Severinia buxifolia Leaves: Isolation, Characterization of Major Metabolites from the Bioactive Fractions and their Antiprotozoal Activity

Safaa Nour[1], Maha Salama[2,3], Engy Mahrous[2]*, Hesham El-Askary[2], Mohamed Hifnawy[2], Mustafa A. Abdel Kawy[2]

[1] Toukh Hospital , Ministry of Health, Egypt.
[2] Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.
[3] Department of Pharmacognosy, Faculty of Pharmacy, British University in Egypt, Egypt.

ABSTRACT

In an effort to explore herbal drugs as treatment for some neglected tropical diseases (NTDs), *Severinia buxifolia* (Poir) Ten, was selected and investigated for its antiprotozoal activity. Based on this objective, the ethyl acetate (EA), *n*-hexane (HE), methylene chloride (MC), *n*-butanol (BU) fractions of the leaves of *S. buxifolia* were screened for the *in vitro* antiprotozoal activity against *Plasmodium falciparum*, *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania infantum*. Hexane and MC fractions showed good antiprozoal activity (IC_{50} for HE8.56, 8.64, 3.37, 10.26 µg/mL and MC 8.20, 12.7, 32.69, 2.63 µg/mL) against *T. cruzi*, *L. infantum*,*T. brucei*,and *P. falciparum* respectively. However, the EA and BU were inactive. Accordingly, two major compounds were isolated from HE identified as and friedelin (**1**) and *β*-sitosterol (**2**) while two coumarins were isolated from MC and identified as seselin (**3**) and (+)-ulopterol (**4**). Identification of the compounds was carried out based on mass spectrometry, proton and carbon 1D NMR, as well as ^{1}H-^{13}C HSQC and HMBC spectral analysis. Among isolated compounds, only seselin showed antiprotozoal activity with IC_{50} of 38.47 and 38.5 µg/mL against *T. cruzi* and *P. falciparum*, respectively and no cytotoxicity. Furthermore, an HPLC fingerprint for each fraction was achieved with the aim of authenticating the plant chemical profile and identified seslin and ulopterol as major constituents of the plant extract.

Keywords: *Severinia buxifolia* , antimalarial, antitrypanosoma, antileishmaniasis, Chinese box-orange.

INTRODUCTION

Neglected tropical diseases (NTD) tend to prevail in developing regions where water quality, sanitation and access to health care are substandard. The WHO estimates that about one-sixth of the world's population (1 billion) suffer from at least one NTD. Among NTDs are several infectious diseases caused by protozo including African sleeping sickness, leishmaniasis and Chagas' disease which are caused by *Trypanosoma brucei*, *Leishmania* spp. and *Trypanosomacruzi* spp, respectively [1]. These diseases lead to 50,000 or more morbidity and mortality per year and are primarily transmitted to humans by insects. Collectively these protozoal infections take a tremendous toll on global health because of the many disabilities associated with them that can persist for a lifetime [1].

Therapeutic regimens for controlling most NTDs are very limited and usually are not cost-effective[1]. Two drugs are used for the treatment of Chagas' disease: nifurtimox and benznidazole which are active only in the acute phase of the disease and dictate long treatment.

***Corresponding author: Engy Mahrous**
engy.abdelhamid@pharma.cu.edu.eg
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Different drugs can be used in the treatment of African sleeping sickness based on the stage of the disease which includes pentamidine and suramin in the first stages and combination therapy of nifurtimox and eflornithine in later stages. Currently, leishmanesis is treated with liposomal Amphotericin-B which is known to possess serious and sometimes lethal side effects. Other treatment options of leishmanesis include pentavalent antimonials and miltefosine.

Resistance to the aforementioned drug strategies represents an urgent need to develop new generations of drugs, which should not only be more effective and safe, but also affordable and available particularly for poor communities. Traditional herbal medicines have long been used in disease control, especially in areas where NTDs are most prevalent and access to modern health facilities is limited. In this context, higher plants are thought to be a potential source of new drugsfor the control of Chagas' disease, leishmaniasis, malaria, and sleeping sickness [1,2]

Family Rutaceae (citrus family) includes many genera that are cultivated mainly for ornamentation purposes such as *Atalantia*, *Clausena*, *Murraya*, and *Swinglea* and their species. Genus *Severinia (Atlantia)* has approximately 20 species with common names such as box orange, boxthorn and Chinese box orange. *Severinia buxifolia* (Poir) Ten. (*Atalantia buxifolia*), known as boxthorn, is an evergreen short shrub native to Indochina and is cultivated as an ornamental plant [3]. It is reputed in China for its efficacy in the treatment of snake-bite, paralysis and chronic rheumatism [4]. Acridone alkaloids, coumarins, sesquiterpenoids, triterpenoids have been reported in boxthorn with different biological activities [5,6].

