The antibacterial and DNA-protective effects of blackberry extract

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ABSTRACT

Emerging of resistance mechanisms to antibiotics used in therapy of infectious diseases fastened the search for new antibiotics molecules. Blackberry fruits are widely used in clinical studies. The study was aimed to evaluate DNA damage protection activity of blackberry fruit and its influence on Stenotrophomonas maltophilia strains causing infectious diseases in hospitals. Protective activity of the fruit extract against UV and H₂O₂ which induced DNA damages was studied. Plasmid pBR322 was exposed to H₂O₂ and UV lights in different times with methanol extract prepared at different concentrations and the results displayed on gel. The results showed that highly concentrated Rubus L. fruit extracts have the capability of protecting DNA from oxidative damage. Disc diffusion and microdilution test studies on S. maltophilia strains showed that methanol extracts have antibacterial activity by inhibiting the maltophilia bacteria. The results obtained can be evaluated in the future in cosmetic industry as a cream and as well as, a new antibiotic molecule in the pharmaceutical industry.

Key words: Rubus L., plasmid DNA, pBR322, UV.

INTRODUCTION

Rubus L. is a fruit that is included in the Rubus genus of the Rosoideae subfamily belonging to the Rosaceae family of the Rosales order. It is a well known fruit commonly called ‘blackberry’ that has medical, cosmetic and nutritive value (Chopra et al., 1956). It has become a popular fruit in recent years due to its pleasant smell, attractive appearance, color and taste (Redalen, 1990). Blackberries, which are included in the berry fruits group, have various uses in the food industry. Therefore, it has a very special place among other fruits. Moreover, it is indicated that some pigments, phenols, flavones, flavonoids, vitamins and fibers contained within blackberries are very high in concentration when compared to other fruits (Pehlivan and Güleryüz, 2004). For example, raspberries and berries are comprising 4-6 g fiber every 100 g. This rate is higher than many fruits such as bananas, apples and pears (Harris, 2002).

Presently, Stenotrophomonas maltophilia is an opportunistic nosocomial infection agent that is increasingly isolated (Dulger, 2007). It is commonly found in natural and hospital environments. It can be frequently isolated from adult oropharynx and phlegm. In hospitals and frequently Intensive Care Units (ICU), S. maltophilia is being increasingly isolated at a rate of 4 to 8% as a nosocomial infection agent (Caylan, 2004; Jones and Sader, 2003). On the other hand, 97% of the infections caused by this bacterium are hospital-acquired. Bacteria can be colonized in disinfectants, in shower heads, on the hands of healthcare personnel, and in dialysis and ventilation devices in the hospital environment (Villarino et al., 1992).

S. maltophilia is a multidrug resistant bacterium that resists many antibiotics with various mechanisms. Therefore, the infection caused by this bacterium is expensive and hard to treat with a high morbidity and mortality rate. The mortality rate of bacteremia caused by this bacterium is higher than 50% (Caylan, 2004; Kanavaki et al., 2005).

Therefore, resistance studies should be conducted frequently in order to determine the ratio of sensitivity-resistance between the relevant bacteria and drugs. Samples collected from medicinal plants have been studied in the search for new antibiotic molecules instead of expensive antibiotic treatments. With this purpose, we evaluated the antibacterial activity of the blackberry plant,
which is of medicinal value, against a nosocomial infection agent, that is, *S. maltophilia* in this study.

The effect of endogenous and exogenous factors can lead to alterations in the molecular integrity of genetic material. This is called “DNA damage” (Atmaca and Aksoy, 2009). The UV (ultraviolet) rays that reach the earth due to the destruction of stratosphere layer leads to diseases such as skin cancer and skin aging (Tepe et al., 2011). Human skin possesses enzymatic or non-enzymatic antioxidant defense systems that suppress reactive oxygen species (ROS). These mechanisms contribute to reducing the harmful effects of VIS (visible light) and UV radiation (Roche et al., 2010). However, exposure to UV radiation may lead to a decrease in cellular antioxidants and UV-induced oxidative DNA damage due to reactive oxygen species (Gutteridge, 1984).

