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PHYTOCHEMICAL SCREENING AND ISOLATION OF A FLAVONE FROM THE LEAVES OF *GEIGERIAAL ATA* (DC). GROWN IN SUDAN

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ABSTRACT
The present study was designed to screen the phytochemicals present in the ethanolic extract of the leaves of *Geigeria alata* (DC) and to isolate the flavonoids present. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides and sterols. A flavone was isolated and characterized via spectroscopic analysis IR, UV, H1NMR and MS.

KEYWORDS: Phytochemical screening, isolation, *a* flavone, *geigeria alata* (DC.)

INTRODUCTION

Flavoniods are natural products widely distributed in plant kingdom, and currently consumed in large amount in the daily diet.[1] They serve a variety of ecological and physiological function on plants.[2] Flavonoids possess a potential pharmacological activities such as anti-oxidant activity, vitamin C sparing activity, Flvonoids have free radical scavenging and antioxidant properties, which are useful for their pharmacological activities including anticancer, and antiaging properties .Flavonoids show interaction with cytochrome P 450, which has antileukemic properties and mild vasodilator properties useful for the treatment of heart diseases .[3] They are not only playing a major functional role in the plant, but also commercially significant in pharmacology, the food industry and in ornamental plants.[4] Flavonids are universal within the plant kingdom, where they afford the most common pigments next to chlorophyll and carotenoids. They generally occur in plant as glycosylated derivatives and their physiological role in the ecology of the plants are diverse. Due to their attractive colors, flavones, flavonols and anthocyanidins may act as visual signals for pollinating insects.[10] In consideration of their astringency, catechins and other flavonols can represent a defense system against insects harmful to the plant.[5]Moreover, flavonoids act as catalysts in the light phase of photosynthesis and/or as regulator of ion channels of phosphorylation[6] They also function as a stress protectant in plant cells by scavenging reactive oxygen spices produced by photosynthetic electron transport system. Because of their UV absorbing properties, flavonoids protect plants against UV radiation.[9]

MATERIALS AND METHODS

Plant material

Leaves of *Geigeria alata* **(**DC) were maintained from a local market at Omdurman city. Plant sample was authenticated at the herbarium of Medicinal and Aromatic Plants and Traditional Medicine Research Institute. The plant material was used for the phytochemical screening and for the extraction, isolation and characterization of its flavonoids contents.

Preparation of plant extract for phytochemical screening

(100g) of powdered air- dried leaves of *Gegeria alata* were extracted with 80% methanol (soxhlet) for 5 hours. The cooled solution was filtered and the volume was adjusted to (100 ml) by addition of enough 80% methanol. This prepared extract (PE) was used for the following tests.

Test for alkaloids

(20ml) of the aliquot of the prepared extract of *Gegeria alata* leaves was evaporated to dryness on a water bath. (5ml) of 2N hydrochloric acid were added and the solution was heated with stirring in a water bath for 10minutes. The mixture was cooled and filtered. To apportion (2ml) of the filtrate, few drops of Mayer reagent were added. Pale yellow precipitate was observed. Also red precipitate was observed when adding Dragendorff's reagent to the extract.

Test for flavonoids

(20ml) aliquot of the prepared extract of the leaves of *Gegeria alata* were evaporated to dryness on a water

bath. The cooled residue was defatted with petroleum ether and the residue was dissolved in (30ml) 80% methanol and filtered. The filtrate was used for the following tests.

a. To (3 ml) of the filtrate few drops methanolic aluminum chloride were added. A dark yellow color was observed
b. To (3 ml) of the filtrate few drops of potassium hydroxide were added. A dark yellow color was observed.
c. To (3 ml) of filtrate few drops of ferric chloride solution were added. A blue coloration was observed.

Test for tannins

(20 ml) aliquot solution of the prepared extract was evaporated to dryness on a water bath and the residue was extracted with n- hexane. The hexane insoluble portion was stirred with (10 ml) of hot saline solution (0.9% w/v of sodium chloride and freshly prepared distilled water). The mixture was cooled and filtered and the volume was adjusted to (10ml) with more saline solution. (5 ml) of this solution were treated with few drops of ferric chloride solution, a blue coloration was formed.

Test for saponins

(1g) of the powdered air dried leaves of *Gegeria alata* was placed in a clean test tube. (10ml) of distilled water were added and the tube was stoppered and vigorously shaken for about 30 seconds. Honey comb was formed.

