PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES OF *MAYTENUS FORSSKAOLIANA*

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تمكن بواسطة كروماتوجرافيا العمود للخلاصات الايثر البترولية والكلوروفورمية لنبات مايتينوس فورسكوليانا من فصل أربعة مركبات هي :- فريديلين ، بيتاأمايرين ، بيتاسيستوستيرول وبيتيولين وقد تم التثبت من التركيب الكيميائي لهذه المواد الأربع بواسطة طيف الأشعة تحت الحمراء ومطياف الكتلة والطنين النووي المغناطيسي لذرة الهيدروجين وذرة الكربون- ١٣ بالإضافة إلى المعطيات الفيزيائية. وقد تبين من التأثير القاتل للبكتيريا لهذه الخلاصات فاعلية ضعيفة (200ميكروجرام/مل) بينما مادة بيتيولين أعطت فاعلية واضحة (20مكغ/مل). وتم دراسة التأثير المضاد للسرطان لمادة بيتيولين وتبين بوضوح وجود فاعلية واعدة (40مكغ/مل) وذلك على خلايا Hela و Hep-2. أما الفاعلية الدوائية (الفارماكولوجية) للخلاصات المختلفة فقد أبانت وجود تأثير مسكن مبطء للتنفس وتأثير مقلل ضغط الأوعية الدموية معتمد على الجرعة في حدود 40.1 مم زئبق (عند جرعة 200 مغ/كغ ، وتأثير مبطء لضربات القلب 67.3% (عند جرعة 50 مغم/كغم) وحفظ لدرجة حرارة الجسم بمعدل 4.2°م بعد ساعتين (عند جرعة ٢٠٠مغم/كغم).

Column chromatography of the petroleum ether and chloroformic extracts of *Maytenus forsskaoliana afforded* four compounds: friedelin **1**, β-amyrin **2**, β-sitosterol **3** and betulin **4**. The structures of these compounds were established on bases of spectral (IR, MS, ^{1}H and ^{13}C-NMR) as well as physical data. Antimicrobial activity for extracts indicated weak potency (200 μg/ml) while betulin **4** showed a pronounced activity (MIC and MBC 20 μg/ml). The cytotoxic activity of betulin **4** significantly indicated promising activity (IC_{50} 40 μg/ml) on both HeLa and Hep-2 cells.The pharmacological activity of the different extracts showed sedative effect, slowing of respiration,dose-dependant decrease in the arterial blood pressure by 40.1 mm Hg (at a dose 200 mg /kg), decrease in the heart rate 67.3 % (at a dose 50 mg/kg)and decrease in the body temperature by 4.2 °C after 2 hrs (at a dose 200 mg/kg).

Key words: *Maytenus forsskaoliana,* celastraceae, friedelin, β-amyrin, β-sitosterol, betulin, cytotoxicity, antimicrobial, general pharmacology .

Introduction

Plants of the Genus *Maytenus* (Family Celastraceae) have been widely known since the isolation of the antitumor maytansine in 1972 (1). In Saudi Arabia, this genus is represented by nine species, widely growing in different areas of the Kingdom (2). It has been reported that plants of this genus are widely used in folk medicine as anti-tumor, anti-asthmatic, anti-ulcer, treatment of stomach problems, anti-inflammatory, analgesic and as antimicrobial (3-6).

Screening the current literature revealed that two of the Saudi *Maytenus* species have been investigated for their chemical constituents; *Maytenus arbutifolia* (3) and *M. undata* (5) , while no chemical or biological records were found on *M. forsskaoliana*. Therefore, fractionation by different chromatographic techniques was undertaken as well as investigation of the biological activity of different extracts including general pharmacological and microbiological screening and cytotoxicity studies were conducted in an attempt to isolate the active compound (s).

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Experimental

Plant material

Maytenus forsskaoliana Sebsebe (Family Celastraceae) was collected in the fruiting stage from the southern region of the Kingdom particularly from Fayfa Mountain (Jabal Fayfa) about 100 km North East of Gizan in 1998. The plant was identified by Dr. Sultan–al-Abedin (Plant Taxonomist, College of Pharmacy, KSU); dried in the shade and then coarsely powdered. A voucher specimen (N0. 14653) was deposited at the Research Center, College of Pharmacy, KSU.