We have previously studied the botanical features of the plant[7] in addition to the volatile constituents of the leaf [8]. In the current study, *S. buxifolia* (Poir.) Ten. leaves ethanolic extract(95%) and its bioactive fractions were phytochemically investigated in the search for new anti-protozoal compounds.The *in vitro* activity of the isolated compounds against *Plasmodium falciparum*, *T.brucei*, *T. cruzi* and *L. infantum* was screened and their possible cytotoxicity was evaluated against human embryonic lung fibroblasts (MRC-5). Moreover, HPLC profiling for the studied fractions was performed as a chemical fingerprint of the plant.

EXPERIMENTAL

Plant Material

Leaves of *S. buxifolia* (Poir.) Ten. used in this study were collected in June 2014 from the Orman Garden, Giza, Egypt. The plant was authenticated by Dr. Mohamed El-Gebaly, senior botany specialist at the Orman Garden, Giza, Egypt. Voucher specimens (26-05-2015) were kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.

Microorganisms:

Plasmodium falciparum, *Trypanosoma brucei*, *T. cruzi*, *Leishmania infantum* were supplied by WHO-TDR supported screening center at the Laboratory of Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical Sciences, Biomedical and Veterinary Sciences of the University of Antwerp, Belgium using the standard protocols used in WHO-TDR Drug Discovery Network.

Chemicals and Equipment

For each assay, appropriate reference drugs were used as positive control: Tamoxifen for MRC-5,(diploid human embryoinic fibroblast) Chloroquine for *P. falciparum*, Suramin for *T. b. brucei*, Benznidazole for *T. cruzi.*, Miltefosin for *L. infantum.* All reference drugs were either obtained from Sigma Chemical Co. (St Louis, MO, USA) or WHO-TDR. Silica gel H (Merck, Darmstadt, Germany) for vacuum liquid chromatography (VLC), silica gel 60 (70–230 mesh ASTM, Fluka, Steinheim, Germany) and silica gel (40-63µm, Fluka) were used for column chromatography. Thin-layer chromatography (TLC) was performed on silica gel GF254 pre-coated plates (E-Merck) using different solvent systems: S1**:** *n*-hexane - ethyl acetate (95: 5 v/v), S2: *n*-hexane - ethyl acetate (90:

10 v/v), S3:*n*-hexane - ethyl acetate (80: 20 v/v), S4**:** Chloroform-Methanol (95:5v/v). The chromatograms were visualized under UV and after spraying with *p*-anisaldehyde/ H_2SO_4 reagent.

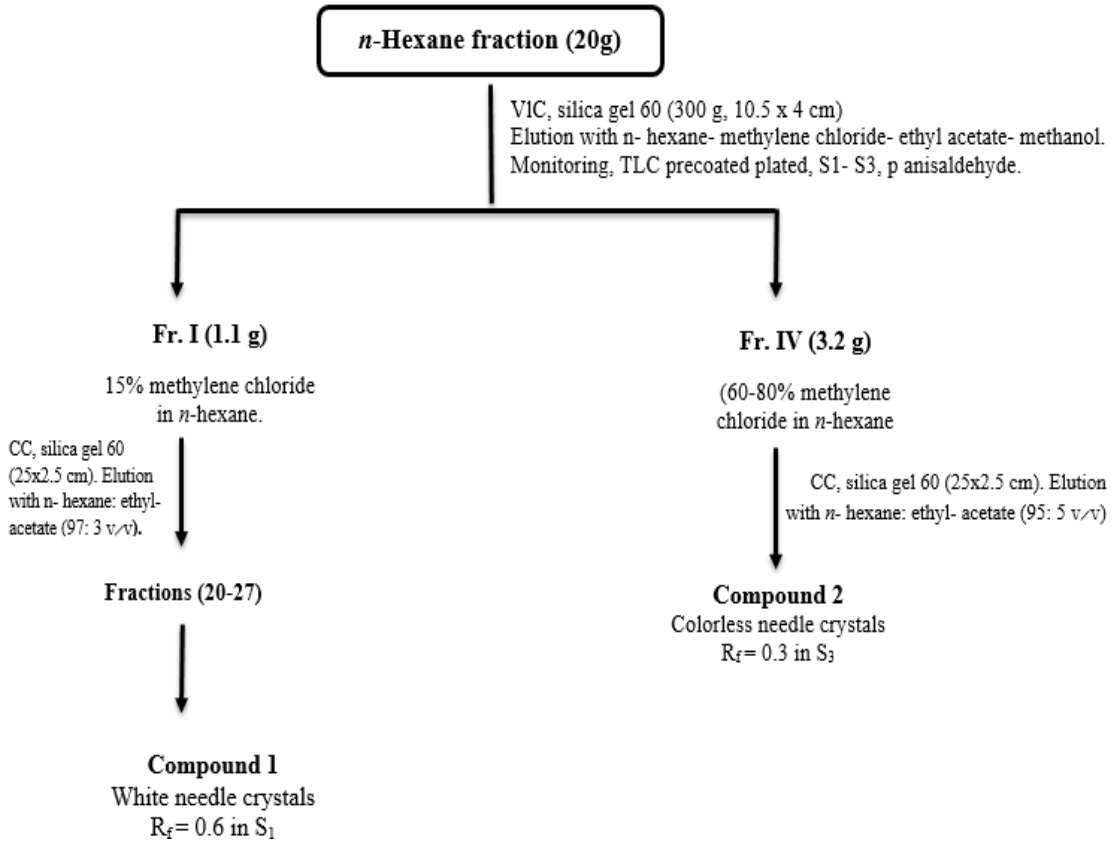
NMR experiments were performed on a Bruker Avance III NMR-spectrometer (Billerica, MA, USA). Spectral data for ^{1}H-NMR (400 MHz), ^{13}C-NMR (100 MHz) were analyzed using Topspin 3.1 Software. NMR spectra were recorded in suitable deuterated solvents (CDCl3 or DMSO) using TMS as an internal standard and chemical shift values were expressed in δ ppm. Mass spectrometer**,** Varian Mat 711, Finnegan SS Q 7000 was used for recording mass spectra. High-performance liquid chromatography (HPLC) using reversed phase C18 column was performed using Agilent Technologies 1100 series HPLC system (Agilent Technologies, Palo Alto, CA), equipped with a quaternary pump and degasser G1322A, series 1200, a G1314A variable wavelength detector and a G1328A manual injector. Agilent ChemStation software was used for data acquisition and processing.

