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Isolation and partial characterization of a flavone from Sudanese *Lepidium sativum* (Cruciferae)

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Abstract
Lepidium sativum is a key species in Sudanese system of medicine where it is used traditionally against a wide range of diseases. The plant contains vitamins A and C beside some minerals. It also contains folic acid; protein; leucine; glutamic acid and methionin. Seeds contains some alkaloids, beside carotene, riboflavan, uric acid, phosphorus, thiamine and niacin.
This study was designed to investigate the major flavonoid of *Lepidium sativum*. The flavonoids were extracted with ethanol and the crude extract was purified by thin layer chromatography where a pure flavone was isolated. The structure of this flavone has been partially characterized by some spectral tools (UV and ^{1}HNMR).

Keywords: *Lepidium sativum* (Cruciferae), isolation, partial characterization

Introduction
Lepidium sativum (Cruciferae) is a plant that can tolerate difficult environmental conditions [1, 2]. This species possesses white-pinkish flowers and has many branches on the upper parts [3, 4]. *Lepidium sativum* is genetically related to mustard and watercress [5, 6].
Lepidium sativum contains vitamins A and C beside some minerals (iron, calcium…etc.). It also contains folic acid; protein (25%); leucine (8.21%); glutamic acid (19.3%) and methionine (0.97%. Seeds are reported to contain some alkaloids, beside carotene, riboflavan, uric acid, phosphorus, thiamine and niacin. It been shown that *Lepidium sativum* oil contains, among others, palmitic, linoleic, stearic, behenic, oleic, arachidic and lignceric acids [7].
In some Asian countries, *Lepidium sativum* is used against some human disorders [8, 9], whereas in African system of medicine it is mainly used against bronchitis. It has been demonstrated that *Lepidium sativum* possesses anti-inflammatory, analgesic, anticoagulant [10], diuretic [11], antihypertensive [12], antirheumatic [13], antidiarrheal, antispasmodic, laxative [14] and hypoglycemic [15] properties.
In some clinical trials concerning the effect of seed administration on bronchial asthma, none of the test subjects showed the presence of adverse effects or any other problems at hematological profile [16].

Materials and methods
Plant material
The seeds of *Lepidium sativum* were collected from Kordofan, western Sudan. The plant was authenticated by The Institute of Medicinal and Aromatic Plants, Khartoum-Sudan.

Solvents
Analytical grade solvents were used. Methanol-HPLC grade-was used for spectroscopic purposes (Loba, India).

Equipments
A Hanofa ultraviolet lamp (λ_{max} 254 / 365 nm) was used in visualizing TLC plates. Ultraviolet absorption spectra were obtained in spectroscopic methanol on UV -Visible Spectrophotometer (Shimadzu).
^{1}HNMR spectra were obtained on a Bruker AM 500 spectrophotometer (Germany) operating at 500 MHz in spectroscopic grade DMSO-d_6. The chemical shifts values are expressed in δ (ppm) units using (TMS) as an internal standard.

Methods
Isolation of flavonoids
Dry powdered seeds of *Lepidium sativum* (1Kg) were macerated with 95% ethanol for 72 hours. The extract was filtered and the solvent was evaporated to dryness under reduced pressure. The ethanol crude extract was applied on silica gel plates (20x20cm) as narrow strips. The bands were developed with BAW (4:1:5;V:V:V). The developed plates were air-dried and examined under UV light (ʎ 366nm). The equivalent bands from each plate were then scratched, combined and slurred with methanol. The solvent was evaporated *in vacuo* to give a yellow powder. On recrystallization from absolute alcohol a chromatographically pure flavonoid-compound I (R_f 0.70) - was obtained.

UV shift reagents
Stepwise procedure
- The UV spectrum of the compound in methanol was first recorded.
- 3 drops of NaOMe reagent were added to the sample and the NaOMe spectrum was recorded, and after 8 minutes the NaOMe spectrum was re-recorded.
- 6 drops of $AlCl_3$ reagent were added to the fresh sample and the $AlCl_3$ spectrum was recorded, 3 drops of HCl were

added and after mixing, the $AlCl_3$/HCl spectrum was recorded.
- Powdered NaOAC was then added to the fresh sample, the mixture was shaked and the NaOAC spectrum was recorded. NaOAC/H_3BO_3 spectrum was then recorded after adding H_3BO_3.

