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# PHYTOCHEMICAL STUDY OF THE LEAVES OF ZIZIPHUS SPINA-CHRISTI L.WILLD

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## ABSTRACT

*Investigation of the leaves of Z.spina-christi L. Willd resulted in the isolation and identification of ceryl alcohol, B-sitosterol, ursolic acid, betulinic acid, fatty acids (myristic, stearic, oleic, linoleic, linoleic and arachidic acids),flavonoidal compounds (taxifolin, dihydrokaempferol , taxifolin-3-O-glucoside and apigenin-7-O-glucoside). In addition, a chromatospectrophotometric method was adopted for quantitative estimation of the flavonoidal content.*

## INTRODUCTION

Several plants of Ziziphus genus (Rhamnaceae) have been used as crude drugs for the cure of biliousness, chronic bronchitis, as analeptic and expectorant.[1,2] It has also been reported that the seeds of Z.vulgaris var. spinosus are effective in treating insomnia and nervous breakdown[3,4]. Wood et al[5,6] attributed the sedative action of the extract of the abovementioned plant to the flavone-C-glycoside and the saponin content.

Ziziphus spina-christic L. Willd is an indigenous large tree with popular edible fruits[7].

The available literature dealt with several species of Ziziphus growing in India and Iran, reported on the isolation and identification of sterols, triterpenes, saponins, flavonoids and cyclopeptide alkaloids.[8,9,10] Z.spina-christi growing abroad was reported to contain B-sitosterol, n-nonacosane, octacosanol, B-sitosterol glucoside, octacosanyl behenate[11] and alkaloids[12,13]. Ikram and Tomlinson isolated betulic and ceanothic acids from the above ground parts of Z.spina-christi[14].

It was therefore deemed interesting to investigate the constituents of the plant growing in Egypt, stressing in the present work on the isolation and characterization of sterols, triterpenes and flavonoidal compounds present in the leaves.

## EXPERIMENTAL

### Plant Material:

The leaves of Z.spina-christi L. Willd were collected in March 1980 from plants growing in different places at Assiut. The identity of the plant was kindly confirmed by Dr. A.Fayed, Assistant Professor, Dept. of Botany, Faculty of Science, University of Assiut. Samples collected were air-dried then reduced to fine powder.

### Preliminary Phytochemical Screening:

The preliminary phytochemical screening of the leaves of Z. spina-christi L. Willd revealed the presence of sterols and/

or triterpenes, flavonoids, tannins, saponins, carbohydrates and/or glycosides and traces of alkaloids and/or nitrogenous bases.

Extraction and Purification:

The air-dried powdered leaves of Z.spina-christi L. Willd ( 2 kg) were successively extracted with pet.ether (b.r. 60-80°C), chloroform and ethanol 70%. Each extract was separately concentrated under reduced pressure and investigated as follows:

Study of the Pet.ether Extract:

The pet.ether extract of the leaves was screened on silica gel G (E. Merck) plates using benzene-ethyl acetate (4:1) solvent system and ethanolic sulfuric acid for location of spots (after heating at 110°C for 5 min.)

Only 9 spots were located, three of them were relevant to those of ceryl alcohol ($R_f$ 0.85), B-sitosterol ($R_f$ 0.56) and ursolic acid ($R_f$ 0.41).

Isolation and Identification:

The pet.ether extract (20 g) was fractionated on column of neutral alumina (Prolabo) and eluted with benzene, benzene-ethyl acetate gradient. Four crystalline substances assigened A,B,C and D were obtained.

Substance A:

The crystals (100 mg) m.p. 65-67°C, were obtained from fractions 13-24 eluted with benzene-ethyl acetate (95:5). $R_f$ values, IR and m.m.p. of the isolated material and authentic ceryl alcohol were found to be identical.

Substance B:

The crystals (120 mg) obtained from fractions 32-49(benzene-ethyl acetate, 90:10) melted at 135-137°C and showed no depre-

ssion when mixed with authentic B-sitosterol. IR spectra and $R_f$ values of both authentic and isolated material were identical. It gave positive Lieberman-Burchard test.

Substance C:

The crystals (90 mg) obtained from fractions 75-88(benzene-ethyl acetate; 70:30) melted at 271-273°C. The IR spectra, $R_f$ values, and m.m.p. of the isolated material and authentic ursolic acid were identical.

Substance D:

The crystals (120 mg) obtained from fractions 95-110 (benzene-ethyl acetate, 50%) melted at 294-296°C and gave positive Lieberman-Burchard test.

