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Anthocyanin and Polyphenol Antimicrobial Proprieties in Forest Fruits and Red Wine Extracts

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ABSTRACT

Plant polyphenols and anthocyanin are structurally diverse compounds used medicinally for centuries due to their antimicrobial properties. In the current study forest fruit and red wine extracts were subjected to alcoholic extraction and rotoevaporation concentration. pH, antocyanidin, total polyphenol concentration were monitored whit antimicrobial action tested on *E.Coli*, *S.Aureus* bacterial cultures. Total polyphenol concentrations reached 2.450 g/L with minimums of 1.400 g/L in red wine samples and 2.332 g/L with a minimum of 1.089 g/L for fruit samples. Maximum antocyanin reached levels of 0.558 g/L for forest fruit and 0.728 g/L for wine extracts. All samples were able to produce variable diameters of inhibition on *S.Aureus* and *E.Coli* bacterial cultures. Results showed that antocyanin and polyphenols can help conventional antibiotic therapy, confirming previous literature indications.

Keywords: Wine Extracts; Fruits; Forest; Polyphenols; Anthocyanin; Shelf Life

Abbreviations: NFE: Native Fruit Extracts; HE: Hydroalcoholic Extracts

Introduction

Polyphenols and anthocyanin are accredited with valuable biological potential, including antimicrobial potential. Current medical practices aim at replacing or enhancing classical allopathic therapy with alternative, effective and safe antimicrobial therapies [1]. Polyphenols have been proven to be effective in the treatment of infectious etiology diseases whit growth limiting effects on certain bacterial metabolisms [2]. Due to previous results regarding polyphenol and anthocyanin antibacterial, these compounds represent a possible alternative or complementary therapeutic option [3,4]. Even though polyphenols and anthocyanin were initially restricted to grapes and derived grape products such as wine, their presence has been later identified in all sorts of vegetable foods [5]. Many of these compounds have beneficial health effects [6]. The addition of phenolic compounds or antimicrobial juice could be a good alternative in lowering health hazards and economic losses caused by microbiological contamination. These techniques could extend the shelf life of processed foods [7,8]. Forest fruits are rich sources of bioactive phenolic compounds and organic acids, which can have antimicrobial action [9]. Previous studies showed that different types of wines could inhibit bacterial growth in *E.Coli* and Salmonella enterica strains. Results cannot be attributed only to polyphenol content since integral wine samples were used [2]. Only a small percentage of polyphenol content is transferred to wine, the vast majority remain in secondary products that result from winemaking technologies [10].

The eastern part of Galati County is part of viticultural areal whit a vast tradition in vine culture, characterized by the production of red wines. Altitude, geographical position, landscape particularities, air temperature variations, pluviometric deficit and atmospheric humidity dynamics represent particularities rarely found in other parts of the country, contributing to the making of wines that have specific organoleptic and chemical composition [11,12].This study tests the antimicrobial potential of different types of fruit extracts by comparison to different red wines specific to Targu Bujor viticulture areal, on the eastern border of Romania

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Material and Methods

Fruit Exact Preparation

All fruit samples were purchased from hypermarket chains or local producers and stored at a constant temperature of -100C ± 20C until use. 100g of the selected fruits were mechanically crushed in order to obtain a homogenized extract of liquid consistence. Mechanical operations were done using an Retsch laboratory mill whit a maximum rotor peripheral speed of 31.4 m/s. Extract preparation aimed at all fruit component (skin, seeds, pulp) preservation. The following types of extracts were developed:

- Alcoholic extract with an alcoholic concentration of 14% native pH.
- Alcoholic extract with an alcoholic concentration of 14% pH adjusted to 7.
- Native pH non-alcoholic extract.
- Non-alcoholic extract pH adjusted to 7.

