International Journal of Academic Multidisciplinary Research (IJAMR)
ISSN: 2643-9670
Vol. 4 Issue 12, December - 2020, Pages: 89-93

In vitro Antimicrobial and Antioxidant Activities of *Pyracantha coccinea* Fruits Ethanol Extract

Dilay TURU[1*], Mustafa Eray BOZYEL[2], Kamil CANDAN[1], Mustafa Ali YAKAN[1], Atakan BENEK[3], Kerem CANLI[1]

[1]Department of Biology, Faculty of Science, Dokuz Eylül University, Buca, İzmir, Turkey
[2]Department of Biology, Faculty of Arts and Science, Çanakkale Onsekiz Mart University, Çanakkale, Turkey
[3]Department of Biology, Faculty of Arts and Science, Kastamonu University, Kastamonu, Turkey
***Corresponding author: dilayturu@gmail.com**

Abstract*: This study aims to observe the antimicrobial and antioxidant activities of ethanol extract from the fruits of Pyracantha coccinea. Antimicrobial activity was tested against 45 bacteria and 3 yeasts by disk diffusion method and antioxidant activity was tested by the DPPH method. The results of this study showed that ethanol extract of fruits exhibited antimicrobial activity against various strains tested, especially against Gram-positive bacteria; Bacillus cereus, Enterococcus faecium, Staphylococcus hominis, Staphylococcus warneri, and Staphylococcus aureus.*

Keywords: *Pyracantha coccinea*, Disk diffusion method, Antimicrobial activity, DPPH, Antioxidant activity.

1. INTRODUCTION

The World Health Organization (WHO) predicts that one of the biggest public health threats of the twenty-first century will be increased antimicrobial resistance and one of the ways to prevent the antibiotic resistance of microorganisms is to use new compounds [1]. Despite the progress in synthetic chemistry, plants are still the source of compounds of important pharmaceutical and economic importance [2]. And today, with modern technology, the use of old plants is increasing, modernized, and used as medicine for humans. The use of drugs produced by biotechnological drug technology in Turkey has gained momentum and importance as it has all over the world [3].

For centuries, plants have been used in the treatment of various diseases [4]. And today, plants are the most important source of traditional medicine. For this reason, people living in many parts of the world have benefited from the plants grown in their surroundings [5]. 80% of the drugs used in the developed countries are of herbal origin [6, 7]. Scientists are investigating the medicinal uses of herbs to discover new substances that can be used to treat diseases [8].

Pyracantha coccinea M.Roem., also known as the firethorn (ateş dikeni), which belongs to the family Rosaceae, is a prickly perennial shrub about 3 m long, with its young branches having a greyish-hairy structure. The leaves are lanceolate or elliptic in shape, the underside of which is hairy or completely glabrous when young. April to June is a multi-flowering shrub, and its flowers are 8 mm in diameter. Its fruit is 5-7 mm in diameter, spherical in shape, red in color, sometimes yellow, and orange in color. It is widely found in Northern, Central, and Southern Anatolia in Turkey, and in southern Europe, Crimea, Caucasus, and northwestern Iran in the world. It grows wild in Turkey, especially in the Black Sea region. It is one of the most used ornamental fruit plants in garden decoration and landscape in the world [9, 10]. It is used as a medicinal plant in traditional medicine due to its diuretic, cardiac and strength-giving properties [11, 12].

In this study, the antimicrobial and antioxidant activity of the fruits of *P. coccinea* was investigated.

2. MATERIALS AND METHODS

Plant sample: *P. coccinea* fruits were collected from the Buca Campus of Dokuz Eylül University in Izmir, Turkey, and identified by Dr. Mustafa Eray Bozyel. The fruits were harvested in September and then left to air-dry.

Microbial strains: There are 16 standard, 7 food isolated, 11 clinical isolated, and 11 multidrug-resistant bacteria species, and 1 standard and 2 clinical isolated yeast species were used. 8 of them are standard Gram-positive bacteria, which are *Bacillus subtilis* DSMZ 1971, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044, *Staphylococcus hominis* ATCC 27844, *Staphylococcus warneri* ATCC 27836, and *Bacillus cereus* RSKK 863. The others are standard Gram-negative bacteria, which are *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSMZ 50071, *Pseudomonas fluorescens* P1, *Salmonella enteritidis* ATCC 13075, *Salmonella typhimurium* SL1344, *Shigella flexneri* RSKK 184, and *Acinetobacter baumannii* CECT 9111. 7 of them non-standard bacteria, which are isolated from food. Three of them are Gram-positive bacteria, which are *Enterococcus durans*, *Enterococcus faecium*, and *Listeria innocua*. The others are Gram-negative bacteria, which are *Klebsiella pneumonia*, *Salmonella infantis*, *Salmonella kentucky*, and *Escherichia coli*. 11 of them are clinical isolated. 7 of them are Gram-positive bacteria, which are *Staphylococcus mutans*, *Streptococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus lugdunensis*, *Enterococcus faecalis*, and 2 strains *Staphylococcus aureus*. The others are clinical isolated Gram-negative bacteria,

