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Determination of antioxidant capacity for some products based on wine and medicinal plants

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Abstract

The aim of this study is to find new natural remedies useful for body against oxidative stress (which induces dysfunction at the cellular level, various ailments in the human body). Its have been investigated as a antioxidant potential or containing compounds with properties to capture the free radicals excess, various medicinal plants: howthorn (*Crataegus oxyacantha L.*), lemon balm (*Melissa officinalis L.)* and garlic (*Allium sativum L.*), used for maceration in wine to obtain some oenoterapic products.

The results obtained from physicochemical analysis (UV–VIS spectrophotometry, HPLC, gas chromatography with mass spectrometry) and biochemical analysis have confirmed remarkable antioxidant potential for all studied plants, because its have a high content in compounds that can capture free radicals.The balance between oxidant action of the free radicals and the level of antioxidants it is essential for life and characterize the resistance capacity of human body, because the antioxidants neutralizing free radicals

Keywords: lantioxidant capacity, herbs, howthorn (*Crataegus oxyacantha L.*), lemn balm (*Melissa officinalis L.)*, garlic (*Allium sativum L.*), wine

1. Introduction

Wine is a very rich source of polyphenols such as: catechin, epicatechin, flavonols (quercetin, rutin, myricetin, etc.), anthocyanins (the most abundant is malvidin-3-O-glucoside), oligomeric and polymeric proanthocyanidins, phenolic acids (gallic acid, caffeic acid, p-coumaric acid, etc.), stilbenes (trans-resveratrol) and many others polyphenols. Many of these compounds (e.g. resveratrol, quercetin, rutin, catechin and their oligomers and polymers proanthocyanidins) have been reported to have multiple biological activities, including cardioprotective, anti inflammatory, anti-carcinogenic, antiviral and antibacterial properties [22, 13, 23].

These biological properties are attributed mainly to their powerful antioxidant and antiradical activity. The results of many published epidemiological studies suggest that regular, moderate consumption of red wine has reduced the incidence of many diseases such as risk of coronary heart disease (CHD), atherosclerosis, cancers, etc [26, 15, 6, 8, 7, 23].

The most intriguing are the studies which reported the possible association between red wine consumption and decrease in risk, and some suppression and inhibition of cancers [4]. Currently, chemoprevention is being used in medicine as a new strategy to prevent cancers.

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Natural phytochemicals, including red wine polyphenols, appear to be very promising substances to block, reverse, retard or prevent the process of carcinogenesis [22].

Many epidemiological studies have found that regular intake of red wine or black wine polyphenols has positive effects on human health. Therefore, determination of the chemical composition, polyphenols content and antioxidant activity of red wine could be very useful for the interpretation of epidemiological studies.

The use of medicinal plants for healing different human affections, dates back to the ancient times – the prehistoric ones – when man living in the middle of nature, fighting through various ways to ensure his existence, has noticed that some plants are good to eat, or heal diseases and some others are toxic. The Global Health Organization recently announced that 75 – 80 % of the world's population treats themselves using natural remedies.

Lemon balm (*Melissa officinalis L.*) is used in tradditional medicine and is native to the eastern Mediterranean Region and western Asia [17]. Dried or fresh leaves and top aerial section of the plant are the parts which are used as medicine [11, 18]. Meftahizade *et al*., (2010), reported that the main constituent of the essential oil are citral (geranial and neral), citronellal, geraniol, β-pinene, α-pinene, β-caryophyllene, comprising 96% of the oil ingredients. Also Carnat *et al*., (1998), reported the chemical composition of essential oil of lemon balm, and found that major components are citral representing 48% of the essential oil, followed by citronellal with 39.47% and caryophyllene with 2.37% in another investigation, the percentage of the main constitute found, are: α-pinene (2.86%), β-pinene (11.37%), linalool (2.74%), citronella (5.86%) borneol (0.62%), neral (12.22%), and geraniol (38.13%), in addition, fresh herb of lemon balm contains total phenolic (2253/100 mg), L-Ascorbic acid (53.2/100 mg) and carotenoids (46.3/100 mg).

Lemon balm (*Melissa officinalis*) has been traditionally used for different medical purposes like tonic, antispasmodic, carminative, diaphoretic, surgical dressing for wounds, sedativehypnotic strengthening the memory, and relief of stress induced headache [3].

