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Isolation, Characterization of dihydroflavonol from *Coriandrum sativum* Seeds

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المستخلص:

تم فصل أول مركب فلافوني من بذور نبات الكزبرة ذو الأهمية الطبية . المستخلص الكحولي أعطي المركب : 7 ، 5 ثنائي هيدروكسيل - ′5 ميثوكسيل ثنائي هيدروالفلافونول ، والذي حددت تركيبته بواسطة طيف الأشعة تحت الحمراء ، طيف الأشعة فوق البنفسجية ، طيف الرنين النووي المغنطيسي و طيف الكتلة .

Abstract:

The authors report on the first isolation of a flavone from the seeds of the medicinally important plant Coriandrum sativum (Apiaceae) . Chromatographic fractionation of the ethanolic extract gave flavonoid (C1) : 5,7,–dihydroxy-5′- methoxy dihydroflavonol.The structure of this compound was elucidated on the basis of its IR, UV,1H NMR and MS.

Key words : dihydroflavonol, Isolation, Characterization, *Coriandrum sativum*.

I-Introduction :

Flavonoids are natural phenolic compounds which appear as secondary metabolites of plant[1] .The name “Flavonoid” is derived from Greek word “Flavus” - it means yellow [2]. They are found in many plant tissues, where they are present inside the cells or on the surfaces of different plant organs. The chemical structure of this class of compounds is based on a C6-C3-C6 skeleton. They differ in the saturation of the heterocyclic ring C, in the placement of the aromatic ring -B at the positions 2, 3 and 4 of ring –C, and in overall hydroxylation patterns [2]. The flavonoids may be modified by hydroxylation, methylation or O- glycosylation of hydroxyl groups as well as C- glycosylation directly to carbon atom of the flavonoid skeleton. In addition, alkyl groups (or phenyls) may be covalently attached to the flavonoid moieties, and sometimes additional ring are condensed to the basic skeleton of the flavonoid core[3].

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Flavonoids mainly occur as glycosides which carry one or more sugar residue[4]. Such glycosides have extreme importance from the phytogenetic aspect in clearing up the origin and evolution of plant [5, 6].w. Flavonoid are a large group of polyphenolic compounds that occur commonly in plants[7] . This group contains more than 8000 known compounds, and its number is constantly growing because of the great structural diversity arising from the various hydroxylation, methoxylation, glycosylation , and acylation patterns. Flavonoids are the pigment responsible for the shade of yellow, orange and red in flowering plants[8]. They are also important factors for plant growth, development and defense. Many flavonoids are endured with biological activities, such as anti-inflammatory, anti-allergic, anti-ischemic, anti-platelet, immune modulatory, and antiumoral activities [9] Based on the degree of oxidation and saturation of the C – ring flavonoid may be divided into the following groups (flavones, flavonols, flavans, flavanones, flavan 3ols, dihydroflavonol, flavan 4 ols, flavan-3.4 diol, aurons, and anthocyanidins[10,11].Natural products such as chalcones, aurones and dihydrochalcones also contain a C6-C3-C6 skeleton and are considered to be minor flavonoids[12]

Coriandrum sativum, also known as cilantro, Chinese parsley or coriander is a herb in the Apiaceae family. Coriander is native to regions spanning from southern Europe and north Africa to south western Asian[13]. It is soft plant growing to 50cm in tall. The leaves are variable in shape, broadly lobed at the base of the plant, and slender and feathery higher on the flowering stems. The flowers are borne umbels in small, white or very pale pink, asymmetrical, with the petals pointing away from the centre of the umbel longer (5-6 mm) than those pointing towards it (only 1-3 mm long) [14, 15]. The fruit is a globular, dry schizocarp 3-5 mm (0.12-0.20 in) in diameter. Although sometimes eaten alone, the seeds often are used as spice or added ingredient in other foods [15]. Coriander like many spices, contain antioxidants, which can delay or prevent the spoilage of food seasoned with the spice. A study found that both the leaves and seeds contain antioxidants, but the leaves were found to have a stronger effect [16, 17]. Chemicals derived from coriander leaves were found to have antibacterial activity against *Salmonella choleraesuis*, and activity was found to be caused in part by those chemicals acting as nonionic surfactants[18].

Coriander has been used as a folk medicine for the relief of anxiety and insomnia in Iran[19]. Coriander seeds are used in traditional Indian medicine as diuretic by boiling

amounts of coriander seeds then cooling and consuming the resulting liquid. In "Holistic" and traditional medicine it is used as a carminative medicine and as a digestive aid[20, 21.]

II - Objectives of this Study:

- Extraction of flavonoids from plant species.
- Isolation of flavonoids via different chromatographic tools.
- Elucidation of structure via sensitive analytical tools.
- Evaluation of the antimicrobial activity of different extracts and pure compounds.

III-Material and methods :

Material:

- **Instruments :** IR spectrophotometer (perkin-Elmer 1310) .
- UV-Visible spectrophotometer (perkin-Elmer lampda 2) .
- NMR spectrophotometer (EM-360-300MHZ) .
- Mass spectrometer(Finnigan – MAT SQ-700) .

Plant material :

The seeds of *Coriandrum sativum* were collected in March 2013 from River Nile state-Sudan. The plant was authenticated by the Botany Department, University of Khartoum and a voucher sample was deposited in the herbarium of the department.

Solvents:

All chemicals, solvents and reagents used were of analytical grade. Chemicals used were supplied by Fisher Scientific (Springfield, NJ), British Drug Houses (England) and Sigma® (Germany). Spectroscopic grade solvents were used for spectral determination and deuterated solvent (DMSO-d6) was used for NMR analyses.