Test for sterols and triterepenes

(20 ml) aliquot of the prepared extract of *Gegeria alata* leaves was evaporated to dryness on a water bath, and the cooled residue was stirred with petroleum ether to remove most of the coloring matters. The residue was extracted (20 ml) chloroform. The chloroform solution was mixed with (0.5 ml) acetic anhydride, followed by two drops of concentrated sulphuric acid. No change in color was observed.

Extraction of flavonoids

Powdered air - dried leaves (1kg) of *Gegeria alata* were macerated with 80% ethanol (5L) at ambient temperature for 48 hours. The solvent was removed *in vacuo* to give a crude product. The crude product obtained from Gegeria *alata* leaves was dissolved in water (100ml) and successively partitioned by n-hexane, chloroform ethyl acetate and n-butanol. The n-butanol fraction was checked for the presence of flavonoids and then was isolated by paper chromatography.

RESULT AND DISCUSSION

The phytochemical screening of the leaves of Geigeria alata revealed the presence of the following phytochemicals demonstrated in the table below.

Class of compound (phytochemical)	Ethanolic extract of leaves
Alkaloids	++
Flavonoids	+++
Tannins	++
Sterols and triteripenes	-
Saponins	++

+++ = abundant, ++ = moderately present, + = present, - = absence

The IR spectrum of the flavonoid revealed the presence (Fig.1) ν (KBr): 613,719(CH, Ar. bending), 1114 (CO), 1419, 1448 (C=C, Ar.), 1743 (C=O), 2922 (C-H, alkanes) and 3346cm^{-1}(OH).

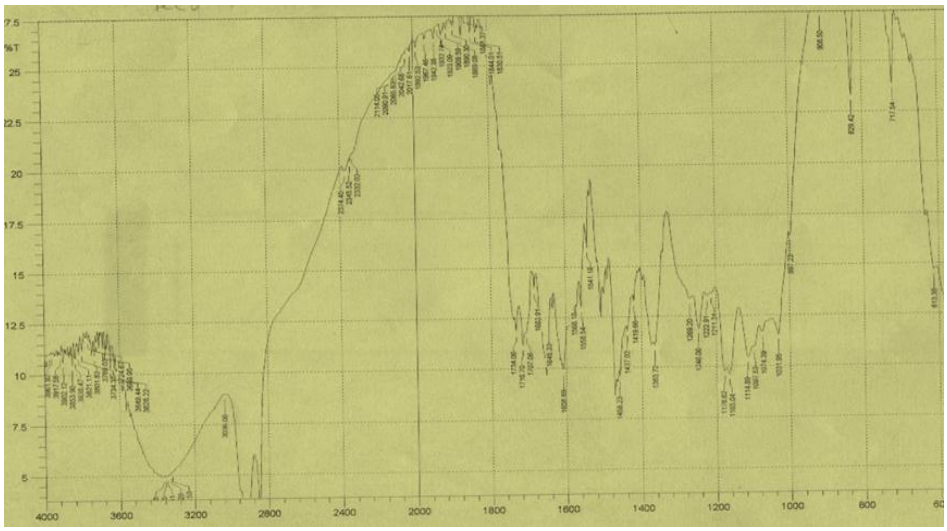


Fig. 1: IR spectrum of the isolatedcompound.

In the UV, the flavonoid absorbs (Fig.2) at λ_{max} 269,337nm. Such absorption is characteristic of flavones.[7]

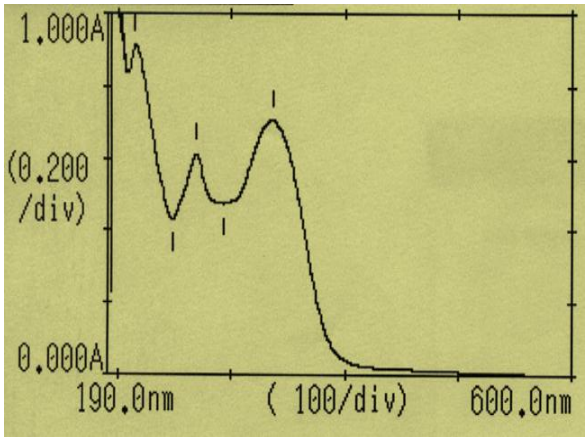


Fig. 2: UV spectrum of the isolated flavonoid.

