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SHORT COMMUNICATION



# Pregnane glycoside from *Huernia saudi-arabica* as latent schistosomicidal mediator

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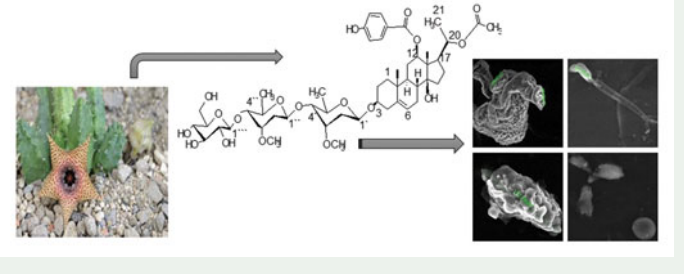
**ABSTRACT**

Development of a novel agent for control of schistosomiasis is a mandate. *In-vitro* anti-schistosomal activity of the aerial parts of *Huernia saudi-arabica* were examined. Chromatographic investigations of the ethanol extract (EE) were afforded three compounds. Pregnane glycoside (CI) 12-$\beta$-*p*-hydroxy-benzoyl-20-*O*-acetyl-boucerin-3-*O*-$\beta$-D-glucopyranosyl-(1→4)-$\beta$-D-cymaropyranosyl-(1→4)-$\beta$-D-cymaropyranoside, in addition to two flavonoids (CII) luteolin-4′-*O*-$\beta$-D-neohesperidoside and (CIII)quercetin-3-rutinoside were recognized *via* spectral analysis. The schistosomicidal effects were evaluated using scanning electron microscope (SEM). *In-vitro* bioassays on the viability (mobility, morphological changes and mortality) of *Schistosoma mansoni* adults, cercariae, miracidia and eggs at different concentrations 2.5, 5, 12.5, 25 and 50 μg/ml of EE and 2.6, 5.2, 13, 26 and 52 μM of CI in incubation times 1,2,4,6,12hrs were carried out. EE and CI evidenced *in-vitro* anti-schistosomal activity with a dose and incubation time-dependent fashion. The effect of EE and CI was evident by the topography damage showed by SEM. EE proved moderate *in-vitro* cytotoxicity with $IC_{50}$ of 8.48 μg/ml.



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## 1. Introduction

Praziquantel (PZQ) is used as the chief means of schistosomiasis control as no effective vaccine is available yet (Hotez et al. 2007). Being the only available drug in use may potentially lead to the development of resistance (Doenhoff et al. 2008). Moreover, PZQ is not effective against immature (juvenile) *Schistosoma* (schistosomula) and also, does not prevent re-infection (Hagan et al. 2000). Schistosomiasis control strategies generally depend on chemotherapy; worthily searching for a drug that also cause death of eggs or miracidia to interrupt *Schistosoma* life cycle will be of great value. To explore new anti-schistosomal drugs that are effective against mammalian stages; schistosomula, juvenile and adult worms of *S. mansoni* and aquatic stages; miracidia, cercariae besides eggs are frequently used in screening through *in-vitro* studies (Keiser 2010). Schistosomicidal activity of ginger and *Nigella sativa* were examined by Mostafa et al. (2011) and Mohamed et al. (2005), respectively. Rodolpho et al. (2010) assessed the inhibitory effect of crude extract of *Harpagophytum procumbens* in removing eggs during infection by *S.mansoni*. *Huernia* (Asclepiadaceae) includes about 70 species which are distributed in the tropical part of the world, South Africa and Saudi Arabia. Several members of the genus *Huernia* have initiate medicinal uses in the treatment of rheumatism, diabetes, leprosy and as antiseptics and disinfectants. *Huernia Sp.Nov.aff.Boleana* has been used as antidiabetic (Alzahrani et al. 2015), anti-inflammatory and for wound healing (Hamam et al. 2018). Previous studies on plants including the genus *Huernia* have reported the isolation of several pregnane glycosides or their esters, some showed anti-tumor activity (Knowles et al. 2000). Consequently, in the current study we report for the first time the anti-schistosomal activity of *H. saudi-arabica*.

## 2. Results and discussion

### 2.1. Isolation and identification of the isolated compounds

Column chromatographic fractionation of the chloroform fraction of the ethanol extract of aerial parts of *Huernia saudi-arabica* allowed the isolation of three compounds (CI-CIII) (Figure S1) which were characterized through their physicochemical and spectral data (Tables S1–S2).

