



ISOLATION, PARTIAL CHARACTERIZATION OF A FLAVONE FROM SUDANESE *PULCARIA CRISPA* STEMS AND BIOLOGICAL ACTIVITY OF DIFFERENT FRACTIONS

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ABSTRACT

Pulicaria crispa is used in folk medicine for the treatment of colds, coughs, colic pain, excessive sweating and as carminative. Phytochemical screening of *pulcaria crispa* stems revealed the presence of flavonoids. A flavonoid - compound I- was isolated from the ethanol extract by column and paper chromatography and its structure was partially characterized on the basis of its spectral data (IR, UV and NMR). The crude extract and different fractions (chloroform, n- butanol and ethyl acetate) of *pulcaria crispa* were screened for their antimicrobial activity against five standard human pathogens. The ethyl acetate fraction of *pulcaria crispa* showed moderate activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. However, the ethanol extract and chloroform fraction were inactive against the tested bacteria, but they gave partial antifungal activity against the fungus *Candida albicans*. The n-butanol fraction exhibited partial activity against *Staphylococcus aureus* and *Candida albicans*.

KEYWORDS: *Pulcaria crispa*, Isolation, Flavonoid, Antimicrobial Activity.

INTRODUCTION

Flavonoids are a group of natural compounds with variable phenolic structures found in plants.^[1] Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection.^[2] Their activities are structure- dependent. The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization.^[3] Recent interest in flavonoids has been stimulated by the potential health promoting properties associated with the antioxidant activity of these polyphenolics. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals.^[4, 5] Accumulation of free radicals may damage target biomolecules.^[6, 7]

Some flavonoids have been isolated from *Pulicaria crispa*.^[8] The presence of these bioactive molecules give a rationale for the various uses of this plant in traditional medicine.^[9, 10] A great number of plant medicines contain flavonoids which possess anti-inflammatory, anti-allergic, anti-mutagenic, anti-thrombotic, and vasodilator activities.^[11-13] Also some have anti-viral and anti-bacterial properties.^[14]

Flavonoids are major constituents of some formulations of alternative medicine.^[15] Several epidemiological studies provide support for a protective role of the consumption of flavonoid-rich diet against cancer^[16, 17], heart disease^[18, 19] and stroke.^[14, 20]

MATERIALS AND METHODS

Materials

Plant material

Stems of *Pulcaria crispa* were collected from White Nile state(Sudan). The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum – Sudan.

Instruments

UV spectra were run on a Shimadzu 2401PC UV-Visible Spectrophotometer. The IR spectra were run on a Perkin- Elmer 1310 Infrared Spectrophotometer. NMR spectra were performed on a Joel ECA 500MHZ NMR Spectrophotometer.

Test organisms

The antimicrobial activity of different fractions of *Pulcaria crispa* was evaluated using the following standard microorganisms: *Bacillus subtilis* (Gram +ve), *Staphylococcus aureus* (Gram +ve), *Pseudomonas*

aeruginosa (Gram –ve), *Escherichia coli* (Gram –ve) and the fungal species *Candida albicans*.

Methods

Phytochemical screening

Stems of *Pulcaria crispa* were screened for major secondary metabolites according to the method described by Harborne.^[21]

Extraction of flavonoids

Powdered stems of a *Pulcaria crispa* (1.5 kg) were macerated at room temperature with ethanol (95%) for 72h. The solvent was evaporated under reduced pressure to dryness to give a crude product.

Isolation of flavonoids

Open column (70× 4 cm) was used for fractionation of the ethanol extract of *Pulcaria crispa*. Silica gel with particle size 60-120 mesh from LOBA chemicals (India) was used as stationary phase. The column was eluted with ethyl acetate: methanol (3:7, fraction I), (1:1, fraction II) and (0:1, fraction III).

Fraction II –being rich in phenolics- was fractionated via paper chromatography using the solvent system: butanol: acetic acid: water (4: 1: 12,v:v:v)- upper layer. The chromatograms were viewed and located under UV light and a flavonoid-compound I- was eluted from paper with methanol.

Antimicrobial activity

Preparation of bacterial suspensions

A (24) hours broth cultures of the test organisms were aseptically distributed onto nutrient agar slopes and incubated for 24h at 37° C. Bacterial growth was washed off with 100 ml sterile normal saline giving approximately 10⁸- 10⁹ C.F.U/ ml. The average number of viable organisms per ml of the stock suspension was determined.

