

## ISOLATION AND CHARACTERIZATION OF A FLAVONE FROM LEAVES OF SUDANESE ARISTOLOCHIA BRACTEATA LINN. (ARISTOLOCHIACEAE)

Prof. Abdel Karim. M.\*<sup>1</sup>, Ayda A.<sup>2</sup> and Khalid M. S.<sup>3</sup>

<sup>1</sup>Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.

<sup>2</sup>Najran University, Faculty of Science and Arts, Dept. of Chemistry.

<sup>3</sup>International University of Africa, Faculty of Pharmacy.

\*Corresponding Author: Prof. Abdel Karim. M.

Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.

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### ABSTRACT

Information on the constituents of medicinal plants used in Sudanese system of medicine is very scarce. Hence, this study was set to investigate the flavonoids of *Aristolochia bracteata* which is a key species in indigenous medicine. This herb which showed many pharmacological effects is used in ethnomedicine as anthelmintic, anti-inflammatory, antipyretic and as purgative. Roots are used by local healers to treat gonorrhoea and syphilis. A flavone was isolated from leaves and its structure was partially elucidated on the basis of its spectral data (UV, <sup>1</sup>HNMR and MS).

**KEYWORDS:** *Aristolochia bracteata*, Isolation, Flavone, Partial Structure.

### INTRODUCTION

*Aristolochia bracteata* is a herb in the family Aristolochiaceae. The herb is used in Sudanese system of medicine as anthelmintic, anti-inflammatory, antipyretic and as purgative. Roots are used by local healers to treat gonorrhoea and syphilis.

*Aristolochia bracteata* showed many interesting pharmacological effects. Leave aqueous extract produced positive inotropic effect on heart of model animals. The extract also showed a wide therapeutic index. The mechanism underlying the positive inotropic effect was also addressed.<sup>[1]</sup>

The acetone and petroleum ether extracts were evaluated for antipyretic activity. It has been reported that these extracts exhibited significant antipyretic activity.<sup>[2]</sup> In another study, the anti-allergic activity of the plant was investigated. The chloroform extract showed potent anti-allergic activity in experimental models. The mechanism underlying this action is probably involving cell membrane stabilization, inhibition of nitric oxide and histamine pathways.<sup>[3]</sup>

*In vivo* studies testified that regular treatment of model animals with *Aristolochia bracteata* extracts significantly improved ESR and Hb value.<sup>[4]</sup> In the disc diffusion bioassay, the ethanol extract of *Aristolochia bracteata* exhibited significant antibacterial activity against a panel of human pathogens.<sup>[5]</sup>

The antioxidant activity of *Aristolochia bracteata* against stable DPPH radicals was assessed and significant radical scavenging capacity was reported.<sup>[6]</sup> Such antioxidant activity lends a rationale for the anti-inflammatory activity of this species. In carrageenan-induced paw edema, the ethanol extract of the leaves demonstrated significant reduction in edema volume.<sup>[7]</sup> Also it has been shown that the chloroform fraction of *Aristolochia bracteata* gave promising trypanocidal effect.<sup>[8]</sup>

In some *in vitro* studies, the methanol extract of *Aristolochia bracteata* exhibited excellent antifungal activity against some standard fungi.<sup>[9]</sup> The plant was also evaluated *in vivo* for anti-ulcer activity. The aqueous extract exhibited significant anti-ulcer effect in ethanol-induced ulcer.<sup>[10]</sup> The wound healing, antiangiogenic and abortifacient activities were also reported.<sup>[11,12]</sup>

### MATERIALS AND METHODS

#### Materials

##### Plant material

The leaves of *Aristolochia bracteata* were collected from Nyala (western Sudan). The plant was identified and authenticated by the Dept. of Botany, University of Khartoum.

### Solvents

All solvents used are of analytical grade. Methanol HPLC grade was used for spectroscopic purposes (BDH, England).

