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Biological Activities of Lupeol

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ABSTRACT

This review covers mainly the past 25 years of research on the biological activities of lupeol, a significant lupane-type triterpene represented in the plant, fungi and animal kingdoms. Anticancer, antiprotozoal, chemopreventive and anti-inflammatory properties, plus the mechanisms of action of lupeol are emphasized. Some insights are provided regarding lupeol as a lead scaffold for synthetic chemical attempts to optimize pharmacological potency. Structure-activity relationship is also discussed.

Keywords: anti-arthritis, anti-inflammatory, antimalarial, antitumor, chemopreventive agent, hepatoprotective

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INTRODUCTION

Throughout human history, natural products have been used as remedies to cure or treat illnesses. In some parts of the world, this tradition has been surpassed by the amazing technological and pharmaceutical developments that have emerged with the promise of easier healing. Humans continue to be affected by several diseases, mainly due to natural forces such as drug-resistant microbes and insects. Consequently, an imperative need exists to connect the ethnopharmacological information with the newest drug-discovery technologies and scientific efforts, in order to discover new active natural metabolites. Humans are continuously learning more about and attaching value to natural products and their therapeutic properties, as well as becoming conscious of the importance of a well-balanced diet along with a healthy lifestyle to gain life quality. In this context, an impressive amount of natural substances have been highlighted by the media due to their wide-ranging properties, such as antioxidant, chemopreventive, cardioprotective and dietary supplement, e.g., resveratrol from red wine, polyphenols from tea, anthocyanins and hydrolyzable tannins from pomegranate, and isothiocyanates from plants of Brassicaceae family such as cauliflower and broccoli (Syed *et al.* 2008; Pan *et al*. 2009). Among these is lupeol, which is a common constituent of grape, hazelnut and olive oils, cocoa butter, mango pulp, white cabbage, and a variety of therapeutic plants. Lupeol exhibits a broad spectrum of biological activities and can be used as chemopreventive to avoid several diseases. Hence, this review focuses on this noteworthy natural compound.

LUPEOL

Definition, structural features, occurrence

Lup-20(29)-en-3β-ol (**Fig. 1**), generally known as lupeol, clerodol, fagarsterol and lupenol, is mainly identified by its ^{1}H and ^{13}C NMR spectral data, which reveal typical signals of a pentacyclic lupane-type triterpene with olefinic protons/carbons at δ 4.68 and 4.57 (brs, H-29)/109.6 and 151.1 (C-29 and 20, respectively), the hydroxymethine proton/carbon at δ 3.19 (dd, 4.8 and 11.6 Hz, H-3)/79.0 (C-3) and seven singlet signals assigned to the tertiary methyl groups at δ 0.77, 0.80, 0.84, 0.95, 0.97, 1.03, 1.20/28.4, 15.8, 16.5, 16.3, 14.9, 18.9, 19.7 (H/C-23 to 28 and 30, respectively) (complete assignment in Fotie *et al.* 2006; Lutta *et al.* 2008). Recently, lupeol structure was elucidated on the basis of X-ray diffraction analysis using the space group $P4_3$ along with the stereochemistry specified by biosynthesis (Corrêa *et al*. 2009).

This triterpene has rare reports in the fungal and animal

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Invited Review

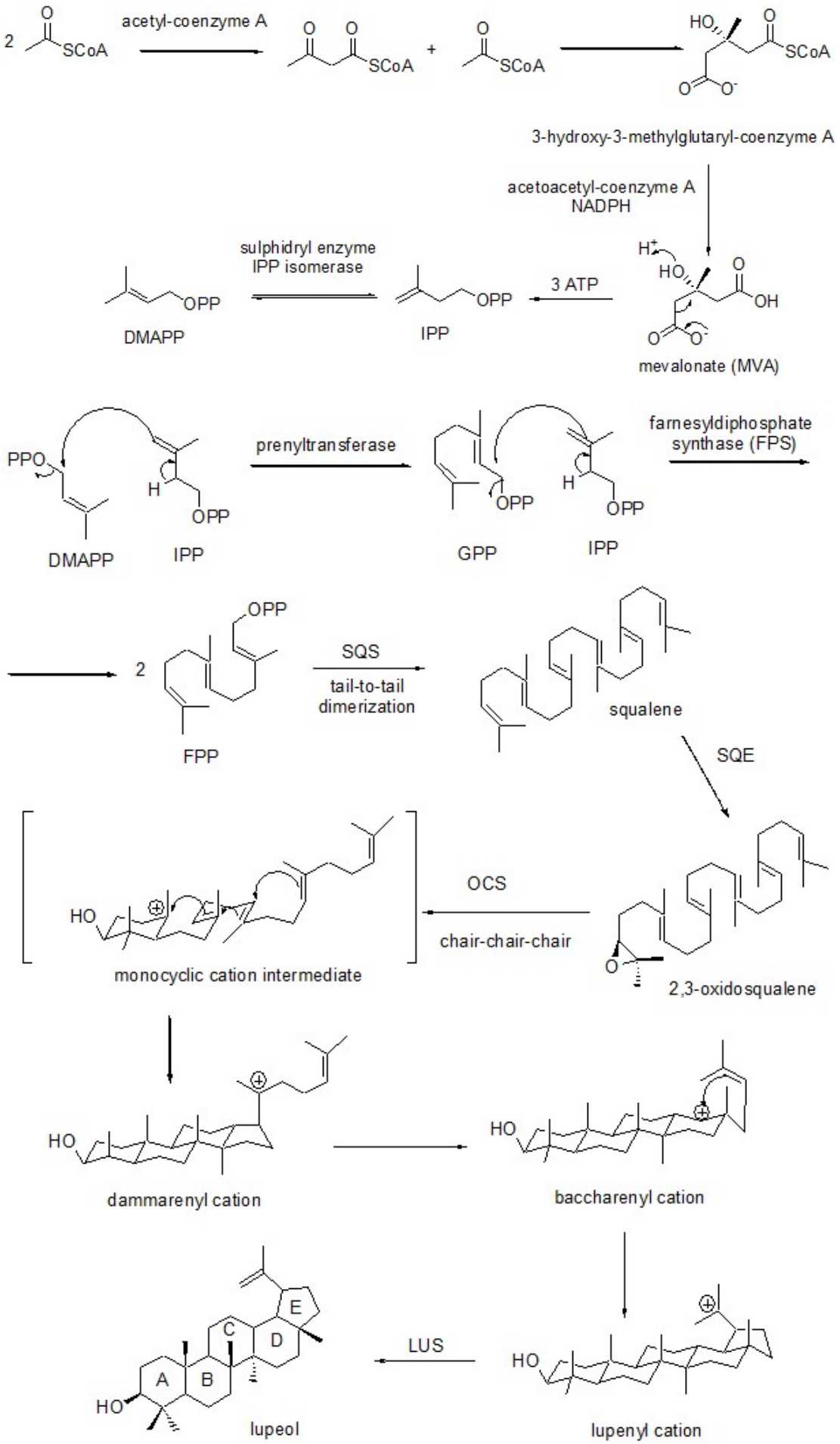


Fig. 1 Mevalonate pathway and biosynthesis of lupeol. IPP = isopentenyl pyrophosphate; DMAPP = dimethylallyl pyrophosphate; GPP = geranyl pyrophosphate; FPP = farnesyl pyrophosphate; SQS = squalene synthase; SQE = squalene epoxidase; OSC = oxidosqualene cyclase; LUS = lupeol synthase.

kingdom (Suzuki and Ikekawa 1966; Kahlos *et al.* 1989; Kim *et al.* 2003; Lutta *et al.* 2008) but is known to have vast occurrence in diverse plant families (Connolly and Hill 2008) and is even found in propolis (Pereira *et al.* 2002). According to Duke (1992), the mango pulp, carrot root, cucumber, soybean and melon seeds, quebracho bark, uva ursi and aloe plants are rich sources of lupeol. Moreover, several herbal medicines have this chemical as one of their principal active constituents. For example, *Crataeva nurvala* (Capparidaceae) bark is employed by the people of India as a lithotriptic agent (Prasad *et al.* 2007a); *Careya arborea* Roxb (Barringtoniaceae) stem bark is used in Ayuverdic therapy to treat tumors as well as an antidote to snake venom (Senthilkumar *et al.* 2008); *Echinops echinatus* Roxb (Asteraceae) roots are employed in India to heal reproductive system disorders (Padashetty and Mishra 2007); natives from the Amazonian region use *Aspidosperma nitidum* (Apocynaceae) to treat uterus and ovary inflammation, and in anticancer, anti-rheumatic and antimalarial therapies (Pereira *et al.* 2006); people from Latin America and Mexico use several species of *Acosmium* (Fabaceae) to treat diabetes, and fever (Souza Júnior *et al*. 2009), whereas *Zanthoxylum riedelianum* Engl. (Rutaceae) is a well-known Brazilian folk plant employed to relief tooth pain (Lima *et al*. 2007).

Lupeol has been studied for more than a century. During the early days, the majority of the published articles were related to its synthesis, phytochemical investigations and biological activities. However, in the beginning of the 21st century, the number of articles on it has increased tremendously. A considerable upswing in publications on lupeol occurred throughout the past 5 years with a mean of 54 articles per year (Scifinder and Web of Science databases), mostly attributable to lupeol's anticancer effects, a fact that once more stimulated the search for further bioactive natural products as lead compounds for drug-discovery programs. Recently, researches regarding biotransformation, chemoprevention, mechanism of action, derivative synthesis and methods of detection and quantitation, in addition to conventional studies, have been carried out with lupeol.

Synthesis and biosynthesis

Triterpenes are considered secondary metabolites, thus they are not vital to the organism that produces them. However, they have a large occurrence and are produced in a great diversity of carbon ring structures that suscitate an insistent question about why a living organism would expend so much energy producing and accumulating these compounds. The answer is not totally understood but it is known they provide unique means for these organisms to interrelate with their environment (Chappel 2002). Thus, for over a half-century scientists have been examining the complete triterpene formation mechanism, which is orchestrated by the triterpene synthases and is considered as one of the most complex reactions occurring in nature (reviewed by Yoder and Johnston 2005; Phillips *et al.* 2006). The basic outlines of the biosynthetic pathway are quite well comprehended; a series of reactions responsible for both triterpenes and steroids biosynthesis occurs in the cytosol and constitutes the mevalonate (MVA) pathway (**Fig. 1**), where a five carbon unit, the isopentenyl pyrophosphate (IPP), and its allyl isomer dimethylallyl pyrophosphate (DMAPP) are formed from acetyl-CoA and sequentially condensed by the farnesyl pyrophosphate synthase (FPS) to farnesyl pyrophosphate (FPP). This precursor is polymerized into squalene by the action of a squalene synthase (SQS). Squalene epoxidase (SQE) oxidizes squalene to 2,3-oxidosqualene, the last common intermediate for triterpenes and steroids, which is then cyclized in a chair-chair-chair conformation by a member of the oxidosqualene cyclases family (OSCs) to continue the triterpene biosynthesis. There is a range of multifunctional or specific OSCs tightly controlling this step cyclization to yield assorted types of triterpenes depending on the plant species (Shibuya *et al.* 2007). Usually, lupeol synthase (LUS) is the OSC that catalyzes the cyclization of 2,3-oxidosqualene through carbocation chemistry occurring by successive electrophilic additions to yield the dammarenyl cation, followed by a rearrangement promoting a ring expansion to afford the baccharenyl cation, which undergoes an electrophilic addition to form the lupenyl cation that is then converted into lupeol by deprotonation of the 29-methyl group (**Fig. 1**; Phillips *et al.* 2006).

Several attempts have been made to understand the role performed by the enzymes in controlling the biosynthetic workflow toward sterols or triterpenes. Isopentenyl pyrophosphate isomerase (IPI) was demonstrated to be essential for the maintenance of IPP and DMAPP levels in different subcellular compartments of *Arabidopsis* and, consequently, plays a decisive role toward the terpenoid and steroid biosynthesis by the MVA pathway (**Fig 1**; Okada *et al.* 2008). Ohyama *et al.* (2007) quantified the total content of steroids and triterpenes in *Arabidopsis* HMGR mutants and discovered this enzyme affects the total amount of those compounds, but the plant synthesizes those products in excess. Once production is in a specific range, one of the mutants presents normal growth while the other, containing much lower amounts of some steroids and triterpenes, shows an abnormal phenotype. It is interesting to notice the large difference in lupeol levels between the mutants and to speculate about its probable function in the phenotypic deviations. No less remarkable, the cloning and functional expression of several OSCs in yeast have revealed new enzymatic functions, disclosed unusual mechanisms of action (Husselstein-Muller *et al.* 2001) and led to the characterization of a very high specific LUS that operates the production of lupeol in the epicuticle of *Ricinus* stem, a strategic location to control herbivorous insects by hampering their traffic (Guhling *et al.* 2006).

The synthesis of lupeol is a stereochemical challenge since its structure comprises ten asymmetric centers. Although some attempts have been made to synthesize it by different routes (MacKelfar *et al.* 1971; Yoder and Johnston 2005), there is a tendency to obtain lupeol from natural sources, for example from lupeol-rich plants such as *Crataeva nurvala* and birch barks or from industrial residues of cork processing (Agarwal and Kumar 2003; Souza *et al.* 2006; Yunusov *et al.* 2006), since this way is, theoretically, less polluting and cheaper.

