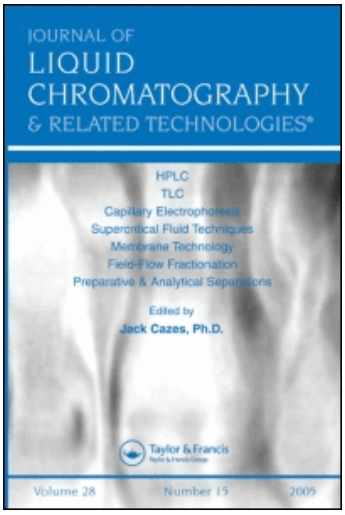

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Comparison of Components from Red and White Wines for Antimicrobial Activity by Biodetection after OPLC Separation

Zsuzsa Király-Véghely[a]; Ágnes M. Móricz[a]; Péter G. Ott[a]; György Kátay[a]; Iván Bélai[a]; Ernő Tyihák[a]
[a] Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary

To cite this Article Király-Véghely, Zsuzsa , Móricz, Ágnes M. , Ott, Péter G. , Kátay, György , Bélai, Iván and Tyihák, Ernő(2009) 'Comparison of Components from Red and White Wines for Antimicrobial Activity by Biodetection after OPLC Separation', Journal of Liquid Chromatography & Related Technologies, 32: 9, 1259 — 1272
To link to this Article: DOI: 10.1080/10826070902854417
URL: http://dx.doi.org/10.1080/10826070902854417

Journal of Liquid Chromatography & Related Technologies®, 32: 1259–1272, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070902854417

Comparison of Components from Red and White Wines for Antimicrobial Activity by Biodetection after OPLC Separation

Zsuzsa Király-Véghely, Ágnes M. Móricz, Péter G. Ott, György Kátay, Iván Bélai, and Ernő Tyihák

Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary

Abstract: Using OPLC separation and subsequent biodetection with *Pseudomonas savastanoi* pv. *phaseolicola* antibacterial compounds have been detected in both red and white wine extracts. However, the microbicidal power of red wine extracts was, in all cases, higher than that of white wines. The red wines contain different types of antimicrobial compounds but trans-resveratrol (TR) and its reduced form (bibenzyl-3,5,4′-triol, BB) in some cases are determining components. When the formaldehyde (HCHO) capturing molecule was used in culture medium (Bio-Arena system) the antimicrobial activity of all antibiotic-like compounds was decreased characteristically, that is, HCHO plays a role in the antibacterial activity of these known and unknown compounds.

Keywords: BioArena, Bioautography, Formaldehyde, OPLC, Red and white wine, Trans-resveratrol

INTRODUCTION

A wide variety of natural biologically active substances are under scrutiny for their clinical potential, both in terms of disease prevention and treatment. Recent data suggest that wines contain a number of biologically active compounds, with the most diverse beneficial effects on human health.[1,2] Among

Correspondence: Ernő Tyihák, Plant Protection Institute, Hungarian Academy of Sciences, Budapest, P.O.B. 102, H-1525 Hungary. E-mail: etyih@nki.hu

these compounds the polyphenolic components, such as phytoalexin TR, may contribute to the various advantageous activities of wines with special emphasis on red wine, which is in general relatively rich in TR.[3] The up to date known healthful effects of TR can be divided into two main groups: chemopreventive/protective (e.g., cardiovascular, antiplatelet, antimutagenic, anti-amyloidogenic) and inhibiting/killing (e.g., antifungal, antibacterial) effects.[4,5] The third effect group of TR is a transition stage between the earlier two groups highlighting the selective anti-leukemic effect of TR[6] or its surprising beneficial effect in three main stages (initiation, promotion, progression) of chemical carcinogenesis.[7] This classification of the favourable activities of TR is based practically on the double effect of TR in which the interaction between TR and endogenous HCHO from labile bonds in biological unit plays a determining role.[4,5] TR is a natural, concentration dependent HCHO capture molecule. The capture of HCHO from a given biological unit (e.g., tissue) with TR (first step) may cause a chemopreventive effect, and the reaction products between endogenous HCHO and TR (second step) may exert a killing/inhibiting effect on pathogens and/or or cancer cells (double effect). It has to point out that the reactions of the first step are needed for the manifestation of the second step. Furthermore, it is an especially interesting new result that the deposition of lipids in atherosclerotic plaques is independent of lipid oxidation and that the chemoprotective action of red wine polyphenols is independent of any antioxidant effect of these compounds.[8]

