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Impact of certain *Solanum* species's natural products as potent cytotoxic and anti-Inflammatory agents

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The present study was conducted to evaluate both the cytotoxic and anti-inflammatory activities of ethanol extracts (T), and both *n*-butanol (B) and total glyco-alkaloid fractions (TGA) of *Solanum seaforthianum* Andr. (SS) and *Solanum macrocarpon* L. (SM) growing in Egypt. Cytotoxic activity was measured using sulforhodamine B (SRB) assay on prostate cancer cell line (PC-3), breast cancer cell line (MCF7), liver cancer cell line (HepG2) and human fibroblast cell line (HFB4) while anti-inflammatory activity was measured using formalin induced paw edema method. The highest cytotoxic potentiality was indicated for those of TGA fraction of *S. seaforthianum* Andr. on PC-3 cell line ($IC_{50} = 0.28 \mu\text{g/ml} \pm 0.01$) followed by its activity on MCF-7 cell line ($IC_{50} = 2.84 \mu\text{g/ml} \pm 0.20$). On the other hand, the potency of TGA fractions of both species showed higher potency followed by *n*-butanol fractions where ethanol extracts showed lowest potency which is emphasizing the cytotoxic potentiality of the glyco-alkaloids. Based on the IC_{50} s indicated for the different extracts and fractions on normal fibroblast cell line, considerable safety was indicated against prostate carcinoma rather than breast or hepatic carcinoma. TGA fraction of *S. macrocarpon* L. and of *S. seaforthianum* Andr. showed the highest anti-inflammatory activity with efficacy of 159 and 156%, respectively as compared to standard indomethacin. That's why the TGA fraction of *S. seaforthianum* Andr. was subjected for isolation of individual alkaloids using different chromatographic techniques and identified using ¹H and ¹³C-NMR spectroscopy beside Co-chromatography with authentic samples as solamargine (A₁), solasonine (A₂) and solasodine (A₃) which are firstly isolated from *S. seaforthianum* Andr. growing in Egypt.

Key words: *Solanum seaforthianum*, *Solanum macrocarpon*, glyco-alkaloid, anti-inflammatory, cytotoxicity, SRB.

INTRODUCTION

The economically transitioning countries showed increased incidence and mortality rates for most cancers

unlike United States and many other western countries (Jemal et al., 2010). Liver cancer incidence for example

in Egyptians was more than 3 times that in US SEER and about 5 to 7 times that in the other Middle East countries consortium populations (Freedman et al., 2006). On the other hand, breast cancer is the most common lethal malignancy especially in the urban areas of the developing countries than rural ones (Dey et al., 2010). In more developed countries, prostate cancer was the most common type of cancer diagnosed among men and was second most common cancer diagnosed among men worldwide (Ferlay et al., 2004; Baade et al., 2009).

Many *Solanum* species are used by humans, and were important sources of food, spice and medicine (Zaidi et al., 1992). The Cytotoxic activities of 20 steroidal glycosides from different *Solanum* species were examined on various cell lines illustrated major structure activity relationship (Nakamura et al., 1996). It was suggested that the cytotoxic activity depends on the kind of oligosaccharide and aglycone moieties of the tested steroidal compounds (Ikeda et al., 2003).

Glycoalkaloids has exhibited apoptotic activity and chemo-preventative effects against known carcinogens and anti-inflammatory activity (Milner et al., 2011). It was suggested that steroidal alkaloids content of *Solanum lycocarpum* St. Hil. fruits account for the anti-inflammatory effect of the crude ethanol extract (Vieira et al., 2003). Also, the aqueous extract of *Sesamum alatum* Moench exhibited anti-inflammatory activity (Lin et al., 1995).

The aim of this study was to evaluate the cytotoxic and anti-inflammatory activities of the different extracts and fractions of *Solanum seafortianum* Andr. and *Solanum macrocarpon* L isolation of the secondary metabolites from the biologically active fractions.