Methods:

Isolation of flavonoids:

Powderd air-dried seeds(1kg) of Coriandrum sativum were macerated with 95% ethanol at room temperature for (3days). The crude extract was suspended in water and partitioned with organic solvents in order of increasing polarity :petroleum ether ,chloroform , ethyl acetate and n- butanol . The n- butanol fraction was rich in phenolics . It was dissolved in methanol and applied on Whatman paper (No 3mm-46x57156o\cm).

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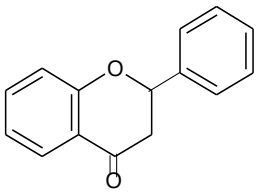
The bands were irrigated with BAW (n- butanol- acetic acid- water; 5:2:6; v:v:v). The developed chromatograms were air- dried and examined under both visible and UV light (ʎ366,245nm). The chromatograms were exposed to ammonia vapor for about 2-3seconds and immediately re-examined to observe possible changes that may eventually appear in colour or fluorescence under a long wavelength UV lamp . The equivalent bands from each paper were then cut out , combined and cut into small strips and slurred with methanol . After several hours of contact with occasional shaking, the liquid was evaporated *in vacus* to dryness .In this way a flavonoid- compound Iwas isolated in chromatographically pure form as brown amorphous powder.

IV-Result and discussion:

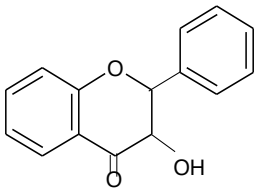
Compound I was isolated as yellow powder from ethanolic extract of the seeds of Coriandrum sativum .The IR sp of compound I displayed absorption bands at ν (KBr): 3382.9 (OH), 2921.9, (C-H, alkane), 1654.81 (C = O), 1554.5, 1433 (C = C, aromatic), 1238.2 (C-O, ether), and 1049. (C – O, phenolic).Since the IR revealed a

C = O function, hence compound II cannot be an anthocanin or catechin.

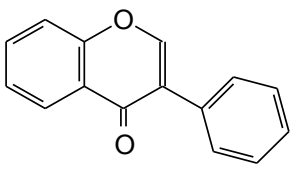
The UV spectrum showed λ max(MeOH) 267nm . Since compound I gave only band II it could be (i)a flavanone (ii) isoflavone (ii)dihydoflavanol or (iv) dihydrochalcone.



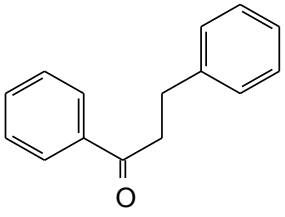
Flavanone



Dihydroflavonol



Isoflavone



Dihydrochalcone

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Isoflavones inconsistently a show shoulder in the 300-340 nm region. Such shoulder was not found in the spectrum of compound I and this indicates absence of isoflavones.

Dihydroflavonol posses a 3- OH function which could be confirmed by the shift reagent sodium methoxide. The sodium methoxide spectrum revealed a 15 nm bathochromic shift with decrease in intensity and this indicative of 3 – OH function, hence compound I is probably a dihydroflavonol.

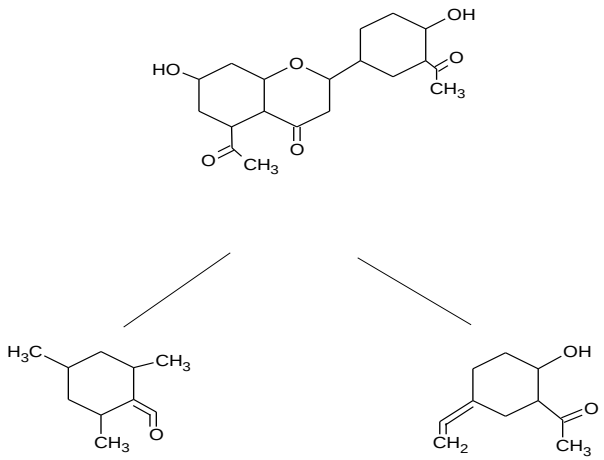
When NaOAc added to methanolic solution of compound I , a18 nm bathochromic shift was observed indicating presence of a 7 –OH function .

The aluminium chloride spectrum revealed a 23nm bathochromic shift .The aluminum chloride was acid stable in indicating a free 5- OH function.

The ^{1}HNMR spectrum gave : δ 3.6 (s,3H) which accounts for a methoxyl group . The resonance at δ 8.4 was assigned for the aromatic protons.

The mass spectru gave m\ z 303 (M^{+} +2) for the a glycone. Other important fragments corresponding to intact aromatic rings were shown at m\z 150 and m\z151. Substitution pattern of these aromatic rings. H′-H′ COSY NMR experiments indicated long rang coupling between the methoxyl protons and $C_{6'}$ and $C_{4'}$-H .Hence the methoxyl function is substituted at $C_{5'}$ of B-ring.

On the basis of the above spectral the following tentative strcture was suggested for this dihydroflavonol.



Scheme I: retro Diels – Alder fission of compound C1

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V- Recommendations:

1. The seeds of Sudanese material of Coriandrum sativum has not yet been investigated and a study of the flavonoids of this material will make a contribution to the chemistry of these plant phenolics .
2. The major compounds were isolated from the studied plant, further studies are necessary for entire exploitation of this natural source of flavonoids.
3. Other phytochemicals (steroids, saponins, and tannins) must be investigated.

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