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Research Article

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Isolation, Partial Characterization of an Isoflavone from Sudanese *Blepharis maderaspatensis* (L.) and Antimicrobial Activity of Fractions

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Abstract *Blepharis* is a genus in the family Acanthaceae. This genus comprises around 129 species distributed in arid and semi-arid regions. Many species belonging to this genus are used in folklore medicine**.** *Blepharis maderaspatensis* is a procumbent or scrambling perennial herb. It is used in folklore medicine against wounds, edema, snake bite and gout. The plant possesses anti-inflammatory and anti-nociceptive properties. An isoflavone has been isolated by thin layer chromatography fr**o**m the whole plant of *Blepharis maderaspatensis*. The partial structure of the isolated flavonoid has been deduced on the basis of its spectral data (UV and ^{1}HNMR). Different fractions of *Blepharis maderaspatensis* have been screened for antimicrobial activity. The ethanolic extract of *Blepharis maderaspatensis* showed moderate against *Bacillus subtilis.* Other fractions showed partial activity against *Bacillus subtilis*. The n-butanol fraction exhibited partial activity against *Staphylococcus aureus* and *Escherichia coli*.

Keywords Isolation, Isoflavone, Sudanese *Blepharis maderaspatensis* (L.), Antimicrobial Activity

Introduction

For decades, medicinal plants and plant-derived products attracted the attention of researchers. This is mainly due to their versatile applications. Medicinal plants are rich source for traditional medicine, modern medicines, pharmaceutical intermediates and synthons for many synthetic drugs [1].

Blepharis is a genus in the family Acanthaceae. This genus comprises around 129 species distributed in arid and semi -arid regions [2-3]. Many species belonging to this genus are used in folklore medicine. *Blpharis edulis* is used by herbal healers against inflammations, gastrointestinal disorders, asthma, fever and cough. A Decoction of the plant is used as a natural remedy for ulcers and wounds. Seeds which contain, among others, glycosides and blepharin [4], are used to increase sperm count [5]. Leaves of *Blepharis boerhaaviafolia* are used to treat ascites, liver disorders, nasal hemorrhage, ulcers, wounds and asthma [6]. *Blepharis molluginifolia* is used for headache, bone fractures, urinary tract infections, skin diseases and allergies [7]. Roasted leaves are used traditionally as a remedy for flatulence and root is an antidote for snake bite [8].

Blepharis maderaspatensis (L.) B. Heyne ex Roth. is a procumbent or scrambling perennial herb. It is used in folklore medicine against wounds, edema, snake bite and gout. The anti-inflammatory and anti-nociceptive properties of the ethanol extract of the whole plant have been reported [9].

Materials and Methods

Materials

Plant material

Blepharis maderaspatensis was collected from a forest reserve around Damazin, Sudan. The plant was authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum.

Instruments

UV spectra were run on a Shimadzu 2401PC UV- Visible Spectrophotometer. NMR spectra were measured on a Joel ECA 500MHZ NMR Spectrophotometer.

Test Organisms

The following standard microorganisms were used to assess the antimicrobial potential: *Bacillus subtilis* (Gram (+ve), *Staphylococcus aureus* (Gram(+ve), *Pseudomonas aeroginosa* (Gram –ve) , *Escherichia coli* (Gram –ve) and the fungal species *Candida albicans*.

Methods

Preparation of Plant Extract for Phytochemical Screening

Powdered air- dried whole plant of *Blepharis maderaspatensis* (200g) was extracted with 95% aqueous ethanol by maceration. This prepared extract (PE) was used for phytochemical screening. Phytochemical screening was accomplished according to the method described by Harborne [10].

Extraction and Isolation of Flavonoids

(1 kg) of powdered air-dried whole plant of *Blepharis maderaspatensis* was macerated with 95% ethanol (5L) for 48hr at room temperature. The extraction process was repeated two more times with the same solvent. Combined filtrates were concentrated under reduced pressure at 40^{o} C yielding a crude product. This crude product was applied on silica gel plates as narrow zones. The plates were developed with 50% acetic acid. After the usual workup a chromatographically pure flavonoid-compound I- was isolated.

Antimicrobial Assay

By using the agar diffusion bioassay, different fractions of the whole plant of *Blepharis maderaspatensis* were assessed for antimicrobial activity against four standard pathogenic bacteria and one pathogenic fungus: (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*).

Preparation of Bacterial Suspensions

Broth culture of the test organisms were distributed into agar slopes and incubated at 37^{o}C for 24 hours. Bacterial growth was harvested and suspended in 100 ml of normal saline to give about 10^{8}- 10^{9} colony forming units per ml. The Average number of viable organism per ml was determined using the surface viable counting technique.

Serial dilutions of the stock suspension were prepared in sterile normal saline. (0.02 ml) of the appropriate dilution was transferred into the surface of dried nutrient agar plates. After drying, the plates were incubated at 37 ^{o}C for 24 hours. Fungal cultures were maintained on Sabouraud dextrose agar incubated at 25^{o}C for four days.

