Biointerface Research in Applied Chemistry
Open-Access Journal (ISSN: 2069-5837)

Review
Volume 14, Issue 5, 2024, 101
https://doi.org/10.33263/BRIAC145.101

Euphorbia resinifera: Chemical Composition and Biological Properties (Short Review)

Oumaima Boutoub [1], Lahsen El Ghadraoui [2], Maria Graça Miguel [1,3,*]

[1] Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; boutoub.oumaima@gmail.com (O.B.)
[2] Laboratory of Functional Ecology and Environment, Faculty of Science and Technology, BP 2202, University Sidi Mohamed Ben Abdallah, Fez 20000, Morocco; lahsen.elghadraoui@usmba.ac.ma (L.E.G.)
[3] MED—Mediterranean Institute for Agriculture, Environment and Development, CHANGE—Global Change and Sustainability Institute, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; mgmiguel@ualg.pt (M.G.M.)
* Correspondence: mgmiguel@ualg.pt;

Scopus Author ID: 35562054500
Received: 7.04.2024; Accepted: 12.05.2024; Published: 21.07.2024

Abstract: This review updates the information on the chemical composition of *E. resinifera* (spurge resin) latex and its biological properties, as well as those from the aerial parts and bee products (honey and propolis), generally without latex. From such review and according to the studies developed so far, it was possible to confirm that the chemical composition of latex and non-latex compounds differs. In the latex, diterpenes, nor sesquiterpenes, triterpenes, and serine proteases (EuRP-61) with 61 kDa predominate despite other minor compounds. The identified protease had anticoagulant, antiplatelet, and peripheral blood cell aggregation inhibitory properties. Phenolics, including flavonoids and tannins, have antioxidant activity and are dominant in the aerial parts of *E. resinifera*. Phenolic acids and flavonoids, particularly flavanones, flavones, and flavonols and their glycosides, were identified in the spurge resin honey. Generally, honey samples were within the acceptable limit of international standards. Antioxidant activity was reported for this monofloral honey. Antimicrobial activity was also detected in hydroalcoholic extracts of propolis.

Keywords: latex; aerial parts; terpenes; serine proteases; phenols; honey; propolis.

© 2024 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Euphorbiaceae is known as the spurge family and is one of the largest families of flowering plants, with over 300 genera and 8000 species. The genera in the spurge family are widely used in traditional medicine for the treatment of several diseases, such as respirational infections, digestion complaints, nutritional diseases, body pain, body and skin irritations, inflammatory infections, snake or scorpion bites, endocrine, pregnancy/birth/puerperium disorders, genitourinary syndromes, and sensory disorders. The genus *Euphorbia* contains several other subgenera and sections with over 2,000 species, characterized by the production of milky irritant latex [1].

Euphorbia resinifera Berg. (Libana maghrabi in Arabic or “Zaqqum” or “Zaggoume” in local language), a large, leafless cactus-like perennial, is endemic to Morocco, generally distributed in the center of the country, in the regions of Azilal and Beni Mellal (Middle Atlas), with some scattered populations in the High Atlas Mountains and the Anti-Atlas [2-4]. *E.*

resinifera appears on sunny slopes, mainly in the rocky and arid rocks of the low limestone mountains between 600 to 1,500 m. In 1966, the Moroccan Ministry of Agriculture and Agricultural Reform considered the geographical area of this species to be the Haouz, Middle Atlas, Umm-er-Rbia, Central High Atlas (M'Goun), and JbelLkest. However, currently, that species occurs mainly in the Beni Mellal-Khenifra region [5].

E. resinifera has a thick, woody stem originating from a number of fleshy, quadrangular, spiny green branches. Their specialized flowering structure is called cyathium, which consists of a cup-like pseudanthial inflorescence. Cyathia appear in small groups at the top of branches, forming clusters of yellow pseudo-inflorescences [6]. *E. resinifera* forms large, cushionlike, glaucous green, 0.80-1.50 m high shrubs, with stems branched only at the base, forming dense bushes of 0.5-2 min diameter [7,8].

After an analysis of variance, it was possible to detect significant differences between the populations of *E. resinifera*, which means high phenotypic variability within this species. This conclusion was obtained after analyzing seventeen qualitative and quantitative morphological characteristics related to the bush, stem, spine, flower, and fruit of the plant of twelve natural populations collected from its geographical range in Morocco [8]. In another publication [9], the same team also studied Morocco's genetic diversity and structure of E. resinifera populations. In the assay, the authors used twelve populations of this species from diverse altitudes and geographical areas, and through molecular markers, the inter-simple sequence repeats (ISSRs) primers (fourteen), they concluded that there exists high genetic diversity, and that variation mainly occurs within populations. The generation of secondary metabolites by spurge resin was attempted through in-vitro propagation methods to produce undifferentiated (callus and cell suspension cultures) and the micropropagation of *Euphorbia resinifera* [10]. This genetic diversity may require a previous selection of plants to achieve adequate yields.

E. resinifera can be the target of infections; for example, Muntañola-Cvetković and Gómez-Bolea [7] described plants with pale brown lesions extending upwards toward the branches and surrounded with brownish margins found in the desert of Marrakech. This appearance was attributed to an infection by a new sporodochial hyphomycete, *Pseudostilbella euphorbia.* These diseases, along with natural fires promoted by local populations for agriculture or forage, utilization for domestic burning, and urban stress, have reduced their healthy population [7,8,11].

E. resinifera has been used in folk medicine in which Moroccan patients mixed the aerial parts with honey or using extracts obtained by decoction method in the treatment of general cancer, whereas fresh latex is used for poisonous punctures, bites, analgesic, and dental pains. Flower infusion or water latex has also been used as antidiabetic despite its toxicity since exposure to latex induces oral, dermal, and ocular symptoms [1,12-16]. In Algeria, this plant treats rheumatism, cysts, and snake bite poisoning [17,18].

Currently, *E. resinifera* is used in the melliferous field because the resin spurge (*E. resinifera*) honey is considered a local product with Protected Geographical Indication (PGI) in the Tadla-Azilal region [5] and considered among the best honey with a unique peppery taste and pungent and powerful aroma [19]. This monofloral honey has also been reported to possess antibacterial and antifungal activity and, therefore, is considered interesting from medical and cosmetic points of view [4].

This review intends to compile the chemical and biological data of latex and non-latex extracts and those of honey, a local Moroccan product with PGI in the Tadla-Azilal region. A

review was recently published [20] that focused more on the biological characteristics of *E. resinifera* than its chemical composition. The chemical composition of various plant parts and those derived from bee products is the primary topic of the current review. At least five new manuscripts have been uncovered since the last review article, indicating the interest in this species.

2. Materials and Methods

The approach of this review was made by seeking the Web of Science (WoS) and PubMed databases. The keyword was "*Euphorbia resinifera*". In the WoS, 66 results were obtained, whereas in PubMed, 39 results were found. In the WoS database, the first results date from 1973, and the last are from 2023. Still in this database, the results included 2 meeting abstracts and 64 complete articles. The first meeting abstract dates from 1973, and the second one from 2014. Figure 1 depicts the evolution of the publication over time, making it clear that there is a significant increase in the number of publications from 2016. The same Figure depicts the evolution of the publications found in the PubMed database using the keyword "*Euphorbia resinifera*"—the first publication dates from 1969. Three articles were in Chinese, and only the abstracts were in English, so they are referred to in the present manuscript but only in the abstracts.

The lower number of publications (37) found in the PubMed database with the sole keyword "*Euphorbia resinifera*" does not mean that the other ones found in the WoS database cannot be observed; such only means that the WoS database has a larger search window than PubMed when the same keyword is used during the seeking process.

Eleven findings in the PubMed database were not found in the WoS one. All findings in the PubMed database and 58 in the WoS one were reported in the present review; therefore, four references are not cited. The main reason is the difficulty of having access to these references. In addition to these references, 15 others not found in these databases were mentioned in this work, as we considered that they would enrich the review work.

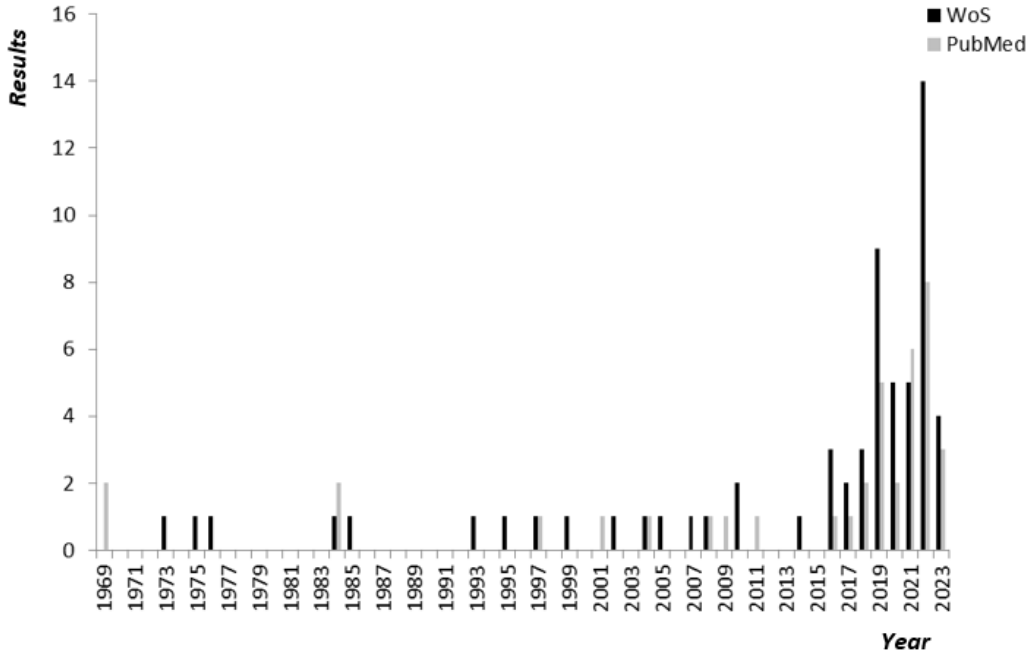


Figure 1. The evolution of the publications on *Euphorbia resinifera* over time was registered in the WoS and PubMed databases.

3. Chemical composition of latex

3.1. Diterpenes and nor sesquiterpenes and their biological properties.

Several diterpenes isolated from *E. resinifera* and other species of the same genus possess medical interest due to their anticancer activity (ingenol 3-angelate) or potent analgesic properties (resiniferatoxin). There were homeopathic mother tinctures of *E. resinifera*. The ingenol content could range from 0.5 to 16.7 μg/mL, determined by high-performance liquid chromatography and after hydrolysis with KOH in methanol [19]. Despite this possible medical interest in these compounds, there has been little information about the biosynthesis of these *Euphorbia* diterpenes. Kirby *et al*. [21] investigated terpene biosynthesis and reported on the distribution of diterpene synthases within the Euphorbiaceae family. The authors discovered genes encoding putative casbene synthases in all of the selected Euphorbiaceae species (*Homalanthus nutans*, *Euphorbia resinifera*, *Euphorbia esula*, *Sapium sebiferum*, and *Ricinus communis*) and a neocembrene synthase in *R. communis*. The authors did not consider gibberellins' biosynthesis [21]. The carbene synthase is important in synthesizing phorbol esters [22]. These authors concluded that carbene synthase from four *Euphorbia* species (*Euphorbia esula*, *Euphorbia resinifera*, *Euphorbia peplus*, and *Euphorbia fischeriana*) was highly identical; nonetheless, carbene synthase from *Jatropha curcas* (family Euphorbiaceae) derives from a different origin than the remaining Euphorbiaceae plants. In a review made by Fattahian *et al*. [23], it was clear that two mechanistically different biogenetic pathways are known in diterpene biosynthesis: one of them will originate the phytanes such as abietanes, kauranes, atisanes, etc.; and the second one will originate the macrocyclic and polycyclic diterpenes such as jatrophane-, casbane-, lathyrane-, tigliane-, ingenane- and daphnane-type. For example, the biosynthesis of 12-deoxy phorbol 13-phenylacetate (tigliane-type) seems to be derived either from cytosol-localized mevalonic acid (MVA) or plastid-localized methylerythritol 4-phosphate (MEP) pathways, though MEP pathway playing a dominant role, according to the next-generation sequencing technologies to build a transcriptome dataset assayed by Zhang *et al*. [24]. The same type of core structures of diterpenes was present in the *E. officinarum*, as would be expected and reviewed recently [25].

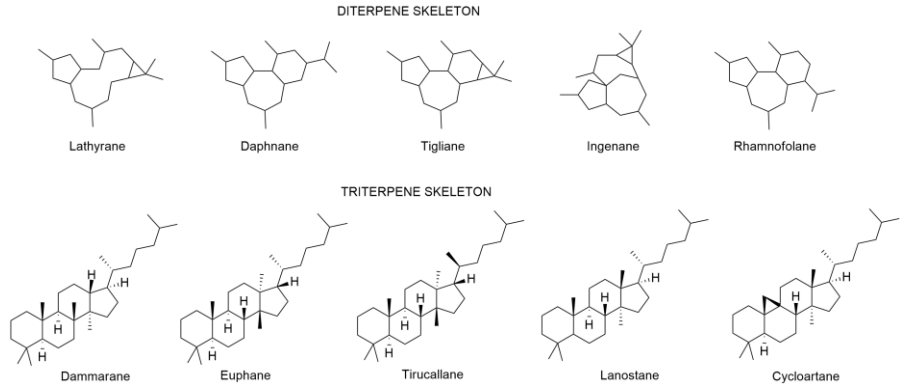
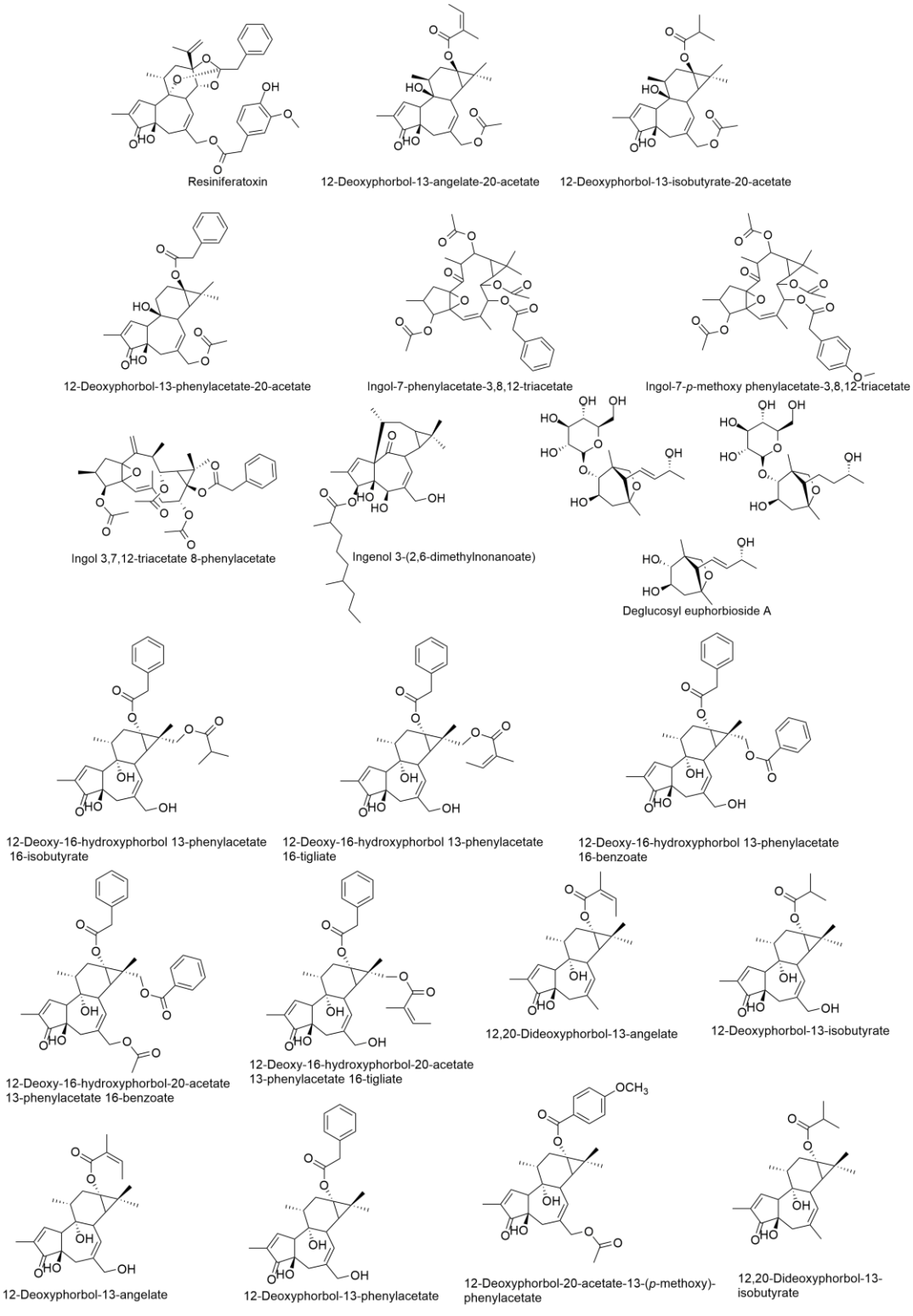


Figure 2. Main diterpene and triterpene skeletons are present in the latex of *E. resinifera* [30].