Results and Discussion

Identification of compound I

Compound I absorbs in the UV (Fig.1) at λ_{max} (MeOH) 266,329nm. This compound revealed both band I (due to cinnamoyl chromophore) and band II (due to benzoyl chromophore). The appearance of band I in the UV spectrum suggests conjugation between the carbonyl function of the flavonoid and the aromatic (B) ring.

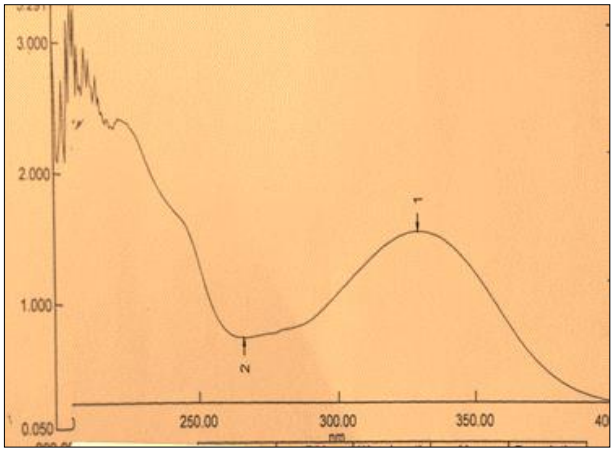
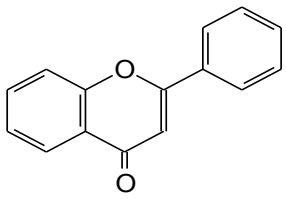


Fig 1: UV spectrum of compound I

Among the different classes of flavonoids such absorption is characteristic of flavones [17].



Flavone

The hydroxylation pattern of this flavones was investigated via different UV shift reagents [17] (sodium methoxide, sodium acetate, aluminium chloride and boric acid). Such shift reagents provide valuable information regarding the location of different hydroxyl groups in the nucleus of flavonoids. While sodium methoxide is used for the specific detection of 3- and 4`- OH, sodium acetate is used for the detection of a 7-OH function. Aluminium chloride can detect hydroxyl functions in positions: 3 and 5. It can also detect catechol moieties. Boric acid is used for the detection of catechol systems. The process of detection is based on the appearance of bathochromic shifts relative to the methanol spectrum of the test sample [17, 18].

The UV shift reagent-sodium methoxide- revealed a bathochromic shift diagnostic of a 4`-OH function (Fig. 2), while the sodium acetate spectrum (Fig. 3) did not afford any bathochromic shift in band II indicating absence of a 7-OH function.