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 was allowed to stand for two hours at room temperature and then incubated at 37 °C for 24 hours.

Preparation of fungal suspension

The fungal cultures was maintained on Sabouraud 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 antibacterial susceptibility

Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) soaked with a solution of each test sample were placed on the surface of the seeded agar. The inoculated plates were incubated at 37 °C for 24 h in the

inverted position. The test was done in duplicates and the diameters (mm) of the inhibition zones were measured and averaged.

Testing for antifungal activity

The above mentioned method was adopted for antifungal activity, but instead of Mueller Hinton agar Sabouraud dextrose agar was used and incubation continued for four days at 25°C. Samples were used here by the same concentrations used above.

Antioxidant activity

The test samples were allowed to react with 2,2- Di (4-tert-octylphenyl)-1-picryl-stable free radical (DPPH) for half an hour at 37° C. the concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiple reader spectrophotometer. Percentage radical scavenging activity by sample was determined in comparison with a DMSO treated control group. All tests were run in triplicate. Propylgalate was used as standard at concentrations 0.5 mM.

RESULTS AND DISCUSSION

Phytochemical screening

Pulcaria crispa stems were screened for major secondary metabolites and the results are displayed in Table 1.

Table 1: Phytochemical screening of *pulcaria crispa* stem.

Test	Result
Saponin	++
Cumarin	+
Alkaloids	+
Flavonoids	+
Tannin	+++

Characterization of compound I

Phytochemical screening of *pulcaria crispa* stems revealed the presence of flavonoids. From the ethanol extract, compound I was isolated by column and paper chromatography and its structure was partially elucidated via a combination of spectral techniques (UV, IR and ¹HNMR).

The IR spectrum of compound I (Fig.1) showed ν (KBr): 630.50(C-H, Ar.), 1273.70 (C-O, ether), 1378.30 (C = C, Ar.), 1772.92 (C = O) and 3368.88 cm⁻¹ (OH).

The appearance of a carbonyl absorption suggests that compound I is neither an anthocyanin nor catechin. These classes are characterized by absence of a carbonyl function.

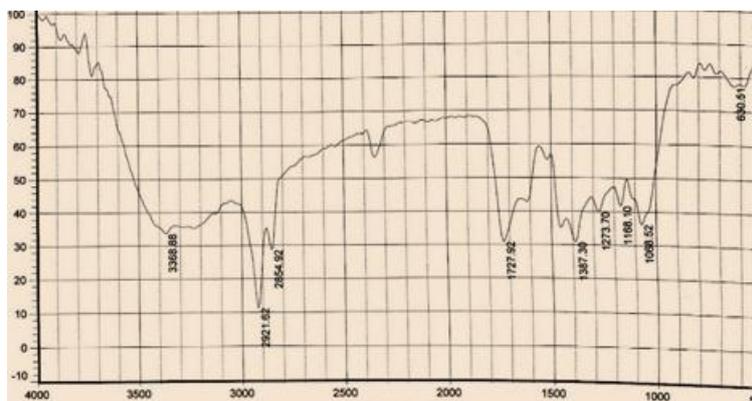


Fig. 1: IR spectrum of compound I.

In the UV, compound I showed both bands I and II (Fig.2) at 262 and 345nm. Such absorption is characteristic of flavones.

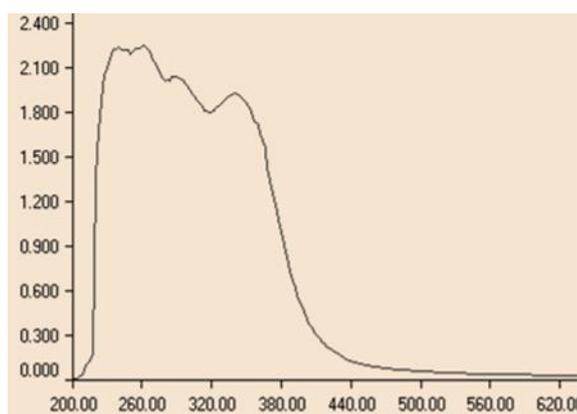


Fig. 2: UV spectrum of compound I in methanol.

When the UV shift reagent – sodium methoxide - was added to a methanolic solution of compound I, a 42nm bathochromic shift in band I without decrease in intensity (Fig.3) was observed indicating the presence of 4'-OH function.

