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Research Article

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Isolation and Partial Characterization of a Flavone from Sudanese *Ammi visnaga* (Umbellifereae)

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Abstract *Ammi visnaga* is widely used in Sudanese herbal medicine where it is used against a wide range of diseases including asthma , urinary calculi and mild anginal symptoms. The plant is diuretic and is claimed to relieve menstruation pain. *Ammi visnaga* contains, among others, khellin, khellinol, amiol, khellol and khellinin. It also contains coumarins and a fixed oil.

This study was undertaken to investigate the major flavonoid of *Ammi visnaga*. The flavonoids were extracted with ethanol and the crude extract was purified by thin layer chromatography where a pure flavones was isolated. The structure of the isolated flavone has been partially characterized by some spectral data (UV and ^{1}HNMR).

Keyword: *Ammi visnaga*, Isolation, Flavonoid, Flavone, Partial Structure

1. Introduction

The gemus *Ammi* is a genus in the family Umbellifereae containing some bioactive constituents like flavonoids and coumarins. The medicinally important *Ammi visnaga* has a wide distribution area including Europe , western Asia, Mediterranean region and north Africa [1-3].

Ammi visnaga is traditionally used by many communities against asthma, urinary calculi and mild anginal symptoms [4]. The plant is reported as diuretic and claimed to relieve menstruation pain [4]. *Ammi visnaga* contains, among others, khellin, khellinol, amiol , khellol and khellinin. It also contains coumarins and a fixed oil [5-9].

The antimicrobial activity of *Ammi visnaga* extracts against a panel of human pathogens has been demonstrated [10]. The essential oil from this species is claimed to exhibit antimicrobial activity [11]. *In vivo* studies have revealed that *Ammi visnaga* induced relaxation of smooth muscles including the urethra and coronary arteries [12].

It has also been shown that the plant has a protective effect against kidney stone formation. Two of its constituents – khellin and visnagin- protected against calcium oxalate- induced cell damage in model animals [13].

2. Materials and Methods

Materials

Plant material

The seeds of *Ammi visnaga* were collected from Nyala- west Sudan. The plant was authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum.

Instruments

UV spectrum was run on a Shimadzu UV – 2401PC UV- Visible Spectrophotometer. NMR spectrum was measured on a Joel ECA 500 NMR Spectrophotometer.

Methods

Extraction and isolation of flavonoids

Dry powdered plant material (1Kg)was macerated with 95% ethanol (5L) for 72hr at room temperature with occasional shaking and then filtered off . The extraction process was repeated two more times with the same solvent. Combined filtrates were concentrated under reduced pressure at 40^{o} C until all ethanol was removed yielding a crude product. Preparative thin layer chromatography was carried out for the ethanol extract using BAW (butanol: acetic: water 4:1:5 upper layer) as solvent system. The developed chromatograms were dried, observed in UV light (366, 254 nm) and then determined with smooth lines using a pencil and scratched.

The equivalent bands from each plate were then slurred with methanol. After filteration, the solvent was evaporated to dryness under reduced pressure .In this way a flavonoid- compound I (R_f 0.66) was isolated and recrystallized from absolute ethanol.

3. Results and Discussion

Characterization of compound I

The UV spectrum (Fig.1) of compound I gave λ_{max} 248 , 303 nm which is consistent with the absorption of flavones. These compounds consistently show band I in the range: λ_{max} 300-356nm. In contrast, the closely related flavonols absorb in the range : λ_{max} 358-390nm(band I) [14].

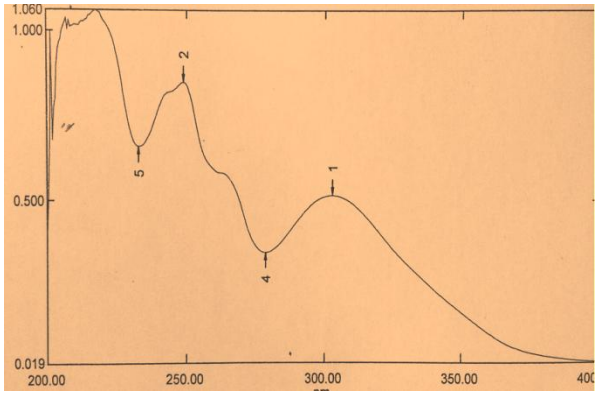


Figure 1: UV spectrum of compound I

The hydroxylation pattern of the isolated flavones has been studied via various UV shift reagents (sodium methoxide, sodium acetate, aluminium chloride and boric acid). These reagents produce diagnostic bathochromic shifts [14] in presence of specific hydroxylation pattern. Sodium methoxide can detect 3- and 4`-OH functions , while sodium acetate is diagnostic of a 7-OH group. Aluminium chloride can detect 3-, 5-OH functions as well as catechol systems. Boric acid is helpful in the specific detection of catechol moieties [14,15].

The sodium methoxide spectrum (Fig.2) did not reveal any bathochromic shift indicating absence of both 3- and 4`-hydroxylation.

