

## Characterization and Some Biological Activity of A Flavonol Isolated from *Cassia Occidentalis* Leaves

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**Abstract-** From the ethyl acetate extract of the leaves *Cassia occidentalis* (Leguminosae) a flavonol: 5,7,2',4'-tetrahydroxyflavonol(I) was isolated. The structure was elucidated via a combination of spectral techniques (UV, IR, <sup>1</sup>H NMR and MS). The flavonol was evaluated *in vivo* for anti-inflammatory and anti-ulcer activity. Treatment of the animal models with compound I inhibited formaldehyde-induced oedema up to 93.99%. The flavonol also showed significant anti-ulcer activity. Treatment with compound I (30)min. before alcohol-induced ulcer completely suppressed ulcer.

**Index Terms:** *Cassia occidentalis*, Isolation, Flavanol, Biological activity

### I. INTRODUCTION

*Cassia occidentalis* belongs to the family of leguminous and sub-family Caesalpiniaceae. It is known as stinking weed in English. The plant is found growing wild throughout India and northern Africa. It is an annual undershrub, about 60-150 cm height [1,2]. *C. occidentalis* is used for treating an array of human disorders including: allergic and inflammatory diseases, and has been reported to have antibiotic and hepatoprotective activities [3-6].

Flavonoids are phenolic compounds widely present in plants and foods of plant origin [7-10]. Flavonoids contain fifteen carbon atoms in their basic nucleus-flavan, arranged in a C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> configuration consisting of two aromatic rings (A and B) linked by a three carbon unit which may or may not form a third heterocyclic ring (C). There are six major subgroups: chalcones, flavones, flavonols, flavanones, anthocyanins and isoflavonoids. Flavonoids encompass a large group of polyphenolic substances with marked physiological potential including: antibacterial, anti-inflammatory, antiallergic, antifungal, antimutagenic, antiviral and vasodilator effects [11-13].

As part of our interest in constituents of medicinal plants growing in Sudan and Saudi Arabia, this study was aimed to:

- Isolation of flavonoids from *Cassia occidentalis*
- Elucidation of the structure of the isolated flavonoids via sensitive spectroscopic tools: UV, IR, <sup>1</sup>H NMR and MS.

- Screening the isolates for anti-inflammatory and anti-ulcer activity.

Hereby we report on the isolation, characterization, anti-inflammatory and anti-ulcer potential of a flavonol aglycone isolated from the ethyl acetate extractives of the leaves of *C. occidentalis*.

### II. MATERIALS AND METHODS

#### Materials

Analytical grade reagents were used. The UV spectra were recorded on a Shimadzu 1601 Spectrophotometer and UV lamp was used for localization of fluorescent spots on TLC plates. The IR spectra were recorded as KBr disks, using Shimadzu IR-8400 Spectrophotometer. Nuclear Magnetic Resonance spectra were run on a JEOL DELTA ESP-400MHZ NMR Spectrophotometer. Melting points were determined on a Kofler Hot-Stage Apparatus and were uncorrected. Mass spectra were measured on a Verian G Spectrometer.

#### Plant material

The leaves of *C. occidentalis* were collected from Riyadh region, Saudi Arabia during November 2009 and identified by Dr. Jacob Thomas, Department of Botany, King Saudi University. A voucher specimen was deposited in herbarium of Department of Botany, King Saudi University.

#### Methods

##### Extraction of active constituents

Powdered air-dried leaves of *Cassia occidentalis* (500g) were macerated with 85% methanol (3 L) at ambient temperature for 2 days.

The solvent was removed under reduced pressure and the residue (56g) was dissolved in (200 ml) water, defatted with petroleum ether (40<sup>0</sup>-60<sup>0</sup>C) (3x200ml) and then extracted successively with chloroform (3x100 ml), ethyl acetate (3x100ml) and n-butanol (3x100 ml).

