Chemical and pharmacological investigations of African *Euphorbia* species

Summary of PhD Thesis

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INTRODUCTION

Plants synthesize a characteristic mixture of chemical constituents to protect themselves against various biotic and abiotic factors (e.g., diseases, pests, pathogens, herbivores, and environmental stresses). In addition, plants and their constituents have been widely used as sources of medicines since ancient times in various forms of traditional medicinal practices. The medicinal properties of plants are generally associated with their specialized metabolites, especially terpenoids, alkaloids and phenolic compounds. Natural compounds exhibit high chemo-diversity with exceptional molecular scaffolds, and thus offer the possibility of synthetic alterations to increase their bioactivity. Therefore, natural resources are highly preferred in developing new drug molecules with therapeutic efficacy. Over half of currently marketed pharmaceutical products are derived or originated from natural sources.

The family Euphorbiaceae (spurges) is one of the largest families of flowering plants with approx. 7500 species belonging to 300 genera worldwide. Important economic plants can be found in this family, such as *Hevea brasiliensis* (pará rubber); *Euphorbia tetragona* and *E. triangularis* (inferior rubber); *Manihot esculenta* (cassava, tapioca); *Croton tiglium* (croton oil); *Ricinus communis* (castor oil); and *Euphorbia resinifera* ('euphorbium'), among others. Members of the largest genus (*Euphorbia*) of Euphorbiaceae are widely distributed throughout mainland of both tropical and temperate regions and characterized by the production of a milky irritant latex. *Euphorbia* species are widely used in different traditional medicines for the treatment of different ailments, e.g., infections, gonorrhea, migraine, intestinal parasites, rheumatism, snakebites, asthma, obstipation, coughs, sores, and skin diseases.

According to the scientific data published earlier in the literature, spurges are sources of compounds with a variety of interesting biological activities. Especially the diterpenes are of considerable interest because of their wide range of potentially valuable biological activities and their broad structural diversity due to their different polycyclic and macrocyclic skeletons and the various aliphatic and aromatic ester groups. Among *Euphorbia* diterpenes, ingenol 3-angelate (ingenol mebutate) has attracted the greatest interest in the past few years as it was approved by the FDA in 2012 and by the EMA in 2013 for the treatment of actinic keratosis, a precancerous skin condition. It has been a considerable time since a natural product without structural modification has been introduced into clinical practice. In addition to ingenol 3-angelate, other promising *Euphorbia* diterpenes are also the subjects of drug development projects. Some phorbol and ingenol derivatives, particularly prostratin, have become of great interest in HIV therapy: they reactivate HIV-1 latency through PKC-dependent NF-κB activation, and avoid the new infection of $CD4^+$ cells.

Phytochemical and pharmacological investigation of *Euphorbia* species in the Department of Pharmacognosy, University of Szeged dates back almost three decades. During this time, many diterpene esters of different skeletal types have been isolated. In continuation of this work, the investigation of three *Euphorbia* species, namely *Euphorbia matabelensis* Pax*, E. trigona* Miller*, and E. gossypina* var. *coccinea* Pax was performed. The present thesis summarizes the results of this preparative work.

AIMS OF THE STUDY

The family Euphorbiaceae is a source of biologically active compounds, especially sesqui-, di- and triterpenoids, flavonoids, lignans, alkaloids and other phenolic constituents. In continuation of the research work performed in the Department of Pharmacognosy dealing with the identification of specialized metabolites of spurges, the objectives of the present research were the isolation and structural characterization of compounds from further *Euphorbia* species, and the investigation of their pharmacological effects. According to these aspects, the objects of this PhD-work were:

- A review of the literature on the genus *Euphorbia*, from the aspect of the chemistry and pharmacological properties of the plants.
- Collection of *Euphorbia* plant samples (altogether six species).
- Preliminary pharmacological investigation of the *E. candelabrum*, *E. trigona*, *E. continifolia* and *E. ramipressa* against four human tumor cell lines of gynaecological origin and against keratinocyte cell line.
- Preparation and fractionation of plant extracts for phytochemical work.
- Isolation and purification of compounds of *Euphorbia matabelensis*, *E. trigona*, and *E. gossypina* var. *coccinea* using a combination of different chromatographic methods.
- Structure determination of isolated compounds by spectroscopic methods (1D and 2D NMR, HR-MS).
- Investigation of the antiproliferative effect of isolated compounds in different test systems.
- Evaluation of the pharmacological and structure-activity relationship of the isolated compounds.
- Evaluation of the chemotaxonomical relevance of the isolated compounds.

MATERIALS AND METHODS

The aerial parts of *E. candelabrum*, *E. cotinifolia*, *E. ramipressa*, and *E. trigona* were collected in the Botanical Garden of the University of Szeged (Hungary), and in the Botanical Garden of the Eötvös Loránd University, Budapest (Hungary), in March 2018. The stems and roots of *E. matabelensis* Pax were collected in Kenya (Matuu subcounty, Machakos county, GPS coordinates 01°04.579’ S, 037°35.065’ E), Africa, in June 2016. The aerial parts of *E. trigona* for preparative work were collected

in June 2018, in the Botanical Garden of Eötvös Loránd University, Budapest (Hungary). And finally, the aerial parts of *E. gossypina* var. *coccinea* were collected in Kenya (GPS coordinates 1◦24024.3177700 S, 36◦42053.8612500 E), Africa, in July 2018. Voucher specimens have been deposited at the Department of Pharmacognosy, University of Szeged, Szeged, Hungary or in case of *E. matabelensis* and *E. gossypina* var. *coccinea* at the Herbarium of the School of Biological Sciences, University of Nairobi, Kenya.

