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Isolation and Identification of Shikonin From *Arnebia Decumbens* L. and its Antibacterial Activity

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Abstract: This study have been comprised three parts : first collected and taxonomy *Arnebia decumbens* L. from south of Iraq, part two isolation and purification Shikonin compound by column chromatography technique and identification it by many techniques, part three investigate antibacterial activity of shikonin. The chemical analysis results showed pure Shikonin compound. The results showed that the Shikonin exhibits greatest antibacterial activity, (50 mm) against *Pseudomonas aeruginosa*, (45 mm) against *Escherichia coli*, (40 mm) against *Staphylococcus aureus*, and (38 mm) against *Klebsiella pneumonia.*

Key words: *Arnebia*; decumbens; Shikonin; Antibacterial activity.

INTRODUCTION

Arnebia decumbanis L. related from Boraginaceae family Perennial growing to 0.3m. It is in flowering from June to August, and the seeds ripen from July to September. The flowers are hermaphrodite[1].

Arnebia species have many biological activities . The root of Its reported as Anti-inflammatory activity. The hexane extract of *Arnebia hispidissima* yielded a mixture of naphthaquinones: arnebin-1, arnebin-7, tiglic acid (ester of dihydroxy alkannin), alkannin, arnebinol and cycloarnebin-7[11].

Afzal and Al-Oreqat[1] investigated shikonin derivatives from *Arnebia decumbens* from Kuwait deserts and identified by NMR.

Chien-Chang *et al*.,[4] were study the Bioassay-directed fractionation of extract of *Arnebia euchroma* led to the isolation of alkannin (1), shikonin (2), and their derivatives and tested they against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). The derivatives of shikonin and alkannin showed stronger anti-MRSA activity than alkannin or shikonin. Some derivatives were also active against vancomycin-resistant *Enterococcus faecium* (F935) and vancomycin-resistant *Enterococcus faecalis* (CKU-17) with MICs similar to those with MRSA.

Singh *et al*.,[11] studied anti-inflammatory activity of shikonin derivatives from *Arnebia hispidissima* ethanolic extract, after chromatography, yielded a number of shikonin derivatives, which were identified as arnebin-5, arnebin-6, teracryl shikonin, arnebinone and acetyl shikonin. All these compounds were evaluated to the anti-inflammatory activity of ethanolic extract and isolated shikonin derivatives.

We chose this plant in this study because its exist in south of Iraq and no any study identified chemical compositions and investigated in biological activity.

MATERIALS AND METHODS

Plant Material: *Arnebia decumbanes* L. is abundantly available in Iraqi deserts. The plant material used in this study were collected from various locations in Basrah in March and April 2005, 2006, 2007 classified by dr. Ali Aboud.

Microorganisms and Media: The test organisms used in this study were as followed: Gram positive *Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli, Klebsiella pneumonia and Pseudomonas aerugenosa*).

These bacteria were obtained from the Bacteriological lab, Biology department , Education college, University of Basrah, Iraq.

Preparation of Plant Extract: The roots of *Arnebia decumbens* L. (50g) were air-dried and then powdered. The powder was extracted with hexane and evaporated invacuo and concentrated by high vaccum. The concentrated extract was purified by column chromatography on silica gel eluted with ethylacetate and spirit petrol (2:1).

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Antimicrobial Susceptibility Testing Hole Diffusion Method: Antibacterial activity tested against Gram-positive bacteria and Gram-negative bacteria by the hole agar diffusion method(Cappuccino and Sherman, 1998). The bacteria were grown on Nutrient agar media. Muller-Hinton agar media were poured into the plates to uniform depth of 5 mm and allowed to solidify.

The bacteria suspensions at 1 x 10^6 cfu ml^{-1} (0.1 light density on 540 nm wave length) were streaked over the surface of Mueller-Hinton agar media using a sterile cotton swab to ensure confluent growth of the organism. The holes made by cookporar , 6 mm in diameter.

100 µL aliquots of the sample 33.3% (v/v), which were then aseptically applied to the surface of agar plates at well-spaced intervals. The plates were incubated at 37 ºC for 24 h and then the inhibition zone diameters were measured.

Results:

Chemical Analysis Results:

Chemical Analysis Results of Shekonin:

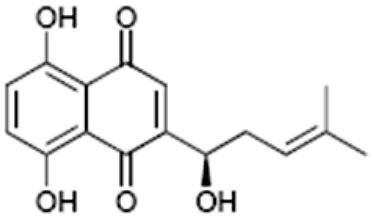

Fig.1: Shikonin structure

Nuclear Magnetic Resonance Spectroscope of Shikonin: The results of NMR spectroscope showe, ^{1}H-NMR ($CDCl_3$ - 500 MHz) δ_H: 12.50 (2H, s,$H_{4,5}$), 7-7.20 (Aromatic Hydrogen 3H, s,$H_{6,7,3}$), 7.00(2H, s,$H_{1,8}$), 5.10 (1H, s, t, H_{10}), 2.60 (2H, m, ,$H_{11,}$), 2.10(2H, m, $H_{2,9}$), 1.68 (2H, s,$H_{15,13}$), 1.60 (2H, s,$H_{16,12}$).

