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ORIGINAL ARTICLE / ÖZGÜN MAKALE

COMPARISON OF SOME BIOLOGICAL ACTIVITIES AND CATECHIN TANNIN CONTENTS OF TWO *JUNIPERUS* AND *PRUNUS* SPECIES

İKİ JUNİPERUS VE PRUNUS TÜRÜNÜN BAZI BİYOLOJİK AKTİVİTELERİNİN VE KATEŞİN TANEN İÇERİKLERİNİN KARŞILAŞTIRILMASI

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ABSTRACT

Objective: *Qualitative and quantitative determination of catechin in fruits of J. communis var. saxatilis, J. oxycedrus subsp. oxycedrus, P. spinosa, and P. cerasifera was analyzed by LC-MS/MS using standards. Antidiabetic, antimicrobial, and antioxidant activities of fruit and cone extracts of these plants were evaluated. Qualitative analysis of secondary metabolites was also done.*

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Material and Method: *Antimicrobial activity was done with MIC. $ABTS^{\cdot+}$ and DPPH• scavenging activies were used antioxidant activity. α-Amylase and α-glucosidase inhibition assays were done for antidiabetic activity.*
Result and Discussion: *JCS (IC_{50} = 578 ± 20 µg/ml), JOO (IC_{50} = 3706 ± 52 µg/ml), and PS (IC_{50} = 313 ± 7 µg/ml) extracts were observed to show a higher activity than acarbose (IC_{50} = 4061 ± 146 µg/ml). J. communis var. saxatilis fruit extract was observed with MIC values between (312.5 - 2500 µg/ml) against all microorganisms. JCS extract has the highest phenolic composition and JOO has the lowest phenolic composition (µg GAE/ mg extract). JCS extract indicated the highest antioxidant activity. It was determined that plant containing the highest catechin (1173.3±5.77 ng/ml) and epigallocatechin (208±7.21 ng/ml) was JCS. In this research, it can be said that JCS with the highest tannin content shows the highest effects.*
Keywords: *Antidiabetic, antimicrobial, catechic, Juniperus, Prunus*

ÖZ

Amaç: *J. communis var. saxatilis, J. oxycedrus subsp. oxycedrus, P. spinosa, ve P. cerasifera meyvelerinde standartlar kullanılarak LC-MS/MS ile analiz edilmiştir. Bitkilerin meyve ve kozalak ekstrelerinin antidiyabetik, antimikrobiyal ve antioksidan aktiviteleri değerlendirilmiştir. Sekonder metabolitlerin kalitatif analizi de yapılmıştır.*
Gereç ve Yöntem: *MIC ile antimikrobiyal aktivite yapıldı. $ABTS^{\cdot+}$ ve DPPH• süpürücü testler antioksidan aktiviteler için kullanılmıştır. α-Amilaz ve α-glukosidaz inhibisyon testleri antidiyabetik aktivite için kullanılmıştır.*
Sonuç ve Tartışma: *JCS (IC_{50} = 578 ± 20 µg/ml), JOO (IC_{50} = 3706 ± 52 µg/ml) ve PS (IC_{50} = 313 ± 7 µg/ml) ekstraktlarının akarbozdan (IC_{50} = 4061 ± 146 µg/ml) daha yüksek aktivite gösterdiği gözlemlenmiştir. Juniperus communis var. saxatilis meyve ekstresi tüm mikroorganizmalara karşı (312.5 -2500 µg/ml) arasında MİK değerleri göstermiştir. JCS en yüksek fenolik bileşime ve JOO en düşük fenolik bileşime sahiptir (µg GAE/ mg ekstrakt). JCS ekstresi en yüksek antioksidan aktiviteyi göstermiştir. En yüksek kateşin (1173.3±5.77 ng/ml) ve epigallokateşin (208±7.21 ng/ml) içeren bitki JCS'dir. Bu araştırmada en yüksek tanen içeriğine sahip JCS'nin en yüksek etkileri gösterdiği söylenebilir.*
Anahtar Kelimeler: *Antidiyabetik, antimikrobiyal, kateşik, Juniperus, Prunus*

INTRODUCTION

Type 2 diabetes mellitus (DM) epidemic is a substantial world health burden. It has been indicated that plant extracts can target the pathophysiology underlying sicks and get numerous mechanisms of action owing to the synergistic effects produced by the combinations of phytochemicals [1]. DM is in conjunction with diversified types of infections, primarily mucous membrane, soft tissue, skin, respiratory tract, urinary tract, and surgical and/or hospital-related infections. There are certain microbes related to each type of infection, and their existence demonstrates certain types of infection. For example, *E. coli* and *Klebsiella* are the most widespread initiative pathogens liable for the improvement of urinary tract infections. Diabetic foot infections (DFI) are usually seen in diabetic patients [2]. DFI are more serious and harder to cure than non-diabetic [3]. Diabetic wound healing is a great difficulty owing to its defenselessness to bacterial infection, as well as less vascularization and extended inflammatory period [4]. Increased oxidative stress in patients with DM and poor glycemic control or insulin resistance is most probably the effect of abnormal metabolisms like hyperglycemia, dyslipidemia, and high free fatty acids levels [5]. Various investigations have shown an important reduction in plasma antioxidants like retinol, lutein, α- and γ-tocopherol, ascorbic acid lycopene, β- and α-carotene, zeaxanthin, and β-cryptoxanthin during diabetes. Therefore, the justification for the therapeutical usage of antioxidants in the therapy and preservation of diabetic complications is powerful [6]. Tannins are potent antioxidants and have been recognized as being antidiabetic, anticarcinogenic, antiviral, antihypercholesterolemic, antiinflammatory, antibacterial, antimutagenic, and cardioprotective [7-8]. Catechin, gallic acid, epicatechin, and ellagic acid, proanthocyanidin are well-known tannins and have been observed to reduce the overexpression of TGF-β, IL-6, AMPK, PARP, and NF-kβ which are the main targets involved in the progression of DM [8]. The members of the *Juniperus* L. genus are a well-known resource of cedarwood oil is commonly distributed in the northern