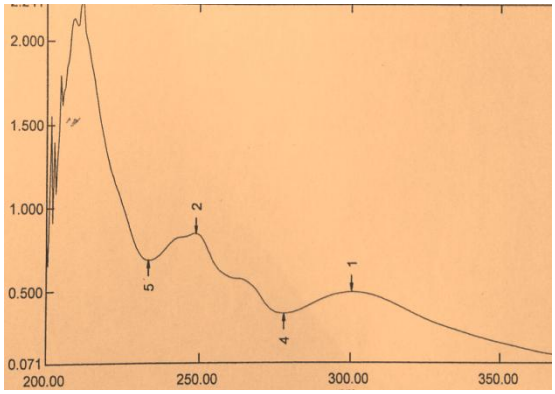


Figure 2: Sodium methoxide spectrum of compound I

No bathochromic shift was detected in the sodium acetate spectrum (Fig.3) suggesting absence of a 7-OH function.

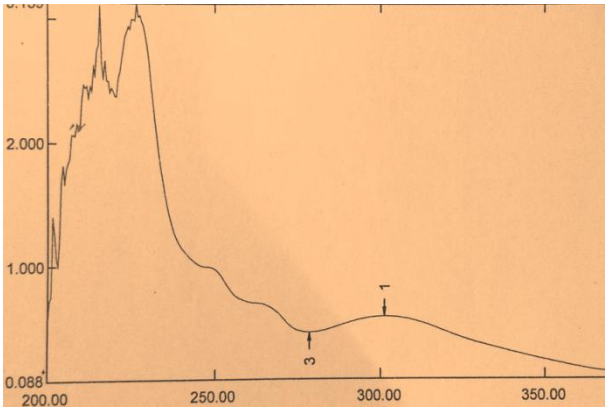


Figure 3: Sodium acetate spectrum of compound I

The aluminium chloride spectrum (Fig. 4) also failed to show a bathochromic shift indicating absence of 3- and 5-OH functions as well as catechol systems.

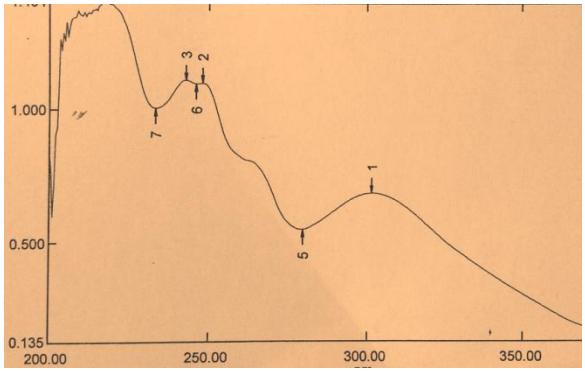


Figure 4: Aluminium chloride spectrum of compound I

The ^{1}HNMR spectrum (Fig. 5) showed: δ1.22(3H), δ1.69(6H) assigned for three methyl groups. The resonance at δ 4.17(3H) is due to a methoxyl function. The signals at δ6.42 and δ6.45ppm accounts for C-6 and C-8 protons respectively. The C-8 proton usually resonates at lower field relative to C-6 proton due to the deshielding influence [14] of the oxygene atom at position -1. Other aromatic protons appeared at δ 7.17, δ7.68 and δ8.12ppm (the resonances at δ2.50 and δ3.30 ppm are due to solvent (DMSO) residual protons and residual water respectively).

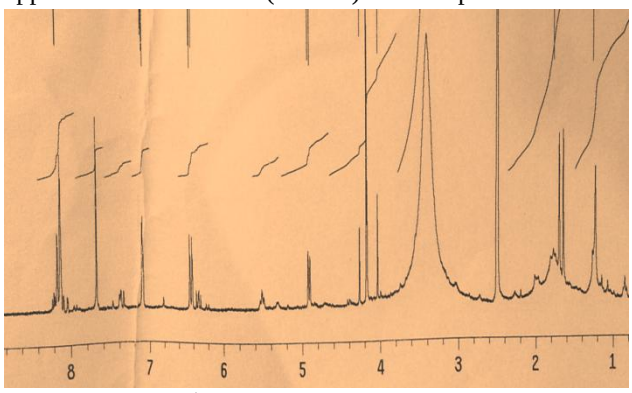
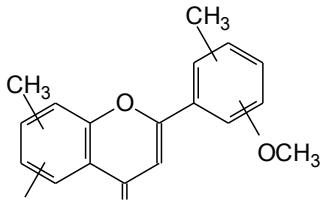


Figure 5: ^{1}H NMR spectrum of compound I

On the basis of the above spectral data the following partial structure was assigned for compound I:



Compound I

A future 2D NMR experiments (^{1}H COSY NMR, HMBC, HSQC) may fully elucidate the structure of this flavone.

Conclusion

The major flavonoid (a flavones) from *Ammi visnaga* has been isolated via chromatographic techniques and a partial structure has been proposed on the basis of some spectral data.

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