Quantitation and detection

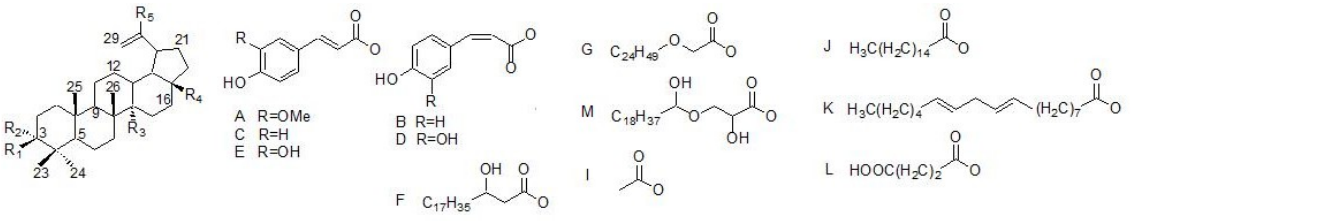
Currently, the use of medicinal plants is massively increasing as a low-cost alternative to the pricey industrial drugs and due to more natural treatment requirements that display fewer side effects. Therefore, several products based on plant species are being manufactured in various pharmaceutical forms, and are being sold in pharmacies and natural product stores. However, it is known that the pharmacological action of a plant is provided by the active components, and the amount of these compounds can differ considerably depending on several factors like the plant tissue used and the season during which the plant is harvested. The development of methods for detection and quantitation of an active substance is fundamental for quality control of either medicinal plants or phytopreparations. Gas Chromatography (GC) and High Performance Thin Layer Chromatography (HPTLC) techniques are the most employed methods to quantitate lupeol in medicinal plants. HPTLC is cost efficient, flexible and quick. Silica gel $60F_{254}$ is used as the stationary phase; the plate development can be carried out with a variety of solvent systems like toluene/methanol (9:1), *n*-hexane/ethyl acetate (5:1), toluene/ethyl acetate/methanol (7.5:1.5:0.7) or toluene/chloroform/ethyl acetate/glacial acetic acid (10: 2: 1: 0.03) and lupeol is detected and quantified by densitometry after reaction with anisaldehyde-sulfuric acid, Lieberman-Burchard reagent or antimony trichloride (Anadjiwala *et al.* 2007; Martelanc *et al.* 2007; Padashetty and Mishra 2007a; Shailajan and Menon 2009). On the other hand, the detection and/or quantitation

of lupeol either in a plant extract or seed oil using GC methods require pre-derivatization of the samples, for example by acetylation or trimethylsilylation; sometimes a sample clean-up employing silica gel columns or liquid-liquid partition is also necessary (Itoh *et al*. 1974; Hooper *et al*. 1982; Dailey *et al*. 1997; Cordeiro *et al*. 1999; Beveridge *et al*. 2002; Yaşar *et al*. 2005; Oliveira *et al*. 2006; Hovaneissian *et al*. 2008; Marín *et al*. 2008). However, Kpoviéssi and collaborators (2008) have completely validated a method for the quantitative determination of lupeol in *Justicia anselliana* by capillary gas chromatography (GC-FID/GC-MS) without derivatization of the extract, which was obtained in a soxhlet apparatus. Finally, the least and also more recent technique used to quantitate and determine lupeol is Reversed-Phase High Performance Liquid Chromatography (RP-HPLC). Mathe *et al.* (2004) developed a RP-HPLC method, using water and acetonitrile (ACN) both containing 0.01% phosphoric acid as mobile phase and an UV detector at 210 nm, in order to determine lupeol and other fourteen pentacyclic triterpenes in an attempt to distinguish the geographical and botanical origins of the commercial oleo-gum-resin frankincense. Martelanc and coworkers (2007) also used a RP-HPLC coupled to UV and mass spectrometer detectors to determine the presence of lupeol in the epicuticular wax of the white cabbage. Li *et al.* (2008) developed an RP-HPLC method to quantify lupeol in *Ilex cornuta* employing a 15 cm C18 column and ACN/water (4:1) as the mobile phase. Martelanc and coworkers (2009) have recently developed a combination of complementary chromatographic techniques to determine lupeol in triterpenoid isomeric mixtures from plant extracts. Using an HPLC coupled to UV at 220 nm and an ion trap LCQ MS-MS/MS system working with APCI ion source in the positive mode and ion trap CID (collision induced dissociation), they obtained good resolution for lupenone, lupeol and cycloartenol, α- and β-amyrin, lupeol acetate and cycloartenol acetate when 93.5% ACN in water was employed as the mobile phase, and the column was heated at 38°C. Furthermore, they also demonstrated that a better separation of isomeric mixtures can be acquired using RP-HPTLC rather than the conventional HPTLC, and proved acetone/ACN 5:1 to be the best developing solvent to resolve lupeol in the majority of the screened extracts.

PHARMACOLOGICAL ACTIVITIES OF LUPEOL

Antiprotozoal

Several of the most severe diseases in the world are caused by protozoa and primarily distress developing nations' populace. Some of these so-called neglected diseases, such as leishmaniasis, trypanosomiasis and malaria, persist without effective treatment either by natural reasons, e.g., resistant strains, or from industrial disinterest due to economics in finding more efficient drugs. Added to these factors, the low purchasing power of the affected people and their inaccessible habitation areas compel people to seek cure in plants, closer and handy resources. In the Amazonian region of Bolivia, the indigenous Chimane population treats cutaneous leishmaniasis with cataplasms of *Pera benensis* fresh stem bark until obtaining the complete healing of the skin lesions. Based on this traditional knowledge, Fournet *et al.* (1992) carried out a phytochemical bioassay-guided study and found plumbagin as the main active constituent (IC_{50} 5.0 µg/mL) alongside a weak action displayed by lupeol against varied strains of *Leishmania* and *Trypanosoma* species (**Table 1**). Furthermore, the bioassay-guided research of a plant used in the treatment of malaria symptoms by a pygmy tribe from Cameroon led to the isolation of an alkaloid-rich fraction along with lupeol and derivatives **13**, **14** and **20** (**Fig. 2**). These last four compounds displayed low individual potencies against two different strains of *Plasmodium falciparum* (**Table 1**) and the suggestion of synergic effect among the metabolites was discussed by the authors (Fotie *et al.* 2006). Biological tests aiming for natural antimalarial agents (reviewed by Schwikkard and van Heerden 2002; Caniato and Puricelli 2003) revealed that lupeol moderates *in vitro* growth inhibition of *Plasmodium falciparum*, but lacks activity in an *in vivo* assay (**Table 1**; Alves *et al.* 1997). Since then, lupeol and related compounds have been tested by several scientists against different strains of some protozoa species (**Table 1**). For example, Srinivasan *et al.* (2002) built and tested a 96-member lupeol-based library. One of the most promising library members was bioassayed on *P. falciparum* NF-54 strain (IC_{50} of 14.8 µM) and *P. berghei*, and the same discrepancy between the *in vitro* and *in vivo* activities was observed. In an attempt to explain the antimalarial mode of action of lupane-type triterpenes, Ziegler and collaborators (2002, 2004) demonstrated that lupeol and related-compounds irreversibly change the erythrocyte membrane shape at concentrations similar to their *in vitro* antiplasmodial IC_{50} values (**Table 1**). They also proposed a structure-activity relationship among the tested compounds for their membrane effects and the way they incorporate into the erythrocyte membrane based on the C-28 group capacity of hydrogen donation, comparing their mechanism of action with some amphiphilic moieties mode. Rather than a targeted toxic effect on the parasite organelles or metabolic pathways (reviewed by Rodrigues and Souza 2008), the antiplasmodial effect of these types of compounds seems to be correlated with alterations in the membrane shape of the host cell, disqualifying them as lead



1 - R_1=OH, R_2=H, R_3=R_4=R_5=Me	lupeol	**12** - R_1=E, R_2=H, R_3=R_4=R_5=Me	3-(*E*)-caffeoyllupeol
2 - R_1=R_2=O, R_3=R_4=R_5=Me	lupenone	**13** - R_1=F, R_2=H, R_3=R_4=R_5=Me	3-*O*-(3'-hydroxyeicosanoyl lupeol
3 - R_1=OH, R_2=H, R_3=R_5=Me, R_4=COOH	betulinic acid	**14** - R_1=G, R_2=H, R_3=R_4=R_5=Me	3-*O*-[(2'-(tetracosyloxy)acetyl] lupeol
4 - R_1=OH, R_2=H, R_3=CH_2A, R_4=COOH, R_5=Me	winchic acid	**15** - R_1=I, R_2=H, R_3=R_4=R_5=Me	lupeol acetate
5 - R_1=OH, R_2=H, R_3=CH_2B, R_4=COOH, R_5=Me	messagenic acid A	**16** - R_1=J, R_2=H, R_3=R_4=R_5=Me	lupeol palmitate
6 - R_1=OH, R_2=H, R_3=CH_2C, R_4=COOH, R_5=Me	messagenic acid B	**17** - R_1=K, R_2=H, R_3=R_4=R_5=Me	lupeol linoleate
7 - R_1=OH, R_2=H, R_3=R_5=Me, R_4=CH_2OH	betulin	**18** - R_1=L, R_2=H, R_3=R_4=R_5=Me	lupeol hemisuccinate
8 - R_1=OH, R_2=H, R_3=R_5=Me, R_4=CHO	betulinaldehyde	**19** - R_1=R_2=O, R_3=R_4=Me, R_5=CH_2OH	30-hydroylupenone
9 - R_1=OH, R_2=H, R_3=R_5=Me, R_4=COOMe	methylbetulinate	**20** - R_1=M, R_2=H, R_3=R_4=R_5=Me	3-*O*-[(1''-hydroxyoctadecyloxy)-2'-hydroxypropanoyl] lupeol
10 - R_1=OH, R_2=H, R_3=R_5=Me, R_4=$CONH_2$	betulinic acid amide	**21** - R_1=OH, R_2=H, R_3=R_4=Me, R_5=CH_2OH	30-hydroylupeol
11 - R_1=D, R_2=H, R_3=R_4=R_5=Me	3-(*Z*)-caffeoyllupeol		

Fig. 2 Structural formula of lupeol and related compounds tested as antiprotozoal and anti-inflammatory agents.

Table 1 Antiprotozoal activities of lupeol and related compounds.

Compound	Protozoan (strain)	Activity	Plant species	Plant family	Reference
1	*Plasmodium berghei*	I[a] *in vivo* at 15 mg/kg	*Vernonia brasiliana*	Asteraceae	Alves *et al.* 1997
	Plasmodium. falciparum (BHz26/86)[b]	45% GI[c] *in vitro* at 25 µg/mL			
	Trypanosoma brucei brucei (TF)[d]	IC_{50}[e] *in vitro* 19.3 µg/mL	*Strychnos spinosa*	Loganiaceae	Hoet *et al.* 2007
	Trypanosoma cruzi[f]	IC_{90}[g] *in vitro* >100 µg/mL	*Pera benensis*	Euphorbiaceae	Fournet *et al.* 1992
	P. falciparum (FCR-3)[h]	IC_{50}[e] *in vitro* 41 µg/mL	*Holahrrena floribunda*	Apocynaceae	Fotie *et al.* 2006
	P. falciparum (3D7)[i]	IC_{50}[e] *in vitro* 45 µg/mL			
	P. falciparum (3D7)[i]	IC_{50}[e] *in vitro* 11.8 µg/mL	*Rinorea ilicifolia*	Violaceae	Ziegler *et al.* 2002
	P. falciparum (K1)[j]	I[a] *in vitro* at 10 and 20.0 µg/mL	*Gardenia saxatilis* *Ziziphus cambodiana*	Rubiaceae Rhamnaceae	Suksamrarn *et al.* 2003, 2006
	P. falciparum (K1)[j]	IC_{50}[e] *in vitro* 5.0 µg/mL	*Cassia siamea*	Fabaceae	Ajaiyeoba *et al.* 2008
	Leishmania[k]	IC_{90}[g] *in vitro* 100 µg/mL	*Pera benensis*	Euphorbiaceae	Fournet *et al.* 1992
2	*P. falciparum* (K1)[j]	I[a] *in vitro* 20.0 µg/mL	*Gardenia saxatilis*	Rubiaceae	Suksamrarn *et al.* 2003
3	*P. falciparum* (K1)[j]	IC_{50}[e] *in vitro* 19.6 µg/mL	*Uapaca nitida*	Euphorbiaceae	Steele *et al.* 1999
	P. falciparum (K1)[j]	I[a] *in vitro* 10.0 µg/mL	*Ziziphus cambodiana*	Rhamnaceae	Suksamrarn *et al.* 2006
	P. falciparum (3D7)[i]	IC_{50}[e] *in vitro* 6.3 µg/mL	*Zataria multiflora*	Lamiaceae	Ziegler *et al.* 2004
	P. falciparum (T9-96)[l]	IC_{50}[e] *in vitro* 25.9 µg/mL	*Uapaca nitida*	Euphorbiaceae	Steele *et al.* 1999
	P. berghei	I[a] *in vivo* at 250 mg/kg/day			
	T. brucei brucei (TF)[d]	IC_{50}[e] *in vitro* 14.9 µg/mL	*Strychnos spinosa*	Loganiaceae	Hoet *et al.* 2007
4	*P. falciparum* (K1)[j]	I[a] *in vitro* at 20.0 µg/mL	*Gardenia saxatilis*	Rubiaceae	Suksamrarn *et al.* 2003
5	*P. falciparum* (K1)[j]	IC_{50}[e] *in vitro* 1.5 µg/mL	*Gardenia saxatilis*	Rubiaceae	Suksamrarn *et al.* 2003
6	*P. falciparum* (K1)[j]	IC_{50}[e] *in vitro* 3.8 µg/mL	*Gardenia saxatilis*	Rubiaceae	Suksamrarn *et al.* 2003
7	*P. falciparum* (K1and T9-96)[j,l]	I[a] *in vitro* 500 µg/mL	*Uapaca nitida*	Euphorbiaceae	Steele *et al.* 1999
	T. brucei brucei (TF)[d]	IC_{50}[e] *in vitro* 4.0 µg/mL	*Strychnos spinosa*	Loganiaceae	Hoet *et al.* 2007
	P. falciparum (3D7)[i]	IC_{50}[e] *in vitro* < 12 µg/mL	Synthetic	-	Ziegler *et al.* 2004
8	*P. falciparum* (K1)[j]	IC_{50}[e] *in vitro* 6.5 µg/mL	*Ziziphus cambodiana*	Rhamnaceae	Suksamrarn *et al.* 2006
	P. falciparum (3D7)[i]	IC_{50}[e] *in vitro* 6.2 µg/mL	*Ziziphus vulgaris*	Rhamnaceae	Ziegler *et al.* 2004
9	*P. falciparum* (3D7)[i]	IC_{50}[e] *in vitro* 3.3 µg/mL	Synthetic	-	Ziegler *et al.* 2004
10	*P. falciparum* (3D7)[i]	IC_{50}[e] *in vitro* 6.4 µg/mL	Synthetic	-	Ziegler *et al.* 2004
11	*P. falciparum* (K1)[j]	EC_{50}[m] 8.6 µg/mL	*Bruguiera parviflora*	Rhizophoraceae	Chumkaew *et al.* 2005
12	*P. falciparum* (K1)[j]	I[a]	*Bruguiera parviflora*	Rhizophoraceae	Chumkaew *et al.* 2005
13	*P. falciparum* (FCR-3)[h]	IC_{50}[e] *in vitro* 198 µg/mL	*Holahrrena floribunda*	Apocynaceae	Fotie *et al.* 2006
	P. falciparum (3D7)[i]	IC_{50}[e] *in vitro* 208 µg/mL			
14	*P. falciparum* (FCR-3)[h]	IC_{50}[e] *in vitro* 69 µg/mL	*Holahrrena floribunda*	Apocynaceae	Fotie *et al.* 2006
	P. falciparum (3D7)[i]	IC_{50}[e] *in vitro* 111 µg/mL			
20	*P. falciparum* (FCR-3)[h]	IC_{50}[e] *in vitro* > 391 µg/mL	*Holahrrena floribunda*	Apocynaceae	Fotie *et al.* 2006
	P. falciparum (3D7)[i]	IC_{50}[e] *in vitro* >391 µg/mL			

