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Antioxidant, Cytotoxic and Antibacterial Activity and Total Phenol Contents of the Roots and the Shoots of *Euphorbia macrostegia* and *Euphorbia microsciadia*

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Abstract

The roots and the shoots of *Euphorbia macrostegia* and *E. microsciadia* were extracted using different solvents; dichloromethane (DCM), methanol (MeOH) and MeOH: water (80:20) and the extracts were screened for their cytotoxic, antioxidant and antimicrobial properties as well as total phenolic content. Cytotoxicity of the extracts was evaluated against human acute lymphoblastic leukemia (MOLT-4) cells by MTT reduction assay. The extracts were also subjected to the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and Folin-Ciocalteu total phenolic assays. The MeOH extract of the shoots of *E. microsciadia* and DCM extract of the roots of *E. macrostegia* were the most cytotoxic ones with IC_{50} values of 10.5 ± 2.6 and 7.0 ± 1.2 µg/mL, respectively. The MeOH extract of the shoots of *E. microsciadia* showed considerable antioxidant activity (IC_{50} = 9.95 ± 1.00 µg plant extracted to scavenge 1 mL of a 0.1 mM DPPH solution), which was consistent with its highest phenolic content (288.50 ± 29.38 mg equivalent of gallic acid in 1g dry plant extract: mg EG/g PE). Determination of minimum inhibitory concentrations (MICs) using the broth dilution method, confirmed that all the extracts from the plants gave various degrees of antibacterial activity against all tested microorganisms. In thin layer chromatography (TLC) investigations, some compounds previously isolated from *Euphorbia* species incldluing cycloartenol, 24-methylenecycloartan-3*β*-ol, *β*-sitosterol and euphol were tested and suggested to be responsible for the above-mentioned biological activities in the plants. Therefore, we suggest *E. macrostegia* and especially *E. microsciadia* as new sources for isolation and identification of various bioactive compounds.

Keywords: *Euphorbia microsciadia*, *Euphorbia macrostegia*, anticancer effect, biological activity, radical scavenging, natural compounds and phytochemical analyses

1 Introduction

Euphorbia is the largest genus of the family Euphorbiaceae (spurges), with more than 2000 known species and is characterized by the presence of milky latex and unique flower structures. It has about 80 species in Iran, two of which are called locally "Dena and Persian spurge", *E. microsciadia* Boiss. and *E. macrostegia* Boiss., respectively. They grow in the mountainous area of Iran (1, 2). Various species of the genus *Euphorbia* have shown different biological activities including enzyme inhibition and cytotoxic activity (3). In addition to their biological activities, these plants are ecologically important in the flora of Iran as weeds, anti-vegetative and poisonous plants (2).

It has been previously shown that a butanol/hexane extract of the aerial parts of *E. microsciadia* had the ability to modulate T-cell responses that suggest its possible beneficial effect on immune host defense (4). A MeOH extract of *E. microsciadia* showed stimulatory effects on the proliferation of the lymphocytes and a *n*-hexane layer of the MeOH extract had significant strong antiproliferative effect against tumor cells (5). The immunosuppressive activity of the plant's extract was attributed to the presences of quercetin-3-*β*-*O*-galactopyranoside, while myricetin-3-*β*-*O*-galactopyranoside was less active constituent among the isolated flavonol glycosides from the aerial part of the plant (6). On the other hand, a dichloromethane (DCM) extract of *E. macrostegia* showed cytotoxicity against two cancer cell lines; MDA-MB-48 and MCF-7 (7). Four cycloartane triterpenoids were isolated from the DCM extract. Two of which; cycloart-23(*E*)-ene-3*β*,25-diol and cycloart-23(*Z*)-ene-3*β*, 25-diol, showed the strongest cytotoxicity against the above mentioned cell lines, respectively. In addition to the cytotoxic activity, the tyrosinase inhibitory activity of constituents of *E. macrostegia* has also been reported recently (8). Among 10 compounds identified in the plants' extract, 2-(4-hydroxyphenyl)-ethylhentriacontanoate, hentriacontan-1-ol, lupenone and cycloart-22-ene-3,25-diol were detected as the most active tyrosinase inhibitors with IC_{50}s of 71.4-78.6 µM. Also, recently we isolated three triterpenoids; 24-methylenecycloartan-3*β*-ol, butyrospermol and cycloartenol and three diglycerides, 1,2-di-O-*α*-linolenoyl-sn-glycerol, 1-O-linoleoyl-3- O-palmitoyl-sn-glycerol and 1-O-*α*-linolenoyl-2-O-palmitoyl-sn-glycerol from

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the hexane soluble part of MeOH/DCM extracts of *E. macrostegia* Boiss. (9).

