KSÜ Tarım ve Doğa Derg 27 (6), 1237-1248, 2024
KSU J. Agric Nat 27 (6), 1237-1248, 2024
DOI:10.18016/ksutarimdoga.vi.1402758

Investigation of Chemical Composition and Biological Activity of *Salix aegyptiaca* L. Roots

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ABSTRACT
The root of *Salix aegyptiaca* L. was extracted using their yield percentage, total condensed tannin concentration, antimicrobial, antioxidant activity and to determine chemical composition by LC-MS/MS. The root extraction was carried out together with water, ethanol and methanol. Accelerated solvent extraction (ASE), conventional extraction (CE), and microwave extraction (ME) were the extraction methods applied during the investigation. The LC-MS/MS methanol extract was used to detect phenolics. The antioxidant activities and total condensed tannin concentrations of root extracts have been done by UV-visible spectroscopy from 517 to 580 nm, severally. The disk diffusion method was used for antimicrobial activity. The maximum extraction yield (17.2%) was obtained in methanol by the ASE technique whereas, the conventional extraction technique obtained the minimum extraction efficiency (9.1%). By triplicate measurement, the total condensed tannin analysis result was found 35.14 mg/L. Using the ASE technique, the methanol extract was the maximum inhibitory zone (26 mm) against *Candida albicans* ATCC 10231. However, in water extract by conventional extraction, a minimum inhibitory zone (11 mm) was obtained against *Staphylococcus aureus* Cowan 1. The highest and lowest DPPH scavenging activity was determined in methanol (ASE) (98.8%) and ethanol (97.5%) extract respectively. The maximum amounts of quinic acid (63895 µg/g) were discovered using LC-MS/MS.

Biochemistry

Research Article

Article History
Received : 10.12.2023
Accepted : 08.02.2024

Keywords
Antioxidants
Antimicrobial
Chemical composition
Condensed tannin
Salix aegyptiaca

Salix aegyptiaca L. Köklerinin Kimyasal Bileşimi ve Biyolojik Aktivitesinin Araştırılması

ÖZET
Salix aegyptiaca'nın kök ekstraktı, verim yüzdesi, toplam kondense tanen konsantrasyonu, antimikrobiyal, antioksidan aktivite ve LC-MS/MS kullanılarak kimyasal bileşiminin belirlenmesi için kullanılmıştır. Kök ekstraksiyonu su, etanol ve metanol ile gerçekleştirildi. Hızlandırılmış solvent ekstraksiyonu (ASE), konvansiyonel ekstraksiyon (CE) ve mikrodalga ekstraksiyonu (ME), araştırma sırasında uygulanan ekstraksiyon yöntemleriydi. Fenoliklerin LC-MS/MS ile tespit edilmesi için metanol ekstraktı kullanıldı. Kök ekstraktlarının antioksidan aktiviteleri ve toplam kondanse tanen miktarları UV-Vis spektrofotometre cihazında sırasıyla 517 nm ve 580 nm olarak ölçülmüştür. Antimikrobiyal aktivite için disk difüzyon yöntemi kullanıldı. Maksimum ekstraksiyon verimi (%17.2) metanolde ASE tekniği ile elde edilirken, minimum ekstraksiyon verimliliği (%9.1) konvansiyonel ekstraksiyon tekniği ile elde edildi. Üçlü ölçümle toplam kondense tanen analizi sonucu 35.14 mg/L bulundu. ASE tekniği kullanıldığında metanol ekstraktı *Candida albicans* ATCC 10231'e karşı maksimum inhibitör bölge (26 mm) olmuştur. Bununla birlikte, su ekstraktında geleneksel ekstrasitasyonla *Staphylococcus aureus* Cowan 1'e karşı minimum inhibitör bölge (11 mm) elde edilmiştir. En yüksek ve

Biyokimya

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 10.12.2023
Kabul Tarihi : 08.02.2024

Anahtar Kelimeler
Antioksidant
Antimikrobiyal
Kimyasal bileşim
Kondense tanen
Salix aegyptiaca

en düşük DPPH giderme aktivitesi sırasıyla metanol (ASE) (%98.8) ve etanol (%97.5) ekstraktında belirlendi. LC-MS/MS kullanılarak maksimum kinik asit miktarları (63895 µg/g) keşfedildi.

Atıf İçin: Abdalrahman, M. R., Göçeri, A., Sofi, S. A., Alma, M. A, Kireçci, E., & Yılmaz, M. A. (2024). *Salix aegyptiaca* L. Köklerinin Kimyasal Bileşimi ve Biyolojik Aktivitesinin Araştırılması. *KSÜ Tarım ve Doğa Derg 27* (6), 1237-1248. DOI: 10.18016/ksutarimdoga.vi.1402758.

