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In Vitro Antimicrobial and Antioxidant Activity of Some Berry Species

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ABSTRACT

The aim of this work was to determine antioxidant and antimicrobial activities of extracts obtained from fresh and dried fruits of *Vaccinium macrocarpon, Morus nigra, Fragaria X ananassa*. Antioxidant and antimicrobial activity of extracts were assayed by DPPH and disc diffusion methods. Antioxidant activity of extracts decreased in the following order: *Fragaria X ananassa* fresh fruit (FAF) > *Vaccinium macrocarpon* fresh fruit (VMF) > *Morus nigra* fresh fruit (MNF) > *Morus nigra* (MND2) > *Morus nigra* (MND1) > *Vaccinium macrocarpon* (VMD1) > *Fragaria X ananassa* (FAD1) > *Vaccinium macrocarpon* (VMD2) > *Fragaria X ananassa* (FAD2). Fresh fruits showed higher antioxidant activity than dried fruits. FAF showed highest antimicrobial activity aganist *E. coli* and MND2 showed higher antimicrobial activity aganist *E. coli* and *S. aureus* in comparison to other extracts. Other extracts showed nearly same antimicrobial activity. All extracts showed no antimicrobial activity aganist *C. albicans*.

Keywords: Mulberry, Strawberry, Cranberry, Antioxidant activity, Antimicrobial activity

INTRODUCTION

Dietary patterns characterized by relatively high intakes of fruits and vegetables are consistently associated with reductions in the incidence of noncommunicable diseases such as coronary heart disease, stroke, cancer, and various chronic disease[1]. Berries provide significant health benefits because of their high levels of polyphenols, antioxidants, vitamins, minerals, and fibers[2]. Most berries are

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delicious and powerful disease-fighting foods and make up the largest proportion of fruit that is consumed in the human diet. Berry fruits are popularly consumed not only in fresh and frozen forms but also as processed and derived products, including dried[3].

Oxidative stress plays an important role in the pathogenesis of most chronic diseases[4]. ROS is said to play an important role in many chronic diseases such cardiovascular diseases, diabetes, inflammation, anaemia, degenerative diseases, cancer[5]. Antioxidants molecules had defensive effects against reactive oxygen species (ROS) in the body. The plants have been known since ancient times as a good antioxidant[4]. Fruits rich in antioxidants can prevent or delay oxidative damage[6]. Fresh fruits are rich in acids, also contain anthocyanins and flavonoids[7]. Anthocyanins and flavonoids have been identified as strong antioxidant[8]. Especially, berry fruits worldwide known and consumed have been well studied Berries, including raspberries, blueberries, black currants, red currants, and cranberries are a rich source of dietary antioxidant[1].

Vaccinium macrocarpon known as cranberry naturally grows North America. Fresh fruits are rich in acids, also contain anthocyanins and flavonoids[7]. Anthocyanins and flavonoids have been identified as strong antioxidant[8]. *Morus nigra* known as mulberry is native to southwestern Asia also cultivated so long time and natural origin is unknown. Mulberries contain vitamins, minerals and anthocyanins[9]. *Fragaria X ananassa* is cultivated variety of strawberries[10]. Strawberries contain various phenolic compounds such as hydroxycinnamic acids, ellagic acid, ellagitannins, flavan-3-ols, flavonols, and anthocyanins[11].

Certain berries rich in tannins have been found to increase bacterial infections. Among the berries, cranberries, cloudberries, red raspberries, strawberries, and bilberries possess clear antimicrobial effects against human pathogens. Berry ellagitannins are strong antimicrobial agents acting as possible anti-adherence compounds in preventing the colonization and infection of many pathogens. Several mechanisms of action in the inhibition of bacteria are involved, such as destabilization of cytoplasmic membrane, permeabilization of plasma membrane, inhibition of extracellular microbial enzymes, direct actions on microbial metabolism, and deprivation of the substrates required for microbial growth[12, 13]. However, there is very little information about the antimicrobial capacity of phenolics present in berries, except in cranberry[3].

The aim of this study was to determine antioxidant and antimicrobial activities of extracts obtained from fresh and dried fruits of *Vaccinium macrocarpon, Morus nigra, Fragaria X ananassa*.

METHODOLOGY

Plant Material

Fresh fruits and two different dried fruits of *Vaccinium macrocarpon, Morus nigra and Fragaria* X *ananassa* were purchased from different local markets (Table 1).

