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Isolation and Partial Characterization of a Flavanone from Sudanese *Mitragyna inermis* (Willd.) O. Kuntze (Rubiaceae) Stem Bark

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Abstract
Genus *Mitragyna* is distributed in tropical and subtropical regions of Africa and Asia. Different species of *Mitragyna* are widely used in ethnomedicine against malaria, fever, worms, diarrhea and cough. *Mitragyna speciosa* is a natural remedy for fatigue. *Mitragyna ciliate, Mitragyna inermis and Mitragyna stipulosa* have been used traditionally against hypertension, inflammation, rheumatism, gonorrhea and bronchpulmonary diseases. *Mitragyna africanus* is used traditionally against mental diseases. In this study stem barks of *Mitragyna inermis* were extracted with 95% ethanol and the crude extract was fractionated over silica gel plates to yield a chromatographically pure flavonoid – compound I. The structure of the isolated compound has been partially characterized by its spectral data (UV and ^{1}HNMR).

Keywords: *Mitragyna inermis***,** flavonoid, isolation, partial characterization

1. Introduction
Mitragyna is a genus in the family Rubiaceae. This genus is distributed in tropical and subtropical regions of Africa and Asia. The genus contains ten species: *Mitragyna parvifolia*, *Mitragyna hirsuta*, *Mitragyna diversifolia*, *Mitragyna tubulosa*, *Mitragyna speciosa*, *Mitragyna rotundifolia*, *Mitragyna ciliate*, *Mitragyna inermis*, *Mitragyna africanus* and *Mitragyna stipulosa* [1].
Different species of *Mitragyna* are widely used in ethnomedicine [2, 3]. against malaria, fever, worms, diarrhea and cough. *Mitragyna speciosa* is a natural remedy for fatigue [4]. *Mitragyna ciliate, Mitragyna inermis and Mitragyna stipulosa* have been used traditionally against hypertension, inflammation, rheumatism, gonorrhea and bronchpulmonary diseases. *Mitragyna africanus* is used traditionally against mental diseases [5].
Some indole alkaloids, triterpenoids and saponins have been reported from the genus *Mitragyna.*The major alkaloid of this genus demonstrated a wide reaching pharmacological potential including cardiovascular, antitumor, and antimicrobial activities [6-9]. It has been documented that mitragynine-the major alkaloid of *Mitragyna*- possesses analgesic properties [10-12]. Different extracts of *Mitragyna speciosa and Mitragyna parvifolia* showed significant antiinflammatory activity [13-16]. It has been reported that *Mitragyna speciosa* exhibited significant antimutagenic activity [17]. *Mitragyna ciliate* extracts induced aortic relaxation in a dose-dependant manner [18]. In some in vivo studies, The extracts of *Mitragyna diversifolia* exhibited antidiarrheal effect [19]. Extracts of *Mitragyna speciosa* also showed antidiarrheal activity [20, 21]. The antimicrobial and antioxidant activities of *Mitragyna speciosa* have been reported [22].

2. Materials and Methods
2.1 Materials
2.1.1 Plant material
Mitragyna inermis barks were collected from Damazin (Sudan). The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum – Sudan.

Instruments
UV spectra were run on a Shimadzu 2401PC UV- Visible Spectrophotometer. ^{1}HNMR spectra were performed on a Joel ECA 500MHZ NMR Spectrophotometer.

Methods
Extraction and Isolation of flavonoid
Air dried powdered *Mitragyna inermis* barks (1 kg) were macerated at room temperature with ethanol (95%) for 72h. The solvent was evaporated under reduced pressure to dryness to give a crude product.
The crude extract was fractionated via thin layer chromatography using 30% acetic acid as solvent. The chromatograms were viewed and located under UV light and a flavonoid-compond I- was eluted from silica with methanol.

Results and Discussion
Mitragyna inermis barks crude extract was fractionated over silica gel plates to yield a chromatographically pure flavonoid – compound I. The structure of the isolated compound has been partially characterized via some spectral data (UV and ^{1}HNMR).

Characterization of compound I

The UV spectroscopy is a valuable analytical tool in the chemistry of flavonoids and can differentiate between flavonoids with double bond in position 2-3 and those lacking such bond. The UV spectra of most flavonoids consist of two major absorption maxima one of which occur in range 220-285 nm (band II) and the other in the range 300-400nm (band I) the appearance of both band I and II in the UV spectrum demonstrates conjunction between the benzoyl and cinnamoyl system.