General:

Evaporation of solvents was done at 45° C under reduced pressure. Infrared spectra were generally obtained in KBr discs using Perkin- Elmer, FTIR, model, 1600 Spectrophotometer. Ultraviolet absorption spectra were obtained in spectroscopic methanol on a Unicum Heyios UV-Visible Spectrophotometer. Melting points were carried out using Thermosystem FP 800 Metler and were uncorrected. EI (70 ev) mass spectra were obtained as solid probe using Shimadzu QP-class-500. ^{1}H and ^{13}C-NMR spectra were recorded on Bruker DRX 500 NMR Spectrophotometer, operating at 400 MHz for protons and at 125 MHz for carbons.

Extraction and Isolation:

A weight of 1 kg of powdered *M. forsskaoliana* was successively macerated in a percolator with petroleum ether, chloroform and ethyl alcohol (96 v/v) 4 L each. The alcoholic extract was dissolved in 200 ml of distilled water and extracted with ethyl acetate. Each extract was evaporated to dryness under reduced pressure.

Column chromatography of petroleum ether extract:

A sample (13 g) of the petroleum ether extract was submitted to column chromatography (150 g silica gel, 60x4 cm)packed in petroleum ether. Elution was started with petroleum ether and polarity was gradually increased with dichloromethane to methanol. The collected fractions(250 ml each) were monitored by TLC using different solvent systems and similar fractions were combined .

Compound 1: Fractions (11-20), eluted with 10 % dichloromethane in petroleum ether on evaporation, yielded an amorphous residue. Repeated crystallization yielded fine needles (70 mg) of compound 1, with R_f 0.56 (hexane- dichloromethane 7:3);mp 259ºC; $[\alpha]_D$ - 22.5º (c 1.0,$CHCl_3$); IR (KBr) ν_{max} :2946, 2882, 2869, 1719, 1464, 1385, 1359cm^{-1}; EIMS m/z (% rel.int.):426(M^+), 341, 303, 283, 273, 259, 246, 232, 218, 205, 191, 189, 179, 163, 149, 123, 109, 95, 69, 55 (100).^{1}H and ^{13}C-NMR (see Table 1& 2).

Compound 2: Fractions (36-50)eluted with dichloromethane on evaporation afforded crystalline scales of compound 2 (200 mg), with R_f 0.45 (chloroform);mp188.8ºC; $[\alpha]_D$ +143º (c 0.04, $CHCl_3$); IR (KBr) ν_{max} 3292, 2945, 2851, 1464, 1386, 1035, 995, 813 cm^{-1}; EIMS m/z (% rel.int.):426 (M^+, 2.2), 411, 285, 257, 218 (100), 207, 203, 189, 175, 161, 119, 107, 95.^{1}H and ^{13}C-NMR (see Tables 1 &2).

Column chromatography of the chloroform extract:

Chloroform extract (7 g) was submitted to column chromatography (150 g silica gel, 4x60cm) packed in hexane. Elution was begun with hexane and polarity was gradually increased with chloroform and finally with methanol. Fractions (250 ml each) were collected, monitored by TLC and similar fractions were collected.

Compound 3: Fractions (10-20), on evaporation and crystallization (methanol), gave compound 3 as needle crystals,with R_f 0.2 (chloroform); mp 138.6 ºC; $[\alpha]_D$ -38º (c 0.02, $CHCl_3$); IR (KBr) ν_{max}: 3440, 2900-2850, 1430, 1370, 1615, 1051, 960 cm^{-1}; EIMS m/z (% rel.int.): 414 (M^+), 399, 396, 381, 329, 273, 303, 255, 231. ^{1}H and ^{13}C- NMR (see Table 1 & 2).