The compounds were isolated by combined chromatographic techniques, including open-column chromatography (OCC), vacuum-liquid chromatography (VLC), preparative layer chromatography (PLC), and high-performance liquid chromatography (HPLC). Normal (NP) or reversed phase (RP) silica gel, and polyamide were applied as stationary phases. The isolated compounds were characterized, and their structures were elucidated by means of spectroscopic methods (1D and 2D NMR, HR-MS).

The pharmacological activities of the isolated compounds were tested in different biological assays. The antiproliferative properties of certain compounds were determined against HeLa, C33a, MCF-7 and MDA-MB-231 cells using the standard MTT assay, and cisplatin and/or doxorubicin as positive controls. Some compounds were tested for their GIRK channel blocking activity, while others for their keratinocyte inhibitory activity.

RESULTS AND DISCUSSION

Preparation of extracts for pharmacological screening

The fresh plant materials of *E. candelabrum* Trémaux ex Kotschy (**ECA**), *E. cotinifolia* (L.) Millsp (**ECO**), *E. ramipressa* Croisat (**ER**), and *E. trigona* Miller. (**ETP**) (100 g, each) were extracted with methanol in an ultrasonic bath at room temperature. After filtration, the extracts were concentrated to dryness *in vacuo*, and then dissolved in MeOH–H_2O 1:1. Thereafter, solvent-solvent partitions were performed with *n*-hexane, $CHCl_3$ and EtOAc. The *n*-hexane, $CHCl_3$ and EtOAc extracts were evaporated to dryness and used for pharmacological investigation.

Isolation of the diterpenes of *E. matabelensis*

The fresh plant materials of *E. matabelensis* (2.5 kg) [stem (EMS); root (EMR)] were crushed with a blender and percolated with MeOH at room temperature. The MeOH extracts were then concentrated *in vacuo*, dissolved in 50% aqueous MeOH and solvent-solvent partitions were performed with $CHCl_3$ and EtOAc. The $CHCl_3$-soluble fractions were separated on a polyamide column (OCC) with gradient system of MeOH–H_2O, to get four-four fractions (RI–IV and SI–IV) from roots and stems (**Fig. 1**). The fractions were monitored by normal phase thin-layer chromatography (NP-TLC), and it could be observed that fractions of stems and roots were differed from each other; therefore, their further

purification was performed separately. Fraction RI was separated by VLC on silica gel and by preparative TLC on reversed-phase silica gel to yield compound **1**. Fraction SII was separated by VLC on silica gel and by NP-HPLC to obtain compounds **2** and **3**.

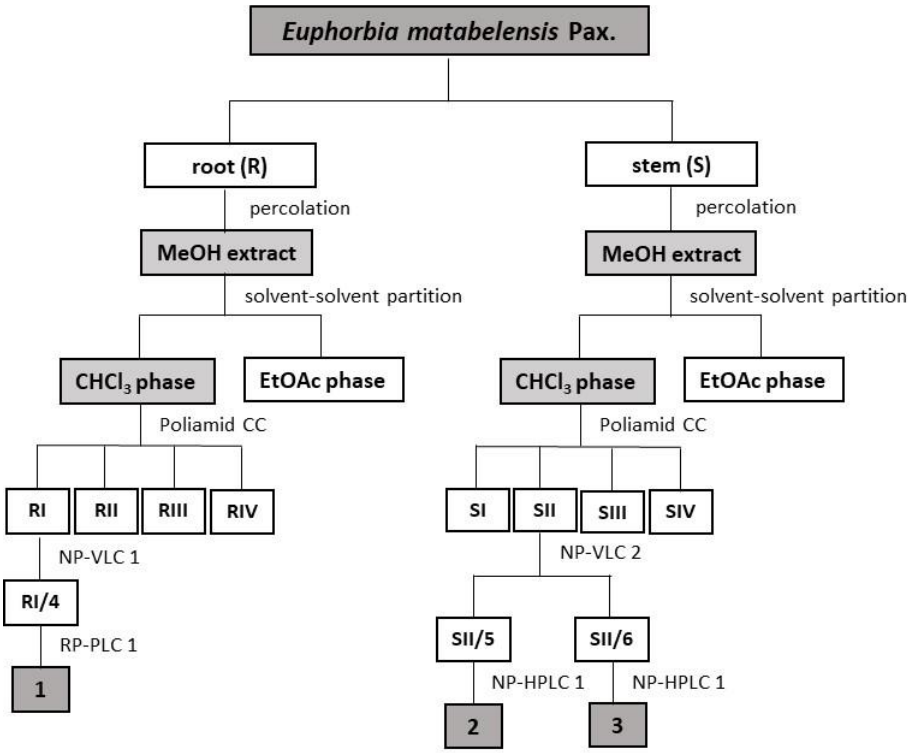


Figure 1. Isolation of the compounds of *E. matabelensis*

Isolation of the diterpenes of *E. trigona*

The fresh plant material of *E. trigona* (5.7 kg, aerial parts) was crushed with a blender and percolated with MeOH at room temperature (**Fig. 2**). The crude MeOH extract was concentrated under vacuo, the residue was dissolved in 50% aqueous MeOH, and partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH, respectively. The fractions were monitored by NP-TLC. Interestingly, based on the TLC determination, instead of the $CHCl_3$ phase, diterpenes were accumulated in the *n*-hexane fraction. *n*-Hexane fraction was further purified at first by polyamide column chromatography, and then by VLC on silica gel with a gradient system of cyclohexane–EtOAc–MeOH (from 99:1:0 to 1:1:1) to yield 20 main fractions (I/1–20). These fractions were further separated by using combined chromatographic techniques (NP-VLC, NP-PLC, and NP-HPLC) to afford 9 compounds (**4**–**12**) (**Fig. 2**).