The IR (KBr) showed absorption bands at 3440 $cm^{-1}$ (OH), 1705 $cm^{-1}$ (C = O), 1640, 1050 and 890 $cm^{-1}$. The mass spectrum showed m/e 456 ($M^+$), 441 ($M^+-CH_3$), 438 ($M^+-H_2O$) and 411 ($M^+$- COOH). NMR 90 MHz ($CDCl_3$) showed five singlets each of 3H(5 $CH_3$) at 0.82,0.99,1.01,1.04 and 1.73 ppm, 3.10 and 3.24 ppm. (2H,m,$C_3$-$C_{19}$-H), 4.63 ppm (1H,d,$C_{29}$-H).

The above data (m.p., IR, mass and NMR) are in well agreement with the published data of betulinic acid[15]

Fatty Acids:

The lipids in the pet.ether extract (10 g) were subjected to saponification[16,17], the liberated fatty acids were methylated and the resulting methyl esters were analyzed by GLC adopting the following operating conditions:

Column/coiled glass, 2 m long, 5 mm i.d., packed with 10% polyethlene glycol adipate on chromosorb P (60-80 mesh); column temp. 190°C, injection port temp. 220°C; detector (FID).

temp. 250°C; nitrogen flow rate, 60 ml/min; hydrogen flow rate, 50 ml/min; air flow rate, 400 ml/min.

Qualitative identification was based on the relative retention time of the resolved peakes with respect to oleic acid and comparing with authentic samples.

Quantitative analysis was based on the internal normalization method using the peak area measured by triangulation. The results are shown in Table 1.

Study of Flavonoids:

A- Chloroformic Extract:

TLC screening of the chlorofomic extract of the leaves on silica gel G plates using chloroform-methanol-formamide (80:19:1) and UV, UV/$NH_3$ and aluminium chloride in ethanol spray reagent (heating the plate at 100°C for 5 min.),revealed the presence of two spots "$Z_1$" ($R_f$ 0.63) and "$Z_2$"($R_f$ 0.55).

Isolation and purification of the two compounds was achieved by using column chromatography (silica gel for column E. Merck) eluted with chloroform-methanol gradient in 20 ml fractions. As a result, dihydrokaempferol "$Z_1$"(150 mg) m.p. 226-8°C was obtained from fractions 30-38 eluted with chloroform-methanol (92:8) and taxifolin "$Z_2$"(170 mg) m.p. 237-9°C was obtained from fractions 45-51 eluted with chloroform-methanol (90:10).

The m.p., m.m.p. and UV data (Table 2) were found identical with those of dihydrokaempferol and taxifolin[18,19] respectively.

B- Alcohol Extract:

TLC screening of the alcoholic extract on silica gel G plates using ethyl acetate-methanol-water (100:16.5:13.5) revealed the presence of two spots ("$Z_3$" and "$Z_4$"), having

$R_f$ values 0.58 and 0.51 respectively.

The alcoholic extract was chromatographed on cellulose column and eluted with chloroform-methanol gradient where two flavonoidal glycosides: "$Z_3$" (250 mg) and "$Z_4$"(200 mg) were obtained from fractions 42-48 ($CHCl_3$:$CH_3OH$,75:25) and 55-60 ($CHCl_3$:$CH_3OH$, 65:35) respectively.

**"$Z_3$" Taxifolin-3-O-glucoside:**

Yellowish crystals, m.p. 145-7°C, purple colour in UV/$NH_3$. UV spectral data are listed in Table 2.

**Acid Hydrolysis:**

Compound "$Z_3$" (10 mg) was refluxed with 50 ml of 10% HCl for 3 hours. The aglycone was extracted with ether, while the sugar moiety in the hydrolysate was examined by PC using n-butanol-pyridine-water (6:3:4) as solvent system, spraying with aniline hydrogenphthalate.

The aglycone and the sugar moiety in the hydrolysate were found identical with taxifolin and glucose respectively.

The above data confirmed that "$Z_3$" is taxifolin-3-O-glucoside.

**"$Z_4$" Apigenin-7-O-glucoside:**

Yellowish-green crystals, m.p. 229-231°C, urple colour in UV and green in UV/$NH_3$. UV spectral data are listed in Table 2.

Acid hydrolysis revealed apigenin as the aglycone and glucose as the sugar.

The above data confirmed that "$Z_4$" is apigenin-7-O-glucoside.

**Quantitative study:**

**Estimation of total flavonoids calculated as taxifolin-3-O-glucoside[20]:**

The air-dried powdered leaves of Z. spina-christi L. Willd (P) (10 g) were defatted with pet.ether, then extracted with methanol. The methanolic extract was then subjected to PC colourimetric assay[20] measuring the colour at 329 nm.