To Cite: Abdalrahman, M. R., Göçeri, A., Sofi, S. A., Alma, M. A, Kireçci, E., & Yılmaz, M. A. (2024). Investigation of Chemical Composition and Biological Activity of *Salix aegyptiaca* L. Roots. *KSU J. Agric Nat 27* (6), 1237-1248. DOI: 10.18016/ksutarimdoga.vi.1402758

INTRODUCTION

The curative antimicrobial substances were used like medicinal plants. Nowadays, there are many kinds of compounds that can be found in nature. The biologically active compounds that can be isolated from plants have always attracted attention (Prince et al., 2011). Aromatic compounds, including flavonoids, tannins, quinones, phenolic acids, and amines, as well as alkaloid compounds with terpenoids, vitamins, and other endogenous metabolites, can be produced by medicinal aromatic plants (Gulcin, 2020). These compounds have insect and herbivore defense properties (Bharathi et al., 2011). There are nearly 330–500 types (genus *Salix*) and ground plants, 200 hybrids, bush, and trees that are extensively spread to many areas of the world such as Africa, America, Europe, and Asia. The types of *Salix* are conventionally used in folk remedies and include a useful source of effective compounds such as salicin, a prodrug for salicylic acid (Savcı et al., 2020; Tawfeek N et al., 2021; Karagecili et al., 2023). The family of *Salicaceae* conventionally contains the genus *Populus* and *Salix* (willow), which are widespread in the northern mild zone (Isebrands et al., 2014). Nowadays, the *Salicaceae* now includes the most effective tropical fruit-consuming plants that do not produce catkins (Thadeo et al., 2014). Therefore, the genus *Salicaceae* currently encloses nearly 56 genera and 1,220 types (Christenhusz et al., 2016). The family of *Salicaceae* are quick-developing trees or bushes (Isebrands et al., 2014). These families are used for many economic objectives such as the manufacture of wood, paper, fences, housing, shoes, arrow shafts, fish traps, pipe, rope, fancy, and gardening like biomasses (renewable energy). Besides, it was used purpose of ecological enrichment owing to the earth erosion control (Kuzovkina et al., 2014). *Salix aegyptiaca* L. plants were noted as one of the first samples of advanced herbal medicine a long time ago. Salicin is one of the chemical compounds of bark willow trees that can be obtained by chemical processes. Salicylic acid is a new material, and acetylated variants of this compound have become famous drugs in the past, including aspirin. Famous salicylate compounds, which include salicylic acid and acetylsalicylic acid, are produced from the *Salix* plant. This class of substances has anti-inflammatory properties that can suppress the production of prostaglandins by cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (Yu et al., 2002; Mahdi et al., 2006). The leaves of *S. aegyptiaca* contain significant levels of phenolics such as *p*-coumaric acid, gallic acid, caffeic acid, catechin, myricetin, vanillin, and epigallocatechin gallate, as well as flavonoids like rutin, quercetin, and salicin (Enayat et al., 2009). Several *Salix* species and their active ingredients, including salicin and salicylic acid, were used in traditional healing to cure a variety of conditions, including fever, chronic and acute inflammation, epilepsy, diabetes, piles, swelling, wounds, earaches, colds, back pain, toothaches, headaches, and cramps. Additionally, they have narcotic, antioxidant, anticancer, cytotoxic, anti-inflammatory, antibacterial, antidiabetic, antiobese, neuroprotective, and hepatoprotective properties. Salicylic acid serves cyclooxygenases (COX I, II) as its primary function since these enzymes are crucial in the production of prostaglandins, which are responsible for regulating pain and inflammation (Tawfeek et al., 2021). *Salix* species have the potential to be important antimicrobials, as demonstrated by the MIC zones and percentages throughout, which indicate their impact on functional foods (Mostafa et al., 2020). Numerous earlier academic works have demonstrated the antibacterial activity of *Salix* plant species and their effective component extracts against a variety of microorganisms, including *Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella enterica, Escherichia coli,* and *Streptococcus mutans,* that form dental biofilms. Meanwhile, *Salix viminalis, L., and Salix atrocinerea s*tudies have shown that the extracts obtained from the species have a strong antioxidant effect thanks to the flavonoids and phenolic compounds in the content (Tawfeek et al., 2021). *Salix* plant is now recognized by the German Pharmacopoeia, and plans are underway for a monograph to be included in the European Pharmacopoeia. The European Scientific Cooperative on Phytotherapy (ESCOP, 1997) advocated taking salicin at a dose of up to 240 mg per day. The current article's purpose is to present *S. aegyptiaca* as a medicinal plant by outlining both its historical uses and the most recent research on its innovative pharmacological and therapeutic uses.

MATERIALS AND METHODS

Plant sample collection

The Sarsang-Dohuk province in northern Iraq is where the *S. aegyptiaca* L. roots were collected in February 2014. Additionally, identification and confirmation were carried out by Dr. Zeravan Abdulkaliq Sadeeq from the University of Dohuk's Faculty of Agriculture/Dohuk-Iraq

Sample Preparation

S. aegyptiaca L. root was stored and dried under normal room conditions and powdered by using an electric steel blender. The powder sample was put through a series of sieves of varying sizes and refrigerated until examination. All studies were carried out in Kahramanmaraş Sütçü İmam University, Faculty of Forestry laboratory.

