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Research Article

Determination of Phenolic Compounds in Willow (*Salix babylonica* L.) Leaf Extract

Soha A. Farag[1], Amira El-Keredy[2], Sally A. Abd El Gawad [1], Adel E. Abouzeid[1]

[1]Department of Animal Production, Faculty of Agriculture, Tanta University, Egypt
[2]Department of Genetics, Faculty of Agriculture, Tanta University, Egypt
* Correspondence: soha.farag@agr.tanta.edu.eg

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Abstract:

Willow (*Salix babylonica* L.) leaf extracts are rich in many bioactive compounds such as phenolic and flavonoid compounds. This study aimed to determine the chemical composition and the total phenolics and flavonoid content in the willow leaf extract. A chemical analysis of the willow leaf was performed. Total phenolics and flavonoids in the leaf extract of willow were determined spectrophotometrically. Separation and identification of phenolic compounds were determined by High Performance Liquid Chromatography (HPLC). The content of crude protein, ether extract, crude fibers, ash, and available carbohydrate were 11.6, 3.4, 23.5, 14.1, and 38.6 %, respectively. Total phenolic and flavonoid contents in leaf extract were 75.30 mg GAE/g and 29.6 mg CE/g dried extract, respectively. Gallic acid, chlorogenic acid, caffeic acid, syringic, coumaric acid, hydroxybenzoic, cinnamic acid, catechin, rutin, naringenin, and quercetin were detected in leaf extract, while the major bioactive compounds in the leaf extract were chlorogenic acid (2.51mg/g) and rutin (1.94 mg/g). It could be concluded that willow leaf extract was a rich source of natural phenolics and flavonoid compounds. Therefore, willow leaf extract can be used as an antioxidant agent in pharmaceutical products, antimicrobial, anti-inflammatory, food preservatives, and animal diets.

1. Introduction

Willow (*Salix babylonica* L.) belongs to the genus *Salix* and the Salicaceae family. The *Salix* genus includes about 330-500 species of different trees and more than 200 hybrid species (Isebrands and Richardson, 2014). Willow species are widely distributed in Asia, Europe, America, and Africa (Argus, 2007). Willow trees tolerate harsh conditions and grow quickly in tropical, semi-tropical, and temperate regions (Isebrands and Richardson, 2014). Willow trees reach a height of 6- 10 meters, and the leaf is often silver-colored, oblong, shaped, usually hairy on the underside, and the color of the leaf turns black when dried (Lauron-Moreau et al., 2015). The willow (*S. babylonica*) tree is a large tree with a broad head and drooping branches and its color is different from green to dark brown. It is also called the weeping willow (Bailey, 1975).

Willow is a global distribution genus that has been reported as a woody economic tree since 1800 (Stott, 2001). Willow is used for the production of fuel, charcoal, bio-energy production, and environmental applications, also it is used as landscape specimens and the production of basketry (Kuzovkina et al., 2008). Willow has antimicrobial activities, antioxidant properties, cholesterol-lowering properties (Seidavi et al., 2020), anti-inflammatory, analgesic, and antipyretic (Noleto et al., 2018). The chemical composition of *S. safsaf* leaf has shown 11.35% crude protein, 50.67% total carbohydrates, 14.51% ash, 2.89% total lipids, and 53.28 % total dietary fiber (Faid et al., 2021). Recent studies revealed the presence of many bioactive secondary compounds have therapeutic properties in the leaf and bark of willow that were not known before the 19th century, these secondary compounds such as phenolic acids, flavonoids, tannins, polyphenols, and salicylate compounds such as salicin, salgin, and salicylic acid (El-shazly et al., 2012; khan *et al.*, 2015). Phenolic compounds possess antioxidant, antimicrobial, and anti-inflammatory activities. Therefore, Phenolic compounds can be used in food preservatives, packaging, pharmaceutical, and cosmetic industries. Leaf extracts of *S. alba* include 29 phenolic compounds (Piatczak et al., 2020). El- 2015) reported that *S. mucronata* leaf extract contains Sayed et al. (high levels of tannins, sterols, total phenols, flavonoids, and cardiac glycosides. The total contents of phenolics and flavonoids in *S. babylonica* leaf extract were 10.88 mg GAE/ g dried extract and 11.4 mg QE/ g dried extract, respectively (Gligoric et al., 2019a).

