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Research Article

Isolation of *β*-amyrin from the Stems of *Solenostemma argel*, Elucidation of its Structure and Determination of Antimicrobial Activity

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ABSTRACT

New triterpenoid alcohol was isolated from the active fraction of the extract of stems of Solenostemma argel and elucidated by physical (m. p.) and spectroscopic methods (UV, IR, ^{1}H and $^{13}CNMR$ and EIMS), as β-amyrin. The cup-plate agar diffusion method was used to estimate the inhibition of the activity of four types of bacteria: two Gram-positive Bacillus subtilis (B.s) and Staphylococcus aureus (S.a), and two Gram-negative Escherichia coli (E.c) and Pseudomonas aeruginosa (p.s), as well as two types of fungal Aspergillus niger (A. n) and Candida albicans (C. a). Among the other solvents used for the extractions (ethyl acetate, n-hexane, n-butanol and water), chloroform extract demonstrated the highest antimicrobial activity.

Keywords: *Microbial growth, inhibition zone, β-amyrin, Solenostemma argel.*

INTRODUCTION

Solenostemma argel that belongs to the Asclepiadaceae family, known for used as anti-seizure (Idris et al., 2011 & Dall et al., 2011), anti-rheumatic and anti-inflammatory agent (Shayoub et al., 2013). It is used in the treatment of some diseases such as diabetes mellitus, jaundice, measles and cold cough (El-Kamali et al., 1997) and in addition has insecticidal effect (Awad et al., 2012).

β-amyrin is one of the most important compounds of triterpens, it is beneficially for inhibiting collagen-induced platelet aggregation (Buon et al., 2018). In addition, *β*-amyrin has anti-exciting action (Kweifio-Okai et al., 1995 & Ching et al., 2010). Solenostemma argel comprise important natural products as saponins, triterpens, steroids, tannins, flavonoids, alkaloids, monoterpine, and steroids. In addition, argelosides, stemmosides were isolated from the leaves (Plaza, 2005).

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Extracts of S. argel were showed anesthetics effect (El-Tahir et al., 2005), antimicrobial properties as well as antibacterial, antifungal (Hegazi et al., 1994), anti-inflammatory activity (Innocenti et al., 2005), anti-cancer and antioxidant activity (Shafek & Michael 2012 & Hanafi & Mansour, 2011).

MATERIALS AND METHODS

General

Nuclear Magnetic Resonance spectra (^{1}HNMR, 500 MHz; ^{13}C NMR, 125 MHz) were recorded on a Bruker Spectrometer 500 MHz's. Infrared (IR) spectra was specified with a Perkin – Elmer FTIR Spectrometer Model 1600, American Spray Probe, Ultra violet absorption was specified with Unicum 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).

Column chromatography (CC) and Thin- layer chromatography (TLC) were used Silica gel 60/230-400 mm. and 60 F254 (Merck), respectively.

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.

Extraction, fractionation and isolation

2.5 Kg of dried stems of *Solenostemma argel* were extracted and fractionated, bioactivity of the different fractions against the microbial activity revealed that the chloroform extract (8.9 gram) was the most active. The active chloroform fraction was examined by column chromatography using 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 6 eluted with ethyl acetate: methanol, 75:25, (1.087mg) was further purified by silica gel thin-layer chromatography.

Antimicrobial activity

Testing for antibacterial Activity

The antibacterial activity of extracts of the stems of *Solenostemma argel* was estimated the inhibition of the activity of four types of pathogenic bacteria: *(E.c), (p.s), (B.s)* and *(S.a),* by using cup-plate agar diffusion method (Kavanagh, 1972). 100ml of molten sterile nutrient agar were added to the one ml of stock suspension of standardized bacterial (108 –109 C.F.U/ ml).

