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Research Article

Chemical and antimicrobial properties of a flavonoid extracted from the *Cleome turkmena* Bobrov

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ABSTRACT

In the process of extracting chemical compounds from the extract of *Cleome turkmena* Bobrov, a flavonoid with a high weight percentage was extracted as pure green crystals. The compound was purified using several chromatographic and recrystallization methods and identified by crystallography and ^{1}H, ^{13}C NMR and it was determined that this flavonoid is Salvigenin with the scientific name 5-hydroxy-4',6,7-trimethoxyflavone. Also, according to the lot of reports about the antibiotic properties of flavonoids, the effect of Salvigenin on inhibiting the growth of several bacteria and fungi was investigated by minimal inhibitory concentration (MIC) and disk diffusion (DD) methods. The best inhibition against *Bacillus pumilus* with MIC value of 64 µg/ml. In disk diffusion method, best result was recorded against *Salmonella typhi*.

Keywords: flavonoids; Phytochemistry; Salvigenin; antibiotic.

1. Introduction

Flavonoid are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables

and certain beverages. They have miscellaneous favourable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease (AD), atherosclerosis, etc.[1–3]. Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is because of their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions. They are also known to be potent inhibitors for several enzymes, such as xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase and phosphoinositide 3-kinase[4–6]. In this regard, and considering the antibiotic properties of salvigenin, a flavonoid derived from the *C. turkmena*, in this study after purification and structure elucidation, we addressed the effects of salvigenin on the the growth of several bacteria and fungi by MIC and disk diffusion method.

2. Experimental

2.1. Plant material

The aerial parts of the wild-growing *C. turkmena* were collected during the full flowering stage were collected from Torbatejam Khorasan Iran, in March 2018. The plant specimen was identified and deposited at the Herbarium of Science and Research Branch, Islamic Azad University, Tehran, Iran. The aerial parts of the plant were air-dried at room temperature (25°C) in the shade for 5 days before the extraction.

2.2. Extraction and isolation

The separation process was carried out using several chromatographic methods [7]. Ground aerial parts (500 g) were extracted with EtOAc : MeOH (1 : 1) (2 × 5 L) at room temperature for 3 days to give 45 g (8.3% yield) of the crude extract which was suspended in EtOH (300 mL) at 55ºC, diluted with H_2O (259 mL) and extracted successively with *n*-hexane (3 × 650)

and $CHCl_3$ (3 × 450 mL). The $CHCl_3$ extract on evaporation at reduced pressure furnished a residue (10 g) which was then subjected to column chromatography on silica gel (200 g) using *n*-hexane with increasing amounts of EtOAc (0-100%) up to EtOAc : MeOH (9 : 1). Fifty-one fractions were collected which were monitored by silica gel-TLC. After being monitored by TLC, fractions 33-35 were combined and recrystallized from hexane-EtOAc to remove the pigments impurities. Recrystallization from *n*-heptane : EtOAc (3 : 1) afforded 54 mg of pure 5-hydroxy-6,7,4'-trimethoxy-flavone.

2.3. MIC macro dilution broth assay

The antimicrobial activity of the extracted Salvigenin was tested by using the disc-diffusion method and determining the minimal inhibitory concentration (MIC) [8] using the macro dilution broth technique. Briefly, an overnight culture of approximately 5×10^5 CFU/ml was inoculated into tubes containing test compound dilutions and incubated at 37° C for 24 h. The MIC was defined as the lowest concentration of test compound able to restrict bacterial growth to a level lower than 0.05 at 650 nm. The extracted Salvigenin was screened against 6 bacterial and 3 fungal strains.

Microorganisms were cultured for 16–24 h at 37 ºC and prepared to turbidity equivalent to McFarland Standard No. 0.5. The suspensions were then spread on a test plate of Muller–Hinton agar. Sterile discs were impregnated with 2 mg of the extract and placed on the surface of the test plate. Positive control discs include; Gentamicin and Nystatin for Gram-negative bacteria, Gram-positive bacteria and fungi, respectively.

2.4. disk diffusion assay

Determinations of antimicrobial activities of dried crystals of Salvigenin were accomplished by agar disk diffusion method. The dried crystals were dissolved in DMSO to a final concentration of 30 mg/ ml and filtered by 0.45 µm Millipore filters for sterilization.

Antimicrobial tests were carried out using the disk diffusion method reported by Murray, Baron, Pfaller, Tenover, and Yolke [9] and employing 100 µl of suspension containing 108 CFU/ml of bacteria and 104 spore/ml of fungi spread on the nutrient agar (NA) and sabouraud dextrose (SD) agar mediums, respectively. The disks (6 mm in diameter) impregnated with 10 µl of the Salvigenin solutions (300 µg/disk) and DMSO (as negative control) were placed on the inoculated agar. The inoculated plates were incubated for 24 h at 37 ºC for bacterial strains and 48 h and 72 h at 30 ºC for mold isolates, respectively. Gentamicin (10 µg/disk) was used as positive controls for bacteria and |Nystatin (100 I.U.) for fungi. The diameters of inhibition zones were used as a measure of antimicrobial activity and each assay was repeated twice.

2.5. Statistical analysis

Regression analyses were performed by SigmaPlot 2008 for Windows version 10.0.

3. Results and discussion

3.1. Structure Elucidation

The isolated compound was identified as 5-hydroxy-4',6,7-trimethoxyflavone or Salvigenin (Figure 1). Structural elucidation was based on crystallography and ^{1}H, ^{13}C NMR and mass spectroscopic data, in comparison with those reported in the literature[10-12].

