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Antimicrobial Compounds from the Rihzomes of *Sansevieria hyacinthoides*

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Abstract

Three known compounds were isolated from the *n*-hexane extract of the rihzomes of *Sansevieria hyacinthoides,* they were two steriods; β-sitosterol (1) and daucosterol (2), and a flavonoid; isokaemferide (3). The structures of the compounds were elucidated on the basis of NMR spectral analysis as well as comparison with available data in the literature. All extracts and the three pure compounds were evaluated for their antimicrobial activity. The compounds showed moderate activity against *Bacillus subtilis, Stapylococcus aureus, Salmonella typhi* and *Candida albicans.*

Key words: *Sansevieria hyacinthoides,* Antimicrobial activity, Infectious diseases, Flavonoid, Steriods

Introduction

A large number of important modern drug, natural products and most of the traditional medicines are derived from medicinal plants and their derivatives. Medicinal plants, also known as herbs, herbal medicines, pharmacologically active plants or phyto-medicine are the dominant form of medicine in most countries. *Sansevieria hyacinthoides* (Linn.) Druce (Amaryllidaceae), an ornamental and medicinal plant is being traditionally used for the treatment of various diseases and aliments especially for the treatment of infectious diseases. It is locally known as Sutahara and is widely grown in many areas of the country (Ghani A, 2003). Roots and rhizomes of the plant are used in the treatment of blood disorders, heart diseases, fever, gonorrhea, itch, leprosy, rheumatism and glandular enlargements (Chopra *et al.,* 1956). Previous studies yielded some compounds from this plant (Ghani A, 2003, Gamboa-Angulo *et al.* 1996). Since very little chemical work has been reported on this plant, we undertook the investigation of its constituents. Bioactivity-guided fractionation of the extract has led to the isolation of three compounds; β-sitosterol (1), daucosterol (2) and isokaemferide (3). Although these known compounds have previously been reported from *Gynura procumbens, Centaurea clementei* and *Dionocarpus famatus* (Collado *et al.,* 1985; Sadikum *et al.,* 1996; Voutquenne *et al.,* 1999,) but this is the first report of these compounds from *S. hyacinthoides.* The minimum inhibitory concentration (MICs) of each compound was determined by disc diffusion methods (Baur *et al.,* 1966) against three *Gram-positive* (*Bacillus cereus, Bacillus subtilis and Staphylococcus aureus*) and three *Gram*-negative bacteria (*Salmonella typhi, E. coli, Pseudomonas aeruginosa*), and a fungus (*Candida albicans*). We now hereby report results from these investigation.

Materials and Methods

Plant material

The rhizomes of *Sansevieria hyacinthoides* were collected from the district of Gazipur, with the help of Bangladesh National Herbarium (BNH). A voucher specimen (No. 31,563) was prepared and deposited at BNH. The fresh rhizomes were taken into laboratory and cut into small pieces and were air dried. The rhizomes were finally dried at 38°C in an oven and ground to powder by cyclotec grinding machine.

General experimental procedure

Melting points were measured on Stuart Scientific SMP3 melting point apparatus and are uncorrected. The IR data for

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all compounds were obtained from dissolved sample in methanol using a Shimadzu FT-IR 8400S spectrometer. UV spectra and absorbance were recorded with a Perkin Elmer Lambda 25 UV-visible spectrophotometer. ^{1}H (400 MHz) and ^{13}C (100.60 MHz) NMR spectra were recorded on a Bruker DPX- 400 (400 MHz) instrument, with chemical shift data reported in ppm relative to the solvent used. General laboratory solvents were distilled from glass vessels before use. Column chromatography (CC) was performed using silica gel (0.063-0.2 mm). Silica gel 60 F_{254} coated on aluminium plates for thin layer chromatography (TLC) was supplied by Merck. Sephadex LH-20 (25-100 μ m) for gel filtration chromatography (GFC) was obtained from Fluka.

Extraction and Isolation

The dried, ground rhizome (74.8 g) was extracted by shaking with methanol for three days. The extracts were filtered and evaporated to a gummy mass in a rotary evaporator under vacuum at a maximum temperature of 40°C. The methanol extract (4 g) was partitioned between water and *n*-hexane and the aqueous part was further portioned between ethyl acetate (EtOAc) and water and then *n*-butanol and water.

The *n*-hexane extract (0.7 g) was fractionated by column chromatography (CC), over silica gel, eluting with *n*-hexane-chloroform (30-100%) and then chloroform-MeOH (0-10%). The eluents were collected in an amount of about 20 mL in a series of test tubes. The eluted fractions were classified according to TLC into several groups.