MATERIALS AND METHODS

Plant materials

Aerial parts of *S. seafortianum* Andr. and *S. macrocarpon* L. used in this study were collected March, 2012 from the Experimental Station for Aromatic, Medicinal and Toxic plants, Faculty of Pharmacy Cairo University, Giza, Egypt. The plants were kindly authenticated by Dr. Mohamed El-Gebaly, botany specialist, National research center (Dokki, Giza, Egypt). Voucher specimens (23082014 I and II respectively) were kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Extracts and fractions preparation

Air-dried powdered samples (1000 g, each) of the aerial parts of *S. seafortianum* Andr. and *S. macrocarpon* L. were, separately, macerated in ethanol (70%) till exhaustion. The extracts were

evaporated to dryness under vacuum. The individual ethanol extracts (104 and 123 g yield for *S. seafortianum* Andr. and *S. macrocarpon* L. respectively) were successively fractionated, using *n*-hexane (37.7 and 35 g yield), chloroform (2 and 3 g yield), ethyl acetate (2 and 4 g yield) and *n*-butanol saturated with water (35 and 43 g yield). While the total glyco-alkaloid fraction preparation adapted from Bushway et al. (1985) where air-dried powdered samples (1000 g, each) of the aerial parts of both species which were macerated with methanol (3x), and the extract filtered; the solvent was eliminated at a reduced pressure. The resulting dry extracts were dissolved in 500 ml of 5% acetic acid and washed several times with *n*-hexane. Then it was extracted with CHCl_3 (v/v). Then filter and adjust supernatant to 10.5 to 11.0 pH with NH_4OH , kept in 70°C water bath for 10 min, cooled and centrifuged. The residue is air dried in desiccator containing anhydrous calcium chloride. Then acid-base purification repeated where 34 and 39 g respectively. The ethanol extracts and both *n*-butanol and TGA fractions of both species evaluated for anti-inflammatory and cytotoxic potentiality.

Experimental models

Carcinoma cell lines

Hepatocellular (HepG2), breast (MCF-7) and prostate (PC-3) carcinoma human cell lines were kindly provided from the Pharmacology and Toxicology Department of Faculty of Pharmacy, Ain-shams University (Cairo, Egypt). Where fibroblast cell lines (HFB4) were provided from Pharmacology Department, National cancer institute (Cairo, Egypt).

Animals

Swiss albino mice (25 to 30 g), used for determination of LD_{50} , and adult male albino rats of Sprague Dawley strain (120 to 150 g), utilized for assessment of the anti-inflammatory activity, were obtained from the animal house colony at the National Research Center (Dokki, Giza, Egypt).

Acute toxicity studies (Determination of median lethal doses, LD_{50} of the tested extracts)

The LD_{50} of each tested ethanol extracts and the fractions (*n*-butanol and TGA) were determined following both intraperitoneal and oral administration according to Kärber (1931), and the animals were observed for any toxic symptoms for 24 h after administration. Preliminary experiments were carried out to determine the minimal dose that kills all animals LD_{100} and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were selected in between these two doses; each dose was injected in a group of six animals by subcutaneous injection. The mice were observed for 24 h and symptoms of toxicity and mortality rates in each group were recorded.

Cytotoxicity assessment using SRB assay

Cytotoxicity was determined using Sulforhodamine B Assay (SRB)

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as described by Skehan et al. (1990). Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96-well plates at 1000 to 2000 cells/well in RPMI-1640 supplemented medium. After 24 h, cells were incubated for 72 h with 0, 0.1, 1, 10, 100 and 1000 µg/ml concentrations of the tested compounds in dimethyl sulfoxide (DMSO). Following 72 h treatment, the cells were fixed with 10% trichloroacetic acid for 1 h at 4°C. Wells were stained for 10 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The plates were air dried for 24 h, and the dye was solubilized with Tris-HCl for 5 min on a shaker at 1600 rpm. The optical density of each well was measured spectrophotometrically at 564 nm with an ELISA microplate reader (ChroMate-4300, FL, USA). The IC₅₀ values were calculated according to the equation for Boltzman sigmoidal concentration–response curve using the nonlinear regression fitting models after 3 repetitions. (Graph Pad, Prism Version 5).