Reactive oxygen species have been reported to cause damage to DNA such as sugar and base modifications, non-basic regions, DNA-protein crosslinking, single and double strand breaks (Atmaca and Aksoy, 2009). Moreover, it was also shown that H$_2$O$_2$ (hydrogen peroxide) causes oxidative DNA (8OhdG) damage *in vitro* (Yokuş and Çakır, 2002).

Some studies have shown that antioxidants could reduce cancer growth and that DNA damage induced by reactive oxygen species could be controlled by phytochemicals (Karaca and Güder, 2009). New compounds are needed in order to prevent oxidative DNA damage. There are some detailed studies concerning this issue on extracts obtained from medicinal and aromatic plants (Feig et al., 1994). For instance, the DNA damage-preventing effect and antioxidant activities of juice extracts obtained from *Cistus incanus* and *C. monspeliensis* have been investigated.

The results revealed that both plants had dose-dependent free radical scavenging capacity and a high capacity to prevent DNA damage (Attaguile et al., 2000). None of the previous studies concerning the blackberry plant investigated its DNA protective activity. Therefore, DNA protective activity of this fruit has been evaluated for the first time in this study in methanol extracts.

**MATERIALS AND METHODS**

**Preparation of blackberry fruit extracts**

*Rubus L.* is a fruit that is included in the *Rubus* genus of the *Rosoideae* sub-family belonging to the Rosaceae family of the *Rosales* order. Blackberry samples were bought from a local store in Gaziantep. They were identified in the Botanic Division of the Biology Department in Gaziantep University. Blackberries were dried in the open in the laboratory and then pulverized with a mechanical grinder. Pulverized fruits were then weighed (30 g each) and placed in the cartridges of Soxhlet device (Gerhardt EV 14). Extraction was performed for 3 h in 150 ml methanol (Merck) per cartridge in the Soxhlet device.

**Disk diffusion test of blackberry fruit extracts on Stenotrophomonas maltophilia**

Mueller Hinton Agar (MHA), physiological saline solution, McFarland, McFarland tubes, swabs and antibacterial disks were used. *S. maltophilia* strains that were isolated and identified in the Microbiology Laboratory of Gaziantep University Medical Research Hospital were used in this study. The disk diffusion method recommended by EUCAST (European Committee on Antimicrobial Susceptibility Testing) was also used in order to determine the antibacterial effect of blackberry fruit extracts on *S. maltophilia*. In the beginning of the study, using the direct colony suspension method, in the medical saline was prepared 0.5 (1.5x108 cfu/ml) McFarland standard suspension of *S. maltophilia*. In this study, blackberry fruit extracts were inoculated into MHA agar plates and incubated. For the test, 100 mg/ml of blackberry methanol extract dissolved in 1000 μl distilled water and prepared different concentrations (1/20, 1/40, 1/80, 1/160, 1/320). Afterwards, concentrations were added dropwise on blank disks. Disks that absorbed the solutions were placed MHA agar plates which inoculated both strains of *S. maltophilia*. Zone diameters were measured from petri dishes that were maintained at 37°C for 24 hours and the results were evaluated.

