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Research Article

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Isolation, Partial Characterization and Antimicrobial Activity of a Flavanone from Sudanese *Cassia arereh* Del. (Caesalpiniaceae)

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Abstract Flavonoids are widely distributed in the plant kingdom and are thus important constituents of the human diet. *Cassia arereh* **Del.** is a small deciduous woody tree in the family Caesalpiniaceae. *Cassia arereh* has many medicinal attributes and potentials. Phytochemical screening of alcoholic extract of *cassia arereh* heartwood indicated presence of steroids, tannins, carbohydrates, saponins, terpenes, and flavonoids. From the ethanolic extract of *Cassia arereh* heartwood a flavanone has been isolated via a combination of chromatographic techniques. The structure of this flavonoid has been partially characterized by some spectral tools. The ethyl acetate extract and compound I have been screened for antimicrobial activity via the agar diffusion bioassay. Compound I exhibited moderate activity against *Staphylococcus aureus*. Compound I and the ethyl acetate extract showed partial activity against *Psuedomonas aeruginosa* and *Escherichia coli*.

Keywords Antimicrobial Activity, Flavanone, *Cassia arereh* Del.

Introduction

For traditional medicine, plants served as a huge resource of drugs for thousands of years. However, only few medicinal plants (1-10%) of the known plants (over 5000 plant species) has been investigated for their pharmacological properties [1].

Flavonoids are a widely distributed group of polyphenolic compounds characterized by a common benzo-pyrone skeleton. In plants flavonoids mainly occur as glycosides. They are widely distributed in the plant kingdom and are thus important constituents of the human diet [2]. For plants, these compounds afford protection against harmful ultraviolet radiation, pathogens, and herbivores. The major flavonoid classes include: flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols, and chalcones [3].

The flavonoids are secondary metabolites with interesting biological activity [4-5]. These plant phenolics exhibit a range of biological activities including: vasodilatory [6-7], antitumor, anti-inflammatory, antibacterial, immune-stimulating, antiviral and antiallergic effects, as well as entity depressive of phospholipase A_2, cycloxyganase and lipoxyganase [8-10].

Cassia arereh Del. is a small deciduous woody tree in the family Caesalpiniaceae. The plant is widely distributed in tropical Africa [11]. *Cassia arereh* has many medicinal attributes and potentials. It is used in folklore medicine to alleviate different diseases including diabetes, gastrointestinal disorders, insect bite, infertility and a range of infections [11-16]. Literature reports claim that *Cassia arereh* contains many biologically interesting molecules including cardiac glycosides, flavonoids, saponins, steroids, terpenoids, alkaloids and tannins [17-21].

Materials and Methods

Materials

Plant Material

Cassia arereh was collected from Kordofan, western Sudan. The plant was authenticated by the Medicinal and Aromatic Plants Research Institute, National Research Center, Khartoum.

Instruments

UV spectra were run on a Shimadzu 2401PC UV- Visible Spectrophotometer. NMR spectrum was measured on a Joel ECA NMR spectrophotometer operating at 500MHz.

Test Organisms

Standard human pathogens used to assess the antimicrobial activity are: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus flavus*.

Methods

Phytochemical Screening

(75 ml) of aliquot of an ethanolic extract of *Cassia arereh* heartwood was evaporated to dryness on a water bath. The cooled residue was defatted by extraction with petroleum ether and defatted residue was dissolved in (30 ml) 95% ethanol, filtered and used for the phytochemical screening as described by Harborne [22].

Extraction of Flavonoids

Powdered air-dried heartwood of *Cassia arereh* (1 Kg) was macerated with 85% methanol (5L) for 24 hours. The solvent was removed under reduce pressure yielding a crude product (70 g) which was suspended in water (100ml) and exhaustively extracted with ethyl acetate. The ethyl acetate was removed under reduced pressure giving a solid (40 g).

Chromatographic Fractionation

Column Chromatography

Cellulose (Merck, Germany) was used as stationary phase, and ethyl acetate/methanol mixture of decreasing polarity was used as mobile phase .The cellulose was soaked in methanol for 1 hour. Five gram of the ethyl acetate extract were dissolved in 6ml of methanol and applied on the top of the column (100x3.5 cm) in a 1:60 ratio (sample: cellulose). Gradient elution commenced by 1:1(methanol: ethyl acetate) ratio followed by increasing ratio of methanol.