hemisphere and is utilized in public medicine. *J. communis* L. (Cupressaceae) is a shrub or small evergreen tree [9]. *J. oxycedrus* L. is a species of plant in the Cupressaceae family, and it is one of ten species within the *Juniperus* genus. It is found throughout the Mediterranean region and can grow as both a shrub or tree. It has been traditionally used to treat a variety of ailments such as hyperglycemia, obesity, bronchitis, tuberculosis, and pneumonia [10]. The *Prunus* L. genus includes many economically important species whose fruits are commonly eaten fresh, frozen, or processed. Traditional medicine often uses these species to treat diabetes [11]. *P. spinosa* L., also known as "blackthorn or sloe," is a deciduous, dense shrub that can grow up to 4 meters tall. It is native to western Asia, northwestern Africa, and Europe and often grows wild in uncultivated regions near roads and canals. The stems of the plant are covered with spines [11-12]. *P. divaricata* Ledeb. (Rosaceae) is a wild plum and extensively grows from the Balkan Peninsula to Anatolia and the Caucasus to Central Asia, containing the Hyrcanian forests in the northern areas of Iran [13]. In an ethnobotanical research, it was seen that the fruits of *J. oxycedrus, P. spinosa,* and *P. divaricata* species are used as antidiabetics in folk medicine [14]. Tannins are polar compounds in polyphenolic structures found in many higher plants. Due to their pharmacological effects, they have been the subject of much research in the fields of food, medicine and medicine. This scope of work; qualitative and quantitative determination of catechin in the fruits of *J. communis* var. *saxatilis* (JCS), *J. oxycedrus* subsp. *oxycedrus* (JOO), *P. spinosa.* (PS), and *P. cerasifera* (PC) was analyzed by LC-MS/MS using standards (+)-catechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin, (-)-and epigallocatechin gallate. Additionally, the 70% methanolic extracts of the fruits and cones of these plants were analyzed for their ability to inhibit α-glucosidase and α-amylase enzymes, as well as for their antimicrobial activity by determining the minimum inhibitory concentration (MIC). The extracts were also tested for their antioxidant activity using the DPPH and ABTS methods. Furthermore, a qualitative analysis of secondary metabolites in these plant species was conducted.

MATERIAL AND METHOD

Plant Materials

JCS, JOO, PS, and PC were previously collected and data about the used parts, places and times of collection of are presented in Table 1. Collection and identification of plants were made by Mehmet Önal (Chief Engineer of the Eastern Anatolia Forestry Research Institute).

Figure 1. The photos of JCS, JOO, PS, and PC by Mehmet Önal.

Table 1. Used parts, places and collection times of JCS, JOO, PS, and PC.

Species	Used part	Places of collection	Times of collection
JCS	Cone	Erzurum/Aziziye/Rizekent Village	14.09.2015
JOO	Cone	Erzurum/Olur/Ormanağzı Village	04.05.2014
PS	Fruit	Erzurum/İspir/Özler Village	02.11.2014
PC	Fruit	Erzurum/Olur/Ilıkaynak Village	06.10.2014

Extraction

Collected fruits and cones were dried in a sun-free environment, away from moisture, by providing appropriate air circulation. Since tannins are polar substances and dissolve in water, 70% methanol was chosen as extraction solvent. After fruits were powdered, 50 grams of each were weighed and transferred to balloon and left to macerate overnight with 70% methanol. Then, it was extracted 3 times for 3 hours at 40°C utilizing a jacketed heater and a reflux cooler. Extracts obtained as a result of each extraction process were filtered and the filtrates were combined. It was concentrated to dryness in a rotavapor at 40°C, 120 rpm.

α-Amylase Inhibition Assay

Inhibitory activity on α-amylase was demonstrated by Nampoothiri et al. (2011) [15] in various modifications [16]. Entire extracts and 1% starch solution in 20 mM sodium phosphate buffer (pH 6.9 including 6 mM sodium chloride) were incubated at 25°C for 10 minutes in a 24-well microplate. Afterwards, incubation 100 µL α-amylase solution (0.5 mg/ml) was annexed and reaction admixtures were incubated at 25°C for 10 minutes. The dinitrosalicylic acid was annexed as a colour reagent. Microplate was heated for 5 minutes and cooled to 25°C. Absorbance at 540 nm was registered.

α-Glucosidase Inhibition Assay

Inhibitory activity on α-glucosidase was established with respect to method of Bachhawat et al. (2011), [17] within several modifications [16]. Entire extracts (20 µL), an enzyme solution (10 µL, 1 U/ml), and potassium phosphate buffer (50 µL, 50 mM, pH 6.9) were admixed in a 96-well plate and incubated at 37°C for 5 minutes. Substrate, p-nitrophenyl-α-D-glucopyranoside, was annexed to each well, also 0.1 M sodium carbonate was annexed. Sum of p-nitrophenol was registered utilising a 96-well microplate reader at 405 nm.

Antimicrobial Activity

Staphylococcus aureus ATCC 6538, *Escherichia coli* ATCC 8739, *Klebsiella aerogenes* ATCC 13048, *Salmonella enterica* ATCC 14028, *Candida albicans* ATCC 10231, *Bacillus subtilis* ATCC 19659, *Pseudomonas aeruginosa* ATCC 9027, and *C. parapsilosis* ATCC 22019 were taken from Microbiologics (San Diego, CA), and used antimicrobial acitivity. Extracts of fruit were studied with a concentration range (5000 to 78.12 µg/ml) and diluted 2-fold initially. Ampicilline and terbinafine (64–0.125 µg/ml) were used as standard drugs and prepared within water and dimethyl sulfoxide. Activity was determined using a slight modification of microdilution methods for aerobic microorganisms (M-7-A7) and fungi (M-27-A3) reported by Clinical Laboratory Standards Institute (CLSI). Standard cultures were kept at -85°C. After incubation, it was got from single colonies that improved on growth medium and transferred to tubes with Mueller Hinton Broth (MHB) (RPMI medium for *Candida* species) and incubated again at 37°C for 24 hours. Afterwards 18-24 hours of incubation, cultures were prepared accordingly McFarland No: 0.5 tube (10^8 cfu/ml for bacteria, 10^6 cfu/ml for yeast culture).

Antioxidant Activity and Total Phenolic Content

Many methods have been developed for the determination of natural compounds in foods, medicinal plants, or biological systems antioxidant activity. However, the most popular ones are ABTS and DPPH radical scavenging capacity assays. Both methods are based on colorimetric measurements of the response of stable radicals to antioxidant activity. The reasons for the popularity of ABTS and DPPH radical scavenging capacity tests can be listed as the stability of the radicals used, ease of measurement, short analysis times, reproducibility of analyzes, and inexpensive spectrophotometric methods. The radicals used in the experiments are readily available commercially.

$ABTS^{\cdot+}$ Scavenging Activity

It was established with respect to method of [18] with slight modifications [19-20]. Prepared 2.45 mM potassium persulfate solution was added to ABTS solution and $ABTS^{\bullet+}$ was obtained. Trolox and *α*-tocopherol were utilised as reference antioxidants. All samples were prepared at diversified

concentrations of extracts. Activity of each specimen was detected via finding color of $ABTS^{\bullet+}$ radical and evaluating reduction in absorbance at 734 nm towards a blank of phosphate buffer. Whole evaluations were established in triplicate.

