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ISOLATION, PARTIAL CHARACTERIZATION OF A DIHYDROCHALCONE FROM SUDANESE *SOLANUM DUBIUM* LEAF (GUBBAIN) AND BIOLOGICAL ACTIVITY OF THE METHANOL EXTRACT

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ABSTRACT

Plants contain many bioactive molecules such as flavonoids, alkaloids and steroids that produce beneficial physiological and biochemical actions in the human body. The presence of such molecules in *Solanum dubuim* was evidenced by a phytochemical screening of *Solanum dubuim* leaves which revealed the presence of flavonoids, alkaloids, tannins, steroids and saponins. In this study a flavonoid -compound(I)- was isolated from the methanol extract of *Solanum dubuim* leaves by thin layer chromatography and its structure was partly characterized on the basis of its spectral data (UV,NMR). The methanol extract of *Solanum dubuim* was evaluated for antioxidant activity. In the DPPH assay, the methanol extract exhibited significant free radical scavenging capacity.

KEYWORDS: *Solanum dubium*, Isolation, Dihydrochalcone Antioxidants Activity.

INTRODUCTION

Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active molecules in higher plants.[1] Herbal medicine is a major component in all traditional medical systems, and a common element in Sudanese system of medicine. Plant materials are used throughout developed and developing countries as home remedies and plant raw materials are used in the pharmaceutical industry and represent a substantial proportion of the global drug market,[2] The natural antioxidants are primarily plant phenolics and many of these antioxidants possess anti-inflammatory, antitumor, antibacterial and antiviral activities.[3]

The *Solanum* genus (*Solanaceae* family) is comprised of one thousand five hundred species and most of the members of the genus are widely used as food and traditional medicine.[4] Some plants of the family Solanaceae such as *Solanum dubium* has been used for the extraction of milk-clotting enzymes.[5-9] *S. dubium* extracts contain compounds that can reduce blood glucose with a short duration of action.

The seed and honey combination is regarded as a potential source of antioxidants for use in several conditions requiring this property.[10]

MATERIALS AND METHODS

Plant material

Solanum dubium leaves were collected from Khartoum – Sudan. The plant was identified and authenticated by direct comparison with a reference herbarium sample.

Instruments

UV spectra were measured on a Shimadzu 240ICP UV-Visble Spectrophotometer. ^{1}HNMR spectra were run on Aeca 300.068MHZ NMR Spectrophotometer.

Methods

Extraction of flavonoids

S.dubium powdered leaves (500 g) were macerated with 80% aqueous methanol for 3 days. The extract was filtered and the residual weight was recorded and % yield was calculated.

Phytochemical screening

S.dubium leaves were screened for major secondary metabolites according to method described by Harbone.[5]

DPPH radical scavenging assay

DPPH radical scavenging was determined according to the method of Shimada et al.[11] with some modification. Briefly, the test samples were allowed to react with 2.2 Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37^o C. The

concentration of DPPH was kept at (300 µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol, after incubation the decrease in absorbance was measured at λ_{max} 517 nm. Percentage radical scavenging activity of samples was determined in comparison with a DMSO - treated positive control. All tests were run in triplicate.

