

## Isolation, Purification and Elucidation of Stigmasterol and $\beta$ -Sitosterol Glucosides from the Stems of *Solenostemma argel* Grown in Sudan and their Application as Insecticides

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### ABSTRACT

Two new compounds were isolated from the chloroform extract fraction of stems of *Solenostemma argel*, purified by column chromatography and elucidated by phytochemical and spectroscopic methods as Stigmasterol and  $\beta$ -sitosterol glucoside. Chloroform had the highest insecticide activity against the growth of the third larval instar of *Tribolium castaneum* used as a test insect, when it was compared with *n*-hexane, ethyl acetate, *n*-butanol and water extracts.

**Keywords:** *Solenostemma argel* stems; *Tribolium castaneum* larvae; Stigmasterol;  $\beta$ -Sitosterol glucosides.

### INTRODUCTION

The plant *Solenostemma argel* (*S. argel*) belongs to the Asclepiadaceae family; it is used in alternative medicine as anti-rheumatic, anti-inflammatory and antispasmodic agent (Innocenti et al., 2010; & Shayoub et al., 2013). It is used as anti-syphilitic when used for prolonged period; in addition, it is used in treatment of diabetes mellitus, hypercholesterolemia, urinary tract infection, cold, cough, jaundice, measles, gastrointestinal cramp and menstrual disturbance (Elkamali &

Elkalifa, 2001). The plant has anti-insecticidal effect (Awad et al., 2012). on mosquito species that causes malaria (Feiha et al., 2009). In addition, it has antimicrobial, antibacterial and antifungal activity (Shafek & Michael, 2013).

According to several studies at stems, leaves and flowers of *S. argel* detected the existence of crystalline compounds, as rutin, flavanones, quercetin and alkaloids flavonoids, kaempferol (Shafek & Michael, 2013; & Tigani & Ahmed, 2009) previous

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studies had include the existence of acylated phenolic glycosides and four new pregnane glycosides from the pericarps monoterpenes, and pregnane glycosides in the leaves (Plaza et al., 2005). A recent study reveals presence of eight compounds in the stems of *S. argel* (Rym et al., 2019). Phytochemical screening showed the presence of flavonoids, tannins, sterols, triterpens and mainly saponins in the leaves, stems and roots at the pre-flowering and flowering stages Researchers in many countries found that the aqueous extract of *S. argel* is effective in the control of the larvae of mosquitoes *Culex spp* and *Anopheles spp* (Hag-EITayeb et al., 2009).

## MATERIALS AND METHODS

### General

Nuclear Magnetic Resonance NMR experiments were collected using Ultra Shield Plus 500 MHz (Bruker) Spectrometer operating at 500 MHz was used for the protons ( $^1\text{H}$ NMR), and 125 MHz for the carbon atoms ( $^{13}\text{C}$  NMR).

Infrared (IR) spectra was specified with a Perkin – Elmer FTIR Spectrometer Model 1600, American Spray Probe, Ultraviolet absorption was specified with Unicam Heyios UV Visible Spectrophotometer. Melting points specified with a thermo system FP800 Mettler FP80. EIMS were obtained using Shimadzu- Liquid Chromatography- Mass Spectrometry (LC/MS). Silica gel 60/230-400 mesh (EM Science) was used for column chromatography. Thin- layer chromatography (TLC) was done using silica gel 60 F254 (Merck).

### Plant material

*Solenostemma argel* stems were taken from Albakheet-Northern State of Sudan during 2017, stems were dried, cleaned and grinded. The powder obtained was stored in room conditions.

### Preliminary Phytochemical screening

The presence or absence of chemical constituents; saponins, tannins, cumarines, alkaloids, triterpenes, steroids, flavonoids and anthraquinones of chloroform extract of *S. argel* was detected by using phytochemical screening, Evans standard method (Evans, 2009).