Total Phenolic Content

It was comparatively evaluated utilising method improved by Folin and Denis and modified by Singleton [21-22] with slight modifications [19-20]. Absorbances read at end of experiment were plotted against concentration. In standard graph, equation is $0.0016x + 0.0105$; r^2 was found to be 0.9977. Stock solutions were designed at a concentration of 1 mg/ml. Whole solutions were administered with FCR and aqueous Na_2CO_3. Absorbance was recorded at 760 nm. Findings are demonstrated in gallic acid equivalents (GAE) and micrograms. Whole evaluations were carried out in triplicate.

$DPPH^{\bullet}$ Scavenging Activity

It was established with respect to method of Blois et al. (2011) [23], with several modifications [19]. 1 mM $DPPH^{\bullet}$ solution was utilised. Trolox and *α*-tocopherol were utilised as reference antioxidants. Standard antioxidants were studied in concentration range of 1-100 µg/ml. 210 µL of stock solutions of extracts and standards at diversified concentrations and 70 µL of $DPPH^{\bullet}$ solution were added to 96 well plate. All measurements were performed in triplicate for all samples.

Analytical Procedure

After method was developed on pure substances, all standard substances were analyzed simultaneously on extracts prepared from fruits and cones of 4 plants belonging to *Prunus* and *Juniperus* genera. All analytical experiments were carried out using below systems:

LC: Spark Holland-SPH1240; MS/MS: AB SCIEX-4000 Q TRAP; Column: Intersil-ODS-3 (5µm, 4.0mm x 250.0mm); Mobile Phase A: MeOH 100%; System: Isocratic; Column Temperature: 30°C; Injection Volume: 10 µL; Flow Rate: 0.8 ml/min; Sample Concentration: 10 mg/ml

Validation of Method

Validation of method was established via testing following criteria: retention time (RT), standard deviation (SD), limits of detection (LOD), limits of quantification (LOQ), regression equation and coefficient (RE/C), and and regain (R).

Qualitative Analysis of Secondary Metabolites

Analysis of Alkaloid

0.5 g of extracts were taken and boiled with 10 ml of 70% ethanol containing 6% H_2SO_4 for 1 minute, cooled and allowed to settle. Mayer and Dragendorff reagents were added and it was checked whether precipitate was formed. After this control was done, ethanolic extract was taken into a small separating funnel, then alkalized by adding a sufficient amount of 25% Na_2CO_3 solution and rinsed with 15 ml of chloroform. Then, it was consumed with 15 ml of 10% acetic acid solution, and acetic acid phase was taken into 3 separate tubes. While one of tubes was kept for control, Mayer reagents were added to second and Dragendorff reagents to third, and it was checked whether precipitate formed [24].

Analysis of Coumarin

1 g of extracts was taken and 10 ml of 1 N H_2SO_4 was added. After boiling for 10 minutes under reflux and filtering while hot, filtrate was shaken with 15 ml of chloroform in a separatory funnel. Chloroform phase was separated and 5 ml of 10% NH_3 solution was annexed to 5 ml of it and shaken. Solution was put to stand for 5 min and then ammonia phase was checked for fluorescence at UV 366 nm [24].

Analysis of Cardioactive Heteroside

Extracts were taken and boiled in 10 ml of 70% ethanol in a water bath for 2 min and filtered. Filtrate was diluted 2 times with water, 1 ml of concentrated lead subacetate solution was added and

filtered. Filtrate was extracted with 10 ml of chloroform and chloroform phase was placed in 3 separate capsules and Keller-Kliani and Baljet reactions were done [24].

Analysis of Saponoside

0.5 g extracts were taken and put in a test tube with 10 ml of hot water and shaken vigorously for about 10 seconds after cooling. If there is saponosite, it was checked whether a foam layer of 1-10 cm height, which remains stable for at least 10 minutes, and which does not disappear when 1-2 drops of 2N HCl is dropped on it [24].

Analysis of Flavonoside

Qualitative analysis of flavonoside of extracts were done according to Cyanidin Reaction [24].

Analysis of Tannin

A 5% infusion was prepared from extracts and examinations were made on prepared infusion [24].

Analysis of Anthocyanoside

Extracts were extracted with 50% ethanol in a low flame, filtered, and filtrate was divided into five and reactions were applied [24].

Analysis of Anthracenoside

0.1 g of extracts were taken, boiled with 5 ml of diluted H_2SO_4 for 2 minutes and filtered while hydrolysis product was hot. Filtrate was cooled and extracted with a small amount of benzene. Upper benzene layer was removed, rinsed with 10% ammonia, and color of lower ammonia layer was observed [24].

Analysis of Cyanogenetic Heteroside

Extracts were placed in a 100 ml flask and only enough water to be heated was added. A filter paper impregnated with picric acid soaked with sodium carbonate solution was hung into flask close to water-soaked material and gently compressed with help of a cork stopper. Erlen was warmed in a light burner flame. Color formed on paper was observed [24].

Analysis of Resin

Extracts are shaken with 90% ethanol in a tube. Insoluble parts are separated by filtration. Ethanol part is taken into another tube. An equal volume of 1% copper acetate solution is added on it. Resulting green color indicates presence of resin [25].

Statistically Analysis

Whole tests were assessed in triplicate. Kruskal-Wallis test was utilised to detect statistical signification. Data were analysed utilising SPSS (IBM SPSS Statistics 20, IBM Corporation, Armonk, NY, USA) at signification level of $P = 0.05$. Percentile inhibition and IC_{50} value findings for extracts are represented as means ± standard deviation. Results of antioxidant activity experiments are given in percent inhibition ± standard deviation, and results of total phenolic component assay assays were represented in gallic acid equivalents ± standard deviation.

RESULT AND DISCUSSION

α-Amylase and α-Glucosidase Inhibition Assays

70% methanolic extracts prepared from fruits and cones of JCS, JOO, PS, and PC were assessed for determining their *α*-glucosidase inhibitory effect. JCS (IC_{50} = 578 ± 20 μg/ml), JOO (IC_{50} = 3706 ± 52 μg/ml), and PS (IC_{50} = 313 ± 7 μg/ml) extracts were observed to show a higher effect than reference compound (IC_{50} = 4061 ± 146 μg/ml). Interestingly, while PS displayed the highest activity, PC showed no inhibition against acarbose; even if their tannin contents were similar (Table 2). JCS and PS, which

have the highest total phenolic content, showed a good effect when compared to acarbose. *α*-Glucosidase inhibitory activity results showed similarity with total phenolic component assay results. Except for JOO extract (15%), other extracts did not show any activity against *α*-amylase at 5 mg/ml concentration. However, it was not found higher than acarbose (59%). In a study, bioactive potential of *Prunus* fruits were evaluated. As in our study, PS (blackthorn) displayed high activity against *α*-glucosidase with 0.78 mg/ml IC_{50} value when compared with acarbose (IC_{50}= 3.73 mg/ml). PC (white cherry plum) had no activity against both enzymes while PS (blackthorn) had'nt displayed any activity towards *α*-amylase when in comparison to acarbose (IC_{50}= 0.11 mg/ml) [26]. JCS hydroalcoholic extract indicated higher activity towards *α*-glucosidase (IC_{50}= 0.0044 mg/ml) than J. *oxycedrus* ssp. *oxycedrus* (IC_{50}= -) as in our study. However, they were not higher than acarbose (IC_{50}= 0.0009 mg/ml). Both of them had no inhibitory activity against *α*-amylase when with regard to acarbose (73.7%) at 3 mg/ml [27]. This was the first comparative study with different species from these two genus: *Prunus* and *Juniperus* towards *α*-glucosidase and *α*-amylase enzymes.