Hawthorn (*Crataegus oxyacantha L.*) is a traditional medicinal plant and has long been used as a folk medicine and is widely utilized in pharmaceutical preparations mainly because of its beneficial health effects and its low toxicity. The pharmacological effects have mainly been attributed to their polyphenolic contents, and oligomeric procyanidins are abundant in hawthorn. The active constituents and the antioxidant effects of the extracts of the leaves and flowers of *C. oxyacantha* have been widely studied [2, 10, 14, 24, 25, 26, 29, 30].

Garlic (*Allium sativum*) is one of the most popular herbs used worldwide to reduce various risk factors associated with several diseases [28,16]. Garlic is well known for its medicinal benefits, especially in helping to prevent cancer and cardiovascular diseases [19]. Alliins (S-alk(en)yl-l-cysteine sulfoxides) are sources of major active compounds in allium plants. Allicin (diallythiosulfinate) is the main biologically active component of freshly crushed garlic (*Allium sativum L.*) cloves [26]. Garlic activity was compared with dietary curcumin and capsaicin [12]. The modulatory effects of garlic on hepatic and blood oxidant–antioxidant status may play a key role in preventing cancer development at extrahepatic sites [1, 9]. The objective of this study was to evaluate the antioxidant capacity for some products based on wine and medicinal plants.

2. Materials and methods

Chemicals. Folin–Ciocalteu reagent, gallic acid were purchased from Merck Co. (Germany). 2,2`-azino-bis (3-ethyl-benzothiazoline-6-sulfonicacid)diammonium salt (ABTS) were purchased from Sigma–Aldrich (St.Louis, MO, USA). All reagents were analytical grade.

Medicinal wine samples. Three variants of products based on wine and herbs, type "*medicinal wine*" (provided by S.C. Hofigal Export Import S.A., Bucharest), were studied: - Medicinal wine with lemon balm, monodoses×50 mL; - Medicinal wine with howthom, monodoses×50 mL; - Medicinal wine with garlic, monodoses×50 mL.

Determination of total phenolic content. The total phenol content in selected wine samples was determined spectrophotometrically according to the Folin–Ciocalteu colorimetric method [7] using gallic acid as a standard polyphenol: 0.1 mL of wine was mixed with 7.9 mL distilled water and 0.5 mL of Folin – Ciocalteu reagent. After 1 min, 1.5 mL of 20 % Na_2CO_3 was added.

The absorbance was measured after 120 min at 760 nm with a Jenway 6300 UV/VIS spectrophotometer. The concentration of the total phenolic compounds in the wines was expressed as gallic acid equivalents (mg/L).

*Measurement of the antioxidant capacity.*The antioxidant capacity of medicinal wine samples was determined by ABTS or TEAC assay. This method is based on the inhibition of the absorbance of the radical cation of 2,2´-azinobis (3-ethylbenzothiazoline6-sulfonate)ABTS$^{\bullet+}$, which has a characteristic long-wavelength absorption spectrum showing a maximum at 734 nm [20].

Results were compared with a standard curve prepared with different concentrations of Trolox, a water-soluble analogue of E vitamin, and were expressed as milimolar Trolox equivalents.

3. Results and Discussion

General characterizations of wines used for medicinal wine. Some relevant analytical parameters of the wines used for medicinal wine are presented in Table 1. The wines differ in many analytical parameters such as the content of alcohol, total extract, total SO_2, reducing sugar, total acidity (as tartaric acid), volatile acidity (as acetic acid) and specific weight.

Table 1. Some relevant analytical parameters in some Romanian wines

Wine	Specific weight (g/cm^3)	Alcohol content (vol %)	Total acidity (tartaric acid) (g/L)	Volatile acidity (acetic acid) (g/L)	Total extract (g/L)	Reducing sugar (g/L)	Total SO_2 (mg/L
Caberrnet Sauvignon (Murfatlar, 2009)	0.9932	11.05	4.57	0.59	34.1	0.72	60.59
Cabernet Sauvignon (Murfatlar, 2010)	0.9936	11.28	7.56	1.8	22.3	0.1	37.42
Cabernet Sauvignon (Vincon Vrancea, 2009)	0.9916	14.04	5.23	0.66	26.15	1.11	25.25
Cabernet Sauvignon (Vincon Vrancea, 2010)	0.9955	12.53	10.02	0.56	31.0	1.34	20.41
Pinot Gris (Veritas Panciu, 2009)	0.9897	15.43	5.42	0.54	21.4	0.36	69.66
Pinot Gris (Veritas Panciu, 2010)	0.9892	14.23	6.52	1.13	19.8	0.16	33.1