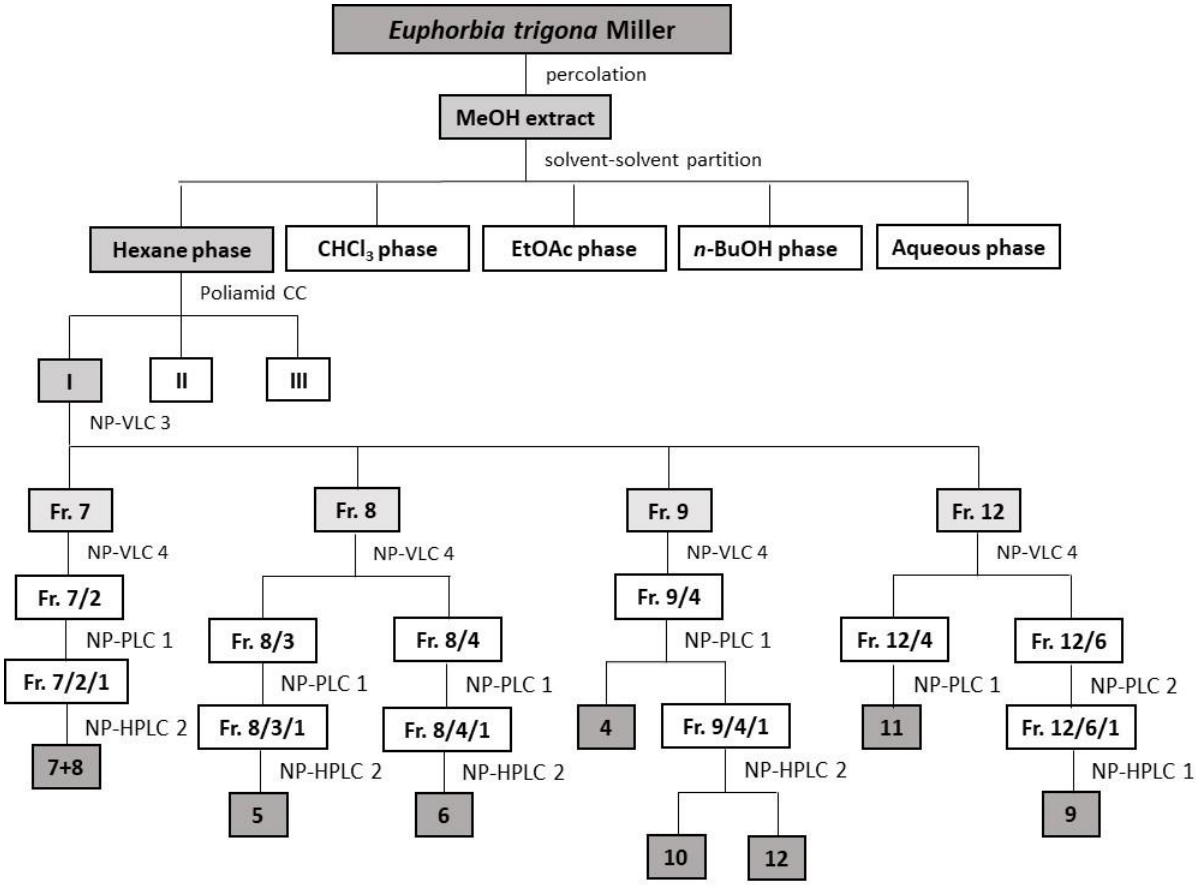


Figure 2. Isolation of the compounds of *E. trigona*

Isolation of the compounds of *E. gossypina* var. *coccinea*

The dried ground aerial parts of *E. gossypina* var. *coccinea* (1 kg) was percolated with MeOH at room temperature (**Fig. 3**). The methanol extract was then concentrated *in vacuo*, dissolved in 50% aqueous MeOH and solvent-solvent partition was performed *n*-hexane, $CHCl_3$ and EtOAc, respectively. After evaporation, the $CHCl_3$ fraction was chromatographed by VLC on silica gel with a gradient system of cyclohexane–EtOAc–MeOH (from 8:2:0 to 6:3:1) to yield 20 major fractions (Fr. 1–20). These fractions were further separated by using combined chromatographic techniques (NP- and RP-VLC, NP- and RP-PLC, and HPLC) to afford 14 compounds (**2**, **13**–**25**) (**Fig. 3**).

