Isolation of a Flavone from the Sudanese Material of Cassia Kordofania (Leguminoseae)

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Abstract- The authors report on the isolation of a flavone (5,7,4′-trihydroxyflavone) from the Sudanese material of Cassia kordofania. The flavonoid was isolated from the ethyl acetate fraction by column chromatography. The structure was elucidated by sensitive analytical tools (UV, IR, 1H NMR, 13C NMR and MS).

Index terms: Cassia kordofania, Isolation, Flavone, Characterization.

I. INTRODUCTION
Flavonoids encompass a large group of polyphenolic substances which are widely distributed in plants and foods of plant origin[1-4]. Flavonoids share a common basic skeleton consisting of two benzene rings joined by a linear three carbon bridge. They are divided into: flavans, flavones, flavonols, flavanones, isoflavones, aurones, chalcones, anthocyanins[1,2]. Recently, due to their health benefits, research on the flavonoids gained an increased pulse[2].

In vitro, flavonoids are effective scavengers of free radicals[5,6]. Several studies have examined the relationship between some measure of dietary flavonoid intake and coronary heart disease (CHD) risk[7-14]. It was found that intake of diet rich in flavonoids is associated with significant reduction in CHD risk[7,11-15]. In general, the foods that contributed most to total flavonoids in these studies were: black tea, apples, and onions.

Different In vitro and in vivo studies revealed that some flavonoids exhibit antimicrobial potential[16-23], others exert antispasmodic activity[24]. Several flavonoids have been shown to have potential as hepatoprotective agents[25]. Flavonoids like gossypin and morin were found to show significant analgesic activity[26]. Cassia kordofania belongs to the Leguminoseae family and the sub-family Caesalpiniaeae. It is Known as Egyption Senna, Tinnevelly Senna , East Indian Senna and Nubia Senna. It grows natively in all regions of Sudan specially in Kordofan-eastern Sudan. Cassia Kordofania is used in Sudanese ethnomedicine for treating an array of human disorders including allergic and inflammatory conditions[27]. Other species belonging to this genus are also medicinally important species. Cassia obtusifolia, which is used traditionally in the treatment of diabetes, showed promising antibacterial potential[28]. Aqueous and methanolic extracts of Cassia fistula (used in ethnomedicine as anti-inflammatory, laxative) were found to possess significant anti-inflammatory effect in both acute and chronic models[29]. Also bark extracts showed significant radical scavenging by inhibiting lipid peroxidation initiated by CCl4 and FeSO4 in rat liver and kidney homogenates[29]. Ethanolic leaves extracts of Cassia fistula showed hepatoprotection against INH/RIF-induced hepatitis in rats[30]. It was shown that treatment with Cassia auriculata leaf extract has a lipid-lowering effect in rats with experimentally-induced, alcohol-related, liver damage[31]. It was also claimed that the Cassia auriculata flowers possess antihyperlipidaemic effect in addition to antidiabetic activity[32]. Seeds of Cassia roxburghii, which is used in ethnomedicine for various liver disorders, exhibited significant hepatoprotective activity[33]. Different extracts of leaves of Cassia occidentalis L. were screened for their antimicrobial activity against seven human pathogenic bacteria and two fungal strains by disc diffusion assay with promising results[34]. The plant also showed significant anti-allergic, anti-inflammatory and anti-lipid peroxidant effects in vivo[35].
II. MATERIALS AND METHODS

Materials
Analytical grade reagents were used. The UV spectra were recorded on a Shimadzu 1601 Spectrophotometer and a UV lamp was used for localization of fluorescent spots on TLC plates. The IR spectra were recorded as KBr discs, using Shimadzu IR-8400 Spectrophotometer. Nuclear magnetic resonance spectra were recorded on a JEOL DELTA ESP400 MHz NMR Spectrophotometer. Melting points were determined on a Kofler Hot-Stage apparatus and were uncorrected. Mass spectra were run on a VARIAN 450 GC-240 MS Spectrophotometer.

Plant material
The leaves of Cassia kordofania were collected from Gazeira province –Sudan during November 2012 and authenticated by Dr. Jacob Thomas, Department of Botany and Microbiology, King Saudi University. A voucher specimen was deposited in the herbarium of this department.