Few data are available on the natural and medicinal properties of willow (*S. babylonica*) leaf. Therefore, the present research aimed to determine the chemical composition of willow leaf and the total content of phenolics and flavonoids in willow leaf extract.

2. Materials and Methods

Willow leaves were collected randomly from several trees in November from the region of the Nile River

in Dakahlia Governorate, Egypt.

2.1. Chemical analysis of willow leaf

The leaf sample was washed, and air dried at room temperature (20°C) for 4-5 days. The dried leaves were ground in an electric grinding machine and kept in clean and dark bags. moisture, crude protein, ether extract, crude fibers, ash and available carbohydrates in willow leaf were determined using the standard method recommended Association of Official Analytical Chemists (A.O.A.C, 2005).

2. 2. Extract preparation

The phenolic extract was prepared from willow leaf according to the method described by Rivero *et al*. (2012) with some modifications. Dried willow leaves (1 kg) were soaked in 8 liters of solvent consisting of distilled water and ethanol (70,30, v/v) and kept at room temperature for 48 hours. The mixture was incubated in a water bath at 40°C for 60 minutes. The extract was filtered through cheesecloth followed by the Whatman No 1 filter paper. Thereafter, the solvent was evaporated by a rotary evaporator (Buchi Rotavapor R-200, Essen, Germany). Then, the resulting extract was lyophilized in a lyophilizer (FDF 0350, Humanlab Inc., Bucheon-si, Gyeonggi-do, Korea). The lyophilized sample was stored at -20 °C until use.

2.3. Determination of total phenolic contents in lyophilized leaf extract,

Total phenolic content was determined spectrophotometrically (Agilent 8453117 UV-Visible Spectroscopy System). The total phenolic contents in lyophilized leaf extract were determined using the Folin-Ciocalteu reagent, following the methods of Singleton et al. (1999) and Dewanto et al. (2002). One mg of lyophilized extract was dissolved in one ml methanol (%) and 500 µl of the dissolved sample was added to 0.5 ml of distilled water and 0.125 ml of Folin-Ciocalteu reagent. After shaking and letting stand for 6 minutes, 1.25 ml of 7% Na_2CO_3 was added to the mixture. After adjusting the solution with distilled water to a final volume of 3 ml, the mixture was thoroughly mixed. After incubation in the dark for 30 minutes, the absorbance at 650 nm was read versus the prepared blank. A standard curve was plotted using different concentrations of gallic acid (standard, from 0-1000 µg/ml). Total phenol content was expressed as gallic acid equivalent (GAE) /g of dry weight. The calibration equation for gallic acid was Y = 0.001x - 0.141

$R^2 = 0.998$

Where,

Y= Absorbance

x= Concentration of gallic acid (mg GAE/g extract)

R^2= Correlation coefficient.

2.4. Determination of the total flavonoids in lyophilized leaf extract

The total flavonoid content of lyophilized leaf extract was determined spectrophotometrically (Agilent 8453117 UV-Visible Spectroscopy System) by a modified colorimetric method according to Sakanaka et al. (2005). Catechol is used as a standard at concentrations of 20–200 µg/ml. Standard solutions (250 µg) were mixed with 1.25 ml distilled water and 75 µl of 5% sodium nitrite ($NaNO_2$) solution, followed by the addition of 150 µl of 10% aluminum chloride ($AlCl_3$) solution 5 min later. After 6 min, 0.5 ml of sodium hydroxide (NaOH) and 0.6 ml of distilled water were added. At 510 nm wavelength the absorbance was measured. Total flavonoid content was expressed as catechol equivalent (CE) and calculated using the following linear equation,

Y =0.004 X - 0.012

$R^2 = 0.999$

Where,

Y= Absorbance

X= Concentration of catechol (mg CE /g extract).