Extract Process

Conventional extraction

For conventional extraction, 80 mL water, ethanol (95%), methanol (95%), and 8 g of *Salix* extract were combined separately. The proportion was extracted to solvent 1:10. The extraction took place for three hours at 40°C. Liquid extract was separated by filtration, and the evaporation process was done (Dhanani et al., 2013).

Microwave extraction

A volume of 80 mL water, methanol (95%), and ethanol (95%) were combined in beakers with 8 g of extract separately. At standard temperatures extraction performed for 25 minutes after filtration extracts were evaporated (Laghari et al., 2011).

Accelerated solvent extraction

Dried 8 g extract was added to a certain flask. The extraction took place at 60 °C for 40 minutes, under pressure and then the sample was evaporated (Comlekcioglu et al., 2013).

Yield determination

The extraction yield, which was defined as the amount of extract residue in mass compared to the initial amount of dry sample, is an evaluation of the solvent's ability to remove particular components from the main material (Zhang et al., 2007). 50 mL each of water, ethanol, and methanol were used to extract 0.5 g of root extract using various extraction techniques. The yield percentage was calculated by using the following Formula given below: (Karaogul et al., 2016b):

$$\text{Yield percentage (\%)} = \frac{x}{y} \times 100$$

where, X is the oven
– dry weight of the extract (in g). Y is the oven
– dry weight of the sample (in g).

Calculating the amount of total condensed tannin

The experiment was conducted UV-Vis spectrophotometer. Extract liquor was ready by stirring 0.05 g of Fe_2SO_4, 95 mL N-butanol, and 5 mL HCl (35%). Condensed tannin calculation, 0.01 g of both plant extract and mimosa tannin was put separately in a test tube and then 10 mL of extract solution was added and placed in a water bath for heating one hour. The absorbance was measured at 580 nm wavelength (Karaogul et al., 2016c; Yavuz et al., 2023).

Quantitative phenolic and flavonoid compound analysis by LC-MS/MS

A total of 27 active substances were measured in *S. aegyptiaca* L. during the study. The method contains the linearity ranges of the researched standard compounds as well as the rectilinear regression equations. It was discovered that correlation coefficients were higher than 0.99. The reported analytical method's limits of detection (LOD) and quantitation (LOQ) were displayed. LOD and LOQ for the substances under study varied from 0.05 to 25.8 and 0.17 to 85.9 g/L, respectively. Additionally, the phenolic compound recoveries ranged from 96.9%to 106.2%. The equation below was used to calculate the conclusion (Ertas et al., 2014a; Ismael, B.Q et al., 2019).

Quantification of compound (µg/g)= $RxU^f \div 100$
Where,
R is the result from LC-MS/MS (µg), U^f is the percent relative uncertainty at 95% confidence level (%)

DPPH radical scavenging activity

Blois (1958) used the DPPH method to examine root extracts of *S. aegyptiaca* L. that had been dissolved in ethanol and methanol. For the study's ethanol and methanol extractions, 0.1 mM DPPH was prepared. Following that, methanol and ethanol were combined with 0.1, 0.2, and 0.3 mL of the sample solvents until a total of 3 mL had been used. One mL of DPPH was then added to each after that. The mixture was vigorously mixed before being left at room temperature for 30 minutes. A Shimadzu UV-Vis 1240 spectrophotometer was used to detect the absorbance at 517 nm. As a reference, butylated hydroxyltoluene (BHT) was used. The following equation was used to quantify radical scavenging activity as the sample's free radical inhibition percentage (Gülçın et al., 2003; Göçeri A et al., 2022).

Inhibition of DPPH radical scavenging activity

$$(\%) = \frac{A-B}{A} X100$$

Where
A is the absorbance of DPPH,

B is the absorbance in the presence of the sample and BHT.

Anti-microbial activity
Microorganisms

The experiment utilized four fungi and seven bacteria. Microorganisms were supplied by the Biology Department's Microbiology Lab at Kahramanmaraş Sütçü University in Turkey. Microorganism information is given in Table 1. (Karaogul et al., 2016a; Göçeri et al., 2020).