Preparative thin-layer chromatography

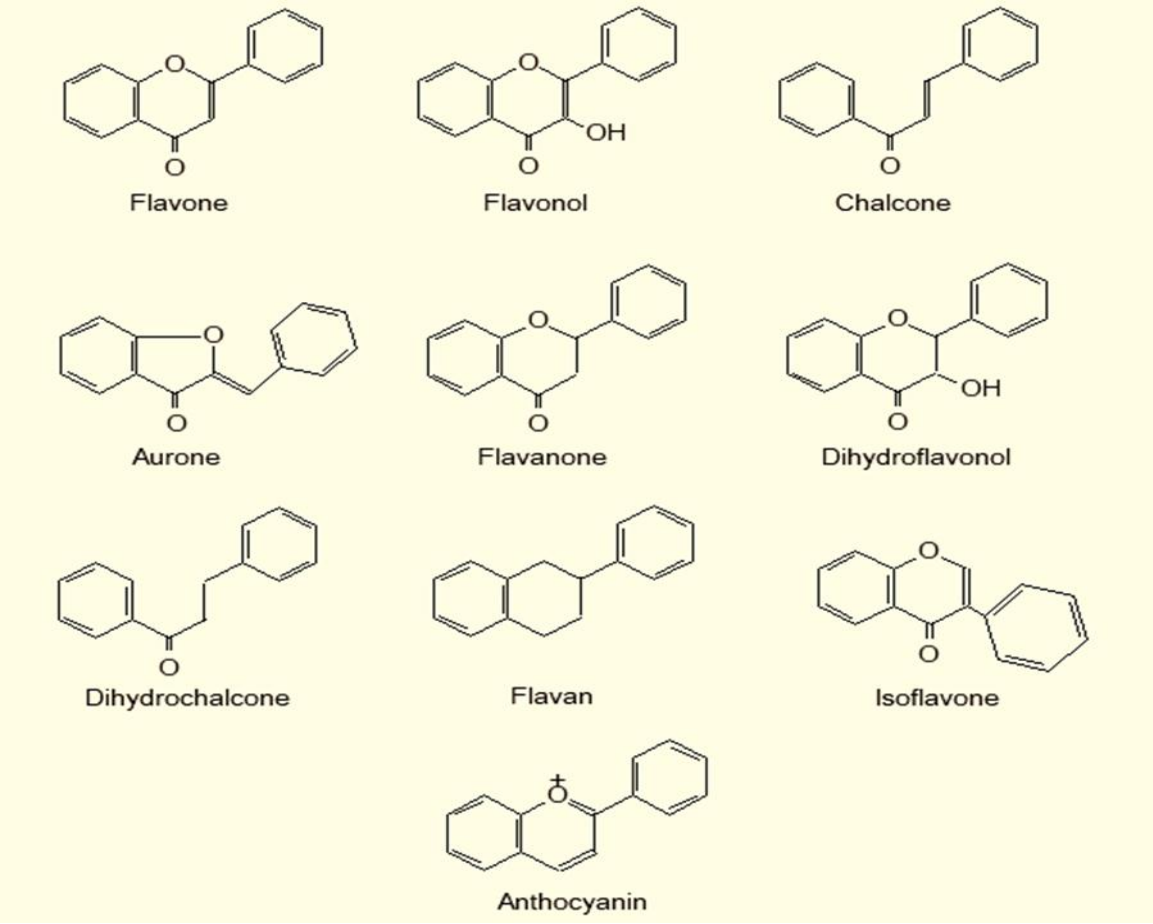
The crude leave methanol extract was chromatographed on preparative TLC plates. The plates were twice developed in a mixture of: toluene: ethyl acetate: formic acid (5:4:1;v:v:v).The chromatograms were examined under UV light and determined. Similar chromatograms were combined. After the usual workup a chromatographically pure flavonoid – compound I – was isolated.

RESULTS AND DISCUSSION

Phytochemical screening of the leaves of *Solanum dubuim* showed the presence of tannins, alkaloids, flavonoids, coumarins, saponins and steroids.

Characterization of compound I

From the methanol extract of *Solanum dubuim*, a flavonoid –compound I -was isolated by thin layer chromatography and its structure was partially deduced on the basis of its spectral data (UV, ^{1}HNMR).In their UV spectra flavones, flavonols, chalcones and aurones give both band I(due to cinnamoyl chromophore) and band II(due to benzoyl chromophore). Other classes: isoflavones, flavanones, dihydrochalcones and dihydroflavonols show only one peak originating from the benzoyl system. Band I, usually 300 – 400nm and band II usually 240 – 280 nm.[5]



The isolated flavonoid – compound I- absorbs at λ_{max} (MeOH) 228nm (Fig.1). Such UV absorption is characteristic of dihydrochalcones. The hydroxylation pattern on the nucleus of this dihydrochalcone has been investigated by using UV shift reagents. The addition of each of these reagents separately to an alcoholic solution of the flavonoid induces structurally significant shifts in the UV spectrum. Shifts of this type are commonly induced by the addition of: sodium methoxide, sodium acetate and aluminum chloride. Sodium methoxide is a strong base and ionizes to some extent all hydroxyl groups on the flavonoid nucleus. However, this reagent is used for the detection of 3-and 4`-OH groups. The sodium methoxide spectrum of compound I (Fig.2)did not reveal any bathchromic shift suggesting absence of 3-and 4-OH functions. Sodium acetate ionizes only the more acidic hydroxyl groups i.e., the 3, 7- and 4'-hydroxyl groups. Because ionization of the 7-hydroxyl group mainly affects Band II (whereas ionization of the 3- and/or 4'-hydroxyl groups mainly affects Band I),

sodium acetate is a particularly useful diagnostic reagent for the specific detection of 7-hydroxyl group. The sodium acetate spectrum(Fig. 3) failed to exhibit any bathochromic shift indicating absence of a 7-OH group.