Compound 4: Fractions (30-38), eluted with chloroform-methanol (99:1) on evaporation and crystallization (hot methanol); yielded fine needle crystals (12 mg) of compound 4 with, R_f 0.11 (chloroform); mp 258-260ºC; $[\alpha]_D$ +20 º (c 0.08, $CHCl_3$); IR (KBr): ν_{max}: 3500, 3425, 3040, 2935, 2915, 2835, 1650, 1470, 1400, 1380, 1350, 1200, 1150, 1020, 1000, 940, 900, 860, 815 cm^{-1}, EIMS m/z(% rel.int.) : 442 (M^+), 235, 217, 205, 202, 193, 171. ^{1}H and ^{13}C-NMR (see Tables 1 & 2).

Pharmacological studies:

Preparation of extracts:

Extracts were prepared in 0.5%(w/v) of sodium carboxymethyl cellulose suspension in a final concentration of 12.5 mg/ml for administration into the animals. The animals were divided into 8 groups (two for control and two for each extract).

Measurement of rectal temperature of mice :

Male Wistar albino mice weighing 25 g each were used, divided into eight groups and prepared for measurement of rectal temperature as previously reported (7). Each extract was administered in two doses (100 and 200 mg/kg, i.p.). Temperature was measured every 15 minutes for 3hrs using a rectal probe and a digital thermometer (Ugo, Basile, Milan) . Two groups of animals served as controls.

Measurement of arterial blood pressure in rats :

Male Wistar rats weighing 250 g each were divided into eight groups and prepared for measurement of the arterial blood pressure as previously reported (8).They were anaesthesized with intraperitoneal injection of urethane in a dose of 1.25 g/kg(used as 25% w/v aqueous solution)and injected intraperitoneally.The right jugular vein was canulated for administration of the extracts and the left carotid artery was canulated and connected with a Gould Statham pressure transducer for measurement of arterial blood pressure . Doses tested were 20 and 50 mg/kg. Each dose was tested in one group of animals and two groups served as controls.

The effect on the heart rate was calculated from the arterial pressure pulse by speeding up the speed of the recording system from 0.05 cm /sec to 1 cm /sec.The blood pressure changes were quantified in mm Hg using the calibration system built in the Narco Biosystem(USA). The Physiograph Coupler (No. 7179) was used for recording.

Measurement of general behavior:

White albino mice weighing 25 g each were divided into eight groups, each group contained four animals. The general behavior was assessed as previously reported(9). Groups 1 and 2 were intraperitoneally injected with 0.5% sodium carboxymethyl cellulose suspension in doses of 0.2 and 0.4 ml/mouse. These two groups served as control animals,while groups 3,4 and 5,6 and 7,8 were intraperitoneally injected with different extracts in doses of 100 and 200 mg/kg.

Antimicrobial activity(10) :

Antimicrobial activity was tested against Gram-positive and Gram- negative bacteria and yeast according to previously reported methods(10).The bacteria were maintained in Nutrient Agar, while Brain Heart Infusion Agar was used for maintenance of *Mycobacterium* and the *Enterococcus* and Sabouraud Agar for yeast. MICs were determined by the two fold dilution methods by using nutrient broth or brain heart infusion with Tween 80 (0.06%) for bacteria and Sabouraud liquid medium for yeast using a final inoculum size of $1.5x10^5$ cells/ml, after 24 hrs, except for *Mycobacterium* after 48 hrs, in agitation at 37 C. The optical density was measured using a micro ELISA reader at 600 nm. The MIC was recorded as the lowest concentration with no visible growth.

Cytotoxicity study:

1-Brine shrimp method:

The assay was conducted as previously reported (11) . The eggs of brine shrimp, *Artemia salina* (Leach), upon being placed in brine solution, hatch within 48 hrs, providing large number of larvae (*nauplii*). Survivors after addition of extracts in concentration 1000, 500, 100, 10 µg/ml were counted after 48 hrs and the % of death at each dose were recorded. Results were expressed as IC_{50} .

