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ISOLATION OF A DIHYDROFLAVONOL FROM SUDANESE *SMILAX REGELII* (SMILACACEAE) AND ANTIMICROBIAL POTENTIAL OF ITS FRACTIONS

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ABSTRACT
The ethanol extract of *Smilax regelii* was purified by thin layer chromatography where a flavonoid - compound I - was isolated and partially characterized by spectral data (UV, ^{1}HNMR). The ethanol extract and the chloroform, ethyl acetate, n-butanol fractons were screened for antimicrobial activity against: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and the yeast *Candida albicans*. The ethyl acetate, chloroform, n-butanol and ethanol fractions showed significant activity against *Staphylococcus aureus*, while the n-butanol and ethyl acetate fractions exhibited significant inhibitory effect against *Bacillus subtilis*.

KEYWORD: *Smilax regelii*, Isolation, Flavonoid, Antimcirobial Activity.

INTRODUCTION

Flavonoids are plant phenolics which are widely distributed in the plant kingdom. They occur naturally as plant pigments in a wide range of fruits and vegetables.[1,2] Basically the Flavonoids are comprising 15 carbons, with two aromatic rings joined by three carbon bridge that forms an oxygenated heterocyclic ring (C_6-C_3-C_6). They are found throughout the plant kingdom and in particular in leaves. Based on the variation of their heterocyclic ring, flavonoids are classified into: anthocyanidins, flavans, flavanones, flavones, flavonols, isoflavones, dihydroflavonols, flavan-3, 4-diols, coumarins, chalcones, dihydrochalcones and aurones. The basic C_6-C_3-C_6 flavonoid skeleton can have numerous substituents (e.g. hydroxyl, acetyl, methoxy and methyl groups) and the majority of the flavonoids exist naturally as glycosides.[3,4]

Smilax regelii is a spiny climbing shrub in the family Smilacaceae. The plant find some applications in phytotherapy A. phytochemical screening revealed that the plant contains the bioactive saponins[5] *Smilax regelii* root extract has been used to flavor soft drinks and baking products. The saponins of *Smilax regelii* have been used as synthons for the synthesis of some steroids.[6] A decoction of the roots has been used traditionally as tonic and aphrodisiac.[7,8]

Many *Smilax* species with similar appearance and chemical constituents share the name "sarsaparilla". This includes *Smilax regelli*, *Smilax officinalis*, *Smilax febrifuge* and other species. Sarsaparilla root has long been used traditionally against skin diseases, rheumatism and physical weakness. Sarsaparilla contains some steroids and saponins.[9] Saponins are known to facilitate the body's absorption of drugs and phytochemicals[9,10]. No toxicity side effects have been reported for sarsaparilla, but large dosages of saponins are associated with gastrointestinal irritation.[11,12] Some flavonoids isolated from sarsaparilla exhibited hepatoprotective and immunomodulatory activity.[13,14]

The antibacterial, antifungal and antimycobacterial properties of sarsaparilla have been documented.[15-18] The *in vitro* and *in vivo* antiinflammatory effect has been reported.[19,20]

MATERIALS AND METHODS

Plant material
Leaves of *Smilax regelli* were collected from Khartoum **(** Sudan**)**. The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

Isolation of flavonoids
Smilax regelli leaves (1.5 Kg) were extracted with 95% ethanol(5L) at room temperature for 72hr. The solvent was removed *in vacuo* giving a crude extract. This

extract was purified over silica gel plates developed by 30% acetic acid. After the usual workup, a flavonoid – compound I(R_f 0.80) – was isolated.

Antimicrobial activity

The antimicrobial screening was performed by using the cup plate agar diffusion assay as described by[21]. Bacterial culture was maintained in Muellar Hinton agar while fungal culture was done on Sabouraud dextrose agar. Wells (6 mm in diameter) were made in the seeded agar using sterile cork borer(No. 4). Test samples(100 mg/ml) were added into wells of the seeded medium and then incubated for 24 h. (at 37°C) - for bacteria – and for 72 h. at 25° for fungal species. The assay was performed in duplicates and the diameters of inhibition zones were measured.

RESULTS AND DISCUSSION

Phtochemical screening

Phtochemical screening of *Smilax regelii* leaves revealed the presence of alkaloids, saponins, flavonoids and steroids.

Identification of compound I

Compound I was isolated from *Smilax regelii*. In the UV, compound I absorbs at λ_{max} 280nm(Fig.1).This absorption is characteristic of: flavanones, dihydroflavonols, dihydrochalcones and isoflavones which are known to be saturated by at $C_2 - C_3$ link.The sodium methoxide spectrum (Fig.2) showed a bathochromic shift indicative of a 3-OH function. The presence of a hydroxyl group at this postion suggests a dihydroflavonol.

Different UV shift reagents(sodium acetate, aluminium chloride and boric acid) were then used to elucidate the hydroxylation pattern of the isolated flavonoid.

The sodium acetate spectrum (Fig.3) did not show a bathochromic shift indicating absence of a 7-OH function. Also the aluminium chloride spectrum(Fig.4) did not reveal any bathochromic shift indicating absence of 3- , 5-OH groups as well as catechol systems. Furthermore, the boric acid spectrum(Fig.5) confirmed the absence of catechol systems(no bathochromic shift was detected).

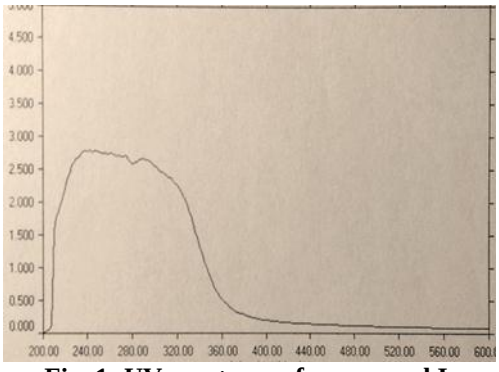


Fig. 1: UV spectrum of compound I.

