Erzincan Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
2021, 14(1), 314-321
ISSN: 1307-9085, e-ISSN: 2149-4584
Araştırma Makalesi

Erzincan University
Journal of Science and Technology
2021, 14(1), 314-321
DOI: 10.18185/erzifbed.804421
Research Article

Investigation of Antioxidant Activities and Hypoglycemic Effect of Black and White Myrtle Fruits

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Geliş / Received: 02/10/2020, Kabul / Accepted: 29/03/2021

Abstract

Fruits, are in black or white colour *Mrytus communis,* is known as locally name murt, hambales, mersin. It is an aromatic and medicinal plant that grows naturally in maquis form in regions with Mediterranean climate. In this research, we aimed to investigate the hypoglycemic effect and antioxidant activity of *Myrtus communis* L. White-black myrtle were collected from Hassa-Hatay and Tarsus-Mersin, respectively in October. Fruits were separated from the leaves and dried in the oven at 40ºC. Fruit extracts were obtained in absolute ethanol (1:15) by using magnetic stirrer at the room temperature for one day. In this study, radical scaving activity-(DPPH), the hydrogen peroxide scavenging activity-(HPSA), metal chelating activity-(MCA), ferric reducing antioxidant power activity-(FRAP), total phenol-flavonoid contents and α-amylase, α-glycosidase inhibition were examined to determine characteristics of samples. DPPH and HPSA values were expressed as µg/ml in terms of SC_{50}. FRAP and MCA features of extract were given as % activity. The hypoglycemic effect was investigated with α-amylase, α-glucosidase inhibition assays. The DPPH, HPSA, FRAP, MCA of white-black myrtle samples were found 56.75-63.52 µg/ml, 193.95-207.73 µg/ml, 81.68-90.87%, and 20.88-27.71%, respectively. When the antioxidant results compare with the used standards such as Buthylated-Hydroxy-Anisole, Rutin, Trolox, white-black myrtle samples have effective antioxidant activity. Samples have shown hypoglycemic effect.

Keywords: Antioxidant activities, Hypoglycemic effect, *Myrtus communis* L.

Siyah ve Beyaz Murtların Hipoglisemik Etkisi ve Antioksidan Aktivitesinin Araştırılması

Latincesi *Myrtus communis* L. olan halk arasında murt, hambeles, mersin diye adlandırılan meyve siyah veya beyaz renkte olmaktadır. Tıbbi ve aromatik bir bitki olan mersin, akdeniz ikliminin yaygın olduğu bölgelerde doğal olarak yetişir. Bu çalışmada, *Myrtus communis* L. nin antioksidan aktivitesini ve hipoglisemik etkisini belirlemek amaçlanmıştır.

Beyaz ve siyah mersin örnekleri sırayla Hassa-Hatay ve Tarsus-Mersin den hasat dönemi olan Ekim ayında toplanmıştır. Meyveler yapraklarından ayrılarak etüvde 40º C de kurutuldu. Kuru örnekler toz haline getirilerek, absolute etanol (1:15) ile 1 gün boyunca manyetik karıştırıcıda karıştırılıp, süzgeç kağıdı ile süzülerek örneklerin özütü elde edildi.

Bu çalışmada Radikal giderme aktivitesi (DPPH), Hidrojen peroksit giderme aktivitesi (HPSA), Demir indirgeme kapasitesi (FRAP) ve Metal şelat oluşturma aktivitesi (MŞA), Total feneol-flavonoid içerikleri ile hipoglisemik etki için α-amilaz, α-glikozidaz inhibisyonu araştırıldı. DPPH ve HPSA değerleri SC_{50} ile FRAP ve MŞA ise % olarak ifade edildi. Hipoglisemik etki ise α-amilaz, α-glikozidaz inhibisyonu ölçümü ile belirlendi. Beyaz ve siyah murt örneklerinde DPPH, HPSA, FRAP, MŞA değerleri sırasıyla 56.75-63.52 µg/ml, 193.95-207.73 µg/ml, 81.68-90.87%, 20.88-27.71% bulunmuştur. Antioksidan sonuçlar Bütilat Hidroksi Anisol (BHA), Rutin (RUT) ve Trolox (TRO) gibi standartlarla karşılaştırıldığında beyaz ve siyah murt örnekleri etkili bir antioksidan aktivite göstermiştir. Örnekler aynı zamanda etkili bir hipoglisemik etki göstermiştir.

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Anahtar Kelimeler: Antioksidan aktivite, Hipoglisemik etki, *Myrtus communis* L.