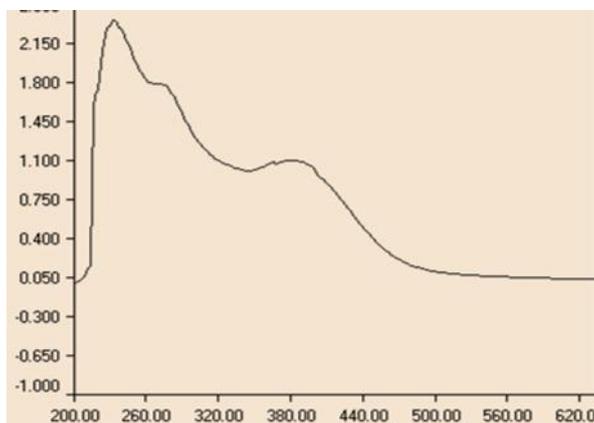


Fig. 3: Sodium methoxide spectrum of compound I.

When a methanolic solution of compound I was treated with excess powdered sodium acetate, no bathochromic shift in (Fig.4) was observed indicating the absence of a 7-OH group.

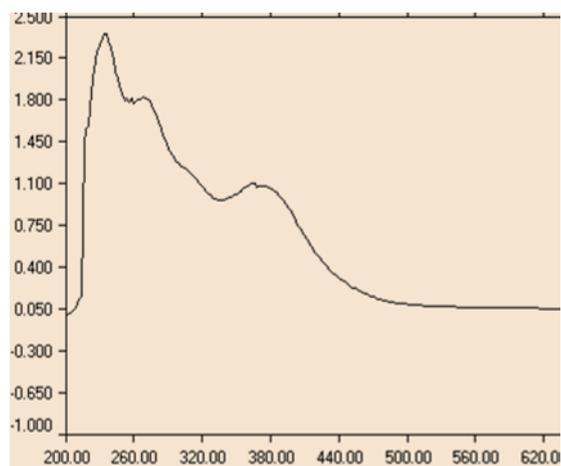


Fig. 4: Sodium acetate spectrum of compound I.

When the previous solution of compound I was treated with excess powdered boric acid, no bathochromic shift was observed (Fig.5). This indicates absence of ortho- dihydroxyl groups.

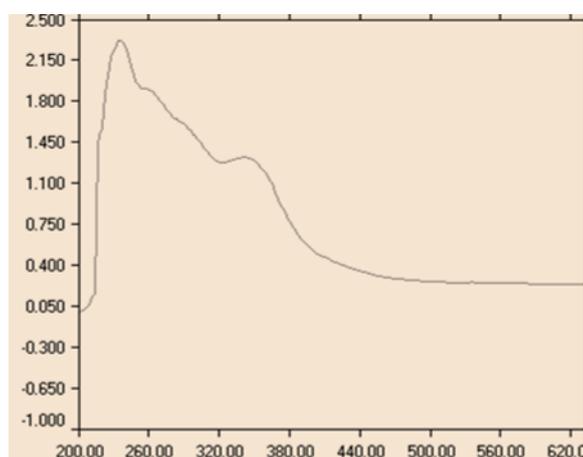


Fig. 5: Sodium acetate/boric acid spectrum of compound I.

The aluminium chloride spectrum of compound I (Fig.6) failed to show any bathochromic shift and this indicates absence of 3-, 5-OH groups and catechol moieties.

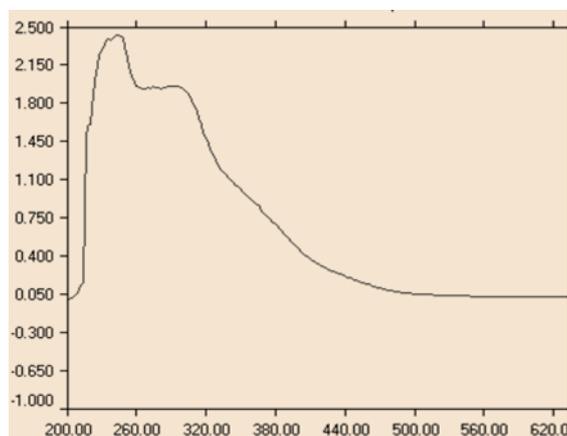


Fig. 6: UV spectrum of compound I in aluminum chloride.