There are recent studies reporting the antimicrobial activities of wines and wine extracts against various pathogens.[9,10] According to some reports the microbicidal power of red wine was higher than that of white wine. Red wines have a higher content of total phenolics and contain a wider spectrum of phenolics than the white wines.[11,12] Some recent studies have indicated that white wine could also be as cardioprotective as red wine if it is rich in tyrosol and hydroxytyrosol.[13,14] It is especially interesting that the Croatian red wines had significant protherapeutic effects on human colon carcinoma cells. Under the same experimental condition and identical concentration applied, investigated white wines showed no or negligible inhibitory effects against human normal and carcinoma cell lines.[15] It is known that the antimicrobial effect of the commercial grape juice, extracts, wines, and wine extracts are detected by conventional methods (e.g., agar well diffusion method[16] without separation of wine extracts). It is observable that the total phenolic content of wine extracts was determined by the Folin-Ciocalteu method, while their phenolic composition was specified by high performance liquid chromatography and diode array detection (HPLC-DAD).[17] It is high time to try the separation of the antimicrobial compounds and to detect them with microbial cells after chromatographic separation of the wines or their extracts. It is known that all conventional

and forced flow planar layer liquid chromatographic techniques can be used theoretically and practically for biological detection, the most efficient version: direct bioautography is mainly performed with modern TLC and linear overpressured layer chromatography (OPLC).[18,19] Column chromatographic techniques are not suitable for such investigations because the adsorbent bed in the column arrangement is not suitable for growth of microbial cells. However, conventional bioautography is not suitable for studying, and understanding all the complicated biochemical reactions involved in these processes. Model experiments are necessary with complex separation and detection systems at the microassay and ultramicroassay levels. One solution is the BioArena system, which integrates the advantages of layer chromatographic separation and the biological detection including the $HCHO/O_3$ idea as well.[20,21]

This paper will concentrate on those studies, performed by OPLC, that have produced information about antimicrobial (antibacterial) activities of red and white wines using conventional and complex bioautographic (BioArena) detection after chromatographic separation, taking into account the earlier experiences in the BioArena system with TR and other compounds as well.[22–25]

EXPERIMENTAL

Materials and Methods

The test substances TR and glutathione, and dye reagent 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Ltd. Budapest, Hungary. All solvents and other chemicals were of analytical grade and were purchased from Merck Co., Ltd. (Darmstadt, Germany) and REANAL Co., Ltd. (Budapest, Hungary).

Preparation of Wine Samples

Wine (20 mL) was extracted three times with 10 mL ethyl acetate. The combined extracts were dried over sodium sulphate and evaporated under vacuum (36°C). The residue was dissolved in 5 mL methanol and e.g., 8 μL samples were applied to the adsorbent layer in 5 mm bands.

Separation Methods

Sample application was carried out onto the dry adsorbent layer (after a precondition at $T = 130°C$, $t = 3$ h) using a microliter syringe or Nanomat sample applicator (CAMAG Co., Muttenz, Switzerland). An automatic OPLC instrument was used, which consisted of the liquid delivery system

and the separation chamber (OPLC-NIT Co., Ltd., Budapest, Hungary). A cassette containing the chromatoplate with samples can be inserted into the chamber. After the separation process the cassette can be pulled out and the chromatoplate is dried and evaluated under UV lamp,[26,27] and it can be used for bioatography/BioArena detection. Chromatographic conditions were as follows: a) OPLC separation of components of wines: chromatoplate, silica gel RP-18 F_{254} (Merck Co., Darmstadt, Germany) with all edges sealed. Conditions: e.g., eluent, aquatic acetic acid (pH, 2.5) – acetonitril, 77:23 V/V; external pressure, 5.0 MPa; flow rate, 100 μL/min; eluent flush, 450 μL; eluent volume, 4500 μL; separation time, 2745 s. b) OPLC separation of components of wines for biological detection in the bioautography/BioArena system: chromatoplate, TLC or HPTLC silica gel 60 F_{254} (Merck Co., Darmstadt, Germany) with all edges sealed. Conditions: e.g., eluent, chloroform-methanol, 80 + 8 (V/V), external pressure, 5.0 MPa; flow rate, 250 μL/min; eluent volume, 9800 μL; eluent flush, 450 μL;, separation time, 2370s.