After incubated of Inoculated nutrient agar at 45 ºC; 20 ml were diffused into sterile petri-dishes, which were cut with a sterile cork in the middle, and by using automatic micro liter pipette drills were filled with 0.1 ml of samples, which were diluted in methanol.

after were diffused about two hours; petri-dishes were incubated at 37 ºC for 18 hours, each extract against each of the test organisms were replicated two times. The growth of inhibition zones were measured, averaged and tabulated (Table (1). Figure (1), (2) and (3) show the active samples.

Testing for antifungal activity

The antifungal activity of the extract of the stems of *Solenostemma argel* was estimated by using the cup-plate agar diffusion method (Kavanagh, 1972). The inoculated medium of Sabouraud dextrose agar was incubated at 25 ºC, for *A. n.* (Three days) and *C. a.*(two days). Preliminary screening for antifungal activity of various extracts of *Solenostemma argel* stems were tabulated. (Table (1).

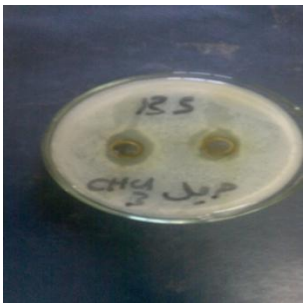

Figure (1): Inhibition zone of bacteria *B s* with $CHCl_3$ extract

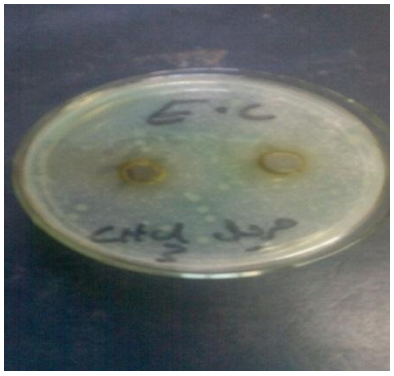

Figure (2): Inhibition zone of bacteria *E c* with CHCl3 extract

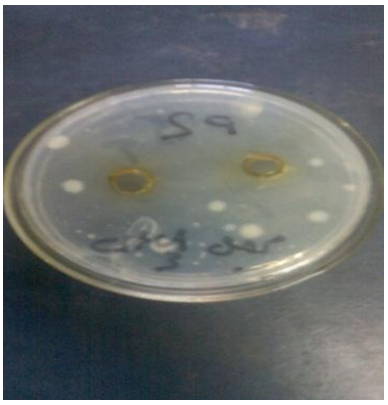

Figure (3): Inhibition zone of bacteria *S a* with $CHCl_3$ extract

Table 1: Preliminary screening for antimicrobial activity of various extracts of *Solenostemma argel* stems

Sample	Size of Inhibition zone (in mm)					
	Bacteria				Fungi	
	Gram positive		Gram negative			
Plant extracts	***E.c***	***p.s***	***S.a***	***B.s***	***A.n***	***C. a***
crude extract	14	13	13	11	17	16
Hexane fra.	12	14	10	14	15	15
$CHCl_3$ fra.	19	18	20	22	20	15
Ethyl acetate fra.	18	20	18	19	18	16
n-butanol fra.	14	15	11	12	14	13
Aqueous extract	10	11	09	11	12	10

(10-14) low activity, (14-18) medium activity and (over 18) high activity

RESULTS AND DISCUSSION

Crude extracts and their fractions against microbial activity are shown in Table (1) obtained results signify a low effectiveness of crude extract, n- hexane and aqueous extract against the four bacteria. Ethyl acetate and chloroform extract showed high effectiveness against *B.s* & *S.a*. Here again n- hexane and the aqueous extract showed low activity against the standard fungi. An increase in the zone of suppress was noted with chloroform extract.

β-amyrin compound

$C_{30}H_{58}O$, white powder. m.p. 197–198 °C. EIMS m/z 426.7, $[M+1]^{+\cdot}$

^{1}H- and ^{13}C NMR (DMSO): Table 2.

Triterpene compound was isolated from *Solenostemma argel* stems subjected to the spectral data and resulting of testing of Lieberman - Borchard reagent that were clarified the compound. The UV spectrum of compound recorded in methanol shows an absorption peak at 231 nm. The IR spectrum of compound shows broadband centered at

3510cm^{1}, 2904 cm^{-1} and 1639 cm^{-1}; that is evidence of the existence of O– H, CH3 and C = C groups, respectively.