##### Isolation of flavonoids

The crude product obtained from ethyl acetate extract (2 g) was chromatographed on a silica gel column (60 mesh, Astem) using MeOH : CHCl<sub>3</sub> (3:2) as eluent. The column fractions F<sub>12</sub> – F<sub>20</sub> (40 ml) were collected and

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combined on the basis of their TLC pattern. Removal of the solvent under reduced pressure gave compound I which was unambiguously determined [14,15] by means of spectroscopic methods; IR, UV, MS, <sup>1</sup>HNMR, <sup>13</sup>CNMR and 2D NMR experiments.

### Biological Screening

#### Induction of inflammation by formaldehyde

Wistar rats (200-220g) were used in this study according to the international regulations for the use of laboratory animals. Formaldehyde (10%) was employed as inflammatory agent [16].

Each animal was marked on its right ankle in circular manner using a non-erasable blue ink. The volume of each paw up to the ankle mark was then measured using a Ugo-Basil plythesmometer. Using 0.45% sodium chloride solution as displacement fluid, the volume of the immersed paw was then read in a digital display.

Groups of three animals were injected with compound I (1mg/kg, i.p).

After 60 minutes, each rat was then injected intraperitoneally with 0.2 ml of 10% formaldehyde using a fine 1 ml hypodermic needle. The control group was injected with 0.25% sodium carboxymethyl cellulose intraperitoneally in a volume equivalent to the test volumes injected. Sixty minutes later, formaldehyde was injected intraperitoneally as described above.

Following formaldehyde injection, paw volumes to the marked sites were read at 1, 2 and 3 hours intervals. The volume of formed oedema was then calculated. Net oedema volumes formed 1 and 3 hour following injection of formaldehyde was used to calculate the influence of compound (I) on the induced oedema.

#### Induction of gastric ulcer by ethanol

The method described by Alsayed [16] was followed. Male Wistar rats (200 g) were fasted for 36 hour with access to drinking water *ad libitum*. The animals were divided into 4 groups. Each animal was administered in 1 ml of 80% ethanol in water intragastrically by gavage needle. The drug under test was administered 30 minutes before administration of alcohol. One hour after administration of alcohol, the animals were killed using diethyl ether. Stomachs were removed, opened along the greater curvature, cleaned with saline and examined with binocular magnifier. Stomach was examined for the total area of lesion. The ulcers were scored according to the method described by Alsayed [16].

### III. RESULTS AND DISCUSSION

The electrospray mass spectrum of (I) exhibited a molecular ion peak (Fig.1) at m/z 302 corresponding to the molecular formula:

C<sub>15</sub>H<sub>10</sub>O<sub>7</sub>. Compound I gave a positive FeCl<sub>3</sub> and AlCl<sub>3</sub> tests indicating a phenolic nature. In the UV it absorbs (Fig.2) at λ<sub>max</sub>(MeOH) 266,364nm due transitions of the benzoyl and cinnamoyl chromophores of the flavonoid to π → π\* and n → π\* on suggests a flavonol skeleton [7,8]. The 3-OH of the flavonol is

demonstrated by the 58 nm bathochromic shift (Fig.3) induced by the UV shift reagent -AlCl<sub>3</sub> [8].

