Phenolic compounds from seeds of *Zizyphus spina-christi*

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Abstract

In this study, three phenolic compounds were isolated from the seeds of *Zizyphus spina-christi* for the first time. The compounds were identified by means of ^{1}H-NMR-, UV and GC-MS as *p*-hydroxybenzoic acid, kaempferol, quercetin-3-O-α-L-rhamnosyl-β-D-glucopyranoside (rutin).

Key words: Kaempferol**,** *p*-hydroxybenzoic acid, rutin, *Zizyphus spina-christi*
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Introduction

Increased attention has been paid to genus *Zizyphus* Family Rhamnaceae due to its significant medicinal uses viz hypoglycemic, hypotensive, anti-inflammatory, antimicrobial, antioxidant, antitumor, liver protective and improve the immune function (Nunes et al. 1987; Adzu et al. 2001; Borgi et al. 2007). Nowadays, the development of drugs from natural sources is recommended to overcome the side effects of many of the synthetic drugs. Recent research on medicinal plants has generated a great deal of information about the biologically active chemical components that are responsible for the claimed medicinal effects.

Zizyphus species (Rhamnaceae family) are commonly used in folklore medicine for the treatment of various diseases such as digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, loss of appetite, fever, pharyngitis, bronchitis, anaemia, diarrhea, and insomnia (Han and Park 1986; Kirtikar and Basu 1984). They are widespread in the Mediterranean region, Africa, Australia, and tropical America. Previous phytochemical studies on the different species of the genus Zizyphus led to the isolation and characterization of cyclopeptide alkaloids, flavonoids, sterols, tannins, and triterpenoid saponins (Ikram et al. 1981; Nawwar et al. 1984). Zizyphus spina-christi (L.) Willd is a wild tree, with spiny branches and small, orange–yellow fruits, commonly found in Jordan, Israel, and Egypt, known in Egypt as Nabq or Sidr (Taeckholm 1974),where it is used to treat the blisters, bruises, chest pains, dandruff, fractures, headache, and mouth problems (Ghazanfar 1994). The fresh leaves are applied on swollen eye at night (Taeckholm 1974). The roots are used to cure and prevent skin diseases (Dalziel 1937). The root bark infusion is used tradionally in northern Nigeria as a remedy for stomach pain and other gastrointestinal tract ailments. It has been used in folk medicine as demulcent, stomachic, for toothaches, as astringent and as mouth wash (Duke 1985). Fruits are used to promote the healing of fresh wounds, for dysentery, bronchitis, coughs and tuberculosis **(** Hutchens 1973). It is also used to relief digestive disorders, obesity, urinary troubles and as a potent anti-microbial agent (Shahat et al. 2001;

Nazif 2002). Zizyphus spina-christi (L.) Willd has many content of peptide and cyclopeptide alkaloids, flavonoids, sterols, tannins, butulinic acid and triterpenoidal saponin glycosides (Ikram et al. 1981; Higuchi et al. 1984; Nawwar et al. 1984; Han et al. 1990; Barboni et al. 1994; Abu-Zarga et al. 1995; Cheng et al. 2000; Shahat et al. 2001;Tripathi et al. 2001).

The present study deals with the investigation and identification of the chemical constituents in 70% methanol extract of the seeds of *Zizyphus spina-christi* L. (Willd).

Materials and methods:

Plant material:

Seeds of *Z. spina-christi* plant were collected from Orman garden, Giza, Egypt in April 2002. The plant was identified by Dr. Kamal El Batanony, professor of Taxonomy and Botany, Faculty of Science, Cairo University.