Biological Detection

For the bacterial biotest, the phytopathogen *Pseudomonas savastanoi* pv. *phaseolicola* race 6, causing halo blight on bean was used. Bacterial cells were grown on a rich medium U (King's broth) at 28°C, 200 rpm on the centrifugal shaker, until they reached late exponential phase ($OD_{560\,nm} = 0.7$), corresponding to 1.5×10^9 cells mL^{-1}. Just before use, 50% vol of fresh medium was added to the culture. Having completed the development, dried chromatoplates were immersed into the bacterial suspension of *Pseudomonas savastanoi* for 20 s. Visualization of the chromatograms with an aquatic solution of MTT was performed, either after a short draining period or after an overnight incubation.[20,23] After staining, the time for evaluation varied (e.g., from 1 h to 6–9 days or more).[21,24]

RESULTS AND DISCUSSION

Separation of Commercial Red Wine Samples by OPLC

In our earlier studies, comparison of OPLC with TLC clearly showed the advantages of the forced flow technique (higher theoretical plate number, better resolution, etc.) over conventional planar layer liquid chromatography.[28] It was established that the glycosides of resveratrol isomers were always present in higher concentrations than free stilbene isomers in red wine samples.

Figure 1 illustrates clearly these advantages of the small modified original OPLC method.[28] For such efficient separation of stilbene

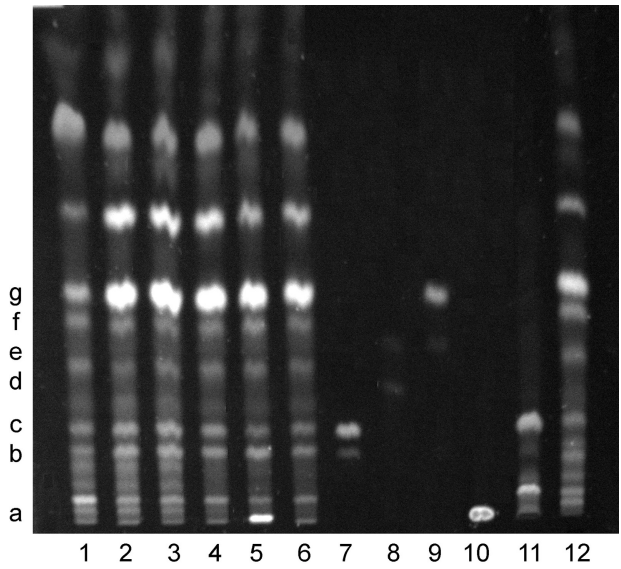

Figure 1. OPLC separation of ethyl acetate extracts of red wines with authentic substances on silica gel 60 RP 18. Chromatographic conditions are in Experimental, photo under UV $\lambda = 366$ nm. Red wines from 2002; 1, Soproni kékfrankos, E 42; 2, Merlot; 3, Merlot 348; 4, Pinot noir P1; 5, Pinot noir M2 + ochratoxin A; 6, Pinot noir 113; 7, trans-resveratrol (2 μg); 8, trans-piceatannol (5 μg); 9, cis-piceid (4 μg); 10, ochratoxin A (1 μg); 11, Chinese tree; 12, Syrah. The signals of authentic substance: a, ochratoxin A; b, cis-resveratrol; c, trans-resveratrol; d, cis-piceatannol; e, trans-piceatannol; g, cis-piceid.

isomers, TLC is not suitable.[28] This figure shows further advantages of OPLC method, e.g., at the start point the ochratoxin A as wine mycotoxin under UV light can be detected.

However, these nice separations can not be used for biological detection because the bioautography is a very sensitive system,[29] and the RP chromatoplates are not suitable for the growth of indicator microbials under the used conditions.

Study of Antimicrobial Components from Wine Extracts

The principle of direct bioautography is that a suspension of a microorganism growing in a suitable medium is applied to a developed chromatoplate, after drying. Incubation of the chromatoplate with the microbes (e.g., bacteria) in a humid atmosphere at the optimum temperature enables growth of bacteria. Using a specific dye, cells that remain alive can be visualized because, e.g., dehydrogenases from living microorganisms

convert a tetrazolium salt into the intensely colored formazan.[29] The antibacterial compounds appear as clear spots against a colored background. It is obvious that a liquid chromatographic system of layer arrangement is suitable for the spreading and direct comparison of the components from different samples (e.g., wine samples) using such a chromatoplate and eluent system, which do not disturb the growth of microbial cells.[29]

BioArena is based on direct bioautography with exploitation of all its advantages. It integrates the benefits of layer chromatography with biological detection and visual and spectroscopic evaluation of spots before and after inoculation. This integration utilizes the possibilities of interactions of microbes with small and large cofactor molecules in the adsorbent bed after chromatographic separation.[22,23]

