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In Vitro Antiproliferative and Antimicrobial Activity of 11-*Epi*artapshin Compound Isolated from *SeriphidiumHerba-alba*

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Abstract: *Seriphidium herba-alba* (Asteraceae family) was known for its therapeutic and medicinal properties, it was used in both traditional and modern medicine. 11-*Epi*artapshin was isolated from *S. herba-alba*. The structure of the compound was elucidated by means of spectroscopic data analysis, including 1D/2D NMR techniques and compared with reported data from the literature. The isolated compound was subjected to antiproliferative potential activity against cancer cell lines of the liver (HepG-2), colon (HCT-116) and breast (MCF-7) using 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction Assay. As well as, the total extract of *S. herba-alba* and the purified 11-*epi*artapshin were tested for antimicrobial activity. 11-*Epi*artapshin showed moderate antibacterial effects against Gram-positive, Gram-negative bacteria and weak antifungal effect. *Seriphidiumherba-alba* total extract has good inhibitory activity against all tested pathogenic bacteria.

Keywords: *Seriphidium herba-alba,* 11-*Epi*artapshin, HepG-2, HCT-116, MCF-7.

1 Introduction

Artemisia is one of the largest and most widely distributed genera belonging to the family Asteraceae. This genus composed of about 500 diverse species distributed mainly in the temperate zones of Europe, Asia, and North America. These species are perennial, biennial and annual herbs or small shrubs [1,2]. Artemisia species have a high economic value in several fields, as food plants and in the treatment of many diseases such as hepatitis, cancer, inflammation, and infections by fungi, bacteria, and viruses. Furthermore, several species of Artemisia are used in folk medicine such as thelminthic, antispasmodic insecticidal, antiatherogenic, hepatoprotective, antihyperglycemic, antihypertensive and in traditional Chinese medicine for the treatment of gynecopathy, amenorrhea, bruise and rheumatic disease [3-6]. After various taxonomic rearrangements, the genus was divided into five large groups; Absinthium DC., Artemisia L., Dracunculus Besser, Seriphidium Besser and Tridantatae [7]. This classification is however not accepted by all authors. Some of these genera (such as Seriphidium) are considered by most authors as members of Artemisia, but others are usually regarded as Independent [8]. Seriphidium was separated from Artemisia as new genus [9,10]. Previous phytochemical reports of the genus Artemisia species are rich in terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols, and acetylene compounds [11,12]. Among these bioactive compounds; artemisinin is a highly oxygenated sesquiterpene, containing 1,2,4-trioxane ring which is responsible for its antimalarial activity [13]. Artemisinin exerts not only antimalarial activity but also profound cytotoxicity against tumor cells [14]. The aim of the current research is the phytochemical study of the isolated compound (11-epiartapshin) from *S. herba-alba*, by extraction, separation and describes the content of active substances compound. As well as, structure elucidation of the isolated compound by means of spectroscopic data analysis. The research work also focused on the evaluation of *S. herba-alba* total extract and 11-epiartapshin for the activity in vitro against the pathogenic strains, evaluate the cancer chemo preventive activity of 11-epiartapshin.

2 Experimental

2.1 General Experimental Procedures

^{1} H and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a JEOL ECA-600 spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C, respectively). All chemical shifts (δ) are given in ppm units TMS as an internal standard and coupling constants (J) are reported in Hz. High performance liquid chromatography (HPLC) was performed on an Agilent pump equipped with an Agilent-G1314 variable wavelength UV detector at 254 nm and a semi-preparative reverse-phase column (Econosphere™, RP-C_{18}, 5 µm, 250 × 4.6 mm, Alltech, Deerfield, IL, USA). Silica gel 60 (230–400 mesh) was used for column chromatography. Pre-coated silica gel plates (Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analyses. Spots were visualized by heating after spraying with 10% H_2SO_4.

2.2 Plant Material

Seriphidium herba-alba was collected in June 2014 from South Sinia (Saint Catherine), Egypt and a voucher specimen (SH-1101) has been deposited in the herbarium of St. Katherine protectorate, Egypt.