The percentage of total flavonoids calculated as taxifolin-3-O-glucosides was found to be 0.25 g% w/w

## DISCUSSION

Phytochemical screening of the leaves of Z.spina christi L. Willd revealed the presence of sterols and/or triterpenes flavonoids, tannins, carbohydrates and/or glycosides and traces of alkaloids and/or nitrogenous bases.

Neumerous reports[12,13] dealt with the isolation and identification of Bsitosterol and betulinic acid, But no reports were found in the available literature dealing with the isolation of ceryl alcohol, ursolic acid nor with the fatty acid composition which reported here.

Four flavonoidal compounds were isolated in crystalline form and identified as dihydrokaempferol, taxifolin, taxifolin 3-O-glucoside and apigenin-7-O glucoside. It is to be noted that two of these flavonoids were isolated for the first time from the genus Zizphus; these are dihydrokaempferol and apigenin 7-O-glucoside. The percentage of the flavonoids was determined using the chromatospectrophotometric method[20]

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Table 1: Fatty acid composition of the lipids of the leaves of Z.spina-christi L. Willd

| Peak No. | Fatty acids | r* | % of fatty acids |
|---|---|---|---|
| 1 | Myristic | 0.38 | 10.34 |
| 2 | Stearic | 1.22 | 12.96 |
| 3 | Oleic | 1.00 | 28.20 |
| 4 | Linoleic | 1.91 | 15.36 |
| 5 | Linolenic | 2.30 | 30.00 |
| 6 | Arachidic | 2.36 | 3.03 |

r* = retention time relative to that of oleic acid.

Table 2: UV spectral data of the isolated flavonoids.

| Flavonoid | MeOH | NaOMe | $AlCl_3$ | $AlCl_3$/HCl | NaOAc | NaOAc/$H_3BO_3$ |
|---|---|---|---|---|---|---|
| $Z_1$ | 289 | 345 | 272(sh) | 280(sh) | 252(sh) | 295 |
| | | | 315 | 312 | 282(sh) | |
| | 330(sh) | 325 | 381 | 379 | 326 | 336(sh) |
| $Z_2$ | 289 | 245(sh) | 280(sh) | 312 | 285(sh) | 290 |
| | | | 310 | | | |
| | 325(sh) | 324 | 372 | 372 | 325 | 335(sh) |
| $Z_3$ | 290 | 245 | 236 | 285(sh) | 290(sh) | 292 |
| | | | 315 | 312 | | |
| | 315(sh) | 326 | 373(sh) | 377 | 329 | 353(sh) |
| $Z_4$ | 262 | 267 | 298 | 276 | 276 | 267 |
| | | 302 | | 300 | 357 | |
| | 332 | 385 | 383 | 384 | 384 | 336 |

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# دراسة فيتو كيميائية لاوراق نبات النبق
## (زيزيفس اسبينا - كريستى ل.)

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اشتملت هذه الدراسة على أجزاء المسح الكيميائى الاولى لاوراق نبات النبق (زيزيفس بينا - كريستى ل.) الذى أظهر احتواء الاوراق على مواد ستيرولية و/أو تربينات ثلاثية. مواد كربوهيدراتية و/أوجلوكوسيدية ، مواد فلافونية ، حمضيات وكميات قليلة من القلوانيات و/أو نيتروجينية.

أظهرت دراسة خلاصة الاثير البترولى باستخدام كروماتوجرافيا العمود وطرق التحليل الطيفية التعرف على كحول سيريل ، بيتاسيتوستيرول ،حمض ارسوليك وحمض بيتيولينيك أما الاحماض الدهنية فقد تمت دراستها باستعمال كروماتوجرافيا الغاز السائل ووجد أن اوراق النبات محل الدراسة تحتوى على احماض الميرستيك الاستياريك ، الاوليك ، اللينوليك و الاراشيديك،و أدت دراسة المواد الفلافونية الى فصل و التعرف على التأكسيفولين ،دايهيدروكامبيفيرول تاكسيفولين-٣-١-جلولكوزيد و ابيجينين-٧-١-جلوكوزيد بالاضافة الى تعيين النسبة المئوية للمواد الفلافونية.

ولاول مرة يتم فصل و التعرف على كحول سيريل ،حمض اورسوليك دايهيدروكامبفيرول و ابيجنين-٧-١-جلوكوزيد من اوراق نبات النبق.

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