R^2= Correlation coefficient.

2.5. Separation, identification and quantification of phenolic compounds of lyophilized leaf extract.

Separation, identification, and quantification of phenolic compounds of the lyophilized extract were determined by High Performance Liquid Chromatography (HPLC) according to Croci *et al.* (2009). 12 phenolic standards have been used for phenolic compounds (gallic acid, chlorogenic acid, catechin, caffeic acid, syringic acid, rutin, coumaric acid, vanillic acid, naringenin, querectin, hydroxybenzoic acid and cinnamic acid). Analyses were conducted using HPLC (Agilent 1260 Series, California, USA). The separation was performed using a C18 column (4.6 mm x 250 mm., 5 µm). The mobile phase consisted of water (A) and 0.02% tri-floroacetic acid in acetonitrile (B) at a flow rate of 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows, 0 min (80%A); 0–5 min (80% A); 5-8 min (40% A); 8-12 min (50% A); 12-14 min (80% A) and 14-16 min (80% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 20 µl for each of the sample solutions. Compounds were identified by comparing their retention times and UV–Vis spectra with those of the standards, while their concentrations were calculated depending on the area under the peak of the standards.

3. Results and Discussion

3.1. Gross chemical composition of willow (*S. babylonica*) leaf,

The chemical composition of the willow leaf is

shown in Table (1). The content of dry matter, crude protein, ether extract, crude fiber, ash, and Carbohydrates were 91.2, 11.6, 3.4, 23.5,14.1, and 38.6 %, respectively.

Table (1): Gross chemical composition of willow leaf.

Component	Concentration (%)
Dry matter	91.2
Crude protein	11.6
Ether extract	3.4
Crude fibers	23.5
Ash	14.1
Avail. Carbohydrates	38.6

These results were in harmony with the findings of Abu Hafsa et al. (2014) who determined the chemical proximate of leaf and stems of *S. tetrasperma* as 91.86 % organic matter, 26.11 % acid detergent fiber, 3.26 % ether extract, and 12.25 % crude protein. Also, Basyony *et al.* (2018) detected 15.9% cellulose, 5.3% hemicellulose, and 38.7% acid detergent in the leaf and stems of willow *(S. safsaf*). In addition, Faid et al. (2021) reported that *S. safsaf* leaf contains 11.35% crude protein, 50.67% total carbohydrates, 14.51% ash, 2.89% total lipids, 53.28 % total dietary fibers, and 14.83 % soluble dietary fibers.

3. 2. Total phenolic and flavonoid contents

Phenolic and flavonoid contents in lyophilized leaf extract are listed in Table (2). The content of total phenolics in lyophilized leaf extract was 75.30 mg gallic acid equivalents (GAE)/g dried extract. The amount of total flavonoids in lyophilized leaf extract was 29.6 mg catechol equivalents (CE) /g dried extract.

Table (2): Phenolic and flavonoid contents in lyophilized leaf extract.

Items	Concentration
Total phenolic (mg GAE/g)	75.3
Total flavonoids (mg CE /g)	29.6

GAE= Gallic acid equivalents, CE= catechol equivalents

Data on the total phenolic and flavonoid content of lyophilized leaf extract present in this study revealed high levels of them. The presence of phenolic compounds in medicinal plant scavenge the free radicals by donating electron or hydrogen atom due to the presence of phenolic hydroxyl groups (Casquete et al., 2015). Also, the leaf and stem of *S. tetrasperma* contain 83.2 g/kg phenolic compounds (AbuHafsa et al., 2014), while *S. safsaf* contains 8.32% phenolic compounds (Basyony et al., 2018) and *S. alba* leaf and stem extract contain 153.75 mg GAE / g total phenolic (Zabihi et al., 2018).