Table 2. *α*-Glucosidase inhibitory activity of extracts

α-Glucosidase Inhibitory Activity	**IC_{50} value (µg/ml)**							
	Mean	**Standard Deviation**	**Median**	**Minimum**	**Maximum**	**Chi-square**	**P**	**Post-hoc**
JCS	578	20	572	563	601	10.385	0.016	*Prunus spinosa* and Acarbose (P=0.013)
JOO	3706	52	3710	3640	3742			
PS	313	7	312	306	320			
PC	-	-	-	-	-			
Acarbose[a]	4061	146	4045	3964	4248			

[a] Positive control for *α*-glucosidase inhibitory effect

Antimicrobial Activity

Minimum inhibitory concentrations (MIC, µg/ml) were given as Table 3. JCS fruit extract was observed with MIC values between (312.5-2500 µg/ml) against whole microorganisms. The most sensitive strains were *Candida albicans* (312.5 µg/ml), and *C. parapsilosis* (312.5 µg/ml) followed by *B. subtilis* 1250 µg/ml), *Klebsiella aerogenes* (1250 µg/ml) and *E. coli* (1250 µg/ml). JOO fruit extract was found more active against *E. coli* (MIC= 625 µg/ml), followed by *B. Subtilis*, *C. albicans*, and *C. parapsilosis* with a MIC= 1250 µg/ml. MIC values were indicated between 156.25-1250 µg/ml for JCS berrries methanolic extract against *Staphylococcus aureus* ATCC 8538P, *S. epidermidis* G1, *Enterococcus hirae* V3, *B. subtilis* P3. Same extract was not found active against Gram-negative strains*; E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9021, *Proteus mirabilis G4*, yeasts *C. albicans* ATCC 10231 and *C. parapsilosis* P7 [28]. Antimicrobial potential of methanolic and aqueous extracts of branches of JCS and JOO were investigated against Gram-positive, Gram-negative, and yeasts by Taviano et al. (2011) [29]. Methanolic and aqueous extracts of *Juniperus* ssp. were found MIC=19.53 µg/ml towards *S. aureus* ATCC 6538P.

MIC values of extracts towards *S. epidermidis* G1 were between 78.12-156.25 µg/ml. MIC values of extracts towards *E. hirae* V3 were >250- 156.25 µg/ml. Any antimicrobial effect in Gram-negative strains and yeasts was not shown. These extracts also indicated a significant inhibiting effect on *S. aureus* biofilm formation (with a reduction of 66–84%) [29]. Antimicrobial effects of hexane, acetone and methanolic berry extracts of JOO were tested via disc diffusion method and hexane and methanol extrats of JOO indicated activity against some microorganisms. Acetone extract of JOO had no effect on any of microorganisms evaluated [30]. Based on result, methanol extract of JOO was observed effective towards *E. coli* with MIC 625 µg/ml. In our study, PS fruit extract was found more effective against *Candida* than bacteria strains. MICs generally were observed >2500 µg/ml for *Prunus divaricata* fruit extract. In literature, *P. divaricata* subsp. *divaricata* leaves exracts was prepared by petroleum ether, dichloromethane, methanol and distilled water and investigated and petroleum ether extract of *P.*

divaricata subsp. *divaricata* showed the highest antimicrobial activity against *E. faecalis* with 11 mm inhibition zone [31]. Antimicrobial effect of methanol extracts of *P. divaricata* subsp. *divaricata* fruits were carried out eight bacteria and two yeast by tube dilution and microdilution methods. Highest antimicrobial effect among clinical isolates Gram negative organisms were observed towards *E. coli* and *K. pneumonia*, while *C. albicans* and *C. parapsilosis* were the least [32]. Antimicrobia effect of PS fruit ethanol extract was investigated by Gram-negative, Gram-positive bacteria, and yeast. No specific inhibitory action was obtained against the evaluated Gram-negative or Gram-positive bacteria. Antimicrobial effect of *PS* ethanol fruit extract showed MICs range of 4.36 mg/ml to 8.72 mg/ml [33]. In addition, comprasion these data with available results, MIC for PS (2500 µg/ml, this paper) is lower than that of PS (8.72 mg/ml) towards *S. aureus* ATCC 6538. For fungal strains, it was found that MIC of *C. albicans* was not similar (625 µg/ml vs. 8.72 mg/ml) to that observed in study [33].

Table 3. Minimum inhibitory concentrations (MIC, µg/ml)

Extracts	*E. coli* ATCC 8739	*S. enterica* ATCC 14028	*S. aureus* ATCC 6538	*B. subtilis* ATCC 19659	*P. aeruginosa* ATCC 9027	*K. aerogenes* ATCC 13048	*C. albicans* ATCC 10231	*C. parapsilosis* ATCC 22019
JCS	1250	2500	2500	1250	2500	1250	312.5	312.5
JOO	625	>2500	2500	1250	2500	2500	1250	1250
PS	2500	2500	2500	2500	2500	2500	625	1250
PC	>2500	>2500	>2500	>2500	>2500	>2500	2500	2500
Ampicillin	0.125>	1.0	0.125>	0.125>	>64	32	-	-
Terbinafine	-	-	-	-	-	-	4.0	32

