SHORT REPORT

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Chemical Constituents and Antimicrobial Activity of *Salix subserrata*

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Abstract: The leaf and bark extracts of *Salix subserrata* showed promising antibacterial, antifungal, and antialgal activities. The bio-guided study of the chemical constituents of the bark and leaves of *Salix subserrata* (Salicaceae) has resulted in the isolation and characterization of eight compounds. These six compounds were identified as (+) catechin (**1**), 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester (**2**), saligenin (**3**), methyl 1-hydroxy-6-oxocyclohex-2-enecarboxylate (**4**), catechol (**5**), propyl acetate (**6**), β-sitosterol (**7**), and β-sitosterol glucopyranoside (**8**), were isolated for the first time from *Salix subserrata.* The above compounds were individually identified by spectroscopic analyses and comparisons with reported data. Preliminary studies indicated that compound **1**, mixture of compounds **3/4**, and **7** showed good antibacterial, fungicidal, and algicidal properties.

Keywords: *Salix subserrata*; antimicrobial activity; Salicaceae; steroid.

1. Plant Source

Salix subserrata (Synonyms *S. safsaf*) Wild. (Salicaceae) is a deciduous bush or small tree 2-10 m high at stream sides in throughout Africa (Gambia, Egypt, Libyan, Zambia and Sudan). The leaf furnishes a laxative for human and veterinary medicine [1]. A black dye is obtained from the leaf to dye mats [1]. Roots are used in medicines that help cure stomach pains, fever, and headaches [1]. Crushed leaves of *S. subserrata* was reported to be used along with milk in treating patients of rabies (Dhukuba Seree) [2].

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The bark and leaves of plant *S. subserrata* was collected from Sharkia (East of Egypt), Egypt, in March 2008, and identified by Prof. Assem Elshazly at the Department of Pharmacognosy, University of Zagazig, Egypt. A voucher specimen has been deposited at the Herbarium of the Pharmacognosy Department, University of Zagazig, Egypt.

2. Previous Studies

Flavonoids such as rutin, luteolin-7-glucoside, quercetrin, and quercetin have been isolated from *S. subserrata* [3].

3. Present Study

The air-dried bark (1 kg) and leaves (0.5 kg) of *S. subserrata* was exhaustively extracted with methanol at room temperature. The resulting crude extracts (after evaporation) of bark and leaves were fractionated with *n*-hexane, chloroform, and EtOAc. The *n*-hexane extract (14 g) was subjected to silica gel column chromatography packed using different solvent systems and yielded twenty fractions (SS-1 to SS-20). *β*-Sitosterol (**7**) was obtained from fraction SS-6 after subjecting to CC eluted with petrol ether:CH_2Cl_2 (9:1) as colorless needles (9 mg). While fraction SS-8 gave propyl acetate (**6**, 7 mg) after CC with petrol ether:CH_2Cl_2 (8.5:1.5). The EtOAc fraction of bark (73 g) subjected to column chromatography (petrolum ether (PE), PE/EtOAc, EtOAc, EtOAc/MeOH) in order of increasing polarity yielding (53) fractions. Fraction SS-1 was subjected to CC eluted with a mixture PE:EtOAc (5:5) yielding catechol (**5**, 7 mg) while column fraction SS-7 [PE:EtOAc (6:4)] gave saligenin (**3**) and methyl 1-hydroxy-6-oxocyclohex-2-enecarboxylate (**4**). Similarly SS-9 was subjected to CC eluted with EtOAc:PE (6:4) gave catechin (**1**, 9 mg). Finally fraction SS-10 [MeOH:CH_2Cl_2 (0.5:9.5) yielded 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester (**2**, 7 mg). The CH_2Cl_2 fraction of bark (73 g) subjected to column chromatography (PE/ CH_2Cl_2, CH_2Cl_2/MeOH) in order of increasing polarity yielding 57 fractions. The column fraction SS-40 [MeOH:CH_2Cl_2 (0.5:9.5)] yielded, *β*-sitosterol glucopyranoside (**8**, 10 mg). Similarly, column fraction SS-48 [CH_2Cl_2:PE (3:7)] gave *β*-sitosterol (**7**, 7 mg). Finally fraction SS-44 [CH_2Cl_2:PE (8:2)] yielded 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester (**2**, 5 mg).

The tested compounds and crude extracts were dissolved in acetone at a concentration of 1 mg/mL. 50 µL of the solution were pipetted onto a sterile filter disc, which was placed onto an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the test organism on the appropriate medium (MPY or NB) [4]. The test organisms were *Escherichia coli* (NB), *Bacillus megaterium* (NB), *Microbotryum violaceum* (MPY) and *Chlorella fusca* (MPY). The radius of zone of inhibition was measured in mm. All the microorganisms belong to the permanent culture collection of the Institut für Mikrobiologie, Technische Universität, Braunschweig.

The whole plant extract of *S. subserrata* was fractionated by silica gel column chromatography to give several fractions, which were further chromatographed on silica gel to give one one flavanol (**1**), one phthalate (**2**), two phenols (**3** and **5**), one cyclic ketone (**4**), and two steroids (**7** and **8**) (Figure 1). These six compounds were identified as (+) catechin (**1**) [5], 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester (**2**) [6], saligenin (**3**) [7], methyl 1-hydroxy-6-oxocyclohex-2-enecarboxylate (**4**) [8], catechol (**5**) [9], propyl acetate (**6**) [10], β-sitosterol (**7**) [11], and β-sitosterol glucopyranoside (**8**) [12]. They were isolated for the first time from *Salix subserrata* and identified by comparison of ^{1}H NMR, ^{13}C NMR (Bruker 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR), and EIMS (VG 7070 mass spectrometer operating at 70 eV) data with reported data.