2.3 Extraction and Isolation

The dried powder of an aerial parts of *S. herba-alba* (2.5 kg) was extracted with CH_2Cl_2–MeOH (1:1) at room temperature. The extract was concentrated in *vacuo*at 45°C to obtain a residue of 200 g of a dark brown residue. The residue was fractionated on a silica gel column (6×120 cm) eluting with *n*-hexane (3 L) followed by a gradient of *n*-hexane:CH_2Cl_2 up to 100% CH_2Cl_2 and CH_2Cl_2–MeOH up to 50% MeOH (3 L of each solvent mixture) followed by a gradient of *n*-hexane:CH_2Cl_2 up to 100% CH_2Cl_2 and CH_2Cl_2–MeOH up to 50% MeOH (3 L of each solvent mixture). The *n*-hexane:CH_2Cl_2 (1:1) fraction (8.3 g) was subjected to a second silica gel column (3×120 cm) eluted with n-hexane:CH_2Cl_2 (6:1) generatingtwo subfractions 1A and 2A. Subfraction 1A (2.3 g) was further purified by HPLC eluted with MeOH:H_2O (20:80). The flow rate was set to 1.5 mL/min and was at 0–70 min to afford 11-*epi*artapshin (25 mg purity > 95% by HPLC), (eluent CH_2Cl_2/MeOH/H_2O 80:3:1, R_f = 0.26).

2.4 Bioassay

2.4.1 Antimicrobial Assay

The antibacterial and antifungal properties of the purified compound(11-*epi*artapshin) in addition to the total extract were tested *in-vitro* against pathogenic bacteria, yeast and fungi in comparison with control drug Thiophenicol (Thiamphenicol, Sanofiaventis, France) as an antibacterial agent, and Treflucan (Fluconazole, Egyptian International Pharmaceutical Industries Company (EIPICO)) as an antifungal agent, by the agar diffusion technique in Petri dishes according to the reported method [21]. The isolated compound and the total extract were tested against Gram-positive bacteria (*Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* ATCC29213), Gram-negative bacteria (*Escherichia coli* ATCC25922), yeast (*Candida albicans* ATCC10321), and fungi (*Fusariumsolani* NRC15). Bacteria and yeast strains are American Type Culture Collection and fungal isolates were obtained from the culture collection of the Department of Chemistry of Natural and Microbial Products, National Research Center, Cairo, Egypt.

Preparation of paper discs:

The isolated compound 11-*epi*artapshin and the total extract were mounted on a paper disc prepared from blotting paper (5 mm diameter) on a concentration of (100µg/5µl DMSO /disc). Total extract was mounted on a concentration of (1mg/5µl DMSO/disc). Thiophenicol and Treflu can were used as positive controls for antibacterial and antifungal activity in a concentration of (100µg/disc). DMSO has been used as a negative control.

Preparation of agar plates:

Spores suspension of pathogenic strains were prepared and adjusted to be approximately (1×10^{6} spores^{-} ^{ml} of fungi and 1×10^{8} of bacteria). 1 ml of fungal and bacterial spore suspensions were inoculated into each plate containing 50 ml of sterile PDA and nutrient agar medium respectively.

Application of the discs:

After the media had cooled and solidified, the discs were applied on the surface of the inoculated agar plates and left for 30 min at 4 ºC for compounds diffusion. The plates were incubated for 24 h at 30 ºC for bacteria and 72 h at 28 ºC for fungi. Diameters of zones of inhibition produced around the discs were measured in (mm) at three different points and the average values are reported as Mean ± SD using MS Excel.

Minimal inhibitory concentration (MIC):

The purified 11-*epi*artapshinwas evaluated for its minimal inhibitory concentration MIC at the final concentrations; 100, 50, 25 and 12.5 µg. And the total extract was tested at different concentrations of 1000, 500, 250, 125 and 62.5µg. The lowest concentration showing inhibition zone around the disc was taken as the minimum inhibitory concentration (MIC).

2.4.2 Antiproliferative of Isolated Compounds

Chemicals Used:

Dimethyl sulfoxide (DMSO), MTT and trypan blue dye was purchased from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza.

Cell lines:

MCF-7 cells (human breast cancer cell line), **HepG-2** cells (human Hepatocellular carcinoma) and **HCT-116** (colon carcinoma) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37 ºC in a humidified atmosphere with 5% CO_2 and were subculture two to three times a week.

Cell viability assay:

For antitumor assays, the tumor cell lines were suspended in the medium at concentration $5x10^4$ cell/well in Corning® 96-well tissue culture plates, then incubated for 24 h. The tested compounds were then added into 96-well plates (three replicates) to achieve twelve concentrations for each compound. Six vehicle controls with media or 0.5 % DMSO were run for each 96 well plate as a control. After incubating for 24 h, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plates and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red then 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96 well plates were then incubated at 37°C and 5% CO_2 for 4 hours. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Then, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graph pad Prism software (San Diego, CA. USA) [22, 23].