Cell culture

PC3 human prostate cancer cell line, MCF-7 human breast cancer cell line, HepG2 human hepatocellular carcinoma cell line and HFB4 human fibroblast cell line were grown in RPMI-1640 medium, supplemented with 10% heat inactivated FBS, 50 units/ml of penicillin and 50 mg/ml of streptomycin and maintained at 37° in a humidified atmosphere containing 5% CO₂. The cells were maintained as “monolayer culture” by serial sub culturing.

Assessment of anti-inflammatory activity by formalin induced paw edema method

Wister albino rats of either sex weighing 150 to 200 g were divided into 8 groups of 5 animals each. They were treated via oral route as follow: the 1st group was given 1% tween 80 and kept as control. The 2nd group administered indomethacin (10 mg/kg body weight) as standard drug according to Young et al. (2005), the tested ethanol extracts and both *n*-butanol and TGA fractions in the form of 1% tween 80 suspensions were given at a dose of 100 mg/kg body weight to last six groups. After 1 h, 0.1 ml of 2% formaldehyde was injected into the footpad of the left hind paw of each rat for induction of paw edema according to Dharmasiri (2003). The initial paw thickness was measured for each animal using Vernier caliper before induction of edema. The increase in this thickness was determined after 30 min, 1, 2 and 3 h after formaldehyde injection. The anti-inflammatory activity was expressed as inhibition percent in paw thickness in treated groups comparing with the control one using the formula proposed by Adedapo (2008). Where the inhibition percentage of different extracts and fractions divided by the indomethacin inhibition percentage to attain the efficacy were compared to indomethacin. The study protocol was reviewed and approved by the institutional review board PC 8385(REC-FOPCU; Research Ethics Committee- Faculty of pharmacy, Cairo University) in Egypt.

Isolation of the constituents

A weighed amount (4g) of the total glyco-alkaloid fraction of the aerial part of *S. seafortianum* Andr. was subjected to fractionation by vacuum liquid chromatography (VLC) on a 50 g Lichoprep Silica gel RP-18 column (25 × 7 cm). Elution was performed starting with methanol 30% in water and the polarity gradually decreased by 5% stepwise addition of methanol. Fractions were collected and monitored by TLC (precoated silica gel plates, Chloroform: Methanol: Ammonia (60:40:1), *p*-anisaldehyde/H₂SO₄). Fractions

with similar chromatographic pattern were pooled, evaporated under reduced pressure, weighed and saved in a desiccator. Collective fractions I (50% methanol in water) and II (95-100% methanol in water) were subjected to rechromatography as follows:

Fraction I: (1.7 g) showing two major spots (R_f value 0.6 and 0.5 in Chloroform: Methanol: Ammonia (60:40:1)), bluish green and turquoise, respectively with *p*-anisaldehyde) was subjected to rechromatography on a Licoprep RP-18 silica column (20 cm×1 cm). Elution was started using 40% methanol in water and the polarity of the eluent further decreased with methanol. Fractions, (10 ml, each) were collected and monitored by TLC (Chloroform: Methanol: Ammonia (60:40:1)) and RP-18 TLC (Methanol: Water: Ammonia (90:10:1)). Fractions (6-25) eluted with up to 60% methanol in water yielded concentration under vacuum 33 mg of white microcrystalline powder (compound A₁). And fractions (28-55) eluted with up to 75% methanol in water yielded concentration under vacuum 18 mg of white microcrystalline powder (compound A₂).