which are *Shigella flexneri*, *Shigella boydi*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*. 11 of them are multidrug-resistant bacteria. 3 of them are Gram-positive bacteria, which are *Streptococcus pneumoniae*, *Staphylococcus aureus* MRSA and *Staphylococcus aureus* MRSA + MDR. Eight of them are Gram-positive bacteria, which are *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Serratia odorifera*, *Proteus vulgaris*, *Providencia rustigianii*, and *Achromobacter* sp. The standard yeast is *Candida albicans* DSMZ 1386, and clinical isolated yeasts are *Candida glabrata* and *Candida tropicalis*.

Microorganism inocula: Conditions for correct incubation; it is 24 hours at 37 ^{o}C for bacteria and 48 hours at 27 ^{o}C for yeasts. Sterile saline solution was prepared for each microorganism and all adjusted to the 0.5 McFarland standard [13].

The extraction procedure: *P. coccinea* fruit samples were dried after collection and the samples were ground by a grinder. Ethanol (Sigma-Aldrich) was preferred as the extraction solvent to transfer all substances into the extract. After adding the milled plant sample into ethanol, it was shaken in a shaker at 160 rpm for 2 days [14]. The extraction process was followed by filtration of the extract into an evaporation flask through filter paper (Whatman No.1), and ethanol was removed at 30°C with a rotary evaporator (Buchi R3) [15]. Finally, the residues were collected and used to prepare the extract.

Antimicrobial activity test: Disk diffusion test, which is a widely used test, was chosen to determine antimicrobial activity [16]. The extract was loaded onto 6 mm Oxoid Antimicrobial Susceptibility Test Disks in three doses of 50, 100, and 200 µl. The discs were allowed to dry for 8 hours at 30°C under sterile conditions to remove the ethanol from the extract. Autoclaved sterilized Mueller-Hinton Agar (BD Difco, USA) was poured into 90 mm sterile petri dishes at an average depth of 4.0 mm (± 0.5 mm). Microorganism suspensions were spread on the surface of the Petri dishes [17]. In the biosafety cabinet, petri dishes with microorganism suspension spread on it were allowed to dry for 5 minutes before the discs were placed, and before 10 minutes the discs were placed on the agar [18]. At the end of the test, petri dishes were incubated and inhibition zone diameters were measured.

Controls: Sterile discs loaded with ethanol were used as a negative control in the study. The ethanol loaded discs were dried under aseptic conditions to remove ethanol from the disc.

Antioxidant activity by DPPH radical scavenging method: It is a method based on measuring the scavenging effects of the extract's antioxidants on DPPH radical. To prepare DPPH, 3.9432 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) was weighed in the precision balance and added into 50 mL of ethanol [19]. The outside of the glass bottle is covered with aluminum foil to protect the DPPH solution from light. The extract was mixed with DPPH solution and incubated at room temperature for 30 minutes in the dark. At the end of the incubation, the absorbance of the samples was measured at 515 nm with a spectrophotometer (Biotek Microplate Spectrophotometer, ABD). Ascorbic acid was used as a control [20].

Statistics: All tests were applied as triplicates. One-way analysis of variance (ANOVA), which is a parametric method was performed (P=0.05) [21]. Pearson correlation coefficient was determined for any possible correlation between the intensity of antimicrobial activity and concentration. R Studio, version 3.3.2 was used for statistical analysis [22].

3. RESULTS AND DISCUSSION

The antimicrobial activity of ethanol extract from the fruits of *P. coccinea* was analyzed. To load extracts, empty sterile disks were used. These disks were applied on a Mueller-Hinton Agar after they were inoculated with microorganisms. An inhibition zone was observed when the extracts had activity against these microorganisms. The diameters of these zones were measured in millimeters as Table 1.

Table 1. Disk diffusion test results for *P. coccinea* (Inhibition zones in mm).