^{13}C-NMR($CDCl_3$ – 500MHz) δ_C: 181.00(C_1), 169.76(C_4), 167.00(C_5), 166.94(C_8), 148.49(C_2), 131.40(C_6, C_7), 136.10(C_3), 111.84(C_{10}), 111.59 (C_9), 132.86(C_{14}), 31.93 (C_{12}), 117.69(C_{13}), 26.63(C_{11}), 22.69(C_{15}) , 22.00(C_6) .

^{13}Cdept-NMR($CDCl_3$ – 500MHz) δ_C: 69.18(C_{15}), 32.85(C_3), 25.76(C_7), 22.35(C_{13}), 20.96(C_6), 17.96(C_{16}) (Morales-Rios *et al.*, 1988; Silverstein and Webster,1997).

Antibacterial Activity Results: The antibacterial activity of Shikonin against microorganisms examined in the present study and their potency were assessed by the presence or absence of inhibition zone diameter.

The results are given in table (4). The data of the study clearly indicated that the Shikonin has a strong antibacterial activity against all bacterial species *Klebsiella pneumoniae* (38 mm), *Escherichia coli* (45 mm), *Staphylococcus aureus* (40 mm), *Pseudomonas aeruginosa* (50 mm).

Discussion: Naphthoquinones compounds like shikonin has been shown to have many biological activity[6,8,9].

Hydrophobic compounds like shikonin are likely to have an influence on biological membranes.

The cytoplasmic membrane of bacteria has two principal functions: (I) barrier function and energy transduction which allow the membrane to form ion gradients that can be used to drive various processes, and (II) formation of a matrix for membrane-embedded proteins[12].

The results showed that the shikonin exhibit high activity against many bacterial species this agreement with previous study[11].

The activity of Naphthoquinones compounds increases with increasing lipophilicity of alkoxy group[3].

The higher antibacterial activities of shikonin might be due to the three hydroxy groups, which give the compound more activity[9]. In this study it is supposed that these compounds may scavenge free radicals (antioxidant activity)[5].

The shikonin may play an important role of the antibacterial activity via the inhibition of protein synthesis[10].

Moreover, the rings compounds acts as a protoplasm toxin to destroy the cell wall system and to precipitate protein in cells[7].

On the other hand, the hydroxyl group was supposed to have essential role in the antibacterial activity to assume another role to increase the activity[2].

Table 1: 500 MHz ^{1}H-NMR data of Shikonin in $CDCl_3$

Chemical shift (ppm)	Assessment ^{1}H
12.50	2H (OH)
7.20	3H- Aromatic
7.00	2H
5.10	1H
2.60	2H
2.10	2H
1.68	2H
1.60	2H

Table 2: 500 MHz ^{13}C-NMR data for Shikonin in $CDCl_3$

Chemical shift (ppm)	Assessment ^{13}C
181.00	1C
148.49	1C
136.10	1C
169.76	1C
167.00	1C
166.94	1C
132.86	1C
131.40	1C
117.69	1C
111.84	1C
111.59	1C
31.93	1C
26.63	1C
22.69	1C
22.00	1C

Table 3: 500 MHz ^{13}Cdept-NMR data for Shikonin in $CDCl_3$

Chemical shift (ppm)	Assessment ^{13}C
137.88	1C
121.22	1C
115.54	1C
114.33	1C
111	1C
55.88	1C
39.92	1C

Table 4: Inhibition zone diameter (mm) of Shikonin against all bacterial species

Bacteria	Inhibition zone
Escherichia coli	45
Pseudomonas aeruginosa	50
Klebsiella pneumoniae	38
Staphylococcus aureus	40