Testing for Antimicrobial Activity

(2 ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45 ^{o}C in a water bath. (20 ml) aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and each plate was divided into two halves. Two cups in each half (10 mm in diameter) were cut using sterile cork borer (No. 4).Agar discs were removed and cups were filled with (0.1 ml) of each test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37 ^{o}C for 24 hour. Tests were performed in two replicates. After incubation the diameters of the resultant growth inhibition zones were measures and averaged.

For antifungal activity, instead of nutrient agar Sabouraud dextrose agar was used. Samples were used here by the same concentrations used above.

Results and Discussion

Phytochemical screening of the whole plant of *Blepharis maderaspatensis* revealed the presence of flavonoids, saponins, alkaloids, coumarins, tannins, sterols and triterpenes.

From the whole plant of *Blepharis maderaspatensis* a flavonoid has been isolated by silica gel TLC. In the UV, the isolated flavonoid-compound I-absorbs at λ_{max} 276 nm (Fig. 1). This absorption is given by: isoflavones, flavanones, dihydrochalcones and dihydroflavonols.

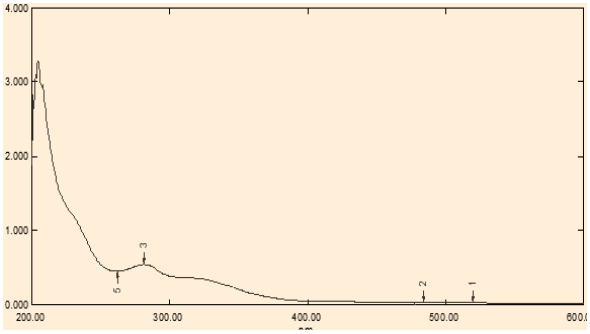
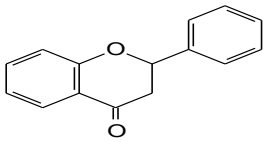
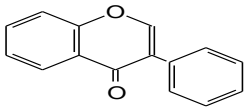


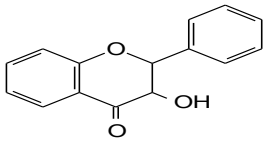
Figure 1: UV spectrum of compound I



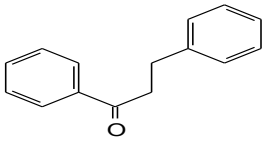
Flavanone



Isoflavone



Dihydroflavonol



Dihydrochalcone

Isoflavone possess a spectrum characterized by a shoulder in the range 300-340n. Such shoulder was detected in the UV spectrum (Figure 1). Hence, the isolated compound is an isoflavone.

The hydroxylation pattern on the nucleus of the isolated flavonoids has been studied via different UV shift reagents. One of them is sodium methoxide which is used for the specific detection of 3- and 4`-OH functions. The sodium methoxide spectrum did not show any bathochromic shift indicating absence of 3- and 4`-OH groups (see Fig. 2).

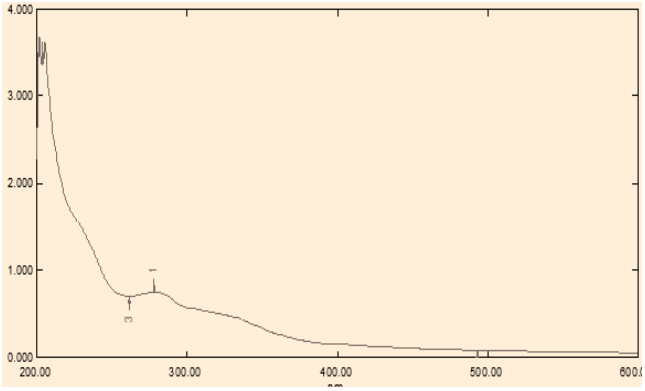


Figure 2: Sodium methoxide spectrum of compound I

The UV spectrum of the shift reagent- aluminium chloride (which is diagnostic of 3- and 5-OH and catechols)- did not show any bathochromic suggesting absence of 3- and 5-OH groups and catechols (Figure 3). Also, the boric acid spectrum (Figure 4) which is diagnostic of catechol systems, did not reveal any bathochromic shift indicating absence of catechols.