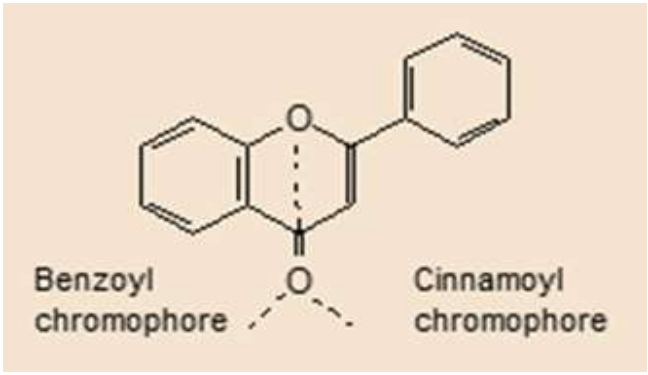


Fig 1

The UV spectrum of compound I (Fig. 3) showed λ_{max} (MeOH) 249m. The appearance of only one band – band II- in this spectrum suggests saturation at the $C_2 - C_3$ position. Consequently compound I is probably a (i) flavanone, (ii) isoflavone (iii) dihydrochalcone or (iv) a dihydroflavonol.

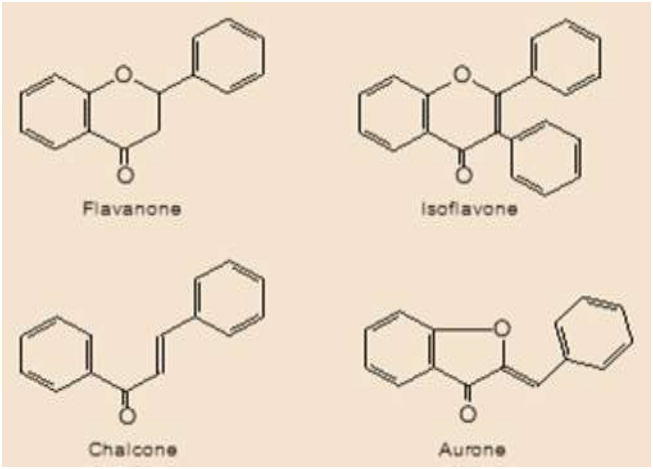


Fig 2

However, isoflavones are easily distinguished by a shoulder in the UV range 300-340nm. Such shoulder has not been detected in the UV spectrum of compound I. On the other hand dihydroflavonols are characterized by a 3-OH function which could be detected by the UV shift reagent- sodium methoxide. Sodium methoxide can give a bathochromic shift with decrease in intensity in presence of a 3-OH. The sodium methoxide spectrum (Fig. 4) failed to give a bathochromic shift.