Disc diffusion assay

Antimicrobial activity was performed using seven bacteria and four fungi (Table 1). By using the disc diffusion method, *Salix* root extracts in ethanol, methanol, and water were tested individually against each type of organism (CLSI, 2022/M100). The dextrose agar from Mueller-Hinton and Sabouraud was sterilized using an autoclave at 121°C for 15 minutes. One each of the 29 sanitized petri plates, the medium was introduced aseptically. Each disc was soaked in 100 µl of plant extract before being permitted to dry. Discs are then placed on an MHA medium, which contains 10 µl of bacteria. A 20 µl suspension of fungus was used to inoculate the SDA medium. 10 µ ml^{-1} gentamicin and ampicillin were used as the positive controls (6 mm in diameter), whereas ethanol, methanol, and water were used as the negative controls. Additionally, 100 µl of a 10 mm-diameter Nystatin unit/disc was employed for antifungal activity. For each test, plates were incubated for 24 hours at 37 degrees and 48 hours at 25 to 27 degrees. The bacteria and fungi activities were assessed by measuring the inhibition zones (Karaogul et al., 2016a; Göçeri et al., 2020). All tests were performed in triplicate.

Table 1. Microorganism information
Çizelge 1. Mikroorganizma bilgileri

Organism code	Organisms	Type	Source
B2	*Bacillus megaterium*	Gram(+)bacteria	DSM32
B11	*Klebsiella pneumoniae*	Gram(-)bacteria	FMC5
B13	*Escherichia coli*	Gram(-)bacteria	DM
B19	*Pseudomonas aeruginosa*	Gram(-)bacteria	DSM50071
B20	*Staphylococcus aureus*	Gram(+) bacteria	Cowan1
B25	*Micrococcus luteus*	Gram(+) bacteria	LA2971
B29	*Bacillus subtilis*	Gram(+) bacteria	IMG22
M1	*Candida albicans*	Fungi	ATCC10231
M2	*Candida utilise*	Fungi	NRRL-Y-900
M3	*Saccharomyces cerevisiae*	Fungi	WET136
M4	*Yarrowia lipolytica*	Fungi	NCIM3589

Statistical analyses

Descriptive statistical methods, such as mean and standard deviation, were used to analyze each result. The statistical program used is Anova SPSS statistical software version 18 (SPSS Inc. Chicago, USA). The mean differences were either found to be significant ($p < 0.05$) or non-significant ($p > 0.05$).

RESULTS and DISCUSSION
Calculation of yield

Table 2 lists the yield percentage results for *S. aegyptiaca* L. The ASE process was used in methanol to provide the highest yield (17.2%). While using the traditional method, water had the lowest yield (9.1%). The findings were confirmed by earlier research published by Anokwuru (2011), which found that methanolic extract produced the maximum yield (17.23%). It was determined that the best method among the extraction methods studied was the ASE.

Total condensed tannin

Table 3 shows the total condensed tannin content of *S. aegyptiaca* roots. A standard calibration curve was used to determine the tannin concentration (R^2=0.999), with values ranging from 6.25 mg/L to 50 mg/L and listed in Table 4. According to the triple measurements, 35.14 mg/L was the average total condensed tannin concentration. The water-soluble antioxidant tannins have a molecular weight of 500-3000 g/mol. Tannins are naturally occurring phenolic compounds found in vegetables, seeds, and fruits. Tannins are frequently used in the production of wine as stabilizers to do things like balance wine color, stop certain enzymes from contaminating grapes, and work as wine-fining substances (Sanz et al., 2008). According to their findings, *Salix sachalinensis* leaves had a condensed tannin concentration of 8.2±0.79 mg g^{-1}. According to Otthudi (2005), tannins exhibit antifungal properties. Its ability to connect with the cell walls of fungus or interact with extracellular and soluble proteins may be the cause of its activity. These chemicals have the potential to sever fungus membranes (Tsuchiya et al., 1996).

Identification of *S. aegyptiaca* root methanol extract compounds by LC-MS/MS

Methanol was used to create the *S. aegyptiaca* root extract, and 27 components (three non-phenolic acids,

ten phenolic acids, and fourteen flavonoids) were analyzed by LC-MS-MS. The chemicals were examined using negative ionization modes in this investigation. The analysis results are shown in Table 5 below. These results were different from previous studies. Because in other studies, salicylic acid was obtained as the main compound (Karawya et al., 2010; Rabbani et al., 2010; Cooper et al., 2014). However, these results conformed with the results of Zhang (Zhang et al., 2014). The findings also revealed increased concentrations of tr-aconitic and malic acids (Table 5).

Table 2. Yield percentage of *S. aegyptiaca* L.
Çizelge 2. S. aegyptiaca L.*'nin verim yüzdesi*

Methods	Solvents	Yield (%)	
		Mean[1]	SD[2]
Conventional extraction	Water	9.1	±0.1
	EtOH	10.6	±0.15
	MeOH	11.7	±0.6
Microwave extraction	Water	10.4	±0.1
	EtOH	12.03	±0.6
	MeOH	12.6	±0.15
Accelerated solvent extraction	EtOH	15	±0.1
	MeOH	17.2	±0.15

EtOH: ethanol, MeOH: methanol, [1]Values presented as mean ± SD of three measurements, [2]SD: Standart deviation

Table 3. Condensed tannin of *S. aegyptiaca* L. Roots
Çizelge 3. S. aegyptiaca L. kökü'nün kondense taneni

