Research Article

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ESTIMATION OF SALICIN IN BARKS AND LEAVES OF SALIX SPECIES BY A TLC-SPECTROPHOTOMETRIC METHOD

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Abstract

Barks and leaves of most Salix species yield a medicinally active glycoside called salicin. To determine the quality of herbal drugs obtained from Salix plants, salicin content of stem-barks and leaves of Salix acmophylla Boiss., Salix aegyptica L., Salix alba L., Salix babylonica L., Salix carmanica Bornm, and Salix excelsa Gmelin, which are growing in Isfahan, was determined.

Two spectrophotometric methods were used in this research. In one method, which is proposed in this work and which is called combined TLC-spectrophotometric method, salicin was separated by using Thin Layer Chromatography technique (TLC), then the intact glycoside was determined spectrophotometrically.

In the other method, called combined TLC-colorimetric method, freshly prepared Erdmann's reagent was used to produce a colored complex with the separated salicin. Then the intensity of the produced color was determined.

The results of both applied methods proved that:

1) The proposed TLC-spectrophotometric method is more sensitive than the TLC-colorimetric one.
2) Stem-barks of all examined species contain more salicin compared to the salicin content of leaves of the same plants.
3) Stem-barks of Salix carmanica Bornm contain the highest quantity of salicin when compared to the salicin content of stem-barks and leaves of other five species examined in this work.

INTRODUCTION

Salicin, [2- (Hydroxymethy) phenyl-ß-D-glucopyranoside], is an alcoholic glycoside occuring in species of the genera Salix and Pouplus (Family: Salicaceae)[1,2]. It has antirheumatic, antipyretic and analgesic actions and has been used similarly to aspirin in doses of 0.3 to 1gm.[2,3]. Recognition of the properties of salicin clarifies many folkloric uses of poplar and willow barks.[2]

Since 1909, when salicin was known[4], some investigations have been done dealing mainly with its isolation and estimation. Because of its polarity, water, boiling water, ethyl alcohol, and different mixtures of ethyl alcohol and water have been usually used for its isolation[5].

The quality of herbal remedies containing vegetable drug preparations of dried Salix can be determined by the total content of salicin[6]. In contrast to most of the other medicinal plants, Salix species used in phytotherapy are not well defined. Several species are quite poor in salicin.

Generally, the bark of Salix alba L. and other species of Salix, notably S. fragilis L., S. purpurea L. and S. pentandra L., is proposed for use as herbal remedy[7]. On the other hand, leaves of many species produce a high yield of salicin, too[6]. From a scientific point of view, there is no reason to avoid the production of vegetable drug preparations obtained from other Salix species.

Estimation of salicin was carried out by different methods. The gravimetric method, which is

rather macro and non-sensitive was used by some investigators[8]. Other methods were also used for the determination of salicin based on determining glucose resulting from its hydrolysis[9]. However, the colored materials which are usually found in plant extracts interfere with the glucose[10].

Some investigators used p-nitroaniline as a color reagent after hydrolyzing with snail enzyme at 46° C[11]. A combined TLC-colorimetric method, using freshly prepared Erdmann's reagent, under specific conditions as a color reagent was also reported[12]. However, the intensity of absorption is dependent upon many factors, including temperature, pH, and time of reaction[13]. GLC[14] and HPLC[6] methods for the estimation of salicin based on its separation on suitable columns were also reported.

In this work, as salicin absorbs intensely in the UV region and can be analyzed spectrophotometrically without changing it to a complex, a TLC-spectrophotometric method for its estimation has been proposed. On the other hand, the combined TLC-colorimetric method was also used for estimation of salicin in the same samples for comparison.

MATERIALS AND METHODS

Materials

Plant Materials: Stem-barks and leaves of six Salix species (i.e: Salix acmophylla L., S. aegyptica L., S. alba L., S. babylonica L., S. carmanica Bornm, and S. excelsa Gmelin.) growing in Isfahan were collected on May 23, 1992. Identity of the samples was confirmed in the Botany Section of Biology Department, Faculty of Sciences, University of Isfahan. The collected samples were air-dried at room temperature and then reduced to powder.

Authentic Salicin: This was purchased from Riedel-de Haen, Germany.