Figure 2 illustrates a bioautogram of red and white wine extracts. It can be seen that TR occupies a central place (with advantageous Rf value) in the bioautogram of red wine extracts, however, the extracts contain other antibacterial components as well. On the contrary, in white wine extracts the amount of TR is low as it is also known from other experiments.[e.g., 28,30,31] After a longer incubation time these differences are more characteristic (Figure 3). Around the starting point in white

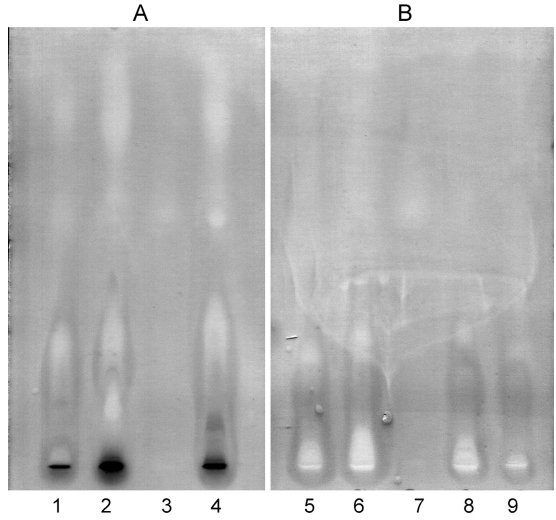

Figure 2. Biodetection after OPLC separation of ethyl acetate extracts of red and white wines. Chromatographic and biodetection conditions are in Experimental. Incubation time between MTT staining and photo was 2 hours; A, red wines: 1, Soproni kékfrankos, 2004; 2, Kékfrankos, Villány, 2004; 3, trans-resveratrol, 0.5 μg; 4, Pinot noir, Sopron, 2004; and B, white wines: 5, Rajnai rizling, D 2003; 6, Savignon blanc, D 2003; 7, trans-resveratrol, 0.5 μg; 8, Rizlingszilvani, D 2003, 9, Egri leányka 2004.

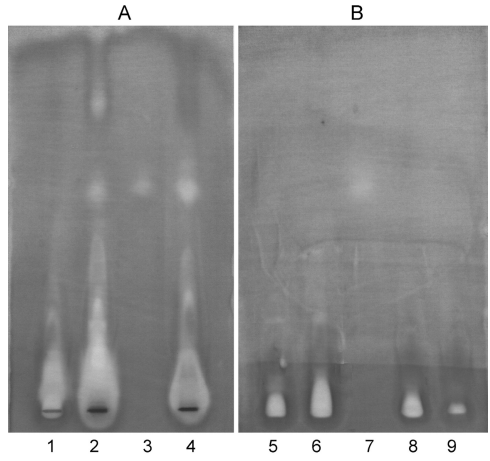

Figure 3. Biodetection after OPLC separation of ethyl acetate extracts of red and white wines. As in Figure 2, but incubation time between MTT staining and photo was 18 hours.

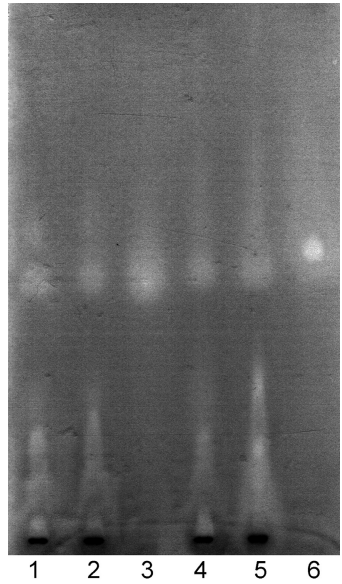

Figure 4. Biodetection after OPLC separation of red wine extracts and authentic substances trans-resveratrol and bibenzyl-3,5,4′-triol. Incubation time between MTT staining and photo was 18 hours. 1, Merlot, D 2004; 2, Kadarka, 2004; 3, trans-resveratrol, 5 μg; 4, Egri bikavér, 2003; 5, Kékfrankos, Villány 2003; bibenzyl-3,5,4′-triol, BB, 5 μg.

wine extracts, a characteristic antibacterial spot (band) is observable, and it can be said that the extracts of red and white wines show basically different reaction profiles at the starting point. Further investigations are needed for the identification of these compounds, researching among others, different phenolic acids[32] or tyrosol and hydroxytyrosol molecules.[13,14] Figure 4 illustrates a characteristic bioautogram with two authentic substances (TR and bibenzyl-3,5,4′-triol (BB), which is a reduced form of TR[33]) and different red wine extracts. It has to be pointed out that, in this case, an HPTLC chromatoplate was used on which TR and BB compounds occupied a well defined place. These red wine samples do not contain the BB compound.