Extraction, Fractionation and Isolation

The air-dried sieved powdered leaves of *S. buxifolia* (Poir) Ten. (2 Kg) were extracted with ethanol 95% (20 L) by cold maceration till exhaustion. The solvent was then removed by vacuum distillation at a temperature not exceeding 60 ºC to yield 300 gm of dry residue. The dry residue (150 g) was suspended in distilled water (1L) and partitioned successively with solvents of increasing polarities viz., *n*-hexane (400 ml x 8), methylene chloride (400 ml x 6), ethyl acetate (400 ml x 8), *n*-butanol (400 ml x 8). The solvents, in each step, were evaporated by distillation under reduced pressure to give fractions of *n*-hexane (57 g), methylene chloride (20 g), ethyl acetate (12 g), *n*-butanol (40g). All fractions were tested for biological activity.

Isolation of the major constituents of n-hexane fraction

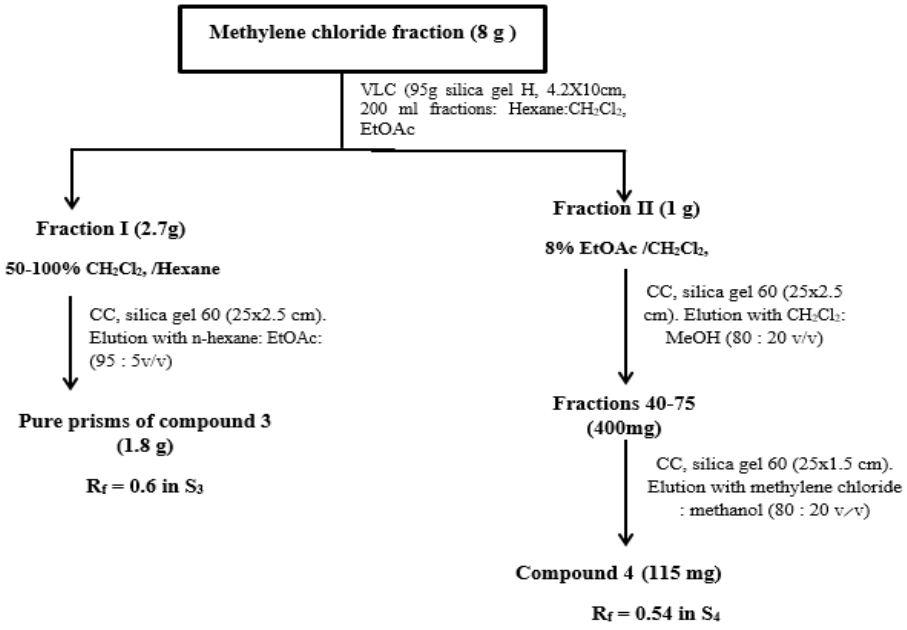
Twenty grams of the *n*-hexane fraction(HE) of the leaves were subjected to fractionation by vacuum liquid chromatography (VLC) on a 200 g silica gel 60 column (10.5 cm × 4 cm). Elution was performed starting with *n*-hexane and the polarity gradually increased by 5% by addition of CH_2Cl_2 till 100%, then stepwise addition of ethyl acetate (EtOAc) up to 50%. Fractions (200 ml, each) were collected and monitored by TLC (using S1-S3, *p*-anisaldehyde) and similar fractions were pooled. Fraction I (1.1 g): **(**15% CH_2Cl_2/*n*-hexane**)** and fraction IV were further purified yo give comprised one major spot (R_f value 0.7, *n*-hexane-EtOAC (80:20 v/v), golden yellow with *p*-anisaldehyde) was further purified on several silica gel columns (25 cm×2.5 cm) using *n*-hexane only and polarity increased gradually by ethyl acetate till 3% (97:3 v/v) to give 250 mg of white needle crystals **(compound 1)**. Fraction IV (1.32 g): **(**60-80% CH_2Cl_2-*n*- hexane), showing one major spot (R_f value 0.3, *n*-hexane-EtOAC (80:20 v/v), purplish violet with *p*-anisaldehyde) was subjected to chromatography on silica gel column (25 cm×2.5 cm) using isocratic elution with *n*-hexane: EtOAc (95:5 v/v) to finally yield 300 mg of white needle crystals **(compound 2)**, as illustrated in scheme 1.