Reagent

Erdmann's reagent: Freshly prepared by adding 10 drops of a mixture of 10 drops of nitric acid and 100 ml of water, to 20 ml pure concentrated sulfuric acid.

Apparatus

Perkin-Elmer Spectrophotometer (UV-VIS), 550 SE, Perkin-Elmer, Germany.

Estimation of Salicin

1. TLC-spectrophotometric method:

One part (10gm) of each powdered leaves and barks of Salix acmophylla L., S. aegyptica L., Alba L., S. babylonica L., S. carmanica Bornm., and S. excelsa Gmelin. was exhaustively extracted by maceration with five parts of 70 percent ethanol for 25hr. Maceration was affected at the first hour on a shaker (200 rpm), and then the mixture was left aside at room temperature for 24hr. Each maceration mixture was filtered, and the filtrate was evaporated under reduced pressure, at a temperature not exceeding 50℃ to about 20 ml. The evaporated extract was then purified by being treated with 10 ml of 10 percent solution of lead acetate, filtered, then excess of lead acetate was removed from the filtrate by addition of 2ml of 10 percent aqueous solution of disodium hydrogen phosphate and again filtered. The filtrate was evaporated to dryness under reduced pressure at a temperature not exceeding 50℃ and the obtained residue of each sample was dissolved in 96 percent ethanol, then enough 70 percent ethanol was added to get a final volume of 20 ml.

A 0.1 ml (equivalent to 0.05gm powdered sample) aliquot of each extract was quantitatively applied as a narrow band on a preparative silicagel HF plate (3 mm in thickness) alongside with a drop of 0.2 percent salicin as a pilot spot. The plates were developed with the solvent system chloroform: methanol: n-butanol: formamide (20: 2: 4: 0.2) for about 12 cm. The developed plate was dried at room temperature then the peripheral pilot spot was located by spraying Erdmann's reagent, while the other part of the plate was protected by a glass sheet. Developed spot areas, present at the level of the pilot spot were separately scraped by means of a spatula. The powdered silicagel obtained from each area was transferred quantitatively into a centrifuge tube and exhaustively eluted with 95 percent ethanol. The solution was then centrifuged, and its volume was adjusted to 10 ml with 95 percent ethanol. Absorbance of the obtained solution was measured at the λmax of salicin (i. e: 269 nm). The percentage of salicin in each sample was

deduced from a pre-established standard curve, and the mean of results of three determinations of each sample was calculated.

To establish the standard curve, aliquot portions of ethanolic solution of salicin, containing 200 to 1600 μgm salicin, were spotted by means of a micropipette on preparative silicagel HF plates (3mm in thickness) and salicin was determined as described before. For each concentration, three determinations were carried out, and when mean absorbances were plotted against corresponding concentrations, a straight line, obeying Beer's law was obtained.

2. TLC- colorimetric method:

In this method, the same primary steps were followed as in the previous one, but after centrifugation, the volume of each cluant was adjusted to 5ml with 95 percent ethanol, cooled in ice, then treated with 5ml freshly prepared Erdmann's reagent. The resulting color reached its maximum intensity after 20 min and remained for 90 min.

Absorbance of the colored complex was measured at its λmax (i. e: 510 nm). Here again, the percentage of salicin in each sample was deduced from a pre-established standard curve, and the mean of results of three determinations for each sample was calculated.

RESULTS AND DISCUSSION

Results of determined percentage of salicin by both TLC-spectrophotometric and TLC-colorimetric methods are shown in Tables 1 and 2 respectively. As it is notable, our proposed method is more sensitive when compared with the TLC-colorimetric method, since the results obtained from our method show significantly (at $P<0.01$) higher amounts of salicin. This may be due to the elimination of colored complex formation step from our method.

The determination of salicin in stem-barks and leaves of six Salix species growing in Isfahan (Tables 1 and 2) revealed that the stem-barks of all species contained higher amounts of salicin than the leaves of the same plants. On the other hand, stem-barks of Salix carmanica Bornm. contained the highest quantity of salicin (4. 106 percent) among all other barks (Figure 1). While, leaves of Salix excelsa Gmelin. contained the highest amount of salicin (1.192 percent) compared with the examined leaves of other species.