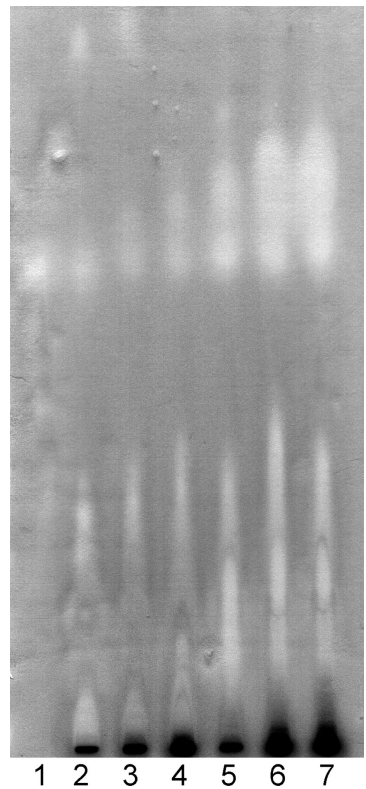

Figure 5. Biodetection after OPLC separation of ethyl acetate extracts of red wines from different technologies. Incubation time between MTT staining and photo was 2 h. 1, trans-resveratrol, 0.5 μg; 2, Pinot noir, Sopron, 2004; 3, Zweigelt, Sopron, 2004; 4, Nero, Sopron, 2004; 5, Kékfrankos, Villány, 2004 (reductive conditions); 6, Cabernet Sauvignon, Villány, 2004 (reductive conditions); 7, Cabernet Sauvignon, 3, Villány 2004 (reductive conditions).

However, in another experimental series a characteristic accumulation of BB was observable in different red wine extracts (Figure 5). These red wines are produced, it is supposed, by intensive reductive technology or under reductive conditions. It is obvious that such a reductive technology decreases the amount of the other biologically active compounds, e.g., yeast origin terpenoids having unsaturated bonds (e.g., trans-trans-farnesol).[33] Figure 6 shows such an experimental series in which the red wine extracts do not contain BB in large amounts using an optimum winemaking technology. According to our experiences, the bacterial killing and antifungal activity of BB is about half of the original TR.[33] It is obvious that the occurrence of BB in wines, mainly in red wines, opens a new horizon in the field of wine topics.

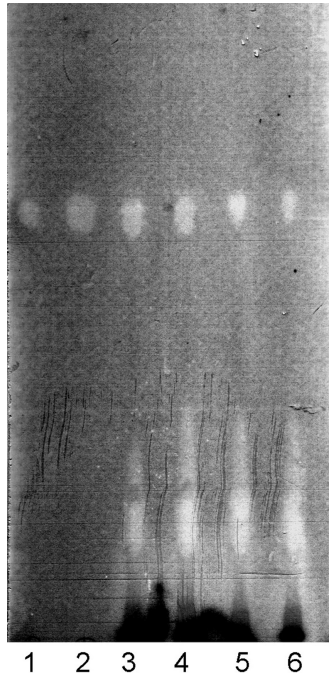

Figure 6. Biodetection after OPLC separation of ethyl acetate extracts of red wines with optimum technology. Incubation time between MTT staining and photo was 20 hours; 1, trans-resveratrol, 0.3 μg; 2, trans-resveratrol, 0.5 μg; 3, Merlot D, 2004; 4, Cabernet Sauvignon, D, 2004; 5, Soproni kékfrankos D 2004; 6, Kadarka, 2004.

Effect of Glutathione on the Antibacterial Activity of the Components of Red Wine Samples

The earlier bioautograms illustrated clearly that red wines gave more antimicrobial spots (bands) than white ones and the figures reveal that TR behaves as a real antibiotic against *Pseudomonas savastanoi* pv. *phaseolicola* with a dominant place on the adsorbent layer. When glutathione as an endogenous HCHO capture molecule is added to the culture medium containing the bacterial cells, the antibacterial activity of all different known and unknown compounds from red wine extracts decreases characteristically on the adsorbent layer (Figure 7A and B). The elimination of HCHO from the spots with glutathione results in a real decrease of the antibiotic intensity (Figure 7B).

Interaction of the TR with the endogenous HCHO molecules (e.g., in chromatographic spots or potentially in grape cells.[34]) is already well known.[5,21,23] This effect is valid for all antibiotic-like compounds from given red wine extracts presented in bioautograms.