Scheme 1: Chromatographic fractionation of the *n*-hexane fraction of *S. buxifolia* Poir.) Ten.

Isolation of the major constituents of the methylene chloride fraction of the leaves

Eight grams of the methylene chloride (MC) fraction was subjected to VLC on 150 g silica gel (10.5 cm × 4 cm). Elution was achieved using *n*-hexane 100%, 50% CH_2Cl_2 then CH_2Cl_2 (100%) then a gradual increase of EtOAc (2%). Fractions (200 ml) were collected and monitored by TLC and similar ones were pooled to afford 5 main fractions. Fraction I (1.5 g) (fractions 50% -100% CH_2Cl_2) revealed one major spot, it was further re-chromatographed on silica gel column (25 cm×2.5 cm) using *n*-hexane: EtOAc (95**:**5 v/v) to give one major pure spot, **Compound 3** (1.8 g), pale yellow prisms (R_f value 0.6, *n*-hexane-EtOAC (80:20 v/v), blue with *p*-anisaldehyde). Fraction II (1g) (8% EtOAc/CH_2Cl_2) showed one major spot ($R_f$value 0.54 in $CHCl_3$-MeOH (95:5 v/v), blue with *p*-anisaldehyde) was purified on silica gel columns (25 x2.5cm) eluted with CH_2Cl_2:MeOH (90:10 v/v) and the polarity of MeOH was gradually increased to yield one pure yellowish-white powder, **compound 4** (115 mg) as illustrated in scheme 2.



Scheme 2: Chromatographic fractionation of the methylene chloride fraction

***In vitro* Anti Protozoal Study:**

Test plate production:

Experiments were performed in 96-well plates (Greiner, Bio-One Ltd, UK), each plate containing 16 samples at 4-fold dilutions in a dose-titration range of 64 mg/mL to 0.25 mg/mL. Dilutions for compounds /fractions were carried out by a programmable precision robotic station (BIOMEK 2000, Beckman, USA). Initially, two-fold serial dilutions were made in 100% DMSO to ensure complete solubility. An immediate dilution step (1/20 dilution: 10 µL compound/ fraction solution in 190 µL cell medium and test system) so that the final in-test concentration of DMSO did not exceed 1%. Each plate comprised medium-controls (blanks: 0% growth), infected untreated controls (negative control: 100% growth) and positive controls (chloroquine, miltefosine, suramin, nifurtimox for *P. falciparum, L. infantum T. brucei and T. cruzi*, respectively) [9]. All tests were run in triplicate.

Plasmodium falciparum:The chloroquine-sensitive *Plasmodium falciparum* GHA-strain was used to test plant extracts and isolated compounds according to procedures reported by Vic et al [1]. Percentage growth inhibition was calculated compared to the negative blank.

Trypanosoma brucei: Trypomastigotes of *T. brucei* Squib-427 strain (suramin-sensitive) were cultured at 37°C and 5% CO_2 in Hirumi-9 medium, supplemented with10% fetal calf serum (FCS). The assay was performed according to procedures reported by Hirumi et al [10].

T. cruzi: Tulahuen CL2 strain (nifurtimox-sensitive) was maintained on MRC-5 cells in minimal essential medium (MEM). The *in vitro* anti-trypanosomal activity was determined according to previously published procedures [11]**.** The color reaction was measured at 540 nm, values were expressed as a percentage of the blank controls.

L. infantum: Amastigotes (MHOM/MA(BE)/67) were collected from an infected donor hamster and used to infect primary peritoneal mouse macrophages (PMM). The *in vitro* anti-leishmanial activity was determined**.** Parasite burdens were determined microscopically after Giemsa staining and expressed as a percentage of the blank

controls without sample [1,2]. Additionally, cytotoxicity against PMM was assessed using the same protocol used for MRC-5 as detailed below.

Cytotoxicity assay

MRC-5 and PMM cells were separately cultivated in MEM medium, supplemented with L-glutamine (20 mM), 16.5 mM $NaHCO_3$ and 5% FCS at 37°C and 5% CO_2. For the assay, 104 MRC-5 cells/well were placed onto the test plates containing the pre-diluted samples and incubated at 37 °C and 5% CO_2 for 72 h. Cell viability was determined fluorimetrically after the addition of resazurin [2]**.** Cytotoxicity was evaluated against human embryonic lung cells (MRC-5).