Paper Chromatography

Paper chromatography was carried out on Whatmann paper (3 mm, Whatmann Ltd, Maidstone, Kent, England). After spotting the material, the chromatograms were irrigated using: butanol: acetic acid:water (4:1:5, upper layer).

Sephadex Column

(10 mg) of flavonoid to ca. 7.5 g of dry Sephadax LH20 was employed for final purification before spectral analysis of the flavonoid obtained via above mentioned fractionation methods.

Antimicrobial Activity

Preparation of Test Organisms

One ml aliquots of 24 hours broth cultures of test organisms were aseptically distributed into nutrient agar slopes and incubated at 37 ºC for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline to produce a suspension containing about 10^8–10^9 colony forming units per ml. The suspension was stored in the fridge at 4 ºC till used. Serial dilutions of stock suspensions were made in sterile normal saline, and 0.02 ml volumes of the appropriate dilution were transferred into the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37 ºC for 24 hours.

Testing for antimicrobial activity

The antimicrobial potential of compound I and the ethyl acetate extract of *Cassia arereh* heartwood was assessed via the agar diffusion assay against five standard human pathogens: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus flavus.*

(2 ml) of the standard bacteria stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45 ºC. Twenty (ml) aliquots of incubated agar were distributed into sterile Petri dishes. The agar was left to settle and each plate was cut using sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with (0.1 ml) of test sample and allowed to diffuse at room temperature. The plates were then incubated in the upright position at 37 ºC for 18 hours. Inhibition zones were then measured (in mm) for two replicates and averaged as indicator of antimicrobial activity.

After incubation, the diameters of the resultant growth inhibition zones were measured, were measured, and averaged for two replicates.

For antifungal activity, the same procedure was adopted, but Sabouraud dextrose agar was used.

Results and Discussion

Identification of compound I

Compound I appeared on paper chromatography as orange - red under UV light. R_f 0.65 and 0.92 in butanol: acetic acid:water (4:1:5) and 15% acetic acid respectively.

The IR spectrum (Fig. 1) of compound I showed ν(KBr): 662(C-H, Ar. bending), 1070(C-O), 1450, 1460(C=C, Ar.), 1730 (C=O), 2863, 2929 (C-H, Aliph.) and 3396 cm^{-1}(OH).