The sodium methoxide spectrum (Fig.3) gave a bathochromic shift (61nm) with decrease in intensity which is diagnostic of a 4`-OH function. The sodium acetate spectrum (Fig.4) did not give any bathochromic shift indicating absence of a 7-OH function.[8]

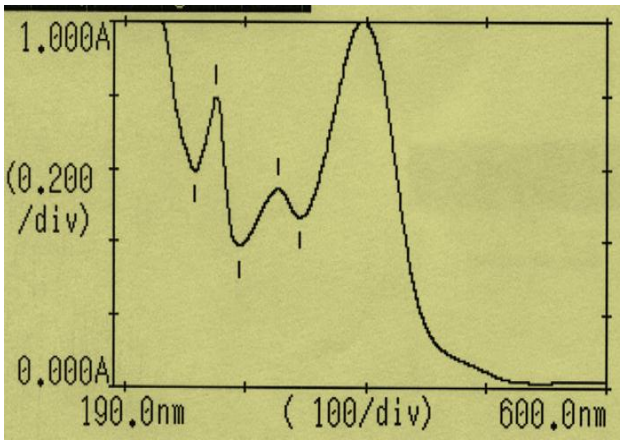


Fig. 3: Sodium methoxide spectrum of the isolated flavonoid.

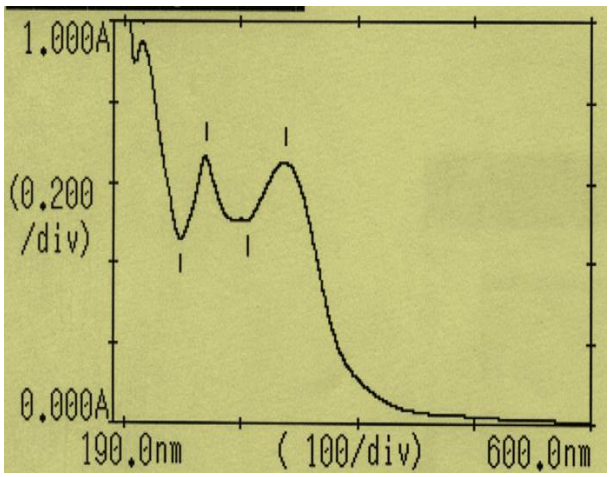


Fig. 4: Sodium acetate spectrum of the isolated flavonoid.

A bathochromic shift was observed in the aluminium chloride spectrum (Fig.5). The spectrum was acid-stable (Fig.6) suggesting a 5-OH function. The boric acid spectrum (Fig.7) did not reveal any bathochromic shift indicating absence of catechol moieties.

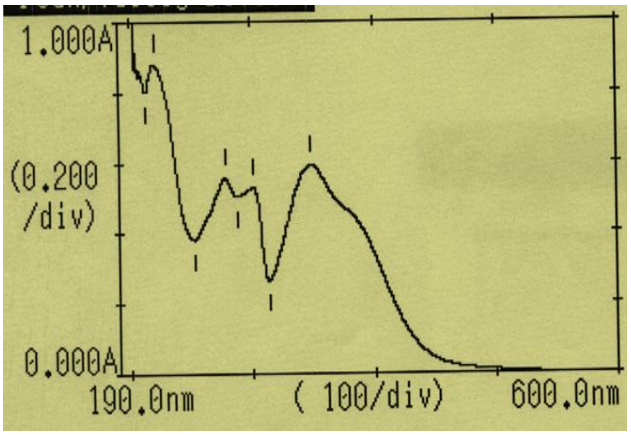


Fig. 5: Aluminium chloride spectrum of the isolated flavonoid.

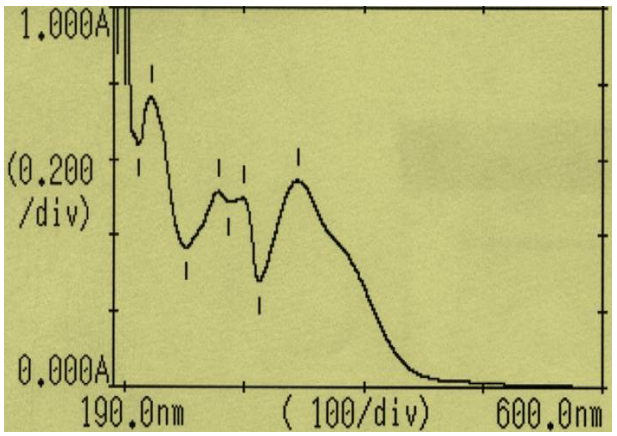


Fig. 6: Aluminium chloride/ HCl spectrum of the isolated flavonoid.