### Chromatographic materials

- Sheets of Whatman paper (No.1 and No.3 mm-46x57cm) from Whatman Ltd. Kent, England.
- Glass jars, 25x45x50 cm were used for chromatographic fractionation.

### Instruments

In paper chromatography, the ultraviolet lamp used in visualizing papers was a multiband UV  $\lambda_{\max}$  (254 / 365 nm) portable ultraviolet lamp, a product of Hanover lamps (6 watt S/Y and L/W). Ultraviolet absorption spectra were obtained in spectroscopic methanol on UV - Visible Spectrophotometer (Shimadzu).

The electron impact ionization (EIMS) mass spectra were obtained on a solid probe using Shimadzu QP-class-500.  $^1\text{H}$ NMR spectra were run on a Bruker AM 500 spectrophotometer (Germany) operating at 500 MHz in spectroscopic grade DMSO- $d_6$ . The chemical shifts values are expressed in  $\delta$  (ppm) units using (TMS) as an internal standard and the coupling constants (J) are expressed in Hertz (Hz).

### Methods

#### Isolation of flavonoids

Powdered shade-dried leaves of *Aristolochia bracteata* were macerated with 95% ethanol at room temperature for 48 hours. The ethanol extract was rich in phenolics. It was applied on Whatman papers (No. 3 mm) as narrow strips. The bands were irrigated with 15% acetic acid. The developed chromatograms were air-dried and examined under both visible and UV light ( $\lambda_{\max}$  366,245nm). 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 vacuo* to dryness. In this way- a flavonoid- compound I was isolated in chromatographically pure form.

### RESULTS AND DISCUSSION

The crude extracts of *Aristolochia bracteata* was fractionated by paper chromatography technique. After the usual workup a flavonoid- compound I - was isolated in chromatographically pure form and its structure was deduced on the basis of spectral data.

#### Characterization of compound I

In UV, compound I absorbs (Fig.1) at  $\lambda_{\max}$  (MeOH) 265,298,312nm. Such absorption indicates conjugation between the 4- keto function and the B aromatic ring of the flavonoid nucleus. This absorption is characteristic of flavones.<sup>[13]</sup>

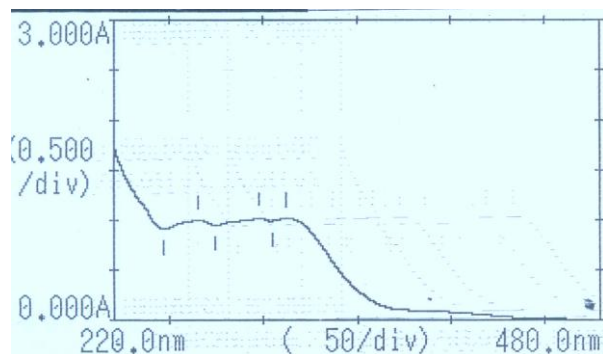
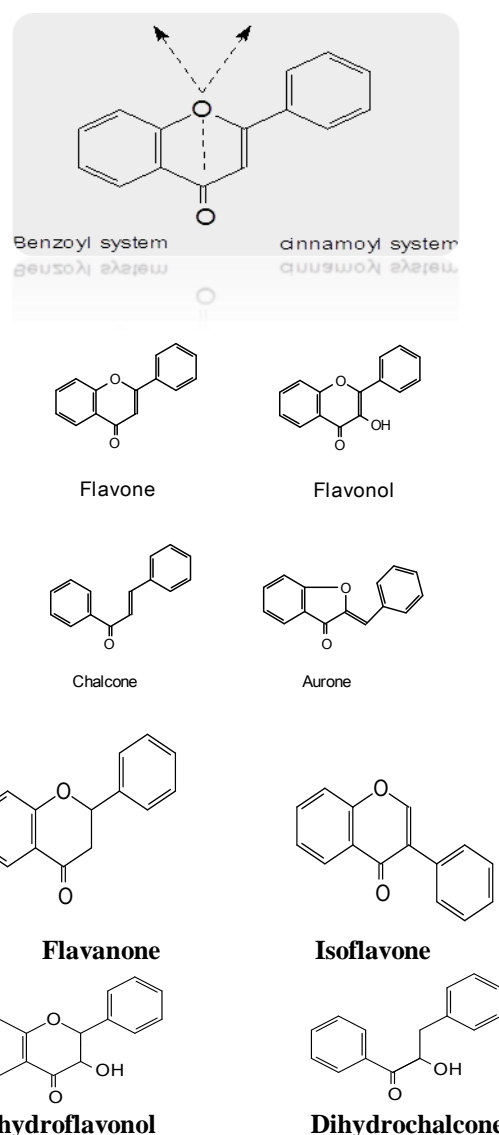