2-Cytotoxic activity using cell lines :

This activity was coducted according to previously reported method(12). HeLa (Human carcinoma of the cervix) and Hep-2 (Human carcinoma of the larynx) cell lines were grown as a monolayer in Dulbecco's modified Eagle's medium, DMEM (Gibco) supplemented with 10 % fetal calf serum (Gibco) and 1% of penicillin –streptomycin mixture (10,000 IU/ml). The cells were maintained at 37º C in 5% CO_2 and 90% humidity.

The cytotoxic activity was assessed using the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide] reduction assay. $2x10^4$ cells(in 50 µl) were added in each well and the optical density was measured using micro ELISA reader at 550 nm. The % viability was plotted against the concentrations of the various extracts as well as betulin 4 and the 50% cell viability (IC_{50}) was calculated from the curve .

Results and Discussion

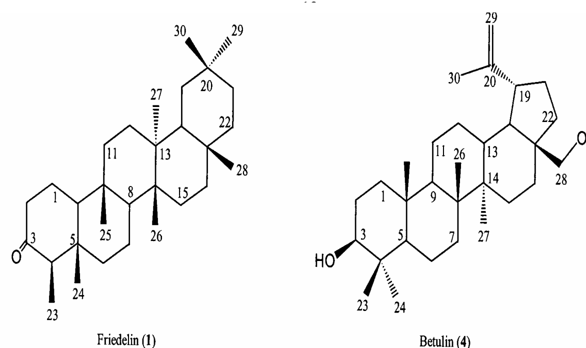
The powdered plant was successively extracted (cold percolation) with petroleum ether, chloroform, ethyl acetate and water. The ethereal extract was fractionated using column chromatography to yield two compounds 1 and 2, while 3 and 4 were separated from the chloroformic extract.

Compound 1 was obtained from the column fractions 11-20 as an amorphous residue, showed positive Liebermann's test and IR absorption peaks at 2946, 2882, 2869 and 1719, 1464, 1385, 1359, 1100 cm^{-1}; with MS parent ion at m/z 426 and other significant fragments at 341, 303, 273, 246, 232, 218, 205, 191; characteristic of 3-Keto-triterpenoidal compounds (13). The ^{13}C-NMR spectrum indicated the presence of a skeleton of 30 carbons; eight methyls, eleven methylenes, four methines and seven quaternary carbons, one of which at (δ 215).These data collectively as well as comparison with reported data(14) confirmed the presence of friedelane skeleton with a carbonyl group.

Thus, 1 was proved to be Friedelin, previously isolated from different *Maytenus* (15-17) species. However, this is the first report on the presence of friedelin in M. *forsskaoliana.*

Continuing elution of column of the petroleum ether extract gave compound 2, with positive Liebermann's test and IR at 3292, 2945, 2851, 1464, and 1386 and 1035 cm^{-1} together with MS parent ion at m/z 426 and other peaks at m/z 218, 203, 207, 189 evidently indicating a pentacyclic triterpenoid compound (13, 18) .The ^{1}H and ^{13}C-NMR spectra of 2 showed thirty carbon resonances, distributed as eight quartets (28.2, 15.5, 15.6, 16.9, 26.0, 28.4, 33.3, 23.7) corresponding to eight methyl groups (δ_H 0.98, 0.78, 0.92, 0.96, 1.12, 0.82, 0.86, 0.82) ; ten triplets (CH_2), five doublets (CH) and seven singlets. The two carbons resonating at δ_c 122.8 (C-12) and δ_c 145.1 (C-13) indicated a double bond (δ_H 5.17); a methine carbon resonating at δ_c 79.0(d) indicated C-3 hydroxyl. These data collectively proved that compound 2 is a 3-hydroxylated oleanane. Direct comparison (co-tlc, mp, ^{13}C-NMR , MS, IR) with reference samples of α- and β-amyrin and literature data (19) unequivocally proved the structure of 2 as β-amyrin. This compound has been previously reported in several *Maytenus* species (20-22) .However this is the first report on the occurrence of β-amyrin in *M. forsskaoliana.*