Statistical Analysis

All results are expressed as mean ± standard deviation (SD). Statistical differences between correlated samples were evaluated using Graph pad Prism software (San Diego, CA. USA).

3 Results and Discussion

3.1 Structure Elucidation

11-*Epi*artapshin was isolated as a colorless oil from the *n*-hexane:CH_2Cl_2 (1:1) fraction of *Seriphidium herba-alba* extract. The ^{1}H- and ^{13}C-NMR spectra of 11-*epi*artapshin gave signals suggested the compound is a trans 12,6-eudesmanolide sesquiterpene bearing a secondary hydroxyl group at C-1 and C-8. From the spectra, it could be deciphered that the sesquiterpene was composed of six methine groups (three oxygenated ones), four methylene groups including one olefinic *exo*-methylene, two methyl groups supported the eudesmane skeleton and three quaternary carbons, one assigned as carbonyl group and one as an olefenic carbon. The ^{1}H-and ^{13}C-NMR spectra of 11-*epi*artapshin indicated the presence of a double bond of an *exo*-methylene group between C-4/C-15 at (δ_H 4.82, 1H, s, H-15a; 4.93, 1H, s, H-15b) which connected to C-15 at δ_C110.4 in the HMQC spectrum. The singlet at δ_H 0.67 and doublet signals (δ_H 1.23) were attributed to methyl groups Me-14, Me-13, according to the one-bond ^{1}H–^{13}C correlations from the HMQC experiment, these hydrogen atoms are directly bonded to the carbons at (δ_C 12.8), C-14 and (δ_C 9.2), C-13. The chemical shift value of the methyl group Me-13 was typical for eudesmanolides having β-methyl group at C-11. An α-Me group would be expected to give this signal above 12 ppm [15] (Table **1**), these methyl groups supported the eudesmanolides sesquiterpene structure. Also, in the down field region, a triplet signal at (δ_H 3.80, 1H, t, *J*=10.98) was assigned to a lactone proton (H-6). However, the characteristic downfield chemical shift of H-7 at (δ_H 2.15) indicated that proton-7 was deshielded by the presence of a carbonyl group at C-12. The ^{1}H-NMR spectrum showed two oxymethine (H-1) and (H-8) appeared at (δ_H 3.41, 1H, dd, *J*=11.7, 4.8 Hz, H-1) and (δ_H 3.82, IH, td, *J*=10.38, 6.12, Hz, H-8). Three up field methylene protons at (δ_H 1.49 (1H, dq, *J*=12.3, 4.14, Hz, H-2a; δ_H 1.74, 1H, m, H-2b); (δ_H 2.1, 1H, m, H-3a; δ_H 2.3, 1H,m,H-3b) and (δ_H 1.15, 1H, t, *J*=11.7Hz, H-9a; δ_H 2.23, 1H, dd, *J*=12.4Hz, 4.1, H-9b).Three sp^3methine groups at (δ_H2.0, 1H, m, H-5); (δ_H2.15, 1H, m, H-7) and (δ_H2.77, 1H, m, H-11). The ^{13}C-NMR spectrum of compound **1** indicated the presence of unequivalent15 carbon atoms DEPT 135 revealed the presence of two methyl groups C-13 and C-14 at (δ_C9.2 and 12.8), three methylene groups at (δ_C 30.4, 33.5 and 45.7 for C-2, C-3 and C-9), four methine groups at (δ_C 52.2, 76.9, 54.8 and 37.3 for C-5, C-6, C-7 and C-11), one sp^3 quaternary carbon at (δ_C 42.12, C-10), two sp^2 quaternary carbon at (δ_C 142.8 and 180.4 for C-4 and C-12), which belong to ester carbonyl carbon. The following carbon signals C-4, C-10, and C-12 were considered quaternary, as they did not exhibit ^{1}H/^{13}C HMBC correlations. Also the spectrum revealed the presence of one sp^2 methylene group C-15 at (δ_C110.4), in addition to two oxygenated sp^3 carbons at (δ_C 77.4 and 64.1 for C-1 and C-8). Interestingly, ^{1}H-^{1}H-COSY spectrum of compound **1** showed cross-peaks between the doublet signal at (δ_H 1.2), Me-13 with the multiplet signal at (δ_H 2.77), which corresponds to the C-11 methine proton. Additionally, there was cross-peaks between the signal at (δ_H 1.49), H-2a with the signals (δ_H 1.74, H-2b; δ_H 2.1, H-3a; δ_H 2.30, H-3b and δ_H 3.41, H-1). The spectrum indicated that signal H-1 at (δ_H 3.4) have correlations with