Resiniferatoxin, a daphnane diterpene (Figure 2), was isolated and identified for the first time by Hergenhahn *et al*. [26] in the fresh latex of *E. resinifera*. The authors verified that during the drying process of latex, the compound concentration decreased due to oxidation. This observation might explain the non-utilization of fresh samples for medicinal purposes [27]. The dried resinous preparation is called euphorbium. It is one of the oldest drugs in the Western medicinal tradition due to its pharmacological properties despite its emetic,

powerfully purgative, and sternutatory effects [28,29]. Euphorbium is used to mitigate toothache in chronic pain and articular tuberculosis [29].

From euphorbium, Hergenhahn *et al*. [31] isolated 3 diterpene fractions, two irritating the mouse ear. In these two fractions, the authors isolated and identified the following tigliane diterpenes: 12-deoxyphorbol-13-angelate-20-acetate, 12-deoxyphorbol-13-isobutyrate-20-acetate, 12-deoxyphorbol-13-phenylacetate-20 -acetate (Figure 3), and a mixture of the ingenane diterpenes ingenol-3-acylates. The lathyrane diterpenes ingol-7-phenylacetate-3,8,12-triacetate and ingol-7-p-methoxy-phenylacetate-3,8,12-triacetate did not present irritant activity.



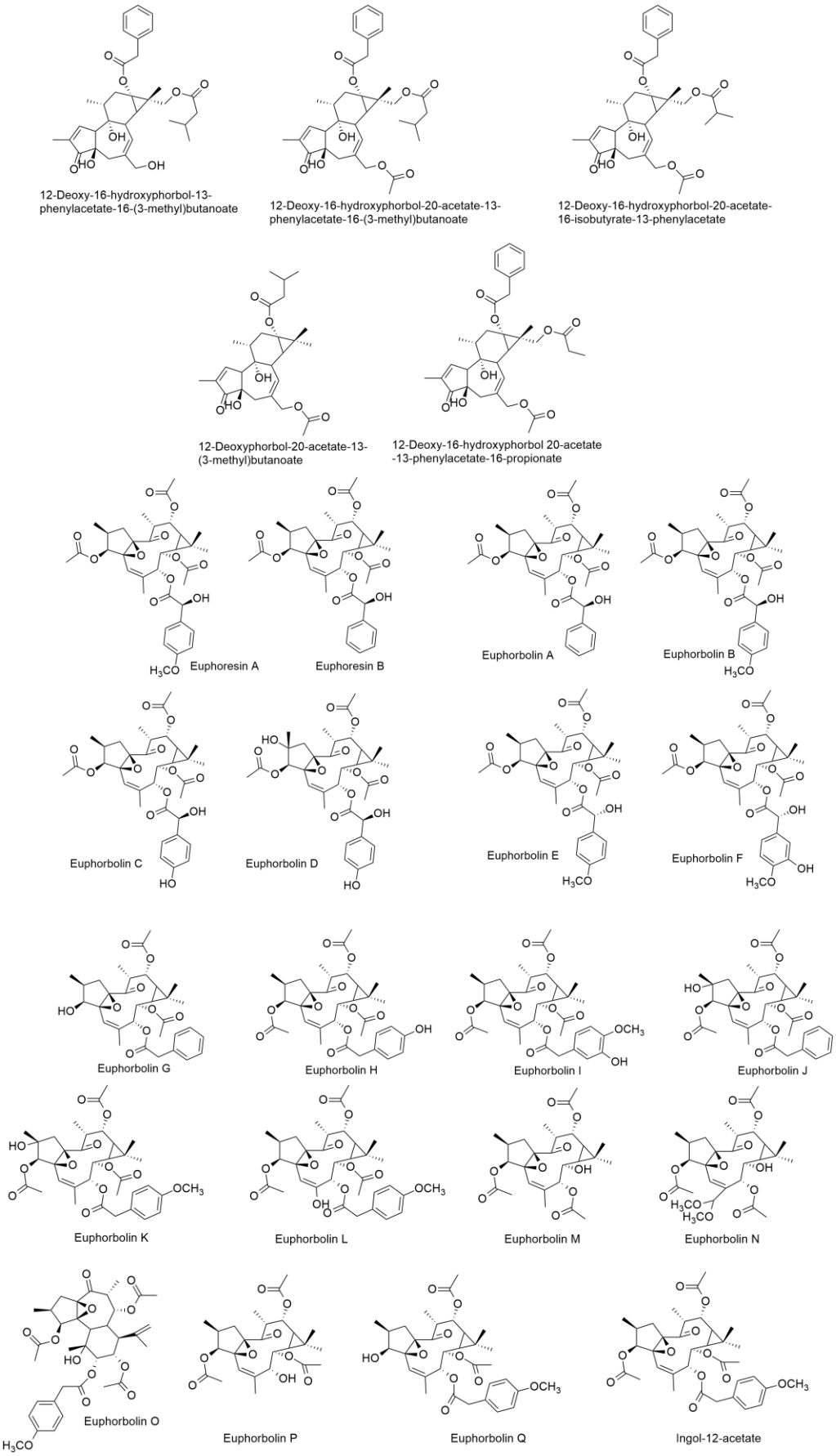


Figure 3. Chemical structures of some diterpenes present in the latex of *E. resinifera*.

These two diterpenes were also reported by Ezzanad *et al*. [32] for latex from *E. resinifera* collected in Demnate, Beni Mellal-Khenifera province (Morocco). The absence of physical and spectroscopic data of ingol-7-phenylacetate-3,8,12-triacetate being the acylation

pattern of the positional isomer deduced from partial hydrolysis experiments makes difficult to be sure about the presence of this isomer and not another positional isomer [29]. Ingol 3,7,12-triacetate-8-(phenylacetate) was spectroscopically confirmed by Fattorusso *et al*. [29] in the fresh latex of *E. resinifera* along with other diterpenes such as ingenol 3-(2,6-dimethylnonanoate), and 12-deoxyphorbol-13-isobutyrate-20-acetate (Figure 3). This identification approach of the spurge resin diterpenoids, including the determination of optical rotations, was essential for the confirmation of the presence of 12-deoxyphorbol-13-isobutyrate-20-acetate in other species of *Euphorbia* growing in different latitudes [33]. Fattorusso *et al*. [29] also isolated and identified three new bisnorsequiterpenes combining spectroscopic data and chemical reactions: the bisnorsequiterpenes glycosides euphorbioside A and euphorbioside B, as well as the aglycone of euphorbioside A (deglucosyl euphorbioside A) (Figure 3) [29]. Later on, Ourhzif *et al*. [34] also isolated euphorbioside monohydrate from *E. resinifera* latex.

12-Deoxyphorbol-13-angelate-20-acetate and 12-deoxyphorbol-13-isobutyrate-20 acetate were also detected by Ourhzif *et al*. [35] in the dichloromethane fraction of the latex of *E. resinifera*, along with and ingol-7-*p*-methoxy-phenylacetate-3,8,12-triacetate and resiniferatoxin (Figure 3). The authors also reported the norsesquiterpenoids deglucosyl euphorbioside A and euphorbioside A but in the *n*-butanol fraction. 12-Deoxyphorbol-13-isobutyrate-20-acetate inhibited the growth of *Aspergillus carbonarius*, whereas 7-*p*-metoxyphenylacetate-3,8,12-triacetate ingol and deglucosyl euphorbioside A had a cytotoxic effect on breast cancer cell line MCF7 with an increase in the level of intracellular reactive oxygen species (ROS), but not on MCF10A normal breast cells [35].

From the latex of *E. resinifera*, Hergenhahn *et al*. [28] isolated 20 factors (RL1-20), with some of these compounds (RL1, RL2, RL9, RL14, RL20) had been previously identified and reported by the same team [26], and from the resin (euphorbium) they isolated 4 factors (RR1-RR4) or compounds. Hergenhahn *et al*. [28] reported that many of these factors of tigliane-, ingenane-, or daphnanae-types sharing some chemical particularities had strong irritant properties. For example, strong irritant qualities were found in tigliane-type 12-deoxy phorbol esters that had either long-chain, partially methyl-substituted acyl residues (10–16 carbon atoms) or short-chain acyl residues (4–5 carbon atoms) or a (substituted) phenyl acetyl group with a 20-acetoxy group in the 13 position; likewise, long-chain 3-esters of ingenane-type ingenol with similar acyl residues (10–16 carbon atoms, partially methyl-substituted) were irritant; or the daphnane type: 9,13,14-ortho phenyl acetate of resiniferonol-20-(4-hydroxy-3-methoxy)phenylacetate (resiniferatoxin), and 9,13,14-ortho phenyl acetate of resiniferonol. Nevertheless, among these irritant diterpenes, others were nonirritant, which were esters of the tigliane-type 12,20- dideoxy phorbol and the lathyrane-type ingol [28]. Thus, in addition to their irritating qualities, naturally occurring diterpene esters with tigliane, daphnane, and ingenane skeletons also have the ability to promote tumor growth and have additional biological impacts on both normal and tumor cells. Long-chain acids are present in the most potent diterpene ester tumor promoters, phorbol and ingenol esters [36, 37]. Some of these diterpenes, nevertheless, might not have much of an effect on tumor growth. Since 12-deoxyphorbol-13-phenylacetate lacks a long-chain acid, it was determined to be one of the RL factors discovered by Hergenhahn *et al*. [28]. This phorbol ester is not known to promote tumor growth. Nevertheless, compared to prostratin (12-deoxy phorbol 13-acetate), 2-deoxy phorbol 13-phenylacetate was significantly more powerful in inducing type 1 human

immunodeficiency virus (HIV-1) gene expression in latently infected ACH-2 T cells. This is likely because of its more lipophilic side chain structure [38].

The authors described resiniferatoxin isolated by Hergenhahn *et al*. [28] from *E. resinifera* as an extremely irritant. This daphnane diterpene is an analog of capsaicin and a potent activator of transient receptor potential cation channel subfamily V member 1 (TrpV1), also known as capsaicin receptor and vanilloid receptor 1. TrpV1 is a nonselective cation channel with high Ca^{2+} permeability composed of up to six transmembrane segments with a pore region between the fifth and the sixth segment, which is mainly expressed on peripheral nociceptive C-fibers and at a lesser extent on Aδ-fibers, but also in cortex, hippocampus, amygdala, and periaqueductal grey of the central nervous system. Endocannabinoids, capsaicin, resiniferatoxin, and temperatures above 43°C can open the channel pore. The activation of TrpV1 leads to a painful and burning sensation; nevertheless, upon prolonged exposure to capsaicin or resiniferatoxin, desensitization occurs, a phenomenon that leads to an analgesic effect but remains unaffected the perception of cold, touch-sense, and locomotor function [39,40]. The molecular key is in the residues Tyr511, Met547, and Thr550 of the transmembrane regions of TrpV1, being its key regulatory site where capsaicin or resiniferatoxin establish bonds [41].

Resiniferatoxin is about 1,000-fold more potent than capsaicin. For this reason, resiniferatoxin has been used in some clinical trials as a potential analgesic to relieve cancer and arthritis pain [42,43] and, in some cases, a pain reliever by desensitizing cardiac sensory fibers expressing TrpV1, which could improve chronic heart failure [44], nevertheless, there are bioanalytical limitations in the quantification of resiniferatoxin in plasma due to the minimal effective dose that is in the range of few nanograms [42]. These authors developed a method using ultra-high-performance liquid chromatography coupled to tandem mass spectrometry with an electrospray ionization source (UHPLC-ESI-MS/MS) in multiple reaction monitoring modes. With this approach, Sharma *et al*. [42] reported that it is possible to have an efficient, rapid, and reliable method to quantify a low concentration of resiniferatoxin, which makes it easier to determine its pharmacokinetic profile, data important in therapeutics.

The analgesia promoted by the resiniferatoxin, isolated from the latex of *E. resinifera*, is due to the interaction with the TrpV1; nevertheless, the latex isolated from a relative of *E. resinifera*, *E. bicolor* Engelm. & A. Gray, also demonstrated analgesic properties through the same action mechanism of that of the spurge resin, assessed through *in vivo* assays (male and female rats). The authors isolated some compounds that belonged to the coumestans, diterpenes, and flavonoid groups. However, they did not present a relationship between any compounds identified in the latex and the agonist action on the TrpV1 [45].

Ziglioli *et al*. [39] reported that apoptosis in prostate cancer cells by the vanilloids, such as capsaicin or resiniferatoxin, can be mediated by a TrpV1-1-dependent (indirect pathway) and a TrpV1-1-independent (direct pathway) mechanism. In the former case, the vanilloids need to interact with the receptor TrpV1-1, which leads to an intracellular calcium increase and late elements of apoptosis, whereas, in the last mechanism, the vanilloids inhibit the electron transport chain because they are analogs of the coenzyme Q, with the consequent electron transport chain inhibition, and great delivery of ROS. Finally, and still in this mechanism, vanilloids also induce apoptosis by interacting with caspases 1 and 3.

Two tigliane diterpenes (Figure 3) were isolated from *E. resinifera* latex from Demnate, Beni Mellal-Khenifera Province (Morocco), and identified as 12-deoxy-16-hydroxyphorbol-

13-phenylacetate-16-isobutyrate and 12-deoxy-16-hydroxyphorbol-13-phenylacetate-16-tigliate [32,46]. The former compound facilitated the transforming growth factor alpha (TGFα) release and promoted neural progenitor cell proliferation at nanomolar levels [46]. Still from the same harvesting area (Demnate, Beni Mellal-Khenifera Province, Morocco), Ezzanad *et al*. [32] isolated and identified other 12-deoxy phorbol esters, confirming the chemical structures of some of them already reported for *E. resinifera* such as 12-deoxy-16-hydroxyphorbol-16-benzoate-13-phenylacetate and 12-deoxy-16-hydroxyphorbol-20-acetate-13-phenylacetate-16-tigliate, confirming the results already reported by Hergenhahn *et al*. [28] for spurge latex; 12-deoxy-16-hydroxyphorbol-20-acetate-16-benzoate-13-phenylacetate and 12,20-dideoxyphorbol-13-angelate also previously reported [26,47]; or in other species such as 12-deoxyphorbol-13-isobutyrate; 12-deoxyphorbol-13-angelate; 12-deoxyphorbol-13-phenylacetate; 12-deoxyphorbol-20-acetate-13-(*p*-methoxy) phenylacetate; 12,20-dideoxyphorbol-13-isobutyrate; 12-deoxy-16-hydroxyphorbol- 13-phenylacetate-16-(3-methyl)butanoate; and 12-deoxy-16-hydroxyphorbol-20-acetate- 13-phenylacetate-16-(3-methyl)butanoate) (Figure 3) [32]. In the same work, three new 12-deoxy phorbol esters were identified by these authors: 12-deoxy-16-hydroxyphorbol-20-acetate-16-isobutyrate-13-phenylacetate, 12-deoxyphorbol-20-acetate-13-(3-methyl)butanoate, and 12-deoxy-16-hydroxyphorbol 20-acetate-13-phenylacetate-16-propionate (Figure 3).