*Compound I (CI)* was obtained as a white amorphous powder (10 mg). The $^{1}$H and$^{13}$CNMR data (Table S1) revealed the presence of three anomeric protons $\delta_H$ 4.83, 4.66, 4.44 and carbon signals at $\delta_c$97.3, 101.2, 106.5 respectively, suggesting the presence a trisaccharide glycoside consisting of two cymarose and one glucose units. The $^{1}$HNMR spectrum showed the presence *p*-hydroxybenzoic and acetyl moieties attached to C-12 and C-20 of the aglycone, respectively (Table S1). This finding was confirmed by comparing the spectral data of CI with those reported in the literature (Al-Massarani et al. 2012). Therefore, the aglycone of CI was deduced as 12-*O*-*p*-hydroxybenzoyl-20-*O*-acetyl boucerin. Based on the mentioned data (Table S1), the structure of CI was tentatively identified as 12-$\beta$-*p*-hydroxybenzoyl-20-*O*-acetylboucerin-3-*O*-$\beta$-D-glucopyranosyl-(1-4)–$\beta$-D-cymaropyranosyl-(1-4)-$\beta$-D-cymaropyranoside, which reported here for the first time from *H. saudi-arabica*.

*Compound II (CII)* give positive Molisch's test indicating its glycosidal nature. $^{1}$HNMR spectrum showed hydroxylation pattern of a flavone skeleton. The identity of sugar moiety was determined to be $\beta$-D-glucose and $\alpha$-L-rhamnose(Table S2). These data along with literature reported by Ko et al. (2002) confirmed the structure of CII as luteolin-4-*O*-$\alpha$-L-rhamnosyl-(1-2)-$\beta$-D-glucopyranoside), this is the first report from *H.saudi-arabica*.

*Compound III (CIII):* Comparison of spectral data ($^{1}$H and $^{13}$CNMR)(Table S2) of CIII with those reported in literature indicated a quercetin skeleton with two sugar moieties(Kim et al. 2005). Based on these data, and by comparison to the published data CIII could be identified as quercetin-3-rutinoside (rutin),which is the first in *H.saudi-arabica*.

Several members of family Asclepiadaceae are reported to be rich in pregnanes and pregnane glycosides (Abdel-Sattar et al. 2002), which are drawing much attention in recent years due to their antitumor (Leo et al. 2005), antitrypanosomal activity (Abdel-Sattar et al. 2009). So, the total ethanol extract and the pregnane glycoside CI were subjected for investigation of their supposed anthelmintic (Anti-bilharizal) activity as an attempt to shed light on the schistomocidal effect of the aerial part of *H.saudi-arabica* and its steroidal pregnane phytochemical.

## 2.2. In-vitro anti-schistosomal activity

Ethanol extract (EE) of *H. saudi-arabica* and its isolated component CI; pregnane glycoside (Figure S1) showed *in-vitro* anti-schistosomal activity against all tested stages (eggs, cercariae, miracidia and adults) (Tables S3–S6) with a dose response patterns (Figure S2 A–D, Tables S3–S6). The earlier significant adulticidal response (100%) was achieved at1h at 25 μg/ml of the ethanol extract (EE) and 52 μM of pregnane glycoside (CI)(Figure S2A,Table S3). While the earlier significant cercaricidal response (100%) was achieved at a dose of 25 μg/ml, 26 μM for (EE) and (CI), respectively at 3 h (Figure S2B, Table S4). The significant prior responses (100%) of both (EE) and (CI) of *H. saudi-arabica* on miracidia were at concentrations of 25 μg/ml, 26 μM respectively and reported at1/2h (Figure S2 C, Table S5). The earliest significant inhibition of egg hatching was achieved at a dose of 50 μg/ml and 52 μM of (EE) and (CI) respectively at 1/2 h (FigureS2 D, Table S6). The minimum concentration as low as 2.5 μg/ml and 2.6μM of both (EE) and (CI) was enough to kill 100% of tested adults, cercariae and miracidia after 24, 6 and 4 hr, respectively and 25 μg/ml of (EE) and 52 μM of (CI) was enough to inhibit hatching of 100% of eggs after 1.5 hr (Table S6). Extract of *Dryopteris filixmas, Tanacetum vulgare, Juglans nigra and Syzygium aromaticum* were displayed robust antischistosomal at minimum actual concentration of 50 μg/ml after 24 hours. *Allium sativum* proved to be the most potent one by a 27.6 and 21.7% reduction in worm burden (Metwally 2015). Where, ethanol extract of *H.saudi-arabia* and the pregnane glycoside were exerted an adulticidal activity with a concentration 25 μg/ml and 50 μM after one hr exposure, respectively. While the minimum concentrations for both was 2.5 μg/ml, 2.6μM after 24 hr exposure respectively. As a result we can acclaim the ethanol extract and the pregnane glycoside as a latent schistosomicidal mediator.