In this study, we report here antimicrobial potential, DPPH radical scavenging activity, total phenolic contents and cytotoxicity of different extracts of the roots and shoots of *E. macrostegia* and *E. microsciadia*.

2 Materials and Methods

2.1 General Experimental Procedures

RPMI 1640 (cell culture medium), fetal bovine serum (FBS), trypsin, and phosphate buffered saline (PBS) were purchased from Biosera (Ringmer, UK). Chloramphenicol and 3-(4,5-Dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St Louis, MO, USA) and penicillin/streptomycin were purchased from Invitrogen (San Diego, CA, USA). Doxorubicin and Folin-Ciocalteu reagents were obtained from EBEWE Pharma (Unterach, Austria) and Fluka, respectively. 2,2'-diphenyl-1-picrylhydrazyl (DPPH), Vanillin and p-iodonitrotetrazolium violet (INT) were purchased from Merck chemical companies. All the solvents were purchased from Merck. Thymol was purchased from Riedel-de Haen (Germany).

2.2 Plant material

Euphorbia microsciadia Boiss. and *Euphorbia macrostegia* Boiss. were collected in July 2012 from the Dena mountain (N 30° 52′ E 51°31′, 2980 m altitude), Yasuj, Iran. The plants were identified by Mr. Mehdi Zare and Dr. Mojtaba Asadollahi, plant taxonomists, in Medicinal and Natural Products Chemistry Research Center (MNCRC), Shiraz University of Medical Sciences, Iran. A voucher specimen for *E. macrostegia* (PC-91-4-1-1.2) and *E. microsciadia* (PC-91-4-2-1.2) has been deposited in the herbarium of MNCRC.

2.3 Extraction procedure

The shade-dried powdered shoots and roots of *E. microsciadia* (120 g) and *E. macrostegia* (120 g) were extracted twice separately and sequentially in 1500 mL of DCM, MeOH and 80% MeOH, by maceration for 24 h. at room temperature. Each filtered extract was concentrated to remove traces of the solvents under reduced pressure using a rotary evaporator to afford the respective dried solvent extracts. The weight of the shoots and the roots extracts of the plants were respectively as follows: DCM (1.04 and 5.8 g), MeOH (12.4 and 4.2 g), and 80% MeOH (4.16 and 1.44 g) for *E. microsciadia* and DCM (2.4 and 1.44 g), MeOH (0.8 and 2.8 g), and 80% MeOH (4.08 and 1.04 g) for *E. macrostegia*.

2.4 Preparation of the extracts, pure compounds, and TLC conditions

The dried MeOH and 80% MeOH extracts were dissolved in MeOH to a concentration of 5 mg/mL for TLC analysis. Also for the bioassays, the extracts were prepared in their extracting solvents in different concentrations. Pure phytochemicals, previously isolated from *Euphorbia* plants in our group, named cycloartenol (**1**), 24-methylenecycloartan-3*β*-ol (**2**), *β*-sitosterol (**3**) and euphol (**4**), were selected for the assessment of their existence in the above extracts (Figure 1). They were extracted from the hexane soluble part of methanol-dichloromethane extracts of the aerial parts of *E. macrostegia* (**1** and **2**) (9), methanol-dichloromethane extracts of *E. erythradenia* (**3**) (10), and acetone extract of the roots of *E. microsciadia* (**4**) (11). They were dissolved in MeOH (5 mg/mL) for spotting on TLC plates. We analyzed the chemical constituents of the extracts compared to the standard phytochemicals **1**-**4** using pre-coated TLC plates (silica gel 60 F254, 0.25 mm film thickness, Merck). Different composition of mobile phase were examined to obtain optimum retention factor (R_f) and resolution. In addition, two TLC reagents thymol-sulfuric acid (0.5 g thymol in 95 mL EtOH and 5 mL 97% H_2SO_4) and vanillin-sulfuric acid (0.3 g vanillin: 28 ml ethanol: 1 ml sulfuric acid), were sprayed followed by heating the developed TLCs to distinguish different phytochemical classes such as glycosides and terpenes in different colors (12).