Column chromatographic separation of the chloroform extract afforded compound 3 with MS molecular ion (M^+) at m/z 414 and other fragments at m/z399, 396 , 329, 273, 303, 255, 231. IR at 3440 and 1051 cm^{-1} (hydroxyl group) , 1615 cm^{-1} , 2900-2850 and 1430, 1370 cm^{-1}. These data suggested a



nine carbon resonances (six quartets, eleven triplets, nine doublets and three singlets), two of these carbons resonated in the olefinic region at δ_c 141.0 (C-5) and δ_c 121.0 (C-6) corresponding to (δ_H 5.35 d) another carbon resonating at δ_c 72.0 (-OH) correlated to the proton signal at δ_H 3.52 (H-3); in addition to six quartet carbons resonating at δ_c 36.7, 34.1, 46.4, 18.9 , 12.32, 19.8 corresponding to six methyl groups (δ_H 0.68, 1.00, 0.91, 0.81 and 0.83). These data collectively confirmed a sitosterol compound (13, 23). The unequivocal identification of the compound was confirmed by direct comparison (co-tlc, mp, IR) with authentic β-sitosterol. This compound has been isolated from numerous species of different families including Celastraceae (24-26). Interestingly, β-sitosterol was reported to have significant activity as anti-hyperlipoproteinemic and to be useful in prostatic hyperplasia and other inflammatory conditions (22).

Continuing elution of the column of the chloroform extract yielded compound 4. The IR spectrum indicated hydroxyl group(s) (3500 and 3425 cm^{-1}) and olefinic methylenes (1650 cm^{-1}).The MS displayed (M^+) at m/z 442 (corresponding to $C_{30}H_{50}O_2$) and significant peaks at m/z 235, 217, 205, 202, 193, 171 characteristic fragmentation of lupane skeleton with an angular hydroxy methylene group (13,28,29). The ^{1}H-NMR spectrum reflected six methyl groups (δ_H 0.96, 0.76, 0.81, 1.07, 0.98 and 1.68) corresponding to δ_C 27.7, 15.5, 17.0, 17.01, 14.8, 19.1; a hydroxymethylene (δ_H 3.22, 3.78 and δ_C 62.7), a hydroxylated methine (δ_H 3.13, corresponding to δ_C 78.9), two olefinic protons (δ_H 4.66, 4.65 corresponding to δ_C 109.6). These data point out to a dihydroxylated lup-20(29)-ene. Screening the literature data for 28 and 30 hydroxy-lupane (14,19), it was found that the

spectral and physical data of compound 4 were in agreement with those reported for 3 β-,28-diol lup-20(29)-ene, trivially known as betulin(28,30-32). This is the first report of betulin in this plant and in the genus *Maytenus*.

Table 1. ^{1}H-NMR of compounds 1-4 (multiplicity)

Position	1	2	3	4
1	1.95, 1.71(ddd)	H_a 1.60(m), H_b, hidden under H-26	1.61(m)	1.67, 0.88(m)
2	2.37, 2.27(ddd)	H_a 1.95 (m), H_b, hidden under H-28	1.99(m)	1.62(ddd)
3	-	3.20(dd)	3.52(tt , J=5.1, 11.7)	3.13(tt)
4	2.25(q)	-	2.31, 2.329(m)	-
5	-	0.79(m)	-	0.69(m)
6	1.73, 1.28(d)	1.36(m), 1.56(m)	5.35 (br d , J= 5.1)	1.48, 1.32(dd)
7	1.49, 1.36(m)	1.31(m),1.55(m)	2.27(m)	1.38(m)
8	1.38(dd)	-	1.84(m)	-
9	-	1.55(m)	1.85(d)	1.29(t)
10	1.53(m)	-	-	-
11	1.45, 1.26(m)	1.86(m), 0.09(m)	1.53(m)	1.40, 1.19(dd)
12	1.33, 1.33(m)	5.17(m)	1.56(m)	1.62, 1.07(dd)
13	-	-	-	1.62
14	-	-	1.51(m)	-
15	1.47, 1.27(m)	1.52-1.63 (m)	1.79(m)	1.71, 1.09(dd)
16	1.58, 1.35(m)	H_a 1.84 (m), H_b, hidden under H-28	2.11(m)	1.91, 1.24(tt)
17	-	-	2.12(m)	-
18	1.56(m)	1.95(m)	0.68(s)	1.62(m)
19	1.37, 1.21(m)	1.02(m), 1.63(m)	1.00 (s)	2.30(dt)
20	-	-	1.99(m)	-
21	1.50, 1.31(m)	1.06(m), 1.32(m)	0.91(d)	1.95, 1.40(m)
22	1.50, 0. 94(m)	1.12(m), 1.42(m)	-	1.86, 1.12(m)
23	0.88(d)	0.98(s)	-	0.96 (s)
24	0.71(s)	0.78(s)	1.98(m)	0.76(s)
25	0.86(s)	0.92(s)	1.65(m)	0.81(s)
26	1.00(s)	0.96(s)	0.81(d)	1.07(s)
27	1.04(s)	1.12 (s)	0.83(d)	0.98(s)
28	1.17(s)	0.82(s)	0.83(m)	3.25, 3.27(dd)
29	0.99(s)	0.86(s)	0.84(t)	5.53,5.56(dd)
30	0.94(s)	0.82(s)	--	1.68 (s)