Two novel ingol-type diterpenes have been isolated from the methanol extract of the latex of *E. resinifera* after separation by diverse chromatographic methods: euphoresin A and euphoresin B. These compounds had weak cytotoxic effects against MCF-7 (breast cancer cell line), U937 (pro-monocytic model cell line), and C6 (rat glial tumor) cells, with values of IC_{50} (half maximal inhibitory concentration) at least ten times higher than those found for taxol [48].

Zhao *et al*. [49] identified 18 diterpenes in the *E. resinifera* latex, including 14 new ingol-type (Figure 2) diterpenoids (euphorblins A-N) (Figure 3), a new rhamnofolane diterpenoid (euphorblin O), and three known analogs (euphorblin P and Q and ingol-12-acetate) (Figure 3). The physical data of euphorblin P and Q were reported for the first time [49]. Euphorbolins B and D and ingol-12-acetate were able to induce lysosomal biosynthesis through the upregulation of the lysosomal genes lysosomal-associated membrane protein 1 (LAMP1), cathepsin B (CTSB), cathepsin A (CTSA), and ATPase H^+ transporting V0 subunit E1 (ATP6 V0E1). According to the authors, such compounds must be deeply studied for Alzheimer's disease since, in this disease, lysosome lysis occurs, and the proteases are released, giving rise β-amyloid accumulation and neuron death [50].

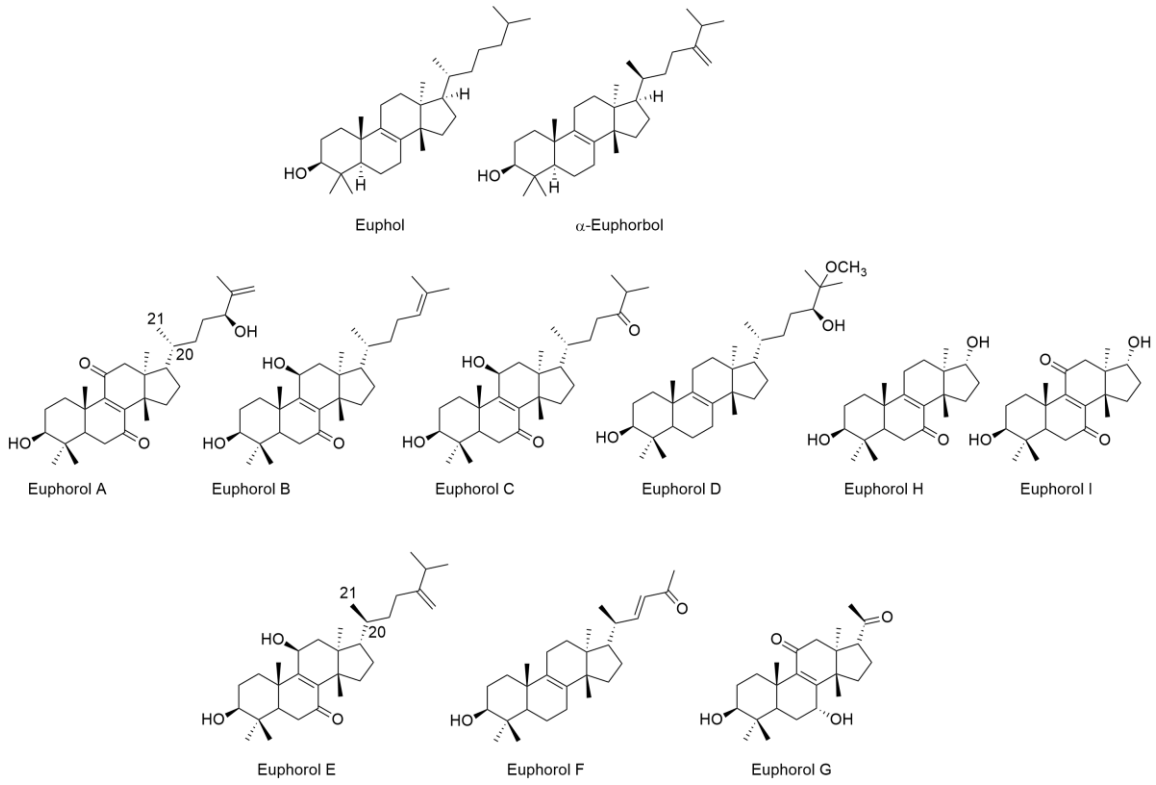
3.2. Triterpenes and their biological properties.

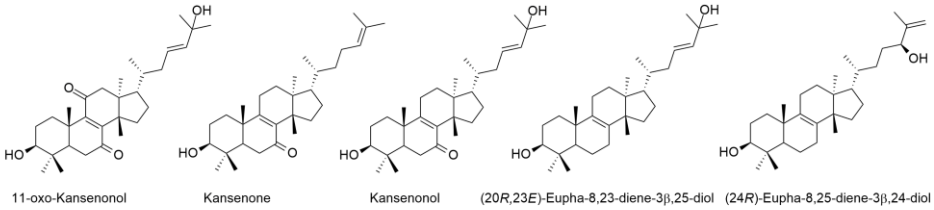
In the latex of *E. resinifera* have been isolated triterpenes with dammarane, euphane, tirucallane, lanostane, cycloartane (Figure 2), and nor triterpenes in which the carbon loss generally occurs at the side chain.

As aforementioned, from the euphorbium, Fattorusso *et al*. [29] isolated diterpenes, new bisnorsequiterpenes, and the triterpenes euphol and euphorbol (Figure 4). Such compounds were also isolated and identified from the resinified latex euphorbium by Newbold and Spring [51], who had already reported that euphorbol had also previously been isolated from euphorbium but in very small amounts. Zhou *et al*. [52] showed that euphol (47.03%) and euphorbol (18.77%), which constitute euphorbium total triterpenes, can significantly lower serum levels of TNF-α, C-reactive protein (CRP), interleukin (IL)-1β, IL-6, IL-18, and rheumatoid factor (RF) in rats in a concentration-dependent manner; additionally, when

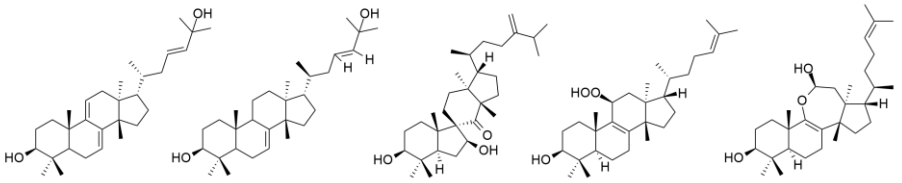
Freund's complete adjuvant (FCA) was used to induce arthritis in rats, the degree of swelling and the histopathological change of the hind paws in rats, as well as the arthritic score, were significantly improved. Acute toxic symptoms, such as irregular respiration, secretion, excretion, or death, were also not observed at doses up to 12 g/kg of euphorbium total triterpenes.

From the air-dried latex of *E. resinifera*, Wang *et al*. [53] isolated six new euphane triterpene derivatives (Figure 4) (euphorol A-D, H and I), three new tirucallane (20*S*-stereoisomer of euphane) triterpene derivatives (euphorol E-G) and seven known compounds, that were previously isolated from other species of the Euphorbiaceae, Melicaceae and Orchidaceae families (11-oxo-kansenonol, kansenone, kansenonol, (20R, 23*E*)-eupha-8,23-diene-3β,25-diol, (24*R*)-eupha-8,25-diene-3β,24 diol, kansenol, and 3β,25-dihydroxy-tirucalla-7,23-diene). All triterpenes isolated and identified were tetracyclic-type but with different grades of oxidation, number, and position of double bonds obtained via oxidation, epoxidation, reduction, hydrolyzation, and dehydration from the same 8-ene dammarane triterpene precursor. According to this observation, the authors [53] proposed a biogenetic pathway for all of those compounds. All compounds were tested for cytotoxicity against the MCF-7, U937, and C6 cancer cell lines. The most effective compound was (24*R*)-eupha-8,25-diene-3β,24-diol, with IC_{50} values of 56.2 µM, 34.6 µM, and 49.6 µM, respectively. However, it was 5–9 times weaker than the positive control taxol [53]. Later on, another team described one new tirucallane triterpene with a spiro [5,6] ring system (euphorol K) and a novel euphane triterpene hydroperoxide (euphorol J), along with kansuinone (Figure 4) [54]. The activity of these compounds against the same tumor cell lines previously studied was investigated, and the authors found that euphorol J had better activity than the remaining compounds, presenting the following IC_{50} values (37.36 µM, 47.17 µM, and 46.89 µM, respectively) [54].

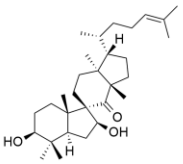




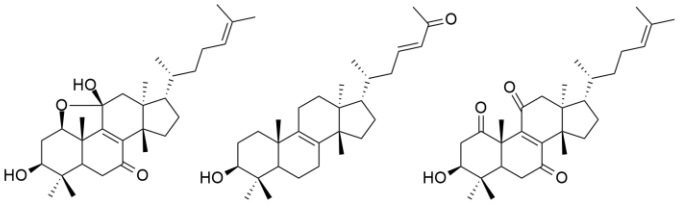
11-oxo-Kansenonol Kansenone Kansenonol (20*R*,23*E*)-Eupha-8,23-diene-3β,25-diol (24*R*)-Eupha-8,25-diene-3β,24-diol



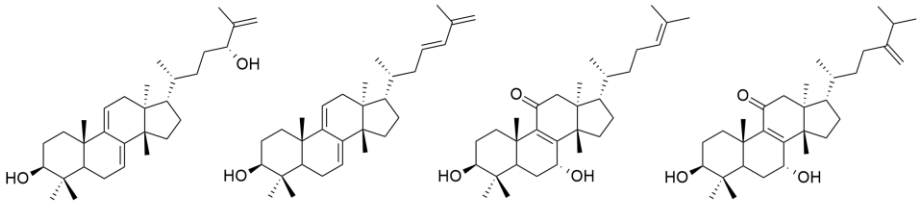
Kansenol 3β,25-dihydroxy-Tirucalla-7,23-diene Euphorol K Euphorol J (putative) Euphorol J (revised)



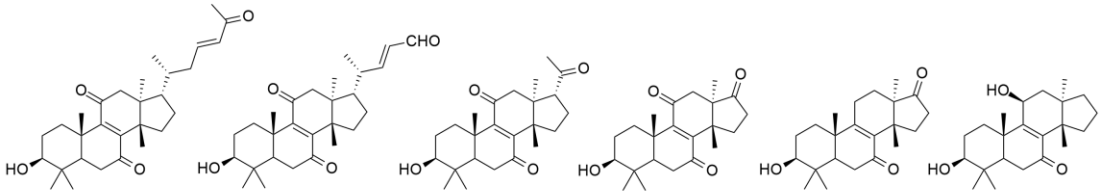
Kansuinone



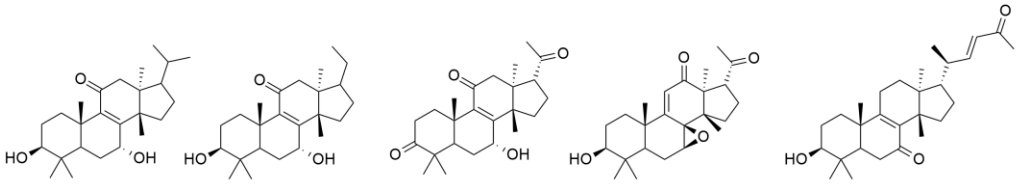
Euphatexol A Euphatexol B Euphatexol C



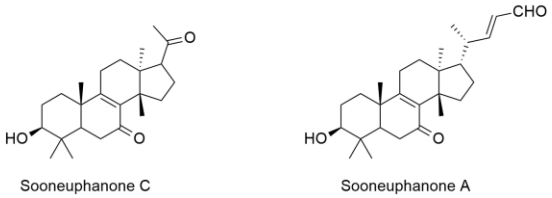
Euphatexol D Euphatexol E Euphatexol F Euphatexol G



Euphorbiumrin A Euphorbiumrin B Euphorbiumrin C Euphorbiumrin D Euphorbiumrin E Euphorbiumrin F



Euphorbiumrin G Euphorbiumrin H Euphorbiumrin I Euphorbiumrin J Euphorol F



Sooneuphanone C Sooneuphanone A

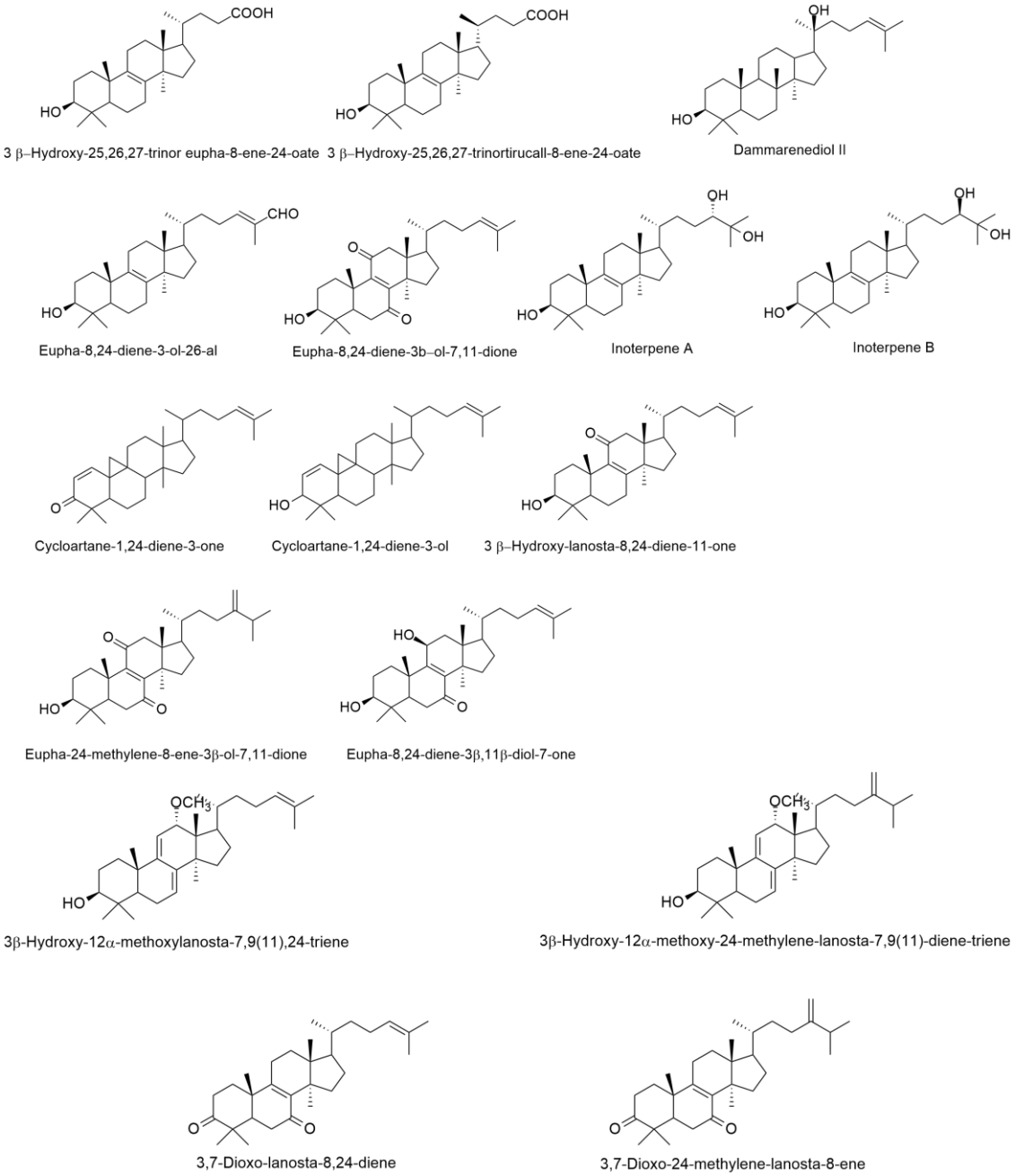


Figure 4. Chemical structures of some triterpenes present in the latex of *E. resinifera*.