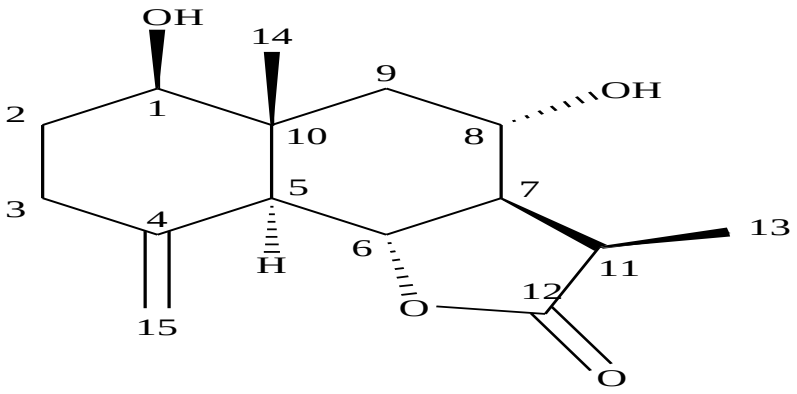
signal H-2a and H-2b. Also, there is a correlation between H-8 and H-9. For more details see (Table **1**). The location of the *exo*-methylene group was assigned to position C-4/C-15 on the basis of the HMBC correlations between H_2-15 (δ_H 4.82, 4.93 withC-3 (δ_C 33.5), C-5 (δ_C 52.2) and C-4 (δ_C 142.8). The presence of γ-lactone ring between C-6 and C-7 was concluded from the HMBC correlations of H-6 with C-11(δ_C37.3); Me-13 with C-11 (δ_C37.3), C-7 (δ_C54.8) and C-12 (δ_C180.4); H-11 with C-12 (δ_C180.4), C-13 (δ_C9.2) and C-6 (δ_C76.9). The location of the methyl groups was established with the aid of long-range hydrogen-carbon correlations from the HMBC experiment which indicated the correlation of Me-14 protons (δ_H 0.67) to the carbon resonances at (δ_C 45.7) and (δ_C 42.1) ppm, which assigned to C-9 and C-10. These correlations in conjunction with HMBC correlations from Me-14 to C- l (δ_C77.4) and C-5 (δ_C52.2) disclosing that C-1, C-9, and C-10 were connected through the quaternary carbon C-10. The last methyl group (C-13) was located at C-11 by the HMBC correlation from Me-13 (δ_H 1.23) to C-7 (δ_C 54.8), C-11 (δ_C 37.3) and C-12 (δ_C 180.4) **(Figure**. **1**). The position of the hydroxyl group at C-1 was determined by correlation of H-1 (δ_H 3.41) with C-14 at (δ_C 12.8) and C-9 at (δ_C 45.7) in the HMBC spectrum. Additionally H-8 (δ_H 3.82) and H-9 showed correlation peaks with C-6 at (δ_C76.9) in the HMBC spectrum. The structure of the isolated compound was deduced from the comparison of its spectral data with those of literature and identified as 11-*epi*artapshin [16-20].

3.2 In Vitro Antibacterial and Antifungal Assay

The agar diffusion assay of 11-*epi*artapshin and *Seriphidium herba-alba* total extract demonstrated different antimicrobial effects against tested micro-organisms as shown in (Table **2**). The results indicated that 11-*epi*artapshinshowed moderate antibacterial effects and weak antifungal effect against the filamentous fungus *F. solani* with zone of inhibition 7 mm. *Seriphidium herba-alba* total extract has good inhibitory activity against all tested pathogenic bacteria with zones of inhibition range from 9 to 11 mm and (MIC) in the range from 125 to 500 µg. Also, the total extract showed an inhibitory effect against the fungal pathogen *F. solani* and *C. albicans* with zone of inhibition 9 and 10 mm, respectively.

