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Isolation and Partial Characterization of a Dihydrochalcone from Sudanese *Leptadenia pyrotechnica* L. (Asclepiadaceae)

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Abstract
This study was carried out to investigate the major flavonoid of *Leptadenia pyrotechnica* and to evaluate the antimicrobial activity of stem fractions. The flavonoids were extracted with ethanol and the crude extract was purified by thin layer chromatography where a flavonoid (compound I) was isolated. The structure of this compound has been partially characterized by its UV and ^{1}HNMR spectra. Different fractions of *Leptadenia pyrotechnica* stem were assessed for antimicrobial activity against: *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa*, *Bacillus subtilis and Candida albicans.* However, all fractions failed to show inhibitory effect on all test organisms.

Keywords: *Leptadenia pyrotechnica*, flavonoids, Isolation

Introduction
Natural medicine, which relies mainly on plants, still plays an important role in developing countries. Medicinal plants are usually affordable and have less side effects compared to modern drugs.
Leptadenia pyrotechnica is a shrub (1.5-3m in height) in the family Asclepiadaceae [1]. Some flavonoids and sugars were detected in the stem and root [2, 3]. Alkaloids were reported from the aerial parts [4], while some pregnane glycosides [5], flavonoids [6], terpenes and sterols were reported from aerial parts [5]. Three cardiac glycosides were reported from the methanol extract of the aerial parts [7]. It has been reported that the methanol extract of the aerial parts exhibited a free radical scavenging capacity [8]. The extract also showed hypolipidemic and anti-atherosclerotic effect [9]. The *in vivo* antidiabetic potential of *Leptadenia pyrotechnica* has been documented [10]. It has been shown that the ethanol extract of the aerial parts exhibited antiinflammatory properties [11].
In traditional medicine, *Leptadenia pyrotechnica* is a medicinal plant of many attributes.Stem is used as antihistaminic and expectorant. It is also used against gout, wounds, kidney disorders and rheumatism. The plant sap is used traditionally against psoriasis,eczema,smallpox and dermatitis [1, 12, 13]. Leave juice is used in the treatment of rheumatism, diarrhea and asthma [1, 12]. Fruit and stem are used traditionally as purgative, carminative and as remedy for chronic renal disorder [14].

Materials and Methods
Plant material
The stems of *Leptadenia pyrotechnica* were collected from a forest around Folla, western Sudan. The plant was identified and authenticated by The Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

Bacterial strains
For antimicrobial screening the following standard human pathogens were used:
- Gram +ve:
Bacillus subtilis and Staphylococcus aureus.
- Gram –ve:
Escherichia coli, Pseudomonas aeruginosa
- Fungal species:
Candida albicans: was used for antifungal screening.
-Media for bacterial culture:
Mueller- Hinton agar.
- Media for fungal culture:
Sabouraud dextrose agar (Oxoid, England)

Equipments
The ^{1}HNMR spectrum was obtained on a Joel- Nuclear Magnetic Resonance spectrophotometer, (Brucker AC-250). A UV spectrophotometer (Shimadzu model UV240) was used for UV measurements.

Solvents
Methanol used for spectrophotometric analysis was obtained from Merck, Germany. DMSO-d_6 was used as NMR solvent and TMS as internal standard.

Methods
Isolation of flavonoids
Powdered stem of *Leptadenia pyrotechnica* (1Kg) was extracted with 95% ethanol for 72h. at room temperature. The solvent was removed under reduced pressure to give the extract. This extract was fractionated over silica gel plates developed with 30% acetic acid. After the usual workup, a flavonoid (compound I) was isolated.

Antimicrobial activity
The antimicrobial activity was screened using the cup plate agar diffusion assay. Muellar Hinton agar was used for bacterial culture while Sabouraud dextrose agar was used for fungal culture. Cups (6 mm in diameter) were made in

the seeded agar. Test samples (100 mg/ml) were added into each well on the seeded medium and allowed to stand on the bench for 1 h. and then incubated at 37ºC for 24 h-for bacteria – and for four days at 25ºC for fungi. The diameters of inhibition zones were measured as indicator of activity. The assay was performed in duplicates.

Results and Discussion

Phytochemical screening

Phtochemical screening of *Leptadenia pyrotechnica* stem revealed the presence of alkaloids, saponins, flavonoids, steroids, carbohydrates and tannins.

Identification of compound I

Compound I was isolated from *Leptadenia pyrotechnica* stem as yellow amorphous powder. The partial structure of this compound was deduced on the basis of its UV and NMR data. In the UV compound I absorbed at (Fig.1) λ_{max} (MeOH) 224,276 nm . This absorption is characteristic of isoflavones, flavanones, dihydrochalcones and dihydroflavonols. However, the UV shift reagent-sodium methoxide failed to show any bathochromic shift indicative of the 3-OH function of dihydroflavonols (Fig.2). Also that characteristic shoulder of isoflavones which usually appears in the UV range 300-340nm was not observed in the UV spectrum of this compound (Fig. 1).In addition those multiplets which appear at δ2.80 and 5.20ppm in the ^{1}HNMR spectrum of flavanones were not detected in the ^{1}HNMR spectrum of this compound(Fig. 6).

However, the ^{1}HNMR showed a 4 proton signal characteristic of two methylene moieties of dihydrochalcones resonating at δ1.64ppm.The resonance at δ1.25ppm is due to two methyl groups. The spectrum also showed δ (ppm): m (2.90-3.80) assigned for sugar moiety (not identified in this study). The signal at δ 4.00 was attributed to a methoxyl group. The aromatic protons appeared at δ6.21 and 6.32ppm.