Table 2. ^{13}C- NMR of compounds1-4 (multiplicity)*

Position	1	2	3	4
1	22.3(t)	38.7(t)	37.5(t)	38.9(t)
2	41.5(t)	27.3(t)	29.3(t)	27.1(t)
3	213.2(s)	79.0(d)	72.0(d)	78.9(d)
4	58.2(d)	38.8(s)	42.1(t)	38.9(s)
5	42.1(s)	55.3(d)	141.0(s)	54.6 (d)
6	41.3 (t)	18.5(t)	121.0(d)	19.6(t)
7	18.2(t)	32.8(t)	31.9(t)	35.2(t)
8	53.1(d)	38.8(s)	31.8(d)	40.3(s)
9	37.4(s)	47.7(d)	50.0(d)	52.0(d)
10	59.4 (d)	37.6(s)	36.7(s)	38.2 (s)
11	35.6 (t)	23.6(t)	21.5(t)	19.6(t)
12	30.5 (t)	122.8(d)	39.9(t)	26.9(t)
13	39.7(s)	145.1(s)	42.5(s)	36.6(d)
14	38.3(s)	41.8(s)	56.9(d)	42.5(s)
15	32.4 (t)	26.2(t)	24.5(t)	27.0(t)
16	36.0 (t)	27.0(t)	28.5(t)	29.6(t)
17	30.0(s)	28.4(s)	56.2(d)	48.1(s)
18	42.8(d)	47.4(d)	36.7(q)	47.7(d)
19	35.3 (t)	46.9(t)	34.1(q)	47.6 (d)
20	28.1(s)	31.1(s)	26.2(d)	157.4(s)
21	32.7(t)	34.8(t)	46.4(q)	30.0(t)
22	39.2(t)	37.2(t)	29.5(t)	34.0(t)
23	7.2(q)	28.2(q)	26.2(t)	28.7(q)
24	15.0(q)	15.5(q)	19.3(d)	15.5(q)
25	17.9(q)	15.6(q)	12.0(d)	17.0(q)
26	20.2(q)	16.9(q)	18.9(q)	17.0(q)
27	18.6(q)	26.0(q)	12.32(q)	14.8 (q)
28	32.1(q)	28.4(q)	19.4(t)	60.3 (t)
29	35.7(q)	33.3(q)	19.8(q)	109.6 (t)
30	32.8(q)	23.7(q)	———	19.1 (q)

* multiplicity was deduced from DEPT

Concerning the pharmacological study, the different plant extracts (petroleum ether, chloroform and alcohol) exhibited sedative effects and slowing of respiration (Table 5). Furthermore, the three extracts seemed to possess dose-dependant decrease in the arterial blood pressure (Table 4); the alcohol extract showed the most effective decrease (40 mm Hg) while pet.ether and chloroformic extracts seemed to be equipotent in their effect. The alcohol extract (in a dose 50 mg/ kg) exhibited potent decrease in the heart rate (67.3 %), while, the petroleum ether extract seemed to be more potent than chloroformic extract in this aspect. Regarding the hypothermic effect, the greatest decrease in the body temperature was induced by the chloroformic extract (4.8 °C) after 2 hrs at a dose of 200 mg/kg (Table 5). These findings represent the first report on the pharmacology of this plant.

