Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(6):3158-3163

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Analysis of Phenolic Compounds in Extracts of *Ziziphus spina-christi* using RPHPLC method

Anwar O. Ghafoor[1], Hemn K. Qadir[2] and Nabil A. Fakhri[3]

[1]*Department of Field Crops, College of Agriculture, University of Salahaddin, Kurdistan Region – Iraq*
[2]*Hawler Medical University, College of Pharmacy, Iraq*
[3]*Department of Chemistry, College of Education/ Scientific Departments, University of Salahaddin, Erbil, Kurdistan Region – Iraq*

ABSTRACT

Ziziphus spina-christi is a medicinal plant with many traditional uses. Separation, identification and quantification of the constituents of the plant are of great interest. Phenolic compounds are present in such plants. The present study describes isolation, qualification and determination of some of these phenolic acids using reversed phase HPLC after the purification of the compounds by passing the plant extract through sephadex LH-20 column. p-Coumaric acid, rutin, apigenin, quercetin, chlorogenic acid and syringic acid were found and isolated in the methanolic extract of the stem, in which rutin content was found at higher concentration (325.0 mg /100g) and apigenin (122.90 mg/100g). Ferulic acid, rutin, p-hydroxybenzoic acid and chlorogenic acid were found and isolated in the extracts of fruit of Ziziphus with maximum rutin content (15.88 mg/100 g). It seems that the phenolic acid contents in stem are higher than that in fruits.

Keywords: *Ziziphus spina-christi*, phenolic compounds, HPLC.

INTRODUCTION

Ziziphus spina-christi locally known as Sidr is a multipurpose tree species belonging to the botanical family Rhamnaceae. Traditional herbal remedies have a long and popular usage in treatment of pain related ailments. It is an important cultivated tree and one of the few truly native tree species of Arabia that is still growing along with many newly introduced exotic plants [1]. Sidr has been used in folk medicine as demulcent depurative, anodyne, emollient, stomachic for toothaches astringents and as a mouth wash. The decoction of bark and fresh fruits is used to promote the healing of fresh wounds and also used as a body wash, while fruits are used for dysentery [2, 3]. The Ziziphus species (Rhamnaceae) are commonly used in folklore medicine for the curing of various diseases. They are wide-spread in the Mediterranean Region, Africa, Australia and tropical America [4].

Numerous investigations have shown high-performance liquid chromatography (HPLC) is one of the most important methods for separating of the complex mixture of the extracts of such plants. The high efficiency of this method and the broad possibilities of the selection of suitable conditions of analysis (sorbent, eluent, method of detection) permit the separation and isolation of many chemicals and numerous isomers present as constituents of these plants. UV and fluorimetric detectors are used most frequently in the HPLC of alkaloids, while electrochemical and refractometric method is used more rarely. In use, a wavelength is selected at which all the component of interest in

the sample absorb. During the elution of the sample in quantitative analysis it is undesirable to change the wavelength setting [5, 6].

The objective of this study is using HPLC for separation and determination of the alkaloid composition of two parts of plant extracts; stems and fruits of (*Z. spina-christi*).

EXPERIMENTAL SECTION

Chemicals

The standard chemicals such as rutin , p-coumaric, ferulic acid, apigenin, quercetin, p-hydroxybenzoic acid, chlorogenic acid and syringic acid and all other chemicals were from (Merck), (Suvchem) and (Youngling industry) companies.

Apparatus

All chemical analysis and extraction were carried out using HPLC instrument with auto-sampler (KOREAN YOUNGLIN INSTRUMENTS 2007), Software(YOUNGLINAutochro-3000 chromatography data system), acm 9000 vacuum degasser and mixer pump, luna column ODS(C18)100R (250×4.6 mm /5Micron), acm9000 Ultra Violet detector with a detection at 280nm .

Plant material

Fresh stems and fruits of *Ziziphus spina-christi* were collected from Iraqi Kurdistan Region- Erbil city center. The samples were freshly obtained.

Chemical extraction
(General Procedure)

A fresh sample of 10 gm of each the stem and fruits was weighed accurately and 150ml of absolute methanol was added to each sample. The resulting mixtures were Soxhlet extracted for 12 hours, then the solution was filtered with filter silite 545 (20-45µL) under vacuum. The resulting filtrates were passed though Sephadex LH-20 column and evaporated under vacuum by rotary evaporator at 60^{o}C to dryness. The resulting dried samples were redissolved in 25 ml of %95 ethanol and acidified to pH 2.0. The samples were ready for HPLC analysis using C18 column with a flow rate 1.0 ml / min. and using a mixture of (butanol: acetic acid: water) in a ratio of (14: 1: 85) as mobile phase [7,8].

RESULTS AND DISCUSSION

The separation and identification of the phenolic compounds was carried out using reversed-phase HPLC with C18 column and detecting at 280 nm.