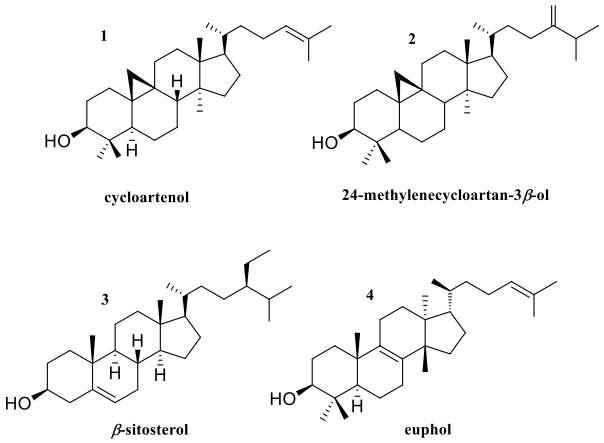

Figure 1: Structures of some compounds previously isolated from *Euphorbia* plants

2.5 Cytotoxic bioassay

Human acute lymphoblastic leukemia (MOLT-4) cells were obtained from the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. MTT assay was performed to assess viability after the exposure of cells to the extracts (13, 14). The cells were cultured in RPMI 1640 medium supplemented with 100 units⁄mL penicillin-G, 10% FBS, and 100 µg⁄mL streptomycin and maintained at 37°C in humidified air containing 5% CO_2. The experiments were performed in 96-well microplates and 3000 cells were seeded in each well. The wells containing growth medium alone were used as blank for background correction. After overnight incubation at 37°C, half of the growth medium was removed and medium supplemented with different concentrations of extracts were added in triplicate. The extracts of *E. microsciadia* and *E. macrostegia* were first dissolved in DMSO and then diluted several times in complete growth medium. Maximum concentration of DMSO in the wells did not exceed 0.5%, a concentration that showed no cytotoxicity in the cells. Standard cytotoxic agents; cisplatin and doxorubicin were used as positive controls. After incubation for further 72 h, the medium was removed and MTT was added to each well at a final concentration of 0.5 mg⁄mL and plates were incubated for another 4 h at 37°C. In the end, formazan crystals were dissolved in 200 µl DMSO. The optical density was evaluated at 570 nm using a microplate reader (Bio-Rad, Model 680) with background correction at 655 nm. The percentage of viability compared to control wells was calculated for each concentration of the extracts and IC_{50} values were estimated with the software Curve Expert (for Windows, version 1.34). Each experiment was repeated at least 3 times and data were presented as mean ± S.E.M.

2.6 DPPH radical scavenging activity

The antioxidant activities of all extracts of *E. microsciadia* and *E. macrostegia* were determined according to the modified method that we have previously described (9, 15-17). Briefly, 5 µL of the extracts was mixed with 195 µL of 0.1 mM DPPH in 96-well microplate. After incubation in the dark at room temperature for 30 minutes, the absorbance of the reaction

mixture was measured at λ 517 nm using a Bio-Rad microplate reader. Inhibition ratio (percent) was calculated from the following equation:

DPPH radical scavenging activity (%) = [($Abs_{Control}$ - Abs_{Sample}) / $Abs_{Control}$] × 100

We used butylated hydroxytoluene (BHT) and quercetin as the standards radical scavenger. The IC_{50} values were calculated by linear regression equations of the DPPH inhibition percentage from different concentrations of the extracts and the standards, using Microsoft Excel and Curve Expert statistical programs (15).

2.7 Determination of total phenolic content

The concentration of total phenolics in the DCM, MeOH and 80% MeOH extracts were determined separately by the Folin-Ciocalteu colorimetric method as described previously (18). Briefly, in each test plate, 5 µL of the 10 mg/mL plant extracts or the standard gallic acid solution, 158 µL distilled water and 10 µL Folin-Ciocalteu reagent were added and the solution was shaken briefly on a vortex mixer well, then after 8.5 min incubation at room temperature 30 µL of a 0.25% sodium carbonate was added to each solution. The reaction mixtures were kept in the dark at room temperature for 2 h and the absorbance of the solutions were measured at λ 765 nm against the blank. The concentrations of the total phenolics were measured against a series of gallic acid standard solutions and expressed as mg equivalent of gallic acid in 1 g plant extract (mg EG/g PE) (18).