### Extraction, fractionation and isolation

2.5 Kg of dried stems of *Solenostemma argel* were extracted and fractionated, bioactivity of different fractions against third larval instar of *Tribolium castaneum* revealed that the chloroform extract fraction (8.9 gram) was the most active. The active chloroform fraction was subjected to column chromatography over gel silica (3.50 cm i.d., 350.75 gm) Elution started with 100% chloroform followed by chloroform/ethyl acetate mixtures with increasing the amounts of ethyl acetate. TLC monitored fractions and similar fractions were pooled to produce 33 fractions. Fraction 9 eluted with 10% ethyl acetate in chloroform showed two spots subjected to PTLC, 10% methanol in ethyl acetate were used as mobile phase, to afford Stigmasterol glucoside (0.402 mg) and  $\beta$ -sitosterol glucoside (0.289 mg).

### Anti-insecticidal activity

Different fractions of plant were evaluated for the anti- insecticidal activity against the third larval instar of *Tribolium castaneum*, as the test insect. Each fraction was separately mixed with Sorghum grains in ratio of 10:100 grams (wt / wt) in petri-dish. The petri-dishes were shaken manually to produce homogenous mixture. Ten larvae were placed in each petri-dish; the experiment was subjected to the complete randomized design (C R D), and mortality of larvae was recorded after 24 hours (Table. 1). Each experiment was measured in triplicates. The mortality percentage of larvae was calculated by the method of (Hag-EITayeb et al., 2009).

**Tables 1: Results of mortality of third larval instars of *Tribolium castaneum*, in 24 hrs**

Fractions	Parameter				Mortality%
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	∑	
n- hexane	2	1	2	5	16.7%
CHCl <sub>3</sub>	3	4	3	10	33.3%
Ethyl acetate	3	4	2	9	30%
n- butanol	1	0	0	1	3.3%
Aqueous extract	0	1	1	2	6.7%
Control	0	1	1	2	6.7%

**Table 2: phytochemical screening of crude extract and chloroform extract**

Components	Parameter	
	Crude extract	CHCl <sub>3</sub>
Saponins	+++	+++
Cumarines	+	+
Alkaloids	++	++
Tannins	++	++
Flavonoids	++	+++
Steroides	++	+++
Triterpenes	+++	+++
Anthraquinones	-	-

(+++) means high concentration, (++) medium conc., (+) low conc. and (-) not detectable

## RESULTS AND DISCUSSION

Each extract of the various fraction of the stems of *Solenostemma argel* was tested against the 3<sup>rd</sup> larval instars of the *Tribolium castaneum* to determine its mortality rate during 24 hours. It was found that the different fractions exhibited relatively high levels of anti-insecticidal activity against the test insect compared with that of the control group. The chloroform extract was the most active and was subjected to preliminary phytochemical study and chromatographic purification. The results of the preliminary phytochemical study are presented in Table. 2. In the chloroform extract fraction of the stems of *S. argel*, coumarins were present in low concentration, alkaloids and tannins in medium concentration, saponins, flavonoids, steroids and triterpenes in high concentration, while anthraquinones were not detected.

### Compound 1

The scientific data recorded for Stigmasterol glucoside were C<sub>35</sub>H<sub>58</sub>O<sub>6</sub> molecular formula, a pale white powder, 270–280 C<sup>0</sup> melting point (m.p.), EIMS *m/z* 574.8 [(M+1) - Glucose], and giving positive result with Liebermann-Burchard test indicating the presence of steroidal.

IR Spectroscopic analysis showed absorption bands at 3437cm<sup>-1</sup>, 2927 cm<sup>-1</sup>, 1564 cm<sup>-1</sup> and 1071 cm<sup>-1</sup>; that is characteristic of O-H stretching, C-H structure, C=C absorption and C-O absorption peak, respectively, that resembling the absorption frequencies observed for Stigmasterol glucoside (Parugrug & Roxass, 2008).

