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Full Length Article

Antibacterial Sensitivity for some Chemically Diverse Steroidal Glycosides *In Vitro*

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ABSTRACT

Antimicrobial sensibility of steroids and steroidal glycosides are being studied widely. The *in vitro* susceptibilities of the steroidal glycosides were tested against *Bacillus cereus*, *Staphylococcus aureus, Serratia marcescens*, *Escherichia coli* and *Pseudomonas putida* using the disc diffusion method. These steroidal glycosides were identified as alpinoside C (1) [kidjolanin3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-oleandropyranosyl-(1→4)-β-D-cymaropyranosyl -(1→4)-β-D-cymaro-pyranoside]; Ghalakoside (2) [cardiac glycoside] and pentandroside A (3) [(22S,25S)-16,22,26-trihydroxycholest-4-en-3-one-16-*O*-β-D-glucopyranosyl-(1→3)-α-D-xylopyranoside], which were isolated from *Oxystelma esculentum* var. *alpine*; *Pergularia tomentosa* and *Tribulus pentandrus*, respectively. Almost all of the selected compounds produced significant antibacterial potentialities against all tested microorganisms and the data were compared to the standard antibiotics. All compounds exhibited remarkable susceptibilities against Gram negative (*S. marcescens*) and Gram positive (*B. cereus*). The minimum inhibitory concentrations (MIC) of compounds ranged from 10 to 50 µg/disc. © 2012 Friends Science Publishers

Key Words: Antibacterial sensitivity; Medicinal plants; Polyhydroxypregnane ester glycoside; Cardiac glycoside; Cholestane glycoside

INTRODUCTION

There is a general tendency to use the metabolites of plant extracts for treatment of diseases allover the world. Steroidal glycosides represent a diverse group of metabolites exhibiting a wide range of biological activities due to their antioxidant properties and ability to modulate several enzymes or cell receptors (Kintia *et al.,* 1986) [1]. As a part of our ongoing study of various Egyptian medicinal plants, we isolated some new steroidal glycosides from *Oxystelma esculentum* var. *alpine* (Hamed *et al.,* 2004a) *Pergularia tomentosa* **(**Al-Said *et al.,* 1988; Hamed *et al.,* 2006) and *Tribulus pentandrus* (Hamed *et al.,* 2004b).

The resistance of pathogenic organisms towards drugs has become a serious problem in the last decades. Therefore, there is an urgent need to discover new compounds possessing potent antimicrobial activities for developing new drugs. Many antimicrobial agents have been introduced into therapy; however the field still needs extensive efforts for the development of new antimicrobial agents of superior activity and less toxicity. Development of new antibacterial agents (natural or synthetic) can be useful in the control of infections caused by pathogenic micro-organisms.

As a matter of fact, phytochemicals were reported to inhibit a number of micro-organisms (Fisher *et al.,* 1984; Gurney & Mantle, 1993; Venera *et al.,* 2009; Zaixiang *et al*., 2010). Moreover, antibacterial activity of steroidal glycosides has been extensively reviewed (Tamura, 1996; Takeya, 1997; Gibbons, 2005). The objective of the present work was to verify, *in vitro*, the antibacterial susceptibility of the major constituent of the steroidal glycosides isolated from the selected plants such as alpinoside C, ghalakoside and pentandroside A.

MATERIALS AND METHODS

Plant materials: All plant materials were identified according to Tàckholm (1974) and Boulos (2000), and the voucher specimens were deposited in Faculty of Science Herbarium at Aswan. The aerial parts of *O. esculentum* var. *alpine* (No. 010497) were collected from conservation Gazal Island, Aswan, Egypt, in December 2001; the root parts *P. tomentosa* L (No.010889) were collected from Wadi Um Hebal (Wadi Allaqi road Eastern Desert, Aswan-Egypt), in January 2001 and the aerial parts of *T. pentandrus* Forssk. (No. 11030) were collected in December 2001 from Wadi Allaqi Eastern Desert, Aswan-Egypt (Table I).

Extraction and isolation of alpinoside C (1): The alpinoside C (1) was isolated from the dried aerial parts (1350 g) of *O. esculentum* var. *alpine* and identified by using extensive spectroscopy methods (Hamed *et al.,* 2004a).

Extraction and isolation of ghalakoside (2): The air-dried material of the root part *P. tomentosa* L (1580 g) was powdered and extracted with 80% ethanol by maceration until exhaustion. The alcohol extract was concentrated under reduce pressure to a syrupy consistency (195 g). The solvent-free residue (195 g) was mixed with 200 mL water, 100 mL methanol, transferred to a separator funnel and partitioned between hexane (38 g), chloroform (90 g) and n-butanol (67 g). 50 g from chloroform fraction was slurred with 20 gm of silica gel (E Merck), and transferred to the top of a column (120 x 4.5 cm) of (600 g) of activated silica gel previously packed by the wet method in chloroform. Elution was started by chloroform and the polarity was gradually increased by using methanol. Fractions were then grouped according to similar contents. Fractions eluted with $CHCl_3$: MeOH (8:2) gave 15 g of ghalakoside (2) (Hamed *et al.,* 2006).

