International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X
Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 3; Issue 5; September 2018; Page No. 12-15

Isolation, partial characterization and biological activity of a Flavonol from Sudanese *Acacia seyal* (Fabaceae)

Abdel Karim M[1*], Ibrahim H[2], Salah El-Deen H[3], El-Hafez M[4]
[1, 2] Faculty of Science, Sudan University of Science and Technology, Khartoum, Sudan
[3] Faculty of Education, Western Kordofan University, Al-Fulah, Sudan
[4] Faculty of Education, University of Zaria, Zaria, Nigeria

Abstract
Acacia seyal is a small to medium tree in the family Fabaceae. The genus *Acacia* dominates large areas in some east African countries. *Acacia seyal* afford "Gum Arabic" which is a heteropolysaccharide. The plant is claimed to possess therapeutic properties. In this study the heartwood of *Acacia seyal* were extracted with ethanol. The ethanolic extract was purified by paper chromatography and a pure flavonol was isolated. The structure of this flavonoid has been elucidated via its spectral data. Furthermore, the antioxidant and the antimicrobial activity of the isolated flavonol have been assessed. Compound I showed significant free radical scavenging properties. Compound I showed excellent activity against *Pseudomonas aeruginosa* and *Bacillus subtilis*. It also exhibited moderate activity ag*ainst Escherichia coli*.

Keywords: *Acacia seyal, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli*

Introduction
Recently there has been a renewed interest in phytochemicals and phytotherapy. Medicinal plants which are used worldwide by different communities for primary health care are plausible candidates for drug leads needed for drug discovery and drug development.
Acacia seyal is a small to medium tree in the family Fabaceae. The genus *Acacia* dominates large areas in east African woodland, bushland and wooded grassland [1, 2]. In east Africa the genus *Acacia* extends from arid deserts to montane forests, where growth forms include small shrubs and large trees [3]. *Acacia seyal* afford "Gum Arabic" which is a heteropolysaccharide. In Sudan this species is cultivated as a cash crop [4]. This gum contains: galactose, arabinose, rhamnose and glucuronic residues beside some minerals [5-7]. Gum Arabic is widely used in food, cosmetic and pharmaceutical industries where it is employed as stabilizer and Sudanese women use *Acacia seyal* stems or stem bark as "smoke bath" for beautification purpose. It is claimed to possess therapeutic properties in addition to its pleasant odor [8-14].

Materials and methods
Materials
Solvents
All solvents used in this study are of analytical grade (Loba Chemicals-India). Methanol HPLC grade was used for spectroscopic measurements (Sigma-Aldrich, England).

Plant material
Acacia seyal heartwood was collected from a forest reserve - Gezira state (Sudan). The plant was identified and authenticated by direct comparison with a herbarium sample.

Equipments
Ultraviolet absorption spectra were obtained in spectroscopic methanol on UV -Visible Spectrophotometer (Shimadzu).
When visualizing chromatograms in paper chromatography a multiband UV (λmax 254 / 365 nm) portable ultraviolet lamb, a product of Hanovia lamps (6 watt S/Y and L/W) was used.. ^{1}HNMR spectra were obtained on a Bruker AM 500 spectrophotometer (Germany) operating at 500 MHz in spectroscopic grade DMSO-d_6. The chemical shifts values are expressed in δ (ppm) units using (TMS) as an internal.

Test microorganisms
Standard microorganisms used for antimicrobial screening are depicted below:

Table 1: Test organisms

Ser. No	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeruginosa*	G-ve
4	*Escherichia coli*	G-ve
5	*Candida albicans*	fungus
6	*Aspergillus niger*	fungus

Methods
Extraction and isolation of flavonoids
Powdered shade-dried heartwood (1Kg) of *Acacia seyal* was macerated with 95% ethanol for 72 hours. The extract was filtered and evaporated to dryness under reduced pressure.
The ethanolic crude extract was applied on paper sheets (Whatman No.3) as narrow strips. The bands were irrigated with BAW (5:1:4;V:V:V). The developed chromatograms were air-dried and examined under both visible and UV light

(ʎ 366,245nm). The equivalent bands from each paper were then cut into small pieces, combined and slurred with methanol. After several hours of contact, with occasional shaking, the liquid was evaporated *in vacuo* to dryness. In this way compound I (R_f 0.70) was isolated in a chromatographically pure form.