Figs. (1-6) represent the chromatograms of the standard phenolic acids (p-coumaric acid, ferulic acid, rutin, apigenin and chlorogenic acid) with retention times 9.217, 7.283, 7.400, 7.400 and 9.340 min respectively. While the Figs 7 and 8 represent the chromatograms of the stem and fruit samples respectively, comparison of the chromatograms of the samples with that of the standards of the phenolic compounds, indicate the presence of the p-coumaric acid, rutin, apigenin, quercetin and chlorogenic acid in the extract of the stem and presence of ferulic acid, rutin, p-hydroxybenzoic acid and chlorogenic acid in the extract of the fruits depending upon the comparison of the retention times of the standards of phenolic compounds and the constituents present in the samples.

The response factor can be used for the determination the concentration of unknown samples, RF (sometimes called a sensitivity factor) can be determined for each standard as follows:

$$RF = \text{standard area} / \text{standard concentration}$$

The response factor (RF) can be used to calculate the sample concentration as follows:

$$\text{Sample concentration} = \text{sample area} / RF$$

If two or more standards are measured (at different concentrations), RF can be calculated as the average value of

response factors of all standards [9].

Depending upon the measurement of the area under peak, the concentrations of the phenolic compounds were determined as shown in Table (1).

Table (1): Concentration of some phenolic acids present in stem and fruit samples.

Standard	Concentration mg/100gm	
	Stem	**Fruit**
Rutin	325.0	**15.88**
apigenin	122.90	**9.45**
p-coumaric acid	25.30	**0.00**
quercetin	**18.08**	**0.00**
Chlorogenic acid	21.70	**12.35**
syringic acid	27.59	6.05

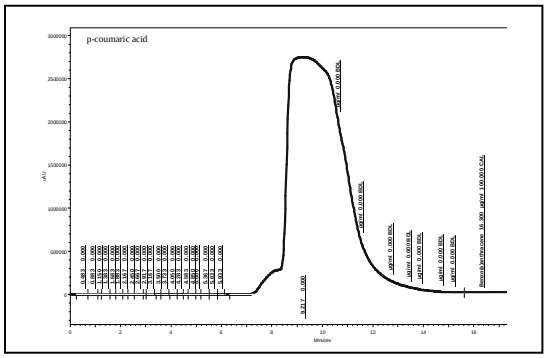


Figure (1): Standard HPLC chromatogram of p-coumaric acid with retention time (9.217) minute

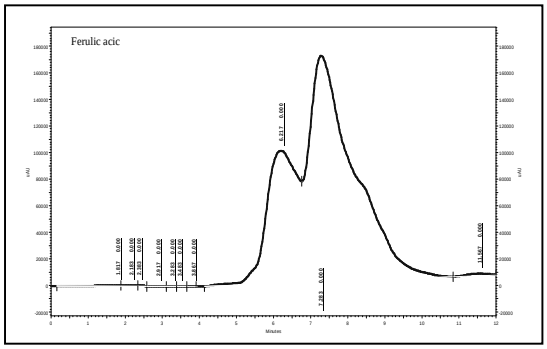


Figure (2): Standard HPLC chromatogram of Ferulic acid with retention time (7.283) minute

Figure (3): Standard HPLC chromatogram of rutin with retention time (7.400) minute

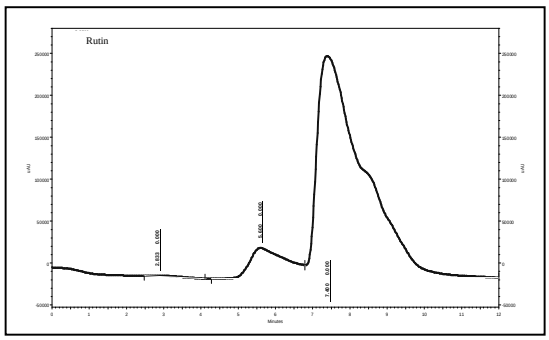


Figure (4): Standard HPLC chromatogram of Apigenin with retention time (7.400) minute