HPLC Profiling for *n*-Butanol, Ethyl Acetate and Methylene Chloride Fractions

The analysis was achieved on a zobrax ODS C_{18} (particle size 5 µ.m, 250 mm x 4.6 mm) (Merck, Germany) equipped with a 5 µm, 10 x 4 mm guard column. The column temperature was maintained at 25°C using mobile phase: solvent A (acetonitrile) and solvent B (0.3% aqueous *ortho*-phosphoric acid (v/v)). Qualitative analysis was done using a linear gradient elution system from 16% to 100% A in B for 25 minutes, at a flow rate of 1 mL/min. Injection volume and UV wavelength were set at 20µl and 310 nm respectively. Salicylic acid standard was obtained from Merck Co., Darmstadt, Germany, while standards of isoquercitrin, hyperoside, chlorogenic acid, kaempferol, quercetin were obtained from Sigma-Aldrich (Steinheim, Germany). Seselin and ulopterol reference standards used for HPLC were isolated and identified in this work. Solutions of the standards as well as the tested extracts for spiking experiments (3.5 mg / 2.5 ml MeOH).

RESULTS AND DISCUSSION

The bioactive fractions; *n*-hexane (HE) and methylene chloride (MC) were processed for the isolation and identification of their major constituents using different chromatographic techniques. Four compounds were isolated (Figure 1); compound (**1**), compound (**2**), compound**(3**) and compound (**4**). Identification of the compounds was based on physicochemical properties, mass spectrometry, ^{1}H and ^{13}C NMR and 2D HSQC and HMBC spectral analysis and comparison to published data.

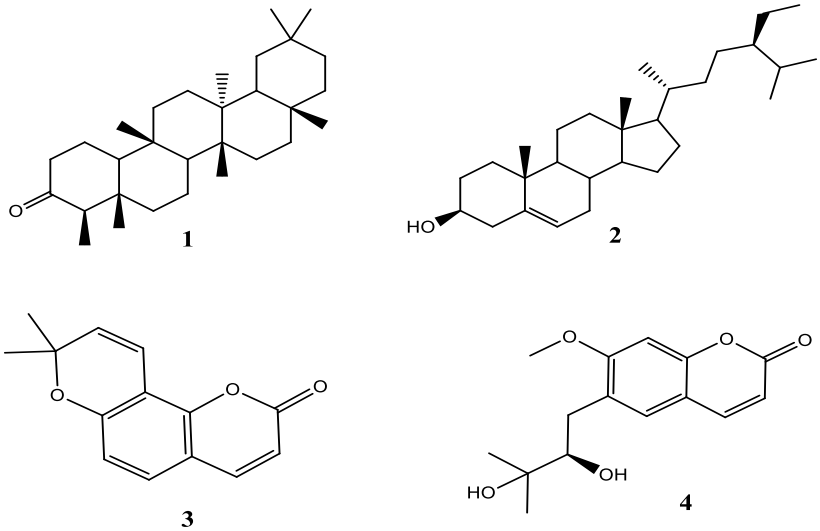


Figure 1: Structures of the isolated compounds

1: Friedelin, 2: β-Sitosterol, 3: Seselin, 4:(+)-Ulopterol

Compound 1 was isolated as white needle crystals (250 mg) from the *n*-hexane fraction. It showed a molecular weight of 426 *m/z* for $M^{+\bullet}$ corresponding to the molecular formula $C_{30}H_{50}O$ and responded positively on TLC plate when reacted with *p*-anisaldehyde/H_2SO_4 (golden yellow)**.** ^{1}H-NMR spectrum revealed the presence of seven methyl groups signals at δ_H1.1 (s, 3H, H-28), 0.98 (s, 3H,H-26), 0.94 (s, 6H,H-29,30), 0.89 (s, 3H, H-25), 0.81(d,3H,J=6.8,H-23),0.66 (s, 3H,H-24), 1.01(s, 3H,H-27) in addition to an unresolved doublet at δ_H 0.81 for eighth methyl group. All chemical shifts are characteristic of the triterpene structure**.** DEPT-Q spectrum revealed the presence of eight methyl groups resonating between 6.84 ppm and 31.8 ppm and seven quaternary carbon atoms resonating in the respective position; δ_C C-3(213.2), C-5 (42.15), C-9 (37.44), C-13(41.5), C-14(39.69), C-17(29.99), C-20 (28.17) ppm indicating possible triterpene with ketone substitution at position 3. Additionally, eleven methylene and four methine carbons were detected confirming its triterpene structure [12]. Upon comparing the results with published data, compound 1was identified as **Friedelin.**

Compound 2 was isolated and purified from the *n*-hexane fraction (300 mg)as white needle crystals**,** molecular formula $C_{29}H_{50}O$. It gave violet color in *p*-anisaldehyde/H_2SO_4indicating its steroidalnucleus. The melting point was 136-140 ̊C. ^{1}H-NMR spectrum showed the proton of H-3 as multiplet resonating at δ_H 3.59 and an olefinic proton (H-6) at 5.37(br). Signals at δ_H 1.029 (s) & 0.699 (s) corresponded to angular methyls CH_3-18 and CH_3-19 respectively [14].DEPT-Q of compound B displayed two sp^2 hybridized carbons at δ_C 146.76 and 121.72ppm, which were assigned for C_5 and C_6, respectively. DEPT-Q spectrum showed twenty-nine carbons including six methyls, eleven methylene, nine methine and three quaternary carbons. These spectral features are in good agreement with the structure of **β-sitosterol** [13].