Aluminum chloride chelates with functional groups such as: the 5-hydroxy-4-keto; 3-hydroxy-4-keto and ortho-dihydroxy1 systems, and this reaction is evidenced by bathochromic shifts of one or both bands in the spectrum. No bathochromic shift was observed in the aluminium chloride spectrum (Fig.4) indicating absence of 3-, 5-hydroxyl groups as well as catechols.

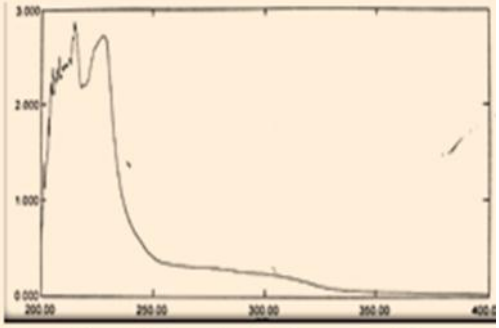


Fig. 1: UV spectrum of compound I

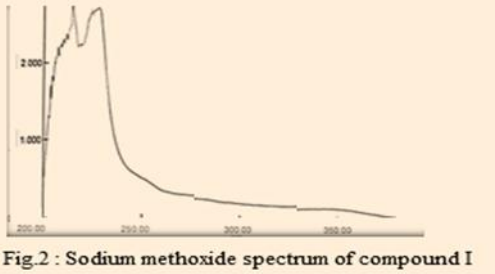


Fig.2 : Sodium methoxide spectrum of compound I

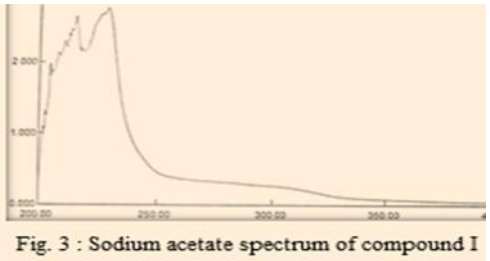


Fig. 3 : Sodium acetate spectrum of compound I

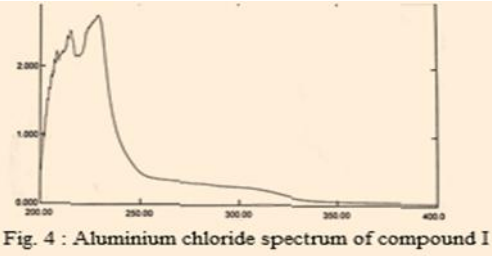
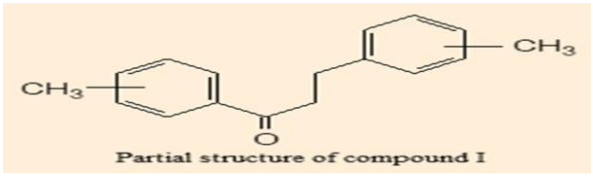


Fig. 4 : Aluminium chloride spectrum of compound I

The ^{1}HNMR spectrum (Fig.5) gave δ_H (ppm): 1.24 (integrating for two methyl groups). The aromatic protons appeared at δ 8.3ppm. T he signals at δ2.50 ppm and δ 3.34 ppm are due to the solvent (DMSO) residual protons and residual water respectively. On the basis of the above argument the following partial structure has been proposed for compound I:



Partial structure of compound I

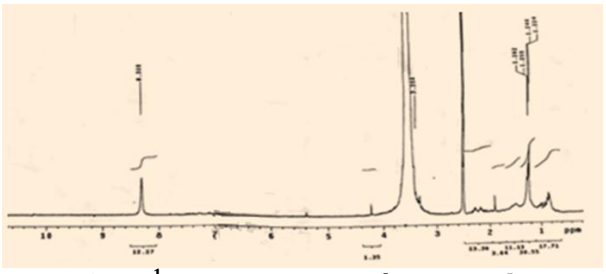


Fig. 5: ^{1}HNMR spectrum of compound I

Antioxidant activity

S. dubium leaf were tested for antioxidant activity. The methanol extract was evaluated for its *in vitro* antioxidant activity using the DPPH method. The extract showed significan antioxidant activity (Table 1).

Table 1: Antioxidant activity of methanol extract.

Sample	RSA % ± SD
Propyl gallate (positive control)	92 ± 0.01
Methanol extract of Solanum dubium	80 ± 0.0

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