From 3*β*-hydroxy-25,26,27-trinor eupha-8-ene-24-oate, the chemical structures were written only according to the name of the compounds described in the abstracts [55-57]. The authors did not have access to the main text. The structure of euphorol J is depicted as [54]; nevertheless, later on, this structure was revised [58], and the structure is depicted as euphorol J revised. In addition, the structure of lnonotusane C is not provided because it is unknown to the authors.

From the latex of *E. resinifera*, the authors Qi *et al*. [59] isolated and identified an unsual euphane triterpenoid due to the presence of a tetrahydrofuran ring formed via C-1 and C-11 (euphatexol A) (Figure 4). An unusual 27-noreuphane triterpenoid was also isolated (euphatexol B) (Figure 4). Both had cytotoxic activity against HepG2, with 87.0% and 87.4% inhibition rate at 1 µM, respectively. However, the positive control, cisplatin, showed a 78.6% inhibition rate at 0.3 µM, better activity than the triterpenes [59]. The same team, as a part of their ongoing program for searching bioactive compounds, reported five new triterpenoids isolated from the latex of *E. resinifera* (euphatexols C-G) (Figure 4) with anti-inflammatory

activity because they were able to inhibit nitric oxide production-induced by lipopolysaccharide (LPS) in RAW264.7 cells. The IC_{50} values ranged from 20.35 µM (euphatexol E) to 48.04 µM (euphatexol D). The IC_{50} for the positive control (dexamethasone) was 20.35 µM [60].

Beyond euphatexol B, a 27-noreuphane triterpenoid previously identified [59], Zhao *et al*. [61] isolated and identified other nortriterpenoids from euphorbium. They named the ten new nortriterpenoids euphorbium in A-J (Figure 4). Along with these components, the authors also found the known compounds euphorol F [53], sooneuphanone C, and sooneuphanone A (Figure 4) [62]. The nortriterpenoids had a dammarane skeleton, with the main differences at C-17 side chain moiety. The authors proposed a possible biosynthetic pathway for the nortriterpenoids. In general, they consider that euphol is the compound from which all nortriterpenoids identified are biosynthesized after degradation of its side chain at C-17, C-22, C-23, C-25, and C-27, which may also undergo epoxidation, oxidation, reduction, hydrolyzation, and dehydration reactions [61], as already suggested by Wang *et al*. [53]. Euphorbiumrin E was found to have the highest activity when it came to inhibiting the tomato yellow leaf curl virus (TYLCV) at a dose of 40 µg/mL, with an inhibition rate of 71.7% [61].

Wang *et al*. [55] isolated and identified ten triterpenes from a methanolic extract of *E. resinifera* latex: 3*β*-hydroxy-25,26,27-trinor eupha-8-ene-24-oate, *iso*-maticadienediol, 25,26,27-trinortirucall-8-ene-3*β*-ol-4-acid, dammarenediol II, eupha-8,24-diene-3-ol-26-al, lnonotusane C, eupha-8,24-diene-3*β*-ol-7,11-dione, inoterpene A, inoterpene B, and eupha-24-methylene-8-ene-3*β*-ol-7,11-dione (Figure 4). According to the authors [55], 3*β*-hydroxy-25,26,27-trinor eupha-8-ene-24-oate was a new natural product. In contrast, *iso*-maticadienediol, 25,26,27-trinor-tirucall-8-ene-3*β*-ol-4-acid, dammarenediol II were first described in the Euphorbiaceae, and eupha-8,24-diene-3-ol-26-al, lnonotusane C isolated for the first time from the genus *Euphorbia*. The structures were confirmed and identified by spectroscopic methods and physicochemical properties after the separation of the compounds through chromatographic methods using silica gel, octadecylsilyl groups (ODS groups or C18 groups), and semi-preparative high-performance liquid chromatography (HPLC). The cytotoxic properties of these compounds were also assayed against MCF-7, U937, and C6 cell lines [55].

Using the same extraction methodologies but with ethanol as extraction solvent, separation, and identification, Li *et al.* [56] reported seven triterpenoids in the latex of *E. resinifera*. Two cycloartane triterpene structures (Figure 2) are new compounds (cycloartan-1,24-diene-3-one and cycloartan-1,24-diene-3-ol) (Figure 4), and one lanostane triterpene structure (Figure 2) was obtained from nature for the first time (3β-hydroxy-lanosta-8,24-diene-11-one): The remaining compounds were: lnonotusane C, eupha-8,24-diene-3β-ol-7,11-dione, eupha-24-methylene-8-ene-3β-ol-7,11-dione, and eupha-8,24-diene-3β,11β-diol-7-one. In this case, no biological activity was reported in the abstract, the sole document consulted in this work.

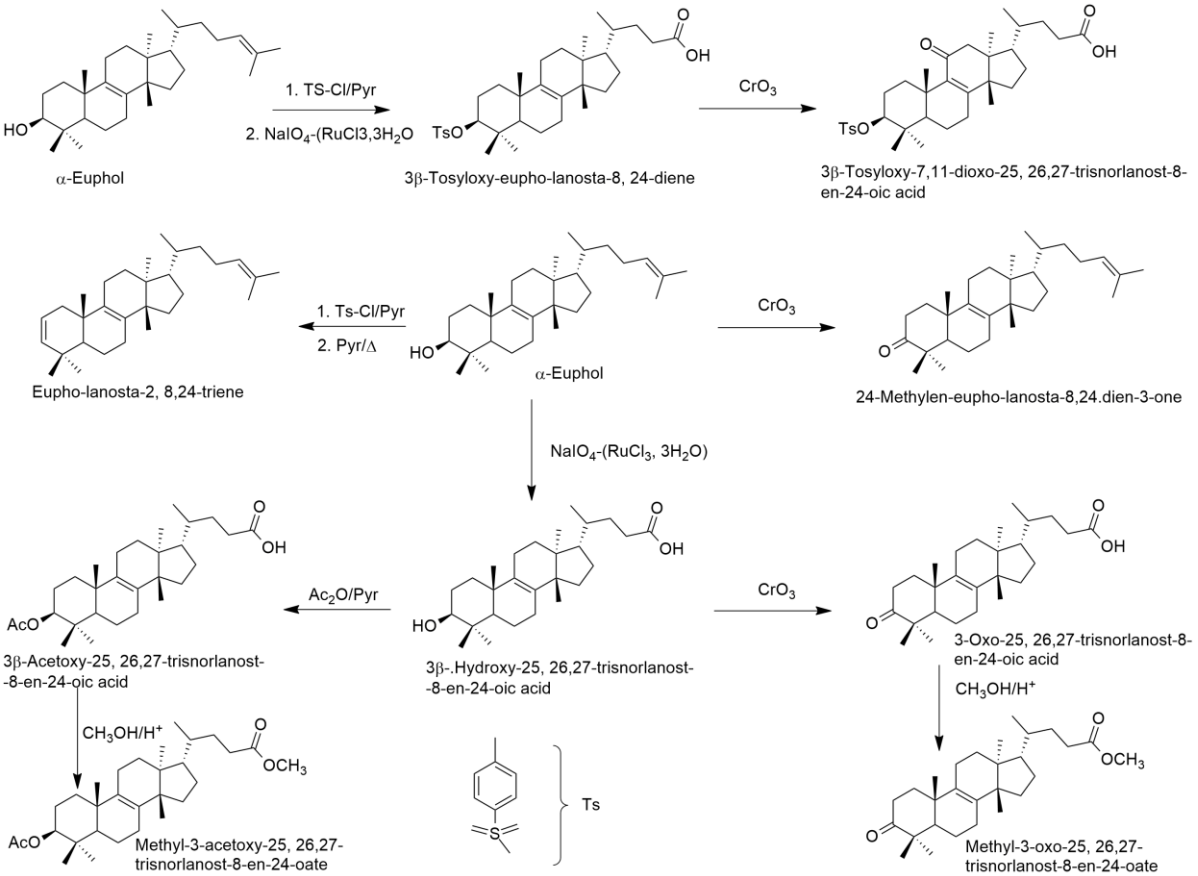
Beyond the lanostane triterpenoids aforementioned, four new ones were reported by Li *et al.* [57]: 3β-hydroxy-12α-methoxylanosta-7,9(11),24-triene, 3β-hydroxy-12α-methoxy-24-methylene-lanost-7,9(11)-diene, 3,7-dioxo-lanosta-8,24- diene, and 3,7-dioxo-24-methylene-lanost-8-ene (Figure 4) in the latex of *E. resinifera*. The first two compounds and the last one were those with moderate inhibition activity of LPS-induced nitric oxide (NO) production by RAW264.7. Their IC_{50} values were 30.4, 37.5, and 28.3 µmol/L, respectively.

3.3. Hemisynthesis of triterpene derivatives from euphol and euphorbol isolated from E. resinifera latex.

The pharmacological properties (anti-inflammatory, antimicrobial, antiplasmodial, and anti-insecticidal properties) described for dammarane triterpenes have led to the development, by hemisynthesis, of new triterpenoid derivatives, generally through tosylation, acylation and oxidation methods, giving rise to much more oxygenated derivatives, that according to some authors, present remarkable biological properties [63-69]. Figure 5 illustrates the hemisynthesis of several α-euphol derivatives after reactions of tosylation, acylation, and oxidation. Figure 6 shows the hemisynthesis of several α-euphorbol derivatives after reactions of tosylation, acylation, and oxidation. It is recommended to consult the previous publications [63-66,69] that provide detailed information about the hemisynthesis reactions.

Recent *in silico* studies of euphol and euphorbol derivatives showed that 3β-tosyloxy-7,11-dioxo-25, 26,27-trisnorlanost-8-en-24-oic acid (Figure 5) had promising antibacterial and insecticidal activities when compared to the remaining derivatives albeit further experimental validation will still be required [69].

The strongest leishmanicidal compounds were 3*β*-tosyloxy-24-methylen-elemo-lanosta-8,24-dien-11-one, 24-methylen-elemo-lanosta-8-en-24-epoxy-3*β*-ol, and 3*β*-acetoxy-24-methylen-elemo-lanosta-8,24-dien-7,11-dione with ED_{50} (the effective dose to give 50% cell viability) values of < 4 µg/mL. Concerning *Trypanosoma cruzi*, the most active compounds were *β*-acetoxy-24-methylen-elemo-lanosta-8,24-dien-7,11-dione and 3*β*-tosyloxy-24-methylen -elemo-lanosta-8,24-dien-11-one, with ED_{50} values < 5 µg/mL [65].



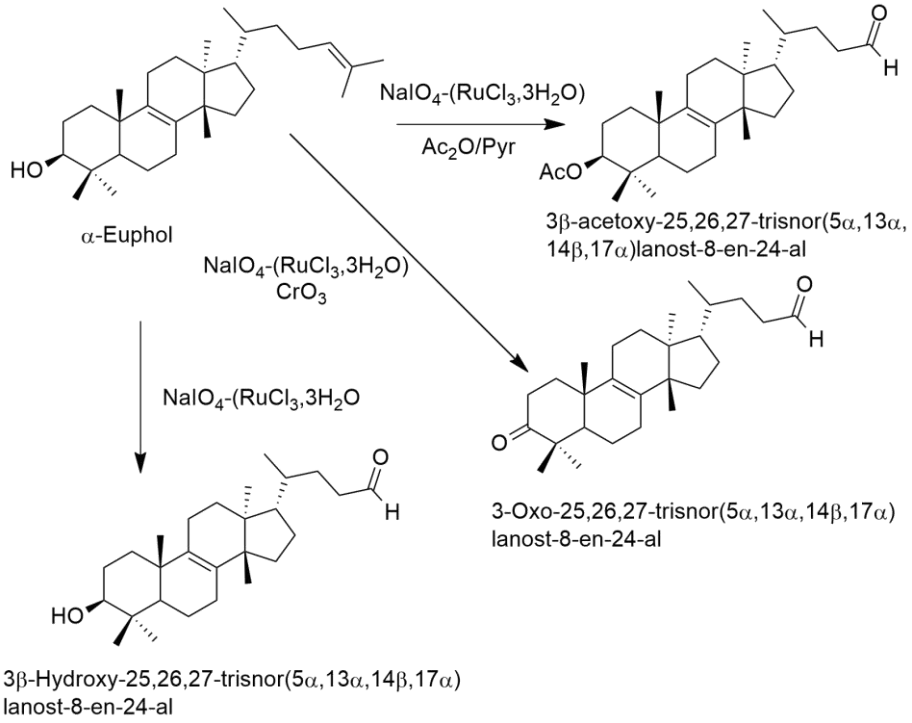
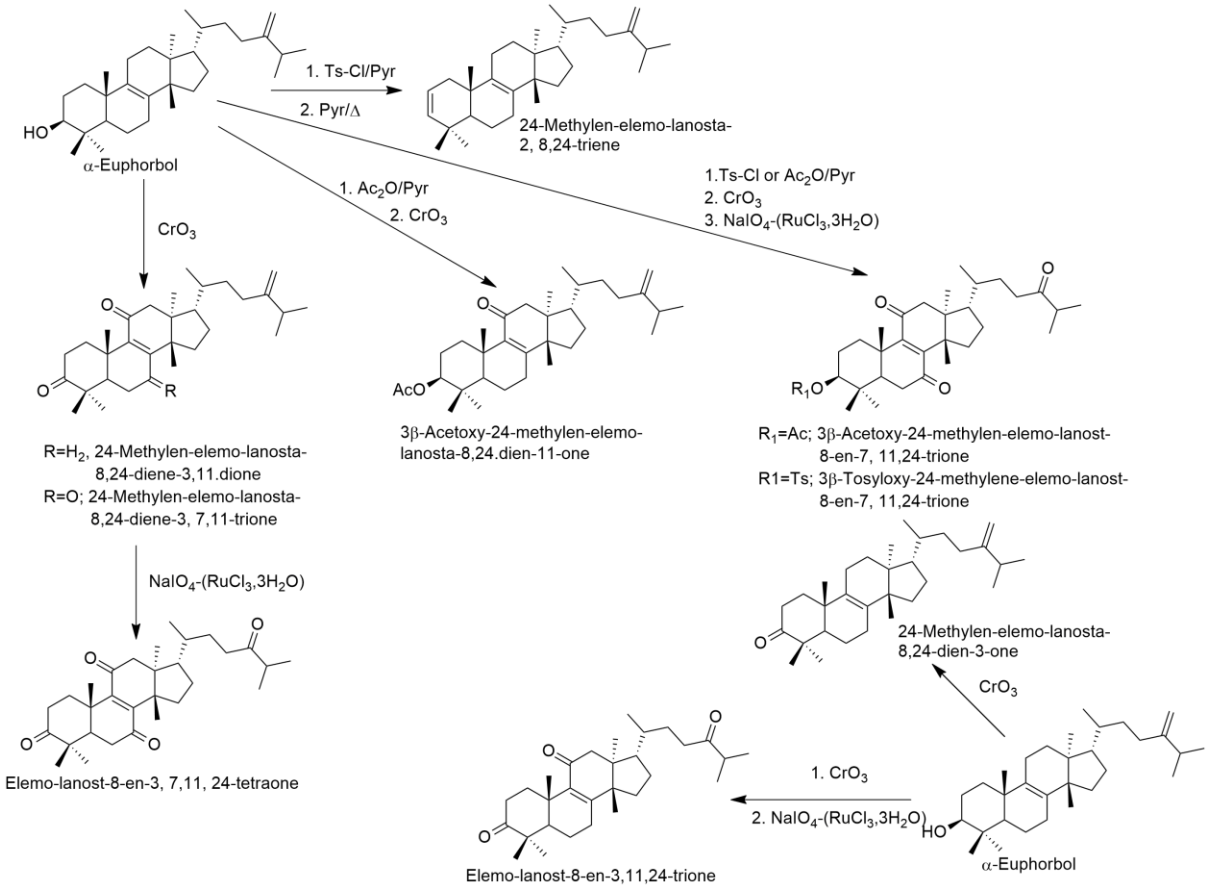


Figure 5. α-Euphol derivatives after reaction of tosylation (Ts), acylation (Ac_2O), and oxidation (CrO_3; $NaIO_4$-$RuCl_3$, 3 H_2O). Ac_2O: acetic anhydride; CrO_3: chromic anhydride; $NaIO_4$-$RuCl_3$: sodium periodate- ruthenium trichloride; Pyr: pyridine; Ts-Cl: tosyl chloride.