2.8 Antibacterial minimum inhibitory concentration using nutrient broth microdilution

To examine the antibacterial activity of the plant extracts, four Gram-negative bacteria (*Escherichia coli:* PTCC1330, *Klebsiella pneumoniae*: PTCC1053, *Pseudomonas aeruginosa*: PTCC 1074, and *Salmonella typhi*: PTCC1609) and three Gram-positive bacteria (*Staphylococcus aureus*: PTCC1112, *Staphylococcus epidermidis*: PTCC1114, *Bacillus subtilis*: PTCC1023) were chosen to measure the minimum inhibitory concentrations (MIC) of the active extracts using nutrient broth micro-dilution (NBMD) assays (19). To perform the test, the stock solution of plant extracts were serially diluted in dimethyl sulfoxide (DMSO) to afford different concentrations of test samples. Chloramphenicol solution in DMSO was also prepared as the positive control. Breifly, 5 µL of the tests sample solutions was added to 95 µL of the fresh media and 100 µL of bacterial suspension culture (OD = 0.1 at 600 nm) in a 96-well microplate. After 24 h incubation at 37 °C in a shaking incubator, 10 µL of a 0.5% INT solution in water was added to each well. Afterwards, at the above-mentioned culture conditions, the microplates were incubated for further 30 min. Finally, the MIC was calculated as the minimum concentration of the test sample extract or antibacterial standard inhibiting the growth of bacterial strain by discoloration of the purple INT solution (6).

3 Results and Discussion

Cytotoxicity of DCM, MeOH and 80% MeOH extracts of the roots and shoots of *E. macrostegia* and *E. microsciadia* were tested against MOLT-4 cells (Table 1). Among the tested extracts of *E. microsciadia*, the DCM extracts showed no activity. The MeOH and 80% MeOH extracts of the shoots of this plant with IC_{50} values of 10.5 ± 2.6 and 17.1 ± 2.9 µg/mL, respectively, showed stronger activity compared to the same extracts from the roots with the IC_{50} values of 46.0 ± 3.5 and 40.0 ± 3.7 µg/mL, respectively. Unlike *E. microsciadia*, all the three extracts of *E. macrostegia* showed strong activity against MOLT-4 cells with IC_{50} values in the range of 7.0- 38.4 µg/mL. Unlike *E. microsciadia*, the root extracts of *E. macrostegia* exhibited lower IC_{50}s compared to the extracts from the shoots of this plant.

The MeOH and 80% MeOH extracts of the shoots of *E. microsciadia* showed the highest radical scavenging potentials in the DPPH free-radical test, with IC_{50} values of 9.95 ± 1.00 and 10.82 ± 1.64 µg/mL, respectively (Table 2). Their activities are more than that measured for butylated hydroxytoluene (BHT: IC_{50} = 51.09 ± 1.35 µg/mL, $P < 0.05$) but less than the quercetin's radical scavenging potential with an IC_{50} value of 2.0 ± 0.16 µg/mL ($P < 0.05$). The other extracts exhibited radical scavenging activity in the range of 28.51- 85.22 µg/mL.

Table 1: Cytotoxic activity of different extracts of *E. macrostegia* and *E. microsciadia*

Sample name	MOLT-4 IC50 (µg/mL) (MeOH extract)	MOLT-4 IC50 (µg/mL) (80% MeOH extract)	MOLT-4 IC50 (µg/mL) (DCM extract)
E. macrostegia, shoots	34.1 ± 4.2	38.4 ± 3.2	13.4 ± 2.3
E. macrostegia, roots	7.9 ± 1.0	34.8 ± 3.3	7.0 ± 1.2
E. microsciadia, shoots	10.5 ± 2.6	17.1 ± 2.9	-
E. microsciadia, roots	46.0 ± 3.5	40.0 ± 3.7 (11)	>100
Doxorubicin (nM)	17.2 ± 2.0	-	-
Cisplatin (µM)	3.1 ± 0.7	-	-

Table 2: DPPH radical scavenging activity and total phenolic contents (mg EG/g PE) of different extracts of *E. macrostegia* and *E. microsciadia*

Sample name	DPPH IC_{50} (µg/mL) (MeOH extract)	Total phenol (MeOH extract)	DPPH IC_{50} (µg/mL) (80% MeOH extract)	Total phenol (80% MeOH extract)
***E. macrostegia,* shoots**	47.64 ± 4.74	78.94 ± 3.89	28.51 ± 2.37	147.12 ± 5.46
***E. macrostegia,* roots**	41.68 ± 2.97	71.65 ± 1.01	35.55 ± 3.14	132.53 ± 4.43
***E. microsciadia,* shoots**	9.95 ± 1.00	288.50 ± 29.38	10.82 ± 1.64	203.61± 28.58
***E. microsciadia,* roots**	45.39 ± 2.93 (20)	37.59 ± 2.07	85.22 ± 2.98	18.29 ± 1.45
BHT	51.09 ± 1.35	-	51.09 ± 1.35	-
Quercetin	2 ± 0.16	-	2 ± 0.16	-

Table 3: Minimum inhibitory concentrations (mg/mL) of different extracts of *E. macrostegia* and *E. microsciadia* by nutrient-broth micro-dilution bioassay