Fraction II: (0.6 g) apparently comprised one major constituent (R_f value 0.89, Chloroform: Methanol:Ammonia (60:40:1), Blue with *p*-anisaldehyde/H₂SO₄), which was purified by rechromatography on a silica gel column using CHCl₃: MeOH 95:5 v/v as eluent afforded 35 mg of white microcrystalline powder (compound A₃). Identification of the isolated compounds: NMR spectra were recorded using Joel SA NMR-spectrophotometer (Japan) ¹H-NMR, 500 MHz, ¹³C, 125 MHz spectra were recorded in suitable deuterated solvents (CDCl₃ or DMSO) using TMS as internal standard and chemical shift values expressed in δ ppm. (National Research Center, Dokki, Giza). Spectra of the isolated compounds were compared with those published by Shabana et al. (2013) and confirmed by Co-TLC with authentic alkaloids kindly provided by prof. Dr. Marawan M. Shabana Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Statistical analysis

Analysis of variance (ANOVA) followed by Turkey's test was performed using Statistical Package for Social Science (SPSS) version 20 to measure statistical significance. Measurements were carried out in triplicate. Data were presented as mean ± S.E.M. (*n*=5) for anti-inflammatory activity where significant levels from zero time at *P*<0.05 were tested and accepted as compared to the control group. Where cytotoxic potentiality data were presented as mean ± standard deviation (SD), the values were considered to be significantly different when *P* Values is less than 0.01.

RESULTS

The results of acute toxicity studies showed no deaths in animal groups which received ethanol extract and *n*-butanol fraction up to 1000 mg/Kg of body weight. LD₁ of *S. seafortianum* Andr. was 1500 mg/Kg b. wt., while at the same dose *S. macrocarpon* L. did not cause any deaths. Also, TGA fraction of both species causes no deaths up to the dose of 500 mg/Kg b. wt.

The results of the cytotoxic activity of doxorubicin, different extracts and fractions on HepG2, MCF7 and PC3 cell lines were shown in Figure 1. Anti-inflammatory activity of ethanol extracts and both *n*-butanol and TGA

Table 1. Anti-inflammatory efficacy of ethanol extracts, both *n*-butanol and TGA fractions of *S. seafortianum* Andr. and *S. macrocarpon* L. as compared to the standard indomethacin.

Treatment	Efficacy			
	30 min	1 h	2 h	3 h
SMT	1.30	1.25	1.01	0.76
SMB	1.25	1.21	1	0.69
SM TGA	1.59	1.58	1.33	1.04
SST	0.78	0.76	0.64	0.50
SSB	0.99	1.11	0.96	0.69
SS TGA	1.56	1.52	1.30	1.04

SMT: Total alcohol extract of *S. macrocarpon* L.; SMB: *n*-butanol fraction of *S. macrocarpon* L.; SM TGA: Total glyco-alkaloid fraction of *S. macrocarpon* L.; SST: Total alcohol extract of *S. seafortianum* Andr.; SSB: *n*-butanol fraction of *S. seafortianum* Andr.; SS TGA: Total glyco-alkaloid fraction of *S. seafortianum* Andr.

Table 2. ¹H NMR chemical shifts (δ ppm) for compound A₃(CDCl₃, 500 MHz, J in HZ) and A₁-A₂(DMSO, 500 MHz, J in Hz).

Proton No.	A ₁	A ₂	A ₃
1	1.70, 1.00 m	1.73, 1.0 m	.72, 0.98
2	2.07, 1.85	2.12, 1.87	2.10, 1.85
3	3.92 m	3.99 m	3.71 m
4	2.77 m	2.71 m	2.74 m
6	5.28br.s	5.3 br.s	5.56 r.s
7	1.50, 1.50	1.90, 1.51	1.35, 1.35
8	1.51	1.54	1.50
9	0.90	0.90	0.89
11	1.45, 1.45	1.45, 1.45	1.45, 1.45
12	1.73, 1.12	1.70, 1.10	1.73, 1.13
14	1.07, 2.07	1.10, 2.10	1.08, 2.09
16	4.38	4.51 m	4.50
17	1.85	1.83	1.87
18	0.72	0.75	0.81
19	0.91	0.97	1.18
20	1.98 m, 1.09 d, (J=7)	2.01 m, 1.17 d, (J=7)	1.91m, 1.09 d, (J=7)
21	0.82, (J=7.6)	0.85, (J=6.8)	0.92 d
22	-	-	-
23	1.73, 1.73	1.70, 1.70	1.73, 1.73
24	1.63, 1.63	1.65, 1.65	1.65, 1.65
25	1.50, 2.77	1.48, 2.83	1.48, 2.77
26	2.77	2.83	2.82
27	0.74 d (J=6.7)	0.76 d (J=8.4)	0.68 d (J=6)
29	-	-	-
1'	4.91 d (J=12)	4.92 d (J=8)	-
1''	5.04br.s	6.39br.s	-
1'''	4.98br.s	5.04br.s	-