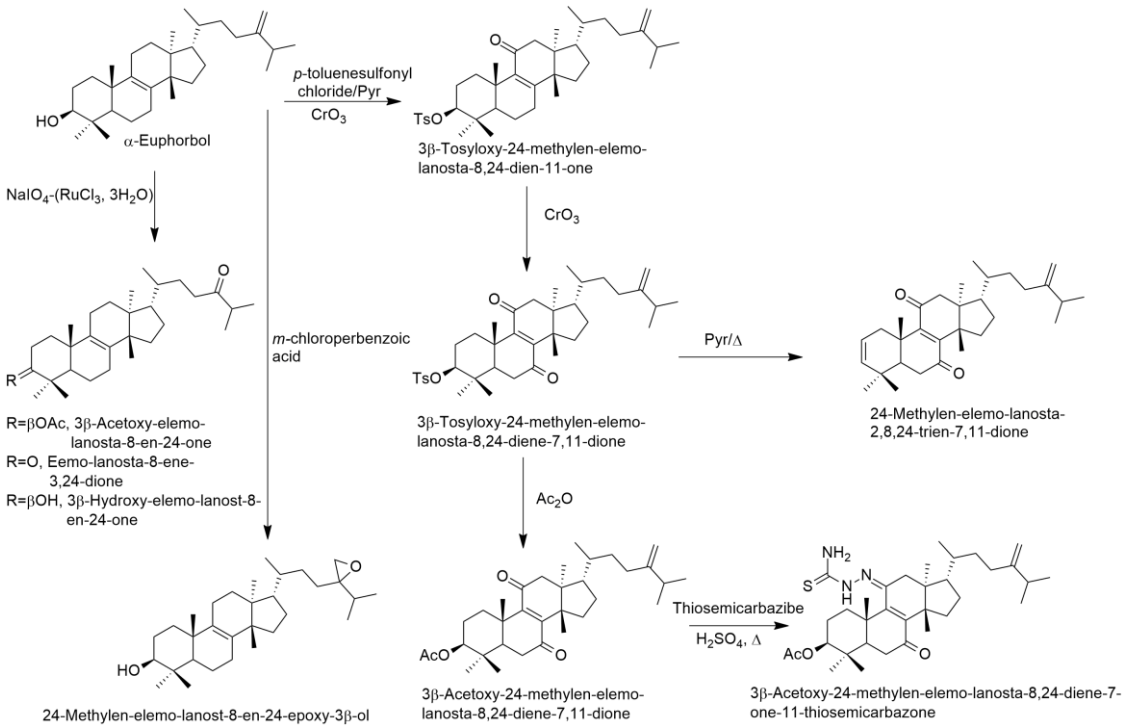


Figure 6. α-Euphorbol derivatives after reaction of tosylation (Ts), acylation (Ac_2O), and oxidation (CrO_3; $NaIO_4$-$RuCl_3$, 3 H_2O). Ac_2O: acetic anhydride; CrO_3: chromic anhydride; $NaIO_4$-$RuCl_3$: sodium periodate-ruthenium trichloride; Pyr: pyridine; Ts-Cl: tosyl chloride.

The larval post-ingestive toxicity of triterpenoid derivatives on insect species *Spodoptera littoralis* was dependent on the hydroxyl/oxo group at C-3 plus ketone at C-24; acetylation or tosylation at C-3 that lowered the activity, while the C-11 thiosemicarbazone increased the toxic activity. Moreover, insect Sf9 cells were more sensitive to the active triterpenoid derivatives than mammalian CHO cells, maybe due to the different membrane composition and/or receptors of both cell types. The active derivatives also had nonselective moderate phytotoxic effects on the radicle elongation of *Lactuca sativa* [64]. In tomato seeds that germinated, the presence of 24-methylen-elemo-lanosta-8,24-dien-3-one and elemo-lanost-8-en-3,11,24-trione at 10 and 50 µg/mL greatly reduced the severity of *Verticillium* wilt without interfering with plant growth [66].

3.4. Other compounds in the latex and plant extracts and their biological properties

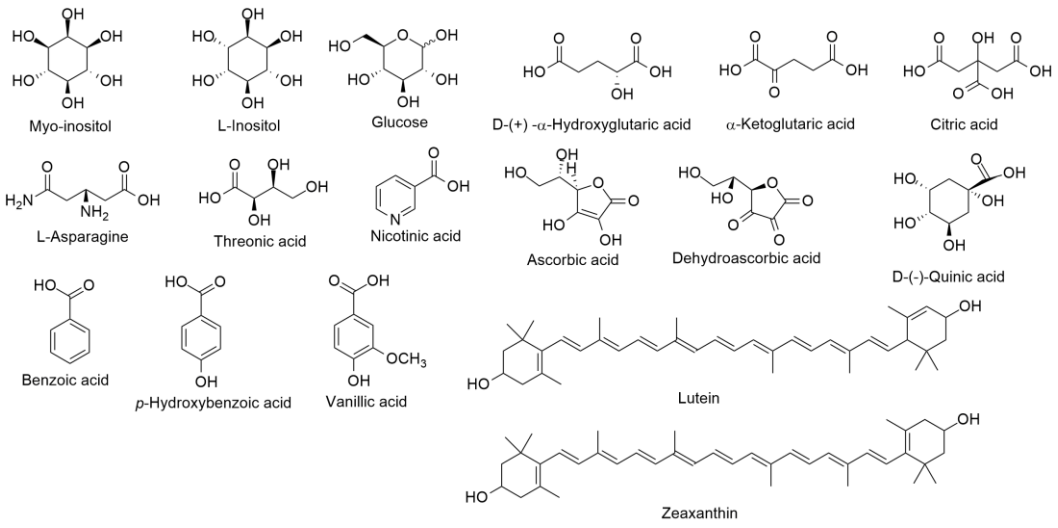
Beyond the diterpene and triterpene aforementioned as being present in the crude drug Euphorbium, Bøe *et al*. [70] found (-)-quinic acid, D(+)-α-hydroxyglutaric acid, *myo*-inositol, L(-)-inositol and probably vanillic acid (Figure 7), nevertheless quinic acid 1,3-lactone although present in one experiment, the authors considered that it was most likely an artifact resulting from the experimental procedure. Phorbic acid (Figure 7) was also found by Nordal and Benson [71] in the crude drug euphorbium. The authors also concluded that this acid is formed in the latex-producing cell system and not in the photosynthetic or chlorophyll-free tissues of *E. resinifera*. Its conclusion was achieved through a radioactive biosynthetic experiment: a branch of *E. resinifera* was kept in a $^{14}CO_2$ atmosphere with 12 hours of light-dark periods for 2 days and then kept under natural conditions in the air outside for 3 days. In these conditions, radioactive phorbic acid was detected in the latex. According to these results, the authors hypothesized that phorbic acid synthesis can be independent of the plants' photosynthetic and respiratory activities, only achieved if the latex of *E. resinifera* contains

enzymes directly involved in the biosynthesis of phorbic acid. Phenolic compounds such as catechol, protocatechuic acid, and 3,4-dihydroxy-phenylacetic acid were also described by Ourhzif *et al*. [35] for the first time in the ethyl acetate fraction of the latex of *E. resinifera*.

By considerably reducing the latency of sleeping and increasing sleeping time at 75 mg/kg, as well as significantly reducing the immobility time and increasing swimming at all assessed doses (25, 50, and 75 mg/kg) in mice, the aqueous extract of *E. resinifera* latex demonstrated antioxidant activity and antidepressant effect. For these properties, the authors believe the possible involvement of the *α2*-adrenoreceptors, 5-hydroxytryptamine receptor 2 ($5HT_2$), dopamine receptor D_2, and γ-aminobutyric acid type A ($GABA_A$) receptors, because, after pretreatment with antagonists, there was a reverse of those effects. The extract contained flavonoids and condensed tannins [72].

Saponarin, a flavone glycoside (Figure 7), was reported to be present in the aqueous extracts of the aerial parts of *E. resinifera*. No diterpene or triterpene were found in the same extracts [3]. Whether the aerial parts used by the authors did not include the latex and only the green part of the plant can explain such observation. The extracts also presented antiradical activities against DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid), ability to decompose H_2O_2, and inhibition of xanthine oxidase activity), hypoglycemic activity (inhibition of α-amylase and α-glucosidase activities), and anti-hyperlipidemic effects (inhibition of lipase activity), with very low acute toxicity (lethal dose 50: LD_{50}) higher than 2 g/kg in treated rats) [3]. However, in another study made by Issiki *et al*. [17], they reported that a concentration higher than 2.5 g/kg of the aqueous extract of *E. resinifera* can cause liver and kidney toxicity. In this study, the authors revealed the presence of flavonoids and tannins, and there were no saponins or alkaloids.

Methanolic extracts of *E. resinifera* contained phenolic acids, flavonoids, and condensed tannins; although the chemical profiling has not been performed, their quantification was made by spectrophotometric method, and this extract had higher amounts of total phenols than *E. echinus*, both species collected in Morocco [73]. The antioxidant activity, measured through the capacity for scavenging the DPPH free radicals, was revealed to be better than the well-known antioxidant ascorbic acid. The authors [73] also described that there was a positive correlation between antioxidant activity and total phenolic content. The antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* was found for the methanolic extract, but an absence of inhibitory activity against *Escherichia coli*.



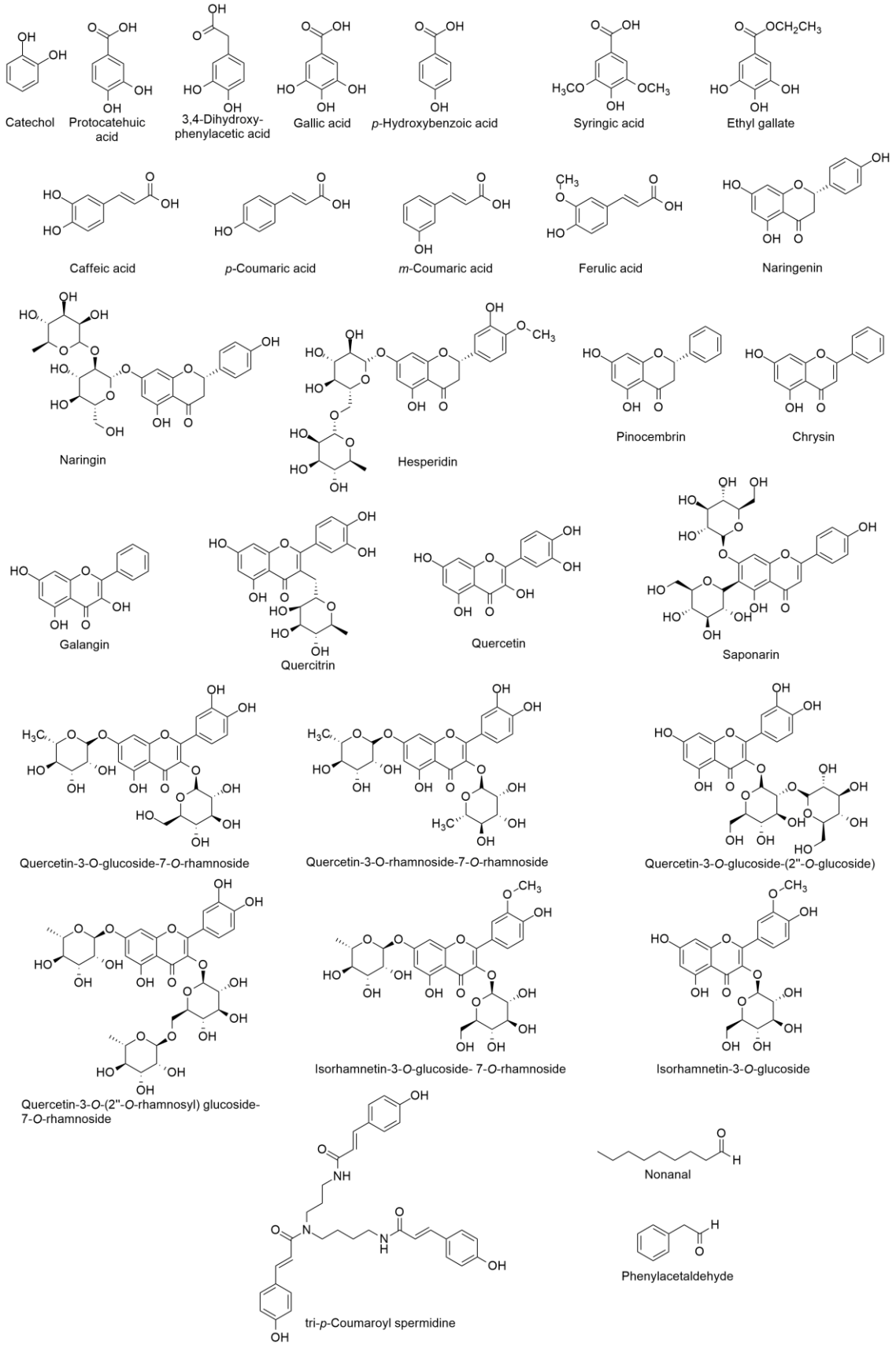


Figure 7. Chemical structure of some phenolics identified in the *E. resinifera* latex (catechol, protocatechuic acid, and 3,4-dihydroxy-phenylacetic acid), in the extract (saponarin), cyathia, and in honey. Other non-phenolic compounds identified in the latex of *E. resinifera* are (-)-quinic acid, D-α-hydroxyglutaric acid, *myo*-inositol, L-inositol; and in cyathia (glucose, citric acid, α-ketoglutaric acid, asparagine, nicotinic acid, threonic acid, ascorbic acid, dehydroascorbic acid, lutein, zeaxanthin, nonanal). See references in this section.

Previously, Boutoub *et al*. [74], through the Response Surface Methodology (RSM) using a full three-level factorial design, were able to optimize the conditions for the extraction of antioxidants and α-glucosidase inhibitors. For this, they made aqueous extracts and then studied the effect of temperature, time, and plant-to-solvent ratio (PSR) and their linear and quadratic interactions on total phenol concentration, total flavonoid concentration, DPPH trapping activity, and α-glucosidase inhibiting activities. The results showed the best extraction temperature between 30 °C and 35 °C. Extraction using PSR of 20 mg/mL for 1 hour at 30 °C yielded extracts with an optimal phenolic content and optimal values of the studied activities for *E. resinifera*. However, other results were obtained for aqueous extracts of *E. officinarum*: extractions for 270 min, at 50°C, using PSR of 10 mg/mL were needed to yield extracts with better activities. Such results reveal the importance of determining the optimal conditions of extraction to obtain extracts with the best biological activities [74].