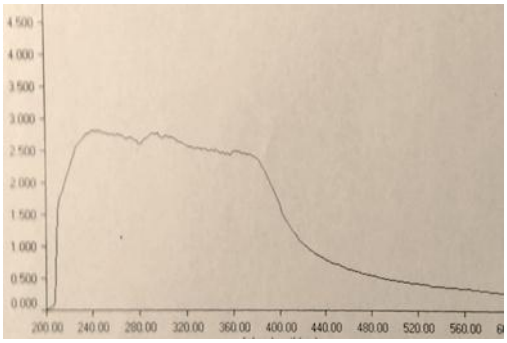


Fig. 2: Sodium methoxide spectrum I.

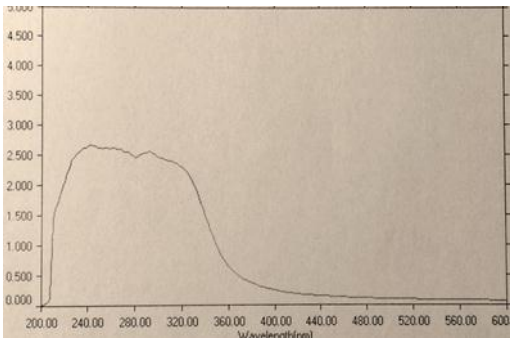


Fig. 3: Sodium acetate spectrum I.

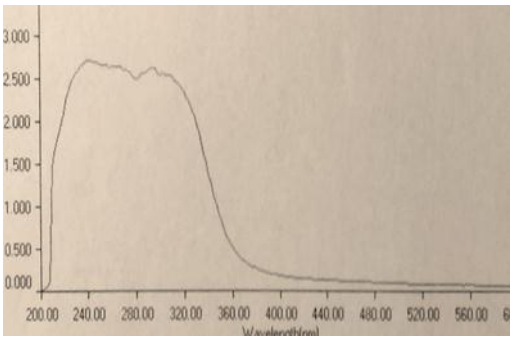


Fig. 4: Aluminium chloride spectrum I.

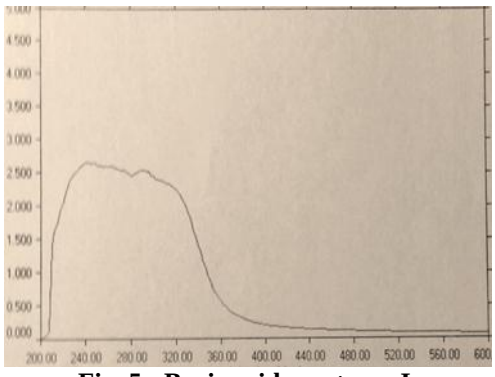


Fig. 5: Boric acid spectrum I.

The ^{1}HNMR spectrum(Fig.6) showed δ(ppm): 1.25,1.60(assigned for two methyl groups); m(3.00-4.00) , 5.17, 5.50,5.62 (assigned for a sugar moiety which was not identified in this study) ; m(6.66-6.75)-Ar. protons. The signals of the solvent(DMSO) residual protons appeared at δ2.50 while the solvent residual water appeared at δ3.30ppm.

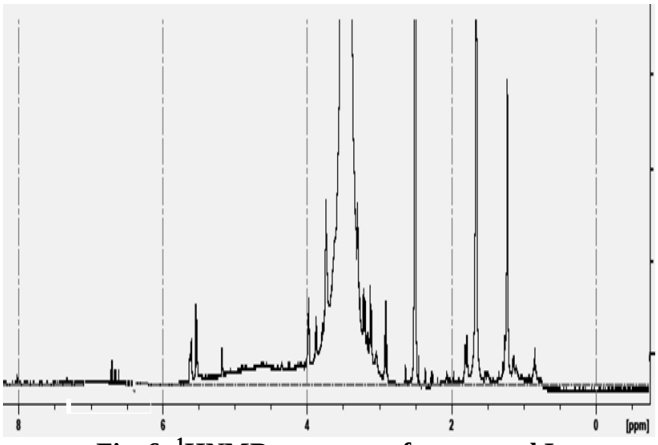
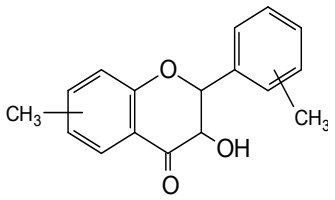


Fig. 6: ^{1}HNMR spectrum of compound I.

The following partial structure was suggested for the aglycone of compound I:



Compound I

Antimicrobial activity

Some fractions of *Smilax regelii* were examined for antimicrobial potential against five standard pathogenic microbes. The diameters of the growth of inhibition zones are shown in Table (1). Ampicilin, gentamycin and clotrimazole were used as positive contols.

The ethyl acetate, chloroform, n-butanol and ethanol fractions showed significant activity against *Staphylococcus aureus*, while the n-butanol and ethyl acetate fractions exhibited significant inhibitory effect against *Bacillus subtilis*. The presence of bioactive molecules such as the flavonoids in this plant may provide a rationale for its marked antibacterial potency.

Table 1: Antimicrobial activity of different fractions.

Extract	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Chloroform	100	21	-	-	-	-
n-Butanol	100	20	20	-	-	-
Ethyl acetate	100	19	22	-	-	-
Ethanol	100	30	-	-	-	-

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

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