**Determination of the DNA protective activity of blackberry fruits**

pBR322 plasmid DNA (vivantis) was used in order to determine the DNA protective activities of the extracts from damages caused by UV and oxidative stress. Plasmid DNA was exposed to damage by applying H$_2$O$_2$ and UV in the presence of the extracts. According to the method specified by Russo et al. (2000), imaging was performed on 1.25% agarose gel. 50 mg methanol fruit extract was weighed and combined with 1000 μl distilled water. The extract was completely dissolved with a vortex device and 1/10 (5 μl fruit extract + 45 μl dH2O), 1/5 (10 μl fruit extract + 40 μl dH2O), 1/2.5 (20 μl fruit extract + 30 μl dH2O) and 1/1.25 (40 μl fruit extract + 10 μl dH2O) dilutions prepared. 5.0 μl hazelnut shell extract was added to the tubes other than the controls. 3.0 μl pBR322 plasmid DNA (172 ng/μl) and 1.0 μl 30% H$_2$O$_2$ were placed in tubes. A UV transilluminator (DNR-IS) device that generates light at 302 nm wavelength and 8000 μW/cm intensity at ambient temperature was used as the light source. After 1.25% agarose gel electrophoresis was conducted for 100 min at 100 V and the photos obtained through imaging in the gel documentation system (DNR-IS,MiniBIS Pro). pBR322 plasmid DNA was used as the control in this test system. The results are shown in Figure 3.

Preparation conditions of controls and fruit extracts:

<table>
<thead>
<tr>
<th>Control</th>
<th>Description</th>
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<tr>
<td>Control 1:</td>
<td>plasmid DNA (3μl) + dH$_2$O (6 μl)</td>
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</tbody>
</table>
Control 2: plasmid DNA (3 μl) + dH2O (6 μl) + 5 min UV
Control 3: plasmid DNA (3 μl) + dH2O (6 μl) + H2O2 (1 μl)
Control 4: plasmid DNA (3 μl) + dH2O (6 μl) + 5 min UV + H2O2 (1 μl)
1/10: plasmid DNA (3 μl) + 1/10 Rubus L. extract (5 μl) + 5 min UV + H2O2 (1 μl)
1/5: plasmid DNA (3 μl) + 1/5 Rubus L. extract (5 μl) + 5 min UV + H2O2 (1 μl)
1/2.5: plasmid DNA (3 μl) + 1/2.5 Rubus L. extract (5 μl) + 5 min UV + H2O2 (1 μl)
1/1.25: plasmid DNA (3 μl) + 1/1.25 Rubus L. extract (5 μl) + 5 min UV + H2O2 (1 μl)

RESULTS

When evaluating the antibacterial results, the study conducted two different strains of S. maltophilia isolated from different patient samples. Only 1/20 and 1/40 concentration among five different concentrations had protective activity against strain defined as number 3 (Figure 1). The inhibition zone diameters was observed 8.7 mm at 1/20 concentration and 4.5 mm at 1/40 concentration. It was concluded that these concentrations have a significant antibacterial effect on this bacterial strain. In the strain of S. maltophilia defined as number 2, zone measurement was only performed at 1/20 concentration (4.5 mm) as seen in Figure 2 and it was seen that it was significantly effective on the sample at this concentration. According to the Eucast disk diffusion method, this 2 (1/20, 1/40) blackberry extracts were categorized as resistant (R< 16 mm) to two different S. maltophilia strains.

DNA protective activity

The DNA protective activity evaluated after fruit extracts at different concentrations (1/10, 1/5, 1/2, 5, 1, 1 and 25) were exposed to UV light for different time periods. Figure 3 shows preparation conditions of controls and fruit extracts under the bands for both different UV exposures. Fruit extracts had protective activity at a lower concentration after UV exposure for 5 min, whereas among the extracts that were exposed to UV for a longer time, that is, 10 min, the extract at a higher concentration had a higher protective activity (Figure 3).

DISCUSSION

The subject of this study, that is, blackberry, has been known for nearly 8000 years as a food and as a therapeutic plant immediately after the end of ice age (Sarkar and Ganguly, 1978). Blackberry contains flavonoids, glycosides, terpenes, acids, tannins, vitamins, steroids, minerals and lipids that have various pharmacological activities such as anti-oxidant, anti-carcinogen, anti-inflammatory, antimicrobial, anti-diabetic, anti-diarrheal and anti-viral activity (Rios et al., 1987). Vitamins A, B and C in small amounts and dietary fiber (soluble or insoluble) structures have great value. Blackberries exhibit anti-microbial, anti-oxidant and anti-inflammatory effects as they contain phenolic compounds in high amounts and especially anthocyanins (Riceevans et al., 1995).