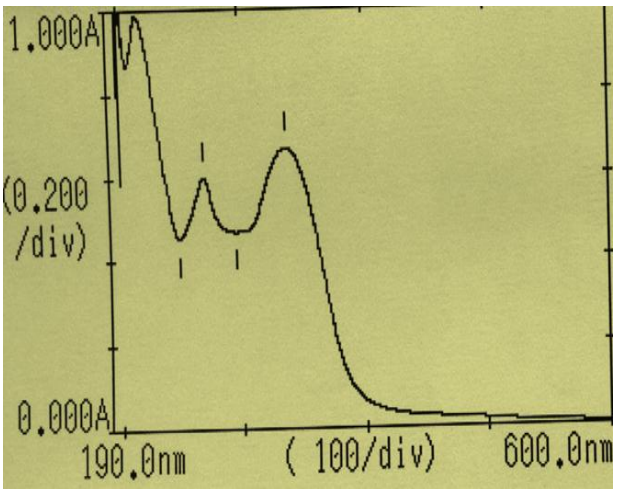


Fig. 7: Boric acid spectrum of the isolated flavonoid.

The ^{1}HNMR spectrum (Fig.8) showed: δ1.23 (6H) assigned for two methyl functions. The resonance at δ 3.75 (3H) accounts for a methoxyl function. The signals at δ 6.19 (1H) and δ 6.48 (1H) were attributed for C_6 – and C_8- protons respectively. The resonances at δ 6.76, 6.91 and were assigned for A ring protons. The B ring

protons, which usually resonate at lower field appeared at δ 7.93ppm.The EI mass spectrum (Fig.9) gave m/z 281 for the molecular ion.

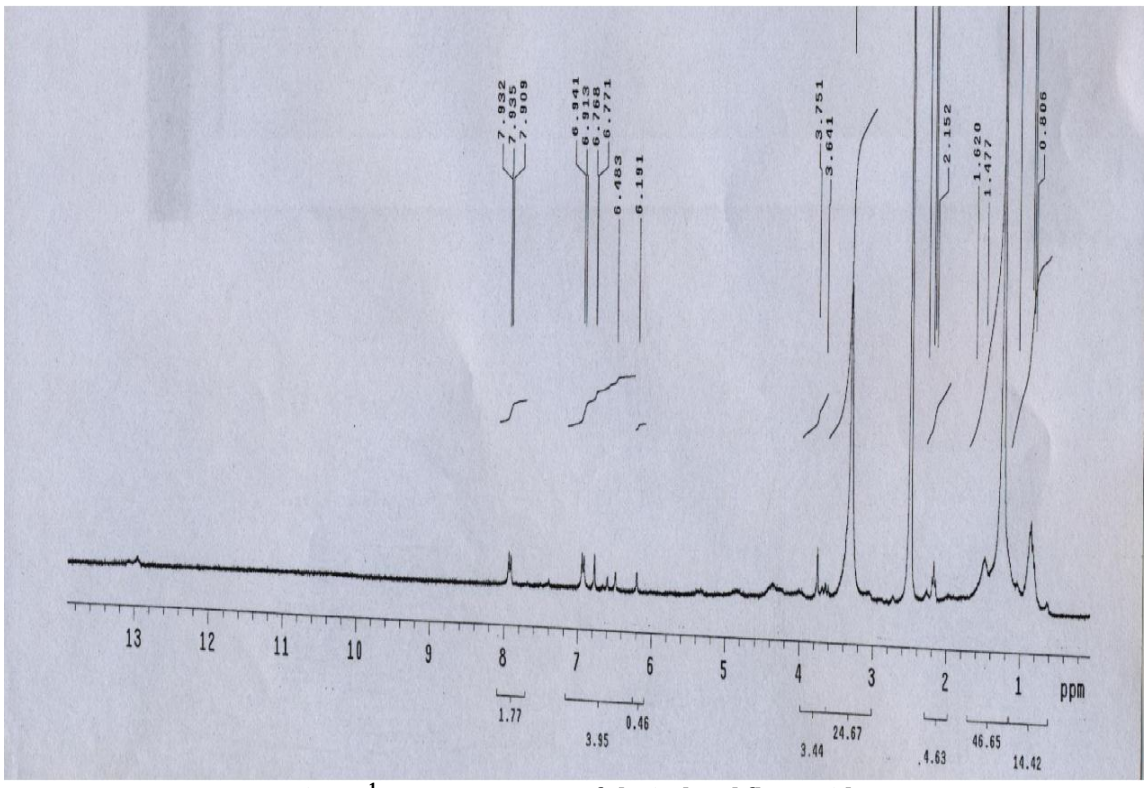


Fig. 8: ^{1}HNMR spectrum of the isolated flavonoid.