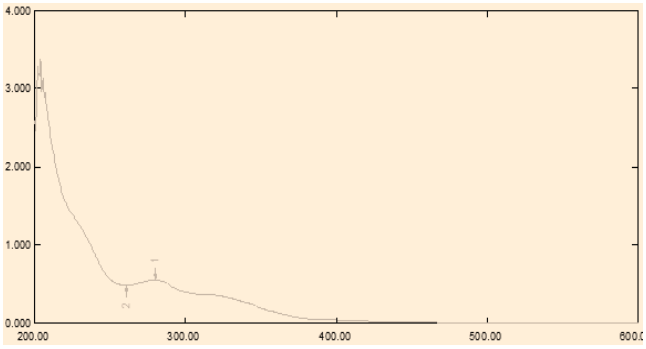


Figure 3: Aluminium chloride spectrum of compound I

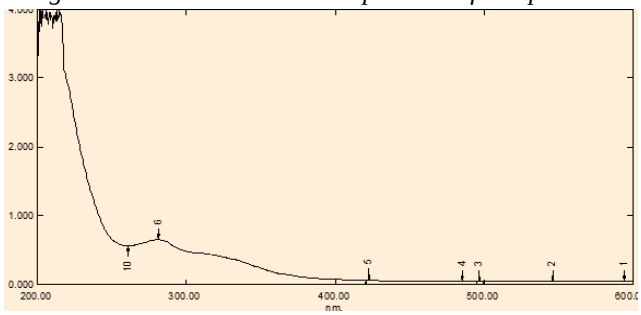


Figure 4: Boric acid spectrum of compound I

Sodium acetate is another useful shift reagent which is diagnostic of 7-OH group .However the sodium acetate spectrum of compound I did not show any bathochromic shift indicating absence of a 7-OH function (Fig. 5).

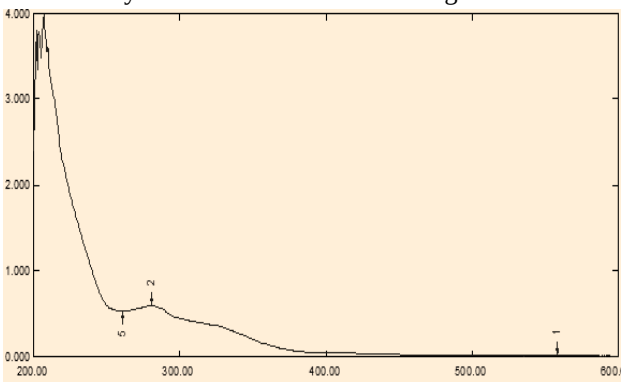
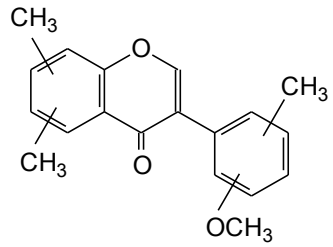


Figure 5: Sodium acetate spectrum of compound I

The ^{1}HNMR spectrum (Fig. 6) showed: δ0.85 (assigned for one methyl group); δ 1.23ppm (assigned for two methyl groups); δ 3.43-3.64-mutiplet (accounting for sugar protons-not identified in this study); δ4.21(assigned for a methoxyl group). The aromatic protons appeared at δ8.00ppm. Signal at δ2.50 ppm is due to solvent (DMSO) residual protons.

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



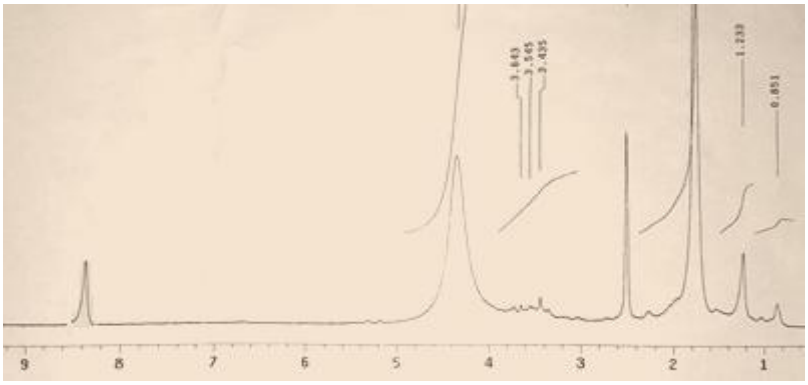


Figure 6: [1]HNMR spectrum of compound I

Antimicrobial Activity

Different fractions of *Blepharis maderaspatensis* were evaluated for antimicrobial activity against five standard microorganisms (Table 1). The results are depicted in Table (2) .Results were interpreted in the following terms: (<9mm: inactive; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Ampicilin, gentamicin and clotrimazole were used as positive controls (Tables 3 and 4). The ethanolic extract of *Blepharis maderaspatensis* showed moderate activity against *Bacillus subtilis.* Other fractions showed partial activity against *Bacillus subtilis*. The butanol fraction exhibited partial activity against *Staphylococcus aureus* and *Escherichia coli*.

Table 1: Test organisms

No	Micro organism	Type	Source
1	*Bacillus subtillus*	G+ve	ATCC 2836
2	*Staphylococcus aureus*	G+ve	ATCC 29213
3	*Pseudomonas aeroginosa*	G-ve	NCTC 27853
4	*Escherichia coli*	G-ve	ATCC 25922
5	*Candida albicans*	fungi	ATCC 7596

* NCTC. National collection of type culture, Colindale. England

*ATCC. American type culture collection, Maryland, USA

Table 2: Inhibition zones of fractions

Fraction (100mg/ml)	Sa	Bs	Ec	Ps	Ca
Ethanolic	---	14	---	---	15
n-Butanol	11	13	10	--	--
Ethyl acetate	--	11	--	--	--
Chloroform	--	10	--	--	--

Sa.: *Staphylococcus aureus*

Ec.: *Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

Ca.: *Candida albicans*

Bs.: *Bacillus subtilis*

Table 3: Antibacterial activity of standard chemotherapeutic agents

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

Table 4: Antifungal activity of standard chemotherapeutic agent

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

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