The group A (27.6 mg) eluted from 55 to 65% $CHCl_3$ in *n*-hexane was further chromatographed over silica gel using $CHCl_3$ (50 ml) followed by $CHCl_3$: MeOH (95:5, 50 ml, 20 fractions, 5 ml each). Fractions 4-7 afforded compound β-sitosterol (1, 5 mg). The group B (60.3 mg) obtained from 90 and 95% $CHCl_3$ in *n*-hexane was subsequently subjected to gel filtration chromatography, eluted with $CHCl_3$ and then $CHCl_3$- MeOH mixtures. It was finally purified by silica gel CC using $CHCl_3$ and $CHCl_3$-MeOH (95:5 v/v) gradient to give a flavonoid; isokaemferide (3, 5.5 mg). The group C (100 mg) eluted with 2-7% MeOH in $CHCl_3$ and subsequently subjected to gel filtration, eluted with $CHCl_3$-MeOH (95:5 v/v) and finally CC using $CHCl_3$ -MeOH (90:10 v/v) to yield daucosterol (2, 10.5 mg).

Antimicrobial screening

In the present study all extracts and pure compounds were tested for antimicrobial activity by disc diffusion method (Baur *et al.,* 1966). Laboratory isolates of 6 bacterial species which include three *Gram*-positive and three *Gram*-negative bacterial strains and a fungus Candida albicans were taken for the test. They bacteria were *Bacillus cereus, Bacillus subtilis, Stapylococcus aureus, Salmonella typhi, E. coli,* and *Pseudomonas aeruginosa*. Each organism was maintained on nutrient agar slant. The samples were dissolved separately in chloroform and applied to sterile filter paper disc at a concentration of 50 μg//disc. Kanamycin disc (30 μg/disc) was used as standard in each study. The sample disc, standard disc and control discs were placed gently on the previously marked zones in the agar plates pre-inocubated with test bacteria. The plates were then kept in a refrigerator at 4°C for about 24 hours upside down to allow sufficient diffusion of the materials from discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24 hours. The antimicrobial potency of the test agents were measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the samples were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

Results and Discussion

The *n*-hexane extract of the rhizomes of *Sansevieria hyacinthoides* was repeatedly fractionated using column chromatography over silica gel, sephadex LH-20, and led to the isolation of three known compounds (1-3). The known compounds were identified by comparison of their physical and spectral data with the literature as β-sitosterol (Sadikum *et al.,* 1996), isokaemferide (Collado *et al.,* 1985) and Daucosterol (Voutquenne *et al.,* 1999).

Compound 1 was a white crystalline compound, m.p. 137-139°C. It showed purple color on TLC when visualized with anisaldehyde-sulphuric acid spray reagent. UVλ_{max} in MeOH: 206 nm. The IR spectrum (liquid film) showed hydroxyl absorption bands at 3440 cm^{-1} (OH) and other bands appeared at 2960 cm^{-1} (CH), 1640 cm^{-1} (C=C) and 1064 cm^{-1} (C-O). The ^{1}H NMR spectrum showed the presence of multiplet signals for oxymethine protons at δ 3.51. The ^{13}C NMR spectrum showed a signal at δ 71.84 due to C_3 -β

Table I: Antimicrobial activity of extracts and isolated compounds (1-3) from S. *hyacinthoides*

Test bacteria and fungus	Methanol extract (50 µg/disc)	n-Hexane extract (50 µg/disc)	Compound 1 (50 µg/disc)	Compound 2 (50 µg/disc)	Compound 3 (50 µg/disc)	Kanamycin (30 µg/disc)
Gram-positive bacteria						
Bacillus cereus	NA	NA	NA	NA	NA	25
B. subtilis	15	14	8	8	9	22
Stapylococcus aureus	16	14	7	7	9	25
Gram-Negative bacteria						
Salmonella typhi	13	14	7	8	8	22
Escherichia coli	7	7	NA	NA	NA	22
Pseudomonas areuginosa	8	9	NA	NA	NA	25
Fungus						
Candida albicans	11	12	NA	NA	9	24

NA=No activity

hydroxyl group (Pretsch *et al.,* 2000), two recognizable olefinic carbon signals at δ 140.78 and 121.74 which were assigned to C_5 and C_6 double bonds, respectively as in Δ^5 spirostene (Agarwal *et al.* 1985). The signals at δ 11.89 and 19.42 corresponded to angular carbon atom C_{18} and C_{19} respectively. The olefinic resonance at δ 5.34 was characteristic of 5 -steroids (Ahmed *et al.*, 1992). The NMR spectra revealed that this compound was having six methyl, eleven methylene and three quaternary carbons with a hydroxyl group and was identical to the reported data for β-sitosterol (Sadikum *et al.,* 1996). Therefore, the structure of the compound 1 was established as β-sitosterol.