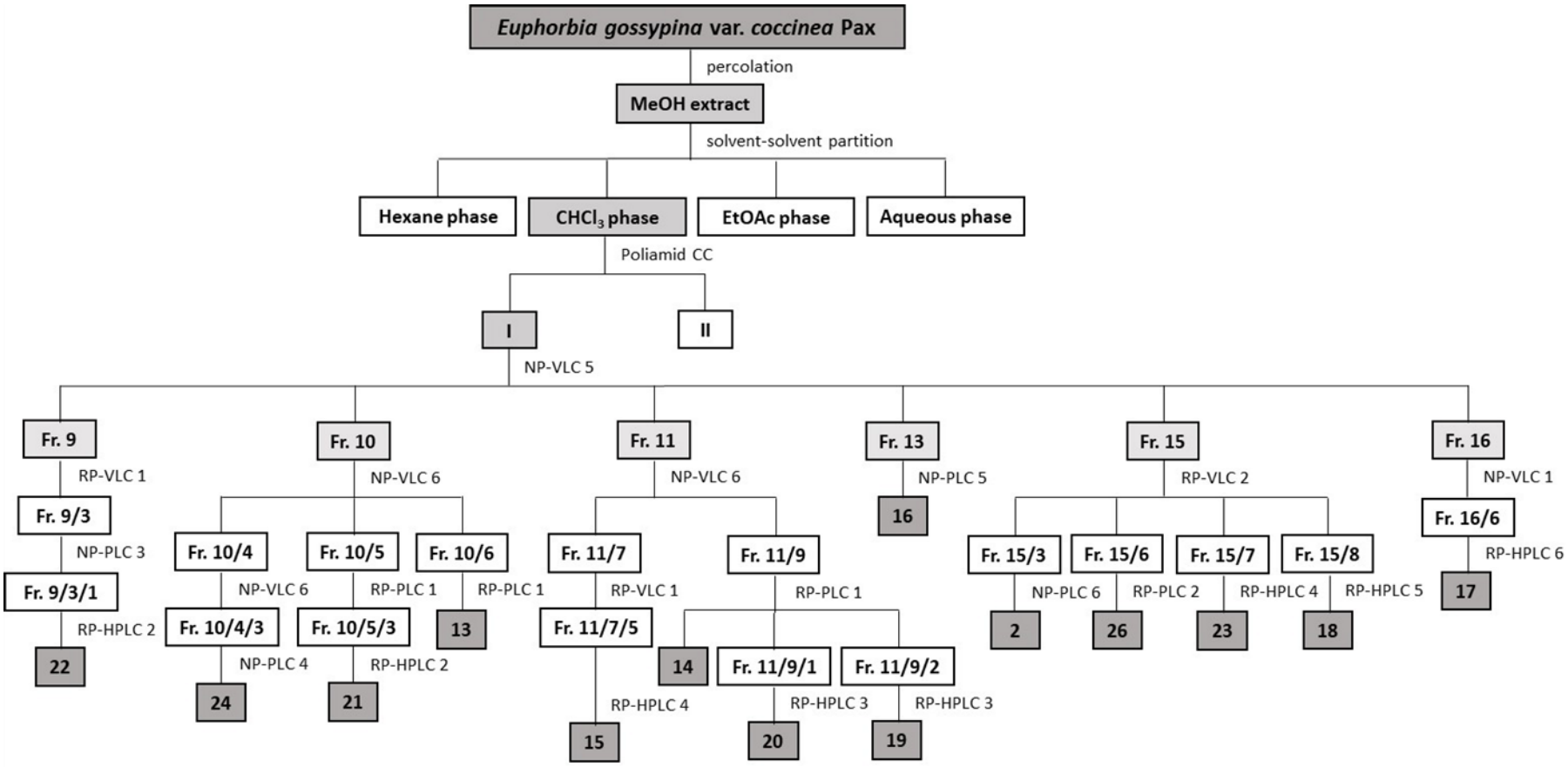
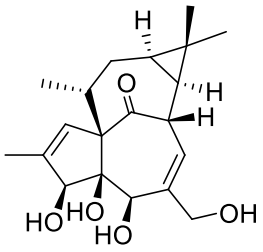


Figure 3. Isolation of compounds from *E. gossypina* var. *coccinea*

Compounds from *E. matabelensis*, *E. trigona* and *E. gossypina* var. *coccinea*

From the methanolic extract of *E. matabelensis* three compounds [one diterpene (**1**) and two flavonoids (**2**, **3**)] were isolated (**Fig. 4**). Purification of the methanolic extract of *E. trigona* resulted in nine diterpenes (**4**–**12**), while twelve compounds, among them nine pregnane glycosides (**13**–**20**, **23**), three lignans (**21**, **22**, **24**), and two flavonoids (**2**, **25**) were determined from *E. gossypina* var. *coccinea*. The structure elucidation of the compounds was carried out by using HR-MS measurements, 1D (^{1}H and ^{13}C) and 2D NMR (^{1}H-^{1}H COSY, HSQC, HMBC, NOESY) spectroscopic methods, and comparison of NMR spectral data with literature values.