The inhibiting/killing effect of HCHO released can be further increased by means of interaction with H_2O_2. Both HCHO and H_2O_2 can be formed intracellularly and extracellularly by almost all cells.[35,36] These two reactive molecules can interact (also endogenously) and the

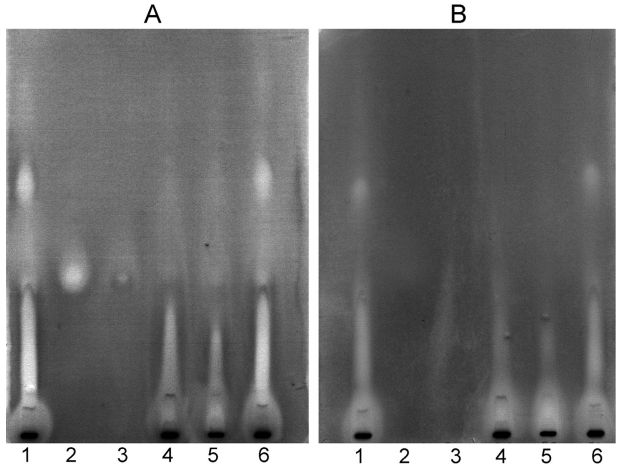

Figure 7. Effect of glutathione on the antibacterial activity of components from red wines. Incubation time between MTT staining and photo was 20 hours. A, control layer: 1, Kékfrankos, Villány, 2004; 2, bibenzyl-3,5,4′-triol (BB), 2 μg; 3, trans-resveratrol, 0.5 μg; 4, Merlot, Sopron, 2004; 5, Kékfrankos, Sopron, 2004; 6, Kékfrankos, Villány, 2004; B, layer treated with 2 mg/mL glutathione: 1–6 are the same with control layer.

very reactive singlet oxygen ($^1O_2$) (red light emission, 530, 633, 705 nm) and excited HCHO (H^*CHO) (blue light emission, 430 nm) can be formed.[36,37] also in chromatographic spots after inoculation. Wentworth et al.[38] and Babior et al.[39] have shown that the antibody catalyzed water-oxidation by means of $^1O_2$ produced different toxicants (e.g., H_2O_3, O_3), which can kill the pathogen cells or cancer cells. However, according to up to date results, the killing/inhibiting effect of TR on antigens is originated mainly from HCHO reactions without O_3.[21]

CONCLUSIONS

The antimicrobial properties of components of different red and white wines against pathogens were investigated. It was observed that all wine samples showed antibacterial properties and the inhibition increased when the polyphenols concentration of wines was bigger. A chromatographic separation (OPLC technique) and subsequent bioautographic detection were used at first in the case of red and white wine extracts. When glutathione as endogenous HCHO capturing molecule was used in the culture medium the antibacterial activity of all antibiotic-like compounds (not only trans-resveratrol) on the adsorbent layer decreased substantially.

The capture of HCHO from a given biological unit with trans-resveratrol (first step) may cause a chemopreventive effect and the reaction products between endogenous HCHO and trans-resveratrol (second step) may exert inhibiting/killing effects on pathogens and/or cancer cells (double effect). It seems that this relationship is also valid for other antibiotic-like compounds from red and white wines.

These investigations were the first preliminary experiments for the comparison of antibiotic-like components from red and white wines. It is valid for the detection of bibenzyl-3,5,4′-triol (BB) in certain red wine samples. However, these preliminary results with this reduced form of TR demand further investigations because it may help the standardization of the winemaking red wine technology.

ACKNOWLEDGMENT

The authors are grateful for the financial support from OTKA T048919.

REFERENCES

1. Soleas, G.J.; Diamandis, E.P.; Goldberg, D.M. Wine as a biological fluid: History, production, and role in disease prevention. J. Clin. Lab. Anal. **1997**, *11*, 287–313.