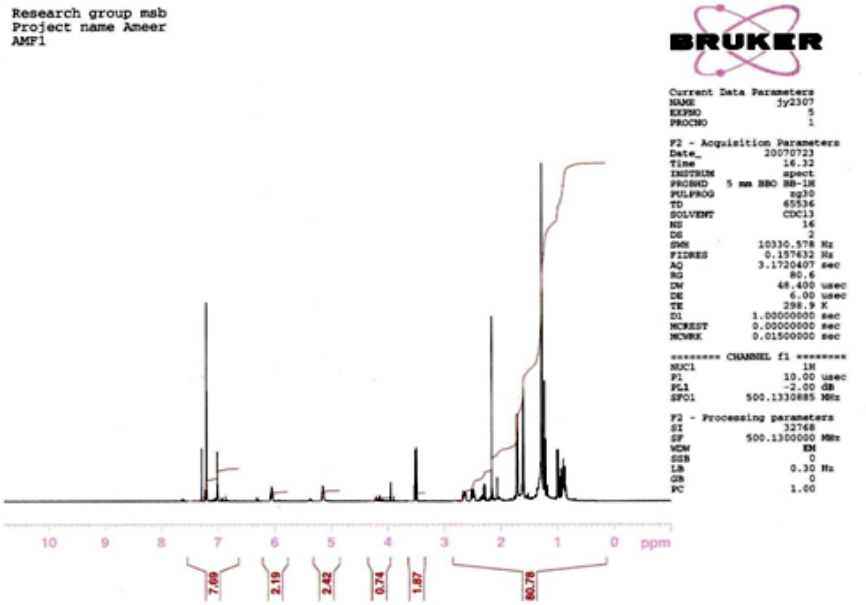

Fig. 2: 500 MHz ^{1}H-NMR spectrum of Shikonin in $CDCl_3$

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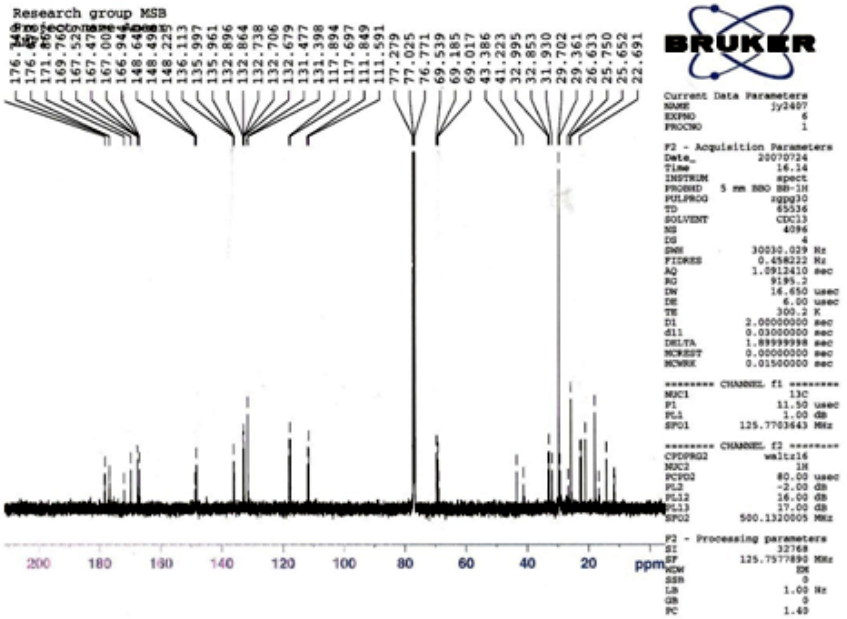

Fig.3: 500 MHz ^{13}C-NMR spectrum of Shikonin in $CDCl_3$

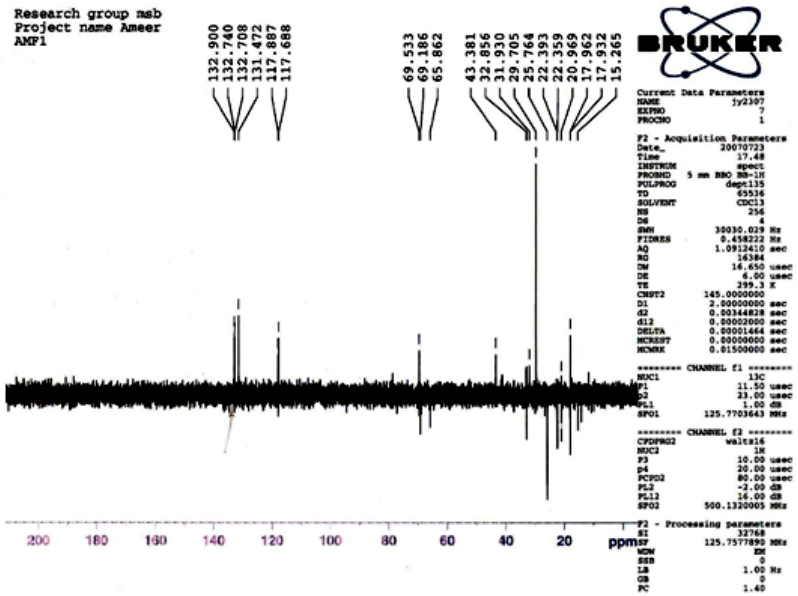

Fig.4: 500 MHz ^{13}C dept-NMR spectrum of Shikonin in $CDCl_3$

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