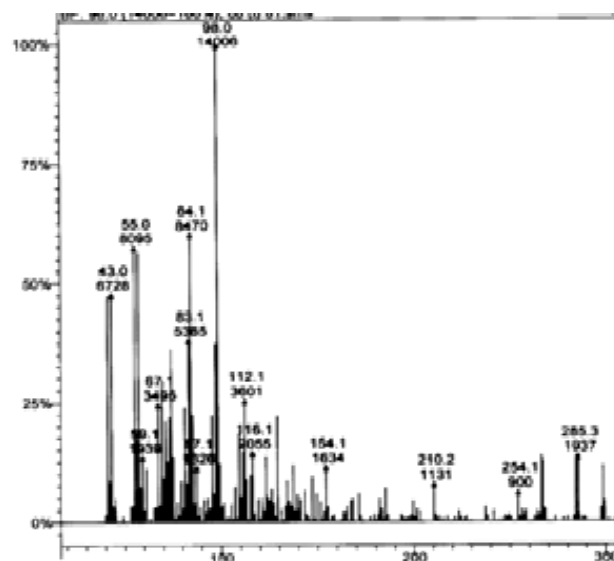


Fig. 1: Mass spectrum of compound I

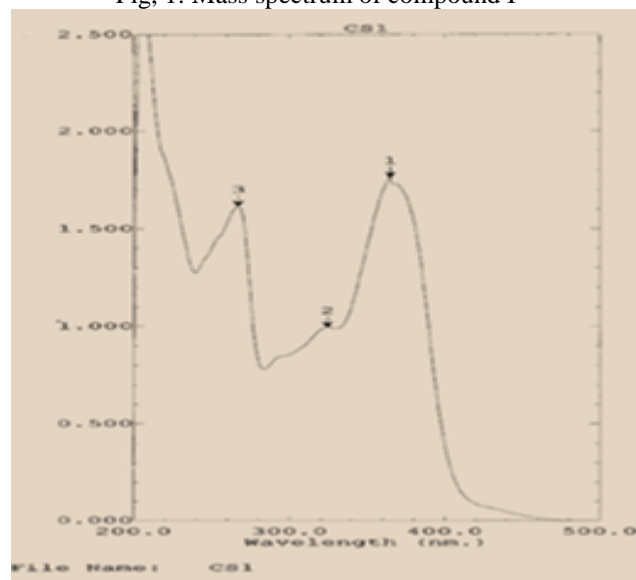


Fig. 2: UV spectrum of compound I

The aluminium chloride spectrum of ( I ) did not degenerate on treatment with HCl. Also

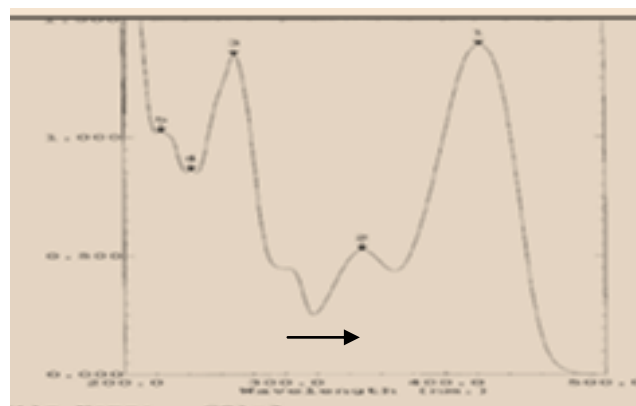


Fig.3: Aluminium chloride spectrum of compound I

the compound gave colour reactions characteristic [7] of flavonols. When sprayed with  $\text{AlCl}_3$  in a silica gel plate it turned yellow and when viewed under UV light it became fluorescent yellow. When fumed with ammonia and viewed under UV light it gave a bright yellow coloration.

The sodium methoxide spectrum (Fig.4) gave a 47 nm bathochromic shift in band I without

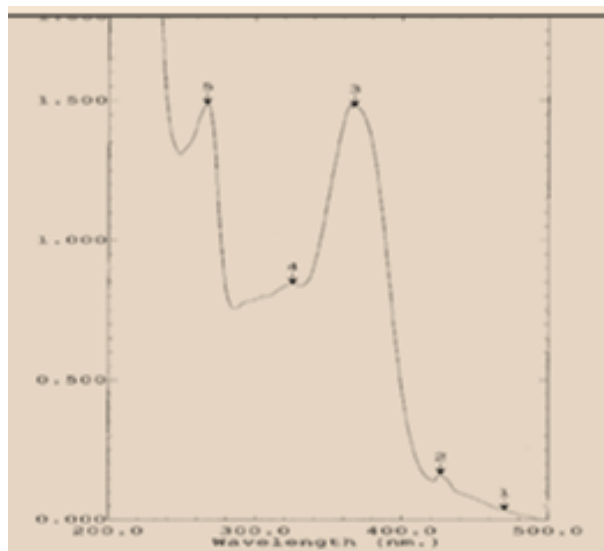


Fig. 4: Sodium methoxide spectrum of compound I

Decrease in intensity and this is diagnostic [8] of a 4'-OH. The shift reagent sodium acetate gave a 9nm bathochromic shift which is indicative of a 7-OH [7]. No bathochromic shift was observed in the boric acid spectrum of (I), thus catechols are absent.