2. Pervaiz, S. Chemotherapeutic potential of the chemopreventive phytoalexin resveratrol. Drug Resist. Updates **2004**, *7*, 333–344.
3. Frémont, L. Biological effects of resveratrol. Life Sci. **2000**, *66*, 663–673.
4. Tyihák, E.; Kátay, Gy.; Király-Véghely, Zs.; Németh, Zs.; Albert, L.; Szende, B. *Vitis vinifera* as a medicinal plant. Acta Hort. **2003**, *597*, 159–165.
5. Tyihák, E.; Király-Véghely, Zs. Interaction of trans-resveratrol with endogenous formaldehyde as one basis of its diverse beneficial biological effects. Bulletin de l'OIV. **2008**, *81*, 65–74.
6. Gautam, S.C.; Xu, Y.X.; Dumaguin, M.; Bremmer, T.A.; Sacco, J. Resveratrol selectively inhibits leukemia cells: A prospective agent for ex vivo bone marrow purging. Cancer Lett. **2000**, *25*, 639–645.
7. Jang, M.; Cai, L.; Udeani, G.O.; Slowing, K.V.; Thomas, C.F.; Beecher, C.W.; Fong, H.H.; Farnsworth, N.R.; Kinghorn, A.D.; Mehta, R.G.; Moon, R.C.; Pezzuto, J.M. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science **1997**, *275*, 218–220.
8. Waddington, E.; Puddey, I.B.; Croft, K.D. Red wine polyphenolic compounds inhibit atherosclerosis in apolipoprotein E-deficient mice independently of effects on lipid peroxidation. Am. J. Clin. Nutr. **2004**, *79*, 54–61.
9. Just, J.R.; Daeschel, M.A. Antimicrobial effects of wine on *Escherichia coli* O157-H7 and *Salmonella typhimurium* in a model stomach system. J. Food Sci. **2003**, *68*, 285–290.
10. Daroch, F.; Hoeneisen, M.; Gonzalez, C.L.; Kawaguchi, F.; Salgado, F.; Solar, H.; Garcia, A. In vitro antibacterial activity of Chilean red wines against *Helicobacter pylori*. Microbios **2001**, *104*, 79–85.
11. Ganan, M.; Martinez-Rodriguez, A.J.; Carrascosa, A.V. Antimicrobial activity of phenolic compounds of wine against *Campylobacter jejuni*. Food Control. Available online 7 October 2008.
12. Lamuela-Raventos, R.M.; de la Tarre-Boronat, M.C. Beneficial effects of white wines. Drugs Exp. Clin. Res. **1999**, *25*, 121–124.
13. Bertelli, A.; Nigliori, M.; Bertelli, A.A.; Origlia, N.; Filippi, C.; Panichi, V., Falchi, M; Giovaninni, L. Effect of some white wine phenols in preventing inflammatory cytokine release. Drugs Exp. Clin. Res. **2002**, *28*, 11–15.
14. Dudley, J.I.; Lekli, I.; Mukherjee, S.; Das, M.; Bertelli, A.; Das, D.K. Does white wine quality for French paradox? Comparison of the cardioprotective effects of red and white wines and their constituents: resveratrol, tyrasol and hydroxytyrasol. J. Agric. Food Chem. **2008**, *56*, 9362–9373.
15. Glavas-Obrovac, L.J.; Opacak, T.; Bares, V.; Subaric, D.; Babic, J.; Ilakovac, V.; Karner, J. Effects of dealcoholized red and white wines on human tumour and normal cells proliferation. Acta Alimentaria. **2006**, *35*, 153–162.
16. Deans, S.G.; Ritchie, G. Antibacterial properties of plant essential oils. Int. J. Food Microbiol. **1987**, *5*, 165–180.
17. Papadopoulou, Ch.; Soulti, K.; Roussis, I.G. Potential antimicrobial activity of red and white wine phenolic extracts against strains of *Staphylococcus aureus, Escherichia coli* and *Candida albicans.* Food Technol. Biotechnol. **2005**, *43*, 41–46.