Salix aegyptiaca L.	Condensed Tannin (mg/L)			Average	Standard deviation	Variation (V)
	35.32	34.85	35.25	35.25	35.14	0.71

Table 4. Standards for mimosa tannin calibration
Çizelge 4. Mimoza tanen kalibrasyonu için standartlar

Mimosa tanin calibration	
Concentration	Absorbance
6.25	0.094
12.5	0.179
25	0.365
50	0.75

The time of collection, plant parts, genesis, extract methods, and solvents could all have an impact on the outcomes (Huang et al., 2005). A high amount of tannic acid (555 µg/g) and a low amount of tr-caffeic acid (8.7 µg/g) were detected in the roots of plants respectively. A substantial amount of salicylic acid (204.9 µg/g) was found in the methanol extract, but no rosmarinic acid was detected. The outcome is shown in Table 5. The research of Bravo (1998), Enayat (2009), Sonboli (2010), and Enayat (2013) supports these findings. The highest hyperoside (275.3 µg g^{-1}) and the lowest coumarin (0.63 µg g^{-1}) compounds were detected respectively. Chrysin, however, was not discovered in the roots of *S. aegyptiaca* L. These substances exist and their results are consistent with other experiments (Qin et al., 2005; Nahrstedt et al., 2007).

Radical scavenging activity in DPPH

The free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), which absorbs characteristically at 517 nm and is purple, is significantly reduced upon exposure to radical scavengers by the contribution of hydrogen atoms or electrons. The extract's best ability to scavenge free radicals can be seen by its absorbance at 517 nm (Taslimi et al., 2017; Gulcin et al., 2023). The best antioxidant mechanism by which lipid oxidation is inhibited is known as free radical scavenging. This work provides a means for serially assessing a compound's or extract's ability to scavenge free radicals and is a popular examination of antioxidant activity (Amarowicz et al., 2004). Table 6 shows the BHT values along with the DPPH radical scavenging activity of the *S. aegyptiaca* L. root extracts made using various extraction methods. The two extracts with the highest and lowest DPPH radical scavenging activity were found to be the ethanol extract by microwave extraction method at 0.3 µl concentration (0.0162 mg mL^{-1}) and the methanol extract by rapid solvent extraction method at 0.1 µl concentration (0.0264 mg/mL). As shown in Table 6, all extracts produce butylated hydroxyltoluene (BHT) in DPPH tests. The presence of polyphenolic chemicals may be the cause of *S. aegyptiaca* L.'s DPPH radical scavenging activity. Because they donate hydrogen atoms, phenolic substances are known to have antioxidant properties (Ho et al., 1994). Due to their scavenging or antioxidative properties (Hatano et al., 1989), phenols are important components of herbs (Duh et al., 1999).

Table 5. *S. aegyptiaca* L. roots' methanol-extracted quantitation by LC-MS/MS
Çizelge 5. S. aegyptiaca L. Köklerinin methanol ekstresinin LC-MS/MS miktarı

No	Chemical Compounds	RT[a]	Parent ion (m/z)[b]	Limit of detection/Limit of quantification (µg/L)e	If	Quantitation µg/g extract
1	Quinic acid	3.3	191	22.3/74.5	4.8	63895
2	Malic acid	3.5	133	19.2/64.1	5.3	38020
3	tr-Aconitic acid	4.1	173	16/52	4.9	816
4	Gallic acid	4.2	170	5/16	5.1	183.91
5	Chlorogenic acid	5.4	353	7.3/24.3	4.9	29.93
6	Protocatechuic acid	5.6	153	26/86	5.1	287
7	Tannic acid	6.4	183	10.2/34.2	5.1	555
8	tr- caffeic acid	7.3	179	4.4/15	5.2	8.7
9	Vanillin	8.7	151	10.1/34	4.9	441
10	*p*-Coumaric acid	9.5	163	15.2/51	5.1	221
11	Rosmarinic acid	9.57	359	10.4/35	4.9	N.D
12	Rutin	10.1	610	17/57	5.0	52.4
13	Hesperidin	9.6	611.1	22/72	4.9	67.94
14	Hyperoside	10.4	463.1	12.4/41.4	4.9	275.3
15	4-OH Benzoic acid	11.7	137	3/10	5.2	201.3
16	Salicylic acid	11.7	137	4/13.3	5.0	204.9
17	Myricetin	11.9	317	10/33	5.9	28.4
18	Fisetin	12.6	285	11/36	5.5	4.83
19	Coumarin	12.5	147	9.1/30.4	4.9	0.63
20	Quercetin	14.4	301	2/7	7.1	19
21	Naringenin	14.6	271	3/9	5.5	105.2
22	Hesperetin	15.2	301	3.3/11	5.3	0.83
23	Luteolin	15.4	285	6/19.4	6.9	4.4
24	Kaempferol	15.4	285	2/7	5.2	8.6
25	Apigenin	17.3	269	0.1/0.3	5.3	1.12
26	Rhamnetin	18.9	315	0.2/1	6.1	0.7
27	Chrysin	21.1	253	0.05/0.17	5.3	ND

RT[a]: Retention time, Parent ion (m/z)[b]: Molecular ions of the standard compounds, R2[c]: Coefficient of determination, RSD[d]: relative standard deviation, U[f] (%): Percent relative uncertainty at 95% confidence level, Values in µg/g (w/w)[g] of plant methanol extract, ND: not detected.