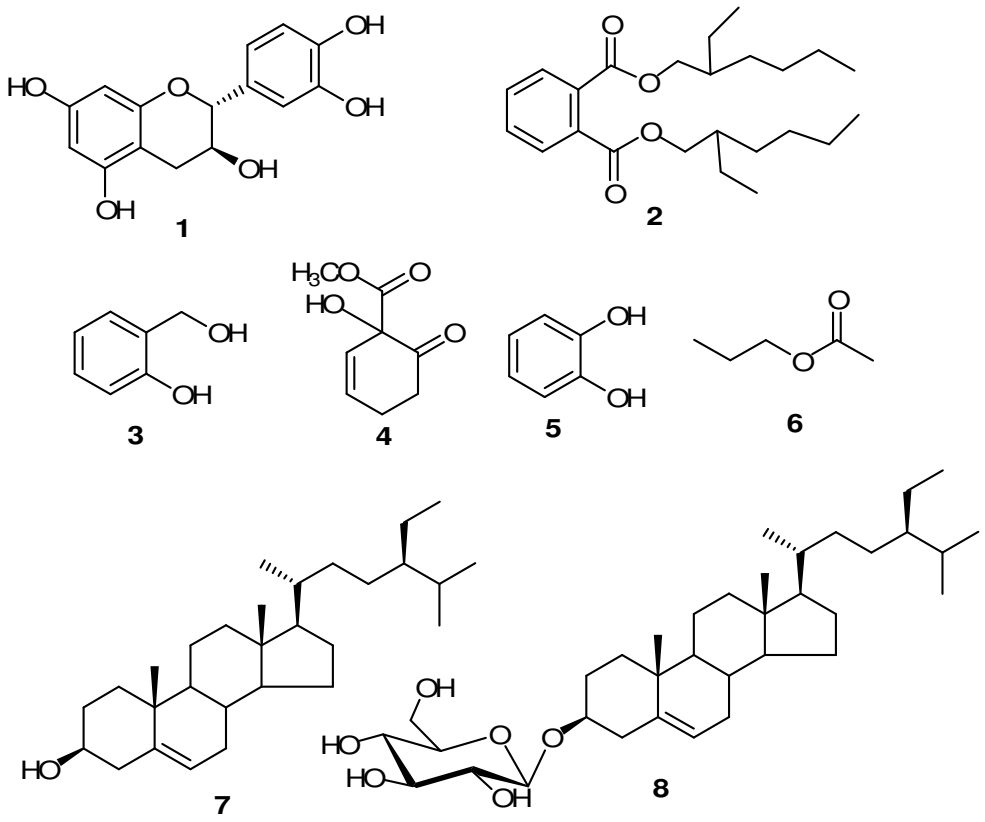

Figure 1. Compounds **1–8** isolated from *S. subserrata*.

The biological activities of the extracts and compounds were tested in an agar diffusion assay for antibacterial (*Escherichia coli, Bacillus megaterium*), antifungal (*Microbotryum violaceum*) and antialgal activities (*Chlorella fusca*). Antibacterial, antialgal, and antifungal activities of CH_2Cl_2 and EtOAc extract of leaves and compounds (**1**, **3**, **4** and **7**) were determined according to Höller et al. [3] (Table 1). The result of antimicrobial activity of the crude extracts of *S. subserrata* showed promising antialgal activity against *Chlorella fusca* except EtOAc extract of leaf but intermediate activity against fungus. Similarly, all extract showed promising antibacterial activity against *Bacillus megaterium.* Surprisingly, both EtOAC extract of both leaf and bark were inactive against fungal *Microbotryum violaceum.*

Compounds **1**, **3**, **4** and **7** showed good activity against the alga *Chlorella fusca* and antibacterial activity against Gram positive bacterium *Bacillus megaterium* and Gram negative bacterium *Escherichia coli.* But these tested compounds showed moderate antifungal activity against *Microbotryum violaceum.*

Table 1. Biological activity of extracts and compounds **1**, **3**, **4** and **7** in an agar diffusion test

Extracts/Compound	antialgal	antifungal	antibacterial	antibacterial
	Chl [a]	Mb	Bm	Ec
CH_2Cl_2 extract (Leaves)	10	5	PI 9	8
EtOAc extract (Leaves)	5	0	PI 7	7
CH_2Cl_2 extract (Bark)	10	6	PI 10	0
EtOAc extract (Bark)	10	0	PI 9	7
1	7	5	PI 7	8
Mixture **3/4**	8	5	PI 8	7
7	8	5	PI 6	5
Penicillin	0	0	18	14
Tetracycline	PI 10	0	18	18
Nystatin	0	20	0	0
Actidione	35	50	0	0
Acetone	0	0	0	0

[a] *Chlorella fusca* (Chl), *Microbotryum violaceum* (Mb), *Escherichia coli* (Ec), and *Bacillus megaterium* (Bm). Application of extracts and pure substances at a concentration of 0.05 mg (50 µL of 1 mg/mL). The radius of zone of inhibition was measured in mm. PI = partial inhibition, i.e. there was some growth within the zone of inhibition.

As a Conclusion**,** plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. But *in vivo* studies on these medicinal plants are necessary and should seek to determine toxicity of active constituents and their side effects. This represents the first preliminary report on the anti-microbial activity of *S. subserrata*. From the above studies, it is concluded that the traditional plants may represent new sources of anti-microbials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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