fractions of both species shown in Figure 2 represent the decrease in paw thickness. While efficacy compared to indomethacin standard is shown in Table 1.

Three compounds were isolated from the TGA fraction of *S. seafortianum* Andr. The ¹H NMR and ¹³C NMR

chemical shifts of the isolated compounds and their assignments are shown in Tables 2 and 3. The compounds were identified as solamargine (A₁), solasonine (A₂) and solasodine (A₃). The structures of the isolated compounds are shown in Figure 3.

Table 3. ^{13}C NMR chemical shifts (δ in ppm) for compound A_3 (CDCl_3 , 125 MHz) and A_1 - A_2 (DMSO, 125 MHz).

Carbon No.	A_1	A_2	A_3
1	37.5	37.0	37.4
2	30.2	29.6	32.0
3	78.2	79.53	73.5
4	39.0	38.3	42.5
5	140.8	140.0	140.0
6	121.8	121.0	120.9
7	32.4	31.8	32.0
8	31.7	31.2	31.5
9	50.4	49.8	50.4
10	37.2	36.6	36.7
11	21.2	20.6	20.9
12	40.1	39.6	39.9
13	40.6	40.1	40.4
14	56.7	56.1	56.5
15	32.6	32.0	31.6
16	76.6	78.5	78.7
17	63.6	62.9	63.2
18	16.5	16.0	15.9
19	19.4	18.9	18.9
20	41.6	41.2	41.4
2	15.7	15.2	14.7
22	97.9	97.48	97.2
23	34.7	34.0	34.2
24	31.0	30.4	30.4
25	31.6	30.8	31.0
26	47.6	47.3	47.5
27	19.8	19.2	18.9
1'	98.6	101.0	-b
2'	77.8	75.6	-
3'	78.0	84.6	-
4'	78.7	69.3	-
5'	76.9	74.3	-
6'	61.3	61.9	-
1''	100.8	102.6	-
2''	72.5	71.7	-
3''	72.7	72.0	-
4''	73.9	73.4	-
5''	69.5	68.9	-
6''	18.6	18.0	-
1'''	101	103.4	-
2'''	72.8	74.2	-
3'''	72.5	77.6	-
4'''	74.1	70.9	-
5'''	70.4	77.3	-
6'''	18.5	61.5	-

DISCUSSION

The results of acute toxicity studies as deduced through determination of LD_{50} of the tested ethanol extract and both *n*-butanol and TGA fractions showed that all could be considered safe, and within the range of the orally administered doses.

The *in-vitro* testing of the cytotoxic potential shown in (Figure 1) the ethanol extracts, TGA and *n*-butanol fractions of the aerial parts of both species on three different human carcinoma cell lines revealed the highest potency for those of TGA fraction of *S. seaforthianum* Andr. on prostate carcinoma cell line PC-3 ($\text{IC}_{50} = 0.28 \mu\text{g/ml} \pm 0.01$) followed by its activity on breast carcinoma cell line MCF-7 ($\text{IC}_{50} = 2.84 \mu\text{g/ml} \pm 0.20$). On the other hand, the potency of TGA fractions of both species shows higher potency followed by *n*-butanol fractions where ethanol extracts show lowest potency which is emphasizing the cytotoxic potential of the glycoalkaloids. From the IC_{50} s listed for the different extracts and fractions on normal fibroblast cell line, the safety was indicated for prostate carcinoma rather than hepatic or breast carcinoma.