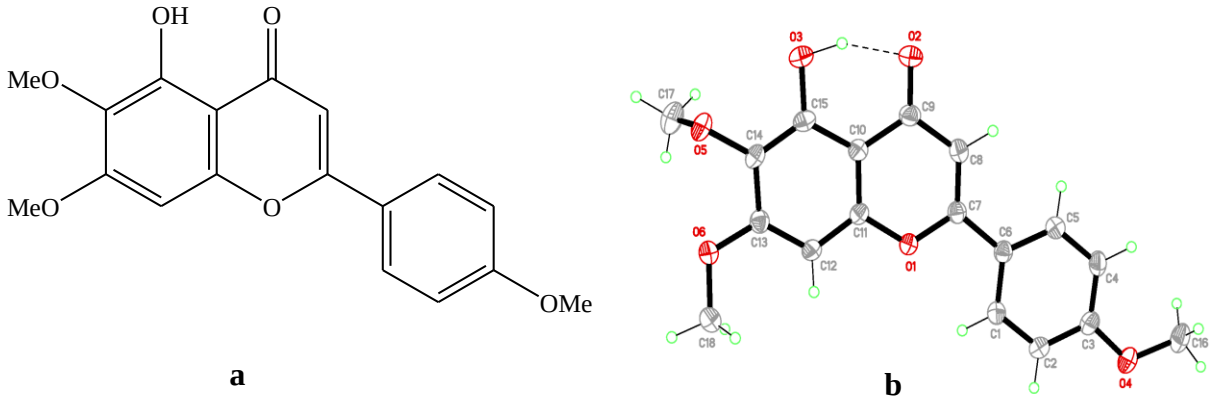

Fig. 1. a structure and **b** crystallography of Salvigenin

5-hydroxy-6,7,4′-trimethoxyflavone (Salvigenin)

$C_{18}H_{16}O_6$, green crystals, m.p. 181-183ºC; 1H-NMR (500 MHz, $CDCl_3$): 3.90 (3H, s, OMe-6), 3.93 (3H, S, OMe-7), 3.98 (3H, s, OMe-4′), 6.55 (1H, s, H-8), 6.59 (1H, s, H-3), 7.02 (2H, dd, *J* = 2.0, 9.0, H-3′ and H-5′), 7.85 (2H, dd, *J* = 1.9, 8.9, H-2′ and H-6′); ^{13}C-NMR (125 MHz, $CDCl_3$): OMe-6 (55.54), OMe-7 (56.30), OMe-4 (60.86), C_8(90.55), C_3 (104.12), C_{10} (106.12), C_5′ and C_3′ (114.51), C_1′ (123.53), C_2′ and C_6′ (127.99),C_5 (132.81), C_6 (153.03), C_9 (153.22), C_7 (158.71), C_4′ (162.6), C_2 (164.00), C_4 (182.65); MS (m/z) (rel.int.): 328 $[M]^+$ (85), 313 $[M\text{-}Me]^+$(85), 285 $[M\text{-}Me\text{-}CO]^+$(37),), 167 (52), 149 (100), 69(80), 57 (60), 43 (44).

3.1. Antimicrobial activity

The antimicrobial activity of Salvigenin was evaluated against a set of 9 microorganisms and its potency was assessed qualitatively and quantitatively by minimum inhibitory concentration (MIC) values and the disk diffusion (DD) method. The results are given in Table 1 and indicate that, at tested concentrations, Salvigenin has mild antimicrobial activity against tested microorganisms. Our findings showed that in the MIC method, The best inhibition against *Bacillus pumilus* one of the tested gram-positive bacteria with MIC value of 64 µg/ml. Moreover, this compound showed mild inhabitation against all gram-negative bacteria strains and *Kocuria varians* with MIC value of 128 µg/ml.

In disk diffusion method, the diameters of inhibition zones were used as a measure of antimicrobial activity. In this method, best result was recorded against *Salmonella typhi* with inhibition zone: 13.5 ± 0.86 mm.

Table 1. Antimicrobial activity of the of the crude extract of Salvigenin

Test microorganisms	Salviagenin		Gentamicin [c]		Nystatin	
	MIC [a]	DD [b]	MIC	DD	MIC	DD
Gram-negative bacteria						
Escherichia coli	128	9.3 ±0.57	128	6.9± 0.55	128	14.3± 1.15
Pseudomonas aeroginosa	170.6	10.3±1.15	128	9.3 ± 1.52	256	15 ± 1
Salmonella typhi	128	13.5±0.86	128	11.6± 1.52	512	14 ± 0.86
Gram-positive bacteria						
Bacillus pumilus	64	11.8±1.25	85.3	8.3 ± 0.57	256	12.1 ± 0.76
Kocuria varians	128	12.5±1.32	128	11 ± 1	128	10±1
Listeria monocytogenes	256	7.6±0.28	256	10.6 ± 1.52	128	14.5 ± 0.86
Fungi						
Aspergillus flavus	256	6.4±0	256	7.16±0.28	256	9±1
Candida glabrata	426.6	6.6±0.34	512	6.4±0	512	7.6±0.28
Aspergillus niger	512	6.4±0	512	6.6±0.34	512	10.3±0.57

[a] *Minimum Inhibitory Concentration (range of concentration: 8–512 µg/ml)*

[b] *DD (Disk diffusion method), Inhibition zones in diameter (mm) around the impregnated disks (Mean ± SD).*

4. Conclusions

The results obtained indicate that from Salviagenin biologic effects may become important in the obtainment of noticeable sources of compounds with health protective potential and antimicrobial activity. They showed mildly significant activity against Gram-positive and Gram-negative bacteria, and fungi.

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