Boutoub *et al*. [6] thoroughly analyzed primary and specialized metabolites and volatile organic compounds (VOCs) from floral cyathia of *E. resinifera*. The non-polar metabolites of cyathia were extracted with chloroform. After ultra-high-performance liquid chromatography (UHPLC), retention times and UV spectra, it was possible to conclude that lutein (30 μg/g of cyathium dry tissue) predominated, immediately followed by carotene and zeaxanthin (approximately 10 μg/g) [6]. Methanolic extracts were obtained, and five groups of primary metabolite classes were reported: carbohydrates, amino acids, organic acids, vitamins, and intermediates of the shikimate pathway. Concerning the carbohydrates' class, glucose predominated, with citrate and α-ketoglutarate being the most abundant in the organic acids' group; asparagine (Asn) was the most concentrated in the amino acids' group; nicotinic acid or vitamin B_3, threonate (a metabolite of vitamin C), and ascorbate and dehydroascorbate were the most representative vitamins; and benzoic acid and *p*-hydroxybenzoic acid (PHBA) were the main intermediates of the shikimate pathway detected in the methanolic extracts of *E. resinifera* cyathia. From the methanolic extract it was also possible to identify secondary metabolites such as numerous flavonol glycosides, particularly quercetin-*3*-*O*-glucoside-*7*-*O*-rhamnoside, quercetin-*3*-*O*-rhamoside-*7*-*O*-rhamnoside, quercetin-*3*-*O*-glucoside-*(2*''-*O*-glucoside), and quercetin-*3*-*O*-*(2*''-*O*-rhamnosyl) glucoside-*7*-*O*-rhamnoside, immediately followed by isorhamnetin-*3*-*O*-glucoside- *7*-*O*-rhamnoside and isorhamnetin-*3*-*O*-glucoside [6]. In the same extract, polyamines were also detected, particularly spermidine derivatives covalently conjugated with phenolic compounds, and tri-*p*-coumaroyl spermidine was the most abundant. Obtained by hydrodistillation, the most abundant volatiles were *n*-nonanal or pelargonaldehyde and phenylacetaldehyde. The antioxidant activity of the aqueous extracts determined by the 1,1-diphenyl- 2-picrylhydrazyl (DPPH) assay revealed anti-DPPH activity with an IC_{50} value of 0.0338 ± 0.0004 mg/mL and 0.0263 ± 0.0004 mg/mL for the anti-superoxide radical activity [6].

Plant latex is a source of proteases exhibiting anticoagulant and fibrinolytic activity, such as a cysteine protease (ficin) from *Ficus carica* [75]. From the *E. resinifera* crude latex, Siritapetawee *et al*. [76] isolated and purified a new protease called EuRP-61. This is a serine protease because its amino acid sequence had homology (between 50 and 70% identities) with the subtilisin-like proteases of other plants, with molecular weight estimated at 61 kDa analyzed by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS). The enzyme had a broad range of pH stability from 1 to 14 and tolerance to denaturation up to a temperature of approximately 65-66ºC. The protein sequence and native crystal structure of EuRP-61 were characterized [76], and the main structural components of

EuRP-61 were composed of three domains: catalytic, protease-associated, and fibronectin type III (Fn3)-like domains. The Fn3-like domain may provide flexibility to the enzyme to bind with various substrates and cell receptors. The active site of EuRP-61 consisted of the catalytic triad of Ser434, His106, and Asp32, similar to other serine proteases. The enzyme has anticoagulant, antiplatelet, and peripheral blood cell aggregation inhibitory properties, being not toxic to human red blood cells in the 4 common blood groups (A, B, O, and AB) (all Rh+) or human peripheral blood mononuclear cells (hPBMCs) [76,77]. These properties of EuRP-61 led Siritapetawee *et al*. [78] to develop a method for improving the antithrombogenicity of a blue nylon monofilament suture (USP 3/0) by coating it with that protease and by using a combination of dipping and ultrasonication methods. The results were promising since the enzyme-coated nylon presented fibrinogenolytic and fibrinolytic activities. Comparing the enzyme-coated and bare nylon, the enzyme-coated nylon prevented the human fibrin clot from adhering to its surface. Analyses of the fibrinogenolytic and fibrinolytic activities were performed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) and microwell plate assays. The results revealed that the adequate protease concentration would be 1 mg/mL for each coating of 20 strips of 10-mm nylon. According to these results, the authors hypothesize using this EuRP-61-coated nylon as a surgical suture, particularly for vessel anastomosis [78].

3.5. Euphorbia resinifera bee products: chemical composition and their biological properties.

In Morocco, *Euphorbia resinifera* honey (resin spurge) is highly appreciated by consumers due to its unique peppery taste, pungency, and powerful aroma, medical and cosmetic properties, mainly due to their antibacterial and antifungal activity [4,79]. For these reasons, several teams have developed their works by searching for the chemical composition of resin spurges as well as their biological activities and sanitary quality. It is very important to guarantee the good sanitary quality of honey because it needs to be safe. Moujanni *et al*. [80] performed a microbiological profile of thirty-seven samples of *Euphorbia resinifera* honey collected in a Protected Geographical Indication area of the Tadla-Azilal region (Morocco). They reported that these samples did not detect total coliforms and fecal coliforms, *Salmonella* spp., *Shigella* spp., sporus of *Bacillus cereus*, or *Clostridium perfringens*. The molds and yeasts were present in 32% and 40% of samples. However, no samples showed a higher value than the recommended limit (102 $CFU.g^{-1}$), so the samples can be considered of good commercial quality parameters [80]. Another important aspect of safe food is the absence of antibiotics, confirmed in 37 samples of *E. resinifera* honey, in which only one presented Trimethoprim at 6.48 µg/kg [81]. According to the authors [81], the antibacterial activity found in the honey samples against *Staphylococcus aureus* and *Escherichia coli* can only be attributed to their physicochemical properties (high osmotic nature, low pH, content of phenolic compounds, hydrogen peroxide, and also to its content of methylglyoxal) and not to the possible presence of antibiotic residues.

In contrast to the absence of antibiotics in *E. resinifera* honey, Massous *et al*. [82] found diverse pesticides, plasticizers, and bisphenols in samples collected in the Béni Mellal-Khénifra region (Morroco). In the area of pesticides, the authors found carbaryl, diazinon, metalaxyl-M, acephate, and cyromazine at concentrations higher than 10 (g/kg). The plasticizers diethyl phthalate, diethanolamine, and dibutyl adipate were present at concentrations higher than 1 mg/kg. Although dibutyl phthalate is lower than 1 mg/kg (0.84 mg/kg), this concentration is substantially higher than the relative specific migration limits, according to the Reg. (EU) No.

10/2011 [83], which is 0.3 mg/kg. Bisphenol A, bisphenol B, and bisphenol AF were present in honey samples but at concentrations lower than the regulatory-specific migration limits, particularly for bisphenol A migration from food contact material, which is 600 ng/g [82]. According to the authors, the presence of plasticizers and bisphenols can be attributed to the plastic components that were released from the honey production equipment, such as honey extractors and uncorkers; although the presence of these compounds cannot be ruled out as environmental contamination, for example, from the nectar used by bees to make honey.

Owing to the importance of resin spurge honey, their botanical and geographical characterization is needed. For this reason, Terrab *et al*. [4] made a palynological and geographical characterization of 29 monofloral resin spurge honey along the protected geographic indication area in the Middle Atlas Mountains (Morocco) and concluded that they presented low to very low pollen content. For this reason, Terrab *et al*. [4] suggest a revision of the established threshold, proposing to lower it to 20% of the pollen of *E. resinifera* and consider it monofloral honey. The other pollen species also detected in the samples were *Ceratonia siliqua*, *Echium plantagineum*, *Olea* sp., *Plantago coronopus*, and *Quercus*. However, Boutoub *et al*. [84] found two resin spurge honey with more than 45% of *E. resinifera* pollen collected in Beni Mellal-Khénifra (Morocco). In the same work, the authors studied the *E. officinarum* honey and their extracts for comparison. All honey samples were within the acceptable limit of international standards, as reported by Moujanni *et al*. [85] for 29 honey samples. Nevertheless, *E. resinifera* honey presented lower moisture HMF but a higher reducing sugar percentage than *E. officinarum* honey.

Regarding the secondary metabolites, gallic acid, 4-hydroxybenzoic acid, and *p*-coumaric acid (Figure 7) were detected in all samples, although in different ratios. The methanolic extracts of honey presented higher biological activities (antioxidant activity measured through DPPH radical-scavenging, nitric oxide scavenging activity, and scavenging ability of superoxide anion radical; and inhibition of acetylcholinesterase, lipoxygenase, tyrosinase, and xanthine oxidase activities) than the entire honey. Nevertheless, *E. resinifera* honey had better anti-inflammatory activities (anti-lipoxygenase activity) than *E. officinarum* honey. Both teams [84,85] found potassium (K) was the most abundant mineral element in *E. resinifera* honey samples. The best activity of the honey extracts would be expected because Boutoub *et al*. [86] previously found that the *E. resinifera* or *E. officinarum* extracts generally had better biological properties than the entire honey, which can support the hypothesis that the antioxidant activity, for example, can be mainly attributed to the secondary metabolites of the plants visited by honeybees.

A much higher number of phenolic compounds (17) were found by Hernanz *et al*. [87] in *E. resinifera* honey than Boutoub *et al*. [84]: gallic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, ethyl gallate, caffeic acid, *p*-coumaric acid, *m*-coumaric acid, ferulic acid, naringenin, naringin, hesperidin, pinocembrin, chrysin, galangin, quercitrin, and quercetin (Figure 7). Some of these compounds were also reported by Boutoub *et al*. [84]: gallic acid, *p*-hydroxybenzoic acid, caffeic acid (in only one sample), *p*-coumaric acid, quercetin, and naringenin. Hernanz *et al*. [87] also reported a correlation between the chromatic attributes and phenolic acids, total phenolic compounds, caffeic acid, *p*-and *m*-coumaric acids, and hesperidin.

The volatile fraction of propolis in which *E. resinifera* polen predominated (58%) was mainly constituted by monoterpene hydrocarbons (35%), particularly α-pinene (16%) [88]. Calcium was the predominant mineral element. The antimicrobial activity of the

hydroalcoholic extract of *Euphorbia resinifera* propolis was evaluated, and the minimum inhibitory concentration (MIC) value ranged from 50 to 150 µL/mL against Gram-positive (*Staphylococcus aureus* ATCC 6538) and Gram-negative (*Escherichia coli* DSM 1077) bacteria.

Hydroalcoholic extract of *E. resinifera* propolis, at the concentration of 250 µL/mL, was able to reduce the adherence of *S. aureus* ATCC 6538 significantly ($p < 0.0001$) in comparison with chlorohexidine (0.2%, v/v), in contrast, it stimulated the adherence of both *S. aureus* MRSA 12 and MRSA 15 in a concentration-dependent manner. It also induced the adherence of *E. coli* DSM 1077 in a concentration-dependent manner; nevertheless, it was not observed on the multiresistant strain of *E. coli* I73194 [88].

4. Conclusions

Euphorbia resinifera Berg. is a large, leafless cactus-like perennial endemic to Morocco, generally distributed in the center of the country, in the regions of Azilal and Beni Mellal (Middle Atlas). This species has been used in folk medicine, in which Moroccan patients often mix the aerial parts with honey or extracts obtained by decoction to treat general cancer. Fresh latex is used for poisonous punctures, bites, and dental pains. The last utilization can be attributed to the presence of resiniferatoxin (daphnane diterpene), which is an analog of capsaicin by acting with capsaicin receptor but about 1,000-fold more potent as an analgesic than that alkaloid; nevertheless, it is extremely irritant. For this reason, resiniferatoxin has been used in some clinical trials as a potential analgesic to relieve cancer and arthritis pain; nonetheless, there are bioanalytical limitations in its quantification in plasma due to the minimal effective dose that is in the range of a few nanograms. Some studies have been developed to achieve an efficient, rapid, and reliable method to quantify low concentrations of resiniferatoxin and to make determining its pharmacokinetic profile easier, data important in therapeutics. Other diterpenes have also been identified in the latex along with nor sesquiterpenes and triterpenes with diverse biological attributes, such as anti-inflammatory, proliferation inhibition of some cancer cell lines, antimicrobial, antiplasmodial, and anti-insecticidal properties. In the latex was also reported the presence of serine proteases named EuRP61, with 61 kDa showed anticoagulant, antiplatelet, and peripheral blood cell aggregation inhibitory properties being not toxic to human red blood cells in the 4 common blood groups (A, B, O, and AB) (all Rh+) hPBMCs. These attributes led to developing antithrombogenicity blue nylon monofilament suture coated with EuRP61. The positive results show that using this EuRP-61-coated nylon as a surgical suture, particularly for vessel anastomosis, is possible.

The aerial parts of *E. resinifera* without latex are constituted by another group of compounds, such as polyphenols. However, the detailed chemical profile of such samples has not been done so far, in contrast to the *E. resinifera* honey in which there is already some chemical detail. In this case, several phenolic acids, flavanones, flavones, flavonols, and some flavonoid glycosides are identified. The *in vitro* biological properties of honey include free radical scavenging activity (superoxide anion radical) and anti-inflammatory activity by inhibiting lipoxygenase activity. Other enzyme inhibition activities were described, such as inhibition of acetylcholinesterase, tyrosinase, and xanthine oxidase activities. These activities were attributed to the secondary metabolites in the honey because the extracts where these compounds were present had better activity than the entire honey. It was demonstrated that extraction conditions are important for aqueous extracts to improve the amounts of phenols and antioxidant activity. The optimal conditions were: extraction temperature between 30°C and

35°C, plant solvent of 20 mg/mL, and extraction time of 1 h if the extraction is made at 30°C. Propolis, for the first time reported, had antimicrobial activity and reduced the adherence of *S. aureus* compared with chlorohexidine.

5. Future trends

Due to the genetic variability found for *E. resinifera*, it is necessary to confirm if this affects the chemical composition and, consequently, the biological properties, despite there already being at least two medicinal products (Euphorbium compositum nasal drops SN®, Euphorbium compositum SN®) [20] for the treatment of rhinitis and improvement of chronic sinusitis. Antiviral activity was also previously reported, particularly against the respiratory syncytial virus (RSV), as long as there is an association between *E. resinifera* and *Pulsatilla pratensis* [89].

The new finding of serine proteases in latex and its possible application as coating in nylon as a surgical suture, particularly for vessel anastomosis, must be quickly confirmed, as well as in other medical applications.

It urges a detailed chemical profile of the aerial parts of *E. resinifera* after finding the best conditions for extraction. The biological properties must be deeply studied before using this plant part after extracting the latex. In these conditions, using both the latex and the non-latex part will be possible.

The biological properties reported for honey must be studied in-depth to confirm those already reported and find other ones. At the same time, it is important to train beekeepers to obtain guaranteed monofloral *E. resinifera* honey in the best collection and storage conditions without being subjected to high temperatures to prevent the formation of HMF (hydroxymethylfurfural). Researchers should start paying attention to studies on *E. resinifera* propolis since, at least *in vitro* tests, it seems useful in inhibiting some microorganisms' growth.

Funding

This research was funded by the FCT (Fundação para a Ciência e a Tecnologia, Portugal) under the project UIDB/05183/2020.

Acknowledgments

The authors acknowledged MED (https://doi.org/10.54499/UIDB/05183/2020; https://doi.org/10.54499/UIDP/05183/2020) and CHANGE (https://doi.org/10.54499/LA/P/0121/2020).

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Kemboi, D.; Peter, X.; Langat, M.; Tembu, J. A Review of the Ethnomedicinal Uses, Biological Activities, and Triterpenoids of *Euphorbia* Species. *Molecules* **2020**, *25*, 4019, https://doi.org/10.3390/molecules25174019.
2. Batanouny K.H. THE WILD MEDICINAL PLANTS IN NORTH AFRICA: HISTORY AND PRESENT STATUS. *Acta Hort*. **1999**, *500*, 183-188, https://doi.org/10.17660/ActaHortic.1999.500.26.