### 2.3. Scanning electron microscopy (SEM)

SEM was a useful tool (Irie et al. 1989) to evaluate surface changes of the studied stages of the parasite up on exposure to *H.saudi-arabica* plant extract. The damage effect of the plant extract and its components was evident by the topography damage of studied stages using SEM (Figures S3–S7). The weak and damaged tegumental surfaces of the treated worms facilitates their attack by the host immune cells and deprive them from nutrients, furthermore tegument damage of the adult worm due to any drug or extract would allow the drug to penetrate to deeper-lying tissues and cause serious consequences (Xiao et al. 2010). The tegument is a major target for anti-schistosomal drugs (Van Hellemond et al. 2006) where most of them act by damaging the worm tegument (Keiser 2010). SEM of *S. mansoni* adult male showing morphological changes of the male worms after 24 hrs exposure to mixture of 2.5μg/ml and 2.6 μM of EE and CI, respectively. Tegumental structures were heavily disturbed, oedematous with reduced numbers of tubercles and spines, among them multiple perforations (arrows) seen clearly in Figure S3: (A, B1, B2&B3). Where, the female worms evidenced tegument shrunken, oedematous with numerous small protuberances. Multiple wide holes (arrows) are clearly evident at the region of the oral and ventral suckers which appear deformed (Figure S4, A&B). Deep grooves appear at the area of the retracted ventral sucker with loss of the tyre-like appearance (Figure S4, C&D). Erosions of the tegument are seen clearly in (E). Furthermore, *S.mansoni* cercariae after 4 hrs exposure to12.5μg/ml, 13 μM of mixture of both EE and CI, respectively presented changes in both body and tail were visible in the form of shrinkage and thickening of the tegument clearly seen in Figure S5:A&B. Moreover, *S.mansoni* miracidium (Figure S6) 2 hrs after exposure to 5 μg/ml and 5.2μM mixture of both EE and CI offered swellings, vesiculation, formation of blebs that completely masking the ciliated surface with preservation of the apical cone. While, ova exposed to 25 μg/ml and 26 μM of mixture of both EE and CI for 2 hrs exhibited elongation with shrinkage of the shell clearly seen in Figure S7A. There is complete loss of the microspecules-like chitinous minute projections of the egg shell surface that is clearly seen in Figure S7 D.

### 2.4. In-vitro screening for cytotoxic activity

The ethanol extract of aerial parts of *H.saudi-arabica* exhibited specific and significant effects on the viability of the tested human cancer cell lines. $IC_{50}$ was calculated as14.8 μg/ml, 8.48 μg/ml and 24.1 μg/ml on HEPG2, MCF7 and CACO2cell lines, respectively, which were comparable to doxorubicin (Leo et al. 2005) (Table S7–S8). Almehdar et al. (2012) proved *in vitro* cytotoxic effect of *Huernia* sp. Nov. Aff. Boleana Gilb. against MCF7(breast), HELA(cervix), HEPG2 (Liver) cancer cell line, and HFB4 (normal melanocytes) with $IC_{50}$ 30.0,30.4,12.5and 30.5 μg/ml, respectively.

## 3. Conclusion

The present *in-vitro* study revealed that adults, cercariae and miracidia stages of *S.mansoni* were efficiently paralyzed and killed as well as their eggs hatching was

inhibited using both EE and CI in a concentration-dependent fashion as the intensity of response increased with the concentration of the drug and the length of incubation time. We can recommend that the anti-bilharzias effect prevailed by the ethanol extract of *H.saudi-arabica* could be attributed to its pregnane glycoside content. Moreover, the ethanol extract exhibits potent anti-schistosomal activity than the isolate which, may explained by the synergistic effects of its constituents. The ethanol extract (EE) shows alike adulticidal effect as the (CI) within half dose. Furthermore, EE and CI exerted similar cercaricidal, miracidicidal response within the same dose and time of exposure. Significant inhibition of egg hatching was achieved comparably from both tested samples. The ethanol extract of *H.saudi-arabica* shows moderate cytotoxic effect especially on breast and liver carcinoma cell line ($IC_{50}$ 8.488 and 14.84 μg/ml) compared to doxorubicin, respectively.

## Disclosure statement

The authors declare that they have no competing interests.

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