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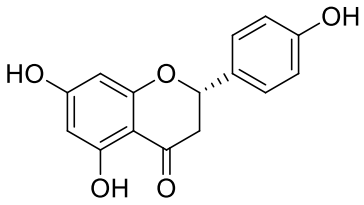

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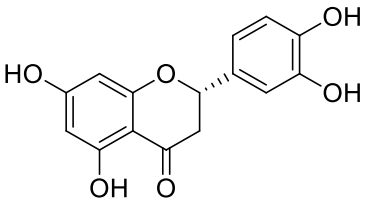

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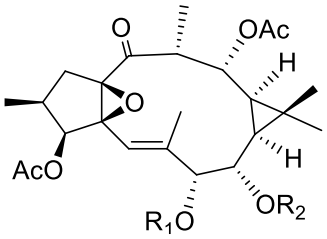
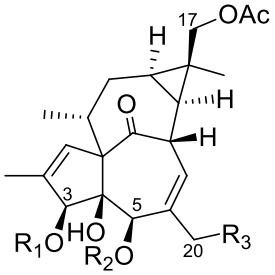
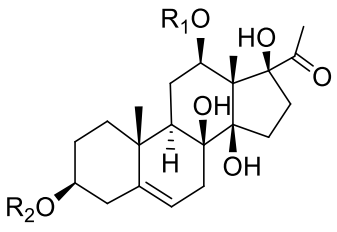


Compound	R_1	R_2
4	Tig	H
5	Tig	Me
6	Bz	Me
7	Ac	Bz
8	Ac	Tig



Compound	R_1	R_2	R_3
9	Ang	H	OAc
10	Ang	Ac	OAc
11	Ang	H	H
12	H	Ang	H



Compound	R_1	R_2 sugar chain			
		Sugar 1	Sugar 2	Sugar 3	Sugar 4
13	Bz	Cym	Cym	Thv	
14	Bz	Dig	Dig	Thv	
15	Bz	Cym	Dig	Thv	
16	Bz	Cym	Cym	Thv	Glc
17	Bz	Dig	Dig	Thv	Glc

18	Bz	Cym	Dig	Thv	Glc
19	Ac	Dig	Dig	Thv	
20	H	Cym	Dig	Thv	
23	Bz				

Sugar 3 (**13**-**15**, **19**, **20**) and sugar 4 (**16**-**18**) are in terminal position.

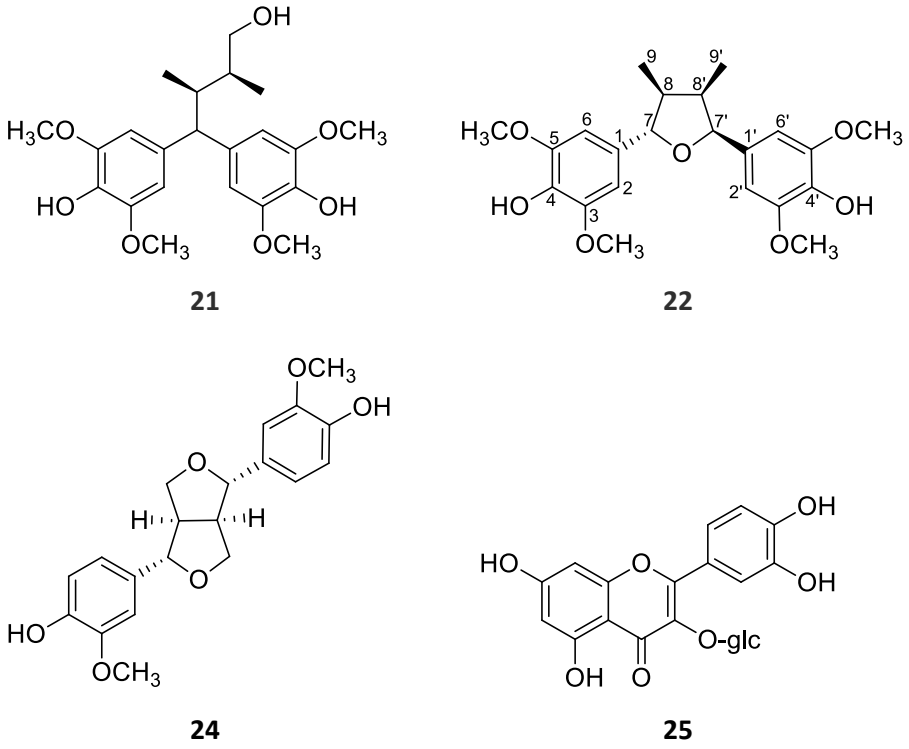


Figure 4. Structures of the compounds (**1**–**25**) isolated from *E. matabelensis*, *E. trigona* and *E. gossypina var. coccinea*

Compounds **13**–**22** were determined to be new natural metabolites, and were named as euphogossypins A–H (**13**–**20**) and gossypilignans A and B (**21** and **22**). Besides new compounds, five ingol-type diterpenoids [ingol 3,12-diacetate 7-tigliate (**4**), 8-*O*-methyl-ingol 3,12-diacetate 7-tigliate (**5**), 8-*O*-methyl-ingol 3,12-diacetate 7-benzoate (**6**), ingol 3,7,12-triacetate 8-benzoate (**7**), and ingol 3,7,12-triacetate 8-tigliate (**8**)], four ingenol esters [17-acetoxyingenol 3-angelate 20-acetate (**9**), 17-acetoxyingenol 3-angelate 5,20-diacetate (**10**), 17-acetoxy-20-deoxyingenol 3-angelate (**11**), and 17-acetoxy-20-deoxyingenol 5-angelate (**12**)], one pregnane aglycone [12-*O*-benzoyl-deacylmetaplexigenin (**23**)], one lignan [9*α*-hydroxypinoresinol (**24**)], and three flavonoids [naringenin (**2**), eriodictyol (**3**) and quercitrin (**25**)] were isolated from the investigated *Euphorbia* species.