Table 1. Absorbance and the calculated percentage of salicin in stem-barks and leaves of six Salix species when TLC-spectrophotometric method was used.

Name of plant	Absorbance of salicin		Percentage of salicin	
	barks	leaves	barks	leaves
Salix acmophylla Boiss.	0.412	0.073	1.672	0.265
Salix aegyptica L.	0.827	0.171	3.752	0.769
Salix alba L.	0.192	0.094	0.413	0.373
Salix babylonica L.	0.462	0.135	1.878	0.584
Salix carmanica Bornm.	0.898	0.192	4.106	0.881
Salix excelsa Gmelin.	0.612	0.253	2.538	1.192

- Each result shows the mean of three determinations.

Table 2. Absorbance and the calculated percentage of salicin in stem-barks and leaves of Salix species when TLC-colorimetric method was used.

Name of plant	Absorbance of salicin		Percentage of salicin	
	barks	leaves	barks	leaves
Salix acmophylla Boiss.	0.164	0.036	1.186	0.142
Salix aegyptica L.	0.312	0.088	2.393	0.567
Salix alba L.	0.051	0.048	0.263	0.240
Salix babylonica L.	0.171	0.070	1.245	0.420
Salix carmanica Bornm.	0.329	0.111	2.532	0.749
Salix excelsa Gmelin.	0.204	0.126	1.514	0.872

- Each result shows the mean of three determinations.

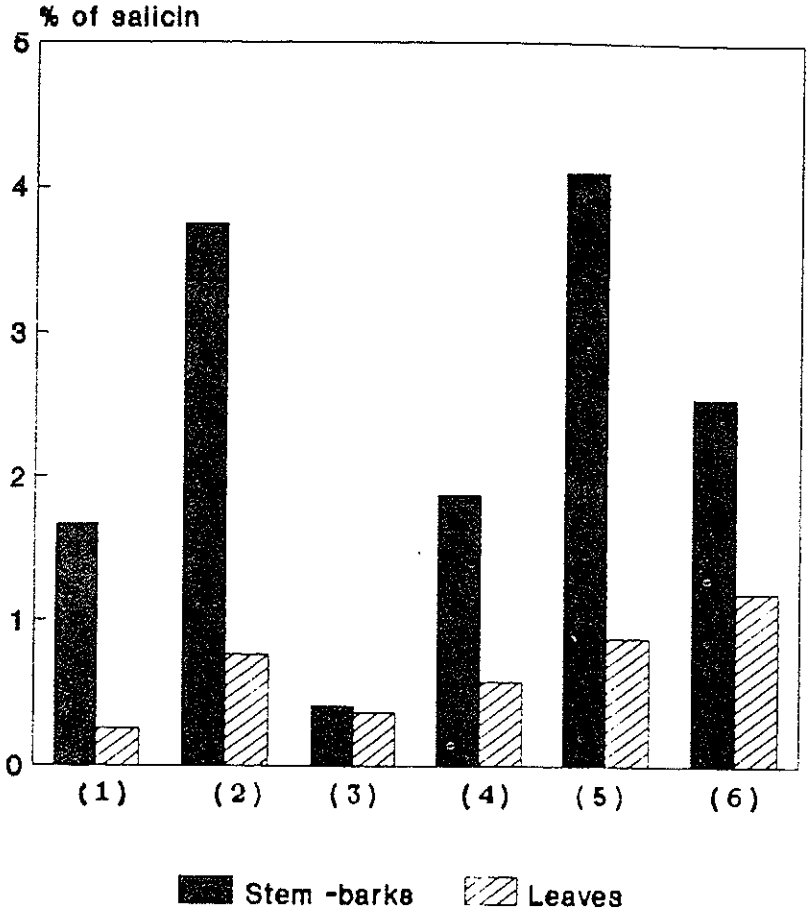

Figure 1. Percentage of salicin glycoside in stem-barks and leaves of different Salix species growing in Isfahan when TLC-spectrophotometric method was used.
[(1)S. acmophylla, (2)S. aegyptica, (3)S. alba, (4)S. babylonica, (5)S. carmanica, (6)S. excelsa.]

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