Table 1. List of sample and abbreviations used in this study

Botanical name	*Fragaria X ananassa*	*Vaccinium macrocarpon*	*Morus nigra*
Fresh fruits	FAF	VMF	MNF
Dried fruits 1	FAD1	VMD1	MND1
Dried fruits 2	FAD2	VMD2	MND2

Chemicals

Methanol, ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were provided from Sigma (Steinheim, Germany). Müeller Hinton agar, Saubaroud Dextrose broth were provided from Merck (Darmstadt, Germany).

Extraction of Plant Material

All fruit samples (100 g) were ground in a grinder and macerated with methanol (200 ml) for four days at room temperature and the extracts were filtered. Then methanol was evaporated with rotary evaporator. All extracts were stored in refrigerator at 4 C^{o} until use.

DPPH Radical Scavenging Activity

The ability to scavenge DPPH radical of extracts was determined according to the method of Yanping Zou[14]. Briefly stock extracts were prepared at 10 mg/mL concentration and diluted to 2,5 mg/mL, 0,625 mg/mL, 0,156 mg/mL with methanol. 10 µL of all dilutions were added 190 µL DPPH solution in a well of 96 well-plate. The mixture was shaken quietly and left in room temperature and dark for 30 minutes. After then the absorbance was measured aganist methanol using a microplate reader at 517 nm.

DPPH radical scavenging activity were calculated according to following:

Antioxidant activity (%) = $[(A_o-A_1) / A_o]X100$

Where A_o is the absorbance of control, A1 is the absorbance of extracts/standard. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage aganist extract concentration. Test were carried out in dublicated. Ascorbic acid was used as positive control.

In vitro Antimicrobial Activity Assay

In this study, disk diffusion method was used to determine of antimicrobial activity of the berries. This method is used for detection whether the samples have inhibition effect on microorganisms[15]. Also used for determination effects of drug and comparison of standards[16].

Microbial Strains And Growth Conditions

The assessment of antimicrobial activity was performed on gram positive bacteria *Staphylococcus aureus* ATCC 25923, gram negative bacteria *Echerichia coli* ATCC 25922 and yeast *Candida albicans* ATCC 10231 was determined by the disc diffusion method. Bacterial cultures were grown at 37oC for 24 hours in Brain Heart Inhibition broth or agar (BHB, BHA, Merck, Darmstadt, Germany), yeast strain was grown at 30 oC for 48 hours in Saubaroud Dextrose broth or agar (SDB, SDA, Merck, Darmstadt, Germany). Microbial cultures for antimicrobial testing were prepared by picking colony from 24 or 48-h-old BHA/SDA plates and it was suspended in saline solution to dilute 10^5-10^6 CFU/mL (%0,89 NaCl). The disk diffusion method was performed on Müeller Hinton agar (MHA, Merck, Darmstadt, Germany) for bacterial strains and SDA for yeast strain.

Disk Diffusion Method

Each of extracts were diluted in sterile distilled water (0,1 w/v). For the disk diffussion assay 0,1 mL of each microbial suspension was spread on a solid growth medium in a Petri dish. Three sterile paper disk (6 mm diameter) were impregnated with 15 uL each plant extract solution and were placed on the surface of agar plate. Plates were incubated for appropriate conditions for microbial strains. Antimicrobial activity was determined with inhibition zone around the disk following incubation. Impregnated discs with ethanol used as positive control[17].

Minimum Inhibitory Concentration (MIC) Assay

Minimum inhibitory concentration (MIC) is described as the lowest concentration of antimicrobial agent is needed to kill the bacteria[18]. MIC of all extracts were determined by microdilution techniques in Mueller-Hinton broth (MHB) for bacteria. Inoculates prepared in the MHB at a density adjusted to 0,5 McFarland turbidity standard and diluted 1/10 for the broth microdilution procedure[15]. The data were given as means±standard deviations and analysed by one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison tests using GraphPad Prism.

RESULTS AND DISCUSSION

Our focus in this study was to complement the previous knowledge of antioxidant and antimicrobial activities of fresh and dry samples of berries.

The antioxidant power of fruit is closely correlated to the presence of efficient oxygen radical scavengers, such as vitamin C and phenolic compounds[19]. Berries are consistently ranked among the top sources of total phenolics and TAC, with levels up to 4 times greater than other fruits, 10 times greater than vegetables[20]. In a study by Tulipani et al. individual contribution was investigated in different strawberry cultivars, where vitamin C was found to be one of the most important components responsible for more than 30% of the TAC of strawberry extracts, followed by anthocyanins contributing 25% to 40%[21]. Viskelis and others reported that significantly larger amounts of anthocyanins were determined in the overripe cranberries of the cultivars[22]. Similarly, antioxidant activity in fresh strawberry was found to be highest in our study, followed by fresh cranberry and mulberry extracts.