Compound 3 was purified from the methylene chloride fraction as pale yellow prisms (1.8 gm). The compound reacted positively with both *p*-anisaldehyde/H_2SO_4 (blue color) and Dragendorff's (persistent yellow). It showed a molecular weight at *m/z* 228 for $M^{+\bullet}$ consistent with the molecular formula $C_{14}H_{12}O_3$, with melting point 119-120ºC. ^{1}H-NMR spectrum showed six doublet signals in the downfield region indicating the presence of six olefinic protons. Additionally, an upfield signal at δ_H 1.45 (s, 6H) can be assigned to two equivalent methyls. ^{13}C-NMR revealed the presence of 13 carbon signals in the molecule while DEPT-135 spectrum showed only the presence of seven positive signals and the absence of negative signals (CH_2). These seven carbons were assigned as six olefinic CH; 112.4 (C-3), 143.9 (C-4),127.8 (C-5),113.4 (C-6), 130.7 (C- 3´), 114.91(C-4´) and one signal for two equivalent CH_3 at δ_C= 28.1 (C-5´, 6´). HMBC correlations indicated a pyranocoumarin skeleton with two germinal methyls [14]. The identity of the compound was further confirmed by comparing its physical and spectral data with available literature and compound 3 was identified as **Seselin,** Figure 1.

Compound 4 was isolated from the methylene chloride fraction as a yellowish-white powder (115 mg). The compound gave a blue color with *p*-anisaldehyde/H_2SO_4. It had a molecular weight of 278 for $M^{+\bullet}$ that corresponded to the molecular formula $C_{15}H_{18}O_5$. Proton and carbon NMR spectra showed signals characteristic for coumarin molecule. Examining splitting patterns and coupling constants led to the assignment of the two doublets at 7.62 and 6.22 ppm as *o*-aromatic protons while the two singlets at 7.32 and 6.79 ppm were assigned as pyron ring protons. The presence of a dihydroxyl*iso*pentyl group was indicated by the presence of two singlets atδ_H 1.28, 1.32 ppm (3H, each), and three other signals at 2.54 ppm (1H,*dd*), 3.65 ppm (1H,*d*), 3.01 ppm (1H,*d*). Methyl protons resonating at δ_H 3.91 were assigned for 7-O-methoxy group. Comparing the results of physical and spectral data of compound **4** with the reported

spectral data [15] confirmed the identity of compound **4** as **Ulopterol,** Figure 1**.**

The results in Table (1) are represented as scores to facilitate the interpretation of IC_{50}; extracts or compounds with score 3-4 are considered active.The *n*-hexane fraction showed good anti-protozoal activity (IC_{50} 8.56 µg/mL, Sc=3) against *T. cruzi*, (IC_{50} 8.64 µg/mL, Sc=3) against *L. infantum***,** (IC_{50}3.37 µg/mL, Sc=3) against *T. brucei***,** (IC_{50} 10.26 µg/mL, Sc=2) against *P. falciparum.* While anti-protozoal activity of methylene chloride was reported as the following; (IC_{50} 8.20µg/mL, Sc=3) against *T. cruzi*, (IC_{50} 12.70 µg/mL, Sc=3) against *L. infantum*,(IC_{50} 32.69 µg/mL) against *T. brucei***,** (IC_{50} 2.63 µg/mL, Sc=4) against *P. falciparum.* However, both *n*-hexane and methylene chloride fractions were considered cytotoxic (CC_{50}19.5 µg/mL and 10.94 µg/mL on MRC-5 cells), respectively. Meanwhile, the ethyl acetate and *n*-butanol fractions were inactive. Nevertheless, the isolated compounds (1-4) from *S. buxifolia* showed only weak inhibitory potential against all tested protozoa except for seselin which exhibited moderate activity (IC_{50}38.47 µg/mL) against *T. cruzi*, (IC_{50}38.5µg/mL) and *P. falciparum* but had an IC_{50}>64 µg/mL against other tested protozoa (Tables 1 and 2). Although seselin showed weaker antiprotozoal activity than the fractions, it was superior to the fractions as it showed no cytotoxicity (CC_{50} > 64µg/mL on MRC-5 cells).

Accordingly, *n*-hexane and methylene chloride fractions can be considered as anti-protozoal agents against all tested protozoa as WHO consideration. On the other hand, the isolated compounds (*β*-sitosterol, Friedelin and (+)-ulopterol) individually exhibited no activity, only Seselin showed moderate activity against *T. cruzi* and *P. falciparum.*This discrepancy in activity might be attributed to the synergistic effect of the many metabolites present in the crude active fractions rather than a strong anti-protozoal activity of one or two component.