Isolation and Identification:

The air dried powder of *Zizyphus spina-christi* seeds (1 kg) was percolated with methanol 70% till exhaustion, then the extract was evaporated under reduced pressure to yield 89 g dried extract. The methanolic extract was suspended in water (300 ml) and partitioned successively with petroleum ether (60-80 °C) (6×400 ml), chloroform (6×300 ml), ethyl acetate (6×400 ml) and butanol (6×400 ml). Each fraction being dried over anhydrous sodium sulphate and concentrated under reduced pressure to give 15 g, 10 g, 12 g , 10 g and 30 g (petroleum ether, chloroform, ethyl acetate, butanol and aqueous dried extracts, respectively). Ethyl acetate fraction was screened by 2D-PC (two dimensional paper chromatography) using n-butanol-acetic acid-water (4:1:5,v/v/v) and acetic acid :water (15:85,v/v) as solvent systems and visualized under UV light before and after spraying with $AlCl_3$ and exposure to ammonia vapour. Three spots were visualized, at different R_f values. Ethyl acetate fraction was subjected to CC using silica gel (E. Merck, type 60-230 mesh, 200 g) as adsorbent and elution was carried out with chloroform-methanol mixtures of increasing polarity. Fractions (50 ml each) were collected and separately concentrated to a small volume. All fractions were screened by PC (Whatmann No. 1) using n-butanol-acetic acid-water (4:1:5,v/v/v) and acetic acid : water (15:85,v/v) as solvent systems; where similar fraction were pooled and the solvents were, separately, evaporated under reduced pressure.

- Elution with chloroform: methanol mixture (95:5) (fractions 8-13) afforded one compound which gave blue colour under UV light changing to bright blue with ammonia. It was purified on Sephadex LH-20 using methanol as eluent to afford compound 1.
- Elution with chloroform: methanol mixture (90:10) (fractions 16-23) afforded one compound which gave dull yellow colour under UV light, on exposure to ammonia vapour and with $AlCl_3$. It was purified on Sephadex LH-20 column using methanol as eluent to afford compound 2.
- Elution with chloroform: methanol mixture (80:20) (fractions 32-36) afforded one compound which gave dark purple colour under UV light changing to yellow on exposure to ammonia vapour and yellow colour with $AlCl_3$. It was purified on Sephadex LH-20 column using methanol as eluent to afford compound 3.

Results and Discussion:

Compound 1 exhibited blue fluorescence under UV light turning bright blue with ammonia and gave green colour with $FeCl_3$, R_f values 75 and 42 in solvent systems of *n*-butanol : acetic acid : H_2O (4 : 1 : 5) v/v/v and acetic acid 15%, respectively, indicating its phenolic nature (Harbone 1973) and suggesting *p*-hydroxy benzoic acid.

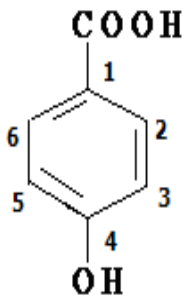
The UV spectrum of 1 exhibited absorption bands at λ 226 and 263 nm. On addition of NaOMe gave a bathochromic shift (Δλ = +13 nm) characteristic for phenolics.

The CI Mass spectrum of 1 gave a molecular ion peak $[M+1]^+$ (m/e 139, 100 %), ($M-CO_2$) 95, 19 %.

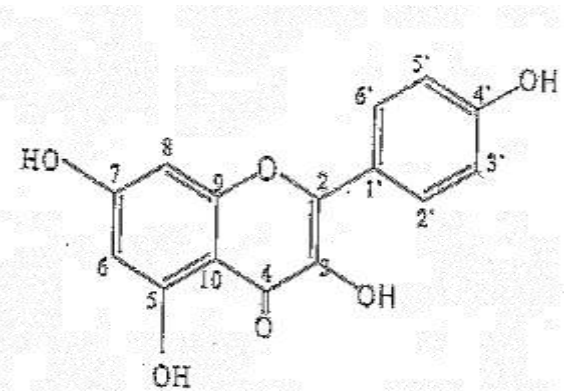
The ^{1}H-NMR spectrum of compound 1 exhibited two broadened doublet signals at δ 7.8 and 6.82 (J = 8.7 Hz) for H-2, 6 and H-3, 5 respectively.

From the above data and comparison with authentic sample, the isolated compound 1 was identified as *p*-hydroxybenzoic acid which is reported from *Z. Spina-christi* fort he first time.