[a]I = inactive
[b]BHz26/86 = chloroquine-resistant strain
[c]GI = growth inhibition
[d]TF = trypomastigote form
[e]IC_{50} = half inhibitory concentration
[f]Epimastigote (vector) and trypomastigote (blood circulating) forms of *T. cruzi* (SC 43C12; C8C11; R107C18; Tulahuen; 1979 C17) strains; [g]IC_{90} = 90% inhibitory concentration
[h]FCR-3 = chloroquine-resistant strain
[i]3D7 = chloroquine-sensitive strain
[j]K1 = multidrug-resistant strain
[k]Amastigote (intracellular) and promastigote forms of *L. amazonensis* (IFLA/BR/67/PH8; MHOM/GF/84/CAY H-142), *L. braziliensis* (MHOM/BR/75/M 2903) and *L. donovani* (MHOM/IN/83/HS-70; MHOM/BR/00/M 2682) strains
[l]T9-96 = chloroquine-sensitive strain
[m]EC_{50} = half maximal effective concentration

molecules for antiplasmodial drug development (Ziegler *et al.* 2006).

When considering the *in vitro* antitrypanocidal activity of some triterpenes (Hoet *et al.* 2007; Gallo *et al.* 2008; Leite *et al*. 2008), the presence of C-28 hydrogen donor groups or a highly oxygenated side chain are structural attributes similar to those required for the *in vitro* antiplasmodial activity. On the other hand, the life cycle of *Trypanosoma* species is a little different from *Plasmodium* species; thus, more studies must be carried out in order to understand the lupane series triterpenes' mode of action against this protozoan genus.

Anti-inflammatory

Inflammation is a cascade of biochemical events, involving the local vascular system and the immune system, characterized by five basic symptoms: *rubor* (redness), *calor* (heat), *tumor* (swelling), *dolor* (pain) and loss of function. It happens as a response to either injurious agents or foreign materials such as chemical irritants, toxins, pathogens, burns and splinters. The synthesis and release of several inflammatory mediators by different types of defense cells are involved in the process, which is regulated by diverse enzymes. In general, the monocytes differentiate into macrophages that synthesize various signaling molecules, among them the protein interleukin-1β (IL-1β), which triggers a second wave of cytokines responsible for the migration of neutrophils to the injured tissue. Moreover, IL-1β enters the blood stream and is carried to the brain where is connected to the surface receptors of the blood-brain barrier cells, eliciting them to produce prostaglandin E2 (PGE2). This mediator crosses the blood-brain barrier and activates neurons and microglia receptors, which trigger the inflammation acute phase. Macrophages also produce reactive intermediates of oxygen such as hydrogen peroxide (H_2O_2) and nitric oxide (NO), important agents in edema development. Inside the neutrophils the enzyme 5-lipoxygenase acts on arachidonic acid to produce other typical type of inflammatory mediators, the leukotrienes (LT), which play a pathological role in allergic and respiratory diseases and are part of a complex response that usually includes the production of histamines. Lymphocytes (B and T cells) produce immunoglobulins (antibodies) and have surface receptors involved in antigen recognition and cell-to-cell interactions with macrophages and other lymphocytes, being res-

Table 2 Plants containing lupeol with anti-inflammatory popular use.

Plant species	Plant family	Studied extract	Folk medicinal use	Reference
Bridelia scleroneura	Euphorbiaceae	Stem bark	Abdominal pain, contortion, arthritis and inflammation	Théophile *et al.* 2006
Leptadenia hastata	Asclepiadaceae	Latex	Anti-inflammatory, wound healing agent	Nikiéma *et al.* 2001
Diplotropis ferruginea	Fabaceae	Stem bark	Inflammation, vaginal and external ulcers	Vasconcelos *et al.* 2008
Pimenta racemosa	Myrtaceae	Leaves	Several inflammatory processes	Fernández *et al.* 2001a
Millettia versicolor	Fagaceae	Leaves	Analgesic, anti-rheumatic and anti-inflammatory	Ongoka *et al.* 2008
Strobilanthus callosus, *S. ixiocephala*	Acanthaceae	Roots	Inflammatory disorders	Agarwal and Rangari 2003
Himathanthus sucuuba	Apocynaceae	Stem bark	Gastritis, hemorrhoids, anemia, arthritis, verminosis and cancer	Miranda *et al.* 2000
Euclea natalensis	Ebenaceae	Root bark	Bronchitis, pleurisy and chronic asthma	Weigenand *et al.* 2004
Croton pullei	Euphorbiaceae	Leaves	Inflammation (the genus)	Rocha *et al.* 2008
Anemone raddeana	Ranunculaceae	Rhizome	Rheumatism and neuralgia	Yamashita *et al.* 2002

ponsible for cellular immunity (for major details, read Medzhitov 2008; Bensinger and Tontonoz 2008). The uncontrolled release of many of those signaling molecules is the basis for the development of different types of inflammatory diseases like asthma and arthritis. Several anti-inflammatory drugs function by preventing the formation of some of the abovementioned mediators or by blocking their actions on the target cells whose behavior is modified by the mediators and, consequently, they are able to break the cross-talk between the signaling pathways.

Several plants employed in folk medicine to treat inflammatory symptoms have been shown to contain lupeol as one of their active principles (**Table 2**), corroborating the popular uses. In order to discover the anti-inflammatory mechanism of action of lupeol and related compounds, some experiments have been done. Bani *et al.* (2006) stated that lupeol decreases the IL-4 (interleukin 4) production by Th2 cells (T-helper type 2), and Vasconcelos and coworkers (2008) have recently confirmed the potent anti-inflammatory activity of lupeol in an allergic airway inflammation model as evidenced by a significant reduction in eosinophils infiltration and in Th2-associated cytokines (IL-4, IL-5, IL-13) levels that trigger the immune responses in asthma. Ding and coworkers (2009) revealed lupeol reduced the LPS-induced IL-6 secretion to 27.6% at a concentration of 1 μM. The topical anti-inflammatory activity of *Pimenta racemosa* extract, containing lupeol, was associated with the reduction of neutrophils into the inflamed tissues (Fernández *et al.* 2001a). Moreira *et al.* (2001) verified the weak immunoestimulatory effect of lupeol on macrophages by measuring their hydrogen peroxide production. Bani *et al.* (2006) observed the suppressive action of lupeol on cytotoxic (CD8+ T) and helper (CD4+) T cells, whose major effector function is the activation of macrophages, that consequently caused inhibition of IL-2 production, diminished the secretion of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon gamma (IFN-γ; which plays a critical hole in the development of arthritis), and reduced phagocytosis. Studies involving several types of induced inflammatory tests revealed lupeol's inability to modulate the edemas induced by dextran, resiniferatoxin and xylene, neurogenic inflammatory agents, as well as by arachidonic acid, a selective assay for 5-lipoxygenase (5-LOX) inhibitors (Huguet *et al.* 2000; Fernández *et al.* 2001a). Additionally, lupeol did not significantly inhibit NO release or synthesis of LTC4, a lipoxygenase metabolite, by macrophages but did show high inhibitory effect on the production of some inflammatory mediators such as PGE2 (IC_{50} 24.3 μM), TNF-α and IL-1β (Fernández *et al.* 2001b). Lupeol also displayed some inhibition of mezerein- (protein kinase C (PKC) activator) and croton oil-induced ear edema in the same range as indomethacin (**Table 3**), although lupeol was inactive when administered 2h before the inflammatory agent (Huguet *et al.* 2000), characterizing a curative but not a preemptive effect. Similar action was observed in ear edema induced by the diterpene PKC activators 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and 12-deoxyphorbol-13-decanoate (DPT) (**Table 3**), which lead Huguet and coworkers (2000) suggest that the anti-inflammatory activity of lupeol-type triterpenoids might depend on inhibition of PKC, without any involvement of neurogenic inflammatory mechanisms. Treatment of arthritic rats with lupeol and its linoleate and eicosapentaenoate esters decreased the level of glycoproteins and lysosomal enzymes, suggesting a reduction of endocytosis by leucocytes and/or stabilization of the lysosomal membrane (Geetha and Varalakshmi 1999; Latha *et al.* 2001). Kim *et al.* (2003) also observed lupeol's capacity of inhibiting the neuraminidase activity (**Table 4**), a glycoprotein present outside the influenza virus particle. A comparative docking study revealed lupeol's ability to elicit the cutaneous wound healing better than the standard drug nitrofurazone due to the complete lupeol enfolding in the entire ATP binding pocket of the glycoprotein glycogen-synthase-kinase-3-β (GSK-3β) and its consequent inhibition (Harish *et al.* 2008). Furthermore, it was verified that lupeol was devoid of antinociceptive, anti-pyretic and ulcerogenic actions (Singh *et al.* 1997; Geetha and Varalakshmi 2001), did not cause collateral effects during topical treatment (Huyke *et al.* 2006), showed a modest cytotoxicity (36.7%) on murine macrophages (Arciniegas *et al.* 2004), displayed minimum hemolysis at 500 mmol/L (Yamashita *et al.* 2002) and caused no mortality in mice after a treatment period of 14 days employing a 2 g/kg dose (Bani *et al.* 2006). These effects indicate a different mode of action in comparison with the known non-steroidal anti-inflammatory drugs that are non-specific cyclo-oxygenase (COX) inhibitors, like aspirin and indomethacin, and cause peptic ulceration as a side effect.

Lupeol and related compounds showed a diversified structure-activity relationship among different types of anti-inflammatory tests. For example, an improvement in activity was observed on bradykinin-, TPA-, DPT-, carrageenan- and 12-deoxyphorbol-13-phenylacetate (DPP)-induced edemas with the presence of C-28 carboxylic or alcohol groups (**Table 3**; Recio *et al.* 1995); lupeol and its hemisuccinyl ester (**Fig. 2**) increased epidermal tissue reconstitution in topical inflammation while acetylation and palmitoylation of the OH-3 group decreased it (Nikiéma *et al.* 2001); an enhancement of the lupeol antiarthritic effectiveness was noticed when its OH-3 group was esterified (**Table 3**; Kweifio-Okai *et al.* 1995b; Latha *et al.* 2001). All of these examples point out a wide mode of action involving different biochemical sites of interaction. Actually, Rajic *et al.* (2000) and Hodges *et al.* (2003) found that lupeol and its palmitate and linoleate esters are selective inhibitors of the serine proteases trypsin and chymotrypsin (**Fig 2; Table 4**) in a competitive and non-competitive way, respectively, while they are inactive or poor inhibitors of some protein kinases as calmodulin-dependent myosin light chain kinase (MLKC), wheat embryo Ca^{2+}-dependent protein kinase (CDPK), Ca^{2+}- and phospholipid-dependent PKC as well as porcine pancreatic elastase. Lupeol and its acetate also inhibited the human serine protease leucocyte elastase (**Table 4**; Mitaine-Offer *et al.* 2002). Furthermore, lupeol did not affect the collagenase release by osteosarcoma cells whereas its linoleate and palmitate esters decreased it by 97 and

Table 3 Anti-inflammatory and anti-arthritic activities of lupeol and related compounds.