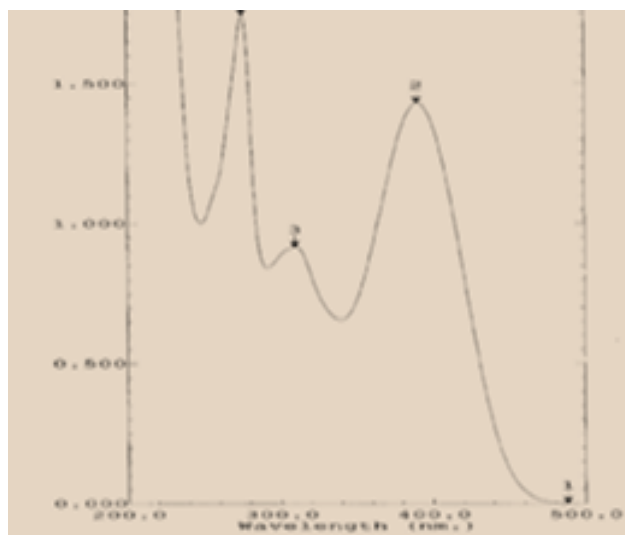


Fig. 5: Sodium acetate spectrum of compound I

The IR spectrum (Fig.6) gave  $\nu(\text{KBr})$  : 638, 810 (C-H, Ar. bending), 1086 (C-O), 1562, 1571 (C=C, Ar.), 1651 ( $\alpha, \beta$ -unsat. carbonyl function) and  $3296\text{cm}^{-1}$  (OH).

