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

Safaa Nour¹, Maha Salama²,³, Engy Mahrous²*, Hesham El-Askary², Mohamed Hifnawy², Mustafa A. Abdel Kawy²

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 (IC₅₀ for HE 8.56, 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 ¹H NMR, as well as ¹H-¹³C HSQC and HMBC spectral analysis. Among isolated compounds, only seselin showed antiprotozoal activity with IC₅₀ 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 protozoa including African sleeping sickness, leishmaniasis and Chagas’ disease which are caused by Trypanosoma brucei, Leishmania spp. and Trypanosoma cruzi 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.
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, leishmaniasis is treated with liposomal Amphotericin-B which is known to possess serious and sometimes lethal side effects. Other treatment options of leishmaniasis 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 drugs for 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 embryonic 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 (95:5 v/v). The chromatograms were visualized under UV and after spraying with p-anisaldehyde/ H2SO4 reagent.

NMR experiments were performed on a Bruker Avance III NMR-spectrometer (Billerica, MA, USA). Spectral data for 1H-NMR (400 MHz), 13C-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 °C 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 (40 g). 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 CH2Cl2 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% CH2Cl2/n-hexane) and fraction IV were further purified to give comprised one major spot (Rf 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% CH2Cl2-*n*-hexane), showing one major spot (Rf 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.
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% CH₂Cl₂ then CH₂Cl₂ (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% CH₂Cl₂) 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/CH₂Cl₂) showed one major spot (R_f value 0.54 in CHCl₃-MeOH (95:5 v/v), blue with p-anisaldehyde) was purified on silica gel columns (25 x2.5cm) eluted with CH₂Cl₂: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.
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.

*T. brucei:* Trypomastigotes of *T. brucei* Squib-427 strain (suramin-sensitive) were cultured at 37°C and 5% CO₂ in Hirumi-9 medium, supplemented with 10% 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.
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 NaHCO₃ and 5% FCS at 37°C and 5% CO₂. 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% CO₂ 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 C18 (particle size 5 µ.m, 250 mm x 4.6 mm) (Merck, Germany) equipped with a 5 µ,m, 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 isoquercitin, 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, ¹H and ¹³C NMR and 2D HSQC and HMBC spectral analysis and comparison to published data.

![Figure 1: Structures of the isolated compounds](image-url)

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⁺ corresponding to the molecular formula C₃₀H₅₀O and responded positively on TLC plate when reacted with p-anisaldehyde/H₂SO₄ (golden yellow). ¹H-NMR spectrum revealed the presence of seven methyl groups signals at δ_H 1.1 (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 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 1 was identified as Friedelin.

Compound 2 was isolated and purified from the n-hexane fraction (300 mg) as white needle crystals, molecular formula C₂₉H₄₈O. It gave violet color in p-anisaldehyde/H₂SO₄ indicating its steroid nucleus. The melting point was 136-140°C. ¹H-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 CH₃-18 and CH₃-19 respectively [14].DEPT-Q of compound B displayed two sp² hybridized carbons at δ_C 146.76 and 121.72ppm, which were assigned for C₅ and C₆, 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/H₂SO₄ (blue color) and Dragendorff’s (persistent yellow). It showed a molecular weight at m/z 228 for M⁺ consistent with the molecular formula C₁₄H₁₂O₃, with melting point 119-120°C. ¹H-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. ¹³C-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 (CH₂). 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 CH₃ 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/H₂SO₄. It had a molecular weight of 278 for M⁺ that corresponded to the molecular formula C₁₅H₁₄O₅. 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 α-aromatic protons while the two singlets at 7.32 and 6.79 ppm were assigned as pyron ring protons. The presence of a dihydroxylisosopentyl group was indicated by the presence of two singlets at δ_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
The results in Table (1) are represented as scores to facilitate the interpretation of IC₅₀; extracts or compounds with score 3-4 are considered active. The n-hexane fraction showed good anti-protozoal activity (IC₅₀ 8.56 µg/mL, Sc=3) against T. cruzi, (IC₅₀ 8.64 µg/mL, Sc=3) against L. infantum, (IC₅₀ 3.37 µg/mL, Sc=3) against T. brucei, (IC₅₀ 10.26 µg/mL, Sc=2) against P. falciparum. While anti-protozoal activity of methylene chloride was reported as the following: (IC₅₀ 8.20 µg/mL, Sc=3) against T. cruzi, (IC₅₀ 12.70 µg/mL, Sc=3) against L. infantum, (IC₅₀ 32.69 µg/mL) against T. brucei, (IC₅₀ 2.63 µg/mL, Sc=4) against P. falciparum. However, both n-hexane and methylene chloride fractions were considered cytotoxic (CC₅₀ 9.5 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 seselin which exhibited moderate activity (IC₅₀ 38.47 µg/mL) against T. cruzi, (IC₅₀ 38.5 µg/mL) and P. falciparum but had an IC₅₀ > 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 (CC₅₀ > 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, quercetin 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 Rₖ 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 (IC₅₀ 38.47 µg/mL) against T. cruzi and P. falciparum while being devoid of cytotoxicity (Table 1 CC₅₀ > 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.:

<table>
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<tr>
<th>Sample/organism</th>
<th>MRC-5</th>
<th><em>T. cruzi</em></th>
<th><em>L. infantum</em></th>
<th><em>T. brucei</em></th>
<th>Pf-K1</th>
<th>PMM cytotoxicity</th>
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MRC-5 = diploid human embryonic lung fibroblast  
*T. brucei* = *Trypanosoma brucei*,  
*T. cruzi* = *Trypanosoma cruzi*,  
*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.

**ACKNOWLEDGMENT**

The authors are also thankful to the staff of the University of Antwerp (An Matheeussen, Natascha Van Pelt and Pim-Bart Feijens) for their generous help in *in vitro* screening work.
Table (2): Activity scores* for the antiprotozoal activity of n-hexane, methylene chloride fractions and the isolated compounds of *S. buxifolia* (Poir)Ten

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<th>Sample</th>
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<th>T.bruc</th>
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</tr>
<tr>
<td>BE</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>EE</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>HE</td>
<td>= 2</td>
<td>= *3</td>
<td>= *3</td>
<td>= *3</td>
<td>= 2</td>
<td>= 1</td>
</tr>
<tr>
<td>MC</td>
<td>= *3</td>
<td>= *3</td>
<td>= 2</td>
<td>= 1</td>
<td>= *4</td>
<td>= 1</td>
</tr>
</tbody>
</table>

*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=Trypanosoma brucei,  
 T. cruzi=Trypanosoma cruzi  
 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= (+)-uopterol

REFERENCES


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

صفاء نور1، مها سلامة2،3، انجي محروس2*، هشام العسكري2، محمد حفناوي2، مصطفى عبدالقوي2

1 مستشفى طوخ، وزارة الصحة والسكان، جمهورية مصر العربية.
2 قسم العقاقير، كلية الصيدلة، جامعة الفجيرة، جمهورية مصر العربية.
3 قسم العقاقير، كلية الصيدلة، الجامعة البريطانية بمصر، جمهورية مصر العربية.

ملخص


الكلمات الدالة: سيفيرينيا بوكسفوليا، مضادات الملاريا، مضادات التريبانوزوما، مضادات الليشمانيا، العوسج الصيني.

*المؤلف المراسل: انجي محروس

engy.abdelhamid@pharma.cu.edu.eg

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