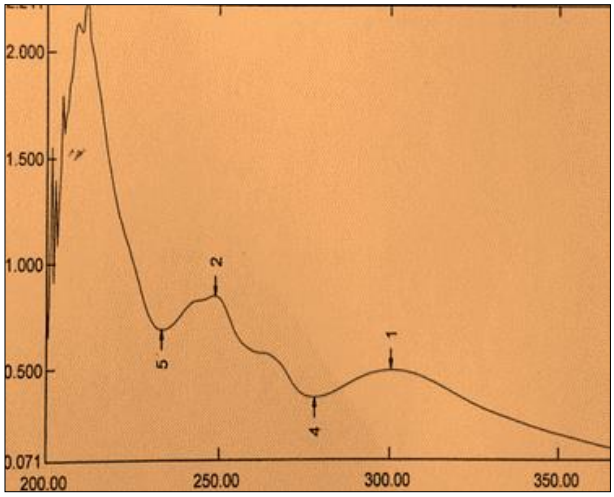


Fig 2: Sodium methoxide spectrum of compound I

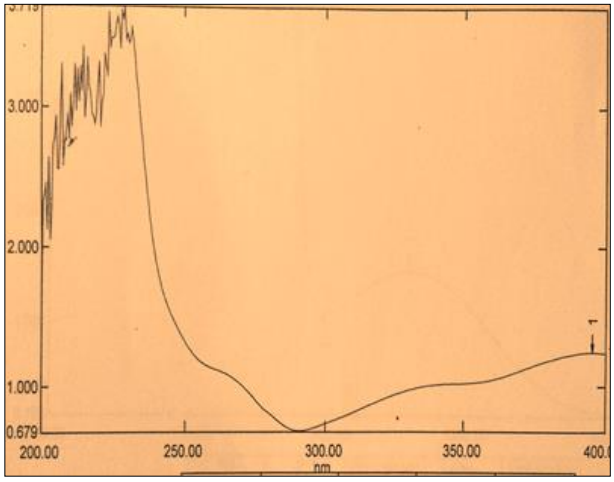


Fig 3: Sodium acetate spectrum of compound I

The aluminium chloride specterum (Fig.4) failed to give a bathochromic shift thus indicating absence 3- and 5-OH groups as well as catechols.

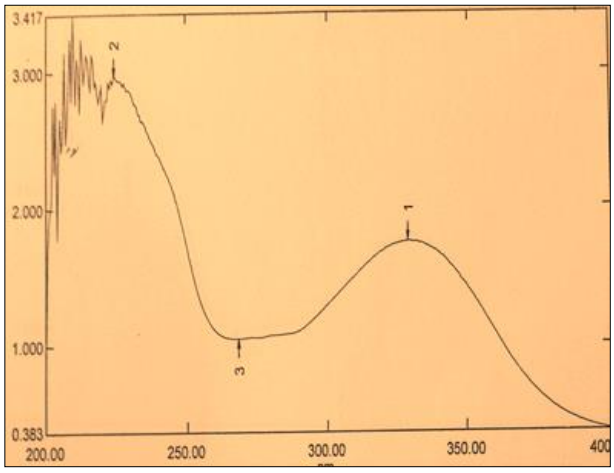


Fig 4: Aluminium chloride spectrum of compound I

The ^{1}HNMR spectrum (Fig.5) showed: δ1. 23(3H) which was assigned for rhamnosyl methyl. The resonance at 1.64(6H) is due to two methyl groups. The multiplet at δ3.40 - 3.88(10H) accounts for a rhamnoglucosyl moiety. The H-1 of the rhamnosyl and glucosyl moieties appeared at δ4.45 and δ 5.18ppm respectively. The aromatic protons resonated at δ6.85 and δ7.18ppm (The resonances at δ2.50 and δ3.30ppm are due to solvent (DMSO) residual protons and residual water respectively).

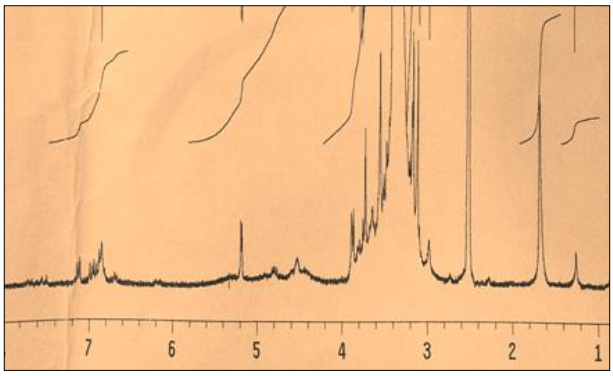
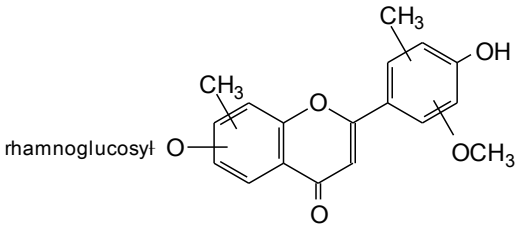


Fig 5: ^{1}HNMR spectrum of compound I

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



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