Microorganisms (rows) **Plants**	**S.a.**	**S.e.**	**B.s.**	**S.t.**	**P.a.**	**E.c.**	**K.p.**
***E. macrostegia, shoots* (DCM extract)**	NA	NA	1.25	NA	NA	1.25	1.25
***E. macrostegia, shoots* (MeOH extract)**	5	1.25	1.25	1.25	5	2.5	2.5
***E. macrostegia, shoots* (80% MeOH extract)**	1.25	1.25	5	1.25	5	NA	NA
***E. macrostegia, roots* (DCM extract)**	NA	NA	1.25	NA	NA	1.25	1.25
***E. macrostegia, roots* (MeOH extract)**	2.5	2.5	1.25	5	5	5	5
***E. macrostegia, roots* (80% MeOH extract)**	5	1.25	2.5	2.5	5	1.25	2.5
***E. microsciadia, roots* (DCM extract)**	NA	NA	1.25	5	5	5	5
***E. microsciadia, roots* (MeOH extract)**	5	1.25	1.25	2.5	2.5	1.25	1.25
***E. microsciadia, roots* (80% MeOH extract)**	2.5	1.25	1.25	1.25	2.5	2.5	2.5
***E. microsciadia, shoots* (DCM extract)**	5	5	5	2.5	NA	NA	5
***E. microsciadia, shoots* (MeOH extract)**	5	2.5	5	NA	NA	NA	2.5
***E. microsciadia, shoots* (80% MeOH extract)**	5	5	5	2.5	NA	NA	2.5
Chloramphenicol	0.0125	0.025	0.0125	0.05	0.05	0.05	0.05

NA: not active (>5). S.a., *Staphylococcus aureus;* S.e., *Staphylococcus epidermidis;* B.s., *Bacillus subtilis;* S.t., *Salmonella typhi;* P.a., *Pseudomonas aeruginosa;* E.c., *Escherichia coli;* K.p., *klebsiella pneumoniae*

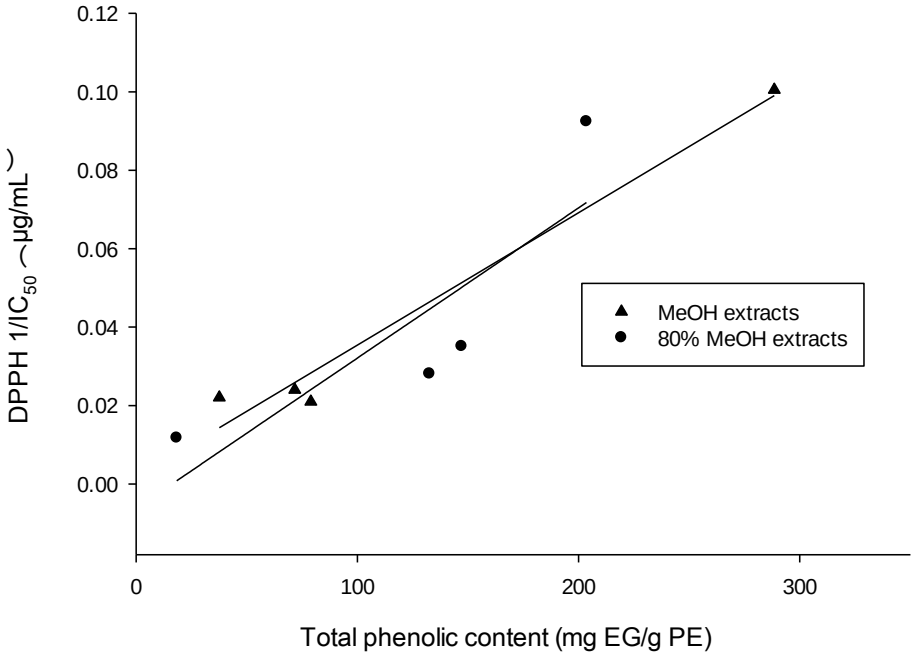

Figure 2: Correlation graphs for DPPH 1 /IC_{50} values and total phenolic contents for MeOH and 80% MeOH extracts

