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# Isolation of flavonoids from *Crotalaria grahamiana* leaves extract

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**Abstract**
Flavonoids are yellow pigments widely distributed as their glycosides obtained from plant sources. Flavonoids may delay or prevent the onset of diseases such as cancer induced by free radicals. In the present work, the air dried leaves of Crotalaria grahamiana belongs to Papilionaceae is chosen for phytochemical and pharmacological studies. Aqueous extract was prepared and fractionated with benzene, diethyl ether and ethyl acetate. Ether fraction results Kaempferol (aglycone) and Ethyl acetate fraction results Populnin (glycoside). The structure was ascertained by UV, NMR, PC and chemical reactions. Ethyl acetate concentrate has been tested for its anti-bacterial activity using two species of bacteria namely staphylococcus aureus and Escherichia coli for two different drug concentrations. The result shows that it is potent enough to act against both Gram positive and Gram negative organisms. The SRBC membrane stabilization studies on the ethyl acetate soluble of *Crotalaria grahamiana* showed destabilization property at lower concentrations. At 75µg the stabilizing effects is observed in the compound. This capacity persists up to 100µg. Beyond that concentration the drug is unable to contain the membrane.

**Keywords:** flavonoids, crotalaria grahamiana, Kaempferol, populnin, anti-bacterial activity

## Introduction
Flavonoids are yellow pigments obtained from plant sources. The important classes are flavones, flavanone, flavonol, flavanonols, aurones and chalcones. Naturally occurring polyphenols have two alternative sugar linkages viz., C-glycosylation or O-glycosylation. The importance of flavonoids in chemotoxonomy has been extensively studied [1, 2].The process of plant synthesizing flavonoid pigments has been obtained from chemical and genetical studies [3, 4].The new types of flavonoids (ie) biflavonoids and 4-aryl coumarin has been reviewed [5]. Luteolin 7-O-glucoside has been isolated from Cassia mimosoides [6]. Pongamia pinnata showed an abundance of flavonoids such as flavanones, chalcones and beta hydroxyl chalcones [7, 8]. Isolation of quercetin and kaempferol from the in vivo and in vitro tissue cultures of Dolichos lablab and their antifungal screening have been reported [9].The analgesic effect of Kaempferol 3-O-sophoroside isolated from Cassia alata has been studied [10]. The aim of this work was to demonstrate the isolation of flavonoids from Crotalaria grahamiana belongs to papilionaceae and analyse its anti-inflammatory and antimicrobial properties.

## Materials and methods
### Materials
Air dried leaves (1kg) of *Crotalaria grahamiana* was chosen for phytochemical and pharmacological studies. 85% of ethanol was used for extraction purpose. The alcoholic concentrate was fractionated with benzene (3×250ml), peroxide free diethyl ether (3×250ml) and ethyl acetate (4×250ml).For screening studies Sheep blood, Alsever solution, 0.85% isosaline, 0.25% hyposaline, phosphate buffer were used. Muller - Hinton agar Method was used for anti-microbial studies.

### Methods
**Isolation of flavonoids: (Kaempferol and populnin)**
Air dried leaves of Crotalaria grahamiana was extracted with 85% ethanol under reflux. The alcoholic extract was concentrated in vacuo and the aqueous extract was fractionated with benzene, diethyl ether and ethylacetate. The benzene fraction did not yield any isolable material. The ether fraction gave kaempferol (aglycon).It had ^Max MeOH nm MeOH 235sh, 266, 294sh, 322sh, 367;+ NaOMe 278, 316, 416; + AlCl3 with and without HCl, 256sh, 269, 303sh, 348, 424; + NaOAc, 274, 303, 387 and + (NaOAc+$H_3BO_3$) 267, 297sh,320sh,372. The EtOAc fraction yield populnin (glycoside). It had $\lambda_{Max}$ MeOH nm MeOH 264, 321, 362; + NaOMe 270, 294sh, 358, 412; + $AlCl_3$ and $AlCl_3$ / HCl 255sh, 266,300sh, 420; + NaOAc 272, 303, 420; + NaOAc / $H_3BO_3$ 272, 303, 420. Both the aglycon and glycoside responded to Wilson's boric acid, Gibb's, Horhammer-Hansel and Shinoda tests. Hydralysis of the glycoside with $H_2SO_4$ gave Kaempferol and glucose. The sugar moiety was identified by Molisch test and Folin-wu method. The $^1H$– NMR spectrum of the glycoside showed a distinct signal at δ 12.60 ppm for 5-OH.The signals at δ 6.75 ppm and δ 7.86 ppm, the protons 3' & 5'resonate respectively. The C-6 & C-8 protons respectively appear at δ 6.37 ppm & δ 6.75 ppm. The anomeric proton at the glucoside H-1" appear at δ 5.41 ppm. The hydroxyl protons at C-3 & C-4' respectively appear at δ 8.34 ppm & δ 8.14 ppm. The rest of the sugar protons appear at δ 3.31-3.45 ppm. On this basis of the above evidences the glycoside has been characterized as populnin (Kaempferol 7-O-glucoside).