Table 6. DPPH scavenging activities in root extracts of *S. aegyptiaca* L.
Çizelge 6. S. aegyptiaca L.'nin kök ekstraktlarının DPPH aktiviteleri

Methods	Solvents[1]	DPPH radical scavenging activity (%)					
		Extract volume (mL)			BHT volume (mL)[2]		
		0.1	0.2	0.3	0.1	0.2	0.3
Conventional[3]	MeOH	98.5	97.9	97.5	90.4	92	90
Microwave[4]	MeOH	98.7	98.3	98.5	90.4	92	90
ASE[5]	MeOH	98.8	98.3	97.6	90.4	92	90
Conventional[6]	EtOH	98.5	98.2	97.5	67.4	66	78.3
Microweve[7]	EtOH	98.4	97.9	97.5	67.4	66	78.3
ASE[8]	EtOH	98.5	98.5	98.1	67.4	66	78.3

[1]MeOH: methanol, EtOH: ethanol, ASE: Accelerated solvent extraction,[2]53 mg/L, [3]0.0183 mg/ml,[4]0.0188 mg/ml,[5]0.0264 mg/ml,[6]0.0162 mg/ml,[7]0.0183 mg/ml,[8]0.0231 mg/ml

With ethanol and methanol extracts and increasing DPPH radical concentrations, the DPPH radical scavenging activity of *S. aegyptiaca* L. roots increased. The experiment by Sulaiman et al. (2013) obtained the same results. The results showed that in methanol extract with microwave extraction, DPPH radical scavenging at 0.2 µl was greater than 0.3 µl but less than 0.1 µl. This was supported by earlier research (Enayat et al., 2009; Sonboli., 2010). The presence of significant levels of phenolic chemicals such gallic acid, quinic acid, malic acid, chlorogenic acid, caffeic acid and salicylic acid may be the cause of the *S. aegyptiaca* L. root's considerable antioxidant activity in methanol and ethanol extracts (Li et al., 2000; Zheng et al., 2001;

Mokbel et al., 2005; Proestos at al., 2005; Farah et al., 2006; Gorzalczany et al., 2008; Shabir et al., 2011; Zhang et al., 2013).

Salix aegyptiaca's antimicrobial activities

Antimicrobial capacity is the applicable reason for controlling microorganisms in contamination treatments and food degradation. There are plenty of experiments to indicate plant extracts' antimicrobial and biological activities. To assess the antibacterial properties of *S. aegyptiaca* L. roots extract, the disc diffusion method was used with ethanol, methanol, and water as solvents. The tests were carried out against 11 microorganisms, which included 3 Gram(-), 4 Gram(+), and 4 fungi (disc concentration: 25 mg/disc). The results are shown in Table 7. Additionally, Table 8 provides a summary of the impacts of synthetic antibiotic activity against bacteria. This study is the first to document the root extracts' antibacterial properties. The varying sizes of the inhibition zones for all of the actions against the organisms made the *S. aegyptiaca* L. extract statistically significant (p<0.05). Test results are shown in Table 7. The *S. aegyptiaca* L. inhibitory zones range from 11-24 and 12-26 mm for bacteria and fungi, respectively. Using the ASE technique, the highest inhibitory zone against *C. albicans* ATCC10231 was 26 mm. While *S. aureus* Cowan1 had a minimum inhibitory zone of 11 mm in a water extract using a traditional extraction method. Additionally, neither *C. utilis* NRRL-Y-900 nor *S. cerevisiae* WET136 had an inhibitory zone. The outcomes demonstrated that the *B. megaterium* DSM32 (24 mm) zone of inhibition was significantly decreased by the methanol extract of the roots made using the ASE method (Table 7). These results are better than those of Hussain et al. (2011) but lower than those of Dığrak et al. (2001). The experiment's utilization of different plant species, locations, heights, and plant parts may be the reason for this dissimilarity. The inhibitory zones against *S. aureus*, *E. coli*, *K. pneumonia*, and *M. luteus* that were produced by conventional and microwave water extracts were also validated by earlier research (Bonjar et al., 2004). The outcomes demonstrated that *M. luteus* LA2971 was impacted by all extracts, except water extract employing conventional extraction, with zone inhibition varying from 12 to 21 mm (Table 7). This outcome was superior to that of Ateş et al. (2003).