Extraction and isolation of pentandroside A (3): The pentandroside A (3) was isolated from the aerial parts (200 g) of *T. pentandrus* and identified by using extensive spectroscopy methods (Hamed *et al.,* 2004b).

Testing the compounds purity: HPLC for compounds 1, 2 and 3 were carried out on a pressure liquid chromatography (Water, Milford, MA) consisting of a model 616 pump, a model 600s controller and a model 996 photodiode array detector operating at 310 nm. Separations were accomplished on 250 x 4.6 mm column prepacked with Eurospher 80 RP 18 (Sulentechnik, Germany); the mobile phase was acetonitrile in 1% H_3PO_4 water in linear gradient during 60 min (Fig. 1).

Selected test strains: *In vitro* bacterial susceptibilities were carried out against two Gram-positive (*B. cereus* & *Staphylococcus aureus*) and three Gram-negative (*S. marcescens, E. coli* & *P. putida*).

The bacteria strains were incubated on a nutrient agar slant (Difco, Detroit, Michigan, USA; 401810; pH 6.8), for 24 h at 37^0C. The agar nutrient was autoclave sterilized and 25 mL portions were dispensed in pre-sterilized Petri dishes (9 cm in diameter). The overnight cultures were preserved in phosphate buffered saline before using.

Screening for antibacterial potentialities: Twenty paper discs (Ø = 4 mm) were impregnated with 2 ml of 10, 25, 50 and 100 µg/mL of the tested compounds and the solvent allowed to evaporate at room temperature. The diluted bacterial culture was spread on sterile nutrient agar medium before placing the compounds impregnated paper discs on the Petri-dishes. Chloroamphenicol, erythromycin, ampicillin, cephaloridine, pencillin G, gentamicin, cortimoxazole and tetracycline HCl were used as reference antibiotics for comparison at concentration of µg/disc. The agar plates were homogeneously inoculated with an inoculum consisting of about 10^6 microorganisms/ml phosphate buffered saline. The Petri-dishes were incubated at 37 ± 2^0C. After 48 h the inhibition clearing zone diameters were measured. The experiments were carried out in triplicate and the results were expressed in average values.

Determination of minimum inhibitory concentrations (MICs): Discs were performed by a modification of the standard agar diffusion method as described by NCCLS (National Committee for Clinical Laboratory Standards, 1995). The compounds were tested at various concentrations. Eight control agents were tested. The MICs were read after 24 h of incubation at 37± under micro-aerobic conditions. The MICs were defined as the lowest compound concentration resulting in a clear zone of growth inhibition from a triplicate experiment.

RESULTS

The growth inhibitions of the selected bacterial strains by different concentrations of the tested steroidal glycosides were recorded and the data were represented in Table II. All compounds have more potent inhibitory effects on both Gram positive bacteria and Gram-negative. All strains were erythromycin, ampicillin, cephaloridine resistance but only three strains were tetracycline HCl resistance.

The alpinoside C (1) of polyhydroxypregnane ester glycoside type showed strong antibacterial activity against *B.cereus, S. aureu, S. marcescens* and *P. putida*; and a moderate activity against *E. coli*. The antibacterial potency of alpinoside C (1) is comparable to references of antibiotics, preventing growth the strains of *B. cereus*, *S. aureu, S. marcescens*, and *P. putida* with a minimal inhibitory concentration of 10 µg/disc and 25 µg/disc for preventing growth of *E. coli*. The lengths of clearing zone diameters were at concentration 100 µg/disc (10.3 & 12.7 mm).

Ghalakoside (2), which have a unique double linkage of 3-deoxy hexose at C-2 and C-3 (Fig. 1 & 2) exhibited strong antibacterial activity against Gram-positive (*B. cereus*) and Gram-negative (*S. marcescens*) whereupon the lengths of clearing zone diameters were at concentrations 50 and 100 µg/disc (13.2, 10.3 mm, respectively). The minimum inhibitory concentration was 10 µg/disc (5.5 mm). It also exhibited weak antibacterial activity against Gram-posative *S. aureus* and Gram-negative *P. putida*. It was inactive against *E. coli* for all tested concentrations.