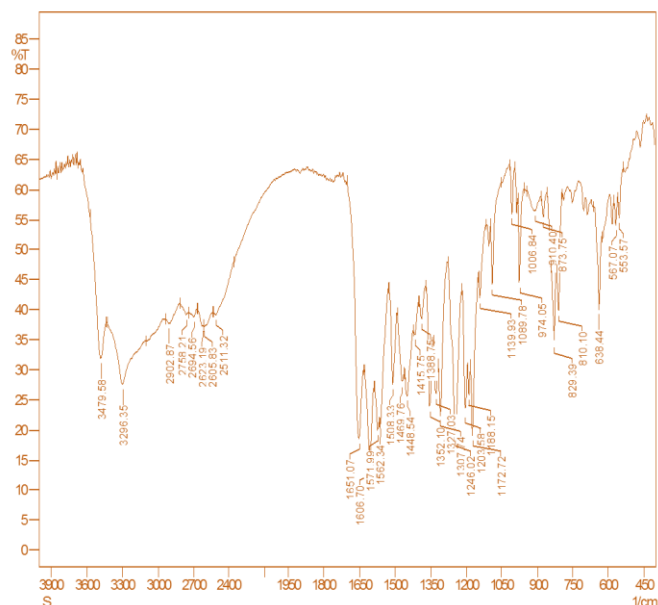


Fig.6: IR spectrum of compound I

The  $^1\text{H NMR}$  spectrum (Fig.7) (400MHz,  $\text{DMSO-d}_6$ ) revealed  $\delta(\text{ppm})$ : 7.24 (1H, d,  $\text{C}_6\text{-H}$ ), 6.30 (3H, m,  $\text{C}_5\text{'}, \text{C}_8\text{-}$  and  $\text{C}_3\text{'}$ -protons), 5.87 (1H, d,  $\text{C}_6\text{-H}$ ), 12.6 (1H, s,  $\text{C}_5\text{-OH}$ ), 10.73 (1H, s,  $\text{C}_7\text{-OH}$ ), 9.80 (1H, s,  $\text{C}_3\text{-OH}$ ).  $^{13}\text{C NMR}$  (400MHz,  $\text{DMSO-d}_6$ ) disclosed a pattern (Fig.8) characteristic of flavonoids. It gave ( $\delta, \text{ppm}$ ): 164.21 (C-2), 103.47 (C-3), 176.74 (C-4), 164.21 (C-5), 98.58 (C-6), 61.47 (C-7), 104.09 (C-8), 157.35 (C-9), 103.47 (C-10), 120.90 (C-1'), 107.35 (C-2'), 136.75 (C-3'), 149.55 (C-4'), 109.73 (C-5'), 120 (C6').  $^1\text{H}-^1\text{H COSY NMR}$  revealed a diagonal relationship between  $\text{C}_5\text{-OH}$  and  $\text{C}_6\text{-H}$ ;  $\text{C}_2\text{-OH}$  and  $\text{C}_3\text{'}$ -proton.

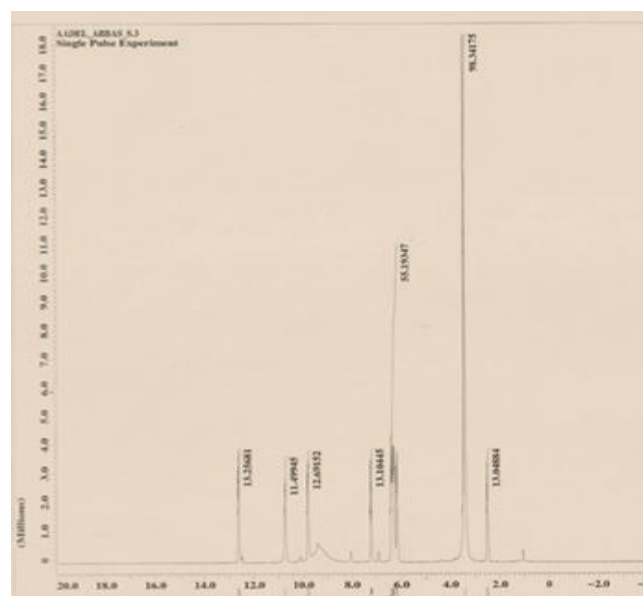


Fig.7:  $^1\text{H NMR}$  spectrum of compound I

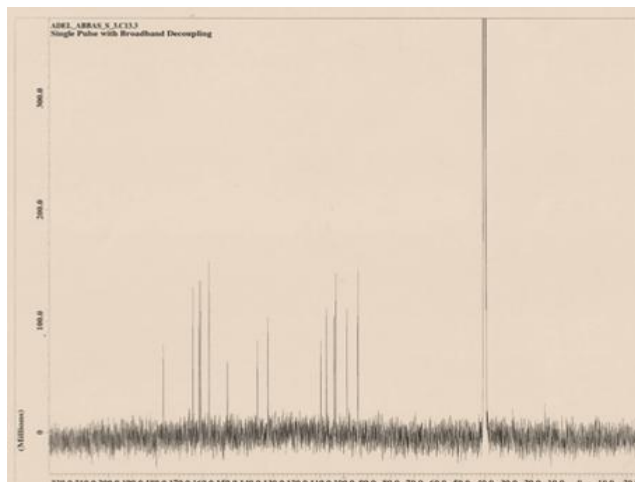
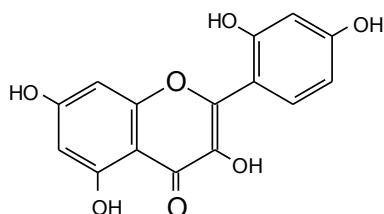
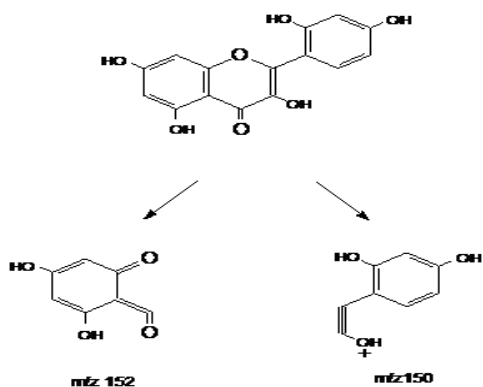