Antimicrobial test

Compound I was screened for antimicrobial activity against six standard human pathogenic bacterial strains: Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and fungal species**:** *Candida albicans* and *Aspergillus niger*. The cup plate agar diffusion bioassay was used.

Preparation of microbial suspensions

Aliquots (1ml) of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline solution to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Fungal cultures were maintained on dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline and the suspension was stored in the refrigerator until used.

Testing of antimicrobial susceptibility

The cup plate agar diffusion bioassay was used to assess the antimicrobial activity of the sample and performed by using Mueller Hinton agar (MHA).

Standardized bacterial stock suspension (2ml) was mixed with (200 ml) of sterile molten nutrient agar which was maintained at 45°C. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle. Each plate was divided into two halves. In each half two cups (10mm in diameter) were cut using sterile cork borer (No 4). Each half was designed for a test solution.

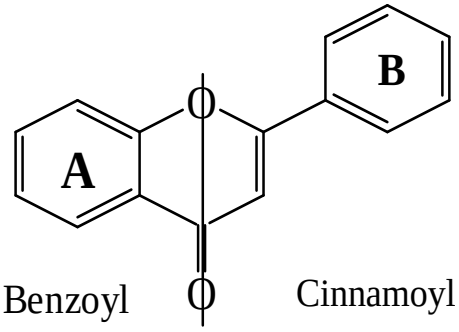
Agar discs were removed, alternate cups were filled with (0.1 ml) samples of each test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37ºC for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

The above mentioned method was adopted for antifungal activity, but instead of agar, dextrose agar was used. Samples were used here by the same concentrations used above.

Results and discussion

Heartwood of *Acacia seyal* were macerated with 95% ethanol for 72hr at room temperature. The solvent was removed *in vacuo* giving a crude product. Paper chromatography gave a chromatographically pure component – compound I. The structure of this compound has been elucidated via its spectral data (UV and NMR).

Flavonoids usually exhibit two absorption bands in their UV spectra; Bands I and II. Band I is associated with the absorption of the cinnamoyl system, while band II originates from the benzoyl system. Flavones, flavonols, chalcones and aurones give band I and II, due to conjugation between the carbonyl function and the aromatic B ring. While flavanones, is flavones, dihydroflavonols and dihydrochalcones give only band II in the range: 230-290nm.These classes of flavonoids lack conjugation between the B ring and the carbonyl function.



The UV absorption of flavones, flavonols, chalcones and aurones is depicted in Table 2.

Table 2: The UV absorption of some flavonoids

Flavonoid class	Band I	Band II
Flavones	330-350	250-270
Flavonols	350-390	250-280
Chalcones	365-390	240-260
Aurones	390-430	240-270

In the UV compound I absorbs (Fig.1) at $ʎ_{max}$247, 316, 364nm. Such absorption is characteristic of: flavones, flavonols, chalcones and aurones. However, aurones absorb above 400nm. Chalcones have dominant band I. Such features were not observed in the UV absorption of compound I. Flavones have band I below 354nm, while flavonols absorb above 358nm. Consequently, compound I is a flavonol.