Compound	Model	Activity % inhibition/reduction (dose)	Reference
1	CFA1[a]	39 (50 mg/kg)	Geetha and Varalakshmi 2001
	CFA2[b]	33 (600 mg/kg)	Agarwal and Rangari 2003
	DPT1[c]	40 (0.5 mg/ear)	Huguet *et al.* 2000
	DPT2[d]	-4 (0.5 mg/ear)	Huguet *et al.* 2000
	DPP[e]	7 (0.5 mg/ear)	Huguet *et al.* 2000
	TPA1[f]	18 (0.5 mg/ear)	Huguet *et al.* 2000
	TPA2[g]	36.2% (0.5 mg/ear)	Fernández *et al.* 2001b
	TPA3[h]	IC_{50} 0.48 mg/ear	Arciniegas *et al.* 2004
	Croton oil[i]	80 (0.42 µM/ear)	Nikiéma *et al.* 2001
	Cotton pellet[j]	33 (600 mg/kg)	Agarwal and Rangari 2003
	Mezerein[k]	56 (0.5 mg/ear)	Huguet *et al.* 2000
	Bradykinin[l]	35 (10 mg/kg)	Huguet *et al.* 2000
	Carrageenan[m]	32.6 (20 mg/kg)	Arciniegas *et al.* 2004
		8 (200 mg/kg)	Agarwal and Rangari 2003
		11 (400 mg/kg)	Agarwal and Rangari 2003
		27 (800 mg/kg)	Agarwal and Rangari 2003
		57 (5mg/Kg)	Nguemfo *et al.* 2009
3	DPT1[c]	51 (0.5 mg/ear)	Huguet *et al.* 2000
	DPT2[d]	2 (0.5 mg/ear)	Huguet *et al.* 2000
	DPP[e]	61 (0.5 mg/ear)	Huguet *et al.* 2000
	TPA1[f]	35 (0.5 mg/ear)	Huguet *et al.* 2000
	Mezerein[k]	48 (0.5 mg/ear)	Huguet *et al.* 2000
	Bradykinin[l]	54 (10 mg/kg)	Huguet *et al.* 2000
	Carrageenan[m]	58 (5mg/Kg)	Nguemfo *et al.* 2009
7	DPT1[c]	54 (0.5 mg/ear)	Huguet *et al.* 2000
	DPT2[d]	45 (0.5 mg/ear)	Huguet *et al.* 2000
	DPP[e]	34 (0.5 mg/ear)	Huguet *et al.* 2000
	TPA1[f]	44 (0.5 mg/ear)	Huguet *et al.* 2000
	Mezerein[k]	45(0.5 mg/ear)	Huguet *et al.* 2000
	Bradykinin[l]	54 (10 mg/kg)	Huguet *et al.* 2000
15	Croton oil[i]	72 (0.42 µM/ear)	Nikiéma *et al.* 2001
	Carrageenan[m]	Inactive (40 mg/kg)	Gupta *et al.* 1969
16	Croton oil[i]	54 (0.42 µM/ear)	Nikiéma *et al.* 2001
17	CFA1[a]	58 (50 mg/kg)	Geetha and Varalakshmi 2001
18	Croton oil[i]	90 (0.42 µM/ear)	Nikiéma *et al.* 2001
19	TPA3[h]	IC_{50} 0.65 mg/ear	Arciniegas *et al.* 2004
	Carrageenan[m]	27.7 (20 mg/kg)	Arciniegas *et al.* 2004
21	TPA3[h]	15.5 (0.5 mg/ear)	Arciniegas *et al.* 2004
	Carrageenan[m]	48.7 (20 mg/kg)	Arciniegas *et al.* 2004
22	Cotton pellet[j]	38 (600 mg/kg)	Agarwal and Rangari 2003
	Carrageenan[m]	1 (200 mg/kg)	Agarwal and Rangari 2003
		27 (400 mg/kg)	Agarwal and Rangari 2003
		53 (800 mg/kg)	Agarwal and Rangari 2003

TPA = 12-*O*-tetradecanoylphorbol-13-acetate; CFA = complete Freund's adjuvant; DPT = 12-deoxyphorbol-13-tetradecanoate; DPP = 12-deoxyphorbol-13-phenylacetate
[a] CFA1 = CFA-induced arthritis (after 19 days)
[b] CFA2 = CFA-induced arthritis (after 21 days)
[c] DPT1 = DPT-ear oedema with simultaneous administration of test compound
[d] DPT2 = DPT-ear oedema 2h pre-treated with test compound
[e] DPP = DPP-ear oedema with simultaneous administration of test compound
[f] TPA1 = TPA-ear oedema 2h pre-treated with test compound
[g] TPA2 = TPA-ear oedema with simultaneous administration of test compound
[h] TPA3 = without specification
[i] Croton oil = Croton oil-ear edema
[j] Cotton pellet = Cotton pellet granuloma in rats
[k] Mezerein = Mezerein-ear oedema with simultaneous administration of test compound
[l] Bradykinin = Bradykinin-paw oedema, 1h pre-treated with test compound
[m] Carrageenan = Carrageenan-paw oedema (after 3h)

78%, correspondingly. These esters also caused more inhibition of cAMP-dependent protein kinases (cAK; IC_{50} values between 4-9 µM) than lupeol (Kweifio-Okai *et al.* 1995a; Hasmeda *et al.* 1999). Ultimately, the inhibition of serine proteases leads to the reduction of protease-mediated cell damage and the inhibition of cAK can prevent the production of PGE2 and the proliferation of B cells, blunting the exaggerated immune responses that occur in some inflammatory processes (Levy *et al.* 1996; Gerits *et al.* 2008), which could explain why the cartilage and subchondral bone suffered less destruction in CFA-induced arthritic rats treated with lupeol 3-palmitate and 3-linoleate. Concomitantly, Sudhahar *et al.* (2007a, 2008) found a drop in the levels of several enzymatic markers, for both cellular damage and oxidative stress, present in cardiac and kidney tissues, and in serum of hypercholesterolemic rats treated with lupeol and its 3-linoleate ester, evidencing their anti-inflammatory effect in that abnormality. Notably, the mechanism of action seems to be similar to the abovementioned since oxidized low-density lipoproteins (LDL) can activate the redox-sensitive molecule NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), which induces transcription of TNF-α and IL-1β that will modulate the inflammatory responses during atherogenesis and resulting atherosclerosis. It is known that incorrect regulation of NF-κB has been linked to several disease states than inflammation where lupeol is active, such as cancer and viral infection.

Table 4 Inhibitory activity of lupeol and related compounds on enzymes.

Compound	Enzyme	Activity IC_{50}	Species	Family	Reference
1	Neuraminidase	5.6 μM	*Microphorus affinis*	Mushroom	Kim *et al.* 2003
	cAK[a]	5 μM	Synthetic	-	Hasmeda *et al.* 1999
	PKC[b]	82 μM	Synthetic	-	Hasmeda *et al.* 1999
	Trypsin	34 μM	*Alstonia boonei*	Apocynaceae	Rajic *et al.* 2000
	Chemotrypsin	22 μM	*Alstonia boonei*	Apocynaceae	Rajic *et al.* 2000
	Topo II[c]	10.4 μM	*Phyllanthus flexuosus*	Phyllanthaceae	Wada *et al.* 2001
	Tyrosinase phosphatase 1B	5.6 μM	*Sorbus commixta*	Rosaceae	Na *et al.* 2009
	DNA polymerase β[d]	6.4 μM	*Solidago canadensis*	Asteraceae	Chaturvedula *et al.* 2004b
	Human leucocyte elastase	1.9 μM	*Maquira coriaceae*	Moraceae	Mitaine-Offer *et al.* 2002
	Mushroom tyrosinase	2.2 mM	*Guioa villosa*	Sapindaceae	Magid *et al.* 2008
	Farnesyltransferase	65 μg/mL	*Lophopetalum wallichii*	Celastraceae	Sturm *et al.* 1996
2	Tyrosinase phosphatase 1B	13.7 μM	*Sorbus commixta*	Rosaceae	Na *et al.* 2009
7	Topo II[c]	38.6 μM	*Phyllanthus flexuosus*	Phyllanthaceae	Wada *et al.* 2001
	Mushroom tyrosinase	1.4 mM	*Guioa villosa*	Sapindaceae	Magid *et al.* 2008
15	DNA polymerase β[d]	20.6 μM	*Solidago canadensis*	Asteraceae	Chaturvedula *et al.* 2004b
	Human leucocyte elastase	66% inhibition at 25 μg/mL	*Maquira coriaceae*	Moraceae	Mitaine-Offer *et al.* 2002
16	Trypsin	6 μM	Synthetic	-	Rajic *et al.* 2000
	Chymotrypsin	37% inhibition at 50 μM			Rajic *et al.* 2000
17	PKC[b]	32 μM	Synthetic	-	Hasmeda *et al.* 1999
	Trypsin	10 μM	Synthetic	-	Rajic *et al.* 2000
	Chemotrypsin	33% inhibition at 50 μM	Synthetic	-	Rajic *et al.* 2000
23	Topo II[c]	Inactive at 200 μM	*Phyllanthus flexuosus*	Phyllanthaceae	Wada *et al.* 2001

[a] cAK = rat liver cyclic AMP-dependent protein kinase catalytic subunit
[b] PKC = rat brain Ca^{2+}- and phospholipid-dependent protein kinase
[c] Topo II = topoisomerase II
[d] lyase activity of rat DNA polymerase β

Antitumor

Cancer is a disease recognized by seven hallmarks: unlimited growth of abnormal cells, self-sufficiency in growth signals, insensitivity to growth inhibitors, evasion of apoptosis, sustained angiogenesis, inflammatory microenvironment, and eventually tissue invasion and metastasis (Mantovani 2009). According to the World Health Organization 84 million people will die of cancer between 2005 and 2015 without intervention. In most developed nations, cancer is the second leading cause of death, falling only behind cardiovascular diseases (WHO 2009). Lupeol and some related compounds have demonstrated antitumor activities in several cancer cell lines. This section discusses about these activities and the compounds' mode of action, including three tables encompassing the compounds' effects on all tested cell lines cited in the text, and on some additional cell lines reported in the literature but not mentioned in the text (**Tables 5**-**7**).

General

Hints at the idea that triterpenes may posses antitumor activity began in the 1970's, when the Cancer Chemotherapy National Service Center reported the tumor-inhibiting effects of an extract from *Hyptis emoryi* containing betulinic acid as its main active constituent (Sheth *et al.* 1972). Then betulin, also a lupeol analogue isolated from the roots of *Sarracenia flava*, demonstrated antitumor activity against human epidermoid carcinoma of the nasopharynx (KB) while lupeol, isolated from the same plant, displayed antitumor activity against lymphocytic leukemia P-388 cells (Miles *et al.* 1974, 1976). Shortly after, betulinic acid isolated from *Vauquelinia corymbosa* also demonstrated antitumor activity against P-388 cells (Trumball *et al.* 1976). When betulinic acid was screened *in vitro* against a panel of human cancer cell lines, strong inhibition was shown against several human melanoma lines with ED_{50} values ranging from 1 to 5 μg/mL (Pisha *et al.* 1995; **Table 7**). The study then moved *in vivo* to mice where betulinic acid was able to completely inhibit tumor growth without causing any toxicity (Pisha *et al.* 1995). A bioassay-guided study of the ethanol extract from *Dendropanax querceti* leaves revealed lupeol as the constituent responsible for the previously observed cytotoxic activity against human hepatocellular carcinoma Hep-G2, human epidermoid carcinoma A-431, and rat hepatoma H-4IIE cells (Moriarity *et al.* 1998). Soon after, a screening of compounds isolated from *Ventilago leiocarpa* revealed no cytotoxic activity for lupeol whose IC_{50} values were higher than 100 μM on all tested cell lines (Lin *et al.* 2001; **Table 5**). The phytochemical study of *Bombax ceiba* and subsequent isolation of lupeol also showed a weak cytotoxicity for this substance in human melanoma SK-MEL-2, human lung carcinoma A549, and murine melanoma B16-F10 cell lines, displaying ED_{50} values greater than 30 μg/mL (You *et al.* 2003; **Table 5**). In addition, lupeol was isolated from the wood of *Vepris punctata* and screened for its cytotoxicity on A2780 human ovarian cancer cell line and exhibited an IC_{50} of 26.4 μg/mL (Chaturvedula *et al.* 2004a). Lupeol caused cytotoxicity in human promyelocytic leukemia HL-60, human leukemia monocyte lymphoma U937 and human neuroblastoma NB-1 cell lines showing IC_{50} values from 19.9 to 16.8 μM. Conversely, lupeol displayed IC_{50} values greater than 20 μM against the human chronic myelogenous leukemia K-562 cell line, G361 and SK-MEL-28 human malignant melanoma cell lines, GOTO human neuroblastoma and W138 human normal fibroblast cell lines (Hata *et al.* 2003a; **Table 5**). More focus was then placed on lupeol's capacity for inhibiting the proliferation of a variety of tumor cells. Lupeol did not affect the proliferation of normal human melanocytes, but it did inhibit the proliferation of human primary WM35 and metastatic 451Lu melanoma cell lines. This study also looked *in vivo*, and lupeol significantly reduced the 451Lu tumor growth in athymic nude mice (Saleem *et al.* 2008; **Table 5**). Lupeol also inhibited the proliferation of MDA-MB-231 human breast cancer cells in a dose dependent manner (Lambertini *et al.* 2005). On the other hand, lupeol and betulinic acid presented weak activity against MCF-7 and other breast cancer cell lines (**Table 5, 7**) while betulin stimulated MCF-7 proliferation at a minimum concentration of 23 nM (Mellanen *et al.* 1996). In other investigation, lupeol inhibited B16 2F2, G361, and NB-1 cell lines' migration in a dose-dependent manner at 10 μM. On the contrary, at that same concentration the growth of nine types of cancerous cells was not affected, and HeLa cervical carcinoma cell growth was only inhibited by 27.6% (Hata *et al.* 2005; **Table 5**). Ding and coworkers (2009) determined the IC_{50} value for lupeol against HeLa, MCF-7 and human hepatoma (SK-Hep1) cell lines as higher than 50 μM.

Table 5 Anticancer activity of lupeol.

