

12.Fernández-Agulló, A., E. Pereira, M.S. Freire, P. Valentão, P.B. Andrade, J. González-álvarez, J.A. Pereira (2013). Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia L.)* green husk extracts. Ind.Crops & Products, 42 (1): 126-132.

13.Fimognari, C. Ferruzzi, L. Turrini, E. Carulli, G. Lenzi, M., Hrelia, P., Cantelli-Forti, G. (2012). Metabolic and toxicological considerations of botanicals in anticancer therapy. Expert Opinion Drug Metab. & Toxicol. 8 (7): 819-832.

14.Fox, J.L. (2006). The business of developing antibacterials, Nat. Biotechnol. 24:1521-1528

15.Gould, I.M. (2009). Antibiotic resistance: the perfect storm, Int. J. Antimicrob. Agents 34: S2-S5

16.Gupta, G., I. Kazmi, M. Afzal, M. Rahman, S. Saleem, M.S. Ashraf, M.J. Khusroo, K. Nazeer, S. Ahmed, M. Mujeeb, Z. Ahmed, F. Anwar (2012). Sedative, antiepileptic and antipsychotic effects of *Viscum album L.* (Loranthaceae) in mice and ratsJournal of Ethnopharmacology, 141(3): 810-816.

17.Gutiérrez-Larraínzar, M., J. Rúa, D. de Arriaga, P. del Valle, M. R. García-Armesto (2013). *In vitro* assessment of synthetic phenolic antioxidants for inhibition of foodborne *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas fluorescens*. Food Control. 30(2): 393–399.

18.Harbourne, N., E. Marete, J.C. Jacquier, D. O' Riordan, (2009) Effect of drying methods on the phenolic constituents of meadowsweet (*Filipendula ulmaria*) and willow (*Salix alba*). LWT - Food Science and Technol.42 (9): 1468-1473.

19.Hasmik, H., C. W. Hazeleger, R. R. Beumer (2012). Inhibition of *Listeria monocytogenes* by pomegranate (*Punica granatum*) peel extract in meat paté at different temperatures. Food Control. 23: 66–72.

20.Hussain, M.A., M.Q. Khan, N. Hussain, T. Habib (2011). Antibacterial and antifungal potential of leaves and twigs of Viscum album L. J.Med. Plant Res. 5 (23): 5545-5549.

21.Hyejung, J., J. Kim, J. Bang, H. Kim, L. R. Beuchat, J.-H. Ryu (2013). Combined effects of plant extracts in inhibiting the growth of Bacillus cereus in reconstituted infant rice cereal. Int. J. Food Microbiol. 160:260–266.

22.Kalemci, S., G. Yildiz, A. Zeybek, S. Ayik, E.S. Cetin, S.C. Micili, A. Bagriyanik, S. Aksun, H. Ersoz, O. Yilmaz (2013). Mistletoe extract helixor treatment attenuates allergic airway remodeling in a mouse model of asthma. Acta Med. Mediterranea. 29 (2): 183-189.

23.Kavak, D.D. , E. Altıok, O. Bayraktar, S. Ülkü (2010). *Pistacia terebinthus* extract: As a potential antioxidant, antimicrobial and possible β-glucuronidease inhibitor, J. Mol.Catalysis B: Enzymes. 64(3–4): 167–171

24.Li, C.-Y., H.-J. Du, X.-H. Su, Y.-J. Zhong, Z.-P. Yuan, Y.-F. Li, B. Liang, (2013). Juglanones A and B: Two novel tetralone dimers from walnut pericarp (*Juglans regia*). Helv. Chim. Acta. 96 (6): 1031-1035.

25.Mirzaei, A., M. Mirzaei, S.A. Khosravani, Z. Salehpour (2013). Radical scavenging potential of iranian *Quercus brantii* and *Juglans regia*. Life Sci. J. 10 (7): 1246-1250.

26.Oancea, S. and Stoica, M. (2010). Antibiotic resistance of bacterial pathogens: the magnitude of the problem from two perspectives. Rom. Biotechnolog. Lett. 15(5): 5519-5529.

27.Paun, G., P. Rotinberg, C. Mihai, E. Neagu, G.L. Radu (2011). Cytostatic activity of *Viscum album L.* extract processed by microfiltration and ultrafiltration. Rom.Biotechnol. Lett. 6(2): 6000-6007.