However, pentandroside A (3) of the cholestane types that isolated from *T. pentandrus* exhibited strong activity against *B. cereus*, *S. aureus, S. marcescens, E. coli*, and *P. putida*. This indicated that the activity was shown to vary dramatically with changes of the aglycones skeleton, in addition to the oligosaccharide chains. The antibacterial potency of pentandroside A (3) is comparable to references of antibiotics, killing strains of both Gram-positive and Gram-negative with a minimal inhibitory concentration of 10-25 µg/disc. Moreover, pentandroside A (3) exhibited strong susceptibility against *E. coli* at all tested concentrations and the largest clearing zone was at 100 µg/disc (14 mm). Also, the results exhibited that pentandroside A (3) was more effective than ampicillin, cephaloridine and pencillin G and similar to cortimoxazole

Fig. 1: HPLC for compounds

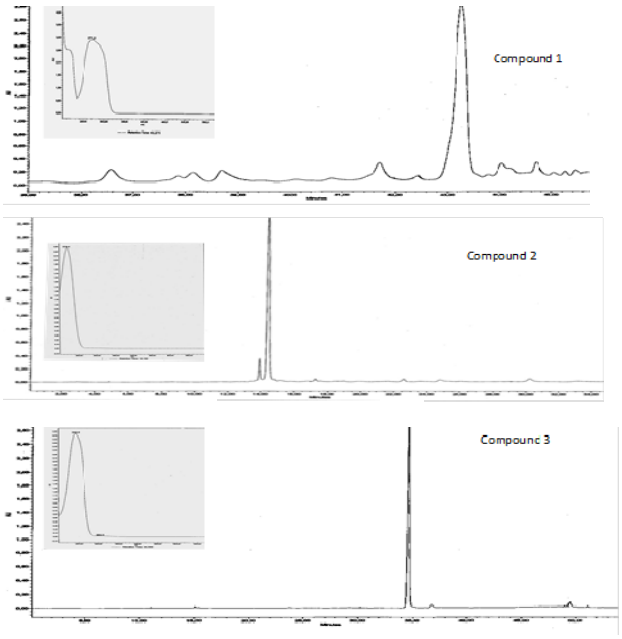
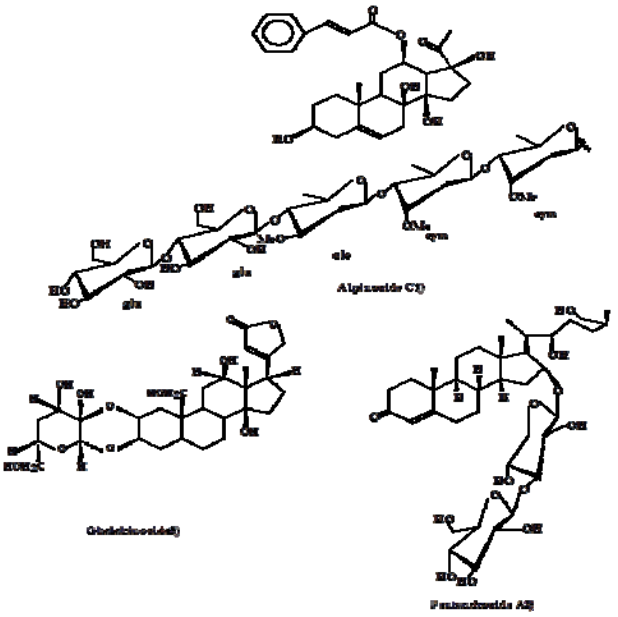


Fig. 2: Structure of the tested compounds



at concentration 25 µg/disc (11 mm of diameter). Based on our results and the literature, cholestane frame seem closely related to antibacterial effects of steroidal glycosides. Also, the presence of α-β-unsaturated lactone in ring A in the tested compound may be increased the activity.

DISCUSSION

In spite of the previous studies reported that the leaves of *Solenostemma argel* contain many derivatives of pregnane and pregnane glycosides (Hassan *et al*., 2001; Hamed, 2001; Plaza *et al*., 2005a &b); a weak antibacterial and antifungal activities were observed with extracts prepared from leaves (Khaled *et al*., 1974; Tharib & El-Migirab, 1986). However, the synthesized amide 3β-acetoxy-17β-(L-prolyl)-amino-5α-androstane, which also represent a pregnane derivative exhibited a clear bactericidal activity against a broad variety of Gram-positive genera (Pettit *et al.,* 2000). This confirmed that the activity was related to the skeleton of the agent. The activities of alpinoside C (1) may be related to the presence of phenolic cinnamate ester at C-12 and/or the chain of oligosaccharides at C-3. Almost all the tested concentrations produced significant antibacterial activity against all the microorganisms being tested. Our results were confirmed with the earlier observations that cardiac steroids were bacterially moderate inhibiting (Huq *et al*., 1999).