1. Introduction

Myrtus communis is scientific name of Myrtle which capaciously used as a medicinal plant and herbal medicine. At the same time, it is cultivated as an ornamental plant for use as a shrub in gardens and parks (Baharvand et al., 2015).
Phenolic substances are usually metabolites in different structures and functions, having an aromatic ring. Also, this aromatic ring contains one or more hydroxyl groups (Naczk and Shadidi, 2004). Polyphenols and flavonoids in plant show efficient antioxidant properties due to their high redox potentials (Tsao and Yang, 2003). Myrtle is traditionally consumed as an antidiabetic agent among the public (Sepici Dincel et al., 2004). It is indicated that myrtle leaf extract had antihyperglycemic and antibacterial properties in the literature. Recently, antioxidant properties of different myrtle extracts and certain ingredients have been described. In addition, Jahori et al., (2014) and Ferchichi et al., (2011) noted the use as a medicament for the treatment of diseases associated with oxidative stress, including diabetes mellitus.
α-amylase, which is the main enzyme, is responsible for the breakdown of starch into simpler sugars (Alexander, 1992; Davies and Henrissat, 1995). Although the activity of the enzyme was directly incorporated into the etiology of diabetes, it increased glucose tolerance in which α-amylase inhibitors were diabetic patients (Lebovit, 1998).
In the recent years, diabetes is a metabolic disease that is widely seen and reduces the quality of life. Studies on plants traditionally used among the people are carried out continuously. Particularly, it are carried out different studies of the plants used against diabetes (Durmuş et al., 2016; Karaman and Elgin Cebe, 2016).
The purpose of this research is to investigate the hypoglycemic effect and antioxidant activity of black and white myrtle.

2. Material and Methods

The samples used in this study were obtained from Hassa-Hatay and Tarsus-Mersin regions in Turkey. Myrtle samples were gathered in harvest period (October).

In this study, 2 different myrtle fruits were used as black myrtle, white myrtle. Samples were dried in drying oven at 40ºC, 96 hours. Dried samples were extracted with absolute ethanol by using a magnettic stirrer.

2.1. Antioxidant activities

2.1.1. Free radical scavenging activity assay (DPPH)

DPPH free radical scavenging activities of myrtle samples were carried out according to literature method (Blois, 1958). In this assay, 1.0 mL of DPPH$^{\bullet}$ (prepared in absolute ethanol as 0.1 mM) and 3.0 mL of sample solution was added and stirred. It was stand up at the room temperature in a dark setting during 30 min. Samples/standards absorbances were recorded at 517 nm. The obtained values were expressed as SC_{50} (μg/mL).

2.1.2. Hydrogen peroxide scavenging activity assay (HPSA)

The hydrogen peroxide scavenging activities were examined in reference to Ruch method (Ruch et al., 1989). In this assays, sample (3.4 mL) and 0.6 mL of H_2O_2 (prepared with pH=7.4 and 0.04 M phosphate buffer as 40

mM) was mixed during 10 min. The final mixtures' absorbance values was recorded at 230 nm. For this reason, phosphate buffer was used as a blank sample. The obtained results were stated as SC_{50} values (µg/mL).

2.1.3. Ferric reducing antioxidant power assay (FRAP)

Ferric reducing antioxidant activities were investigated by using Oyaizu method (Oyaizu, 1986). For this reason, 2.5 mL of PBS (pH 6.6, 0.2 mol L^{-1}) and 2.5 mL of potassium ferricyanide (1.0%) were added into samples/standards (2.5 mL) tubes, respectively. The final mix tubes were incubated at 50°C for 20 min and then 2.5 mL of TCA (10%) were mixed with the all test and standard tubes. 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%) were mixed with 2.5 mL of this solution. The FRAP was calculated with using the absorbance values at 700 nm following the formula:

$FRAP\ (\%) = (A_s/A_c) \times 100$

A_c: Absorbance of control, A_s: Absorbance of samples/standards

2.1.4. Metal chelating activity assay (MCA)

The metal chelating activities of the standards/samples antioxidant materials were determined by following the Dinis Method (Dinis et al., 1994). The sample/standard solutions (0.4 mL) mixed with $FeCl_2$ (0.05 mL, 2 mM). The mixtures were kept during 10 min. 0.2 mL of ferrozine (5 mM) and 3.3 mL of absolute ethanol were added to all test and standard tubes. The all tubes were forcefully shaken and the absorbance values of the samples were recorded at 562 nm. The MCA were obtained by using this formula:

$Ferrous\ Ions\ Chelating\ Activity\ (\%) = [1 - (A_s/A_c)] \times 100$

2.1.5. Total phenolic content (TPC)

The total phenolic content in the sample extract was investigated according to the Slinkard and Singleton by using Folin-Ciocalteu reagent (Silinkard and Singleton, 1977). 0.5 mL of standard/sample solution in ethyl alcohol (1 mg/mL) were mixed with 7.0 mL of deionized water. Then, 0.5 mL of Folin C reagent was added and all tubes were vortexed for 3 min. 2.0 mL of Na_2CO_3 (2.0%) was added and tubes were stand up at the room temperature in a dark environment during 2 hours. Absorbance values of tubes were recorded at 760 nm. TPC of the sample was calculated by using gallic acid calibration curve (R^2:0.9964).

2.1.6. Total flavonoid content (TFC)

Total flavonoid content of sample extract was carried out in reference to the literature method by using aluminium chloride (Chang et al., 2002). 0.5 mL of samples in ethyl alcohol (1 mg/mL) was combined with 1.5 mL of deionized water. Then, 0.1 mL of $A1C1_3 \cdot 6H_2O$ (10.0%), 0.1 mL of potassium acetate (1 mL) and 2.8 mL of deionized water were added, respectively. All tubes were waited at room temperature during 30 min. After this period, absorbance values were measured at 415 nm. TFC of the sample was calculated by using catechin calibration curve (R^2=0.9970).

2.2. Hypoglycemic effect

2.2.1. α-amylase inhibition assay

α-amylase inhibition assay as i*n vitro* was analyzed by following the Bernfeld method with minor modifications (Bernfeld, 1955; Guder, 2016). The starch (0.5 %) solution was obtained by boiling and blending potato starch (0.25 g) in deionized water (50 mL) for 15 min. The α-amylase (EC 3.2.1.1) enzyme (0.5 unit/mL) solution was procured by blending α-amylase (0.001 g) in phosphate buffer solution (PBS) (pH 6.9, 20 mM, 100 mL) containing NaCl (6.7 mM). Samples (5-100 µg/mL) and acarbose were dissolved at various concentrations in PBS. The solution of color reagent contains DNS (20 mL, 96 mM), sodium potassium tartrate (5.31 M, 8 mL) in NaOH (2.0 M) and distilled water (12

mL). 1 mL of samples (TAMC or acarbose) and 1.0 mL of enzyme solution were mixed and waited at 25°C for 30 min. 1.0 mL of starch solution was added with 1 mL of this mixture and incubated at 25°C during 3 min. After this period, 1.0 mL of color reagent was added. The closed tubes were stand up in water bath (at 85°C) during 15 min. The cooled reaction mixtures were diluted with distilled water (9.0 mL) and the absorbance value was recorded at 540 nm. IC_{50} values were used for expression of α-amylase inhibition activities. Linear regression analysis was used for the determination of IC_{50} values. For the blank tube, the color reagent solution was mixed with starch solution. This mixture was waited into the boiling water bath. The other procedures were performed as the test tube. Control tube was prepared with PBS (1.0 mL). Positive control (acarbose solution) was used for compared with the sample result.

2.2.2. α-glucosidase inhibition assay

A previously described bioassay method was used for measurement α-glucosidase inhibition of samples (McCue et al., 2005). The enzyme solution was prepared by using 20 µL of α-glucosidase (EC 3.2.1.20) enzyme (0.5 unit/mL) and 120 µL of PBS (pH 6.9, 0.1 M). *p*-nitrophenyl-α-D-glucopyranoside as substrate solution was used (5.0 mM in the PBS). 10 µL of acarbose and samples at different concentrations were dissolved in PBS (5–100 µg/mL). Enzyme solution was mixed with this solution and waited during 15 min at 37°C. End of the incubation period, 20 µL of substrate solution was added and kept for at 37°C during 15 min again. The reaction was stopped with addition of 80 µL of $NaCO_3$ solution (0.2 M). Enzyme inhibition values were measured via absorbance value at 405 nm. IC_{50} values were used for expression of α-glucosidase inhibition activities. Linear regression analysis was used for the determination of IC_{50} values.