The 80% MeOH extract of the roots of *E. microsciadia* had the weakest antioxidant activity against DPPH radicals, as the result of the highest IC_{50} value (85.22 ± 2.98 µg/ml). The increasing order of total phenol contents (18.29 ± 1.45 to 288.50 ± 29.38 mg EG/ g PE) of the plant extracts were in agreement with the decreasing order of the IC_{50} (85.22 ± 2.98 to 9.95 ± 1.00 µg/mL) of DPPH test (Figure 2). The most prominent total phenolic contents was measured in the shoots of *E. microsciadia*, while the roots of *E. microsciadia* had the least TP contents. Antimicrobial activity of the DCM, MeOH and 80% MeOH extracts of the plants were measured against four different Gram-negative and three Gram-positive bacteria (Table 3). The MeOH extracts of the shoots and roots of *E. macrostegia*, and methanol and 80% MeOH extracts of the roots of *E. microsciadia*, were the most active antibacterial extracts that inhibited the growth of all tested microorganisms at MIC values between 1.25-5 mg/mL (Table 3). In addition, DCM extract of *E. macrostegia* was only active at MIC 1.25 mg/mL against the growth of *B. subtilis*, *E. coli*, and *K. pneumonia*. Generally, among the microorganisms, *B. subtillis* was the most susceptible ones to almost all extracts.

We have analyzed the bioactive plant extracts using TLC in comparison to *Euphorbia* derived phytochemicals. The TLC analyses of DCM, MeOH and 80% MeOH of the roots and the shoots of *E. macrostegia* and *E. microsciadia*, resulted in several purple colored spots after vanillin-sulfuric acid reagent treatments after development by chloroform: acetone (95:5) mobile phase (Figure 3). Two spots with R_f values between 0.45 and 0.80, were present in all extracts, while the chromatogram of the methanol roots extract of *E. macrostegia* was the most chemically diverse among them. On the other hand, all of the tested extracts were analyzed by silica-gel TLC using more polar mobile phase (chloroform: formic acid: methanol: water (5:0.2:3:0.5)).

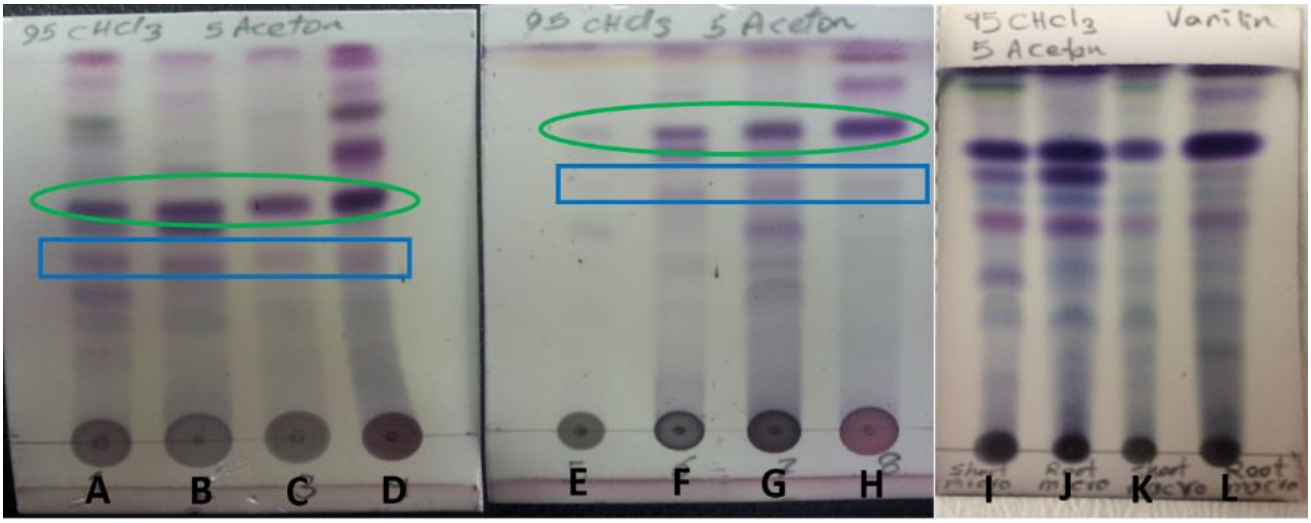

Figure 3: Spots in daylight after spray with vanillin-sulfuric acid using silica gel- TLC plates and chloroform: acetone (95:5) mobile phase. Spots are the MeOH extracts of the shoots (A) and the roots (B) of *E. microsciadia*, the MeOH extracts of the shoots (C) and the roots (D) of *E. macrostegia*, the 80% MeOH extracts of the shoots (E) and the roots (F) of *E. microsciadia*, the 80% MeOH extracts of the shoots (G) and the roots (H) of *E. macrostegia*, DCM extracts of the shoots (I) and the roots (J) of *E. microsciadia*, and DCM extracts of the shoots (K) and the roots (L) of *E. microsciadia*