Microorganisms	50µL	100µL	200µL
B. subtilis DSMZ 1971	-	-	-
C. albicans DSMZ 1386	-	7	8
E. aerogenes ATCC 13048	-	-	-
E. faecalis ATCC 29212	-	-	7
E. coli ATCC 25922	-	-	-
L. monocytogenes ATCC 7644	-	-	-
P. aeruginosa DSMZ 50071	7	8	9
P. fluorescens P1	-	-	7
S. enteritidis ATCC 13076	-	-	-

S. typhimurium SL 1344	-	-	-
S. aureus ATCC 25923	7	8	9
S. epidermidis DSMZ 20044	-	-	8
S. hominis ATCC 27844	7	8	10
S. warneri ATCC 27836	7	8	10
B. cereus RSKK 863	7	8	10
S. flexneri RSKK 184	-	-	8
A. baumannii CECT 925	-	-	7
E. durans (FI)	-	-	-
E. faecium (FI)	-	9	10
K. pneumoniae (FI)	-	-	-
L. innocua (FI)	-	-	8
S. infantis (FI)	-	-	-
S. kentucky (FI)	-	-	-
E. coli (FI)	-	-	8
S. aureus (CI)	-	7	8
S. mutans (CI)	-	-	8
S. hominis (CI)	-	-	7
S. haemolyticus (CI)	-	-	7
S. lugdunensis (CI)	-	-	-
S. boydi (CI)	-	-	-
A. baumannii (CI)	7	7	7
S. felxneri (CI)	-	-	-
S. aureus (CI-2)	-	-	-
E. faecalis (CI)	-	-	-
K. pneumoniae (CI)	-	-	-
C. tropicalis (CI)	-	-	-
C. glabrata (CI)	-	-	-
E. coli (MDR)	-	-	-
K. pneumoniae (MDR)	-	-	-
A. baumannii (MDR)	-	-	-
E. aerogenes (MDR)	-	-	-
S. odorifera (MDR)	-	-	-
P. vulgaris (MDR)	-	-	-
S. pneumoniae (MDR)	-	-	-
S. aureus (MRSA)	-	-	7
S. aureus (MRSA+MDR)	-	-	8
P. rustigianii (MDR)	-	-	-
Achromobacter sp. (MDR)	-	8	9

"-": No inhibition, CI: Clinic isolated, FI: Food isolated, MDR: Multidrug resistant

The antioxidant activity of ethanol extract from the fruits of *P. coccinea* was analyzed by the DPPH radical scavenging method. Concentrations of the extract were set from 1,075µg/mL to 200µg/mL. The results of the DPPH radical cleaning activity of *P. coccinea* and ascorbic acid are shown in Table 2.

Table 2. DPPH radical scavenging activity results of *P. coccinea* and Ascorbic acid (%)

Concentrations (µg/mL)	*P. coccinea* (%)	Ascorbic acid (%)
200,000	91,432	94,665
100,000	94,715	93,391
50,000	95,169	92,077
25,000	94,953	90,086
12,500	93,479	69,943
6,250	92,582	35,794
3,125	88,056	17,698
1,075	73,578	8,739

In our study, *P. coccinea* fruits ethanol extract antimicrobial activity was determined against 48 microorganisms with disc diffusion method at 50µL, 100 µL, and 200 µL. According to our results, *P. coccinea* has antimicrobial activity against 22 of them. Most of these are Gram-positive microorganisms such as *B. cereus*, *E. faecium*, *S. hominis*, *S. warneri*, and *S. aureus*.

S. aureus is known as one of the common nosocomial infections in medical intensive care units [23]. Several researchers study the antimicrobial activity of some plant extracts on *S. aureus* strains. In our study, we observed a 9 mm zone against the standard, 8 mm zone against MRSA+MDR and clinic isolated, and a 7 mm zone against *S. aureus* MDR strains. *P. coccinea* is active against *S. aureus* when compared to some other higher plants [24].

In this study, ethanol extract showed high DPPH radical scavenging activity compared to ascorbic acid up to a concentration of 100µg/mL and low DPPH radical scavenging activity compared to ascorbic acid at a concentration of 200µg/mL.

In another study, it has been found that the DPPH scavenging activity of *P. coccinea* fruits ethanol extract in the concentration level of 250µg/mL is 78,73% [25]. According to our results, *P. coccinea* appears to have high DPPH radical scavenging activity.

4. CONCLUSION

Our study makes it clear that *P. coccinea* has the potential for a possible medical drug candidate. Especially it has antimicrobial activity against *S. aureus* strains and also has a big antioxidant potential against DPPH radical. However, further researches are needed to analyze the active substances and their activity mechanisms in detail. Data obtained as a result of studies using biotechnological drug technologies can reveal the true potential of this plant.

5. REFERENCES

[1] Canlı, K., Yetgin, A., Benek, A., Bozyel, M. E, & Altuner, E. M. (2019). In vitro antimicrobial activity screening of ethanol extract of Lavandula stoechas and investigation of its biochemical composition. *Advances in Pharmacological Sciences, 2019*, Article ID 3201458, 1-6.