3.3 Antiproliferative Activity

To evaluate the antiproliferative activity of 11-*epi*artapshin, their anti-proliferative potential against MCF-7 cells (human breast cancer cell line), HepG-2 cells (human Hepatocellular carcinoma) and HCT-116 (colon carcinoma) were assessed using a cell viability assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT). The cells were treated at concentrations 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.6 µg/mL, 7.8 µg/mL and 3.9 µg/mL for 24 h. The anticancer drug, Doxorubicin, was used as a positive control. 11-*epi*artapshin showed IC_{50} (390 ± 8.9, 340 ± 8.7, 225 ± 3.7) against MCF-7, HepG-2, and HCT-116 cells, respectively (**Figure**. **2**).



Chemical structure of 11-*epi*artapshin

Table 1: ^{1}H- (600 MHz), ^{13}C-NMR (150 MHz), DEPT, COSY and HMBC spectral data of 11-*epi*artapshin recorded in $CDCl_3$.

Position	δ_H (*J* in Hz)	δ_C ppm	**COSY** $^{1}H\leftrightarrow{}^{1}H$	**HMBC** $^{1}H\leftrightarrow{}^{13}C$
H-1	3.41 dd (11.7, 4.8)	77.4 (CH)	H-2a, H-2b	C-9, C-14
$H\text{-}2_a$	1.49 dq (12.3, 4.14)	30.4 (CH_2)	H-1,H-3a, b, H-2b	C-3, C-1
$H\text{-}2_b$	1.74 m		$H\text{-}2_a$ H-3a,b	C-1, C-3, C-10
$H\text{-}3_a$	2.1 m	33.5 (CH_2)	H-2a,b, H-3b	C-2, C-4, C-5, C-15
$H\text{-}3_b$	2.30 m		H-2a,b, H-3a	C-2, C-4, C-5, C-15
H-4	…….	142.8 (C)	…….	…….
H-5	2.0 m	52.2 (CH)	H-6	C-4,C-10,C-14,C-15
H-6	3.80 t (10.98)	76.9 (CH)	H-5, H-7	C-5, C-8, C-11
H-7	2.15 m	54.8 (CH)	H-11, H-8, H-9b	C-6, C-8, C-13
H-8	3.82 td (10.38, 6.12)	64.1 (CH)	H-9a,b, H-7	…….
$H\text{-}9_a$	1.15 t (11.7)	45.7 (CH_2)	H-9b, H-8	C-8, C-10, C-14
$H\text{-}9_b$	2.23 dd (12.4, 4.1)		H-9a, H-8	C-8, C-10, C-14
H-10	……	42.1 (C)	…….	…….
H-11	2.77 m	37.3 (CH)	H-13, H-7	C-6, C-7, C-12, C-13
H-12	……..	180.4 (C)	…….	…….
H-13	1.23 d (7.56)	9.2 (CH_3)	H-11	C-7, C-11, C-13
H-14	0.67 s	12.8 (CH_3)	…….	C-1,C-5, C-9, C-10
$H\text{-}15_a$	4.82 s	110.4 (CH_2)	…….	C-3, C-4, C-5
$H\text{-}15_b$	4.93 s			C-9, C-14

Table 2: Anti-microbial activity (diameters of growth inhibition zones) of 11-*epi*artapshin and *S. herba-alba* - total extract.

compounds	Inhibition zone diameter (mm)				
	Gram positive		Gram negative	Yeast	Fungi
	B. subtilis	*S. aureus*	*E. coli*	*C. albicans*	*F. solani*
11-*Epi*artapshin	7±0.72	8±0.15	8±0.71	N.A.	7±0.14
S. herba-alba total	10±0.71	11±0.71	9±0.70	9±0.70	10±1.40
Thiophenicol	20±0.70	18±1.42	15±0.70	N.A.	N.A.
Treflucan	N.A.	N.A.	N.A.	22±0.42	12±0.71

Notes: The agar diffusion technique was followed and the inhibition zone diameter (IZD) expressed in (mm). Thiophenicol and Treflucan were used as positive controls at a concentration of 100 µg/disk. N.A. No activity.

Table 3: Minimum inhibitory concentration of 11-*epi*artapshin and *S. herba-alba* total extract against the pathogenic strains.