28.Pobłocka-Olech, L. and M. Krauze-Baranowska (2007). Chromatographic analysis of salicylic compounds in different species of the genus Salix. J. Separation Sci., 30 (17): 2958-2966.

29.Pobłocka-Olech, L. and M. Krauze-Baranowska (2008). SPE-HPTLC of procyanidins from the barks of different species and clones of Salix Journal of Pharmaceutical and Biomedical Analysis, 48 (3): 965-968.

30.Pobłocka-Olech, L., M. Krauze-Baranowska, D. Głód, A. Kawiak, E. Łojkowska (2010). Chromatographic analysis of simple phenols in some species from the genus salix.Phytochemical Analysis. 21 (5): 463-469.

31.Pop, C., F.Ranga, F. Fetea, C. Socaciu (2013). Application of three alternative technologies (spray drying, fluid bed drying and freeze drying) to obtain powdered formulas from plants with antimicrobial potential, Bull. USAMV Cluj-Animal Science and Biotechnology. 70(1-2): *in press*

32.Raiciu, A.D., D.E. Mihele, C. Ioniță, V. Nistorică, Ș. Manea (2010). Antimicrobial activity of *Ribes nigrum*, *Rosmarinus off.*, *Betula pubescens*, *Salix alba*, *Vaccinium myrtillus* gemoderivatives. Farmacia. 58 (6): 735-748.

33.Rıos, M.C. (2005). Medicinal plants and antimicrobial activity, Recio J.Ethnopharmacol. 100: 80–84.

34.Sevastre, B., N.K. Olah, D. Hanganu, O. Sárpataki, M. Taulescu, R. Mănălăchioae, I. Marcus, C. Cătoi (2012). *Viscum album L.* alcoholic extract enhance the effect of doxorubicin in ehrlich carcinoma tumor cells. Rom. Biotechnol. Lett. 17 (1): 6975-6981.

35.Shen, X., Q. Suna, L. Haiqua, Z. Yong, P. Yingji, H. Cheng-An, V.C.H. Wuc (2014). Antimicrobial effect of blueberry (*Vaccinium corymbosum L.)* extracts against the growth of *Listeria monocytogenes* and *Salmonella Enteritidis*. Food Control. 35: 159–165

36.Stan, R., A. Hangan, L. Dican, B. Sevastre, D. Hanganu, C. Catoi, O. Sarpataki, C. Ionescu (2013). Comparative study concerning mistletoe viscotoxins antitumor activity. Acta Biol. Hung. 64 (3): 279-288.

37.Sulaiman, G.M., N.N. Hussien, T.R. Marzoog, H.A. Awad (2013). Phenolic content, antioxidant, antimicrobial and cytotoxic activities of ethanolic extract of *Salix Alba*. Am. J. Biochem & Biotechnol. 9 (1): 41-46.

38.Vicaş, S.I., D. Rugină, L. Leopold, A. Pintea, C. Socaciu (2011). HPLC Fingerprint of bioactive compounds and antioxidant activities of *Viscum album* from different host trees Not. Bot. Horti Agrobot. 39 (1): 48-57.

39.Waihenya, R.K., M.M.A Mtambo', G. Nkwengulila, U.M. Minga (2002). Efficacy of crude extract of *Aloe secundiflora* against *Salmonella gallinarum* in experimentally infected free-range chickens in Tanzania, J. Ethnopharmacol. 79(3): 317–323.

40.WHO Report (2001). General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. WHO, Geneva, Switzerland

41.Wong, P.Y.Y. and D.D. Kitts (2006). Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts. Food Chemistry. 97: 505–515.

42.Zakavi, F., G. L. Hagh, A. Daraeighadikolaei, A. Farajzadeh Sheikh, A. Daraeighadikolaei, Z. L. Shooshtari. (2013). Antibacterial effect of Juglans regia bark against oral pathologic bacteria. I. J. of Dentistry. art 854765, doi.org/10.1155/2013/854765

43.Zuo, G.Y., G.C. Wang, Y.B. Zhao, G.L. Xu, X.Y. Hao, J. Han, Q. Zhao (2008). Screening of Chinese medicinal plants for inhibition against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). J. of Ethnopharmacol. 120(2): 287–290.