The HPLC was carried out in an attempt to identify other compounds in the active & inactive fractions and to authenticate these fractions as anti-protozoal agents. Methylene chloride, ethyl acetate and *n*-butanol fractions were analyzed by reversed phase HPLC (RP-HPLC) and the isolated compounds: seselin and ulopterol were individually analyzed under the same conditions. Furthermore, chlorogenic acid, quinic acid, caffeic acid, hyperoside, quercetin,quercetrin and salicylic acid were also injected to check for their presence in any of the tested fractions. Chromatograms of the fractions and isolated compounds are shown in Figure 2. Seselin ,ulopterol and salicylic acid were detected at R_t 23.66, 9.4, 12.1 min. respectively. Seselin and ulopterol were present in both ethyl acetate and methylene chloride but not in the *n*-butanol fraction, while salicylic acid was detected only in the ethyl acetate fraction (Figure. 2). None of the other compounds was identified in any of the fractions analyzed.

CONCLUSION

The present work describes the isolation of four compounds from bioactive non-polar fractions of the leaves of *S. buxifolia*and investigate their antiprotozoal activity. HPLC analysis revealed that the two major compounds in the active MCE were seselin and ulopterol. When tested for anti-protozoal activity, both the MCE and HE fractions were active against all tested protozoa but showed cytotoxicity against MRC-5 cell lines. However, among the isolated compounds, only seselin showed moderate activity (IC_{50} 38.47 µg/mL) against *T. cruzi* and *P. falciparum* while being devoid of cytotoxicity (Table 1 CC_{50}> 64.00 µg/mL on MCR-5). Therefore, seselin may be a good lead compound for the development of anti-protozoal drugs that exhibit a good safety profile.

Table (1): *In vitro* Antiprotozoal activity of the ethanolic extract, *n*-hexane, methylene chloride and the isolated compounds of *S. buxifolia* (Poir) Ten.:

Sample/organism	MRC-5	*T. cruzi*	*L. infantum*	*T. brucei*	*Pf-K1*	PMM cytotoxicity
	IC-50 (μg/mL)					
1	> 64.00	> 64.00	> 64.00	> 64.00	> 64.00	> 64.00
2	> 64.00	> 64.00	> 64.00	> 64.00	> 64.00	> 64.00
3	> 64.00	**38.47**	> 64.00	> 64.00	**38.5**	> 64.00
4	> 64.00	> 64.00	> 64.00	> 64.00	> 64.00	> 64.00
EE	> 64.00	> 64.00	> 64.00	> 64.00	> 64.00	> 64.00
BE	> 64.00	> 64.00	> 64.00	> 64.00	> 64.00	> 64.00
HE	**19.5**	**8.56**	**8.64**	**3.37**	**10.26**	**32.00**
MC	**10.94**	**8.20**	**12.70**	**32.69**	**2.63**	**32.00**

MRC-5 = diploid human embryonic lung fibroblast *T. brucei=Trypanosomabrucei*, *T. cruzi= Trypanosomacruzi* ,*L. infantum = Leishmania infantum*, Pf- K1= *Plasmodium falciparum* (K1 strain - asexual forms), PMM =Peripheral mouse macrophages, BE= Butanol fraction EE= ethyl acetate fraction MCE= methylene chloride fraction , 1= Friedelin,2= β-sitosterol, 3= Seselin, 4=(+)-ulopterol

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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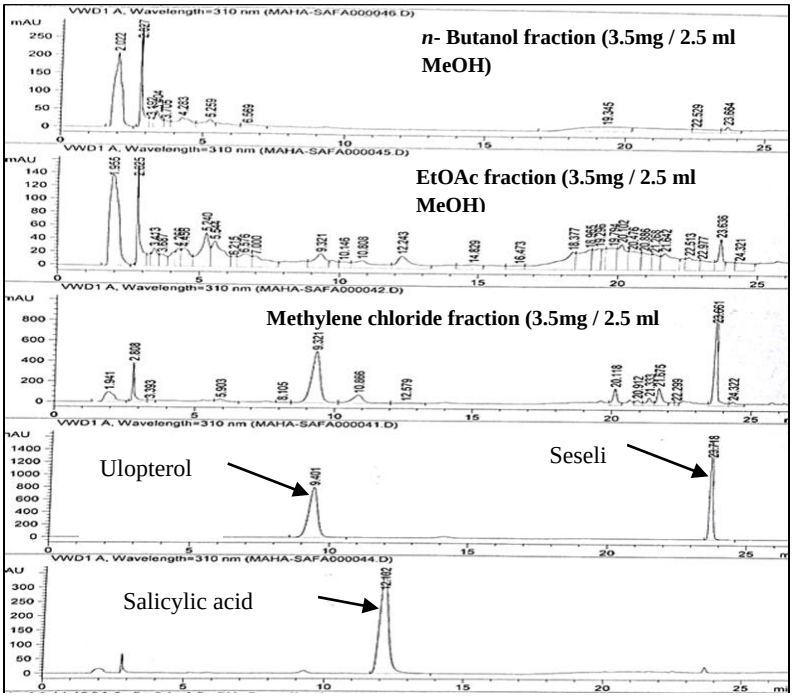

Figure 2: HPLC fingerprint of; *n*-butanol, ethyl acetate, and methylene chloride fractions, together with the major isolated compounds of the leaves of *S. buxifolia* (Poir) Ten. All extracts were prepared at concentration of 3.5mg / 2.5 ml of methanol