The phenol and flavonoids contents in *S. mucronata* leaf extracted with 85% methanol, 70% methanol, and distilled water gave 131.39 mg GAE /g and 67.69 mg RE /g, 129.92 mg GAE /g and 62.65 mg RE /g, and 89.49 mg GAE /g and 28.72 mg RE /g, respectively (El-Sayed et al., 2015). The amount of total phenolic and flavonoid in *S. safsaf* leaf extract was 12.35 mg GAE /g and 8.67 mg QE /g, respectively (Faid et al., 2021). The total content of phenolics and flavonoids in species of the genus *Salix* leaf extract was 10.26–87.06 mg GAE/g dried extract and 11.4–32.82 mg QE/g, respectively (Gligoric et al. 2019a). The variations between our findings and other previously published data can be related to environmental and growth conditions, extraction solvent polarity, species and the age of the plant (Förster et al., 2010).

3.2. Identification and Quantification of phenolic compounds,

Phenolic compounds of lyophilized leaf extract are listed in Table (3). Quantification phenolic compounds in lyophilized leaf extract of the present study were as follows, gallic acid (0.24 mg/g), chlorogenic acid (2.51 mg/g), caffeic acid (0.01mg/g), syringic acid (0.12 mg/g) coumaric acid (0.16 mg/g), hydroxybenzoic (0.31 mg /g), and cinnamic acid (0.39 mg/g). While, flavonoid compounds detected in willow leaf extract were catechin (0.87 mg /g), rutin (1.94 mg /g), naringenin (0.20 mg /g), and querectin (0.65 mg /g), while vanillic acid was not detected in leaf extract.

Table (3): Phenolic compounds identified and quantified in lyophilized leaf extract.

Component	mg/g lyophilized extract
Phenolic acids	
Gallic acid	0.24
Chlorogenic acid	2.51
Caffeic acid	0.01
Vanillic acid	ND
Syringic acid	0.12
Coumaric acid	0.16
Hydroxybenzoic acid	0.31
Cinnamic acid	0.39
Flavonoids	
Catechin	0.87
Rutin	1.94
Naringenin	0.20
Querecetin	0.65

ND = Not detected (Below the detection limit)

Our results of identified phenolic compounds are

compatible with that observed by Gligoric et al. (2019a) who reported that the content of gallic acid, chlorogenic acid, hydroxybenzoic acid, syringic acid, epicatechin, coumaric acid, rutin, and tranc-cinnamic acid in *S. babylonica* leaf extract were 0.20, 1.62, 0.46, 0.20, 1.55, 0.13, 2.05, and 0.25 mg/g of leaf, respectively. Meanwhile the values in case of extraction of *S. caprea* leaf by maceration with 60% ethanol for 24 hours were changed to 0.077 g/100g gallic acid, 0.100 g/100 g chlorogenic acid, 0.015 g/100 epicatechin, 0.259 g/100 g drug rutin, 0.113 g/100 g naringenins, 0.096 g/100 g vanillic (Gligoric et al., 2019b). *S. Safsaf* leaf extract contains gallic acid (9.25 mg/100g), chlorogenic acids (5.41 mg/100g), cinnamic (6.15 mg/100g), naringin (7.35 mg/100g), rutin (10.49 mg/100g), and catechin (3.58 mg/100g) (Faid et al., 2021). However, the amount of gallic acid, chlorogenic acid, vanillic acid, epicatechin, naringenin, cinnamic acid, coumaric acid in species of the genus *Salix* leaf extract ranged from 0.060 - 0.111, 0-0.019, 0.0002 to 0.017, 0.0004–0.087, 0.091–0.330, 0.0008–0.09, and 0.020 g/100 g of dried extract, respectively (Gligoric et al., 2019a). The differences between the current data and those reported in the literature may be due to the influence of genetic and environmental factors.

4. Conclusions

Phytochemical analysis of willow (*Salix babylonica*) leaf extract revealed that leaf extract is a rich natural source of phenolics and flavonoid compounds. Willow leaf extract contains phenolic compounds such as gallic acid, chlorogenic acid, caffeic acid, syringic acid, coumaric acid, hydroxybenzoic, cinnamic acid, catechin, rutin, naringenin, and quercetin. Therefore, willow leaf extract can be used as an antioxidant agent in pharmaceutical products, antimicrobial, anti-inflammatory, and food preservative, and could be supplemented in animal diets.

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