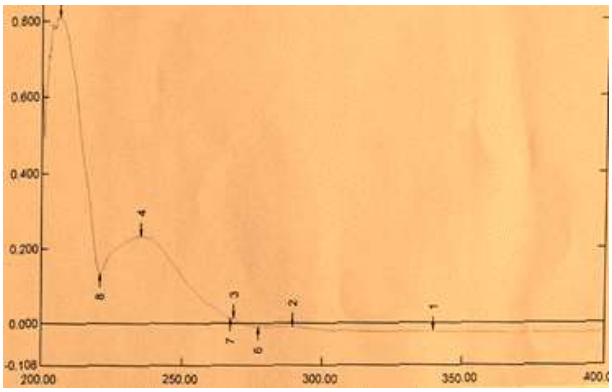


Fig 1: UV spectrum of compound I

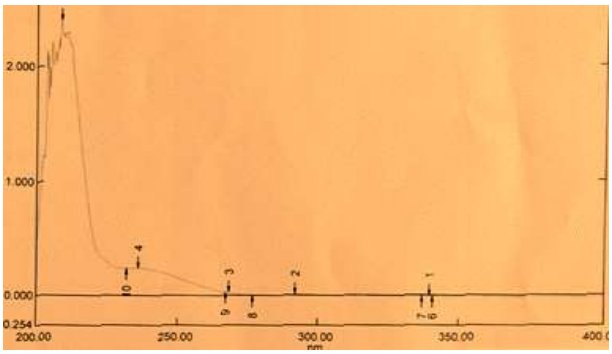


Fig 2: Sodium methoxide spectrum of compound I

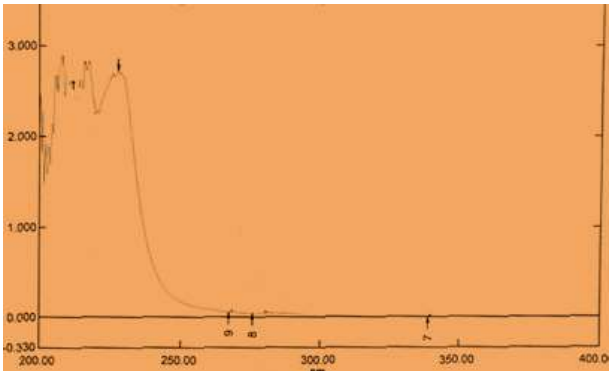


Fig 3: Sodium acetate spectrum

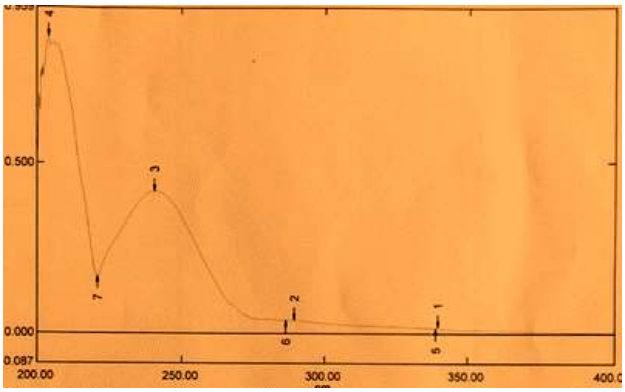


Fig 4: Aluminium chloride spectrum of compound I

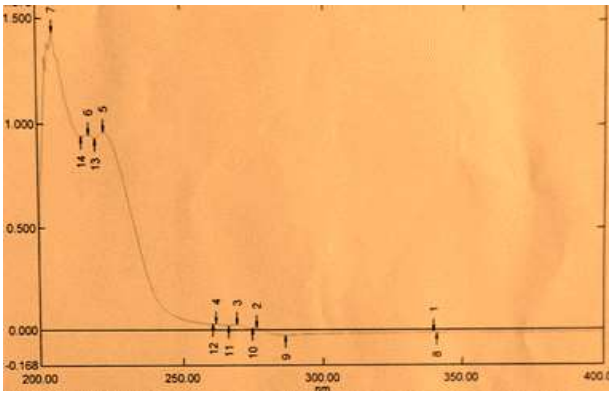


Fig 5: Boric acid spectrum of compound I

In their ^{1}HNMR spectra flavanones show a double mutiplet around 2.8ppm and 5.2ppm. The mutual spin-spin splitting of the magnetically unequivalent protons at C_3 suffers further splitting by the neighboring C_2 protons to give a double doublet (usually merging into a multiplet) around 2.8ppm. The signal of C_2 proton is split by one of the protons at C_3 into a doublet. Such doublet suffers further splitting by the other proton at C_3 to yield a double doublet (usually appearing a multiplet) around 5.2ppm. However, these multiplets were detected in the ^{1}HNMR spectrum of compound I (Fig. 8). Thus the isolated flavonoid is a flavanone.

The hydroxylation pattern of compound I has been studied via various UV shift reagents: sodium acetate (which gives a bathochromic shift in presence of a 7-OH); aluminium chloride (showing a bthochromic shift diagnostic of 3-, 5-OH and catechol systems) and boric acid (diagnostic of catechol moieties).

When a methanolic solution of compound I was treated with the shift reagent: sodium acetate, no bathochromic shift was observed indicating absence 7-hydroxylation (Fig. 5).

The aluminium chloride spectrum failed to show a bathochromic shift. This clearly suggests absence of 3-, 5-OH as well as catechol systems (Fig. 6). The boric acid spectrum behaved in the same manner and did not show any bathochromic shift suggesting absence of catechol systems (Fig. 7).

The ^{1}HNMR spectrum showed δ(ppm): 1.25,1.52(assigned for two methyl groups), multiplet centered at 2.00(assigned for C_3 protons), mutiplet:3.60-4.50(sugar protons-not identified in this study), multiplet centered at 5.20(accounting for C_2 proton). The aromatic protons appeared as a multiplet centered at 7.40ppm. Signals at 2.50 and 3.35 are due to solvent (DMSO) residual protons and residual water respectively.

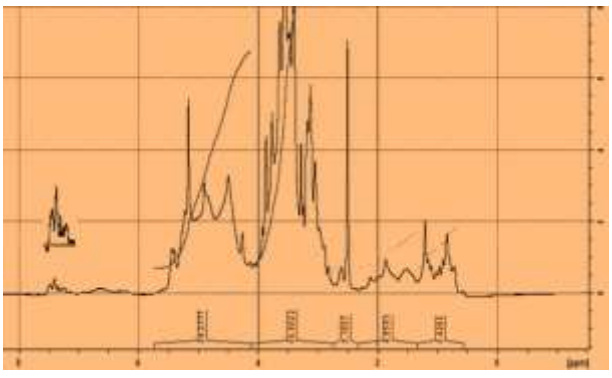
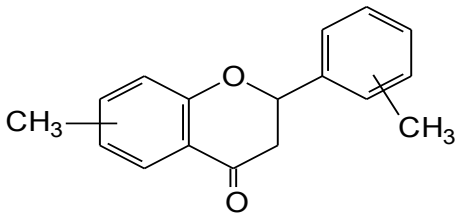


Fig 6: ^{1}HNMR spectrum of compound I

On the basis of the above argument, the following partial structure was proposed for compound I:



Compound I

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