Total Phenolic Content and Antioxidant Activity

Many investigations have also indicated that there is a relationship between diabetes and free radicals. Again, studies have shown that antioxidant-effective compounds have antimicrobial effects on microorganisms [34]. Antioxidant effect of aqueous and ethanolic extracts prepared from *Juniperus communis* fruits was tested with various methods, it was noted that they showed strong antioxidant effects, especially at 20, 40, and 60 µg/ml concentrations. Especially, it was observed that ethanolic extract had better DPPH$^{\bullet}$ scavenging capacity and there was a statistically important difference with aqueous extract when compared ($p<0.05$) [35]. *J. communis* var. *communis* (JCc) and *J. communis* var. *saxatilis* (JCs) extracts indicated DPPH radical scavenging activity, which was much higher in *J. communis* var. *communis* ($IC_{50} = 0.63 \pm 0.09$ mg/ml) than in *J. communis* var. *saxatilis* ($IC_{50} = 1.84 \pm 0.10$ mg/ml). When compared in terms of total phenolic content, findings showed compatibility with antioxidant effect results (JCc 59.17 ± 1.65 mg GAE/g extract; JCs 17.64 ± 0.09 mg GAE/g extract) [28]. *P. domestica*, *P. cerasifera*, and *P. spinosa* extracts were contrasted in terms of antioxidant effect and phenol component *P. spinosa* extract was observed to be superior to other two species as compared with antioxidant effect ($p<0.01$) [36]. Difference of our study is that these four species are compared in terms of antioxidant effect, associated with tannin content, tested in terms of antidiabetic effect and antimicrobial effect. 70% methanolic extracts prepared from fruits and cones of PC, PS, JOO, and JCS were carried out for determining their antioxidant activity and total phenolic content. Findings of total phenolic content experiments were similar to those of antioxidant capacity tests. JCS extract has to highest phenolic composition and JOO has to lowest phenolic composition (µg GAE/ mg extract). [(JCS)19.38>(PS)17.21>(PC)2.94>(JOO)2.08 µg GAE/mg extract].

Results of this experiments were represented in Table 4. In ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ scavenging effect tests α-tocopherol (TK) and trolox (TR) were utilised as reference, *J. communis* var. *saxatilis* extract showed the best activity and *J. oxycedrus* subsp. *oxycedrus* showed lowest activity compared to other extracts. [(TR)99.14>(TK)47.92>(JCS)14.29>(PS)11.14>(PC)7.84>(JOO)3.65 %; at 100 µg/ml for ABTS$^{\bullet+}$)]. [(TR)92.78>(TK)90.78>(JCS)10.63>(PS)8.09>(PC)6.65>(JOO)3.48 %; at 100 µg/ml for

DPPH•)]. Findings of antioxidant effect experiments are presented in Table 5. When all results are evaluated, it is seen that phenolic compounds are effective in antioxidant effect of extracts. On the basis of findings of this investigation, it can be said that JCS with the best tannin content shows the highest effects.

Table 4. Total phenolic content results of *Juniperus* and *Prunus* plant extracts

Extracts	**Total Phenolic Content (µg GAE/mg extract ± SD*)**
JCS	19.38 ± 0.000854
JOO	2.08 ± 0.000416
PS	17.21 ± 0.000681
PC	2.94 ± 0.003161

*****SD: Standard deviation

Table 5. $ABTS^{\bullet+}$ and DPPH• scavenging activity results of *Juniperus* and *Prunus* plant extracts.

$ABTS^{\bullet+}$ Scavenging Activity (% Inhibition ± SD*)		
	% Inhibition (60 µg/ml)	**% Inhibition (100 µg/ml)**
JCS	9.32 ± 0.006149	14.29 ± 0.01195
JOO	1.10 ± 0.00315	3.65 ± 0.011817
PS	7.69 ± 0.002023	11.14 ± 0.002205
PC	4.36 ± 0.006338	7.84 ± 0.006894
Trolox	57.21 ± 0.005551	99.14 ± 0.000557
***α*-Tocopherol**	28.04 ± 0.004752	47.92 ± 0.0065
DPPH• Scavenging Activity (% Inhibition ± SD*)		
	% Inhibition (60 µg/ml)	**% Inhibition (100 µg/ml)**
JCS	7.86 ± 0.001429	10.63 ± 0.015451
JOO	1.31 ± 0.039647	3.48 ± 0.016417
PS	6.21 ± 0.033602	8.09 ± 0.003219
PC	2.64 ± 0.071712	6.65 ± 0.044022
Trolox	92.14 ± 0.001877	92.78 ± 0.0006
***α*-Tocopherol**	77.05 ± 0.004244	90.78 ± 0.000252

*SD: Standard deviation

Quantitative Analysis Secondary Metabolites

As a result of analyzes made; amounts of catechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate were determined in ng/ml. While catechin was not found in extracts of PC, PS, and JOO species; it was determined that these plants contained the most epigallocatechin (17.6±0.65, 15.1±0.56, and 20.3±2.01 ng/ml, respectively) compared to other substances. It was determined that plant containing the highest catechin (1173.3±5.77 ng/ml) and epigallocatechin (208±7.21 ng/ml) was JCS. Epigallocatechin gallate was found to be the least common substance in PC, PS, JOO, and JCS with 5.3±1.02, 4.2±0.19, 3.6±0.10, and 3±0.14 ng/ml, respectively. Tannin amounts determined as a result of analyzes are given in Table 6. Validation of method was performed by testing following criteria: retention time, LOD, LOQ, regression equation and coefficient, and regain. Results of validation method

was given in Table 7. LC-MS/MS chromatograms of standard compounds were represented in Figures 2-5. HPLC-MS analysis method parameters and Q1 and Q3 ion values of standard compunds are given in Table 8. MASS Spectrums of standard compounds are given in Figures 6-9. Catechin content was analysed in fresh fruit of PS and PC by using HPLC. It was found as 2.12 and 1.722 mg kg^{-1} fw, respectively [36]. However, catechin was not found in these two species in our study by LC-MS/MS. Qualitative analysis by using HPLC-DAD, all standards we used in our study except for epigallocatechin gallate were determined in PS [37]. Catechin was found in cones of *J. oxycedrus* and *J. communis*, while epigallocatechin was not found according to HPLC-MS analyse [38]. In contrast to, catechin and epigallocatechin was found in JCS in another research [39]. It was the first comprehensive and comparative study about qualitative and quantitative analyses of catechic tannins contents of these species.

Table 6. Tannin amounts determined as a result of analyzes.

Extracts (70% MeOH) (10 mg/ml)	(+)-catechin (ng/ml)	(-)-epigallocatechin (ng/ml)	(-)-epigallocatechin gallate (ng/ml)	(-)-epicatechin gallate (ng/ml)
JCS	1173.3±5.77	208±7.21	3±0.14	9.2±0.52
JOO	-	20.3±2.01	3.6±0.10	8.9±0.25
PS	-	15.1±0.56	4.2±0.19	9.6±1.38
PC	-	17.6±0.65	5.3±1.02	10.2±0.64

Table 7. LOD, LOQ values and calibration equation for standards (Concentration range 15.6-2000 ng/ml).