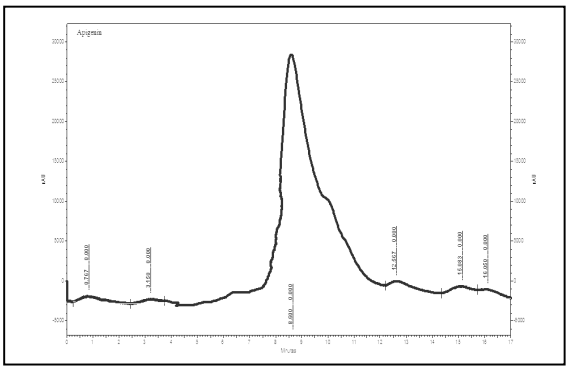
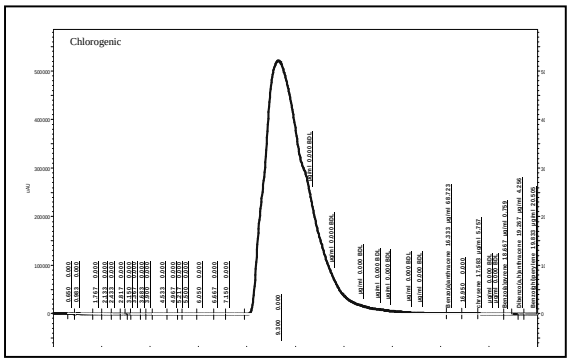


Figure (5): Standard HPLC chromatogram of Chlorogenic with retention time (9.3400)



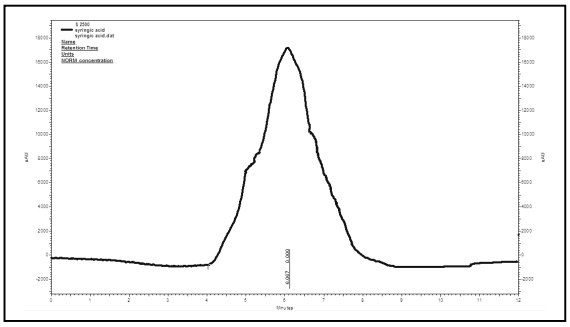


Figure (6): Standard HPLC chromatogram of Syringic acid with retention time (6.067).

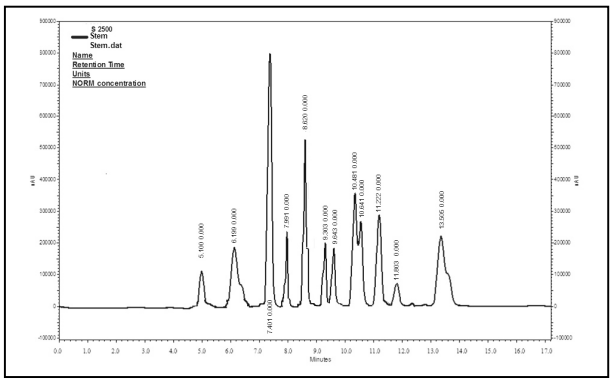


Figure (7): HPLC chromatogram of stem sample.

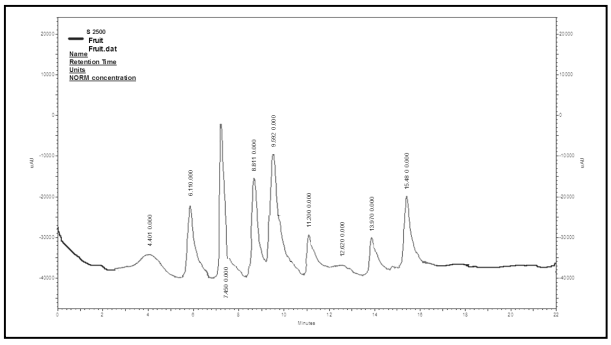


Figure (8): HPLC chromatogram of fruit sample.

CONCLUSION

From the mentioned Figs. and Table (1) it seems that there are differences in the type and concentration of the phenolic acids present in the stem and fruit samples of *Ziziphus spina-christi*

REFERENCES

[1] CC Townsend; E Guest; Flora of Iraq, Ministry of Agriculture and Agrarian Reform- Iraq, **1980**,Volume 4, Part 1, 429- 437.
[2] A M Waggas; R H Al-Hasani; *American-Eurasian Journal of Scientific Research*, **2009**, 4 (4), 263-267.
[3] B Adzu; A Haruna; *African Journal of Biotechnology*, **2007**, 6 (11), 1317-1324.
[4] H Motemedi; A Safari; S Maleki; S.M. Seyyednejad, *Asian Journal of Plant Science*, **2009**, 8(2), 187-190.
[5] BD Kabulov; AL D'yakonov ; SV Zalyalieva, *Chemistry of Natural Compounds*, **1991**, 27(5), 521-540.

[6] JD Fair; CM Kormos; *J Chromatogr*., **2008**, A12 (2), 49-54.
[7] JA Adamovics. Chromatographic Analysis of Pharmaceuticals, 2nd Edition, Marcel Dekker Inc., New York, **1997**, 1- 427.
[8] JP Wolff; FX Mordret; A Dieffenbacher, *Pure & Applied Chem*., **1991**, 63(8), 1173-1182.
[9] LR Synder; J Kirkland; JL Glaich; Practical HPLC Method Development, 2nd Edition, Wiley –Interscience Inc., New York, **2000**, 1-745.