Fig. 1: UV spectrum of compound I.

In their UV spectra flavones give both band I (due to cinnamoyl chromophore) and band II (due to benzoyl chromophore), a feature which is shared by flavonols, chalcones and aurones.<sup>[13]</sup> Other classes: isoflavones, flavanones, dihydrochalcones and dihydroflavonols afford only one peak originating from the benzoyl system. Band I, usually 300 – 400nm and band II, usually 240 – 280 nm. The UV absorption of some important classes of flavonoids is depicted in Table 1.

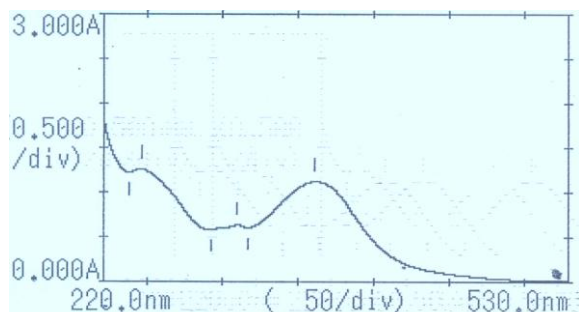
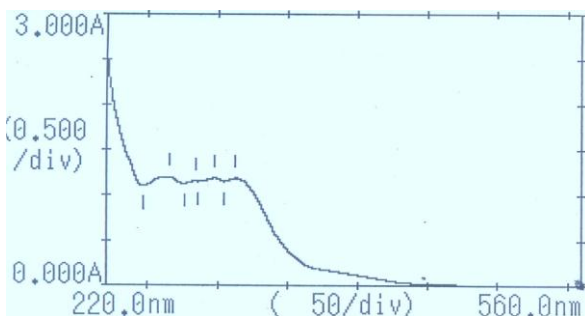
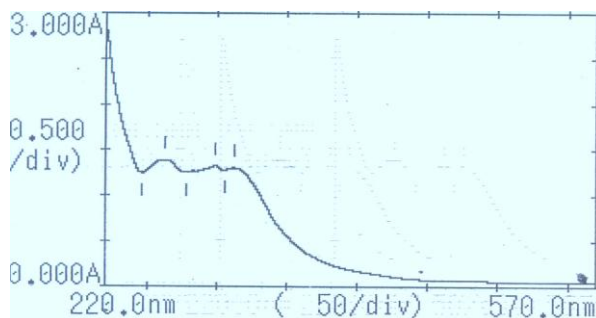
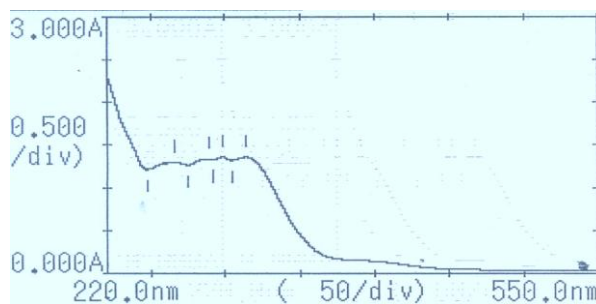