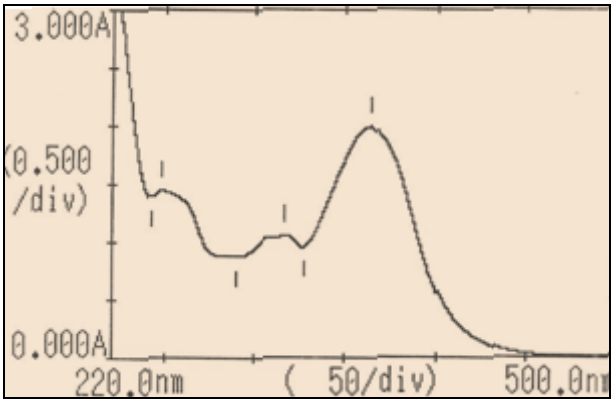


Fig 1: UV spectrum of compound I

In considering structural elucidation of flavonoids, the use of the UV shift reagents (sodium methoxide, sodium acetate, aluminium chloride and boric acid) provide valuable information regarding the hydroxylation pattern in the nucleus of the flavonoid. The UV shift reagent-sodium methoxide-revealed a bathochromic shift accompanied with decrease in intensity indicating a 3-OH function (Fig.2).

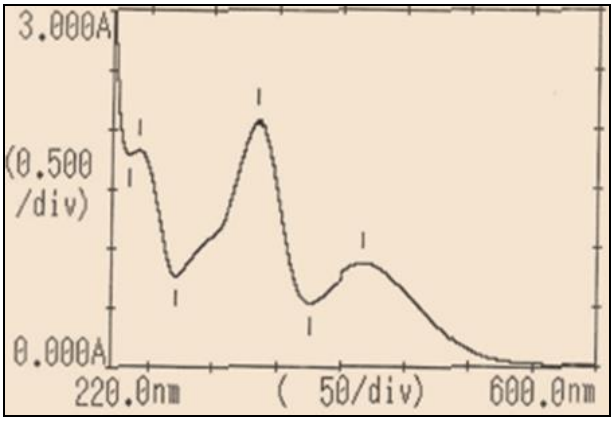


Fig 2: Sodium methoxide spectrum of compound I

Sodium acetate is another useful shift reagent and it is diagnostic of a 7-OH group. The sodium acetate spectrum (Fig. 3) did not afford any bathochromic shift indicating absence of a 7-OH function.

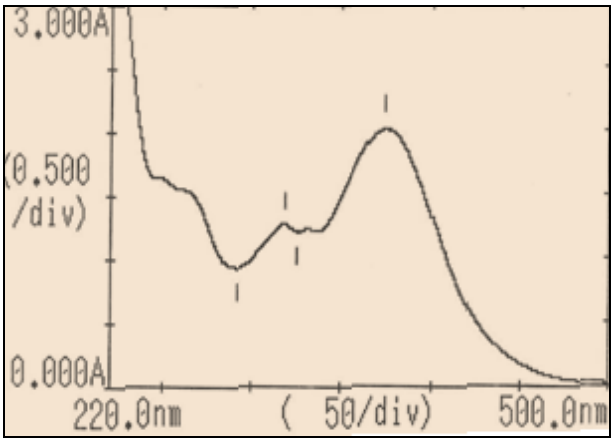


Fig 3: Sodium acetate spectrum of compound I

The aluminium chloride spectrum (Fig. 4) gave a bathochromic shift indicative of a 5-OH group (the spectrum was stable in acidic medium). The boric acid spectrum (Fig.5) which is diagnostic of catechol systems did not reveal any bathochromic shift suggesting absence of such catechols.