Methods
Extraction and isolation of the active constituents
Powdered air-dried leaves of Cassia Kordofania (2.5Kg) were macerated with 95% ethanol (5 L) at ambient temperature for 48hr. The solvent was removed under reduced pressure and the residue (25g) was fractionated by column chromatography using silica gel (60-120 mesh) column using MeOH : CHCl₃(1:1,v:v) as eluent. The column was monitored by TLC and five ml fractions were collected. Depending on their TLC pattern fractions (F₂-F₁₈) were pooled together. Removal of the solvent under reduced pressure gave a pure component-compound I - which reacted positively with ferric chloride and vanillin-H₂SO₄, indicating its strong phenolic nature. Compound I(25mg) is a yellow powder, m.p.319⁰(chloroform);UVλ_max(MeOH) 343, 265, 226, (MeOH-OMe) 248, 274, 392, (MeOH-NaOAc) 364, 374, (MeOH-NaOAc- H₂BO₃) 266, 342,(MeOH-AlCl₃) 266, 305, 397 (MeOH-AlCl₃-HCl) 266, 304 ,398 . IR (KBr): ν (cm⁻¹) : 638, 723, 1089, 1614, 1660 and 3319. GC-MS, m/z : 273(M⁺+3H), 152, 118, 124.

III. RESULTS AND DISCUSSION
The electronspray mass spectrum of (I) exhibited(Fig.1) a peak at m/z 273 corresponding to (M⁺+3H). Compound I gave a positive FeCl₃ and AlCl₃ tests indicating a phenolic nature. In the UV it absorbs(Fig.2) at λ_max(MeOH) 265,343nm due to π → π* and n → π* transitions of benzoyl and cinnamoyl chromophores of flavonoid moiety. This absorption suggests a flavone skeleton[1,2]. Furthermore, the compound gave colour reactions characteristic of flavones[1]

Fig.1: Mass spectrum of compound I
Fig. 2: UV spectrum of compound I

Fig. 3: Aluminium chloride spectrum of compound I

Fig. 4: Sodium methoxide spectrum of compound I

Fig. 5: Sodium acetate spectrum of compound I

The aluminium chloride spectrum (Fig. 3) of (I) gave a 54 nm bathochromic shift without degeneration on treatment with HCl indicating a 5-OH function [2]. The sodium methoxide spectrum (Fig. 4) gave a 49 nm bathochromic shift in band I without decrease in intensity and this is diagnostic of a 4'–OH [1,2]. The shift reagent sodium acetate gave a 9 nm bathochromic shift (Fig. 5) which is indicative [2] of a 7-OH function. No bathochromic shift indicative of catechol moieties [1] was observed in the boric acid spectrum (Fig. 6).
The IR spectrum (Fig7) showed absorption bands at $\nu$(KBr) : 3410 (OH), 1655 ($\alpha,\beta$-unsaturated carbonyl group), 1614 (aromatic C=C) cm$^{-1}$ functionalities. The $^1$H NMR spectrum (Fig.8) exhibited resonances at: $\delta$6.22 (d,2H) and $\delta$ 6.46(d,2H) accounting for C$_6$- and C$_8$- protons respectively. In flavonoids the C$_6$- proton usually resonates at higher field relative to C$_8$- proton[36]. The doublet at $\delta$ 6.95(2H) accounts for C$_3$- and C$_5$- protons, while the resonance at $\delta$ 8.03(d,2H) is characteristic of C$_2$- and C$_6$- protons. The latter protons resonate downfield relative to C$_3$- and C$_5$- protons due to the deshielding effect of the neighbouring heterocyclic C ring [36].
The structure was further supported by the retro Diels–Alder fission depicted in scheme (I) and by its $^{13}$C NMR spectrum (Fig. 9) which revealed a pattern characteristic of a C$_{15}$ skeleton [37,38]. The chemical shifts ($\delta_c$) are depicted in table (1).

<table>
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<th>Carbon No.</th>
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<td>5'</td>
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<tr>
<td>6'</td>
<td>122</td>
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The retro Diels-Alder cleavage gave evidence for the proposed structures since the ions, m/z 102, 118, 135, 152 which correspond to intact aromatic rings were detected in the electron beam.

The above cumulative data limits the structure of compound I to the following:

The retro Diels-Alder cleavage is shown in scheme (I).

**REFERENCES**


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