Standards	RT (dk)	SD	LOD (ng/ml)	LOQ (ng/ml)	RE/C	R (1000 ppb)
(+)-Catechin	2.41	1.56	4.67	15.57	y = 84.1x-185 r = 0.9999	905
(-)-Epigallocatechin	2.34	0.99	2.96	9.87	y = 169x+1.27e+003 r = 1.0000	927
(-)-Epigallocatechin Gallate	2.37	1.55	4.65	15.50	y = 71.8x-73.1 r = 0.9996	932
(-)-Epicatechin Gallate	2.30	1.10	3.30	11.02	y = 268x-684 r = 0.9999	1080

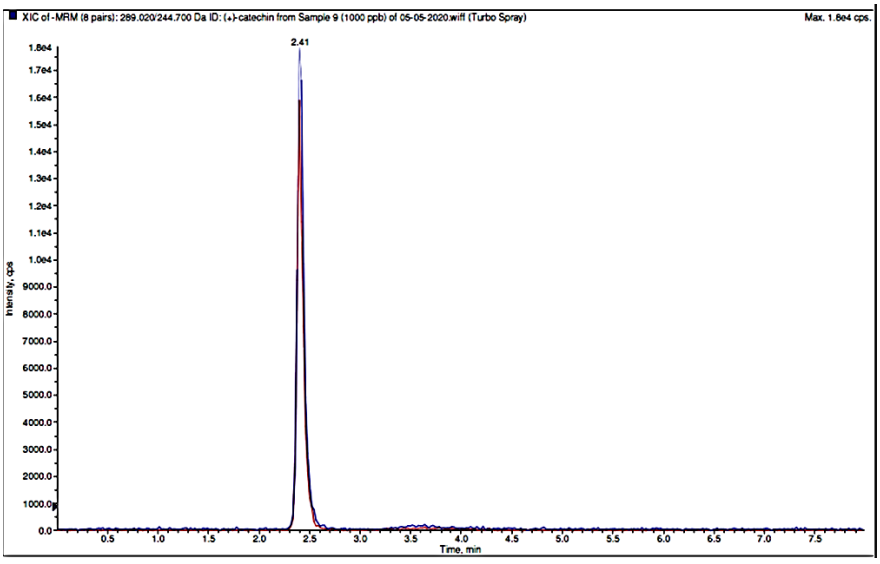

Figure 2. LC-MS/MS chromatogram of (+)-catechin.

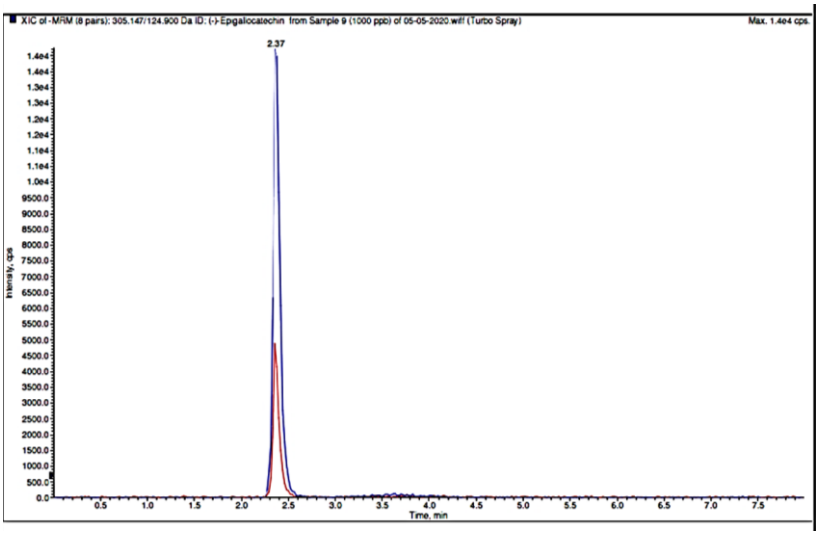

Figure 3. LC-MS/MS chromatogram of (-)-epigallocatechin.

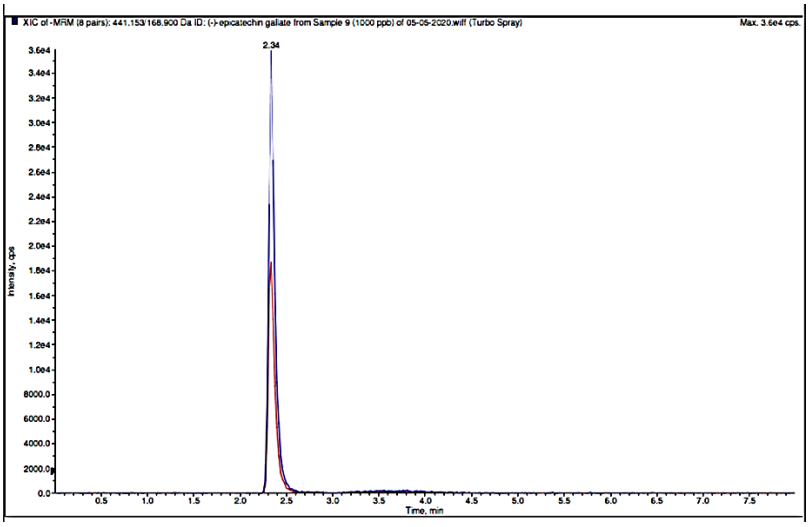

Figure 4. LC-MS/MS chromatogram of (-)-epicatechin gallate.

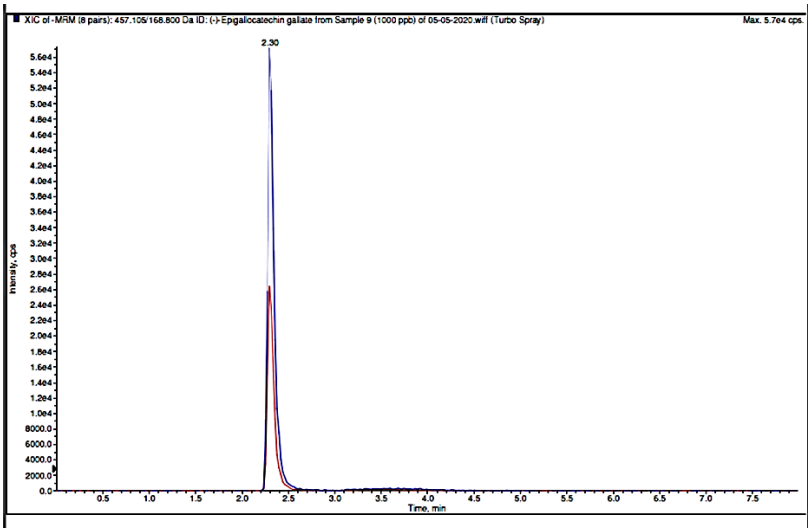

Figure 5. LC-MS/MS chromatogram of (-)-epigallocatechin gallate.