18. Tyihák, E.; Botz, L.; Ott, P.G.; Nagy, J.; Kocsis, B.; Király-Véghely, Zs.; Mincsovics, E. The combination of the overpressured layer chromatography and bioautography and its applications to the analysis of molecules influencing cell proliferation. Chem. Anal. (Warsaw) **2003**, *48*, 543–553.
19. Tyihák, E.; Móricz, Á.M.; Ott, P.G. Bio-detection and determination of biological activity of natural compounds, in *Thin-Layer Chromatography in Phytochemistry*; Waksmundzka-Hajnos, M., Sherma, J., Kowalska, T., Eds.; CRC Press: 2008, Ch. 9, 193–213.
20. Tyihák, E.; Mincsovics, E.; Kátay, Gy.; Király-Véghely, Zs.; Móricz, Á.M.; Ott, P.G. BioArena: An unlimited possibility of biochemical interactions in the adsorbent layer after chromatographic separation. J. Planar Chromatogr. **2008**, *21*, 65–70.
21. Tyihák, E.; Móricz, Á.M.; Ott, P.G. Use of the BioArena system for indirect detection of endogenous ozone in spots after TLC or OPLC separation. J. Planar Chromatogr. **2008**, *21*, 77–82.
22. Móricz, Á.; Otta, K.-H.; Ott, P.; Tyihák, E. Separation and detection of aflatoxins using overpressured-layer chromatography and bioautography. J. Planar Chromatogr. **2003**, *16*, 417–420.
23. Tyihák, E.; Ott, P.; Móricz, Á.M.; Kátay, Gy.; Király-Véghely, Zs. Antibiosis, antibiotics and the formaldehyde cycle: The unique importance of planar chromatographic techniques to progress in these fields. J. Planar Chromatogr. **2004**, *17*, 84–88.
24. Móricz, Á.M.; Ott, P.G.; Billes, F.; Otta, K.-H.; Tyihak, E. The influence of L-ascorbic acid on the antibacterial-toxic activity of aflatoxins on adsorbent layer. J. Appl. Microbiol. **2007**, *103*, 2525–2532.
25. Tyihák, E.; Móricz, Á.M.; Ott, P.G.; Hajnos, M.L.; Glowniak, K. New approach to mechanism of action of paclitaxel by means of BioArena system. J. Planar Chromatogr. **2008**, *21*, 331–336.
26. Tyihák, E.; Mincsovics, E. Overpressured Layer Chromatography–Optimum Performance Laminar Chromatography, in *Planar Chromatography–A Retrospective View for the Third Millenium*; Nyiredy, Sz., Ed.; Springer: Budapest, 2001, 137–176.
27. Mincsovics, E.; Ferenczi-Fodor, K.; Tyihák, E. Overpressured layer chromatography, in *Handbook of Thin-Layer Chromatography,* 3rd Ed.; Dekker: New York, 2003, 175–205.
28. Király-Véghely, Zs.; Kátay, Gy.; Tyihák, E.; Merillon, J-M. Separation of stilbene isomers from red wine by overpressured-layer chromatography. J. Planar Chromatogr. **2004**, *17*, 4–8.
29. Botz, L.; Nagy, S.; Kocsis, B. Detection of microbiologically active compounds, in *Planar Chromatography – A Retrospective View for the Third Millenium*; Nyiredy, Sz., Ed.; Springer: Budapest, 2001, 489–516.
30. Revilla, E.; Ryan, J.-M. Analysis of several phenolic compounds with potential antioxidant properties in grape extracts and wines by high-performance liquid chromatography-photodiode array detection without sample preparation. J. Chromatogr. A **2000**, *881*, 461–469.
31. Opie, L.H.; Lecour, S. The red wine hypothesis: from concepts to protective signalling molecules. Europ. Heart J. **2007**, *28*, 1683–1693.

32. Vaquero, M.J.R.; Alberto, M.R.; Manca de Nadra, M.C. Antibacterial effect of phenolic compounds from different wines. Food Control **2007**, *18*, 93–101.
33. Tyihák, E.; Királyné Véghely, Zs.; Botz, L.; Szende, B.; Bélai, I.; Kátay, Gy. Preparation of wines having high concentrations of stilbene derivatives and terpenoic compounds with unsaturated bonds by addition of suitable extracts and natural identical compounds. 2004, Hung. Pat. No. HU200202979 A1.
34. Király-Véghely, Zs.; Tyihák, E.; Albert, L.; Németh, Zs.I.; Kátay, Gy. Identification and measurement of resveratrol and formaldehyde in parts of white and blue grape berries. Acta Biol. Hung. **1998**, *49*, 281–289.
35. Tyihák, E.; Trézl, L.; Szende, B. Formaldehyde cycle and the phases of stress syndrome. Ann. N.Y. Acad. Sci. **1998**, *851*, 259–270.
36. Trézl, L.; Pipek, J. Formation of excited formaldehyde in model reactions simulating real biological systems. J. Mol. Struct. (Theochem.) **1988**, *170*, 213–223.
37. Tyihák, E.; Rozsnyay, S.; Sárdi, É.; Gullner, G.; Trézl, L.; Gáborjányi, R. Possibility of formation of excited formaldehyde and singlet oxygen in biotic and abiotic stress situations. Acta Biol. Hung. **1994**, *45*, 3–10.
38. Wentworth, Jr., P.; McDunn, J.E.; Wentworth, A.D.; Takeuchi, C.; Nieva, J.; Jones, T.; Bautista, C.; Ruedi, J.M.; Gutierrez, A.; Janda, K.D.; Babior, B.M.; Eschenmoser, A.; Lerner, R.A. Evidence for Antibody-Catalyzed ozone formation in bacterial killing inflammation. Science **2002**, *298*, 2195–2199.
39. Babior, B.M.; Takeuchi, C.; Ruedi, J.; Gutierrez, A.; Wentworth, Jr., P. Investigating antibody-catalyzed ozone generation by human neutrophils. PNAS **2003**, *100*, 3031–3034.

Received December 22, 2008
Accepted January 27, 2009
Manuscript 6469C