Table (2): Activity scores* for the antiprotozoal activity of *n*-hexane, methylene chloride fractions and the isolated compounds of *S. buxifolia* (Poir)Ten

Sample	MRC-5	*T.cruz*	*L.infantum*	*T.bruc*	Pf-K1	PMM cytotoxicity
	Activity scores					
1	< 1	= 1	< 1	< 1	= 1	< 1
2	< 1	< 1	< 1	< 1	< 1	< 1
3	< 1	< 1	< 1	< 1	< 1	< 1
4	< 1	< 1	< 1	< 1	< 1	< 1
Ex	< 1	< 1	< 1	< 1	< 1	< 1
BE	< 1	< 1	< 1	< 1	< 1	< 1
EE	< 1	< 1	< 1	< 1	< 1	< 1
HE	= 2	= *3	= *3	= *3	= 2	= 1
MC	= *3	= *3	= 2	= 1	= *4	= 1

*Score 3-4 are considered active against any protozoa Ex: total extract
PMM: Process Maturity Model (rating scale).

MRC-5 = diploid human embryonic lung fibroblast *T. brucei=Trypanosomabrucei*, *T. cruzi= Trypanosomacruzi* ,*L. infantum = Leishmania infantum*, Pf- K1= *Plasmodium falciparum* (K1 strain - asexual forms), PMM= peripheral mouse macrophages, BE= Butanol fraction EE= ethyl acetate fraction MCE= methylene chloride fraction , 1= Friedelin,2= β-sitosterol, 3= Seselin, 4=(+)-ulopterol

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أوراق سيفيرينيا بوكسفوليا: فصل وتعريف المركبات الرئيسية من المستخلاصات النشطة بيولوجيًا ونشاطها المضاد للطفيليات الأولية

صفاء نور[1]، مها سلامة[2،3]، انجي محروس[2*]، هشام العسكري[2]، محمد حفناوي[2]، مصطفى عبدالقوي[2]

[1] مستشفى طوخ، وزارة الصحة والسكان، جمهورية مصر العربية.

[2] قسم العقاقير، كلية الصيدلة، جامعة القاهرة، القاهرة، جمهورية مصر العربية.

[3] قسم العقاقير، كلية الصيدلة، الجامعة البريطانية بمصر، جمهورية مصر العربية.

ملخص

في محاولة لاستكشاف أدوية عشبية لعلاج بعض الأمراض الاستوائية المهملة، تم اختيار نبات سيفيرينبا بوكسيفوليا(بوار تن. (العوسج الصيني) بغرض الكشف عن نشاطها المضاد للطفيليات. واستنادًا إلى هذا الهدف، تم اختبار النشاط المضاد للطفيليات لخلاصة أوراق النبات المذابة في مذيبات عضوية متعددة (الهكسان، كلوريد الميثيلي، خلات الإيثيل والبيوتانول) في المختبر خارج الجسم الحي وتحدديا ضد طفيليات *Plasmodium falciparum*.المسببة للملاريا، *Leishmania infantum*، *Trypanosoma brucei Trypanosoma cruzi* . وقد أظهرت مستخلصات الهكسان وكلوريد الميثيلين نشاطًا جيدًا مضادًا للطفيليات بمنع نمو 50% من الطفيليات عند تركيزات 8.56و 8.64 و 3.37 و 10.26 ميكروغرام / مل لمستخلص الهكسان و 8.20و 12.7 و 32.69 و 2.63 ميكروغرام / مل مستخلص كلوريد الميثيلين ضد طفيليات التريبانوزوما كروزي *Trypanosoma cruzi* ووالليشمانيا انفاتم *Leishmania infantum* والتريبانوزوما بروسي *Trypanosoma brucei* والبلاسموديم فالسيبارم *Plasmodium falciparum* على التوالي. بينما كان مستخلصات خلات الإيثيل والبيوتانول غير نشطة. ووفقاً لذلك، تم عزل مركبين رئيسيين من جزء الهكسان وتعرفهما باسم friedelin [**(1)**] و β-sitosterol**(2)** وكذلك تم فصل مركبين من عائلة الكومارين من جزء كلوريد الميثيلين وتم تعريفهما باسمseslin **(3)** وulopterol (+) **(4)**. وحده مركب seslin**(3)** أظهر تأثير مضاد للطفيليات وبدون سمية علي الخلايا في المختبر. تم التعرف على التركيب الكيميائي للمركبات التي تم فصلها بالإستعانة بنتائج التحليل الطيفي وإعتماد نتائج تحليل الكتلة والتحليل بالرنين المغناطيسي الأحادي والثنائي الأبعاد. وعلاوة على ذلك، تم تحليل خلاصات النبات باستخدام الكروماتوجرافيا السائلة فائقة الأداء وتحديد البصمة المعرّفة بهدف توثيق التركيب الكيميائي للنبات.

الكلمات الدالة: سيفيرينا بوكسيفوليا، مضادات الملاريا، مضادات التريبانوزوما، مضادات الليشمانيا، العوسج الصيني.

[*] ***المؤلف المراسل: انجي محروس***

engy.abdelhamid@pharma.cu.edu.eg

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