Fruit extracts were centrifuged using a Hettich universal 320 r centrifuge at a 2433 RCF for 15 min, retaining only the clear upper liquid layer. These samples were divided in to 4 sample categories:

Native fruit extracts (NFE): Raspberries, Blackberries, Strawberries, Blueberries

Hydroalcoholic extracts (HE): Raspberries Alc, Blackberries Alc, Strawberries Alc, Blueberries Alc,

Fruit extracts whose pH had been adjusted to neutral pH (FE 7): Raspberries pH 7, Blackberries pH 7, Strawberries pH 7, Blueberries pH 7.

Hydroalcoholic extracts from fruits whose pH had been adjusted to neutral pH (HE 7): Raspberries Alc pH7, Blackberries Alc pH 7, Strawberries Alc pH 7, Blueberries Alc pH 7.

Wine Samples and Wine Extracts Preparation

Wine samples selected for their antimicrobial potential were procured from Bujoru viticulture and wine Research Station (S.C.D.V.V.). All wine varieties are kept in climate controlled room, at a constant temperature of 10 0C ± 2 0C.

Wine Extracts: Ethanol removal from the selected wine samples and their concentration was carried out with via roto-evaporation techniques, at a constant temperature of 500C for 15 and 45 minutes. The purpose for this operation being the removal of ethylic alcohol natively present and anthocyanin and polyphenol concentration attempt.

Extracts were divided on experimental grounds into 3 categories as follows:

(W.S) Wine samples: Black maiden, Merlot, Cabernet Sauvignon, Burgund

(W.E 15) 15 minutes: Black maiden 15, Merlot 15, Cabernet Sauvignon 15, Burgund 15.

(W.E 45) 45 minutes: Black maiden 45, Merlot 45, Cabernet Sauvignon 45, Burgund 45.

Plate Seeding Technique

Antimicrobial activity was determined by extracts seeding on: Staphylococcus aureus and Escherichia Coli bacteria cultures. Bacterial strains were isolated from patients undergoing treatments at "Anton Cincu" Tecuci Municipal Hospital. The use of these types of strains were isolated in order to test multi-resistant pathogens. Pathogen replication was carried out by diffusimetric method in specific nutrient media. Uniform diffusion in the pathogen culture medium required the use of a cellulosic support, soaked with 100 µL of sample. The sensitivity status of the bacteria was determined by inhibition zone diameter measurements to the nearest whole millimeter. Guidelines defines the bacteria as resistant (≤9 mm), moderately sensitive (10-11 mm), or sensitive (≥12 mm) to a reference antibiotic in our case amoxicillin [13].

Total Polyphenol Content in Wine, Wine Extracts and Fruit Samples

Total polyphenol content was carried out by the use of Florin-Ciocaltau method [14].

pH Determination

pH variations were monitored by means of potentiometric titrations.

Anthocyanin Determination

Anthocyanin concentrations in wine and fruit samples were determined spectrophotometrically with maximum absorbance at specific wavelength of 520 nm. The spectrophotometric method used uses the pH variation for the formation of colored anthocyanin compounds. All quantitative determinations of anthocyanin concentration were performed in triplicate.

Results

Variation of total polyphenols concentration depending on the nature of the analyzed sample

Total polyphenols concentration varied dependent on sample nature.

Total Polyphenol Content in Wine and Wine Extracts: Wine samples, reached a maximum average concentration identified for Burgund variety (Figure 1). Wine extracts, suffered overall increases in total polyphenol average concentration. Samples subjected to 15

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minutes evaporation procedures registered maximum values in the range of 2.235-3.419 g/L. 45 minute extracts showed a more pronounced increases of total polyphenol concentrations. Average values for this parameter in extracts reached maximum values of 3.419 g/L (Burgund).