[2] Zhang, W. J., & Björn, L. O. (2009). The effect of ultraviolet radiation on the accumulation of medicinal compounds in plants. Fitoterapia, 80(4), 207-218.

[3] Dundar, M., & Akbarova, Y. (2011). Current state of Biotechnology in Turkey. Current opinion in biotechnology, 22, S3-S6.

[4] Jones, F. A. (1996). Herbs–useful plants. Their role in history and today. European journal of gastroenterology & hepatology, 8(12), 1227-1231.

[5] Bozyel, M. E., & Merdamert-Bozyel, E. (2020). Ethnomedicinal Uses of Genus Lavandula (Lamiaceae) in Turkish Traditional Medicine. International Journal of Academic and Applied Research (IJAAR), 4 (2), 5-16.

[6] Baytop, T. (1999). Türkiye'de bitkiler ile tedavi: geçmişte ve bugün. Nobel Tıp Kitabevleri.

[7] Keles, O., Ak, S., Bakırel, T., & Alpınar, K. (2001). Türkiye'de yetişen bazı bitkilerin antibakteriyel etkisinin incelenmesi. Turkish Journal of Veterinary and Animal Sciences, 25(4), 559-565.

[8] Rajakaruna, N., Harris, C. S., & Towers, G. H. N. (2002). Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka. Pharmaceutical Biology, 40(3), 235-244.

[9] Akguc, N., Ozyigit, I., Yasar, U., Leblebici, Z., & Yarci, C. (2010). Use of Pyracantha coccinea Roem. as a possible biomonitor for the selected heavy metals. International Journal of Environmental Science & Technology, 7(3), 427-434.

[10] Akar, T., Anilan, B., Gorgulu, A., & Akar, S. T. (2009). Assessment of cationic dye biosorption characteristics of untreated and non-conventional biomass: Pyracantha coccinea berries. Journal of Hazardous Materials, 168(2-3), 1302-1309.

[11] Fico, G., Bilia, A. R., Morelli, I., & Tomè, F. (2000). Flavonoid distribution in Pyracantha coccinea plants at different growth phases. Biochemical Systematics and Ecology, 28(7), 673-678.

[12] Akar, T., Celik, S., & Akar, S. T. (2010). Biosorption performance of surface modified biomass obtained from Pyracantha coccinea for the decolorization of dye contaminated solutions. Chemical Engineering Journal, 160(2), 466-472.

[13] Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. Journal of applied microbiology, 86(6), 985-990.

[14] Altuner, E. M., Canli, K., & Akata, I. (2014). Antimicrobial screening of Calliergonella cuspidata, Dicranum polysetum and Hypnum cupressiforme. Journal of Pure and Applied Microbiology, 8(1), 539-545.

[15] Canlı, K., Yetgin, A., Akata, I., & Altuner, E. M. (2016). In vitro antimicrobial screening of Aquilaria agallocha roots. African Journal of Traditional, Complementary and Alternative Medicines, 13(5), 178-181.

[16] Andrews, J. M. (2007). BSAC standardized disc susceptibility testing method (version 6). Journal of Antimicrobial Chemotherapy, 60(1), 20-41.

[17] Silici, S., & Koc, A. N. (2006). Comparative study of in vitro methods to analyse the antifungal activity of propolis against yeasts isolated from patients with superficial mycoses. Letters in applied microbiology, 43(3), 318-324.

[18] Benek, A., (2020). Bazı karayosunlarının antimikrobiyal, antioksidan ve antibiyofilm aktivitelerinin belirlenmesi. Unpublished MSc Thesis. Kastamonu University, Turkey.

[19] Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C. D., Coube, C. S., & Leitão, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy research, 15(2), 127-130.

[20] Tunç, K., Semerci, A. B., & Okur, İ. (2020). Antioxidant activity of the fruits of Pyracantha coccinea using ethanolic extract method. Food and Health, 6(1), 35-40.

[21] Canli, K., Yetgin, A., Akata, I., & Altuner, E.M. (2017). Antimicrobial Activity and Chemical Composition Screening of Epilobium montanum Root. Indian Journal of Pharmaceutical Education and Research. 51(3s), 239-243.

[22] Core R Team (2016). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

[23] Richards, M. J., Edwards, J. R., Culver, D. H., & Gaynes, R. P. (1999). Nosocomial infections in medical intensive care units in the United States. Critical care medicine, 27(5), 887-892.

[24] Nair, R., & Chanda, S. (2007). Antibacterial activities of some medicinal plants of the western region of India. Turkish Journal of Biology, 31(4), 231-236.

[25] Keser, S. (2014). Antiradical activities and phytochemical compounds of firethorn (Pyracantha coccinea) fruit extracts. Natural Product Research, 28(20), 1789-1794.