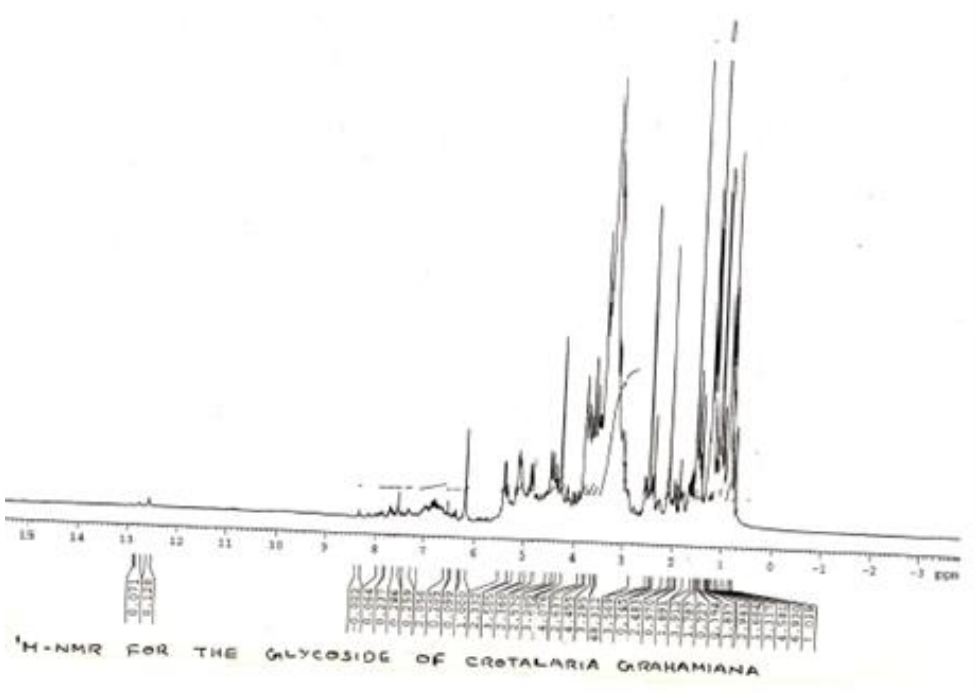
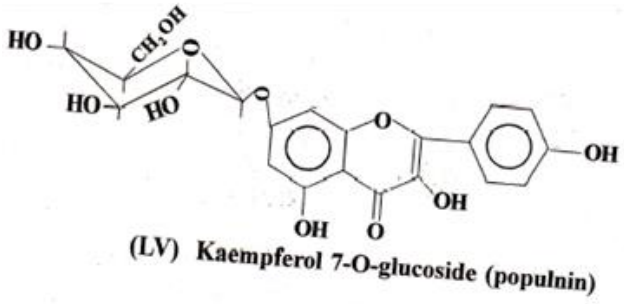



**Fig 1**




**(LV) Kaempferol 7-O-glucoside (populnin)**

**Fig 2**

**Anti-inflammatory activity in SRBC**

Fresh sheep blood was mixed with equal volume of Alsever solution. Then the blood was centrifuged at 3000 rpm and washed with isosaline (0.85%) 3 times and a 10% suspension was made with isosaline. Then different concentrations of glycoside was prepared. Assay mixture contained the drug, 1ml of phosphate buffer (0.15M; pH7.2), 2ml of hyposaline (0.25%) and 0.5ml of 10% RBC suspension. In another tube 2ml of distilled water was taken. All the tubes were incubated at 37'C for 30 min. Then they were centrifuged and the haemoglobin content in the supernatant was estimated using a photoelectric colorimeter at 560 nm. The concentration was plotted against transmittance (graph 1). The screening study of SRBC membrane stabilization for anti-inflammatory activity showed a maximum stabilization at 75 µg. This capacity persists up to 100 µg.

| S.No. | Concentration of drug in µg | Transmittance (%) |
|---|---|---|
| 1. | 10 | 20 |
| 2. | 25 | 18 |
| 3. | 50 | 13 |
| 4. | 75 | 14 |
| 5. | 100 | 14 |
| 6. | 200 | 11 |

**Fig 3**

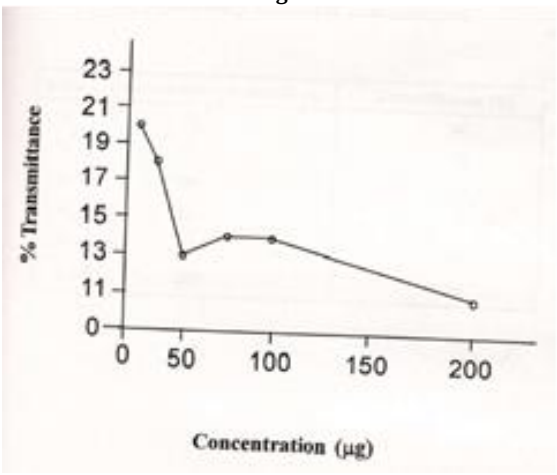



**Fig 4**

**Antimicrobial studies of populnin**

Muller-Hinton agar was prepared by suspending 38g in 100ml distilled water and boiled to dissolve the medium completely. The two species of bacteria used were Escherichia coli and Staphylococcus aureus. The MH agar plate was divided into four sections smeared each with pure flavone solution. It was incubated for 18hrs. The two controls E. coli and staphylococcus aureus smeared to four different areas. The antibacterial activity of EtOAc soluble of Crotalaria grahamiana was totally potent enough to act against both Gram positive and Gram negative organisms. Even at lower concentration at 25 µg staphylococcus aureus showed bactericidal property. The same efficiency observed even in the higher concentration of 75 µg. E.coli has also been having control of a concentration of 25 µg. This tendancy holds good at higher concentrations too.

**Fig 5**

**Conclusions**

Phytochemical investigation on *Crotalaria grahamiana* results the isolation of two compounds Kaempferol and populnin. The structures of the isolated flavonoids were ascertained by UV, NMR and chemical reactions. The EtOAc soluble exhibited good anti-bacterial and anti-inflammatory activity.

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