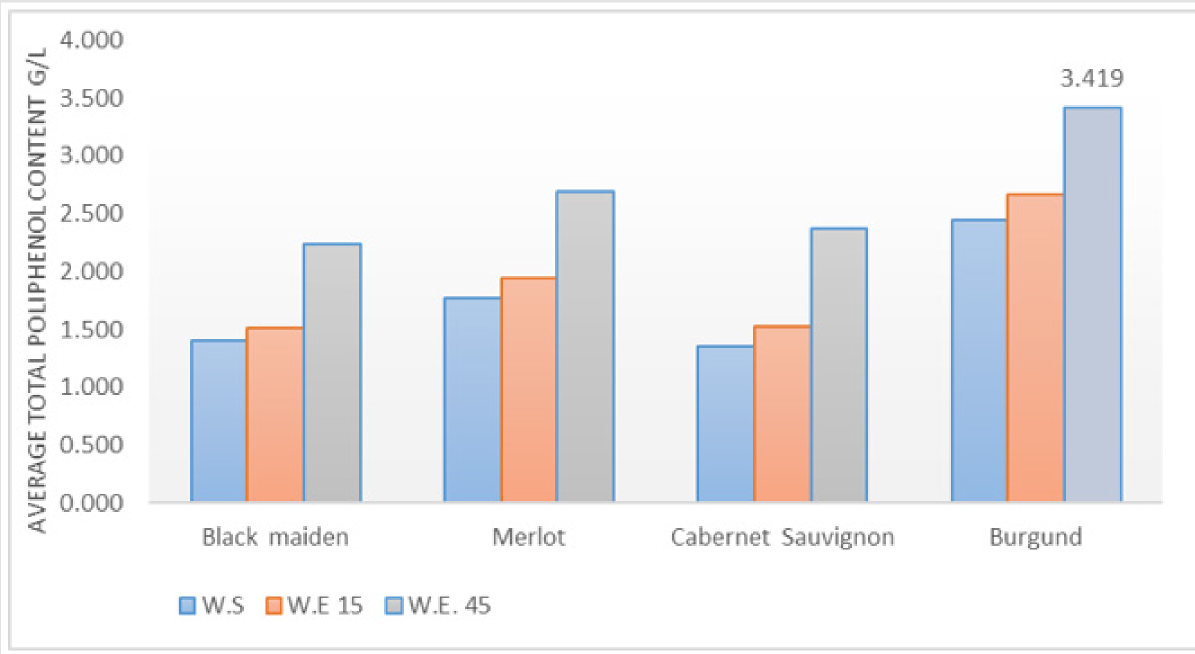

Figure 1: Total polyphenol variation according to extraction time and variety.

Total Polyphenol Fruit Extract Concentration: Average recorded values did not exceed 1.223 g/L. for all fruit samples submitted to analysis in the current study. Concentration variations are shown in Figure 2. The highest values were recorded for strawberry samples (0.731 g/L). On the other hand, according to Figure 2, highest values for polyphenols were seen in alcoholic extracts whit the exception of raspberries. pH variation influenced polyphenol concentrations, causing concentration decreases in all sample categories. Specific pH values for NF and HE influence polyphenol concentrations. pH adjustment to neutral values determine possible molecule denaturation. Compound specific stability were situated at values of 1.8-4.5. Adjustment attempts aimed at reduction of specific pH influence on bacterial strains.