Table 8. HPLC-MS analysis method parameters and Q1 and Q3 ion values of standard compounds.

Q1 MASS (Da)	Q3 MASS (Da)	DEFINITION	DP (Volt)	EP (Volt)	CE (Volt)	CXP (Volt)
289.02	244.7	(+)-Catechin	-65	-10	-20	-13
289.02	108.8	(+)-Catechin	-65	-10	-32	-7
305.147	124.9	(-)-Epigallocatechin	-85	-10	-30	-7
305.147	178.8	(-)-Epigallocatechin	-85	-10	-22	-17
441.153	168.9	(-)-Epicatechin Gallate	-80	-10	-26	-1
441.153	288.7	(-)-Epicatechin Gallate	-80	-10	-22	-5
457.105	168.8	(-)-Epigallocatechin Gallate	-80	-10	-24	-13
457.105	124.7	(-)-Epigallocatechin Gallate	-80	-10	-58	-9
Ion spray voltage	-4500					
Temperature	250 °C					
Mode	Negative					

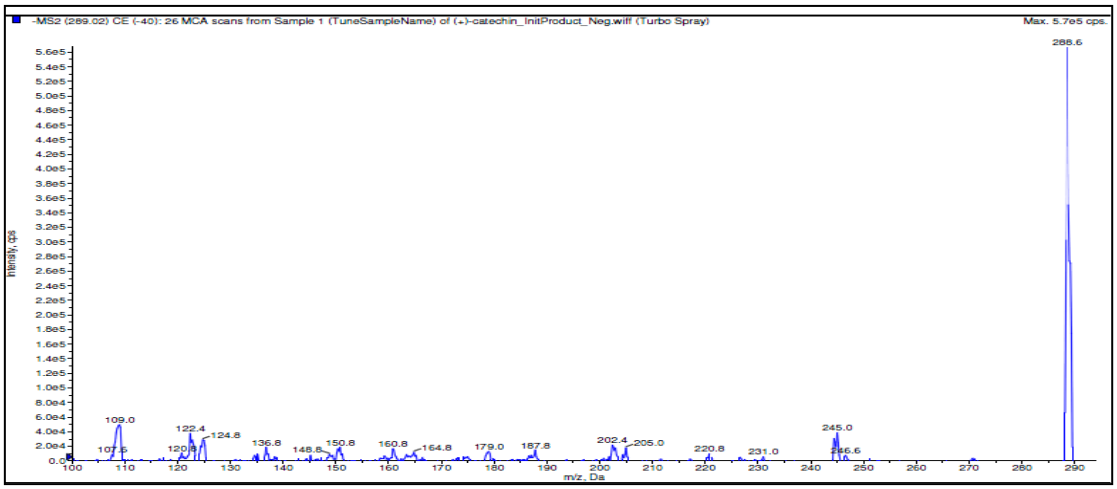

Figure 6. MASS spectrum of (+)-Catechine.

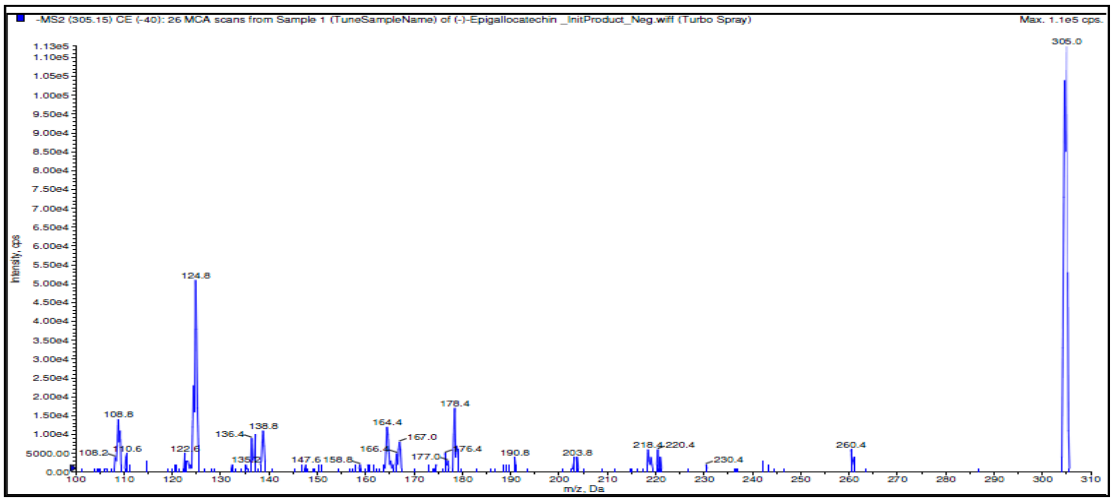

Figure 7. MASS spectrum of (-)-Epigallocatechin.

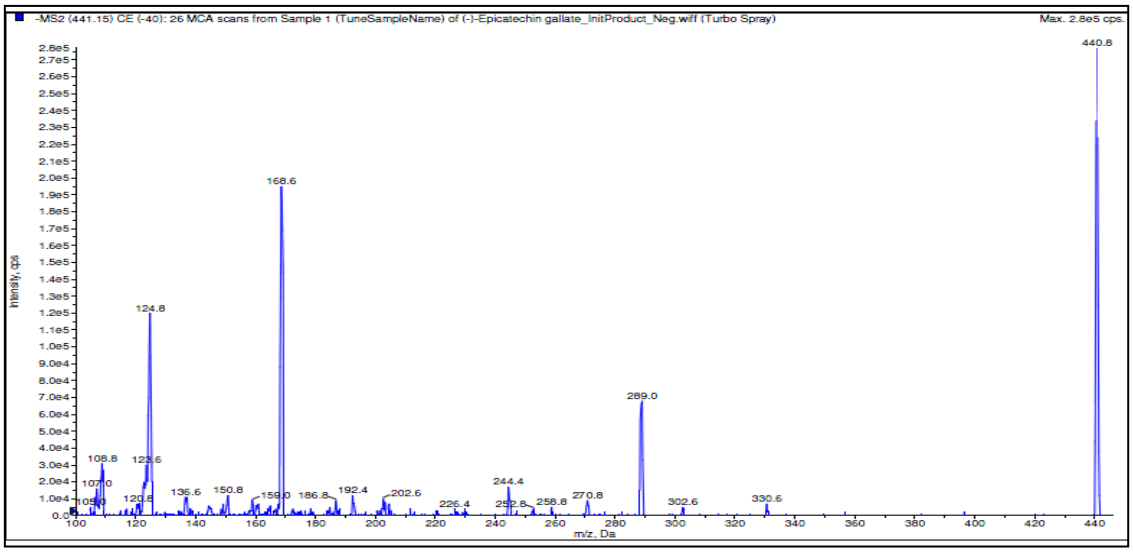

Figure 8. MASS spectrum of (-)-epicatechin gallate.