10 Compounds are diterpenes and except ingenol (**1**) they are polyesters, substituted with acetyl, tigloyl, benzoyl and angeloyl groups. Interestingly, ingenols are substituted with tigloyl, while ingenanes with angeloyl groups. Compounds **7**, **8** and **10** are the most highly esterified diterpenoids, with 4 ester groups. The pregnane glycosides (**13**–**20**) are cynanforidine or metaplexigenin derivatives substituted with deoxy sugars, cymarose, digitoxose and thevetose. In case of compounds **16**–**18**,

glucose is the terminal sugar. Moreover, all pregnanes but **20** are esterified by benzoyl or acetyl group at C-12. The lignans isolated from *E. gossypina* var. *coccinea* belong to different subgroups, compound **21** is a new 7,7-diarylbutanol *seco*-lignan, compound **22** is a new tetrahydrofuran lignan derivative, while the known **24** is a furofuran-type lignan.

All compounds were isolated for the first time from *E. matabelensis* and *E. gossypina var. coccinea*, and all compounds with the exception of **10** and **11** from *E. trigona*.

Pharmacological activity of the isolated compounds

Antiproliferative activity:

The three compounds from *E. matabelensis* (**1**–**3**) were investigated for their antiproliferative activity against 4 human tumor cell lines (HeLa and C33a [cervix adenocarcinomas], MCF-7 and MDA-MB-231 [breast carcinomas]) by means of MTT assay with cisplatin as a positive control [IC_{50} values range from 3.69 μM on C33a to 19.13 μM on MDA-MB-231]. In comparison with cisplatin, ingenol (**1**), naringenin (**2**) and eriodictyol (**3**) exhibited insignificance growth inhibition activity against the utilized cell lines at 30 μM concentration.

As pregnane glycosides and lignans known to have antiproliferative activities, all isolated compounds from *E. gossypina* were also tested for their antiproliferative activity against HeLa cell line using the MTT assay. Doxorubicin and cisplatin were used as positive controls (IC_{50}s 0.02 ± 0.003 μM and 2.07 ± 0.07 μM, respectively). Among the tested compounds, only euphogossypin A (**13**) showed weak antiproliferative activity (IC_{50} 52.4 ± 0.23 μM), while the others proved to be inactive.

Keratinocyte inhibitory activity:

The extracts prepared from *E. candelabrum*, *E. cotinifolia*, *E. ramipressa*, and *E. trigona* were evaluated at concentrations of 5 and 0.5 μg/mL (**Fig. 5**). Ingenol mebutate administered at 5 μg/mL for 24 h exerted the strongest cytotoxic effect, and after a 48-h treatment, lower cytotoxicity was measured. The treatment of keratinocytes with ingenol mebutate at a 0.5 μg/mL concentration resulted in a weaker, but still significant, cytotoxic effect after 24 h and, interestingly, after 48 h, the inhibitory activity was comparable to the treatment when 5 μg/mL was used for 48 h. The extracts with a 5 μg/mL concentration were applied for 24 h, and ETP1 had similar, but lower, cytotoxic activity than that of ingenol mebutate. Interestingly, the 48-h treatment of cells with 5 μg/mL extracts ECA1, ECO2, ER1, ER2, and ETP1 showed a very similar cytotoxic property as ingenol mebutate. Cytotoxic activity was the lowest when the extracts were used at a 0.5 μg/mL concentration for 24 h, but in the case of ER2 and ETP1, cytotoxic activity was significant compared to the control and quite similar to that of ingenol mebutate. After administration of the extracts at a 0.5 μg/mL concentration for 48 h, only ETP1 displayed cytotoxicity comparable to that of ingenol mebutate. Based on these results, the *n*-hexane extract of *E. trigona* (ETP1) could be considered the most promising one for further investigation.