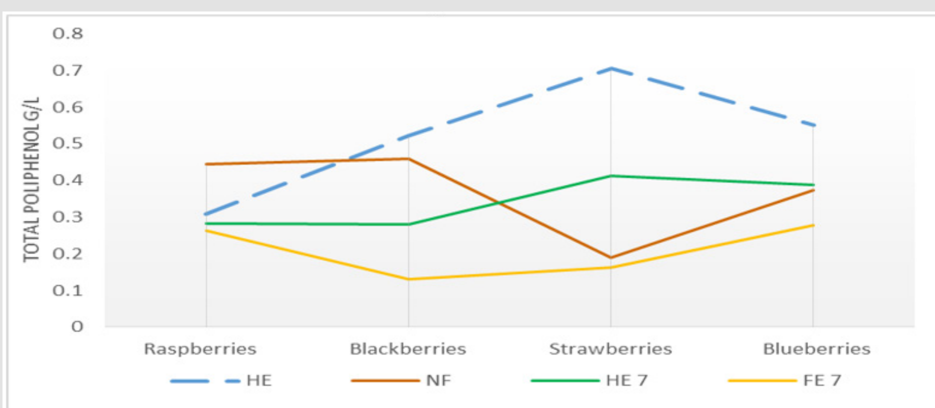

Figure 2: Total polyphenol content registered for fruit extracts.

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Anthocyanin Content Variation

Concentration of Anthocyanins in Berry Extracts: The concentration of anthocyanins varies within fairly wide limits, with values in the range of 0.558 - 0.003 g/L. Maximum concentrations are obtained for strawberry alcoholic extracts and the lowest values of the concentrations of anthocyanins correspond to all categories of cherry extracts (Figure 3).

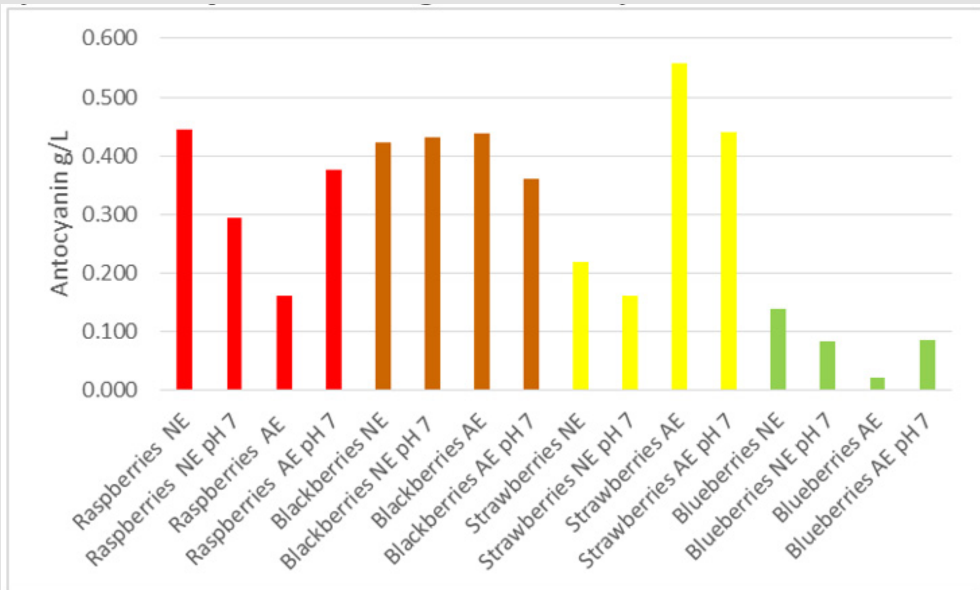

Figure 3: Antocyanin content variation in fruit extracts.

Anthocyanin Concentration in Wine Samples and Wine Extracts: In the case of WE 45, anthocyanin concentration varied in the range of 1.413 – 1.736 g/L. Concentration procedures caused an increase in anthocyanin concentration directly proportional to extraction times whit increases, between 35.08% and 47.17% were recorded. Initial highest concentrations were obtained for Burgundy WS, with average concentration of 1.127 g/L (Figure 4). By comparison, strawberry sample maximum concentration were by about 50,48% lower than the maximum concentration of WS.