3. Benrahou, K.; Guourrami, O.E.; Mrabti, H.N.; Cherrah, Y.; Faouzi, M.E.A. Investigation of Antioxidant, Hypoglycemic and Anti-Obesity Effects of Euphorbia Resinifera L. *J. Pharmacopunct.* **2022**, *25*, 242-249, https://doi.org/10.3831/KPI.2022.25.3.242.
4. Terrab, A.; Moujanni, A.; Essamadi, A.K.; Hernanz, D.; Díez, M.J.; Berjano, R. A palynological and geographical characterization of labeled resin spurge honey: *Euphorbia resinifera*. *Palynology* **2022**, *46*, 1-10, https://doi.org/10.1080/01916122.2021.1933639.
5. Ettaqy, A.; Taha, A.; ElGhiouane, A.; ElKhou, A.; Boulli, A.; Abbas, Y. New data on the ecological distribution of *Euphorbia resinifera* O.Berg in the Beni Mellal-Khenifra region. *E3S Web Conf.* **2020**, *183*, 01001, https://doi.org/10.1051/e3sconf/202018301001.
6. Boutoub, O.; Jadhav, S.; Zheng, X.; El Ghadraoui, L.; Al Babili, S.; Fernie, A.R.; Figueiredo, A.C.; Miguel, M.G.; Borghi, M. Biochemical characterization of *Euphorbia resinifera* floral cyathia. *J. Plant Physiol*. **2024**, *293*, 154184, https://https://doi.org/10.1016/j.jplph.2024.154184.
7. Muntañola-Cvetković, M.; Gómez-Bolea, A. *Pseudostilbella euphorbiae*, a new sporodochial hyphomycete, isolated from *Euphorbia resinifera*. *Can. J. Bot.* **1995**, *73*, 590-597, https://doi.org/10.1139/b95-062.
8. Abd-dada, H.; Bouda, S.; Aitbella, Y.; Haddioui, A. Phenotypic diversity of natural populations of an endemic Moroccan plant (*Euphorbia resinifera* O. Berg). *Hacquetia* **2023**, *22*, 81-90, http://dx.doi.org/10.2478/hacq-2022-0015.
9. Abd-dada, H.; Bouda, S.; Khachtib, Y.; Bella, Y.A.; Haddioui, A. Use of ISSR markers to assess the genetic diversity of an endemic plant of Morocco (*Euphorbia resinifera* O. Berg). *J. Genet. Eng. Biotechnol*. **2023**, *21*, 91, https://doi.org/10.1186/s43141-023-00543-4.
10. Mallon, R.; Onrubia, M.; Lota, F.; Corral, P.; Lorrain, B.; Michoux, F. In vitro propagation methods of the succulent plant Euphorbia resinifera and quantification of its major compounds. *Planta Med.* **2014**, *80*, P2F2, https://doi.org/10.1055/s-0034-1394836.
11. Nemmaoui, A.; García-Lorca, A.M.; Aguilar, F.J.; Aguilar, M.A. Time series of vegetation for a destroyed forest model: The case of Tadla Azilal (Morocco). *Agrociencia* **2013**, *47*, 267–280.
12. Bourhia, M.; Abdelaziz Shahat, A.; Mohammed Almarfadi, O.; Ali Naser, F.; Mostafa Abdelmageed, W.; Ait Haj Said, A.; El Gueddari, F.; Naamane, A.; Benbacer, L.; Khlil, N. Ethnopharmacological Survey of Herbal Remedies Used for the Treatment of Cancer in the Greater Casablanca-Morocco. *Evid. Based Complement. Altern. Med*. **2019**, *2019*, 1613457, https://doi.org/10.1155/2019/1613457.
13. Mancera, N.; Wadia, H.P. Corneal Edema Associated With Systemic Dopaminergic Agents. *Cornea* **2019**, *38*, 1040-1042, https://doi.org/10.1097/ico.0000000000001941.
14. El Hachlafi, N.; Benkhaira, N.; Ferioun, M.; Kandsi, F.; Jeddi, M.; Chebat, A.; Addi, M.; Hano, C.; Fikri-Benbrahim, K. Moroccan Medicinal Plants Used to Treat Cancer: Ethnomedicinal Study and Insights into Pharmacological Evidence. *Evid. Based Complement. Altern. Med.* **2022**, *2022*, 1645265, https://doi.org/10.1155/2022/1645265.
15. Talbaoui, A.; El Hamdaoui, L.; Bouyahya, A.; El Moussaouiti, M.; Bakri, Y. Chemical Composition, *in vitro* Cytotoxic, and Antibacterial Activities of Moroccan Medicinal Plants *Euphorbia resinifera* and *Marrubium vulgare*. *Biointerface Res. Appl. Chem*. **2020**, *10*, 7343-7355, https://doi.org/10.33263/BRIAC106.73437355.
16. Benmehdi, H.; Bounoua, N.; Amrouche, A.; Lahcene, D.; Maazouzi, A. Phytochemical study, antioxidant and antimicrobial activities of Euphorbia resinifera L. *Int. Res. J. Pharm*. **2013**, *4*, 44-50.
17. Issiki, Z.; Daoudi, F.; Mtairag, E.M.; Rais, S.; Marnissi, F.; Zaid, Y.; Naya, A.; Oudghiri, M. ACUTE A SUB-CHRONIC TOXICITY AND IMMUNOMODULATORY ACTIVITY OF AN AQUEOUS EXTRACT OF EUPHORBIA RESINIFERA IN RODENTS. *Int. J. Pharm. Sci. Res*. **2019**, *10*, 2962-2969.
18. Belhaj, S.; Chaachouay, N.; Zidane, L. Ethnobotanical and toxicology study of medicinal plants used for the treatment of diabetes in the High Atlas Central of Morocco. *J. Pharm. Pharmacogn. Res*. **2021**, *9*, 619-662.
19. Girin, M.A.; Paphassarang, S.; David-Eteve, C.; Chaboud, A.; Raynaud, J. Determination of ingenol in homoeopathic mother tinctures of Euphorbia species by high-performance liquid chromatography. *J. Chromatogr*. **1993**, *637*, 206-208, https://doi.org/10.1016/0021-9673(93)83215-E.
20. Hmidouche, O.; Bouftini, K.; Chafik, A.; Khouri, S.; Rchid, H.; Rahimi, A.; Mimouni, M.; Maarouf, E.; Zaakour, F.; Nmila, R.; Khouchlaa, A. Ethnomedicinal Use, Phytochemistry, Pharmacology, and Toxicology of *Euphorbia resinifera* O. Berg. (B): A Review. *J. Zool. Bot. Gard*. **2023**, *4*, 364-395, https://doi.org/10.3390/jzbg4020029.
21. Kirby, J.; Nishimoto, M.; Park, J.G.; Withers, S.T.; Nowroozi, F.; Behrendt, D.; Rutledge, E.J.G.; Fortman, J.L.; Johnson, H.E.; Anderson, J.V.; Keasling, J.D. Cloning of casbene and neocembrene synthases from

Euphorbiaceae plants and expression in *Saccharomyces cerevisiae*. *Phytochemistry* **2010**, *71*, 1466-1473, https://doi.org/10.1016/j.phytochem.2010.06.001.
22. Jadid, N.; Mardika, R.K.; Nurhidayati, T.; Irawan, M.I. Reverse Transcription-PCR analysis of *geranylgeranyl diphosphate synthase* (JcGGPPS) in *Jatropha curcas* L. and *in silico* analysis of *Casbene Synthase* (JcCS) among Euphorbiaceae. *AIP Conf. Proc.* **2016**, *1744*, 020042, https://doi.org/10.1063/1.4953516.
23. Fattahian, M.; Ghanadian, M.; Ali, Z.; Khan, I.A. Jatrophane and rearranged jatrophane-type diterpenes: biogenesis, structure, isolation, biological activity and SARs (1984–2019). *Phytochem. Rev.* **2020**, *19*, 265-336, https://doi.org/10.1007/s11101-020-09667-8.
24. Zhang, K.; Fan, W.; Huang, Z.; Chen, D.; Yao, Z.; Li, Y.; Yang, Y.; Qiu, D. Transcriptome analysis identifies novel responses and potential regulatory genes involved in 12-deoxyphorbol-13-phenylacetate biosynthesis of *Euphorbia resinifera*. *Ind. Crops Prod.* **2019**, *135*, 138-145, https://doi.org/10.1016/j.indcrop.2019.04.030.
25. Boutoub, O.; Ghadraoui, L.E.; Miguel, M.G. Biological Properties of Latex, Aqueous Extracts and Bee Products of *Euphorbia officinarum* L.: A Short Review. *Molecules* **2022**, *27*, 7200, https://doi.org/10.3390/molecules27217200.
26. Hergenhahn, M.; Adolf, W.; Hecker, E. Resiniferatoxin and other esters of novel polyfunctional diterpenes from Euphorbia resinifera and unispina. *Tetrahedron Lett.* **1975**, *16*, 1595-1598, https://doi.org/10.1016/S0040-4039(00)72207-6.
27. Appendino, G.; Szallasi, A. Euphorbium: Modern research on its active principle, resiniferatoxin, revives an ancient medicine. *Life Sci.* **1997**, *60*, 681-696, https://doi.org/10.1016/S0024-3205(96)00567-X.
28. Hergenhahn, M.; Kusumoto, S.; Hecker, E. On the active principles of the spurge family (Euphorbiaceae). *J. Cancer Res. Clin. Oncol.* **1984**, *108*, 98-109, https://doi.org/10.1007/BF00390980.
29. Fattorusso, E.; Lanzotti, V.; Taglialatela-Scafati, O.; Tron, G.C.; Appendino, G. Bisnorsesquiterpenoids from *Euphorbia resinifera* Berg. and an Expeditious Procedure to Obtain Resiniferatoxin from Its Fresh Latex. *Eur. J. Org. Chem.* **2002**, *2002*, 71-78, https://doi.org/10.1002/1099-0690(20021)2002:1<71::AID-EJOC71>3.0.CO;2-C.
30. Forzato, C.; Nitti, P. New Diterpenes with Potential Antitumoral Activity Isolated from Plants in the Years 2017–2022. *Plants* **2022**, *11*, 2240, https://doi.org/10.3390/plants11172240.
31. Hergenhahn, M.; Kusumoto, S.; Hecker, E. Diterpene esters from 'Euphorbium' and their irritant and cocarcinogenic activity. *Experientia* **1974**, *30*, 1438-1440, https://doi.org/10.1007/BF01919683.
32. Ezzanad, A.; De los Reyes, C.; Macías-Sánchez, A.J.; Hernández-Galán, R. Isolation and Identification of 12-Deoxyphorbol Esters from *Euphorbia resinifera* Berg Latex: Targeted and Biased Non-Targeted Identification of 12-Deoxyphorbol Esters by UHPLC-HRMS^{E}. *Plants* **2023**, *12*, 3846, https://doi.org/10.3390/plants12223846.
33. Popplewell, W.L.; Marais, E.A.; Brand, L.; Harvey, B.H.; Davies-Coleman, M.T. Euphorbias of South Africa: two new phorbol esters from *Euphorbia bothae*. *S. Afr. J. Chem.* **2010**, *63*, 175-179.
34. Ourhzif, E.-M.; Ketatni, E.M.; Akssira, M.; Troin, Y.; Khouili, M. Crystal structure, Hirshfeld surface analysis and DFT studies of *Euphorbioside* monohydrate a major bisnorsesquiterpene isolated from *Euphorbia resinifera* latex. *J. Mol. Struct.* **2021**, *1241*, 130511, https://doi.org/10.1016/j.molstruc.2021.130511.
35. Ourhzif, E.-M.; Ricelli, A.; Stagni, V.; Cirigliano, A.; Rinaldi, T.; Bouissane, L.; Saso, L.; Chalard, P.; Troin, Y.; Khouili, M.; Akssira, M. Antifungal and Cytotoxic Activity of Diterpenes and Bisnorsesquiterpenoides from the Latex of *Euphorbia resinifera* Berg. *Molecules* **2022**, *27*, 5234, https://doi.org/10.3390/molecules27165234.
36. Marston, A.; Hecker, E. Structure-Activity Relationships of Polyfunctional Diterpenes of the Tigliane Type, V*. Preparation and Irritant Activities of Amino Acid and Peptide Esters of Phorbo. *Z. Naturforsch.* **1983**, *38b*, 1015-1021.
37. Zayed, S.; Sorg, B.; Hecker, E. Structure Activity Relations of Polyfunctional Diterpenes of the Tigliane Type, VI[1]. *Planta Med.* **1984**, *50*, 65-69, https://doi.org/10.1055/s-2007-969623.
38. Bocklandt, S.; Blumberg, P.M.; Haner, D.H. Activation of latent HIV-1 expression by the potent anti-tumor promoter 12-deoxyphorbol 13-phenylacetate. *Antiviral Res.* **2003**, *59*, 89-98, https://doi.org/10.1016/S0166-3542(03)00034-2.
39. Ziglioli, F.; Frattini, A.; Maestroni, U.; Dinale, F.; Ciufifeda, M.; Cortellini, P. Vanilloid-mediated apoptosis in prostate cancer cells through a TRPV-1 dependent and a TRPV-1-independent mechanism. *Acta Biomed.* **2009**, *80*, 13-20.