The IC_{50} s on normal cell line (HFB4) indicated that all extracts and fractions except TGA fractions of both species had possible selectivity against cancer cells rather than normal cells compared to doxorubicin ($\text{IC}_{50} = 4.0 \pm 0.21 \mu\text{g/ml}$ on HFB4). The high toxicity of TGA fractions on HFB4 could justify the anti-proliferative activity of glycoalkaloids.

All the tested extracts and fractions exhibited anti-inflammatory activity as indicated by the percentage of edema inhibition, where the isolated TGA fraction of *S. macrocarpon* L. and of *S. seaforthianum* Andr. showed the highest anti-inflammatory activity with potency 159 and 156%, respectively as compared to standard indomethacin drug following oral dosing. The potency of TGA SS fraction is followed by SSB fraction and SST extract. While the high potency of TGA SM is followed by SMT extract and SMB fraction, respectively. These results are in agreement with the result obtained for *Solanum torvum* Swartz. by Ndebia et al. (2006).

CONCLUSION

TGA fractions of both *S. seaforthianum* Andr. and *S. macrocarpon* L. growing in Egypt possessed significant anti-inflammatory and cytotoxic activity on PC-3, MCF-7 and HepG2, respectively, and highest safety was indicated for prostate carcinoma when comparing the IC_{50} with that for the normal fibroblast cell lines, and safety considerations should guide the use of these compounds as preventative or therapeutic treatments against carcinomas. As far as the available literature is concerned, this is the first report on isolation of