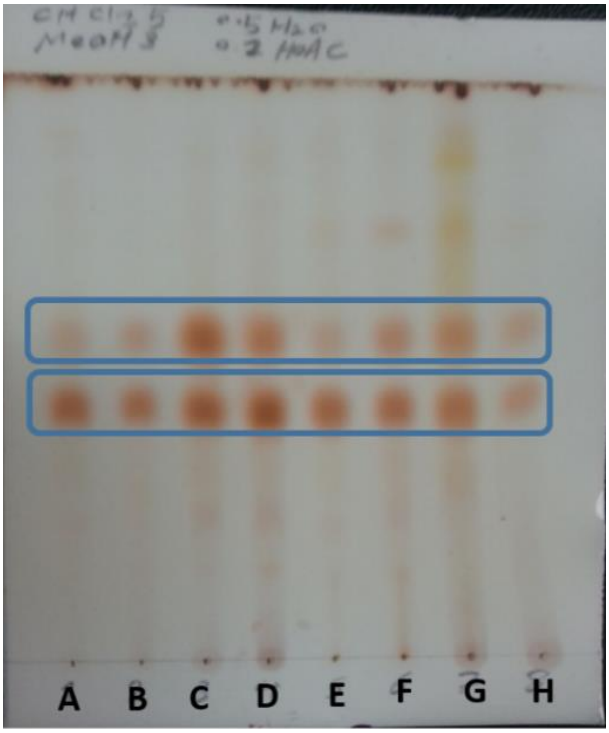

Figure 4: Spots in daylight after spray with thymol using a TLC plate and chloroform: formic acid: methanol: water (5:0.2:3:0.5). The extracts numbers are the same as described in Figure 3

Then, after visualizing the chromatogram by thymol spray reagent followed by heating, all the TLCs except DCM one, exhibited two major pink colored spots that suggested the glycosides character of compounds (Figure 4). While, the pink colored compounds were appeared as green-gray color spots (except the 80% extracts of roots and shoots of *E. macrostegia*) when the chromatogram sprayed with vanillin-sulfuric acid reagent (Figure 5). Co-TLC analyses of the extracts along with common phytochemicals **1**, **2**, **3** and **4** were performed using silica gel (Figure 6, (1)) and $AgNO_3$ - silica gel stationary phases (Figure 6, (2), (3)). In literature, compound **1** and **4** showed antioxidant and cytotoxic activity, while compound **3** showed antibacterial activity (20-24). Compounds **1** and **2** are present in almost all extracts in different ratios (Figure 6 (1), (3)), while the presence of compound **3** was confirmed in the MeOH extracts of the shoots of *E. microsciadia*, the roots of *E. macrostegia*, 80% MeOH extracts of the roots of *E. microsciadia* and the shoots of *E. macrostegia* and all the DCM extracts. To distinguish compounds **1** and **2**, we used silica gel TLC plates impregnated with 5% $AgNO_3$, using chloroform: acetone (97:3) as the mobile phase, followed by treatment with the thymol-sulfuric acid reagent spray. In $AgNO_3$ -silica gel TLC plate, compound **1** is clearly present in all MeOH and 80% MeOH extracts of the roots of the plants (Figure 6, (2)).

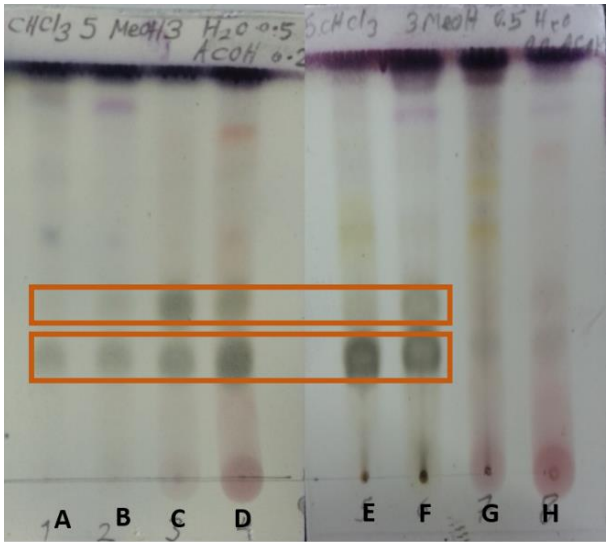

Figure 5: Spots in daylight after spray with vanillin-sulfuric acid reagent using an TLC plate and chloroform: formic acid:methanol:water (5:0.2:3:0.5). The extract numbers are the same as described in Figure 3