Minimum Inhibitory Concentration MIC (µg/disc)					
compounds	Gram positive		Gram negative	Yeast	Fungi
	B. subtilis	*S. aureus*	*E. coli*	*C. albicans*	*F. solani*
11-*Epi*artapshin	100	25	50	-	50
S. herba-alba -total	125	250	500	500	62.5
Thiophenicol	3.13	3.13	25	-	-
Treflucan	-	-	-	12.5	50

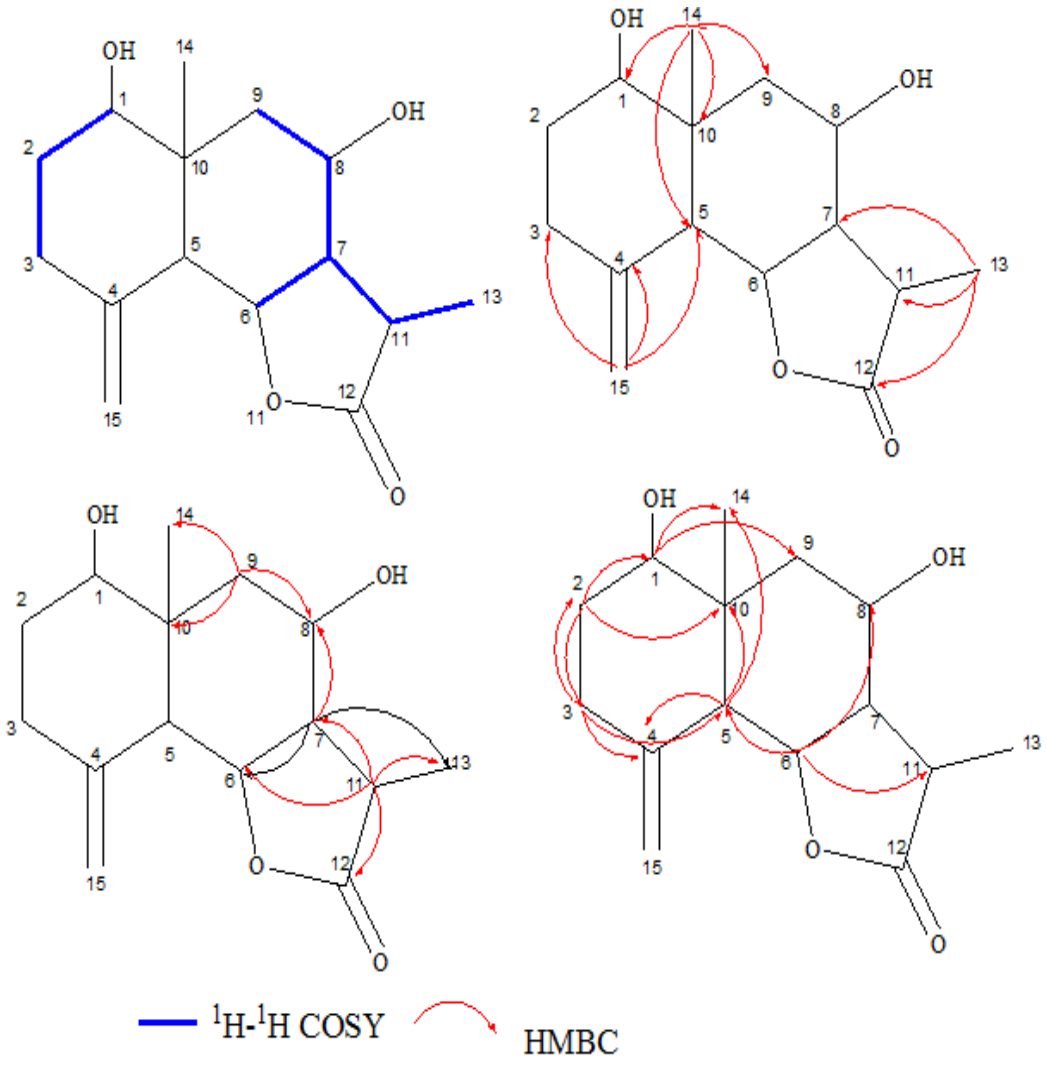


Fig. 1: Key 2D NMR correlations of **11-*epi*artapshin**

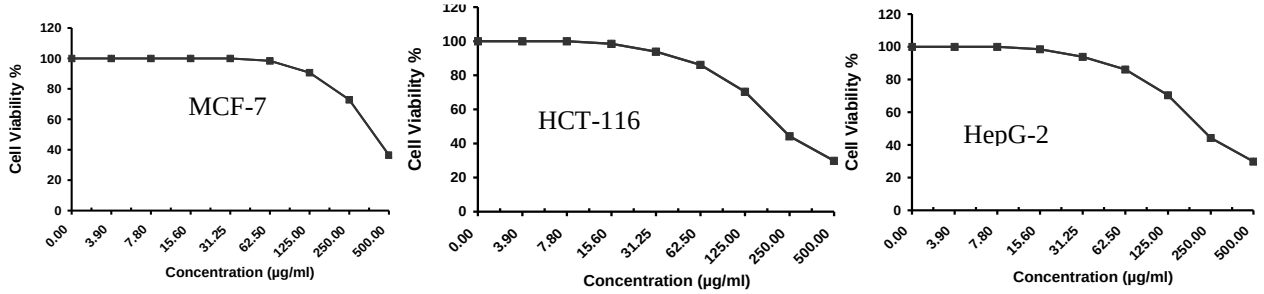


Fig. 2: Relationship between different doses of 11-*epi*artapshin (3.9–500 µg/mL) and the cell viability of **MCF-7**, **HCT-116**and **HepG-2** using MTT reduction assay