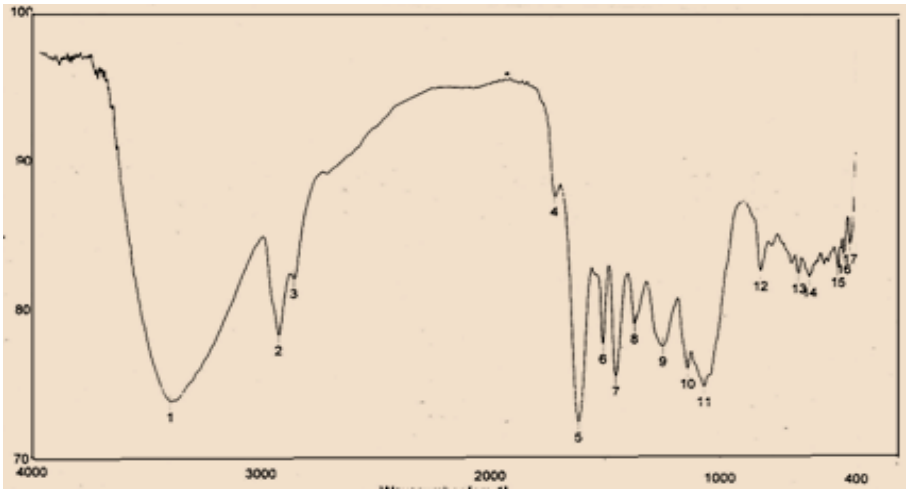


Figure 1: IR spectrum of compound I

In the UV compound I absorbs at λ_{max}(MeOH) 280 nm (Band II). Such absorption is shown by flavanones, isoflavones, dihydrchalcones and dihydroflavonols. Isoflavones give a shoulder in the range 300-340 nm. Such shoulder was not observed in the UV spectrum of compound I. Dihyroflavonols have a 3- OH function which could be ascertained by the UV shift reagent sodium methoxide. Sodium methoxide is a strong base and is used as a diagnostic reagent for C_3-OH and $C_{4`}$-OH. In both cases it gives bathochromic shifts. However, in case of 3-hydroxylation the shift is accompanied by decrease in intensity. The sodium methoxide spectrum of compound I (Fig. 3) revealed a 63 nm bathochromic shift without decrease in intensity indicating a 4`-OH function. Consequently compound I is either a dihydrochalcone or a flavanone. These two classes are distinguishable via their ^{1}H NMR spectra. The H^1 NMR spectrum of compound I (Fig. 4) showed signals for C_3- and C_2- protons of flavanones which resonated as multiplets around δ2.8 and δ5.0 ppm respectively. Furthermore the spectrum showed: δ1.40 ppm assigned for a methyl protons. The signal at δ 2.00 accounts for an acetyl function. The multiplet at δ3.60-4.40 ppm is due to a sugar moiety (not identified in this study). The aromatic protons resonated as a multiplet at δ 5.80-6.80 ppm. Other aromatic protons appeared as doublets centered at δ 7.20 and δ7.70 ppm.

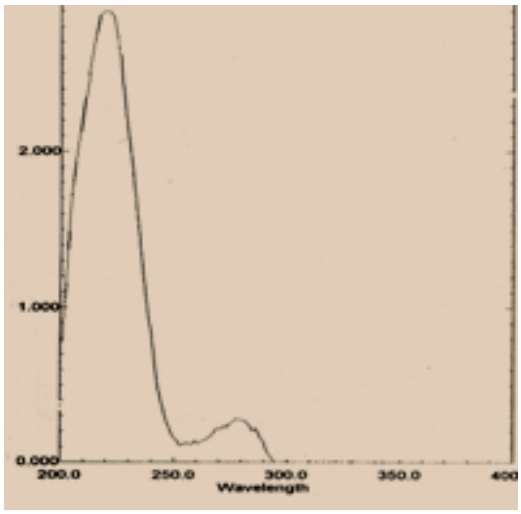


Figure 2: UV specrum of compound I

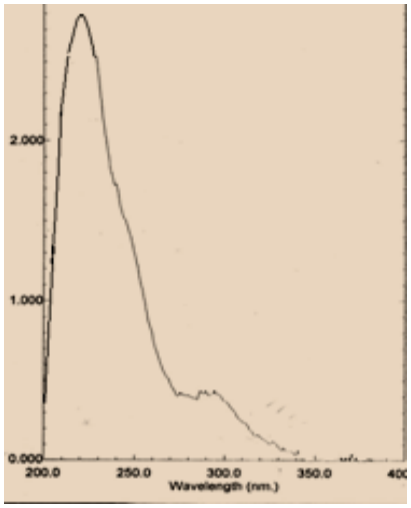


Figure 3: Sodium methoxide spectrum of compound I

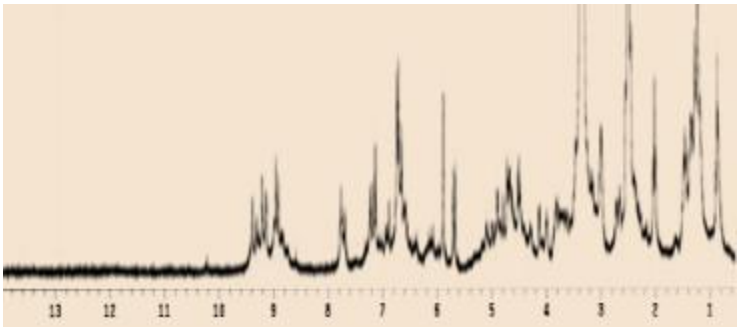


Figure 4: ^{1}H NMR spectrum of compound I

In the chemistry of flavonoids UV shift reagents are usually used to illustrate the hydroxylation pattern on the nucleus of flavonoids. These are: sodium acetate (diagnostic of 7-OH); aluminium chloride (diagnostic of 3- and 5-OH and catechols); boric acid (diagnostic of catechols). These reagents produce bathochromic shifts in presence of certain hydroxylation patterns.