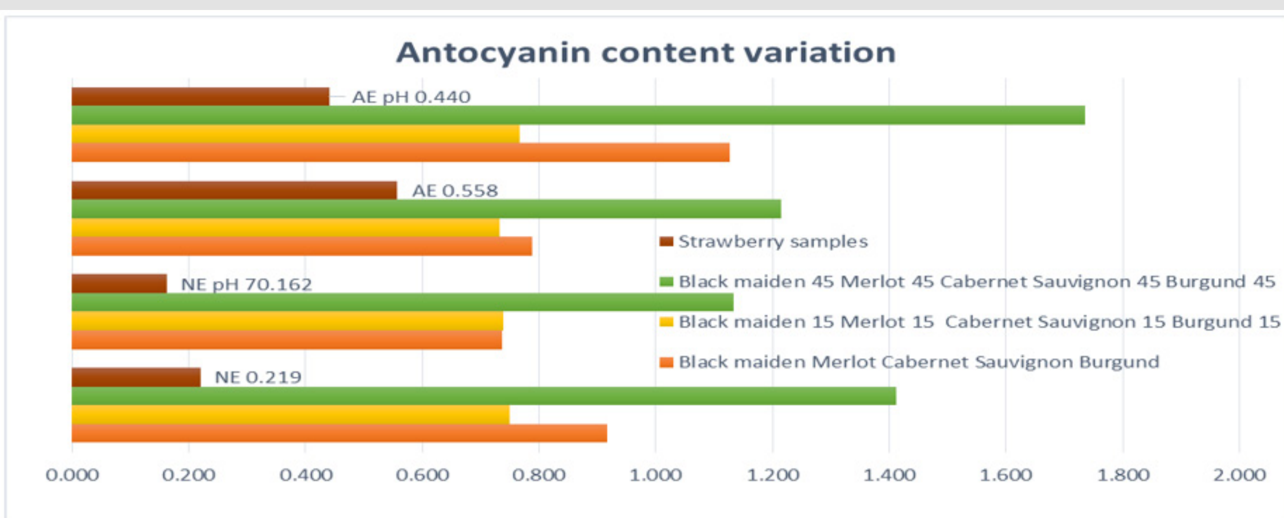

Figure 4.

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Antimicrobial Action Specific to the Samples Taken in the Study: Incubation at 370C for 48 hours, revealed that selected samples were able to sensitize *E.Coli* and *S.Aureus* bacterial cultures, whit variable inhibition diameters as seen in Figure 5. According to Figures 6 & 7 a comparative analysis between inhibition diameters recorded for S. Aureus and E. Coli found that S. Aureus strains were more sensitive to wine sample treatments. Maximum inhibition diameter, of 14.77 mm, were seen in Burgund 45 WE, on S. Aureus strains. Values of 12.40 mm, were obtained on *E.Coli* strains specific to the same type of wine extract. *S.Aureus* strain were by 16.04% more sensitive to the proposed wine extracts. Fruit extract treatments on mentioned bacterial strains followed a similar trend, as previously observed in wine and wine extracts. Most conclusive results were observed for alcoholic fruit extracts with and without pH correction. Observable inhibition diameters were seen only in alcoholic fruit extracts, with maximum values specific for Raspberry AE. His type of extract was able to produce inhibition radius values of 11.6 mm for *S.Aureus* and 9.9 mm for *E.Coli* (Figure 8). Raspberry AE values produced the same inhibition diameters as seen in Cabernet Sauvignon WE, but these AE were not nearly as effective as Burgund WE. The rest of the fruit extracts, produced some inhibition effects whit average diameter variations between 7.4 mm (*S.Aureus*) and 6.1 mm (*E.Coli*).