Cell line	Derivation	Activity[a]	Reference
451Lu	Human metastatic melanoma	38 μM[b]	Saleem *et al.* 2008
WM35	Human primary melanoma	34 μM[b]	Saleem *et al.* 2008
B16-F10	Mouse melanoma	> 30 μg/mL[c]	You *et al.* 2003
B16 2F2	Mouse melanoma	38 μM[f]	Hata *et al.* 2002
B16-F1	Mouse melanoma	104 μM[g]	Gauthier *et al.* 2006
SK-MEL-2	Human malignant melanoma	>30 μg/mL[c]	You *et al.* 2003
G 361	Human malignant melanoma	>50 μM[h]	Cmoch *et al.* 2008
		> 20 μM[i]	Hata *et al.* 2003a
		2.5%[d]; 59.5%[e]	Hata *et al.* 2005
SK-MEL-28	Human malignant melanoma	> 20 μM[i]	Hata *et al.* 2003a
MCF-7	Human breast adenocarcinoma	> 50 μM[h]	Cmoch *et al.* 2008
K562	Human chronic myelogenous leukemia	>100 μM[j]	Lin *et al.* 2001
		> 20 μM[i]	Hata *et al.* 2003a
CEM	Human T-lymphoblastic leukemia	27.6 μM[h]	Cmoch *et al.* 2008
U937	Leukemic monocyte lymphoma	16.8 μM[i]	Hata *et al.* 2003a
HL60	Human promyelocytic leukemia	19.9 μM[i]	Hata *et al.* 2003a
A2780	Human ovarian cancer	26.4 μg/mL[k]	Chaturvedula *et al.* 2004a
Calu-1	Human lung carcinoma	> 100 μM[j]	Lin *et al.* 2001
A549	Human lung carcinoma	165 μM[g]	Gauthier *et al.* 2006
		> 50 μM[h]	Cmoch *et al.* 2008
		-0.1%[d]; 12.7%[e]	Hata *et al.* 2005
		> 30 μg/mL[c]	You *et al.* 2003
As-PC1	Human pancreatic adenocarcinoma	35 μM[l]	Saleem *et al.* 2005b
MIAPaCa 2	Human pancreatic carcinoma	0.9%[d]; 6.9%[e]	Hata *et al.* 2005
DLD-1	Human colorectal adenocarcinoma	125 μM[g]	Gauthier *et al.* 2006
HeLa	Human cervical carcinoma	>100 μM[j]	Lin *et al.* 2001
		> 50 μM[h]	Cmoch *et al.* 2008
		27.6%[d]; -1.4%[e]	Hata *et al.* 2005
LNCaP	Human prostate cancer	75 μM[l]	Prasad *et al.* 2008a
		21 μmol/L[l]	Saleem *et al.* 2005a
PC-3	Human prostate cancer	500 μM[l]	Prasad *et al.* 2008a
CRW22Rv1	Human prostate cancer	18.5 μmol/L[l]	Saleem *et al.* 2005a
RPMI 8226	Human multiple myeloma	37.5 μM[h]	Cmoch *et al.* 2008
Saos 2	Human osteogenic sarcoma	0[d]; -1.3%5[e]	Hata *et al.* 2005
SH-10-TC	Human stomach cancer	0.4%[d]; 5.4%[e]	Hata *et al.* 2005
ACHN	Human renal adenocarcinoma	-6.3%[d]; -3.4%[e]	Hata *et al.* 2005
T24	Human bladder carcinoma	9.3%[d]; 1.5%[e]	Hata *et al.* 2005
HT1080	Human fibrosarcoma	8.4%[d]; -0.6%[e]	Hata *et al.* 2005
GOTO	Human neuroblastoma	> 20 μM[i]	Hata *et al.* 2003a
NB-1	Human neuroblastoma	19.7 μM[i]	Hata *et al.* 2003a
		4%[d]; 60.3%[e]	Hata *et al.* 2005
Vero	Green monkey kidney tumor	> 100 μM[j]	Lin *et al.* 2001
Raji	Human Burkitt's lymphoma cells	> 100 μM[j]	Lin *et al.* 2001

[a] Activity expressed in IC_{50} value, which represents the concentration that inhibited cell growth by 50%, unless otherwise noted
[b] cytotoxicity measured by MTT assay after 72 h treatment
[c] ED_{50} = concentration that produces 50% reduction in cell growth percentage relative to a negative control; cytotoxicity assessed by SRB assay
[d] lupeol's cell growth inhibition at 10 μM for 72 h; cytotoxic method not specified by the authors
[e] lupeol's cell migration inhibition at 10 μM for 6 h
[f] cytotoxic method and treatment time not specified by the authors
[g] cytotoxicity assessed by resazurin method after 48 h treatment
[h] cytotoxicity assessed by Calcein AM assay after 72 h treatment
[i] cytotoxic method not specified by the authors and treatment time of 72 h
[j] cytotoxicity assessed by [^{3}H]-thymidine assay after 72 h treatment
[k] cytotoxicity measured by Neutral Red staining after 48 h treatment

Mechanisms of action

As far as lupeol's mechanism of action in cancer cells, the first understanding of lupeol's cytotoxic activity was attributed to its ability to inhibit topoisomerase II (topo II) (Moriarity *et al.* 1998), an essential enzyme in eukaryotic cells replication whose role is to relax supercoiled DNA by catalyzing a transient break in double stranded DNA. Therefore, lupeol was screened for its capacity for inhibiting the conversion of supercoiled plasmid DNA to relaxed DNA by topo II. It was found that lupeol selectively inhibited topo II catalytic reaction (IC_{50} shown in **Table 4**) but did not affect topo I activity at a dose of 200 μM. Betulin, which holds an extra hydroxyl group at C-27 (**Fig. 2**), acted similar to lupeol, whereas lup-20(29)-en-3β, 24-diol, that also has an extra hydroxyl group but at C-24, caused no inhibition against both enzymes (**Fig. 3; Table 4**). It was demonstrated that lupeol interfered with binding of topo II to DNA, preventing the binary complex formation between them, a different mechanism of action comparing with other anticancer drugs such as etoposide, which stabilizes that complex (Wada *et al.* 2001).

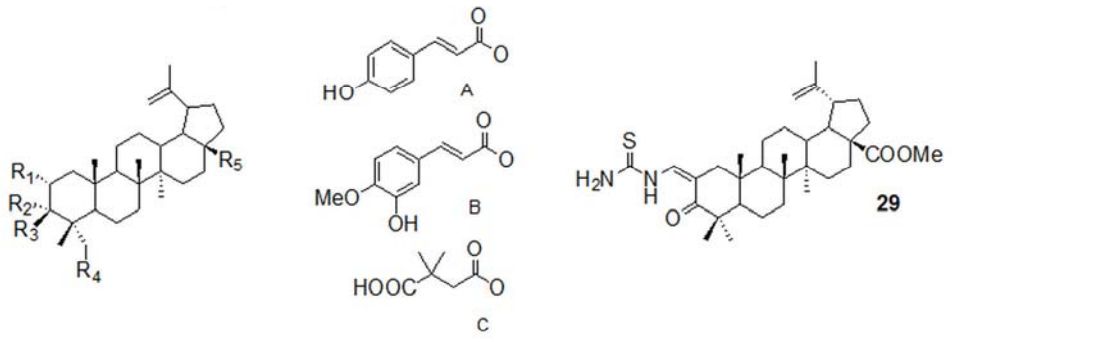
Lupeol was also able to inhibit the lyase activity of DNA polymerase β with an IC_{50} value of 6.4 μM (Chaturvedula *et al.* 2004b). Inhibitors of this lyase activity might be expected to sensitize cancer cells to DNA-damaging agents and to potentiate their cytotoxicity, being regarded as promising adjuvant drugs to anticancer therapy (Sobol *et al.* 2000). Mizushina *et al.* (2003) also examined the activity of lupeol and some related compounds on topo II, DNA polymerase α and β. They observed that lupeol, betulin, and lupeol acetate showed IC_{50} values greater than 500 μM on all tested enzymes. However, betulinic acid, supporting a C-28 carboxyl group (**Fig. 2**), was much more active revealing IC_{50} values of 26.2, 32.3 and 80 μM on DNA polymerase α, DNA polymerase β, and topo II, respectively.

Lupeol inhibited the farnesyltransferase enzyme, making it a potential anticancer agent in tumors where the Ras

Table 6 Anticancer activity of some lupeol analogues.

Compound	Cell line	Derivation	Activity[a]	Reference
2	B16 2F2	Mouse melanoma	25.4 μM[b]	Hata *et al.* 2002
7	A549	Human lung carcinoma	3.8 μM[d]	Gauthier *et al.* 2006
	DLD-1	Human colorectal adenocarcinoma	6.6 μM[d]	Gauthier *et al.* 2006
	B16-F1	Mouse melanoma	13.8 μM[d]	Gauthier *et al.* 2006
	CEM	Human T-lymphobastic leukemia	250 μmol/L[e]	Urban *et al.* 2007
	B16 2F2	Mouse melanoma	27.4 μM[b]	Hata *et al.* 2002
9	A549	Human lung carcinoma	19 μM[d]	Gauthier *et al.* 2006
	B16-F1	Mouse melanoma	26 μM[d]	Gauthier *et al.* 2006
	DLD-1	Human colorectal adenocarcinoma	25 μM[d]	Gauthier *et al.* 2006
15	B16 2F2	Mouse melanoma	22.7 μM[b]	Hata *et al.* 2002
	A2780	Ovarian cancer	22.6 μg/mL[c]	Chaturvedula *et al.* 2004a
24	A549	Human lung carcinoma	74.2 μmol/L[f]	Bi *et al.* 2007
	BEL-7402	Human hepatoma	63.9 μmol/L[f]	Bi *et al.* 2007
	SF-763	Human cerebroma	54.7 μmol/L[f]	Bi *et al.* 2007
	B16	Mouse melanoma	80.5 μmol/L[f]	Bi *et al.* 2007
	C6	Mouse neuroglioma	82 μmol/L[f]	Bi *et al.* 2007
25	CEM	Human T-lymphoblastic leukemia	10 μM[g]	Cmoch *et al.* 2008
	MCF-7	Human breast adenocarcinoma	21.8 μM[g]	Cmoch *et al.* 2008
	A549	Human lung carcinoma	43 μM[g]	Cmoch *et al.* 2008
	HeLa	Human cervical carcinoma	14.5 μM[g]	Cmoch *et al.* 2008
	RPMI 8226	Human multiple myeloma	6.7 μM[g]	Cmoch *et al.* 2008
	G 361	Human malignant melanoma	32.3 μM[g]	Cmoch *et al.* 2008

[a] Activity expressed in IC_{50} value, which represents the concentration that inhibits cell growth by 50%, unless otherwise noted (for more data about betulinic acid anticancer activity, see Eiznhamer and Xu 2005)
[b] cytotoxic assay and time of treatment were not mentioned by the authors
[c] cytotoxicity measured by Neutral Red staining after 48 h treatment
[d] cytotoxicity assessed by resazurin method after 48 h treatment
[e] cytotoxicity measured by MTT assay after 72 h treatment
[f] cytotoxicity measured by MTT assay, time not specified by authors
[g] cytotoxicity assessed by Calcein AM assay after 72 h treatment



22 - $R_1 = R_2 = R_4 = H$, $R_3 = OH$, $R_5 = Me$, H19–α — 19α–H lupeol
23 - $R_1 = R_2 = H$, $R_3 = R_4 = OH$, $R_5 = Me$ — lup-20(29)-en-3β,24-diol
24 - $R_1 = R_2 = H$, $R_3 = R_4 = OH$, $R_5 = COOCH_2CHCH_2$ — lup-20(29)-en-28-oic acid, 3β,24-dihydroxi-,2-propen-1-yl ester
25 - $R_1 = R_2 = R_4 = H$, $R_3 = OAc$, $R_5 = COOH$ — betulinic acid acetate
26 - $R_1 = OH$, $R_2 = R_4 = H$, $R_3 = A$, $R_5 = COOH$ — 3-*O*-*trans*-*p*-coumaroylalphitolic acid
27 - $R_1 = R_4 = H$, $R_2 = R_3 = O$, $R_5 = CHO$ — betulonal
28 - $R_1 = R_2 = R_4 = H$, $R_3 = B$, $R_5 = Me$ — lup-20(29)-en-3β-isoferulate
30 - $R_1 = R_2 = R_4 = H$, $R_3 = C$, $R_5 = COOH$ — 3-*O*-(3',3'-dimethylsuccinyl) betulinic acid
31 - $R_1 = R_4 = H$, $R_2 = R_3 = O$, $R_5 = COOH$ — betulonic acid

Fig. 3 Structural formula of lupeol analogues tested as anticancer and antimicrobial agents.

oncogene plays a role (**Table 4**; Sturm *et al.* 1996). Lupeol has also been demonstrated to induce the estrogen-receptor α (ER-α) expression, which may explain its growth inhibitory action in MDA-MB-231 breast cancer cells (Lambertini *et al.* 2005).

Another mechanism lupeol has been proven to act through is angiogenic inhibition. Angiogenesis is the formation of new blood vessels from pre-existing vessels and is known to play an important role in tumor growth and metastasis (Käβmeyer *et al.* 2009). Lupeol caused a noticeable *in vitro* inhibition of tube formation by human umbilical vein endothelial cells (40-60%) at a concentration of 30 μg/mL (You *et al.* 2003).

Much focus has been placed on lupeol-induced apoptosis. The first evidence for apoptosis in cancer cells treated with lupeol was shown in human promyelotic leukemia HL-60 cells, where apoptotic bodies were observed along with DNA fragments characteristic of apoptosis (Aratanechemuge *et al.* 2004). This process known as "programmed cell death" is used to remove ineffective or irreparable damaged cells. Once the apoptotic signals are triggered, cells undergo organized degradation by proteolytic enzymes, the caspases, which are then cleaved from their pro-form to their active form at the start of apoptosis (Riedl and Shi 2004). Recently,

Table 7 Anticancer activity of betulinic acid.