Additionally, ethanol and methanol extracts utilizing ASE and conventional extractions had higher inhibitory zones against *B. subtilis*, ranging from 16.3 to 22.3 mm. This outcome was better than Ayepola et al. (2008). According to Angioni (2006), who reported that the antimicrobial activity of plant extract varies from different searches conducted in various zones, the antibacterial activity of a plant extract can vary depending on the age of the plant, collection time, freshness, physical factors (water, temperature) and extraction process. This could be due to several things, including the influence of the temperature, the quality, quantity, and content of the extracted product, as well as different bacterial strain sources. However, with microwave extraction in methanol, and ethanol as well as in water extract with traditional extraction, the inhibitory zone values against *B. subtilis* were 13–15 mm (Ayepola et al., 2008). The antibacterial properties of *S. aegyptiaca* L. roots in methanol extracts were comparable to those found by Ali et al. (2010), against *S. Aureus*, *K. Pneumonia, E. coli*, *and P. aeruginosa*. The outcomes against *K.* Pneumonia, *E. coli*, and *S. aureus* in ethanol extracts using all extraction techniques were also comparable to those of Hussein et al. (2011). Whereas, the outcomes were better than that of Al-Kadum et al. (2008). The dissimilarity among results could be owing to solvents, plant species, and plant parts used in this investigation. Using organic solvents is better than using water for antimicrobial activity. Methanol and ethanol root extracts have shown powerful antimicrobial effects against all strains. According to some research, water is not as effective as other solvents, like methanol or ethanol, for the extraction of antibacterials (Parekh et al., 2005). The G(-) bacteria were shown to be more resistant than the G(+) bacteria in all of the outcomes (Palombo et al., 2011). That could be differences between bacteria's cell structures. Because of their exterior membrane, G(-) bacteria are thought to be more resistant (Kaye et al., 2004). When compared to Gentamicin 10 g/ml, *S. aegyptiaca* L. displayed poor antibacterial activity, however, it was effective against practically all bacteria when compared to Ampicillin 10 g/ml (refer to Table 8). The roots' antibacterial properties may be explained by the presence of more phenolic compounds such as salicylic acid, chlorogenic acid, 4-hydroxybenzoic and coumaric acid (Proestos et al., 2005; Shabir et al., 2011), rutin and caffeic acid (Coneac et al., 2008), quinic acid (Farah et al., 2006), gallic acid (Akiyama et al., 2001), malic acid (Mokbel et al., 2005). According to methanol extracts and the ASE technique, the maximum inhibition zone against *C. albicans* was 26 mm (Table 7).

Additionally, employing methanol and ethanol extracts for traditional and microwave extraction procedures, respectively, 12 mm of the *Y. lipolytic* inhibition zone was obtained. Furthermore, it was shown that *S. cerevisiae* and *C. utilis* had higher resistance to the plant extracts. Antifungal activity results are compiled in Table 7. The roots of *S. aegyptiaca* L. displayed varying levels of antifungal activity. As a result of employing the ASE approach, methanol extracts provided the maximum inhibition zone (26 mm) against *C. albicans*, according to the data.

Table 7. Root extract inhibition zones (mm) against microorganisms
Çizelge 7. Mikroorganizmalara karşı kök ekstraktı inhibisyon bölgeleri (mm)

Microorganisms		Inhibition zone (mm)[1]							
		Extraction							
		Conventional			Microwave			Accelerated solvent	
		Water	EtOH	MeOH	Water	EtOH	MeOH	EtOH	MeOH
B2	Mean	11.6	18	19	ND	16.3	17	19	24
	SD	±0.57	±0.6	±0.6	ND	±0.6	1	±0.6	1
B11	Mean	ND	15	14.6	11.3	15.3	15.3	16.3	17.3
	SD	ND	1	±0.6	±0.6	±0.6	±0.6	±1.2	±1.2
B13	Mean	ND	14.3	17.0	ND	14	13	16	18.6
	SD	ND	±0.6	±1.0	ND	±1.52	1	1	±0.6
B19	Mean	ND	17	16	ND	13.6	15.3	18	17.3
	SD	ND	1	1	ND	±0.6	±1.2	1	±0.6
B20	Mean	11	16	14.6	ND	14	17.3	15	19.3
	SD	0.0	±0.6	±0.6	ND	1	±0.6	±0.6	±1.52
B25	Mean	ND	18.3	16.0	12	14.3	12	19.3	21
	SD	ND	±0.6	1	1	±0.6	±0.6	±0.6	±1.73
B29	Mean	13	16.3	19.3	ND	15	15	19	22.3
	SD	0.0	±0.6	±0.6	ND	±0.6	1	±1.73	±1.52
M1	Mean	15.3	23.3	23.3	18	20.3	23.3	24.3	26
	SD	±0.57	±1.5	±0.6	±0.6	±0.6	±1.5	±1.5	±4.04
M2	Mean	ND	ND	ND	ND	ND	ND	ND	ND
	SD	ND	ND	ND	ND	ND	ND	ND	ND
M3	Mean	ND	ND	ND	ND	ND	ND	ND	ND
	SD	ND	ND	ND	ND	ND	ND	ND	ND
M4	Mean	ND	14	12	13	12	14.3	17	20
	SD	ND	±0.6	±0.57	±0.6	±0.6	±0.6	±0.6	±0.6