Concerning the antimicrobial activity (10) , the different extracts showed activity against Gram positive and Gram negative bacteria as well as *Candida albicans* with MIC and MBC 200 µg/ml; while compound 4 showed a pronounced activity against Gram positive and Gram negative bacteria with MIC and MBC 20 µg/ml.

Regarding the cytotoxic activity, the brine shrimp bioassay (11) was tried as primary screening; the chloroformic extract showed the highest activity followed by pet.ether then alcohol extract (IC_{50} 190, 400 and 1000 µg/ml, respectively). Moreover, the cytotoxic activity against HeLa and Hep-2 cells (Table 6), all extracts (petroleum ether, chloroform, ethyl acetate and alcohol) exhibited the same IC_{50} (200 µg/ml) on Hep-2, whereas compound 4 showed IC_{50} 40 µg/ml on both HeLa and Hep-2 while the different extracts showed different non-significant activity on HeLa cells in the order: compound 4>ethanol >ethyl acetate >chloroform>petroleum ether (40, 72.8, 96, 184.9 and 200 µg/ml respectively).

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Table 3. Effects of *M.forsskaoliana* extracts (i.p.) on general bebaviour of mice.

Type of Extract	Dose	Effect on Behaviour
Petroleum ether extract	200 mg/kg	Slow and shallow respiration. Skin itching. Sedation in a dose-dependent manner. Death of one animal after 24 hrs of administration.
Chloroform extract	100-200 mg/kg	Slow and shallow respiration. Skin itching. Mild tremors. Inhibition of muscle tone. Sedation in a dose-dependent manner. Both doses of extract were lethal to all animals 24 hrs of administration
Alcohol extract	200 mg/kg	Slow respiration. Decrease in heart rate. Skin itching. Sedation in a dose-dependent manner. Induced death after 24 hrs.

Table 4. Effects of different extracts on blood pressure and the heart rate of the rat.

Extract	Dose (mg/kg)	Decrease in Blood Pressure (mm Hg)	% Decrease in the heart rate
Petroleum	20 mg	7.5±2.1	9.1±1.8
ether extract	50 mg	27±3.1	23.8±4.1
Chloroform	20 mg	8±1.7	Zero
extract	50 mg	30±3.6	8.3±2.7
Alcohol	20 mg	22.5±1.5	17±1.6
extract	50 mg	40.1±3.7	67.3±6.9

* Mean ± S.E.M.

Table 5. Effects of *M.forsskaoliana* extracts (i.p) on body temperature of mice*.

Type of extract	Dose	Decrease in body temperature after 30 minutes**	Decrease in body temperature after 120 minutes**
Petroleum	100 mg/kg	2.4±0.2 °C	1.7±0.2°C
ether extract	200 mg/kg	3.0±0.1 °C	2.5±0.3°C
Chloroform	100 mg/kg	3.2±0.1 °C	3.4±0.2 °C
extract	200 mg/kg	3.8 ±0.1 °C	4.8±0.1 °C
Alcohol	100 mg/kg	2.6 ±0.1 °C	3.6±0.2 °C
extract	200 mg/kg	3.1 ±0.2 °C	4.0±0.1 °C

* Mean ± S.E.m.
**Temperature of control animals was 36.5 ± ° C

Table 6. Cytotoxic activity of the different extracts against HeLa and Hep-2 cells.

Samples	*HeLa*	*Hep-2*
Petroleum ether extract	200	200
Chloroform extract	184.9	200
Alcohol extract	72.8	200
Ethyl acetate extract	96.1	200
Betulin 4	40	40

The results are expressed as IC_{50} (µg/ml).

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