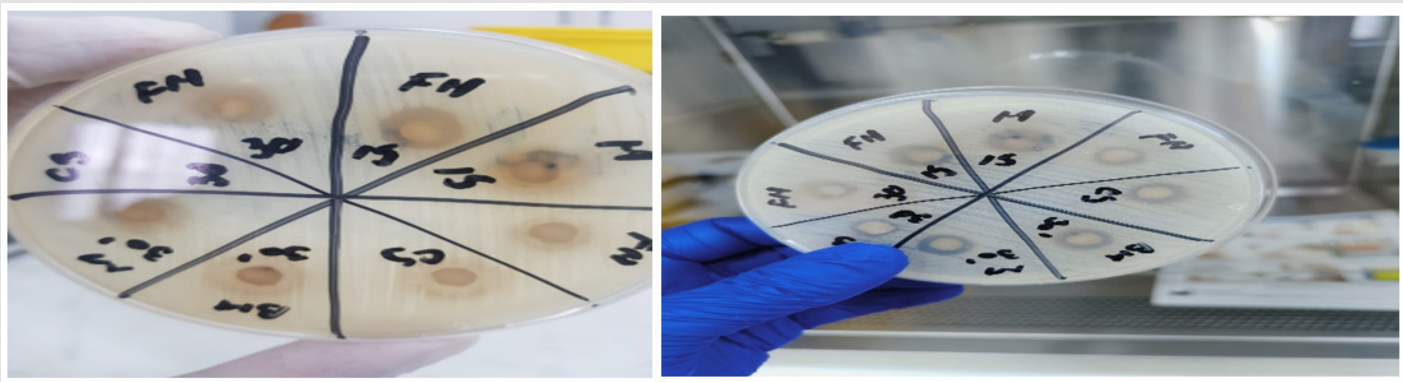

Figure 5: Inhibition diameter shown by *S.Aureus* and *E.Coli* bacterial strains.

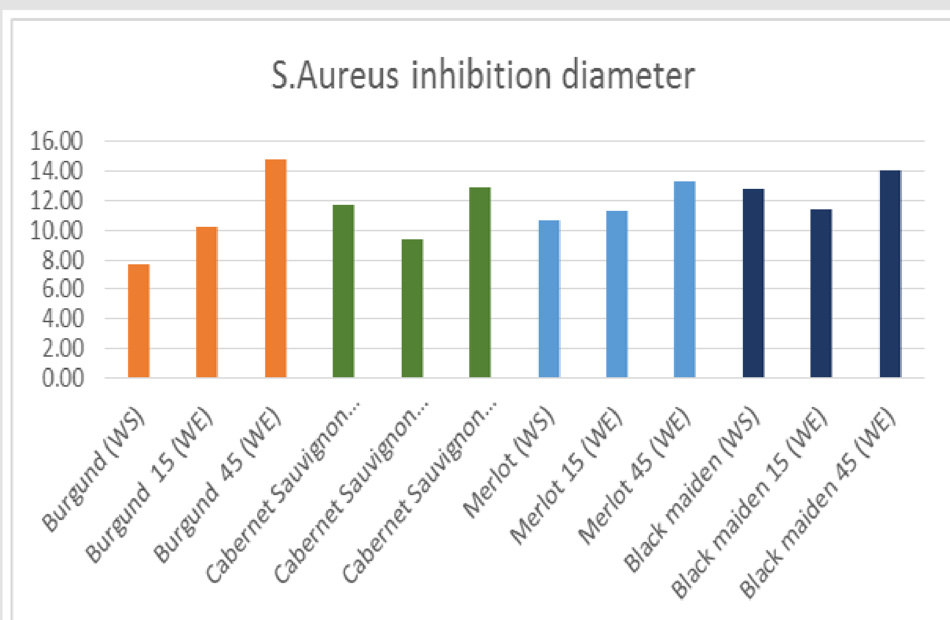

Figure 6: Inhibition diameter specific to *S.Aureus*.