The hydroxylation pattern of this flavonoids has been illustrated by using different UV shift reagents. The UV reagent – sodium acetate which is diagnostic of 7-OH did not induce any bathochromic shift (Fig.3) indicating absence of a 7-OH function. Also the aluminium chloride spectrum (diagnostic of 3-, 5- OH and catechols) failed to show a bathochromic shift suggesting absence of 3-, 5-OH groups and catechol moieties (Fig.4).The boric acid spectrum (Fig.5) did not reveal any bathochromic shift confirming absence of catechol systems.

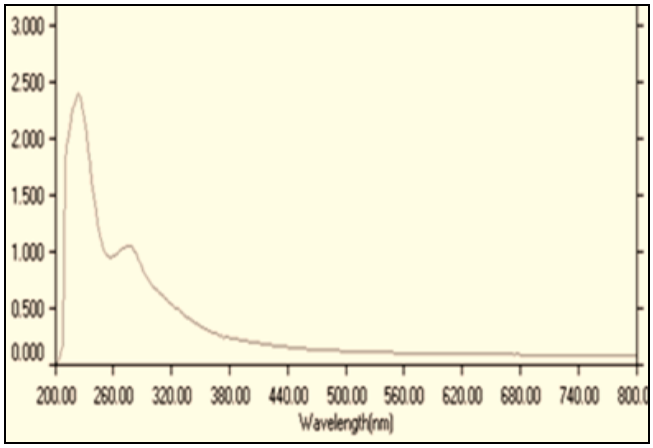


Fig 1: UV spectrum of compound I

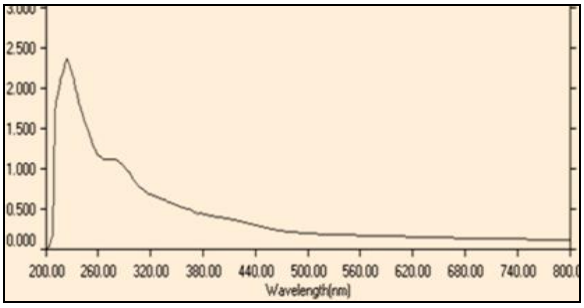


Fig 2: Sodium methoxide spectrum of compound I

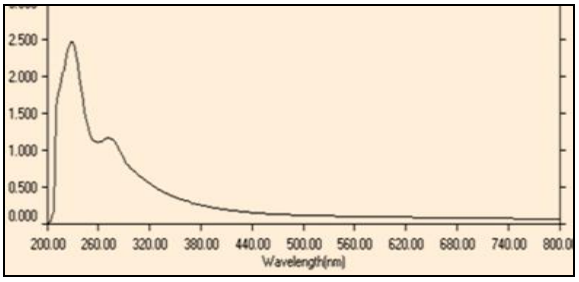


Fig 3: Sodium acetate spectrum of compound I

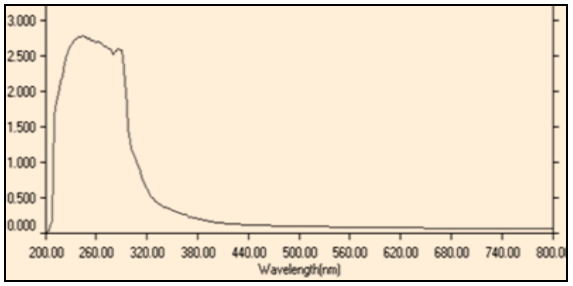


Fig 4: Aluminium chloride spectrum of compound I

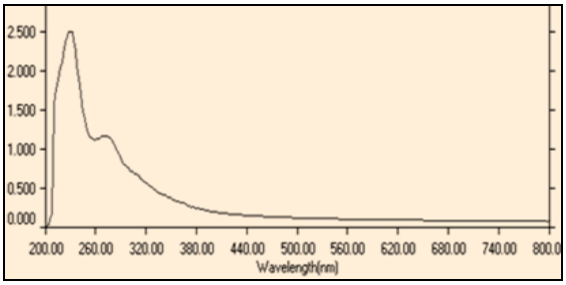


Fig 5: Boric acid spectrum of compound I

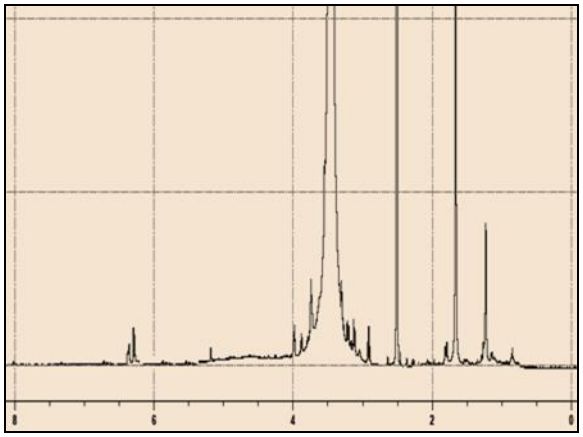
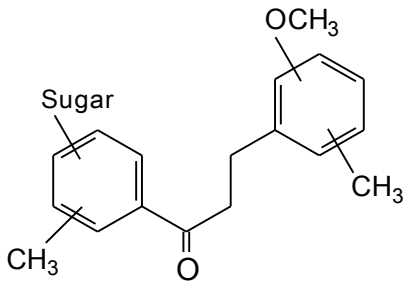


Fig 6: ^{1}HNMR spectrum of compound I

The following partial structure was proposed for compound

I:



Compound I

Antimicrobial activity

Different fractions of *Leptadenia pyrotechnica* were assessed for antimicrobial activity against: *Staphylococcus aureus* , *Escherichia coli* , *Pseudomonas aeruginosa*, *Bacillus subtilis and Candida albicans.* However, all fractions failed to show inhibitory effect on all test organisms.

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