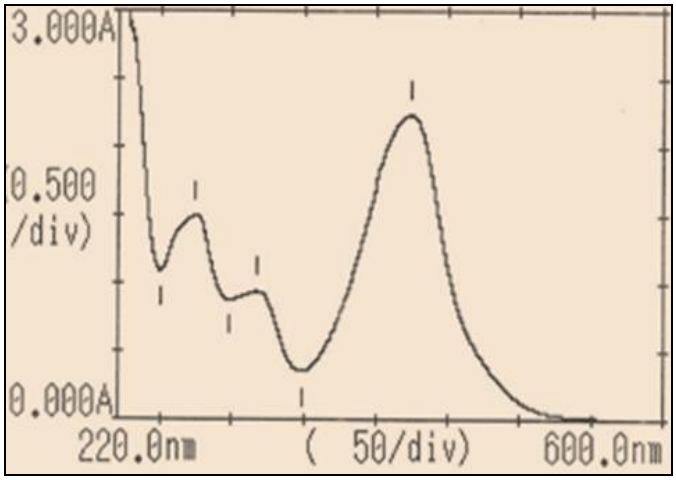


Fig 4: Alumnium chloride spectrum of compound I

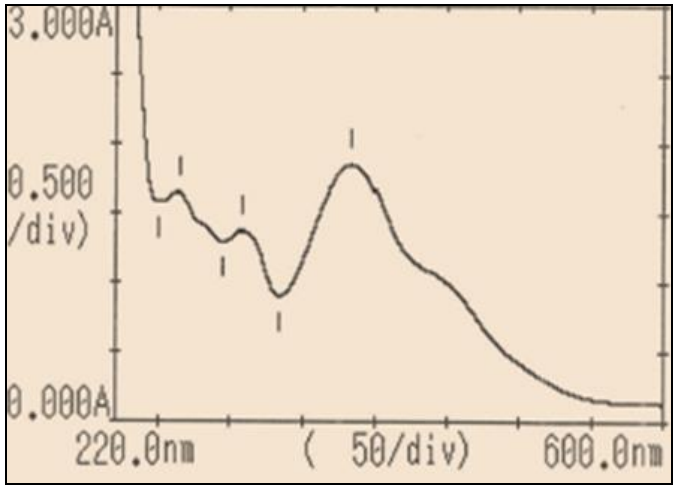
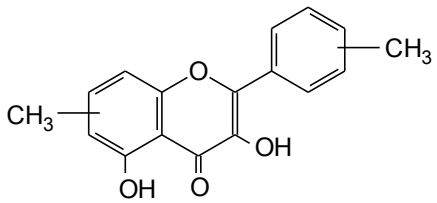


Fig 5: Boric acid spectrum of compound I

The ^{1}HNMR spectrum (Fig. 6) showed: δ1.15(6H) which was assigned for two methyl groups. The multiplet at δ3.16-3.72 accounts for a sugar moiety (not identified in this study). The C_6-H appeared at δ6.87ppm while other aromatic protons resonated at δ7.54, 7.67 and δ7.88ppm.

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



Compound I

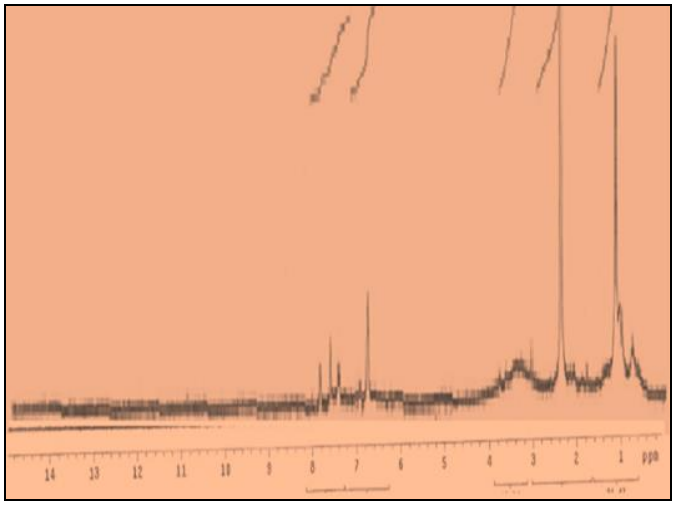


Fig 6: [1]HNMR spectrum of compound I

Antimicrobial assay

Compound I has been screened for antimicrobial activity against six human pathogens. The results are depicted in table 3.Tables 4 and 5 display the antimicrobial activity of standard drugs.