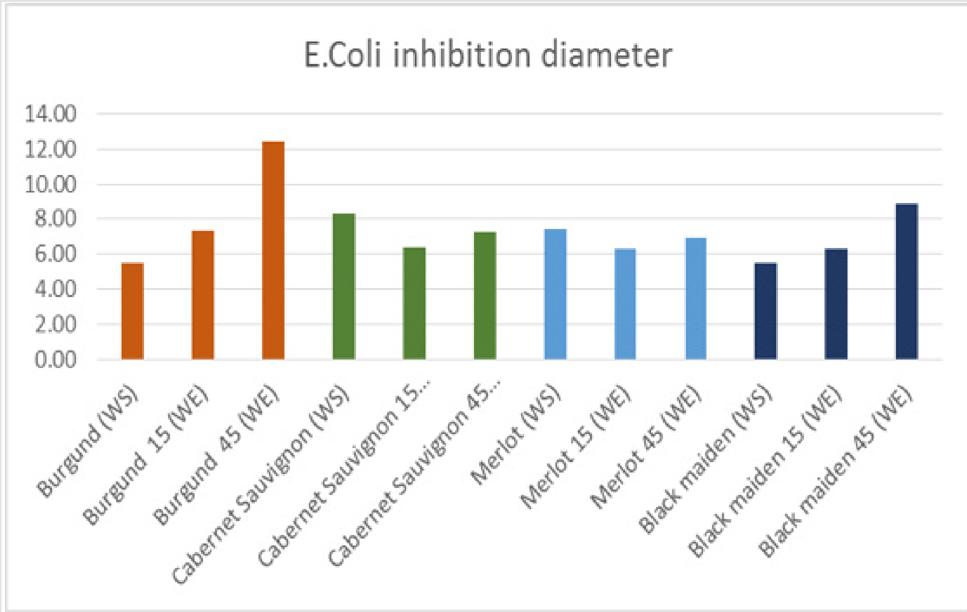

Figure 7: Inhibition diameter specific to *E.Coli*.

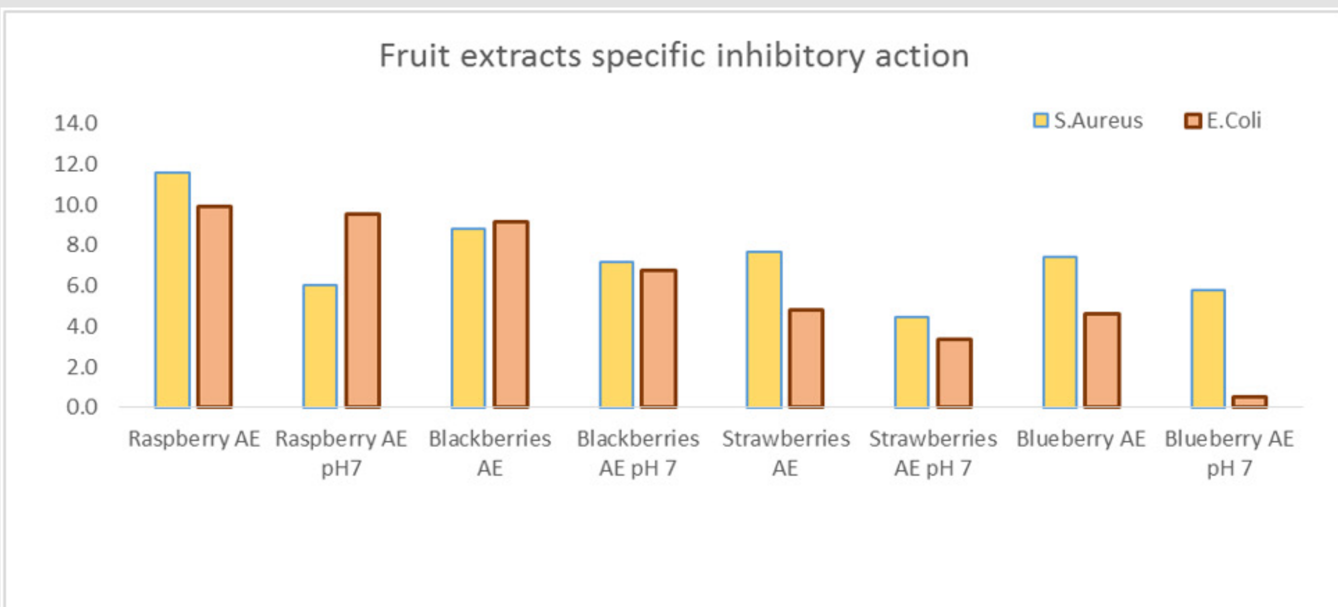

Figure 8: The inhibitory activity shown by alcoholic fruit extracts.