The activity of ghalakoside (2) and its hygroscopic property seem to be an explanation of the use of *P. tomentosa* L as a poultice in the treatment hemorrhoids. Due to cytotoxic potential of ghalakoside thereof should be used with great caution (Al-Said *et al*., 1988). Although the previous study was tested aglycone of cardiac, there was no variation in activity compared to cardiac glycoside. The furostanol and spirostanol types that previously isolated from *T. terrestris* do not have antibacterial activity against *S. aureus*, *P. aeruginosa* and *Mycobacterium intracellulare*, the spirostanol types were fungally active and furostanol types were fungally inactive (Huffored *et al.,* 1988; Bedir *et al.,* 2002; Zhang *et al.,* 2006).

In conclusion, results of the present study demonstrated that steroidal glycosides isolated from Egyptian traditional medicinal plants have potent properties against a number of Gram-positive and Gram-negative bacteria. Thus, pharmacological screening of medicinal plants remains important to provide a scientific basis for the continued traditional use of plants and to provide society with potential sources of new, effective and safe drugs. Identification the mode of action and its *in vivo* toxicity warrants further study to explain the traditional uses of these plants as antibacterial agents.

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Table I: Botanical names, synonyms, local names and traditional medical uses of the selected medicinal plants

Plant (family)	Synonyms	Local names	Traditional medicine uses
Oxystelma esculentum var. *alpine* (Asclepiadaceae)	*Oxystelma alpine* Decne.; *Periploca esculenta* L.; *Sarcostemma esculentum*	Libbein; Libbeina; Ollaiq; Olleiq; Moddeid, Meddaad; Motteit	A decoction is used as a gargle; the latex as a dressing for ulcers and the roots in the treatment of jaundice. The sap mixed with *Styrax benzoin*, *Citrus aurantifolia* & *Santalum album* juice, is drunk to treat cholera; the bark, with vinegar & spices, is smeared on the body (Perry & Metzger, 1980).
Pergularia tomentosa L. (Asclepiadaceae)	*Daemia cordata* R. Br.; *Daemia tomentosa* L.	Ghalaka; Laban El-Homara.	As a depilatory, poultice, laxative, anthelmintic, abortive, hemorrhoids and in the treatment of some skin diseases (Iben El-Bitar, 1809; El-Antaki, 1923; Chopra *et al.,* 1956).
Tribulus pentandrus Forssk. (Zygophyllaceae)	*Tribulus alatus* Delile.; *Tribulus longipetalus* Viv.; *Tribulus alatus* Delile. var. *odontopteris* Kralik.	Goodoob; Qoyoob; Qatoob; Qotoob onthawy; Gathah	Related species *Tribulus terrestris* is a famous pharmaceutical herb of the worled wild distribution and some sex function-enhancing preparation, namely Tribestan, Vitanone, & Tribusaponin based on saponins of the aerial parts are commercially available (Tomova *et al.,* 1981). Also, it possesses aphrodisiac properties (Adaikan *et al.,* 2000; Gauthaman *et al.,* 2002).

Table II: Antibacterial potentialities of the tested steroidal glycosides against different strains of bacteria

Test compounds	Concentration (µg/disc)	Zone inhibition (mm) Bacteria strains				
		S. aureus	P. putida	B. cereus	S. marcescens	E. Coli
Alpinoside C	100	10.0 ±0.10	11.0 ± 0.50	9.5 ± 0.50	6.7 ± 0.33	12.7± 0.05
	50	6.7 ± 0.53	8.0 ± 0.00	8.2 ± 0.25	5.7 ± 0.13	10.3 ± 0.03
	25	5.6 ± 0.25	7.0 ± 0.00	7.0 ± 0.10	5.7 ± 0.23	8.7 ± 0.13
	10	5.3 ± 0.13	6.0 ± 0.30	5.5 ± 0.0	-	6.3 ± 0.03
Ghalakoside	100	7.0 ± 0.25	13.0 ± 0.80	13.2 ± 0.55	-	10.3 ± 0.15
	50	-	12.0 ± 0.62	10.3 ± 0.40	-	8.3 ± 0.13
	25	-	10.5 ± 0.70	7.0 ± 0.19	-	7.3 ± 0.03
	10	-	7.0 ± 0.00	5.5 ± 0.15	-	-
Pentandroside A	100	8.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.11	14.0 ± 0.00	11.0± 0.10
	50	7.0 ± 0.30	9.0 ± 0.33	8.5 ± 0.15	11.5 ± 0.16	9.3 ± 0.03
	25	6.0 ± 0.30	7.0 ± 0.33	7.8 ± 0.12	10.5 ± 0.10	9.0 ± 0.00
	10	-	6.0 ± 0.25	6.6 ± 0.15	8.0 ± 0.90	7.7± 0.13
Chloroamphenicol	3	15	-	-	17	15
Erythromycin	15	25	-	-	18	25
Ampicillin	10	-	-	-	-	-
Cephaloridine	30	-	-	-	-	-
Gentamicin	10	-	10	12	17	15
Cortimoxazole	25	20	10	18	11	28
Tetracycline HCl	30	-	-	-	16	20

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