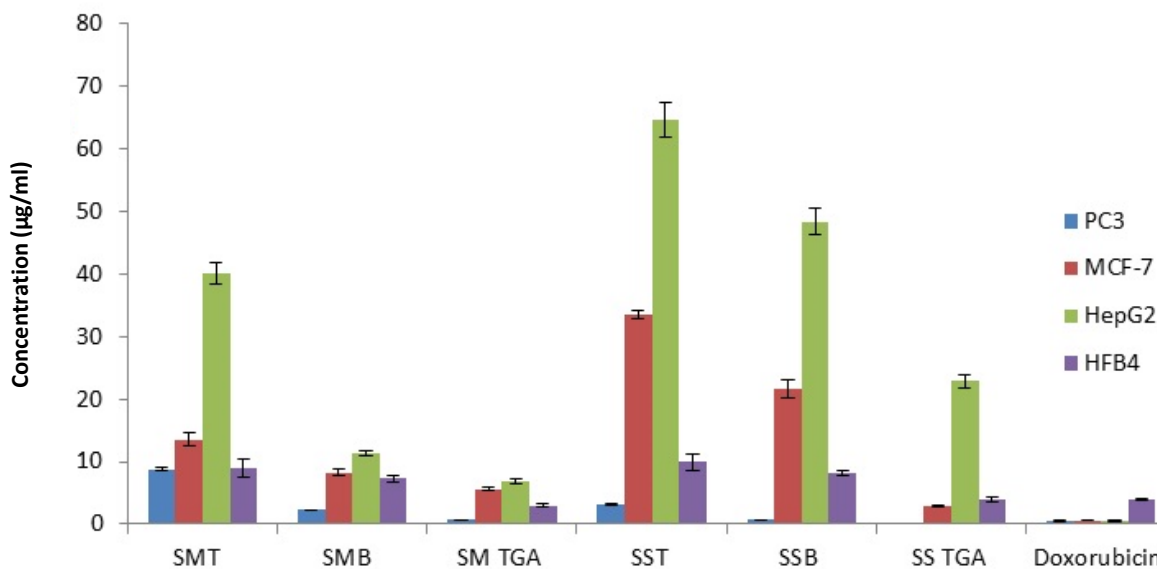


Figure 1. Histogram representing the IC₅₀s results in SRB assay of ethanol extracts and the both *n*-butanol and TGA fractions of *S. Seaforthianum* Andr. and *S. macrocarpon* L. on PC3, MCF-7 HepG2 and HFB-4cell lines. SMT: Total alcohol extract of *S. macrocarpon* L. SMB: *n*-butanol fraction of *S. macrocarpon* L. SMTGA: Total glyco-alkaloid fraction of *S. macrocarpon* L. SST: Total alcohol extract of *S. seaforthianum* Andr. SSB: *n*-butanol fraction of *S. seaforthianum* Andr. SS TGA: Total glyco-alkaloid fraction of *S. seaforthianum* Andr.

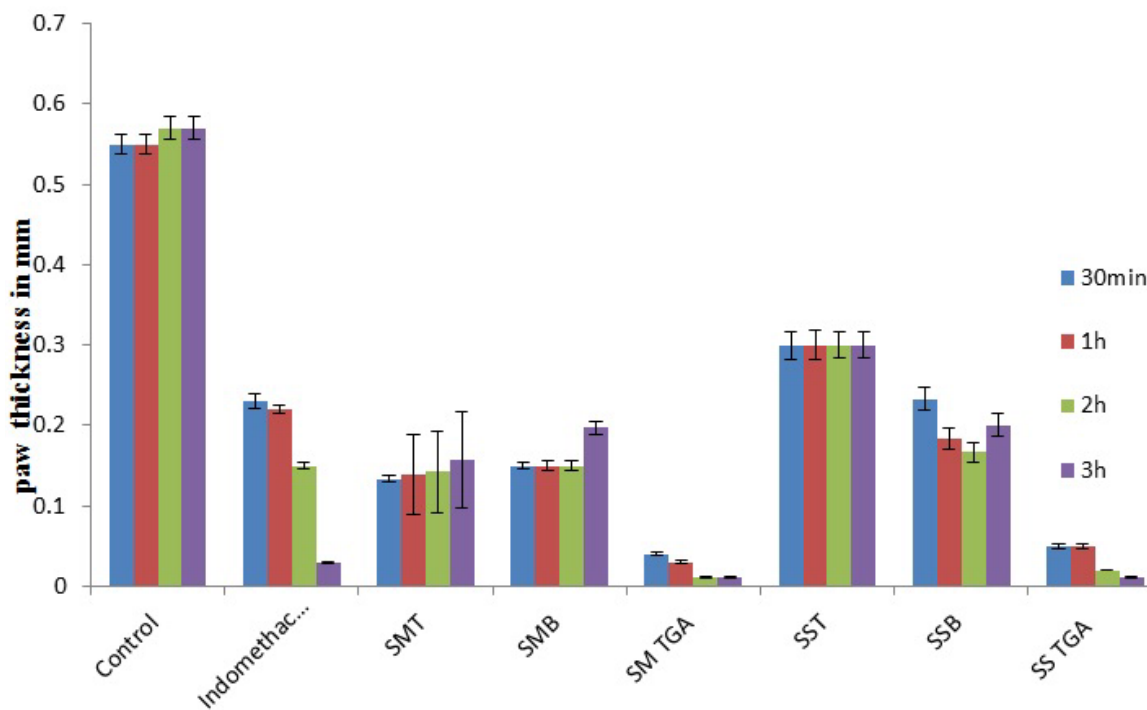


Figure 2. Histogram representing the anti-inflammatory activity *S. Seaforthianum* Andr. and *S. macrocarpon* L. following oral administration in rats. (Mean± S.E., n=5). SMT: Total alcohol extract of *S. macrocarpon* L. SMB: *n*-butanol fraction of *S. macrocarpon* L. SM TGA: Total glyco-alkaloid fraction of *S. macrocarpon* L. SST: Total alcohol extract of *S. seaforthianum* Andr. SSB: *n*-butanol fraction of *S. seaforthianum* Andr. SS TGA: Total glyco-alkaloid fraction of *S. seaforthianum* Andr.