Cell line	Derivation	Activity[a]	Reference
MEL-1	Human melanoma	1.1 μg/mL[b]	Pisha *et al.* 1995
MEL-2	Human melanoma	2.0 μg/mL[b]	Pisha *et al.* 1995
MEL-3	Human melanoma	2.7 μM[c]	Šarek *et al.* 2003
MEL-4	Human melanoma	4.8 μg/mL[c]	Pisha *et al.* 1995
G 361	Human malignant melanoma	> 50 μM[i]	Cmoch *et al.* 2008
B16	Mouse melanoma	30.5 μM[c]	Šarek *et al.* 2003
		53.5 μmol/L[j]	Bi *et al.* 2007
B16-F1	Mouse melanoma	16.1 μM[h]	Gauthier *et al.* 2006
B16F	Metastatic mouse melanoma	4.6 μM[c]	Šarek *et al.* 2003
MDA231	Human breast cancer	10.4 μg/mL[d]	Kessler *et al.* 2007
MDL13E	Human breast cancer	11.5 μg/mL[d]	Kessler *et al.* 2007
BC-1	Human breast cancer	>20 μg/mL[b]	Pisha *et al.* 1995
HBL100	Human breast cancer	5.0 μg/mL[f]	Kumar *et al.* 2008
MCF-7	Human breast cancer	194 μM[c]	Šarek *et al.* 2003
		NR[e]	Kessler *et al.* 2007
		>>50 μM[i]	Cmoch *et al.* 2008
BT474	Human breast cancer	12.1 μg/mL[d]	Kessler *et al.* 2007
BT483	Human breast cancer	12.8 μg/mL[d]	Kessler *et al.* 2007
BT549	Human breast cancer	5.5 μg/mL[d]	Kessler *et al.* 2007
		>250 μM[c]	Šarek *et al.* 2003
MDA-MB-238	Human breast cancer	195 μM[c]	Šarek *et al.* 2003
SKBR3	Human breast cancer	16.2 μg/mL[d]	Kessler *et al.* 2007
T47D	Human breast cancer	13.0 μg/mL[d]	Kessler *et al.* 2007
		2.4 μM[g]	Rzeski *et al.* 2006
ZR-75-1	Human breast cancer	NR[e]	Kessler *et al.* 2007
MOLT-4	Human leukemia	1.9 μg/mL[j]	Rajendran *et al.* 2008
K562	Human leukemia	53.9 μM[c]	Šarek *et al.* 2003
		3.3 μg/mL[f]	Kumar *et al.* 2008
CEM	Human T-lymphoblastic leukemia	>250 μM[c]	Šarek *et al.* 2003
		30 μmol/L[f]	Urban *et al.* 2007
		40 μM[i]	Cmoch *et al.* 2008
Jurkat 1E.6	Human T-cell leukemia	6.9 μM[g]	Rzeski *et al.* 2006
CEM-DNR 1/C2	Human T-lymphoblastic leukemia, daunorubicin resistant	>250 μM[c]	Šarek *et al.* 2003
CEM-DNR bulk	Human T-lymphoblastic leukemia, Daunorubicin Resistant	>250 μM[c]	Šarek *et al.* 2003
CEM-VCR 1/F3	Human T-lymphoblastic leukemia, vincristin resistant	19.1 μM[c]	Šarek *et al.* 2003
CEM-VCR 3/D5	Human T-lymphoblastic leukemia, vincristin resistant	24.1 μM[c]	Šarek *et al.* 2003
CEM-VCR bulk	Human T-lymphoblastic leukemia, vincristin resistant	68.5 μM[c]	Šarek *et al.* 2003
KB	Human prostate cancer	>20 μg/mL[b]	Pisha *et al.* 1995
LNCaP	Human prostate cancer	>20 μg/mL[b]	Pisha *et al.* 1995
		11.9 μg/mL[d]	Kessler *et al.* 2007
		244 μM[c]	Šarek *et al.* 2003
PC3	Human prostate cancer	12.3 μg/mL[d]	Kessler *et al.* 2007
22Rv1	Human prostate cancer	10.1 μg/mL[d]	Kessler *et al.* 2007
DU145	Human prostate cancer	11.6 μg/mL[d]	Kessler *et al.* 2007
		241 μM[c]	Šarek *et al.* 2003
		9.8 μg/mL[j]	Rajendran *et al.* 2008
		>20 μg/mL[f]	Kumar *et al.* 2008
FTC238	Human thyroid carcinoma	5.2 μM[g]	Rzeski *et al.* 2006
N417	Human lung cancer	6.2 μg/mL[d]	Kessler *et al.* 2007
MBA9812	Human lung cancer	7.6 μg/mL[d]	Kessler *et al.* 2007
GLC-2	Human lung cancer	8.8 μg/mL[d]	Kessler *et al.* 2007
GLC-36	Human lung cancer	9.6 μg/mL[d]	Kessler *et al.* 2007
GLC-4	Human lung cancer	10 μg/mL[d]	Kessler *et al.* 2007
H187	Human lung cancer	8.7 μg/mL[d]	Kessler *et al.* 2007
H322	Human lung cancer	12.3 μg/mL[d]	Kessler *et al.* 2007
H460	Human lung cancer	6.1 μg/mL[d]	Kessler *et al.* 2007
SW 1573	Human lung cancer	NR[e]	Kessler *et al.* 2007
LU-1	Human lung cancer	>20 μg/mL[b]	Pisha *et al.* 1995
L132	Human lung cancer	3.2 μg/mL[j]	Rajendran *et al.* 2008
A549	Human lung carcinoma	10.3 μM[h]	Gauthier *et al.* 2006
		>>50 μM[i]	Cmoch *et al.* 2008
		79.3 μM[c]	Šarek *et al.* 2003
		4.3 μM[g]	Rzeski *et al.* 2006
		8.3 μg/mL[d]	Kessler *et al.* 2007
		97.5 μmol/L[j]	Bi *et al.* 2007
		3 μg/mL[f]	Kumar *et al.* 2008
CaSki	Human cervical cancer	9.6 μg/mL[d]	Kessler *et al.* 2007
HeLa	Human cervical cancer	14.3 μg/mL[d]	Kessler *et al.* 2007
		>47.6 μM[i]	Cmoch *et al.* 2008
HPCC	Human cervical carcinoma	4.5 μM[g]	Rzeski *et al.* 2006
SiHa	Human cervical cancer	11.8 μg/mL[d]	Kessler *et al.* 2007
HPOC	Human ovarian carcinoma	5.5 μM[g]	Rzeski *et al.* 2006
OVCAR-3	Human ovarian cancer	164 μM[c]	Šarek *et al.* 2003

Table 7 (Cont.)

Cell line	Derivation	Activity[a]	Reference
PA-1	Human ovarian cancer	10.0 μg/mL[j]	Rajendran *et al.* 2008
		11.5 μg/mL[f]	Kumar *et al.* 2008
SW620	Metastatic human colon cancer	>250 μM[c]	Šarek *et al.* 2003
Caco-2	Human colon cancer	19.6 μM[c]	Šarek *et al.* 2003
COL-2	Human colon cancer	>20 μg/mL[b]	Pisha *et al.* 1995
SW620	Human colon cancer	13.3 μg/mL[f]	Kumar *et al.* 2008
Hep2G	Human hepatocellular carcinoma	3.6 μM[c]	Šarek *et al.* 2003
BEL-7402	Human hepatoma	43.4 μmol/L[j]	Bi *et al.* 2007
A431	Human epidermoid carcinoma	>20 μg/mL[b]	Pisha *et al.* 1995
U373	Human glioma	>20 μg/mL[b]	Pisha *et al.* 1995
C6	Human glioma	7.0 μM[g]	Rzeski *et al.* 2006
C6	Mouse neuroglioma	90.7 μmol/L[j]	Bi *et al.* 2007
RPMI 8226	Human multiple myeloma	34.6 μM[i]	Cmoch *et al.* 2008
		4.3 μM[g]	Rzeski *et al.* 2006
SF-763	Human cerebroma	92.1 μmol/L[j]	Bi *et al.* 2007
HPGBM	Human glioblastoma multiforme	3.9 μM[g]	Rzeski *et al.* 2006
U87MG	Human glioblastoma	>228 μM[c]	Šarek *et al.* 2003
Saos2	Human rhabdomyosarcoma	>250 μM[c]	Šarek *et al.* 2003
NIH3T3	Mouse immortalized fibroblasts	>250 μM[c]	Šarek *et al.* 2003
		4.3 μg/mL[f]	Kumar *et al.* 2008
DLD1	Human colorectal cancer	15 μM[h]	Gauthier *et al.* 2006
		NR[e]	Kessler *et al.* 2007
HCT81	Human colorectal cancer	16.4 μg/mL[d]	Kessler *et al.* 2007
CO115	Human colorectal cancer	12.2 μg/mL[d]	Kessler *et al.* 2007
HT-29	Human colorectal cancer	>250 μM[c]	Šarek *et al.* 2003
		1.8 μg/mL[j]	Rajendran *et al.* 2008
		NR[e]	Kessler *et al.* 2007
		2.7 μM[g]	Rzeski *et al.* 2006
LS180	Human colorectal cancer	11.7 μg/mL[d]	Kessler *et al.* 2007
RKO	Human colorectal cancer	9.5 μg/mL[d]	Kessler *et al.* 2007
SW1463	Human colorectal cancer	3.8 μg/mL[d]	Kessler *et al.* 2007
SW480	Human colorectal cancer	15.1 μg/mL[d]	Kessler *et al.* 2007
SW837	Human colorectal cancer	11.3 μg/mL[d]	Kessler *et al.* 2007
T84	Human colorectal cancer	11.3 μg/mL[d]	Kessler *et al.* 2007
TE671	Human rhabdomyosarcoma-medulloblastoma	4.4 μM[g]	Rzeski *et al.* 2006
U2OS	Human osteosarcoma	>250 μM[c]	Šarek *et al.* 2003
HT-1080	Human sarcoma	>20 μg/mL[b]	Pisha *et al.* 1995
U-937	Human lymphoma	0.7 μg/mL[j]	Rajendran *et al.* 2008
MIAPaCa	Human pancreatic cancer	>20 μg/mL[f]	Kumar *et al.* 2008
SKNAS	Human neuroblastoma	3.9 μM[g]	Rzeski *et al.* 2006

[a] Activity expressed in IC_{50} value, which represents the concentration that inhibits cell growth by 50%, unless otherwise noted (for more data about betulinic acid anticancer activity, see Eiznhamer and Xu 2005)
[b] ED_{50} values
[c] TCS_{50} values = concentration with 50% tumor cell survivor, cytotoxicity measured by MTT assay after 72 h treatment
[d] EC_{50} values = betulinic acid concentration needed for half maximal cell death, which was measured with propidium iodide exclusion after 48 h treatment
[e] NR = not reached after 48 h treatment
[f] cytotoxicity measured by MTT assay after 72 h treatment
[g] cytotoxicity measured by MTT assay after 96 h treatment
[h] cytotoxicity assessed by resazurin method after 48 h treatment
[i] cytotoxicity assessed by Calcein AM assay after 72 h treatment
[j] cytotoxicity measured by MTT assay, time not specified by authors

a proteomics study using two dimensional gel electrophoresis investigated the effect of betulin in A549 cells, a human lung cancer cell line. Betulin treatment at 20 μM for 24 h caused up-regulation of two protein-members of the Krebs cycle, aconitate hydratase and malate dehydrogenase, and of arginine/serine-rich 1 (SFRS1), linked with DNA fragmentation. Down-regulation of heat shock protein 90-alpha 2 was also observed. Ultimately, these results corroborated the betulin-induced apoptosis via the mitochondrial pathway (Pyo *et al*. 2009). Using a different approach, Prasad and coworkers (2009) demonstrated lupeol action in human epidermoid carcinoma A431 cells was also associated with the caspase dependent mitochondrial cell death pathway by activation of Bax, caspases, apoptotic protease activating factor 1 (Apaf1), decrease in B-cell lymphoma 2 (Bcl-2) expression and consequent cleavage of poly(ADP)ribose polymerase (PARP). A negative modulation of Akt/PKB signaling pathway by inhibition of Bad (Ser 136) phosphorylation and 14.3.3 protein expression was also observed. Reduction of cell survival was linked with the overexpression of IκB and consequent inhibition of NF-κβ. Yet, lupeol was shown to cause growth inhibition of hepatocellular carcinoma SMMC7721 cell line in a dose-dependent manner inducing apoptosis by activation of caspase 3 expression, down-regulation of death receptor 3 (DR3) and overexpression of FADD mRNA (Zhang *et al*. 2009).

Depending on the specific cancer type, lupeol and related compounds may display slightly different mechanisms of inducing apoptosis and each case is discussed in the next sub-items.

Anti-prostate cancer

Prostate cancer is a disease where lupeol and related compounds hold particular promise. Lupeol was demonstrated to not affect the viability of human prostate epithelial cells, but displayed IC_{50} values of 21 and 18.5 μM against the human prostate cancer cell lines LNCaP and CWR22R*v*1, respectively. The *in vivo* model using CWR22R*v*1 cells implanted into nude mice corroborated the lupeol anticancer activity by a significant tumor volume reduction after treatment of mice with 1mg of lupeol i.p. three times a week.