The proton NMR spectrum shows the presence of methyl signals at δH 0.89 at C23, C24, C26 and C27, δH 0.84 at C25, δH 0.70 at C28 and δH 0.87 at C29 and C30.

The proton signal at δH 3.34 ppm as double triplet correlated to the carbon signal at δC 32.08 ppm is assigned to C-3. The proton signal at δH 5.12 corresponding to the carbon signal at δC 109.35 ppm along with the quaternary carbon signal at δC 150.90 ppm are assigned to the C = C functional group between C-12 and C-13. According Vesterberg (Vesterberg et al., 2010), the compound was elucidated as 3b-hydroxylolean-12-ene its beta amyrin (Figure 2).

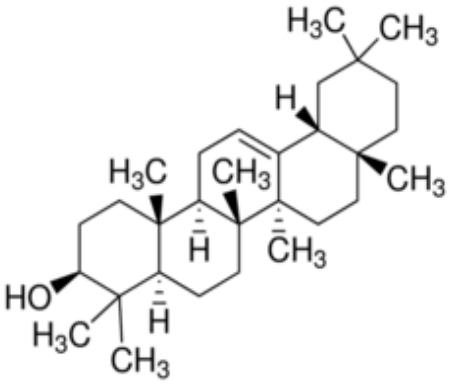

Figure (2): beta amyrin

Table 2: NMR spectral data of isolated beta amyrin in DMSO

	Parameter	
Position	**δ H (ppm)**	**δ C (ppm)**
C-1	**1.56; 1.31**	**25.14**
C-2	**1.55; 1,47**	**25.76**
C-3	**3.34 (dd, j = 4.4; 10.8)**	**79.30**
C-4	**-**	**38.71**
C-5	**0.94 (d, j = 11.0)**	**34.28**
C-6	**1.63; 1.38**	**27.04**
C-7	**1.56; 1.31**	**27.45**
C-8	**-**	**38.63**
C-9	**0.94**	**35.59**
C-10	**-**	**40.01**
C-11	**1.63; 1.38**	**27.99**
C-12	**5.12 (t, j = 3.2**	**109.35**
C-13	**-**	**150.9**
C-14	**-**	**42.83**
C-15	**1.56 (td, j = 4.0)**	**29.38**
C-16	**1.56 (td, j = 4.3)**	**29.45**
C-17	**1.04**	**43.01**
C-18	**1.04**	**38.05**
C-19	**1.93 (dd, j = 4.0)**	**29.63**
C-20	**-**	**79.02**
C-21	**1.56; 1.31**	**29.72**
C-22	**1.56; 1.31 m**	**29.85**
C-23	**0.89 (s)**	**25.76**
C-24	**0.89 (s)**	**25.14**
C-25	**0.84 (s)**	**25.40**
C-26	**0.89 (s)**	**18.01**
C-27	**0.89 (s)**	**18.33**
C-28	**0.70 (s)**	**19.32**
C-29	**0.87 (s)**	**20.94**
C-30	**0.87 (s)**	**22.72**
1'	**4.77 (b s)**	**171.40**
2'	**4.49 (b s)**	**21.49**

CONCLUSION

The present study showed that chloroform extract was very active against microbial growth and gave a new picture about the presence of some major and trace elements in the plant.

New triterpenoid beta amyrin had been isolated, which was identified by spectroscopic methods and comparison of their determined physicals properties with those cited in the literature.

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REFERENCES

Awad, K. T., Khalid, O. A., Tagelsir, I. M., & Sidahmed, O. (2012). Argel (solenostemma argel Del Hayne) applications for control of the date palm green scale insect (Astrolicanium phoenicis rao) and yield enhancement. ARPN *J. Agric. and Biol. Sci.*, 6-7.