The ^1H NMR spectrum (Fig.7) showed a signal at δ 1.23 ppm assigned for a methyl group. The signal at δ 2.1 ppm accounts for an acetyl function. The multiplet at δ 4.23-5.45 ppm is due to a sugar moiety (not identified in this study).

The aromatic protons appeared as a multiplet (δ 6.68-7.65 ppm). Signals at δ 2.50 and δ 3.35ppm are due to solvent(DMSO) residual protons and residual water respectively.

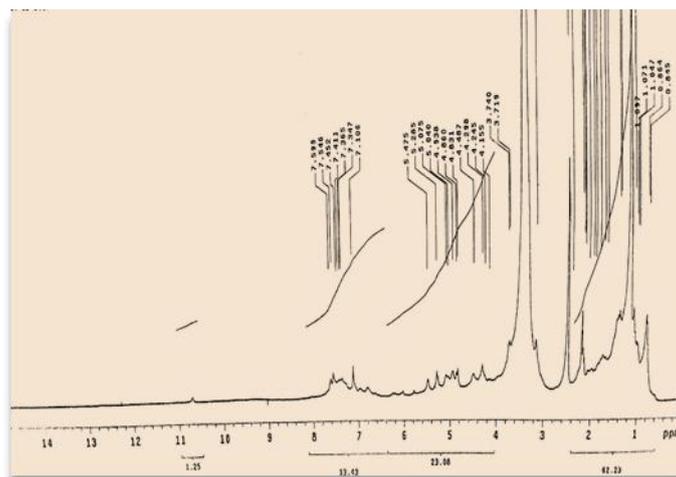
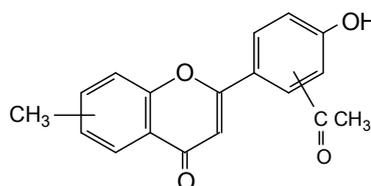


Fig. 7: ^1H NMR spectrum of compound I.

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



Antimicrobial activity

The crude extract and different fractions of *pulcaria crista* (ethyl acetate, chloroform and n-butanol) were screened for their antimicrobial activity against five standard microorganisms (Table 2). The results are depicted in Table (3). Results were interpreted in the

following conventional terms: (>9mm: inactive ;9-12mm:partially active ;13-18mm: active;<18mm:very active). Tables (4) and (5) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 2: Test organisms.

Ser. No	Micro organism	Type	Source
1	<i>Bacillus subtilus</i>	G+ve	ATCC 2836
2	<i>Staphylococcus aureus</i>	G+ve	ATCC 29213
3	<i>Pseudomonas aeruginosa</i>	G-ve	NCTC 27853
4	<i>Escherichia coli</i>	G-ve	ATCC 25922
5	<i>Candida albicans</i>	fungi	ATCC 7596

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

*ATCC. American type culture collection, Maryland, USA

Table 3: Antimicrobial activity of some extracts.

Sample	Ec	Ps	Sa	Bs	Ca
Ethanol extract	–	–	–	–	10
Ethyle acetate fraction	12	10	13	–	12
Choroform fraction	–	–	–	–	11
Butanol fraction	–	–	10	–	10

Table 4: Antibacterial activity of standard drugs.

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	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)	Ca
Clotrimazole	30	38
	15	31
	7.5	29

Sa. *Staphylococcus aureus*

Ec.: *Escherichia coli*

Pa.: *Pseudomonas aeruginosa*

Bs: *Bacillus subtilis*

Ca: *Candida albicans*

The ethyl acetate fraction of *pulcaria crispera* showed moderate activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. However, the ethanol extract and chloroform fraction were inactive against the tested bacteria, but they gave partial antifungal activity against the fungus *Candida albicans*. The n-butanol fraction exhibited partial activity against *Staphylococcus aureus* and *Candida albicans* (Table 3).

Antioxidant assay

The antioxidant capacity of crude extract has been measured. Evaluation of the antioxidant activity was carried out by measuring the capacity of the test sample against stable DPPH radical. The change in color is measured spectrophotometrically at 517 nm. As depicted in Table (6) the ethanol extract of *pulcaria crispera* stem exhibited significant antioxidant activity.

Table 6: Radical scavenging activity of ethanol extract.

Sample	Antioxidant activity
Propyl gallate	92.00%
Ethanol extract	82.00%

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