Additionally, the levels of PSA, the commonly used diagnostic biomarker for prostate cancer, were significantly lower in the lupeol-treated mice throughout the treatment (Saleem *et al.* 2005a). On the other hand, different studies concerning human prostate cancer showed a weak inhibition of PC-3 cell proliferation by lupeol (Prasad *et al.* 2008a; **Table 5**) whereas some related compounds such as betulinic acid and 3-*O*-*trans*-*p*-coumaroylalphitolic acid (**Fig. 3**) displayed ED_{50} values of 15 and 4 μM, respectively, suggesting the presence of C-28 carboxyl group and esterification of C-3 hydroxy group by coumaric acid as structural features for better activities (Lee *et al*. 2003). After PC-3 cell treatment with betulinic acid, its mechanism of action by apoptosis with concomitant suppression of NF-κB was confirmed by a considerable shift in the ratio of Bax/Bcl-2, pro- and anti-apoptotic proteins respectively, and the cleavage of PARP, a DNA nick sensor which is cleaved during apoptosis and so considered a biomarker of this process (Scovassi and Diederich 2004; Rabi *et al.* 2008). Prasad and coworkers (2008a) demonstrated that lupeol acted in a similar way in PC-3 cells. After 48 h of treatment at a dose of 500 μM, lupeol induced a G2/M cell cycle block and alterations to several of the key players involved in the transition between those phases of the cell cycle. Apoptosis occurred only after 96 hours of treatment with a decrease in Bcl-2 mRNA levels and an increase in Bax, Apaf-1, caspase-9, and caspase-3 mRNA, characteristic features of apoptosis via the mitochondrial pathway (Khan *et al.* 2007). Additionally, injection of lupeol led to the arrest of prostate enlargement in testosterone-treated mice by ROS (reactive oxygen species)-mediated apoptosis via the mitochondrial pathway, which was also observed in lymph node carcinoma of the prostate (LNCaP) cells treated with lupeol at 75 μM for 48 h (Prasad *et al.* 2008b). However, it was demonstrated that LNCaP cells treated with lupeol at 1-30 μM for 48 h did not present alterations in the expression of Bcl-2, Bax and procaspase-3, but they showed reduction in the expression of procaspases-6, -8 and -9. Moreover, the levels of cleaved PARP and acinus protein were increased and significant dose-dependent inductions in the expression of Fas (death receptor protein) and Fas receptor-associated FADD protein (death adapter protein) were observed suggesting a lupeol-induced apoptosis through Fas receptor-mediated apoptotic pathway (Saleem *et al.* 2005a) and arising the possibility that lupeol may act by different mechanisms on the same cell according to the employed dose. Most recently investigations showed lupeol caused significant inhibition of androgen-insensitive (PC-3 and DU 145) as well as androgen-sensitive (LNCaP and CWR22Rv1) human prostate cancer (CaP) cells (5-50 μM for 48 h) without producing any adverse effect on the viability of normal prostate epithelial cells. Lupeol treatment induced G2/M cell cycle arrest by a dose-dependent way, displaying reduction in the protein levels of cyclins -A, -D1, -D2, -E2 and cyclin dependent kinase 2 (CdK-2), and increase in cyclin-dependent kinase inhibitor 1A (p21); modulation of microtubule assembly by down-regulation of microtubule-regulatory molecules such as stathmin and surviving, and the antiapoptotic cellular FLICE-like inhibitory protein (cFLIP) was also observed (Saleem *et al.* 2009a). Furthermore, it was revealed lupeol capability's to decrease the expression of CaP cells' modulator proteins at transcriptional and translational levels such as ERBB2, an activator of androgen receptor, CdK-1 and metalloproteinase-2 (MMP-2), known to be associated with proliferation and/or survival of CaP cells and to act as downstream targets of β-catenin signaling pathway. These findings suggested that lupeol treatment initiates the molecules events very early (24 h post treatment) that ultimately result in loss of β-catenin levels. The fact of lupeol has also decreased the expression of NF-κB and TNF highlighted lupeol potential against the inflammatory processes common in human prostate cancer (Saleem *et al*. 2009b).

Experiments aimed at the synthesis of betulinic acid derivatives for further preclinical development, yielded compounds modified at the C-3 position containing nitrogen or fluorine. These products presented better activities (IC_{50} values from 0.4 to 2.5 μg/mL) than the original chemical (**Table 7**) against DU 145 (prostate), PA-1 (ovary) and MOLT-4 (leukemia) cancerous cell lines, confirming the lupane-type triterpenes' potential as a scaffold to originate more potent anticancer drugs (Rajendran *et al.* 2008).

Anti-pancreatic cancer

Pancreatic cancer is one of the most fatal cancers. Lupeol has shown growth inhibitory activity on AsPC-1 human pancreatic adenocarcinoma cell, which is highly resistant to currently available chemotherapeutic drugs, displaying an IC_{50} of 35 μM. Lupeol treatment of AsPC-1 cells using doses between 30-50 μM induced apoptosis and the mechanism of action was proved to occur in a similar way of lupeol mechanism on PC-3 cells, with cleavage of PARP, and considerable increase in the levels of Bax and active caspases -3, -8, and -9. Reductions in the expression of the Ras oncoprotein and in the activation of the NF-κB signaling pathway as well as modulation of the protein expression of several other signaling molecules such as protein kinase Cα and ornithine decarboxylase were also observed and provided evidence for lupeol as a potent multi-target anticancer agent (Saleem *et al.* 2005b). In addition to these results, a recent study has demonstrated the *in vitro* and *in vivo* modulating effect of lupeol on TRAIL-induced apoptosis in the chemoresistant pancreatic cancer cell lines AsPC-1 and PANC-1 by increasing the expression level of active caspase-8 and down-regulating the expression of cFLIP. Based on the outcomes, the authors also suggested the development of lupeol to prevent pancreatic cancer as well as to be used as adjuvant to known therapeutic agents in the treatment of this cancer (Murtaza *et al*. 2009).

Anti-head and neck squamous cell carcinoma

An investigation focusing on head and neck squamous cell carcinoma (HNSCC) demonstrated the lupeol's ability to effectively and selectively inhibit proliferation both *in vitro* and *in vivo* of the human tongue squamous cell carcinoma cell line (CAL27), and primary (TU159) and metastatic (MDA1986) oral squamous cell carcinoma cell lines in a slightly different way than lupeol's in PC-3 prostate cells, by mediating G1 arrest and cell apoptosis. In addition, lupeol inhibited migration of these cells, causing suppression of local metastasis by modulation of the NF-κB activity and strongly potentiated cisplatin's anticancer effect in a combined treatment with this drug (Lee *et al.* 2007). Betulinic acid has also displayed anti-tumor activity in HNSCC significantly reducing the cell numbers of the human tongue squamous cell carcinoma cell lines SCC9 and SCC25 by activation of the caspase cascade. Additionally, when used in combination with cisplatin, it presented more than an additive effect regarding the growth inhibition of those cell lines (Thurnher *et al.* 2003).

Anti-melanoma

Using B16 2F2 cells, a sub-cell line derived from B16 mouse melanoma cells with high differentiation ability, Hata and coworkers (2000) demonstrated that lupeol induced melanin biosynthesis, an indicator of melanoma cell differentiation, and inhibited cell proliferation at concentrations of about 5 and 20 μM, respectively. Further, several lupeol analogues were tested on the same cells in order to investigate the relationship between their structures and corresponding activities. When the IC_{50} values were compared, lupenone (25.4 μM) was more active than lupeol (38 μM) revealing that the presence of a ketone group at C-3 improved cell differentiation-inducing activity. However, the results with betulinic acid (IC_{50} 7.9 μM) and betulonal (**Fig 3**; IC_{50} 4.1 μM) suggested the presence of a carbonyl group at C-28 as an essential requirement for the increase in melanogenesis (Hata *et al.* 2002). Upon investigating the

mechanism by which lupeol induced B16 2F2 melanoma cell differentiation, it was determined lupeol was acting through activation of p38 MAPK. It was established that the groups at C-3 and C-28 also played important roles in compounds' apoptotic effects and selectivity against the tested tumor cell lines (Hata *et al.* 2003b). Shortly after, it was demonstrated that lupeol at 10 μM for 12 h induced the disassembly of actin stress fibers in B16 2F2 cytoplasm cells by decreasing the levels of phospho-cofilin, which is involved in the assembly of actin stress fibers and consequently promoted the formation of dendrites, a morphological marker of cell differentiation (Hata *et al.* 2005). Recent studies showed that a short-term treatment of B16 2F2 cells using lupeol at 10 μM for 8 h produced the same type of cell differentiation observed by Hata and coworkers (2005), but 48 h of treatment induced up-regulation of enzymes that triggered the pigment cell differentiation (Ogiwara and Hata 2009). Noteworthy, Magid *et al.* (2008) had previously observed that lupeol and betulin displayed weak inhibition of mushroom tyrosinase (**Table 4**), a key enzyme in the catalysis of L-DOPA oxidation to further production of melanin, corroborating the lupeol's ability to induce melanogenesis. Yet, lupeol treatment of metastatic melanoma 451Lu cells caused an increase in cleaved PARP and Bax levels as well as decreased procaspase-3 and Bcl-2 levels both *in vitro* and *in vivo* assays, indicating apoptosis by the mitochondrial pathway. Lupeol also induced a specific cell cycle arrest at G1/S, which triggered alterations in some G1 cell cycle regulatory proteins such as cyclin D1, -D2 and CdK-2. Additionally, lupeol induced an increase in WAF1/p21, a protein that regulates entry into the S phase (Saleem *et al.* 2008).

Regarding the specific inhibition of melanoma by betulinic acid, morphological changes such as a sub-G1 cell cycle peak and DNA fragmentation demonstrated the compound was inducing apoptosis (Pisha *et al.* 1995). On the other hand, betulinic acid-induced apoptosis in neuroblastomas depended on the activation of caspases-3 and -8; an augmented expression of the proapoptotic Bax and Bcl-x_s proteins was also observed but there was no variation in the expression of the antiapoptotic Bcl-2 and Bcl-x_L. Before the caspases were cleaved to their active forms, the compound also induced a disturbance of the mitochondrial membrane potential and generated ROS (Fulda *et al.* 1997). More details about the betulinic acid anticancer mechanisms of action can be read in Fulda's review (2009).

Nutraceutical/chemopreventative agent

Cancer chemopreventive

The term nutraceutical, or so-called functional food, refers to an extract, food or a bioactive compound derived from food capable of benefiting an organism and providing protection or treatment against a disease in addition to its basic nutritional value (Helmenstine 2009). While the word chemopreventive, or chemopreventative, refers to a larger concept regarding the agent, including chemicals, drugs or food supplements that prevent or interfere with a disease by blocking or suppressing its process (Surh 2003). The first report about lupeol as a cancer chemopreventive agent involved the induction of Epstein-Barr virus early antigen (EBV-EA) by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA), in Raji lymphocytes. Lupeol demonstrated 85.3, 34.5, and 16% inhibition at 1×10^3, 5×10^2 and 1×10^2 mol ratio/TPA, respectively. These inhibitory activities were stronger than those presented by lupenone and lupeol acetate (Takasaki *et al.* 1999). In addition, derivatives from lupeol, lupenone and betulin bearing extra carbonyl, alcohol or ester groups were tested for their inhibitory effects on the same assay but all of them displayed IC_{50} values higher than 290 mol ratio/32 pmol TPA (Tanaka *et al.* 2004b). Lupeol treatment of mice before benzo[α]pyrene -induced clastogenicity reduced aberrant cells, micronuclei presence and cytotoxicity in the bone marrow cells as well as caused an increase in the mitotic index, revealing the lupeol's antigenotoxic potential (Prasad *et al.* 2008d). Lupeol was also investigated for its ability to provide protection against metal toxicity, which can lead to cancer. Rats exposed to cadmium when treated with lupeol showed an improvement in the antioxidant enzyme levels and peroxidative status (Nagaraj *et al.* 2000). The modulating effect of lupeol on antioxidant enzymes, lipid peroxidation and glutathione levels was also observed by Saleem *et al.* (2001) and Prasad *et al.* (2008c) in assay models using the ubiquitous carcinogen benzoyl peroxide- and testosterone-induced oxidative stress. A methanolic extract of *Careya arborea* bark, containing lupeol and betulinic acid, increased the antioxidant and hepatoprotective parameters as well as the superoxide dismutase and catalase enzymes' levels in liver and kidney tissues of Erlich ascites carcinoma tumor-bearing mice (Senthikumar *et al.* 2008). In addition to its antioxidant modulating action, topical lupeol pretreatment on TPA-induced mouse skin cancer significantly reduced skin edema, hyperplasia and tumor incidence as well as inhibited PI3K (phosphatidyl inositol kinase) activation, Akt (protein kinase B) phosphorylation, NF-κB and IKKα activation, phosphorylation and degradation of IκBα (Saleem *et al.* 2004). All of this evidence contributed to the idea that lupeol plays an indirect antioxidant role against oxidative stress in the early stages of tumor promotion and is an effective prophylactic agent against skin, liver and prostate cancer (Khan *et al.* 2008). In fact, besides the suppression of NF-κB activation, another crucial approach to chemoprevention is to impede the DNA damage caused by carcinogens, which can be detoxified by induction of the cellular stress response, which includes the phase II enzyme system (Surh 2003). Indirect antioxidants activate the Keap1/Nrf2/ARE pathway resulting in transcriptional induction of the phase II enzymes, which act catalytically, are not consumed, have long half-lives, and are unlikely to evoke pro-oxidant effects (Dinkova-Toskova and Talalay 2008). Lately, it was demonstrated that lupeol, when co-administered with the carcinogen DMBA, was capable of preventing alterations on cell proliferation in mouse skin by inducing p53 and cyclin B-mediated G2/M cell cycle arrest, and targeting apoptosis by activation of caspases (Nigam *et al*. 2009).

Cardioprotective

Lupeol has been investigated for its cardioprotective effects and was demonstrated to provide 34.4% protection against *in vitro* LDL oxidation (Andrikopulos *et al.* 2003). Lupeol and lupeol acetate have also shown hypotensive activity, which may make them possible preventative agents in this cardiac disorder and other consequent cardiovascular diseases (Saleem *et al.* 2003). In addition, supplementation of lupeol or lupeol linoleate was effective against the cardiac oxidative injury caused by cyclophosphamide, a drug used in the treatment of cancer and autoimmune disorders (Sudharsan *et al.* 2005). A study showed lupeol and lupeol linoleate can ameliorate the lipidemic-oxidative abnormalities in the early stages of hypercholesterolemic atherosclerosis in rats (Sudhahar *et al.* 2006). Sudhahar and coworkers (2007b) corroborated this effect and revealed the triterpene's mode of action by a restoration of several transmembrane enzymes, total cholesterol, triglycerides and phospholipids to normal levels, preventing hypertrophic cardiac histology. Reddy and collaborators (2009) also demonstrated lupeol's antidyslipidemic activity in hamster at 100 mg/Kg body weight. In addition, they synthesized 10 lupeol ester derivatives and found a nicotinic acid derivative that exhibited better lipid lowering profile at a dosage twice lower than lupeol along with an antihyperglycemic effect, which revealed the lupeol's potential as a scaffold for developing drugs targeting coronary diseases and diabetes.