The UV shift reagents – sodium acetate did not show any bathochromic shift indicating absence of a 7-OH group (Figure 5). Also no bathochromic shift was observed in the aluminium chloride spectrum (Figure 6). This indicates absence of 3- , 5-OH groups. The boric acid spectrum also did not afford a bathochromic shift suggesting absence of catechols (Figure 7).

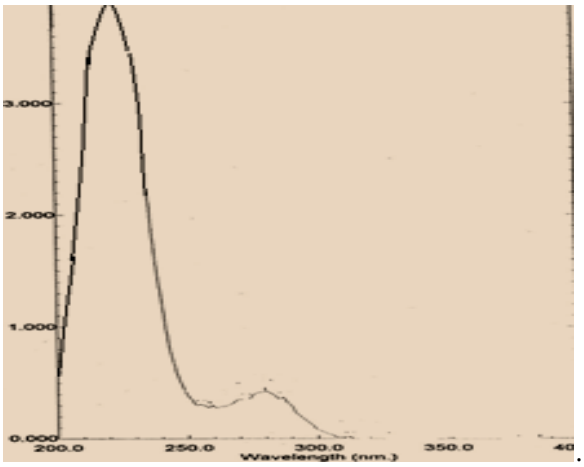


Figure 5: Sodium acetate spectrum of compound I

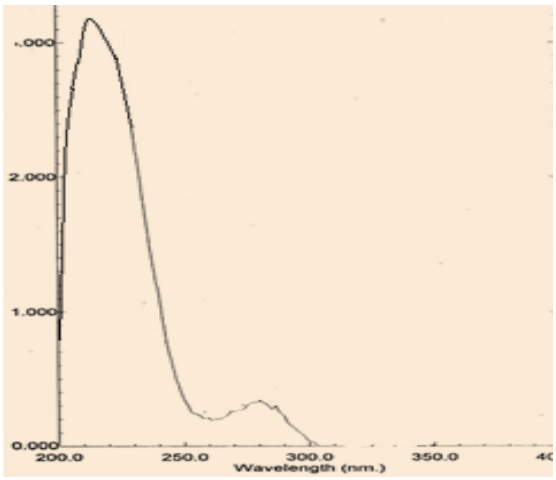


Figure 6: Aluminium chloride spectrum of compound I

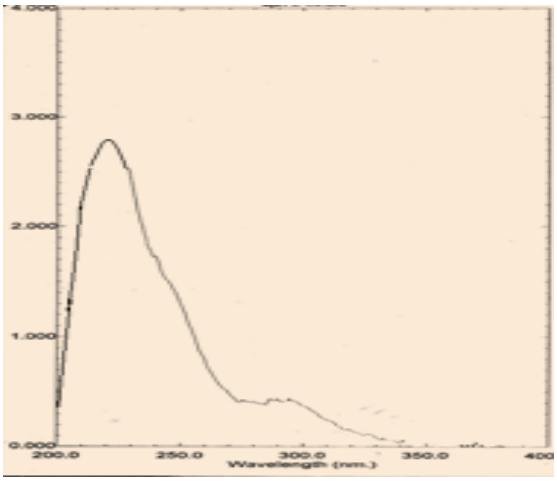
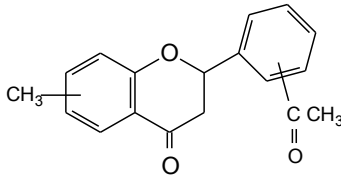


Figure 7: Boric acid spectrum of compound I

On the basis of the above argument, the following partial structure was proposed for the aglycone compound I:



Antimicrobial Activity

The ethyl acetate extract and compound I have been screened for antimicrobial activity via the agar diffusion bioassay. Results are shown in Table 1. Compound I exhibited moderate activity against *Staphylococcus aureus*. Compound I and the ethyl acetate extract showed partial activity against *Psuedomonas aeruginosa* and *Escherichia coli*.

Table 1: Inhibtion zones (mm) of compound I and ethyl acetate extract

Microorganism	DMSO	Ethyl acetate extract	Compound I
Escherichia coli	-	10	11
Staphylococcus aureus	-	11	15
Psuedomonas aeruginosa	-	11	10
Aspergillus flavus		-	-
Candida albicans	-	-	-

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