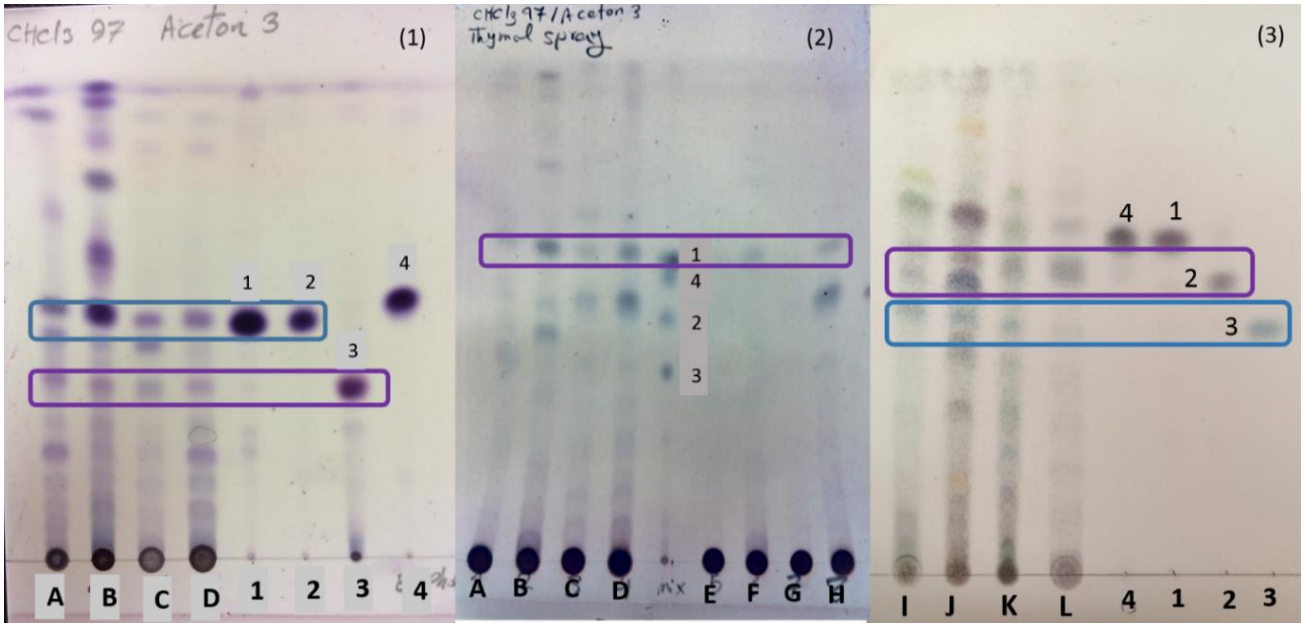

Figure 6: (1) Spots in daylight using chloroform: acetone (97:3) as the mobile phase (left) spray with vanillin-sulfuric acid reagent using an TLC plate, (2) and (3) spray with thymol reagent using $AgNO_3$ coated TLC plate. cycloartenol [**1**] 24-methylenecycloartan-3β-ol [**2**], β-sitosterol [**3**] and euphol [**4**]. The extract numbers are the same as described in Figure 3

4 Conclusion

The MeOH and DCM extracts of the roots of *E. macrostegia* are good candidates to isolate cytotoxic compounds. While the shoots DCM, and 80% MeOH extracts of the plant are suitable for extraction of cytotoxic and radical scavengers. However, the shoots of *E. microsciadia* is the best for exploring cytotoxic, and antioxidants when the plant is extracted with MeOH and 80% MeOH. The shoots MeOH extracts and roots MeOH and 80% MeOH extracts of *E. macrostegia* are the best for exploring antibacterial agents, while the last two solvent extracts of *E. microsciadia* shoots are preferred for isolating moderate antibacterial agents. The above-mentioned results indicated that the semi-polar to polar substances are responsible for the studied biological activity of the shoots of *E. microsciadia* in addition to the nonpolar to polar phytochemicals in both roots and shoots of *E. macrostegia*. It seems that terpenoid and glycosylated phytochemicals are the major phytochemicals in the DCM, MeOH and 80% MeOH extracts of *E. macrostegia* and *E. microsciadia*, which need to be isolated and identified. The antibacterial, cytotoxic and antioxidant activity of the plant extracts may be attributed to the presence of compounds **1-4** as these activities have been reported for them previously in the literature. For instance, compound **1** and **4** showed antioxidant and cytotoxic activity, while compound **3** showed antibacterial activity (12, 14, 19, 21, 22). However, the other compounds detected in the TLCs maybe isolated, identified, and further tested for the above biological activities in future.

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Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

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