B2: *B. Megaterium*, B11: *K. Pneumoniae*, B13: *E. Coli*, B19: *P. Aeruginosa*, B20: *S. Aureus*, B25: *M. Luteus*, B29: *B. Subtilis*, M1:*C. Albicans*, M2:*C. Utilis*, M3:*S. Cerevisiae*, M4:*Y. Lipolytica*, ASE: A. solvent extraction, ND: not detected, EtOH: ethanol, MeOH: methanol, [1]The values presented as mean ± SD of three replications; Mean differences were statistically classified as significant ($p< 0.05$). P- value (0.00)

Table 8. Synthetic antibiotics' effects on microorganisms
Çizelge 8. Sentetik antibiyotiklerin mikroorganizmalar üzerindeki etkileri

Microrganism	Inhibition zone (mm)[1]		
	Ampicillin 10 µg/ml	Gentamicin 10 µg/ml	Nystatin/ units
B. megaterium	7±0.6	35±0.6	-
K. pneumoniae	-	44±2	-
E. coli	-	34.3±1.52	-
P. aeruginosa	6±0.0	34.3±1.2	-
S. aureus	6.3±0.6	36.3±1.52	-
M. luteus	9.3±0.6	35±1.15	-
B. subtilis	6.3±0.6	39±1.15	-
C. albicans	-	-	15±0.6
C. utilise	-	-	18±0.6
S. cerevisiae	-	-	14±0.6
Y. lipolytica	-	-	13±0.0

(–): no inhibition zone.[1]The values presented as mean ± SD of three replication

This did not line up with the findings of the Bonjar et al. (2004) study, which showed no inhibition of *C. albicans* growth. *Laurus nobilis* extract according to Dığrak et al. (2001), was ineffective against *C. albicans*. The plant species, extract type, and concentration all affect the extracts' antifungal activity. The inhibition zones for *C. albicans* with all investigated techniques for methanol and ethanol extracts ranged from 20.3 to 26 mm. According to Aneja et al. (2009) and Sulaiman et al. (2013), this was accurate. According to their findings, *Amomum subulatum* fruit extracts inhibited *C. albicans* (29.3 mm) growth. *E. arborea* and *M. piperita* extracts also demonstrated antifungal efficacy against *C. albicans* mm and *A. Niger* 18-23 mm, according to Ertürk (2006). The *C. albicans* and *Y. lipolytic* demonstrated moderate antifungal activity in the water extracts with traditional and microwave extractions 13–18 mm respectively. This outcome was somewhat comparable to that of Ertürk et al. (2006), who noted that *M. officinalis*, *P. nigrum*, *C. annual*, and *C. cyminum* extracts had antifungal activity against *C. albicans* with inhibition zones ranging from 10 to 16 mm.

Additionally, these findings showed that *C. utilize* and *S. cerevisiae* were more resistant to all extracts carried out using different techniques. Similarly, Bonjar et al. (2004) also supported this finding. Tested methanol and ethanol extracts had stronger antifungal effects than the common antifungal nystatin against *C. albicans* and *Y. lipolytica*. The outcomes of the water extract, however, were comparable to those of the synthetic medicine nystatin (Table 7). This could be explained by the presence of gallic acids (Akiyama et al., 2001; Panizzi et al., 2002) and quinic acid (Zhang et al., 2013) or phenolic and flavonoids compounds (Zidon et al., 2005). It could be the presence of chlorogenic, salicylic, caffeic, and 4-hydroxybenzoic acids. The antibacterial capabilities of each of the phenolic compounds found have been confirmed (Proestos et al., 2005).

CONCLUSIONS

Salix aegyptiaca L. Roots were examined for their chemical composition and biological properties. According to the latest information, it seems obvious that *S. aegyptiaca* has good chemical effects as well as (especially) antioxidant, and antimicrobial activity. It is also recommended that isolated chemicals discovered in this study be put to use in the creation of fresh antibacterial and antioxidant medications. These plant chemicals could possess anti-inflammatory and antioxidant properties, their potential to prevent oxidative stress and inflammation, which are major causes of disease, including cancer, needs further investigation. Because of these features, considerable biological and chemical investigations should be conducted on human metabolism and must be focused on future studies. Eventually, this research shows that *S. aegyptiaca* could be utilized in new medicinal drugs and supply the main information for further research on therapeutic plants. It would be recommended that *S. aegyptiaca* farming must be available place.

ACKNOWLEDGMENTS

The authors would like to thank Kahramanmaraş Sütçü İmam University Committee of Scientific Research Projects. This study is supported by: Kahramanmaraş Sütçü İmam University. Project Number: 2014/2-60 YLS

Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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