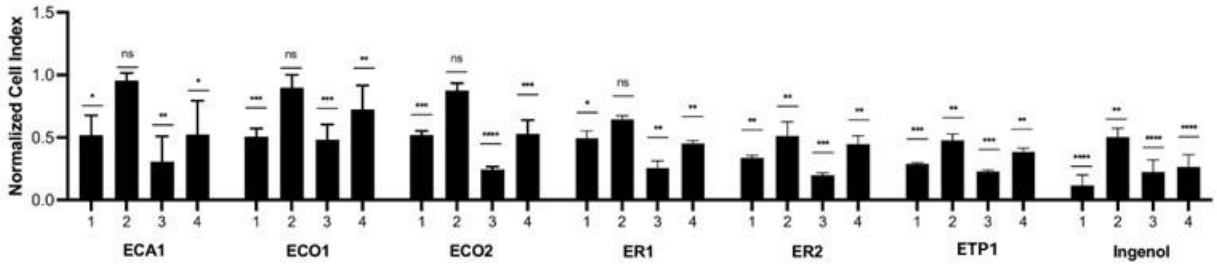


Figure 5. Inhibitory activity of different *Euphorbia* extract against keratinocytes. ECA1: *E. candelabrum n*-hexane extract, ECO1: *E. cotinifolia n*-hexane extract, ECO2: *E. cotinifolia* $CHCl_3$ extract, ER1: *E. ramipressa n*-hexane extract, ER2: *E. ramipressa* $CHCl_3$ extract, ETP1: *E. trigona n*-hexane extract; 1: 5 µg/mL, 24 h, 2: 0.5 µg/mL, 24 h, 3: 5 µg/mL, 48 h, 4: 0.5 µg/mL, 48 h; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Since the isolated compounds from *E. trigona*, especially the ingenol-derivatives (**9**–**12**), are structurally very similar to ingenol mebutate, it was rational to test whether they have any effect on the viability of keratinocytes. The HPV-Ker cell line was treated with the isolated ingol- and ingenol-type diterpenoids in the concentration range of 5×10^{-9}–5×10^{-4} M, and the viability was measured for 72 h, data at 24 and 48 h were used for calculations (**Table 1**).

Table 1. IC_{50} values (µM ± SD) of the ingol- and ingenane-type diterpenoids (**4**–**12**).

Compound	IC_{50} value (µM)	
	24h	48h
4	14.19 ± 2.85	1.72 ± 0.14
5	17.29 ± 1.65	14.48 ± 3.78
6	inactive	
7+8	4.50 ± 0.93	0.66 ± 0.05
9	0.39 ± 0.09	0.32 ± 0.05
10	0.32 ± 0.02	0.87 ± 0.07
11	4.32 ± 0.92	-
12	14.83 ± 3.83	7.93 ± 1.71
Ingenol mebutate	0.84 ± 0.01	0.96 ± 0.03

Ingenol mebutate, which was used as a positive control, showed IC_{50} values of 0.84 and 0.96 µM at 24 and 48 hours of treatment, respectively. The ingenol-type compounds 17-acetoxyingenol 3-angelate 20-acetate (**9**) and 17-acetoxyingenol 3 angelate 5,20-diacetate (**10**) possessed the same order of magnitude as ingenol mebutate (**Fig. 6**). Moreover, the IC_{50} values of **9** (0.39 µM and 0.32 µM) and **10** (0.32 µM and 0.87 µM) were slightly lower on the HPV-Ker cell line than that of ingenol mebutate. For 17-acetoxy-20-deoxyingenol 5-angelate (**12**), about one and two order of magnitude higher IC_{50} values were recorded after 24 and 48 hours of treatment (14.83 and 7.93 µM) than for ingenol mebutate (**Table 1**).

In case of the ingol-type diterpenoids **4**, **5** and **7**+**8** the measured IC_{50} values were one or two orders of magnitude higher than that of ingenol mebutate, except for **7**+**8** at 48 h (0.66 µM) (**Table 1**).

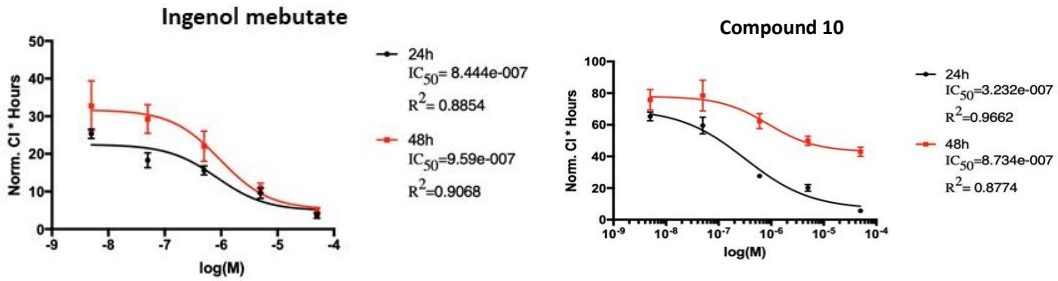


Figure 6. RTCA (real-time cell analysis) measurement of CI (cell index) values of HPV-Ker cells treated with ingenol mebutate and compound **10**. Normalized CI * hours values were plotted as a function of concentration of the indicated diterpenoid (logM).

Based on the pharmacological results in our study, structure–activity relationship (SAR) investigations could also be performed. The ingenol derivatives **9**–**12** are structurally close to ingenol mebutate. The main difference between ingenol mebutate and the isolated compounds is the presence of an acetoxy group at C-17 in these compounds instead of a 17-methyl group in ingenol mebutate. In the less active 17-acetoxy-20-deoxyingenol 5-angelate (**12**), the angeloyl group at C-3, and hydroxy group at C-5, are replaced compared to ingenol mebutate and to the compounds **9**–**11**; therefore, it was concluded that the presence of the angeloyl group at C-3 seems to be essential for the cytotoxic activity. Since compound **11** differs from ingenol mebutate in only the presence of an acetoxy group at C-17, and its activity was lower, the acetoxy group alone, presumably, is not able to increase the activity. In the case of the most active compounds **9** and **10**, one (at C-20 in **9**) or two (at C-5 and C-20 in **10**) additional acetyl groups are attached to the diterpenoid core; therefore, acetylation of the molecule results in increased cytotoxic activity. It is in accordance with the previously determined SAR statement that the carbonyl moieties of the ester groups are essential for the desired biological effects and the activation of PKC, which likely happens through interaction with Gly23 NH in the C1 domain. Moreover, besides the activation of PKCδ, ingenol mebutate was found to reduce the expression of PKCα, which is the PKC isoform responsible for the promotion of cell survival. Thus, further studies are required to evaluate the beneficial effect of our compounds that might result in more effective isoform-specific regulation.