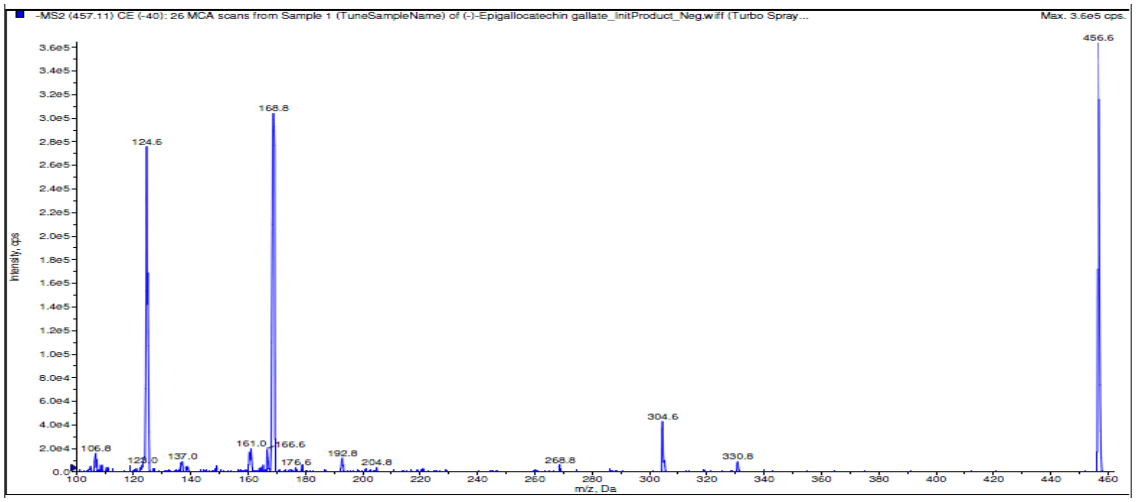

Figure 9. MASS spectrum of (-)-Epigallocatechin gallate.

Qualitative Analysis of Secondary Metabolites

Results of qualitative analysis of secondary metabolites of extracts is given in Table 9. Among extracts, alkaloid was observed in all extracts except PC with Dragendorff reagent. No coumarin and anthracenoside were observed among samples. Among extracts, cardioactive heteroside was observed in all extracts with Keller-Kliani reaction. Saponoside has been seen only in *Juniperus* species. Among extracts, flavonoside and tannin were observed in all extracts with Cyanidin reaction (for flavonoside), $FeCl_3$, gelatin reaction, brominated water, and Stiasny reactions (for tannin). Anthocyanoside has been seen in all extracts with lead acetate and amyl alcohol reactions. Cyanogenetic heteroside and resin were observed in all extracts except PC.

This is the first detailed research of the *in vitro* antimicrobial, antioxidant, antidiabetic effects, along with qualitative and quantitative analysis of JCS, JOO, PS, and PC. On the basis of the data of this research, it can be said that *J. communis* var. *saxatilis* with the highest tannin content shows the highest effects. We conclude that these species may be utilized as antimicrobial, antidiabetic, and antioxidant agents.

Table 9. Results of qualitative analysis of secondary metabolites of extracts.

Secondary metabolites	Chemical Reaction	JCS	JOO	PS	PC
Alkaloid	Mayer	-	+	-	-
	Dragendorff	+	+	+	-
Coumarin	UV 366	-	-	-	-
Cardioactive Heteroside	Keller-Kliani reaction	+	+	+	+
	Baljet reaction	-	-	-	-
Saponoside	Foaming test	+	+	-	-
Flavonoside	Cyanidin Reaction	+	+	+	+
Tannin	$FeCl_3$	+	+	+	+
	Gelatin reaction	+	+	+	+
	Brominated water reaction	+	+	+	+
	Stiasny reaction	+	+	+	+
Anthocyanoside	Diluted H_2SO_4	-	-	+	-
	NaOH-HCl	-	-	+	-
	Lead acetate reaction	+	+	+	+
	Amyl alcohol reaction	+	+	+	+
	Heat with diluted H_2SO_4 reaction	-	-	+	-
Anthracenoside	Borntrager reaction	-	-	-	-
Cyanogenetic Heteroside	Picric acid reaction	+	+	+	-
Resin	Abietat reaction	+	+	+	-

+ present, - absent

Phenolic compounds are a group of naturally occurring plant compounds that are known for their antioxidant properties. These compounds are found in a wide range of fruits, vegetables, and other plant-based foods. Recent studies have suggested that phenolic compounds may have a beneficial effect on individuals with diabetes. Diabetes is a metabolic disorder characterized by high blood sugar levels. Phenolic compounds have been found to improve insulin sensitivity and regulate blood sugar levels. Overall, while more research is needed to fully understand the relationship between phenolic compounds and diabetes, evidence suggests that incorporating phenolic-rich foods into the diet may have potential benefits for individuals with diabetes. In this study, the fruits of *J. communis* var. *saxatilis*, *J. oxycedrus* subsp. *oxycedrus*, *P. spinosa*, and *P. cerasifera* were analyzed for their catechin content using LC-MS/MS. The antidiabetic, antimicrobial, and antioxidant activities of the fruit and cone extracts of these plants were also evaluated. The JCS, JOO, and PS extracts showed higher activity than acarbose in terms of antidiabetic activity. *J. communis* var. *saxatilis* fruit extract showed good antimicrobial activity against all microorganisms tested. The JCS extract had the highest phenolic composition and antioxidant activity, and also contained the highest levels of catechin and epigallocatechin. It can be concluded that JCS with the highest tannin content exhibited the highest effects in this research.

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AUTHOR CONTRIBUTIONS

Concept: H.Y., H.C.D., B.A., M.Ö., E.T., A.C., M.N., G.G., S.K., Z.G.; Design: H.Y., E.T., A.C., M.N., G.G., S.K.; Control: H.Y., B.A., M.Ö., E.T., A.C., M.N., G.G., S.K., Z.G.; Sources: H.Y., M.Ö., S.K.; Materials: H.Y., M.Ö., E.T., A.C., M.N., G.G., S.K., Z.G.; Data Collection and/or Processing:

H.Y., H.C.D., B.A., M.Ö., E.T., A.C., M.N., G.G., S.K., Z.G.; Analysis and/or Interpretation: H.Y., B.A., M.Ö., A.C., G.G., S.K.; Literature Review: H.Y., B.A., G.G., S.K.; Manuscript Writing: H.Y., B.A., M.Ö., A.C., G.G., S.K.; Critical Review: H.Y., H.C.D., B.A., M.Ö., E.T., A.C., M.N., G.G., S.K., Z.G.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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