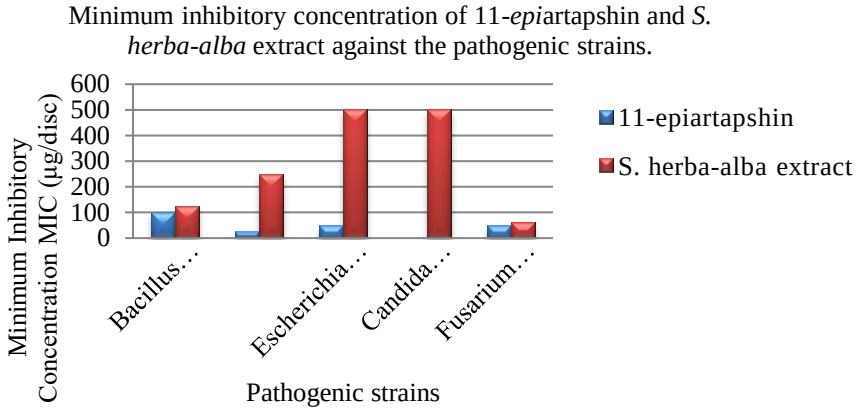


Fig. 3: Anti-microbial activity of 11-*epi*artapshin and *S. herba-alba* extract against the pathogenic strains.

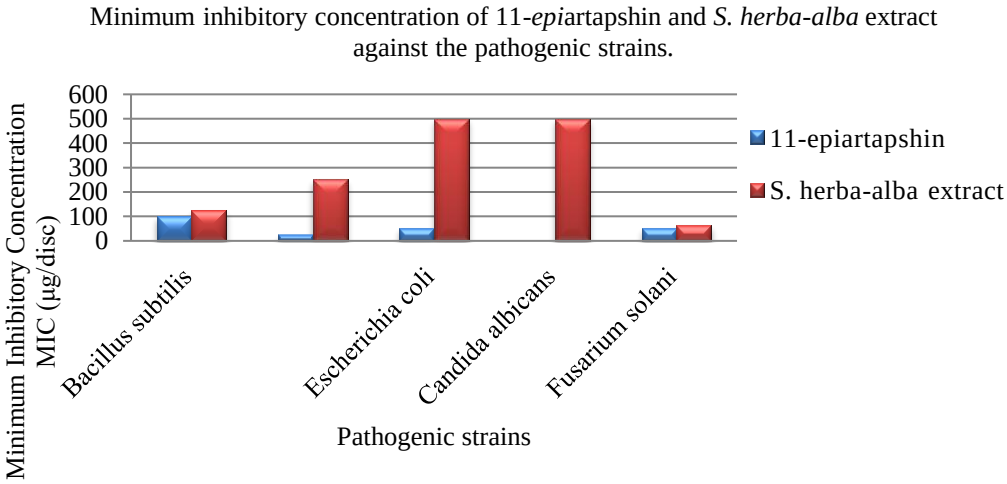


Fig. 4: The minimum inhibitory concentration of 11-*epi*artapshin and *S. herba-alba* extract against the pathogenic strains.

4 Conclusions

Seriphidium herba-alba afforded 11-*epi*artapshin, it has moderate antibacterial properties, a weak antifungal effect against the filamentous fungus *F. solani* and a weak antiproliferative properties.

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