**Table 1: The UV absorption of some flavonoids.**

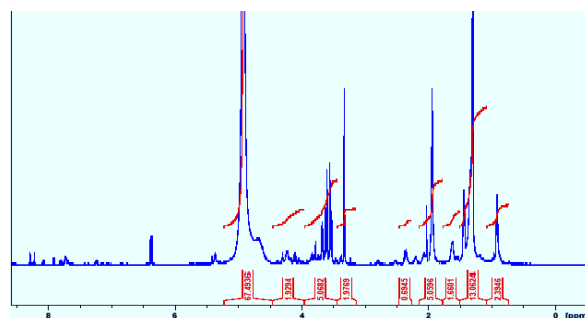
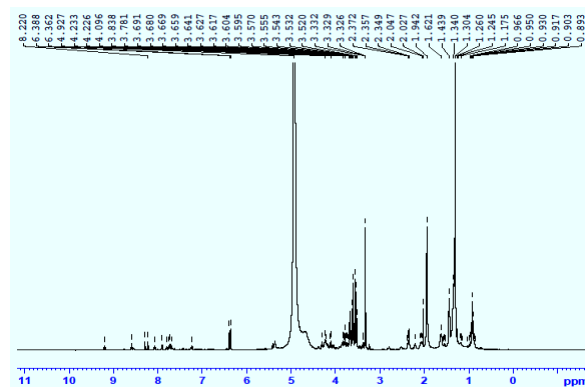
Flavonoid class	Band I	Band II
Favone	320-350	250-270
Flavonol	350-390	250-280
Chalcone	365-390	240-260
Aurone	390-430	240-270

Very significant structural features have been obtained by utilizing the so- called UV shift reagents which produce shifts in the UV absorption maxima in accordance with the location of the various hydroxyl functions in flavonoid nucleus; these reagents are: sodium methoxide (which is diagnostic of 3- and 4'-OH functions); sodium acetate (diagnostic of 7-OH function); aluminium chloride (diagnostic of 3- , 5-OH and catechol systems) and boric acid (diagnostic of catechol systems).

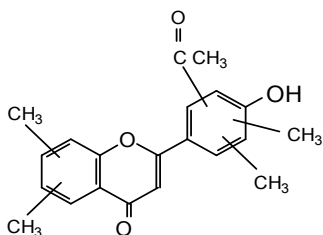
The sodium methoxide spectrum (Fig. 2) showed a bathochromic shift without decrease in intensity indicating a 4'-OH function. When sodium acetate was added to a methanolic solution of compound I, no bathochromic shift diagnostic of a 7- OH function was observed (Fig. 3). Other shift reagents-boric acid (Fig. 4), aluminium chloride (Fig.5) - failed to give any detectable bathochromic shifts. The boric acid spectrum thus suggests absence of catechols systems, while the aluminium chloride spectrum indicated absence of 3- and 5-hydroxylation as well as catechol moieties.

**Fig. 2: Sodium methoxide spectrum of compound I.****Fig. 3: Sodium acetate spectrum of compound I.****Fig. 4: The boric acid spectrum of compound I.****Fig. 5: The aluminium chloride spectrum of compound I.**

The above UV data suggests that compound I is a flavone which is hydroxylated at the 4'-position. The  $^1\text{H NMR}$  spectrum (Figures 6a and 6b) showed:  $\delta$  1.34 (12H) assigned for 4 methyl functions;  $\delta$  1.90 (3H) characteristic of an acetyl group;  $\delta$  3.55-3.83 (m) assigned for a sugar moiety;  $\delta$  6.36 (1H) assigned for  $\text{C}_6$ -proton. Other aromatic protons appeared as a multiplet centered at  $\delta$  7.80 ppm. The EI mass spectrum gave  $m/z$  338 for  $(\text{M}^+ + 2\text{H} - \text{aglycone})$

**Fig. 6a:  $^1\text{H NMR}$  spectrum of compound I.****Fig. 6b:  $^1\text{H NMR}$  spectrum of compound I.**

On the basis of the above cumulative data, the following partial structure was proposed for the aglycone of compound I :



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