Compound 2 was an amorphous solid. It was detected as purple color on TLC when visualized with anisaldehyde-sulphuric acid spray reagent. UVλ_{max} (MeOH) nm: 220. The IR spectrum (liquid film) showed hydroxyl absorption bands at 3440-3410 cm^{-1} and other absorption band at 1665 cm^{-1} due to C=C absorption.. The ^1H and ^{13}C NMR spectra gave a pattern very similar to that of ß-sitosterol with the exception than an additional signal for a glucose moiety. The ^1H NMR spectrum showed a doublet at d 4.31 with J=7.3 Hz for anomeric proton signal which gave confirmation of β-anomeric configuration. The ^{13}C NMR spectrum indicated the presence of 35 carbon resonances. Out of these, six carbon signals were of the glycosidic group corresponding to a glucose moiety and 29 carbon signals for steroidal nucleus of the aglycone moiety. From this data and also by comparison with literature (Voutquenne *et al.,* 1999) compound 2 was identified as daucosterol.

Compound 3 was isolated as a yellow amorphous solid. The UV spectrum showed absorption maxima (MeOH) at 227, 296 and 315 nm. The IR spectrum showed absorption bands at 3420 cm^{-1} (OH), 2925, 1640 cm^{-1} (C=O) and other bands at 1629, 1514, 1442, 1393, 1350, 1275, 1259, 1196,1168, 1061, 1009, 945, 893, 829 cm^{-1} .

The ^1H NMR spectrum of compound 3 showed resonance for a methoxyl group at δ 3.86 (s), a phenolic hydroxyl proton (δ 12.76) which was strongly hydrogen bonded. Two aromatic signals for four protons at δ 7.14 (2H, d, J=8.4 Hz) and d 6.78 (2H, d, J=8.4 Hz) suggested a flavone with 4'-substituent B-ring and also showed two singlets at δ 6.23 (s) and 6.45 (s) for H-6 and H-8 positions of A-ring. The ^{13}C NMR spectrum showed 16 carbons including one methoxyl carbon resonating at δ 59.1 indicating that both positions ortho to the methoxyl were subsitituted (Sultana *et al.* 1999). The spectrum revealed chemical shifts that suggested 3,5,7, 4'-oxygnated flavone nuclus. On this basis and in comparison with published data (Collado *et al.,* 1985) the compound 3 was identified as isokaemferide.

Antimicrobial activity

The crude methanol and *n*-hexane extracts of S. *hyacinthoides* showed significant inhibition activity (Table I) against tested bacteria and fungus. The n-hexane extract was fractionated and yielded three compounds (1-3). The antimicrobial activities of compounds 1, 2 and 3 were moderate against *Bacillus subtili, Stapylococcus aureus* and *Salmonella typhi* (Table I), while only compound 3 showed moderate antifugal activity against *Candida albicans.* The

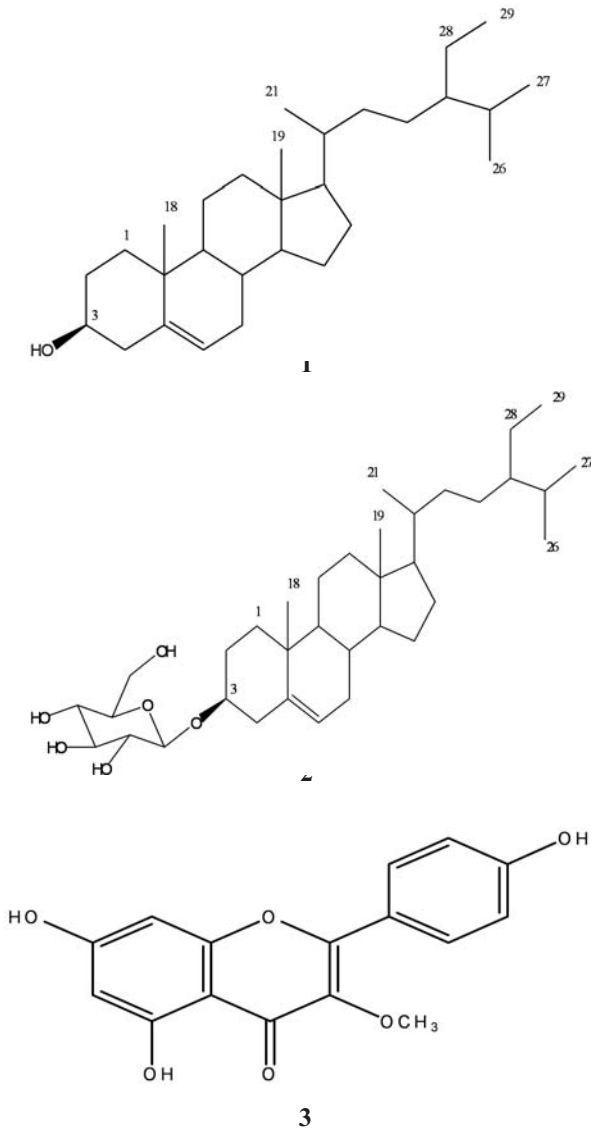

Fig. 1: The structures of isolated compounds from S. *hyacinthoides*

present results may provide some explanation for synergetic effect of crude extracts as well as the medicinal uses of this plant.

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