Table 2. The total polyphenols content of medicinal wines

No	Medicinal wine	TP (mg gallic acid equivalent/L)
Sample 1	Caberrnet Sauvignon (2009) + lemon balm	2318 ± 13
Sample 2	Caberrnet Sauvignon (2010) + lemon balm	2368 ± 33
Sample 3	Caberrnet Sauvignon (2009) + howthorn	2326 ± 13
Sample 4	Caberrnet Sauvignon (2010) + howthorn	2717 ± 15
Sample 5	Pinot Gris (2009) + garlic	1781 ± 89
Sample 6	Pinot Gris (2010) + garlic	1815 ± 32

Table 3. Antioxidant capacity of tested medicinal wines

No	Medicinal wine	Antioxidant capacity (mM Trolox)
Sample 1	Caberrnet Sauvignon (2009) + lemon balm	26.5 ± 3.69
Sample 2	Caberrnet Sauvignon (2010) + lemon balm	30.3 ± 1.44
Sample 3	Caberrnet Sauvignon (2009) + howthorn	25.2 ± 4.77
Sample 4	Caberrnet Sauvignon (2010) + howthorn	29.4 ± 1.94
Sample 5	Pinot Gris (2009) + garlic	13.8 ± 1.16
Sample 6	Pinot Gris (2010) + garlic	20.5 ± 1.13

Total polyphenols in medicinal wine. Phenolic compounds are responsible for the antioxidant activity of medicinal wines. The concentration of the total phenolic (TP) compounds in the medicinal wines was expressed as gallic acid equivalents (mg/L). The concentration of the total phenolic content was determined by using calibration curve of gallic acid. The equation of standard curve was $y = 0.4317x – 0.024$ and $R^2 = 0.9934$. The total polyphenols content of medicinal wines, obtained using the Folin–Ciocalteu reagent, is presented in Table 2.

The total polyphenol concentration was found to vary between 1781 mg/L Pinot Gris (Veritas Panciu, 2009) + garlic and 2717 mg/L Caberrnet Sauvignon (Vincon Vrancea, 2010) + lemon balm. Our results confirm a variation in phenolic content among medicinal wine samples tested and these data showed that the wine samples from *Cabernet sauvignon* grapes generally had the highest total phenolic content. The phenol composition has an important role in stabilizing lipid oxidation and is associated with antioxidant activity.

Measurement of the antioxidant capacity. The total antioxidant capacity of medicinal wine samples was determined by the bleaching of pre-formed ABTS radical cations. Table 3 shows that medicinal wines studied presented a substantial antioxidant capacity.

The TEAC values for medicinal wines are in the range between 30.3 mM Caberrnet Sauvignon (Vincon Vrancea, 2010) + lemon balm and 13.8 mM Pinot Gris (Veritas Panciu, 2009) + garlic.

A good correlation was obtained in our work between the total phenolic content of the medicinal wine samples tested and values of ABTS (Figure 1). To model the results were used TableCurve 2D software (Version 5.01.01).

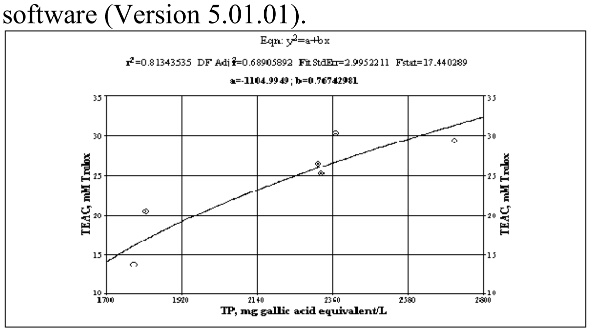

Figure 1. Correlation between TP and TEAC

4. Conclusion

The levels values of the total phenolic compounds in the medicinal wines reported in this study showed that the medicinal wine samples from *Cabernet Sauvignon* grapes generally had the highest phenolic compounds content; so we can suppose that from these grapes is possible to produce in medicinal wines with phenolic compounds amount available to induce physiological effects.

The antioxidant potential of a medicinal wine can't be exactly foreseen on the base of its phenolic content since it doesn't depend only on phenolic content but also on the phenolic specific composition and the specific analytical methods applied.

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