3. Research Findings

Table 1. Antioxidant activities of Black and White Myrtle Samples and Standards

	Free Radical Scavenging Activity-DPPH (SC_{50})	HPSA (SC_{50})	FRAP (%)	Metal chelating activity-MCA (%)
White Myrtle	56.75	193.95	81.68	20.88
Black Myrtle	63.52	207.73	90.87	27.71
BHA	18.56	8.76	94.19	93.11
RUTIN	10.56	17.78	95.27	94.39
TROLOX	190.51	26.46	86.46	88.58

Table 2. Total Phenolic and Total Flavonoid Contents of Black and White Myrtle Samples

Samples	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg CAE/g)
White Myrtle	682.25	212.34
Black Myrtle	797.20	395.24

Table 3. Hypoglycemic effects (IC_{50}-µg mL^{-1}) of Black and White Myrtle Samples and Acarbose

Parameters	White Myrtle	Black Myrtle	Acarbose
α-Glucosidase Inhibition (IC_{50}-µg mL^{-1})	1603.61	1355.64	79.12
α-Amylase Inhibition (IC_{50}-µg mL^{-1})	784.92	571.84	94.89

4. Results

Ferric reducing antioxidant power, metal chelating activities, DPPH radical scavenging activities and hydrogen peroxide scavenging activities of standards and samples are found at the intervals of 10.56 – 190.51 µg/mL, 8.76 – 207.73 µg/mL, 81.68 – 95.27 % and 20.88 – 94.39 %, respectively (Table 1). According to obtained results, DPPH radical scavenging activities of samples are higher than Trolox. HPSA results of samples are not as effective as standards. FRAP activities of Black Myrtle are more efficient than Trolox. Metal chelating activities of samples are not as well as standards. We can say that black myrtle exhibits more effective antioxidant activity than white myrtle. On the other hand, comparing DPPH radical scavenging activity and FRAP, black myrtle showed more activity than trolox. Also, BHA and RUT have a higher value than the standard in all activities examined. Methanol extract of myrtle fruit supplied from Mediterranean region of Turkey has the influential of radical scavenging and antioxidant activity (Asif et al., 2011). Radical scavenging activity of the essential oil of myrtle flowers from Morocco was the highest level at 200 µg/ml of concentration. This essential oil containing mainly monoterpene hydrocarbons and their derivatives are known to possess good antioxidant activity (Derwick et al., 2011). Moreover, the essential oil obtained from *M. communis* leaves has the effective DPPH radical scavenging activity (Mimika-Dukic et al., 2010). Trolox equivalent antioxidant capacity analyzes indicated that the black *M. communis* berries has the high radical scavenging activities. This effective activity may be due to its rich flavonoids and anthocyanin content (Montoro et al., 2006).

Total flavonoid and phenolic contents of samples were stated as mg GAE/g and mg CAE/g, respectively. In reference to obtained results, black myrtle sample has exhibited higher total phenolic and flavonoid contents than white myrtle sample (Table 2). Antioxidant activities of plant extracts are related to their phenolic content (Wang et al., 1999). Flavonoids and anthocyanins are essential components in the myrtle fruits and extracts. The phenolic content and essential oil of some species such as *P. lentiscus* L. and *M. communis* L. in Zakynthos (Greece) were determined. The effective antioxidant properties of both plants indicate that they are rich in phenolic contents (Chyssavgi et al., 2008).

In vitro hipoglycemic effect were applied for determination samples α-glucosidase and α-amylase inhibition activity assays. Acarbose was used as control. White and black myrtle samples show lower hypoglycemic effect than acorbose. α-glucosidase and α-amylase inhibition of black myrtle is more effective than white myrtle (Table 3). Antioxidants are important to biologists and clinicians due to their protective effects against human body damage (Asparganah and Ariamanesh, 2015). Anti-hyperglycemic effect of *M. communis* leaves extract had been reported on diabetic mice (Benkhayal et al., 2009). *In vitro* α-glucosidase effect of some sample extracts such as *Taraxacum officinale, Urtica dioica, Myrtus communis* and *Viscum album*, and some synthetic inhibitors such as Amaryl (glimerid), Betanorm (gliclazide) and Glucobay (acorbose) was investigated by Önal et al. (2005). They found that *Myrtus communis* showed more effective inhibitory activity (Önal et al., 2005). Myrtle fruits were extracted and given to experimental animals in different doses. It was observed that different doses of extracts decreased blood glucose levels (Demir et al., 2011). Similarly, in a study conducted on rats, it was reported

that murt leaf extraction decreased blood glucose levels (Baz, 2014). In another study, the effect of mrytle oil on diabetic rabbits was studied and its has proven to be effective on blood glucose levels (Sepici Dincel et al., 2007). In a study in animals with experimentally created diabetes, the ethanolic, aqueous and hydroalcoholic extracts of myrtle leaf were presented to investigate effects on its blood glucose level. According to this research, myrtle leave extracts have been found to reduce blood glucose levels (Panjeshahin et al., 2016).

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