A low IC_{50} value (the concentration of extract, which is required to scavenge 50% of DPPH free radical) means strong antioxidant activity. FAF showed the highest antioxidant activity with IC_{50} value of 0,327 mg/mL, while FAD2 showed the lowest antioxidant activity with IC_{50} value of 3,331 mg/mL in DPPH assay. All extracts showed low antioxidant activity compared to standard. It was determined that fresh extracts showed higher antioxidant activity. Antioxidant activities of extracts decreased in the following order: FAF>VMF>MNF>MND2>MND1>VMD1>FAD1>VMD2>FAD2 (Table 2).

It was thought that, drying and storage conditions may have affected the antioxidant capacity of dry extracts. In the phytochemical studies on these plants, it have been reported that this species contained phenolic compounds such as flavonoids and anthocyanins intensively. Therefore, the antioxidant activity of these fruits might be resulting from the phenolic contents of them.

Table 2. Antioxidant activities of extracts

Extracts / Standards	DPPH activity IC_{50} ($mgmL^{-1}$)
FAF	0,327±0,002c
FAD2	3,331±0,024j
FAD1	2,189±0,005h
VMF	0,408±0,007cd
VMD2	2,332±0,054i
VMD1	1,640±0,021g
MNF	0,454±0,005d
MND2	1,145±0,045e
MND1	1,318±0,004f
Ascorbic acid	0,002±0,000a
Butylated hydroxyanisole	0,057±0,000b

Low IC_{50} value indicates high antioxidant activity.

Each value in the table is represented as mean ± SD (n = 3)

Different letter superscripts in the same column indicate significant differences ($P < 0.05$)

In this study, disc diffusion method was used to determination of antimicrobial activity of strawberries, blueberries and black mulberies on *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *C. albicans* ATCC 10231. Inhibition zones were measured and shown in Table 3.

This study showed that, dry strawberry (FAD2) has the highest level of antimicrobial activity was observed against *E. coli*. There was no inhibition zone against *C. albicans*. The dry blueberry (VMD2) has inhibition zone of 7 mm diameter against *S. aureus*.

In addition to disk diffusion method, broth dilution method was also performed to observe effect against on *E. coli* and *S. aureus*. Fresh strawberry (FAF), fresh blueberry (VMF) and dry black mulberry (MND1) were found that most effective on *S. aureus*. Dry black mulberry (MND1) was also found that most effective extract against to *E. coli* (Table 4).

One of the study showed that the highest antimicrobial activity of blueberry against *E. coli* and it has 18,67±1,15 mm inhibition zone also the lowest antimicrobial activity was found that against *S. aureus* and has 11,00±2,00 mm diameter[23]. Howell reported that high-molecular weight proanthocyanidins (condensed tannins) from cranberry juice inhibit the adherence of uro-pathogenic fimbriated *E. coli* and thus offer protection against urinary tract infections[24]. Compared with our study, it was observed that blueberries did not produce any antimicrobial product against *S. aureus* and *C. albicans,* which showed that higher antimicrobial activity against *E. coli*. Another study showed that blueberry inhibited the growth of *E. coli* and *S. aureus,* but did not inhibit the *C. albicans*[25]. It was shown that, Black mulberry has more effectively inhibition against to Gram positive bacteria than Gram negative bacteria[26]. All of the extracts did not have any effect on the growth of the yeast species *(C. albicans)* studied.

Table 3. Inhibition zone around disks

Extracts / Control	*E. coli*	*S. aureus*	*C. albicans*
Ethanol	9 mm	9 mm	10 mm
FAF	6 mm	-	-
FAD2	10 mm	-	-
FAD1	11 mm	-	-
VMF	10 mm	-	-
VMD2	9 mm	-	-
VMD1	9 mm	-	-
MNF	-	-	-
MND2	8 mm	7 mm	-
MND1	9 mm	-	-

Table 4. MIC results

Extracts/Control	MIC (mg/mL)	
	S. aureus	*E. coli*
Ethanol	0,1	0,1
MNF	0,003125	0,05
FAF	0,0015625	0,05
VMF	0,0015625	-
MND2	0,00625	0,0125
FAD2	0,0125	0,1
VMD2	0,0125	0,05
MND1	0,0015625	0,00625
FAD1	0,003125	0,1
VMD1	0,05	-

In conclusion, fresh samples of fruits showed higher antioxidant and antimicrobial capacity than dried samples. Strawberries also showed higher effects than other berry samples. The antioxidative and antimicrobial activity depends on the cultivar, growth conditions, storage of raw material, and the method of isolation of active substances. Drying can affect the amount and activity of antioxidant ingredients. Further studies are needed to verify the antioxidant and antimicrobial activity of the compounds of berries.

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