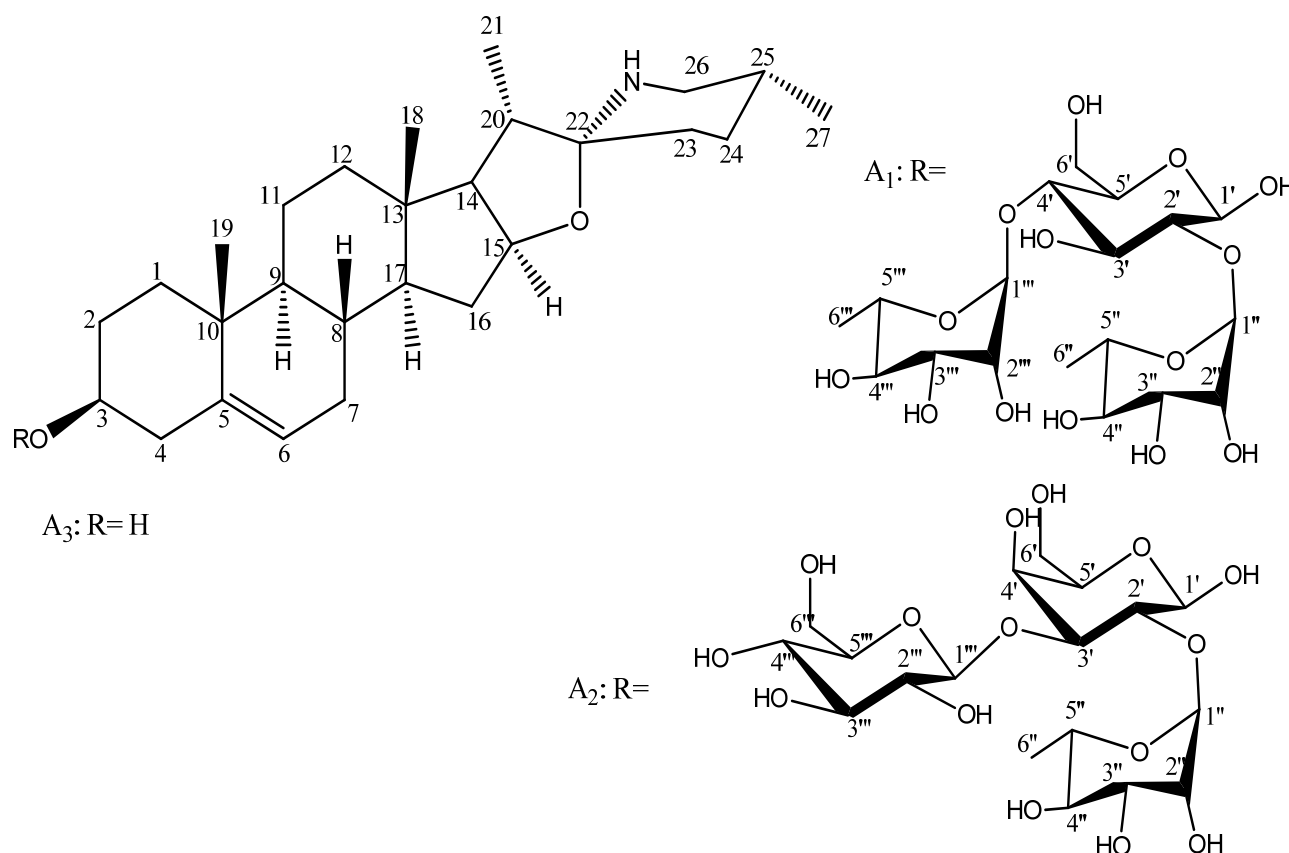


Figure 3. Structures of the isolated compound A₁-A₃

solamargine, solasonine and solasodine from *S. seaforthianum* Andr. growing in Egypt.

Conflicts of interest

The authors have not declare any conflict of interest.

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