In the literature, there are many studies regarding the antibacterial effects of blackberries. Cavanagh et al. (2003) reported that drinks containing fruit extracts of the blackberry plant have bacteriostatic properties. Blackberry juice inhibited Bacillus cereus, Bacillus subtilis, Streptococcus marcescens and Escherichia coli growth from 50 to 75% (Cavanagh et al., 2003).

In a study conducted by Riaz et al. (2011), antibacterial activity of methanol extracts obtained from various areas of the blackberry plant against eight bacterial strains (Salmonella typhi, E. coli, Streptococcus aureus, Micrococcus luteus, Proteus mirabilis, B. subtilis Citrobacter spp., Pseudomonas aeruginosa) was investigated and it was found that all extracts inhibited bacterial growth (Riaz et al., 2011).

In the previous years, methanol extracts of the plant were used as an antiseptic and disinfectant for wound healing and treating cough in the public (Rios et al., 1987). Therefore, we obtained methanol extracts of the blackberry fruit and determined its antibacterial and DNA protective effect by identifying the activity of these methanol extracts at various concentrations.

In the USA, Miyasaki et al. (2013) investigated the isolation and characterization of antimicrobial compounds produced by several plant extracts including blackberry (Rubus chingii) against Acinetobacter Baumannii. Six antimicrobial compounds that have an activity against A. Baumannii, which causes nosocomial infections were identified in the fruit extracts. Among these antimicrobial compounds, norwogonin was present in the highest amount. The minimum inhibitory concentration (MIC) and bactericide concentration of norwogonin against A. Baumannii were found as 128 μg/ml and 256 128 μg/ml, respectively (Miyasaki et al., 2013).

In our study, two different strains of S. maltophilia, which has multidrug resistance and causes nosocomial infections were evaluated and it was found that blackberry methanol fruit extract at different concentrations exhibited a very high antibacterial effect. In a similar study, it was found that aqueous and ethanol extracts of the blackberry plant had antibacterial activity against Helicobacter pylori (Abachi et al, 2013).

Recently, researchers have been conducting detailed studies on extracts obtained from medicinal and aromatic plants in order to investigate new compounds that control oxidative DNA damage that leads to cancer (Bayil et al., 2016).
Spormann et al. (2008) found that blackberry fruit juice rich in phenols and anthocyanins protects against cancer by reducing the nuclear factor-KB binding activity of lipid peroxidation and oxidative DNA damage in cells (Spormann et al., 2008). Tate et al. (2006) found that blackberry extracts protect from UV (ultraviolet) light. In the study, it was observed that plant extracts protected from UV-induced DNA damage and inhibit mutagenesis in *S. typhimurium* (Tate et al., 2006).

In our study, we exposed blackberry fruit extracts to UV during different time periods and identified their protective activity on DNA. Fruit extracts at a high concentration had protective activity on DNA after exposure to UV for a long time as shown in the bands in Figure 3b. Divya et al. (2015) investigated the protective effects of blackberry extracts against ultraviolet-B (UVB) radiation. The experiment was conducted on hairless mice exposed to UVB radiation. Findings revealed that blackberry extract suppressed UVB-induced hyperplasia on the skin and reduced inflammatory cell infiltration on the skin of hairless mice. In this study, it was found that blackberry extracts protect against oxidative stress and inflammation on the skin and
contribute to skin cancer prevention (Divya et al., 2015).

In our study, protective activity of blackberry extracts against UV even at low concentrations was observed as shown in Figure 3 and we recommend the use of these extracts as a natural UV protection in the cosmetic industry and especially in sunscreens.

REFERENCES


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