The NMR spectrum of the compound 1 shows the existence of six methyl signals at δ<sub>H</sub> 0.91 ppm at C19 and C21 and δ<sub>H</sub> 0.96 ppm, δ<sub>H</sub> 0.82 ppm, δ<sub>H</sub> 0.79 ppm, δ<sub>H</sub> 0.94 ppm at C18, C26, C27 and C29, respectively. The proton signal at δ<sub>H</sub> 3.42 ppm as double triplet correlated to the carbon signal at δ<sub>C</sub> 70.53 ppm was assigned to C-3. The olefinic signals at δ<sub>H</sub> 5.18, δ<sub>C</sub> 129.30 and δ<sub>H</sub> 5.04, δ<sub>C</sub> 121.58 were assigned to C-22 and C-23, respectively. The proton signal at δ<sub>H</sub> 5.32 (H-6), corresponding to the carbon signal at δ<sub>C</sub> 121.3 ppm along with the quaternary carbon signal at δ<sub>C</sub> 140.9 ppm were assigned to the double bond between C-5 and C-6. The doublet at δ<sub>H</sub> 4.23 (H, 1') corresponding to the carbon signal at δ<sub>C</sub> 98.28 ppm was assigned to C-1' of glucopyranosyl moiety. The NMR data measured for compound (Table 3) were closely similar to those reported for

stigmasterol glucoside (Feiha et al., 2009). Figure. 1 shows the elucidated structure of compound 1(stigmasterol glucoside).

### Compound 2

Steroid base indicates by Liebermann-Burchard test, the scientific data recorded for  $\beta$ -sitosterol glucoside were  $C_{35}H_{60}O_6$  molecular formula, white crystalline solid, 144 - 146  $C^0$  (m.p.). EIMS  $m/z$  576.8 / 613(M + 2H<sub>2</sub>O + 1)<sup>+</sup>.

IR spectroscopic analysis showed absorption bands at 3422  $cm^{-1}$ , 2926, 1568  $cm^{-1}$  and 1050  $cm^{-1}$ , that clearly indicating the O-H stretching, aliphatic C-H str.vib. aromatic C=C (str. vib) and CO phenolic str. Vib., respectively. That resembling the absorption frequencies observed for  $\beta$ -Sitosterol Glucoside (Parugrug & Roxass, 2008).

The <sup>1</sup>H NMR spectrum of the compound shows the presence of six methyl

groups at  $\delta_H$  0.52, 0.96, 0.91, 0.82, 0.78 and 0.67 ppm, at C18, C19, C21, C26, C27 and C29, respectively.

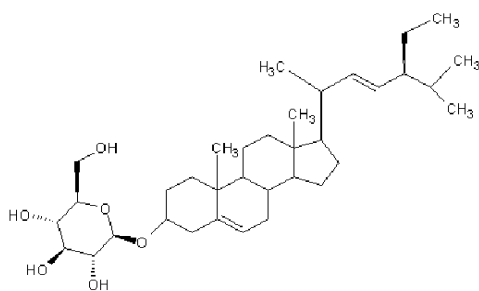
The proton signal at  $\delta_H$  3.64 ppm as double triplet correlated to the carbon signal at  $\delta_C$  77.42 ppm is assigned to C-3. A signal at 137.99 was assigned for C5 and a signal at 127.75 ppm was assigned for C6; which created C5=C6 double bond, 19.32 ppm for C-22 and 36.70 ppm for C-23,  $\delta_C$  77.42 ppm for C3  $\beta$ -hydroxyl group 24.35 and 12.59 for angular methyl carbon atoms for C19 and C18 respectively.

The NMR data measured for compound 2 were closely similar to those reported for  $\beta$ -sitosterol glucoside (Feiha et al., 2009). Figure. 1 shows the elucidated structure of compound 2( $\beta$ -sitosterol glucoside).