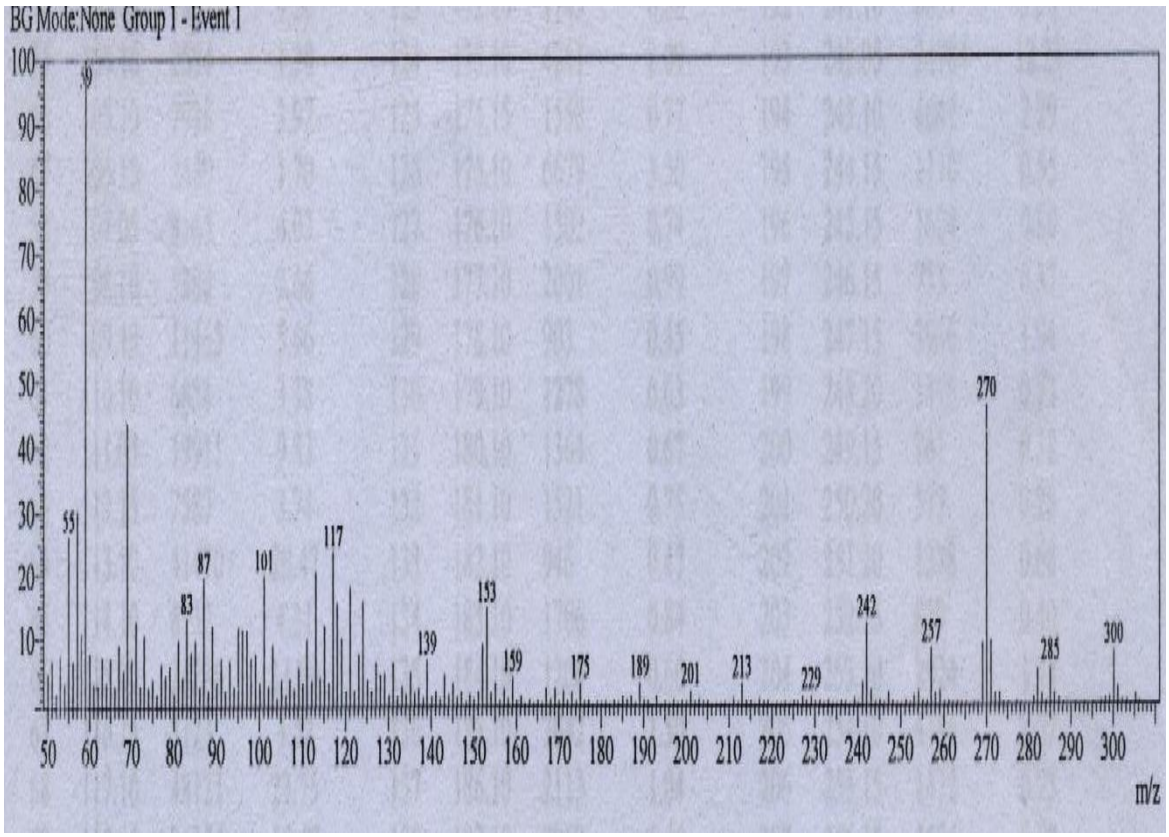
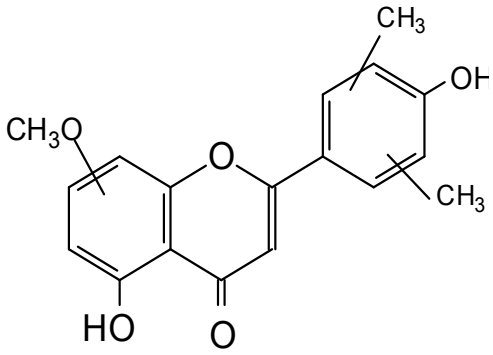


Fig. 9: Mass spectrum of the isolated flavonoid.

On the basis of the above argument, the following tentative structure was suggested for the flavonoid:



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