From the above results, it could be concluded that ingenane-type diterpenes should be considered therapeutically relevant natural products. In case of other compounds (e.g., pregnane glycosides and lignans) other pharmacological investigations (e.g., antihyperglycaemic or phytoestrogen) can be more promising.

SUMMARY

The aim of this work was the isolation and structure determination of specialized metabolites from African *Euphorbia* species, *E. matabelensis*, *E. trigona,* and *E. gossypina* var. *coccinea*. First, the contents of the plant materials were investigated by using a CC, TLC-based screening method. The isolation was carried out by a multistep separation procedure, including OCC, VLC, PLC and NP- and RP-HPLC. In some cases, compounds with very similar structures were separated: pregnane glycosides (**13**–**15** and **16**–**18**) are differing only in the connecting deoxy sugars (cymarose or digitoxose) or glucose moieties, while the ingenane diterpenes **11** and **12** differ in the position of the angeloyl group. The structures of the isolated compounds were elucidated by means of spectroscopic methods (HR-MS and NMR). As a result of 1D (^{1}H and JMOD), and 2D NMR studies (COSY, HMBC, HSQC, HMQC), complete ^{1}H and ^{13}C assignments were made for the characterization of the compounds.

As a result of our work, altogether 25 compounds were isolated from the three investigated *Euphorbia* species; one diterpene (**1**) and two flavonoids (**2**, **3**) from *E. matabelensis*, nine diterpenes (**4**–**12**), among them five ingols (**4**–**8**) and four ingenols (**9**–**12**) from *E. trigona*, and eight new pregnane glycosides (**13**–**20**), two new lignans (**21** and **22**), one known pregnane (**23**), one known lignan (**24**), and two known flavonoids (**2** and **25**) from *E. gossypina* var. *coccinea*. 10 compounds are diterpenes and except ingenol (**1**) they are polyesters, substituted with acetyl, tigloyl, benzoyl and angeloyl groups. Interestingly, ingenols are substituted with tigloyl, while ingenanes with angeloyl groups. Compounds **7**, **8** and **10** are the most highly esterified compounds among the isolated diterpenoids, with 4 ester groups. The pregnane glycosides (**13**–**20**) are cynanforidine or metaplexigenin derivatives substituted with deoxy sugars, cymarose, digitoxose and thevetose. In case of compounds **16**–**18**, glucose is the terminal sugar. Moreover, all pregnanes but **20** are esterified by benzoyl or acetyl group at C-12. The lignans isolated from *E. gossypina* var. *coccinea* belong to different subgroups, compound **21** is a new 7,7-diarylbutanol *seco*-lignan, compound **22** is a new tetrahydrofuran lignan derivative, while the known **24** is a pinoresinol-type lignan.

Based on the diterpene composition, *E. trigona* displays a close relationship with *E. hermentiana*, *E. antiquorum*, *E. canariensis*, *E. candelabrum E. royleana,* and *E. kamerunica* as all species belong to the same Section and Subsection of genus *Euphorbia*, all of them are succulents, and they accumulate similar diterpenes.

All compounds (**1**–**3**) from *E. matabelensis*, and all ingol derivatives (**4**–**8**) and the ingenane diterpenes **9** and **12** from *E. trigona* were detected for the first time from the plants. The chemical constituents of *E. gossypina* var. *coccinea* have not been investigated previously.

The *in vitro* pharmacological activities of the isolated compounds were tested in different test systems. Among them, the ingenol-type 17-acetoxyingenol 3-angelate 20-acetate (**9**) and 17-

acetoxyingenol 3 angelate 5,20-diacetate (**10**), differing from ingenol mebutate only in the esterification pattern, showed higher cytotoxic activity on keratinocytes after 24 and 48 h of administration than the positive control ingenol mebutate.

Compounds isolated in our experiments enlarge the natural compound library and verify the botanical relationship of some of the *Euphorbia* species. Our investigations open up new opportunities for natural product-based drug discovery and development; especially ingenol esters **9** and **10** are promising for design new drugs for the treatment of actinic keratosis.

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LIST OF PUBLICATIONS RELATED TO THE THESIS

I. **Hammadi R**, Kúsz N, Mwangi PW, Kulmány Á, Zupkó I, Orvos P, Tálosi L, Hohmann J, Vasas A. Isolation and pharmacological investigation of compounds from *Euphorbia matabelensis* *Natural Product Communications* **2019**, *14*, 1–5. DOI: 10.1177/1934578X19863509 **IF: 0.482**

II. **Hammadi R**, Kúsz N, Dávid CZ, Behány Z, Papp L, Kemény L, Hohmann J, Lakatos L, Vasas A. Ingol and ingenol-type diterpenes from *Euphorbia trigona* Miller with keratinocyte inhibitory activity
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III. **Hammadi R**, Kúsz N, Dávid CZ, Mwangi PW, Berkecz R, Szemerédi N, Spengler G, Hohmann J, Vasas A.
Polyoxypregnane ester derivatives and lignans from *Euphorbia gossypina* var. *coccinea* Pax. *Plants* **2022**, *11*, 1299. DOI: 10.3390/plants11101299 **IF: 4.658***

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1. **Hammadi R**, Kúsz N, Hohmann J, Vasas A.
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