Buon, (2018). Antitumor effects of beta amyrin in Hep-G2 liver carcinoma cells are mediated via apoptosis introduction, cell cycle distruption and activation of JNK and P38 signalling pathways, *Jul-Aug*, *23*(4), 965-970.

Ching, J., Chua, T., Chin, L., Lau, A., Pang, Y., Jaya, J., Tan, C., & Koh, H. (2010). b-Amyrin from Ardisia elliptica Thunb. Is more potent than aspirin in inhibiting collagen-induced platelet aggregation, *Indian J. Exp. Biol*. *48*, 275–279.

Dall, I. G., Minesso, A. S., Micucci, B. P., & Chiarini, M. A. (2010). Evaluation of muscarinic M3-receptor autagonism of *solenostemma argel leaves plant med*., *76*, 634.

El-Kamali, H. H., & Elkalifa, H. H. (1997). Treatment of malaria through herbal drugs in the central Sudan. *Fitoterapia*, *6*, 527-528.

El-Tahir, M. M., El-Tayeb, I. B., & Shaddad, S. A. I. (2005). The pharmacological actions of the aqueous extract of the leaves of *Solenostemma argel* (Hayne) on isolated rabbit aortic strip and guinea pig atria. *J. Anim. Vet. Advances*, *4*(10), 831-834.

Hanafi, N., & Mansour, S. Z. (2011). Antitumor efficacy of aqueous extract of Salenostemma argel leaves and/or gamma-radiation exposure against Ehrlich carcinoma in Swiss albino mice, *Inter. J. Bio Sci. Techn*., *4*(1), 1-11.

Hegazi, A. A., El-Enbaawy, M., Abd El-Hady, F. K., & Ata, N. S. (1994). Studies for determining antimicrobial activity of Solenostemma argel (Del.) Hayne. 3-Extraction with petroleum ether and ether. *Journal of the Egyptian Veterinary Medical Association*, *54*(5), 401-411.

Idris, T. I. M., Ibrahim, A. M., Mahdi, E. M., & Taha, A. K. (2011). Influence of Argel soil applications on flowering and yield of date pam, *Agric. & Biol. J. North America*, *2*(3), 538-542.

Innocenti, G., Dall'Acqua, S., Sosa, S., Altinier, G., & Della Loggia, R. J. (2005). Topical anti-inflammatory activity of Solenostemma argel leaves. *Ethnopharmacollogy*, *102*, 307-310.

Kavanagh, F. (1972). *Analytical Microbiology, F. Kavanagh (Ed.) 11*, Academic Press, New York and London, pp 11.

Kweifio-Okai, G., De Munk, F., Macrides, T. A., Smith, P., & Rumble, B. A. (1995). Antiarthritic mechanisms of lupeol triterpenes. *Drug Dev. Res*., *36*, 20–24.

Plaza, A., Perrone, A., Balestrieri, M. L., Felice, F., Balestrieri, C., Hamed, A. I., Pizza, C., & Piacente, S. (2005). New unusual pregnane glycoside with antiproliferative activity from Solenostemma argel. *Steroids*, *70*(9), 594–603.

Shayoub, M., Haj, E, A., Makawy, A., Rasha, R., & Mona, A. (2013). Adverse reaction of *Solenostemma argel* leaves, extraction and alkaloids tablets administered to patients. *Global J. Trad. Med. Sys*., 14-18.

Shafek, R. E., & Michael, H. N. (2012). Antibacterial and antioxidant activity of two new kaempferol glycosides isolated from solenostemma argel stem extract, *Asian J. plant Sci*., *11*(3), 143-148.

Vesterberg, Viji V., Shobha, B., Kavitha, S. K., Ratheesh, M., Kripa, K., & Helen, A. (2010). Betulinic acid isolated from *Bacopa monniera* (L.) wettst suppresses lipopolysaccharide stimulated interleukin-6 production through modulation of nuclear factor-jB in peripheral blood mononuclear cells, *Int. Immunopharmacol*. *10*, *Bull Soc. Chem*., *37*, 742.