Hepatoprotective

Lupeol and analogues have also displayed hepatoprotective effects. Betulin was the first compound shown to be hepatoprotective in rat liver as evaluated by bile production and secretion upon treatment (Flekhter *et al.* 2000). Lupeol showed some effectiveness in lessening the action of aflatoxin B_1 (Preetha *et al.* 2006), a secondary fungal metabolite known for its hepatotoxic and hepatocarcinogenic effects (Bennett and Klich 2003). In this study, rats pretreated with lupeol had the serum and liver enzyme levels restored to almost normal at the same time that the activities of enzymatic antioxidants and the non enzymatic antioxidants GSH, vitamin C, and vitamin E levels were brought back to those of the control. Additionally, treatment with lupeol substantially normalized degenerative alterations in the hepatocytes with granular cytoplasm. Lupeol also reestablished antioxidant enzyme activities in mouse liver affected by 7,12-dimethylbenz(α)anthracene (DMBA)-induced oxidative stress. Noteworthy, the observed decrease in ROS levels along with restoration of mitochondrial transmembrane potential, reduction in DNA fragmentation and subsequent inhibition of apoptosis indicated a divergent mechanism that lupeol plays when acting as an anticancer agent (Prasad *et al.* 2007b). Lupeol treatment induced growth inhibition and apoptosis in hepatocellular carcinoma SMMC7721 cells by down-regulation of the death receptor 3 (DR3) expression. Therefore, lupeol was revealed as a promising chemopreventive agent for that type of cancer (Zhang *et al.* 2009).

Antimicrobial

First tested against *Mycobacterium tuberculosis*, lupeol did not show any antibacterial activity. However, betulinaldehyde and betulinic acid both presented minimal inhibitory concentrations (MIC) of 25 µg/mL (Suksamrarn *et al.* 2006). In another investigation, lupeol and betulinic acid were inactive against three bacteria species but revealed MICs of 63 and 16 µg/mL, respectively, against *Enterococcus faecalis* (**Table 9**; Shai *et al.* 2008). Lupeol was also inactive against eight bacterial species displaying MICs > 200 µg/mL (**Table 9**; Mathabe *et al.* 2008). Additionally, lupeol, betulin and betulinic acid were inactive against several other bacteria species, including some resistant strains (Chaaib *et al*. 2003; Woldemichaela *et al*. 2003; Weigenand *et al.* 2004; Silva *et al*. 2008). Conversely, lupeol showed significant zones of inhibition in the cultures of 18 hospital strains of the Gram-negative bacteria *Pseudomonas aeruginosa* and *Klebsiella pneumonia* at a concentration of 30 µg/100 µL (Ahamed *et al*. 2007). Zones of inhibition were also observed in *P. aeruginosa*, *Salmonella typhi* and *Escherichia coli* cultures using lupeol-, betulinic acid- and betulonic acid-impregnated disks at a concentration of 10 mg/mL (Lutta *et al.* 2008) while lupeol acetate did not display any activity against Gram-negative bacteria and fungi, but displayed a strong antimicrobial effect against Gram-positive bacteria (Freire *et al.* 2002). As reported for betulinic acid, the antibacterial activities of lupeol are also conflicting and one of the hypotheses lay in some microorganisms' ability to biotransform the substances yielding different metabolites that possess different activities (Eiznhamer and Xu 2004). Regarding this subject, a recent study presented compounds originated from lupeol biotransformation by *Penicillium roqueforti* (Severiano *et al.* 2008).

As antifungal agents, lupeol and analogues showed effects quite similar to their antibacterial activities concerning the effectiveness. Lupeol displayed moderate zones of inhibition in *Aspergillus niger*, *Aspergillus flavus*, *Rhizoctonia phaseoli*, and *Penicillium chrysogenum* cultures at 1 mg/disc (Singh and Singh 2003) while *A. niger* was significantly inhibited by 20(29)-lupene-3β-isoferulate at 0.01 mg/mL (Lall *et al.* 2006), confirming that stronger inhibition can be reached when C-3 position is esterified. However, Nguyen and coworkers (2007) synthesized several ester derivatives from lupeol in the C-3 position (COMe, $COCHMe_2$, COPh, COCH: CHPh), which only yielded weak antimicrobial compounds. Lupeol failed to display appreciable activity against *Candida albicans* but demonstrated high and selective activity against *Sporothrix schenckii* and *Microsporum canis*. However, betulinic acid was more active against these species as well as *Candida guilliermondi* (**Table 8**). The authors explained those different activities based on both compounds' cytotoxic LC_{50} values against monkey kidney (Vero) cells, 89.5 and 10.9 µg/mL, respectively, suggesting a cytotoxic effect for betulinic acid and a cytostatic action for lupeol (Shai *et al.* 2008). Additionally, lupeol was inactive against *Cryptococcus neoformans*, *Cladosporium cladosporioides and Cladosporium sphaerospermum* (Marqui *et al.* 2008) and betulinic acid demonstrated only a moderate activity against *Microsporum audouinii*, *Trichophyton soudanense* and *Trichophyton mentagrophytes* (**Table 8**; Kuiate *et al.* 2006).

Lupeol has shown weak anti-viral activities in several studies, but it has been served as lead drug for the generation of more effective compounds. For example, when tested against Influenza A and herpes simplex virus type 1 (HSV-1), lupeol demonstrated an EC_{50} value greater than 234 and 663 µM, respectively, whereas its derivative 2-methylidene-thioureido-methylbetulonate displayed EC_{50} values of 13 and 142 µM, correspondingly (compound **29** in **Fig. 3**; Flekhter *et al.* 2004). On the contrary, lupeol isolated from *Strobilanthes cusia* root revealed an EC_{50} of 11.7 µM against HSV-1 and caused 100% inhibition of virus plaque formation at 58.7 µM (Tanaka *et al.* 2004a). However, betulinic acid exhibited a much better activity against HSV-1 with an EC_{50} value of 5.7 µM for reducing virus plaque formation, a 50% cytotoxic concentration (CC_{50})

Table 8 Antifungal activities of lupeol and betulinic acid.

Fungal species	Lupeol MIC µg/mL	Betulinic acid MIC µg/mL	Reference
Sporothrix schenckii	12; S^a = 7.4	16; S^a = 0.69	Shai *et al.* 2008
Microsporum canis	16; S^a = 5.5	12; S^a = 0.92	Shai *et al.* 2008
Aspergillus fumigatus	93.5; S^a = 0.95	24; S^a = 0.46	Shai *et al.* 2008
Candida albicans	250; S^a = 0.36	16; S^a = 0.69	Shai *et al.* 2008
Cryptococcus neoformans	180; S^a = 0.49	32; S^a = 0.34	Shai *et al.* 2008
Candida guilliermondi	94; S^a = 0.95	15.6; S^a = 0.71	Shai *et al.* 2008
Candida spicata	250; S^a = 0.3	47; S^a = 0.23	Shai *et al.* 2008
Microsporum audouinii	NT[c]	100	Kuiate *et al.* 2007
Trichophyton soudanense	NT[c]	25	Kuiate *et al.* 2007
Trichophyton mentagrophytes	NT[c]	12.5	Kuiate *et al.* 2007
Aspergillus niger	AI^b = 0.73	NT[c]	Singh and Singh 2003
Aspergillus flavus	AI^b = 0.68	NT[c]	Singh and Singh 2003
Rhizoctonia phaseoli	AI^b = 0.58	NT[c]	Singh and Singh 2003
Penicillium chrysogenum	AI^b = 0.63	NT[c]	Singh and Singh 2003

[a] S = Selectivity of compound was calculated by LC_{50}/MIC. LC_{50} is the concentration of drug that resulted in 50% reduction of cells compared to untreated cells.
[b] AI = inhibition area of test sample/inhibition area of standard
[c] NT = not tested

Table 9 Antibacterial activity of lupeol, betulinic acid, and betulinaldehyde.

Bacteria species	Lupeol MIC μg/mL	Betulinic acid MIC μg/mL	Betulinaldehyde MIC μg/mL	Reference
Pseudomonas aeruginosa ATCC 27853	250	250	NT	Shai *et al.* 2008
Escherichia coli ATCC 25922	250	250	NT	Shai *et al.* 2008
Straphylococcus aureus ATCC 29213	250	250	NT	Shai *et al.* 2008
Enterococcus faecalis ATCC 29212	63	16	NT	Shai *et al.* 2008
Staphylococcus aureus ATCC 25923	> 200	NT	NT	Mathabe *et al.* 2008
Salmonella typhi ATCC 0232	> 200	NT	NT	Mathabe *et al.* 2008
Vibrio cholera	> 200	NT	NT	Mathabe *et al.* 2008
Escherichia coli ATCC 35218	> 200	NT	NT	Mathabe *et al.* 2008
Shigella spp. batch 0.57 (*S. dysentery*; *S. flexneri*; *S. sonnei*; *S. boydii*)	> 200	NT	NT	Mathabe *et al.* 2008
Mycobacterium tuberculosis	Inactive	25	25	Suksamrarn *et al.* 2006

NT = not tested

value of 35.5 μM and a therapeutic index of 6.2 (Kurokawa *et al*. 1999; note: activities were transformed to μM for better comparison). Betulinic acid also showed activity against human immunodeficiency virus (HIV) replication in H9 lymphocytes displaying an EC_{50} value of 1.4 μM (Fujioka *et al.* 1994). A second study confirmed this result and determined an IC_{50} value of 12.9 μM for viral replication in H9 cells (Kashiwada *et al.* 2000). Due to these results, extensive research was carried out to develop the C-3 modified derivative 3-*O*-(3', 3'-dimethylsuccinyl)-betulinic acid, so-called DSP or bevirimat (**Fig. 3**), the first-in-class HIV maturation inhibitor in phase II clinical trial. The SAR (structure-activity relationship) study of the C-3 position indicated that the side chain, an ester group with a terminal carboxylic acid, and an isovaleryl domain all contribute to the potent anti-HIV activity of the compound (Yu *et al*. 2007).

Diverse

In addition to the major roles of being antiprotozoal, anti-inflammatory, antitumor, and chemopreventive agents, lupeol and related compounds also possess a diverse array of other activities. Lupeol is one of the components of an antiallergic formulation patented by Kovalenko *et al.* (2008). Lupeol reduced the activity of α-amylase (Ali *et al.* 2006) and inhibited tyrosinase phosphatase 1B (Na *et al*. 2009; **Table 4**), enzymes considered attractive targets in the treatment of diabetes mellitus. Lupeol also showed moderate inhibitory activity against glutathione *S*-transferase and acetylcholinesterase (Kosmulalage *et al*. 2007).

Lupeol and lupeol linoleate were proven to be effective antiurolithiatic agents by preventing the formation of vesical calculi and decreasing the size of pre-formed stones (Anand *et al.* 1994; Vidya *et al.* 2002). In addition, the lupeol and betulinic acid's antiurolithiatic mechanism of action were revealed due to their capacity of minimizing crystal-induced renal peroxidative changes and subsequent tissue damage (Malini *et al.* 2000). Lupeol deterred the foraging activity of the leaf-cutting ant *Atta sexdens rubropilosa* (Salatino *et al.* 1998). Lupenone and 3-*epi*-lupeol (**Fig. 3**) showed allelochemical properties by inhibiting the root growth in *Lycopersicon exculentum* and *Echinochloa crus-galli*. Conversely, the two compounds stimulated the root growth in *Amaranthus hypochondriacus* (Macías-Rubalcava *et al.* 2007). Lupeol also serves a function in anti-aging creams, lotions, gels, and lip balm at levels of 0.2-3% w/w due to its ability to maintain skin texture and integrity by promoting epidermal regeneration and replenishing cutaneous antioxidant enzymes depleted by environmental toxins (Majeed and Prakash 2005). Lupeol acetate isolated from *Hemidesmus indicus* neutralized viper and cobra venom activities as well as potentiated snake venom antiserum action in a mouse model. The compound antioxidant properties (lipid peroxidation and superoxide dismutase activity) along with its capacity of reducing PGE2 production and cytokine release from macrophages were suggested to play a role in the snake venom induced inflammatory process culminating with an antagonistic effect and prevention of pro-inflammatory mediators production (Chatterjee *et al.* 2006). Yet, lupeol and some analogues have demonstrated ability to function as antifertility agents. This was revealed by the effect produced by lupeol acetate, which reduced male albino rats' fertility by 100% (Gupta *et al.* 2005), and by an extract from *Echinops echinatus*, with lupeol as its main component, which decreased testosterone levels and testicular weight in male rats (Padashetty and Mishra 2007b). Lupeol presented a gastroprotective effect on ethanol-induced gastric damage in mice in a dose-response manner (Lira *et al.* 2009). Finally, lupeol and its related compounds have also demonstrated to possess some activity in the nervous system. For example, lupeol significantly enhanced [^{3}H]-glutamate uptake by astrocyte cultures and may play a role in treatment for neurodegenerative disorders (Martini *et al.* 2007). Muceniece *et al.* (2008) found that betulin is able to bind to the brain neurotransmitter γ-aminobutyric acid (GABA) receptors and antagonize the convulsant action of bicuculline, whereas lupeol and betulinic acid displayed no binding affinity, classifying betulin as a lead compound to the development of new anticonvulsant drugs.

CONCLUSION

As this review demonstrates, lupeol and some analogues have been shown to possess a range of folk and proven biological activities, further a potential to be consumed as dietary supplement to prevent cancer, coronary and hepatic diseases. Due to their widespread distribution in diverse plant families, these compounds are also easier to obtain than most treatments currently available, which justify future studies aiming the development of new methods of quantitation and detection in order to control the quality of marketed medicinal plants and phytopreparations. Additionally, lupeol revealed capability of interacting with multiple molecular targets, affecting and modulating the inflammation process, carcinogenesis and cellular stress response. Lupeol also displayed low cytotoxicity on healthy cells and acted synergistically when used in combined therapies, which make it worthy of exploration to be employed alone or as adjuvant to clinically used antineoplastic, anti-inflammatory, anti-hypertensive and antiurolithiatic drugs. Regarding this aspect, proteomics investigations should be carried out in order to uncover differentially expressed proteins during these conjugated therapies aiming the discovery of new targets and markers of drug efficacy. In addition, studies concerning lupeol pharmacokinetics should be done to improve its solubility, absorption and systemic availability. Finally, lupeol does not appear to be a promising antiprotozoal drug, but it revealed to be a valuable scaffold to originate more effective antimicrobial derivatives.

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