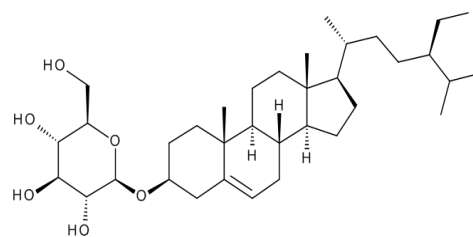
**Table 3: <sup>1</sup>H, <sup>13</sup>C NMR spectral data ( $\delta$  ppm) of compounds 1 and 2**

Pos	1		2	
	<sup>1</sup> H <sup>a</sup>	<sup>13</sup> C	<sup>1</sup> H <sup>a</sup>	<sup>13</sup> C
1	.76 (m, 2H)	38.79	1.39 (m,2H)	37.28
2	1.50 (m, 2H)	33.82	1.39 (m, 2H)	29.44
3	3.42 (dd, j = 8.4)	70.53	3.64 (m, 1H)	77.42
4	1.80 (br d, j = 10.2)	36.76	1.39 (m, 2H)	42.23
5	-	140.92	-	137.99
6	5.32 (br d, j = 4.5)	121.65	5.33 (d, j = 7.8, 1H)	127.75
7	1.46 (m, 2H)	29.73	1.39 (m,2H)	31.85
8	1.55 (s, 1H)	31.88	1.39 (m,1H)	31.90
9	0.99 (s, 1H)	50.10	1.39 (m,1H)	51.07
10	-	36.94	-	37.06
11	1.39 (m,2H)	36.69	1.39-2.36(m,2H)	22.19
12	1.93 (m, 1H)	22.58	1.39-2.36(m,2H)	38.89
13	-	42.21	-	42.99
14	1.04 (m,1H)	40.47	1.39 (m,1H)	55.88
15	1.79 (m, 2H)	56.74	1.39 (m, 2H)	34.79
16	1.81 (m, 2H)	12.31	1.39 (m,2H)	44.29
17	1.14 (m, 1H)	55.81	1.39 (m, 1H)	55.68
18	0.96 (s, 3H)	12.06	0.52 (s, 3H)	12.59
19	0.91(s, 3H)	19.57	0.96 (s, 3H)	24.35
20	2.02 (m, 1H)	24.36	1.39 (m, 1H)	54.95
21	0.91 (d, j = 7.2, 3H)	31.20	0.91 (d, j = 5.6, 3H)	12.59
22	5.18 (dd, j = 8.4 & 15)	129.30	0.82 (m, 2H)	19.32
23	5.04 (dd, j = 8.4 & 8.1)	121.58	0.82 (m, 2H)	36.70
24	1.84 (s, 2H)	31.85	1.51 (m,1H)	49.15
25	1.63 (m, 1H)	37.31	1.94 (m,1H)	25.44
26	0.82 (d, j = 6.3)	21.42	0.82 (d, j = 7.2, 3H)	21.38
27	0.79 (d, j = 6.9)	28.99	0.78 (d, j = 8.0, 3H)	21.15
28	1.44 (s, 2H)	25.36	0.82 (m, 2H)	29.85
29	0.94 (d, j = 8.1)	20.18	0.67 (d, j = 8.0, 3H)	21.41
1,	4.23 (d, j = 7.8)	98.28	5.03 (m, 1H)	101.30
2,	3.37 (m, 1H)	70.53	3.06-3.64 (m,1H)	73.90
3,	3.14 (m, 1H)	77.23	3.06-3.64 (m,1H)	77.42
4,	3.06 (m, 1H)	73.99	3.06-3.64 (m,1H)	70.57
5,	3.04 (m, 1H)	73.08	3.06-3.64 (m,1H)	77.16
6'	3.66 (m, 1H)	61.53	3.06-3.64 (m,2H)	61.56
2'OH	4.77 (s, 1H)			
3'OH	4.75 (s, 1H)			
4'OH	4.83 (s, 1H)			
6'OH	3.89 (t, j = 6.0)			

<sup>a</sup> J in parentheses in Hz



(Stigmasterol glucoside)

( $\beta$ -Sitosterol -3-O- Glucoside)**Figure (1): structure of components****CONCLUSION**

Chloroform extract fraction of the stems of *Solenostemma argel* included two compounds that were identified spectroscopically as stigmasterol and  $\beta$ -Sitosterol glucoside. Chloroform extract fraction had the highest inhibitory activity of the test larva of *Tribolium castaneum* compared with those solvents of extract fractions.

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