Compound I showed excellent activity against *Pseudomonas aeruginosa* and *Bacillus subtilis.* It also exhibited moderate activity ag*ainst Escherichia coli.*

Table 3: Antimicrobial activity of compound

	Ec	Ps	Sa	Bs	Ca	An
Compound I	14	17	11	17	12	12

Table 4: Antibacterial activity of standard chemotherapeutic agents

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 5: Antifungal activity of standard chemotherapeutic agent

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Ec = *Escherichia coli*
Pa = *Pseudomonas aeruginosa*
Sa = *Staphylococcus aureus*
Bs = *Bacillus subtilis*
Ca = *Candida albicans*
An = *Aspergillus Niger*

Antioxidant assay

The antioxidant capacity of compound I has been measured. Evaluation of the antioxidant activity was carried out by measuring the capacity of the test compound against stable DPPH radical. The change in colour is measured spectrophotometrically at 516nm. As depicted in Table (6) compound I exhibited significant anti- oxidant activity.

Table 6: Radical scavenging activity of compound I

Sample	Antioxidant activity (%)
Trolox	96.50
Compound I	96.01

References

1. Coe M, Beentje H. A field guide to the Acacias of Kenya, Oxford University Press, Oxford, 1991.
2. Pratt DJ, Gwynne MD. Rangeland management and ecology in East Africa, Hodder and Stoughton, London, 1977.
3. New TR. A biology of Acacias, Oxford University Press, Oxford, 1984.
4. Lelon JK, Jumba IO, Keter JK, Chemuku W, Oduor FDO. Assessment of physical properties of gum Arabic from *Acacia senegal* varieties in Baringo District, Kenya. Afr J Plant Sci. 2010; 4:95-98.
5. Williams PA, Phillips GO. Gum arabic. In: *Handbook of Hydrocolloids.* GO PHILLIPS, PA Williams (eds) CRC Press, Boca Raton, FL, 2000, pp155-168.
6. AL-Assaf S, Phillips GO, Williams PA. Studies on Acacia exudate gums: Part 1: The molecular weight of *Acacia senegal* gum exudates. Food Hydrocoll. 2005; 19:647-660.
7. Ali AA, Ali KE, Fadlalla AE, Khalid KE. The effects of gum arabic oral treatment on the metabolic profile of chronic renal failure patients under regular haemodialysis in Central Sudan, Nat Prod Res. 2008; 22:12-21.
8. Ali BH, Ziada A, Blunden G. Biological effects of gum arabic: a review of some recent research. Food Chem Toxicol. 2009; 47:1-8.
9. Ikram ME, Eiman SE, Jihad HE, Saad MHA. Comparative studies of antioxidant and antimicrobial activities of Acacia seyal stem, stem wood and stem bark dry distillates, Am J Pharm Tech Res. 2016; 6:440-50.
10. Abdalbasit AM, Noha MFM, Fatima ON, Alfatih AH. Ethnobotanical study of three trees: indigenous knowledge on trees used as cosmetic in khartoum state, sudan, Asian J Pharm Sci. Tech. 2014; 4:178-82.
11. Orwa CA, Mutua A, Kindt R, Jamnadass R, Simon A. Agroforestree database: a tree reference and selection guide, version 4.0. Kenya: World Agroforestry Centre ICRA, Nairobi, 2009.
12. Weldegebriel, Berihe KK, Mulata H. Effect of feeding Acacia pods (Acacia seyal) with or without wheat bran on feed intake and digestibility of tigray highland sheep in hay based feed, J Biol Agric Health. 2014; 4:26-33.
13. Roothaert RL, Franzel S. Farmers preferences and use of local fodder trees and shrubs in Kenya, Agro Syst. 2001; 52:239-59.
14. Ikram ME, Eiman SE, Jihad HE, Saad MHA. Chemical composition, antioxidant and antimicrobial activities of *Acacia seyal* stem dry distillate, Int J Curr Res. 2016; 8:40010-6.
15. Shuttleworth S. The ancient history of Sudanese perfumes. African Aromatic South Africa, 2011. https://alisonbate.ca/2011/08/28/the-ancient-history-of-sudanese-perfumes/.