Fig.8: <sup>13</sup>C NMR spectrum of compound I

On the basis of the above cumulative data the following structure was suggested for compound I:



Additional evidence in favour of the above structure comes from the retro Diels – Alder fission shown in Scheme I, where the fragments m/z 150 and m/z152 corresponding to intact A and B rings were recorded in the electron beam.



Scheme I : Retro Diels - Alder fission of compound I

Next we investigated compound I for its pharmacological potential. The compound was screened for anti-inflammatory and anti-ulcer activity. Such studies could cite a rationale for the ethnomedical use of *C. Occidentalis*.

Groups of three animals (Wistar rats, 200g) were injected with compound

I (1g/kg, i.p). After 60 minutes, each rat was then injected intraperitoneally with 0.2 ml of 10% formaldehyde to induce oedema. A control group was injected with 0.25% sodium carboxymethyl cellulose.

The volume of oedema formed in test animals (after 1 and 3 hour) after treatment with formaldehyde and compound I is shown in table (1). The percentage inhibition of ulcer is depicted in table (2).

Table.(1):Effect of compound( I)on formaldehyde-induced oedema.

Treatment	Net volume (in ml) of oedema after :	
	1 hour	3 hours
Formaldehyde	2.3	2.33
Compound I	0.64	0.14

Table.(2): Anti-ulcer activity of compound I

Compound I	%age inhibition after:	
	1 hour	3 hours
	72.17	93.99

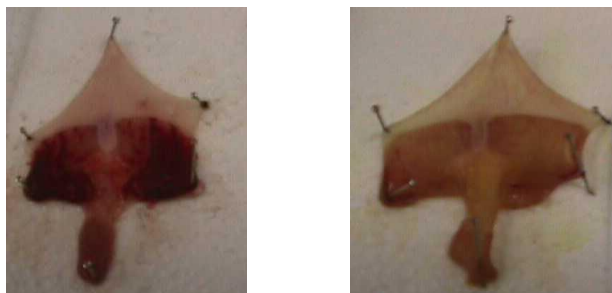
It seems that treatment of the animals with compound I inhibited oedema formation up to 93.99% as shown in table ( 2 ). This flavonol exhibited marked anti - inflammatory activity. The actual mechanism for such inhibition was not yet clarified. However, various mediators, such as prostaglandins, leucotriens and oxygen radicals, are known to be involved in formaldehyde – induced inflammation [18, 19].

The anti-inflammatory activity of these flavones may be attributed to inhibition of any of these mediators the exact mechanism will be addressed in a future study.

Treatment of rats with alcohol alone induced some ulcers giving a total of 5. All of the ulcers were longitudinal across the stomach mucosa. The flavonol showed anti-ulcer potential against alcohol- induced ulcers. Treatment of the animals with compound I (30) min. before alcohol intake completely suppressed alcohol – induced ulcers (see Fig.1). These ulcers are known to involve various mechanisms such as induction of free oxygen radicals, increase in acid secretion, breakdown of the mucosal barrier [20].

The actual mechanism operated by compound in suppressing such ulceration was not examined in this study. However, it may involve inhibition of all or one of the above-mentioned mediators released under influence of alcohol. Future experiments may clarify this point.

On a broad basis, these data orient attention to the potential anti-inflammatory and ant-ulcer activity of this phytochemical . The study also sites a rationale for the ethnomedical use of the plant.



(a)

(b)

Fig.1: Gastric mucosa (a): before treatment with compound I(b)after treatment

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