40. Wank, I.; Kutsche, L.; Kreitz, S.; Reeh, P.; Hess, A. Imaging the influence of peripheral TRPV1-signaling on cerebral nociceptive processing applying fMRI-based graph theory in a resiniferatoxin rat model. *PLoS ONE* **2022**, *17*, e0266669, https://doi.org/10.1371/journal.pone.0266669.
41. Gavva, N.R.; Klionsky, L.; Qu, Y.; Shi, L.; Tamir, R.; Edenson, S.; Zhang, T.J.; Viswanadhan, V.N.; Toth, A.; Pearce, L.V.; Vanderah, T.W.; Porreca, F.; Blumberg, P.M.; Lile, J.; Sun, Y.; Wild, K.; Louis, J.-C.; Treanor, J.J.S. Molecular Determinants of Vanilloid Sensitivity in TRPV1. *J. Biol. Chem.* **2004**, *279*, 20283-20295, https://doi.org/10.1074/jbc.M312577200.
42. Sharma, P.K.; Avula, B.; Panda, A.; Wang, Y.-H.; Khan, I.A.; Murthy, S.N. A sensitive bioanalytical method for quantitative determination of resiniferatoxin in rat plasma using ultra-high performance liquid chromatography coupled to tandem mass spectrometry and its application in pharmacokinetic study. *J. Pharm. Biomed. Anal*. **2019**, *165*, 284-291, https://doi.org/10.1016/j.jpba.2018.12.024.
43. Brown, K.C.; Modi, K.J.; Light, R.S.; Cox, A.J.; Long, T.E.; Gadepalli, R.S.; Rimoldi, J.M.; Miles, S.L.; Rankin, G.; Valentovic, M.; Denning, K.L.; Tirona, M.T.; Finch, P.T.; Hess, J.A.; Dasgupta, P. Anticancer Activity of Region B Capsaicin Analogs. *J. Med. Chem.* **2023**, *66*, 4294-4323, https://doi.org/10.1021/acs.jmedchem.2c01594.
44. Salem, J.B.; Nkambeu, B.; Arvanitis, D.N.; Beaudry, F. Resiniferatoxin Hampers the Nocifensive Response of *Caenorhabditis elegans* to Noxious Heat, and Pathway Analysis Revealed that the Wnt Signaling Pathway is Involved. *Neurochem. Res*. **2022**, *47*, 622-633, https://doi.org/10.1007/s11064-021-03471-2.
45. Basu, P.; Tongkhuya, S.A.; Harris, T.L.; Riley, A.R.; Maier, C.; Granger, J.; Wojtaszek, J.; Averitt, D.L. Euphorbia bicolor (*Euphorbiaceae*) Latex Phytochemicals Induce Long-Lasting Non-Opioid Peripheral Analgesia in a Rat Model of Inflammatory Pain. *Front. Pharmacol*. **2019**, *10*, 00958, https://doi.org/10.3389/fphar.2019.00958.
46. Ezzanad, A.; Gómez-Oliva, R.; Escobar-Montaño, F.; Díez-Salguero, M.; Geribaldi-Doldan, N.; Dominguez-Garcia, S.; Botubol-Ares, J.M.; Reyes, C.d.l.; Durán-Patrón, R.; Nunez-Abades, P.; Macías-Sánchez, A.J.; Castro, C.; Hernández-Galán, R. Phorbol Diesters and 12-Deoxy-16-hydroxyphorbol 13,16-Diesters Induce TGFα Release and Adult Mouse Neurogenesis. *J. Med. Chem*. **2021**, *64*, 6070-6084, https://doi.org/10.1021/acs.jmedchem.1c00156.
47. Hergenhahn, M.; Fürstenberger, G.; Opferkuch, H.J.; Adolf, W.; Mack, H.; Hecker, E. Biological assays for irritant tumor-initiating and -promoting activities. I. Kinetics of the irritant response in relation to the initiation-promoting activity of polyfunctional diterpenes representing tigliane and some daphnane types. *J. Cancer Res. Clin. Oncol*. **1982**, *104*, 31–39, https://doi.org/10.1007/bf00402051.
48. Wang, S.-Y.; Li, G.-Y.; Zhang, K.; Wang, H.-Y.; Liang, H.-G.; Huang, C.; Huang, J.; Wang, J.-H.; Yang, B.-F. New ingol-type diterpenes from the latex of *Euphorbia resinifera*. *J. Asian Nat. Prod. Res*. **2019**, *21*, 1075-1082, https://doi.org/10.1080/10286020.2018.1498084.
49. Zhao, N.-D.; Ding, X.; Song, Y.; Yang, D.-Q.; Yu, H.-L.; Adelakun, T.A.; Qian, W.-D.; Zhang, Y.; Di, Y.-T.; Gao, F.; Hao, X.-J.; Li, S.-L. Identification of Ingol and Rhamnofolane Diterpenoids from *Euphorbia resinifera* and Their Abilities to Induce Lysosomal Biosynthesis. *J. Nat. Prod*. **2018**, *81*, 1209-1218, https://doi.org/10.1021/acs.jnatprod.7b00981.
50. Yuan, S.; Jiang, S.-C.; Zhang, Z.-W.; Fu, Y.-F.; Yang, X.-Y.; Li, Z.-L.; Hu, J. Rethinking of Alzheimer's disease: Lysosomal overloading and dietary therapy. *Front. Aging Neurosci*. **2023**, *15*, 1130658, https://doi.org/10.3389/fnagi.2023.1130658.
51. Newbold, G.T.; Spring, F.S. 65. The isolation of euphol and α-euphorbol from euphorbium. *J. Chem. Soc*. **1944**, 249-252, https://doi.org/10.1039/JR9440000249.
52. Zhou, M.; Tan, W.; Hasimu, H.; Liu, J.; Gu, Z.; Zhao, J. Euphorbium total triterpenes improve Freund's complete adjuvant-induced arthritis through PI3K/AKT/Bax and NF-κB/NLRP3 signaling pathways. *J. Ethnopharmacol.* **2023**, *306*, 116146, https://doi.org/10.1016/j.jep.2023.116146.
53. Wang, S.; Liang, H.; Zhao, Y.; Wang, G.; Yao, H.; Kasimu, R.; Wu, Z.; Li, Y.; Huang, J.; Wang, J. New triterpenoids from the latex of *Euphorbia resinifera* Berg. *Fitoterapia* **2016**, *108*, 33-40, https://doi.org/10.1016/j.fitote.2015.11.009.
54. Wang, S.-Y.; Huang, C.; Sun, R.-K.; Lu, L.-N.; Liang, H.-G.; Gao, L.; Huang, J.; Wang, J.-H.; Yang, B.-F. New tirucallane triterpenoids from the dried latex of *Euphorbia resinifera*. *Phytochem. Lett*. **2019**, *29*, 220-224, https://doi.org/10.1016/j.phytol.2018.12.012.
55. Wang, S.Y.; Li, G.Y.; Zhang, K.; Wang, H.Y.; Liang, H.G.; Huang, C.; Huang, J.; Wang, J.H.; Yang, B.F. [Triterpenoids from Uygur medicine latex of Euphorbia resinifera]. *Zhongguo Zhong Yao Za Zhi* **2018**, *43*, 3688-3693, https://doi.org/10.19540/j.cnki.cjcmm.20180625.003.

56. Li, Y.-J.; Ji, T.-F.; Zhao, J.; Gu, Z.-Y. [Chemical constituents of triterpenoids from Euphorbia resinifera.] *Zhongguo Zhong Yao Za Zhi* **2021**, *46*, 4433-4437, https://doi.org/10.19540/j.cnki.cjcmm.20210621.201.
57. Li, M.-M.; Qi, Y.-R.; Feng, Y.-P.; Liu, W.; Yuan, T. [Four new lanostane triterpenoids from latex of Euphorbia resinifera.] *Zhongguo Zhong Yao Za Zhi* **2021**, *46*, 4744-4748, https://doi.org/10.19540/j.cnki.cjcmm.20210623.201.
58. Yuan, F.-Y.; Xu, F.; Fan, R.-Z.; Li, W.; Huang, D.; Tang, G.-H.; Yuan, T.; Gan, L.-S.; Yin, S. Structural Elucidation of Three 9,11-*Seco* Tetracyclic Triterpenoids Enables the Structural Revision of Euphorol J. *J. Org. Chem.* **2021**, *86*, 7588-7593, https://doi.org/10.1021/acs.joc.1c00631.
59. Qi, Y.; Liu, W.; Chen, Y.; Guan, M.; Yuan, T. Euphatexols A and B, two unsual euphane triterpenoids from the latex of *Euphorbia resinifera*. *Tetrahedron Lett.* **2019**, *60*, 151303, https://doi.org/10.1016/j.tetlet.2019.151303.
60. Li, M.-M.; Qi, Y.-R.; Feng, Y.-P.; Liu, W.; Yuan, T. Euphatexols C-G, five new triterpenoids from the latex of *Euphorbia resinifera*. *J. Asian Nat. Prod. Res.* **2022**, *24*, 311-320, https://doi.org/10.1080/10286020.2021.1935894.
61. Zhao, N.-D.; Li, Y.-L.; Song, Y.; Yang, B.-J.; Ding, X.; Gao, F.; Ye, J.; Hao, X.-J.; Zhang, Y.; Li, S.-L. Ten new nortriterpenes from *Euphorbia resinifera* and their anti-tomato yellow leaf curl virus activities. *Fitoterapia* **2021**, *153*, 104989, https://doi.org/10.1016/j.fitote.2021.104989.
62. Gao, J.; Aisa, H.A. Terpenoids from *Euphorbia soongarica* and Their Multidrug Resistance Reversal Activity. *J. Nat. Prod*. **2017**, *80*, 1767–1775, https://doi.org/10.1021/acs.jnatprod.6b01099.
63. Mazoir, N.; Auhmani, A.; Daoubi, M.; Collado, I.G.; Benharref, A. Hemisynthesis of New Triterpene Derivatives using Oxidation by CrO_3 and $NaIO_4$-($RuCl_3$, $3H_2O$). *Synth. Commun*. **2007**, *37*, 1289-1299, https://doi.org/10.1080/00397910701226756.
64. Mazoir, N.; Benharref, A.; Bailén, M.; Reina, M.; González-Coloma, A. Bioactive triterpene derivatives from latex of two *Euphorbia* species. *Phytochemistry* **2008**, *69*, 1328-1338, https://doi.org/10.1016/j.phytochem.2008.01.004.
65. Mazoir, N.; Benharref, A.; Bailén, M.; Reina, M.; González-Coloma, A.; Martínez-Díaz, R.A. Antileishmanial and Antitrypanosomal Activity of Triterpene Derivatives from Latex of Two Euphorbia Species. *Z. Naturforsch*. **2011**, *66*, 360-366, https://doi.org/10.1515/znc-2011-7-807.
66. Smaili, A.; Mazoir, N.; Rifai, L.A.; Koussa, T.; Makroum, K.; Kabil, E.M.; Benharref, A.; Faize, M. Triterpene derivatives from *Euphorbia* enhance resistance against *Verticilium* wilt of tomato. *Phytochemistry* **2017**, *135*, 169-180, https://doi.org/10.1016/j.phytochem.2016.12.017.
67. Smaili, A.; Mazoir, N.; Aicha Rifai, L.; Koussa, T.; Makroum, K.; Belfaiza, M.; Benharref, A.; Faize, M. Induced resistance to wild fire disease of *Nicotiana benthamiana* using seed treated with triterpene derivatives from *Euphorbia*. *J. Plant Pathol.* **2018**, *100*, 75-83, https://doi.org/10.1007/s42161-018-0031-0.
68. Daoui, O.; Mazoir, N.; Bakhouch, M.; Salah, M.; Benharref, A.; Gonzalez-Coloma, A.; Elkhattabi, S.; Yazidi, M.E.; Chtita, S. 3D-QSAR, ADME-Tox, and molecular docking of semisynthetic triterpene derivatives as antibacterial and insecticide agents. *Struct. Chem*. **2022**, *33*, 1063-1084, https://doi.org/10.1007/s11224-022-01912-4.
69. Yamari, I.; Mouhib, A.; Es-Sounni, B.; Nejjari, R.; Mazoir, N.; Bakhouch, M.; Mouzdahir, A.; Benharref, A.; El Kouali, M.h.; Chtita, S. Oxidative functionalization of triterpenes isolated from *Euphorbia resinifera* latex: semisynthesis, ADME-Tox, molecular docking, and molecular dynamics simulations. *Chem. Phys. Impact* **2023**, *7*, 100372, https://doi.org/10.1016/j.chphi.2023.100372.
70. Bøe, J.E.; Winsnes, R.; Nordal, A.; Bernatek, E. New constituents of *Euphorbia resinifera* Berg. *Acta Chem. Scand*. **1969**, *23*, 3609, https://doi.org/10.3891/acta.chem.scand.23-3609.
71. Nordal, A.; Benson, A.A. Phorbic Acid biosynthesis in the latex vessel system of euphorbia. *Plant Physiol*. **1969**, *44*, 78-84, https://doi.org/10.1104/pp.44.1.78.
72. Badaoui, M.; Moubtakir, S.; Terrafe, C.; Aboufatima, R.; Chait, A. Antianxiety and antidepressant effects of aqueous latex extract of Euphorbia resinifera. *Bangladesh J. Pharmacol*. **2022**, *17*, 32-41, http://dx.doi.org/10.3329/bjp.v17i2.59258.
73. Elagdi, C.; Bouaouda, K.; Rahhal, R.; Hsaine, M.; Badri, W.; Fougrach, H.; Hajjouji, H.E.L. Phenolic compounds, antioxidant and antibacterial activities of the methanolic extracts of *Euphorbia resinifera* and *Euphorbia echinus*. *Sci. Afr.* **2023**, *21*, e01779, https://doi.org/10.1016/j.sciaf.2023.e01779.
74. Boutoub, O.; Aazza, S.; El-Guendouz, S.; El Ghadraoui, L.; Miguel, M.G. Response surface methodology (RSM) for optimization of Euphorbia resinifera and Euphorbia officinarum Extracts with antioxidant and anti-diabetic activities. *Pharmacogn. Mag.* **2022**, *18*, 940-952.

75. Siritapetawee, J.; Teamtisong, K.; Limphirat, W.; Charoenwattanasatien, R.; Attarataya, J.; Mothong, N. Identification and characterization of a protease (EuRP-61) from *Euphorbia resinifera* latex. *Int. J. Biol. Macromol.* **2020**, *145*, 998-1007, https://doi.org/10.1016/j.ijbiomac.2019.09.190.
76. Siritapetawee, J.; Khunkaewla, P.; Thumanu, K. Roles of a protease from *Euphorbia resinifera* in human anticoagulant and antithrombotic activities. *Chem. Biol. Interact.* **2020**, *329*, 109223, https://doi.org/10.1016/j.cbi.2020.109223.
77. Siritapetawee, J.; Attarataya, J.; Charoenwattanasatien, R. Sequence analysis and crystal structure of a glycosylated protease from *Euphorbia resinifera* latex for its proteolytic activity aspect. *Biotechnol. Appl. Biochem.* **2022**, *69*, 2580-2591, https://doi.org/10.1002/bab.2307.
78. Siritapetawee, J.; Rodhirun, S.; Siritapetawee, S. Improving the antithrombogenecity of nylon monofilament for surgical suture by coating a protease from *Euphorbia resinifera* latex. *Materialia* **2022**, *25*, 101547, https://doi.org/10.1016/j.mtla.2022.101547.
79. Chakir, A.; Romane, A.; Marcazzan, G.L.; Ferrazzi, P. Physicochemical properties of some honeys produced from different plants in Morocco. *Arab. J. Chem.* **2016**, *9*, S946-S954, https://doi.org/10.1016/j.arabjc.2011.10.013.
80. Moujanni, A.; Terrab Benjelloun, A.; Eddoha, R.; Nasser, B.; Benbachir, M. Microbiological quality of Moroccan labeled Euphorbia resinifera honey. *J. Microbiol. Biotechnol. Food Sci.* **2017**, *6*, 1188-1194, https://doi.org/10.15414/jmbfs.2017.6.5.1188-1194.
81. Benjamaa, R.; Moujanni, A.; Terrab, A.; Eddoha, R.; Benbachir, M.; Moujahid, A.; Nasser, B.; Darkaoui, S.; Zyate, N.; Talmi, A.; Essamadi, A.K. Relationship Among Antibiotic Residues And Antibacterial Activity Of The Endemic Spurge Honey (Euphorbia Resinifera O. Berg) From Morocco. *Emir. J. Food Agric.* **2020**, *32*, 795-807, https://doi.org/10.9755/ejfa.2020.v32.i11.2190.
82. Massous, A.; Ouchbani, T.; Lo Turco, V.; Litrenta, F.; Nava, V.; Albergamo, A.; Potortì, A.G.; Di Bella, G. Monitoring Moroccan Honeys: Physicochemical Properties and Contamination Pattern. *Foods* **2023**, *12*, 969, https://doi.org/10.3390/foods12050969.
83. European Commission. Commission Regulation No. 10/2011 on plastic materials and articles intended to come into contact with food. *Off. J. Eur. Union* 2011, 12, 1–89.
84. Boutoub, O.; El-Guendouz, S.; Manhita, A.; Dias, C.B.; Estevinho, L.M.; Paula, V.B.; Carlier, J.; Costa, M.C.; Rodrigues, B.; Raposo, S.; Aazza, S.; El Ghadraoui, L.; Miguel, M.G. Comparative Study of the Antioxidant and Enzyme Inhibitory Activities of Two Types of Moroccan *Euphorbia* Entire Honey and Their Phenolic Extracts. *Foods* **2021**, *10*, 1909, https://doi.org/10.3390/foods10081909.
85. Moujanni, A.; Partida, L.; Essamadi, A.K.; Hernanz, D.; Heredia, F.J.; Terrab, A. Physicochemical characterization of unique unifloral honey: *Euphorbia resinifera*. *Cyta-J. Food* **2018**, *16*, 27-35, https://doi.org/10.1080/19476337.2017.1333529.
86. Boutoub, O.; El-Guendouz, S.; Estevinho, L.M.; Paula, V.B.; Aazza, S.; El Ghadraoui, L.; Rodrigues, B.; Raposo, S.; Carlier, J.; Costa, M.C.; Miguel, M.G. Antioxidant activity and enzyme inhibitory potential of *Euphorbia resinifera* and *E. officinarum* honeys from Morocco and plant aqueous extracts. *Environ. Sci. Pollut. Res.* **2021**, *28*, 503-517.
87. Hernanz, D.; Palomar, M.Á.; Moujanni, A.; Essamadi, A.; Heredia, F.J.; Terrab, A. Phenolic compounds and color of labeled resin spurge honey and their correlations with pollen content. *LWT* **2022**, *169*, 113987, https://doi.org/10.1016/j.lwt.2022.113987.
88. Boutoub, O.; El-Guendouz, S.; Matos, I.; El Ghadraoui, L.; Costa, M.C.; Carlier, J.D.; Faleiro, M.L.; Figueiredo, A.C.; Estevinho, L.M.; Miguel, M.G. Chemical Characterization and Biological Properties Assessment of *Euphorbia resinifera* and *Euphorbia officinarum* Moroccan Propolis. *Antibiotics* **2024**, *13*, 230, https://doi.org/10.3390/antibiotics13030230.
89. Glatthaar-Saalmüller, B.; Fallier-Becker, P. Antiviral action of Euphorbium compositum and its components. *Forsch Komplementärmed Klass Naturheilkd* **2001**, *8*, 207-212, https://doi.org/10.1159/000057223.