Discussion

As previously mentioned total polyphenol concentrations had quite a wide range of variation extreams belonging to Merlot and Burgund varieties. Concentrations identified are in concordance the values identified in the reports from the specialized literature [15]. Total polyphenol concentrations in different red wine samples of Italian origin ranged from 1.365-3.326 g/L [16,17], lower than currently seen in our WE for this study. An increase in total polyphenols concentrations was possible which was directly proportional to evaporation times. A 28% (Burgund variety) to 37%(Merlot variety) concentration increases were observed dependent to evaporation times when compared to initial wine samples. Results were similar to those reported by Ševcech, J et al. (2015), when low temperatures polyphenol extraction techniques were used [5,18]. Concentrations obtained in our study are slightly higher than those reported by literature and that ranged from 0.394 to 1.879 g/L [19,20]. Highest concentrations for total polyphenols, in fruit samples, were obtained from alcoholic strawberry extracts. Average concentration values of 0.731 g/L were obtained, representing a 78.62% decrease when compared to WE 45 (Burgund variety) and by 70.26 % lower when compared to initial Burgund WS.Results obtained by our study identify strawberries as a rich source of polyphenols even though concentrations did not reached levels as high as those obtained by Gasperotti, et al.(2015). Anthocyanins followed the same trend in regards of concentrations increase as seen in WE 45 vs WS.

Highest values for WS of 1.127 g/L compared to 1.736 g\L WE 45, representing a 35% increase in anthocyanin content. Fruit samples reached lower values when compared to those of WS and WE 45 whit maximum values of 0.558 g/L (Strawberry AE) followed by 0.444 g/L (Raspberry AE). Results similar to those reported by previous studies [21]. Previous works have demonstrated the ability of wine samples, wine and fruit extracts to inhibit *S.Aureus* and *E.Coli* bacterial growth [22,23]. Wine extracts subjected to longer evaporation times produced larger inhibition diameters when compared to initial wine samples. In fruit samples, good inhibition diameters were mainly observed in native pH alcoholic extracts [15]. Raspberrie AE produced bacterial sensitization but inhibition diameters were not as large as those seen for WE. Polyphenol specific antimicrobial action from wine samples was also demonstrated in various Greek origin wines. Better results for S. Aureus bacterial strain sensitization when compared to those observed in the present study, when bacteria reference strains were used [24].

Conclusion

Present study identifies a possible antimicrobial action determined by polyphenol and antocyanins present in wine and fruit extracts subjected to different treatments (evaporation at different times and pH variations). In our selected fruit extracts total polyphenol content reached concentrations previously mentioned by literature [15,16,25]. Results lead to extraction method refinement for polyphenols and anthocyanins present in fruit samples. Most likely candidates for extraction method refinement being: Raspberry alcoholic extracts because this type of extract registered highest bacterial inhibition diameters although total polyphenol and anthocyanin concentrations were not the highest recorded in our study. Previous microwave extraction techniques in sour cherry samples obtained total polyphenol and anthocyanin concentrations up to 2 mg\L [26], whit possible method adjustments for anthocyanin and polyphenol raspberry extraction.Results showed an increase of antimicrobial activity directly proportional to wine sample evaporation times, corroborated whit total polyphenol concentration increase. Polyphenol structural denaturation was avoided by low temperature evaporation (<500C), whit the best results obtain for Burgund varieties. Results obtained by us, confirm previous reports regarding polyphenol and anthocyanin content, opening perspectives that involve the use of fruit and wine extracts as a complementary therapeutic choice [27-32].

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