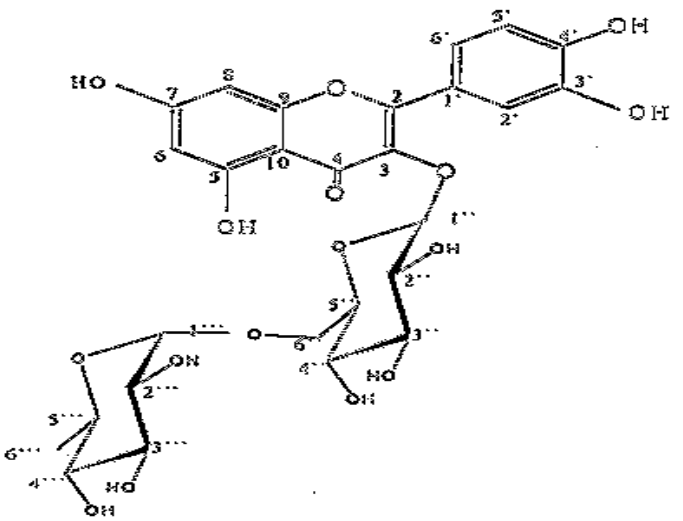
Compound 2 showed single spot with R_f values = 65 and 7 in solvent systems: *n*-butanol : acetic acid : water (4 : 1 : 5 v/v/v) and 15% acetic acid, respectively. It gave dull yellow colour under UV the colour doesn't change on exposure to ammonia vapour or with $AlCl_3$.



Kaempferol



p-hydroxybenzoic



Rutin

Figure 1. Compounds of Kaempferol, p-hydroxybenzoic and Rutin

The UV spectrum of compound 2 is characteristic for flavonol nucleus exhibiting absorption bands at λ 268 and 367 nm. On addition of NaOMe, a bathochromic shift in band I (Δλ = +50 nm), as well as an increase in intensity was observed, indicating a free OH at C4′. The bathochromic shift in band II (Δλ = +7 nm) on addition of NaOAc indicated a free OH group at position C-7. The lack of hypsochromic shift with HCl confirmed the absence of orthodihydroxy pattern in ring B
These data are in agreement with those reported for kaempferol (Mabry *et al*.,1970). In addition, comparison with an authentic sample, confirmed that the isolated compound 2 is kaempferol, previously isolated from *Z. spina-christi* leaves (Ali et al. 1984).

Compound 3 showed a single spot with R_f values = 32 and 44 in solvent systems *n*-butanol : acetic acid : water (4 : 1 : 5 v/v/v) and 15% acetic acid, respectively. It exhibited a dark purple colour under UV which is turned to yellow on exposure to ammonia vapour and $AlCl_3$.

The UV spectrum of compound 3 is characteristic for flavonol nucleus exhibiting absorption bands at λ 258 and 359 nm. Addition of NaOMe leads to a bathochromic shift in band I (Δλ = +51 nm), as well as increase in intensity indicating a free OH at C4′. Bathochromic shift in band II was shown on addition of NaOAc indicating free OH group at position 7. Hypsochromic shift with HCl confirmed the presence of orthodihydroxy pattern in ring B. These data are similar to those reported for quercetin-3-*O*-glycosidal compounds (Mabry et al. 1970).

Acid hydrolysis and paper chromatography of the hydrolysate of 3 showed that the aglycone was quercetin, while glucose and rhamnose constituted the sugar moiety.The ^{1}H-NMR spectrum of 3 showed the characteristic aglycone pattern of quercetin, in which two *meta* coupling protons resonated at δ 6.2 and δ 6.4 assigned for H-6 and H-8, respectively. It showed a doublet at δ 7.5 (*J* = 2.1 Hz) assigned for H-2′, and a doublet of doublet at δ 7.49 (*J* =2.1, 8.4 Hz) assigned for H-6′, while H-5′ appeared as a doublet at δ 6.7 (*J* = 8.4Hz). The doublet signal at δ 5.2 corresponded to anomeric glucose proton H-1′′ with β- linkage, while those at δ 4.3 (1H, H-1′′′), and sugar protons appeared from δ (4.3 to 3.3) and at δ1.1 (3 protons of methyl) revealed rhamnose with α-linkage. From above data and comparison with authentic sample, the isolated compound was identified as quercetin-3-O-α-L-rhamnosyl-β-D-glucopyranoside (rutin), which was isolated before from *Z. spina-christi* leaves and fruits (Shahat et al. 2001).

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