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REVIEW ARTICLE **OPEN ACCESS**

Review on Lupeol: Extraction Techniques, Analytical Methods and Pharmacology

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ABSTRACT
Lupeol is a pentacyclic triterpenoid that can be found in fruits, vegetables, and medicinal plants. Lupeol can also be found in olives, figs, mangoes, strawberries, red grapes, Japanese pear, tomato, pepper, carrot, white cabbage, and cucumber along with a variety of other plants. Lupeol is a potential triterpenoid that acts against many pharmacological activities including anti-inflammatory, antimicrobial, anti-angiogenic, anti-protozoal, antiproliferative, anti-invasive, antioxidant, cholesterol-lowering, anticancer, and treatment of kidney disorders, diabetes, arthritis, wound healing, and cardiovascular disease. There are numerous techniques available for the extraction of lupeol from different plant species. Significantly popular extraction techniques include high hydrostatic pressure, microwave assistance, sonication, maceration, soxhlet, exhaustive percolation, and cold percolation. The present review summarises the pharmacology, extraction techniques, and a variety of analytical methods used to detect and quantify lupeol and its combination. Lupeol can be identified and quantified using many analytical methods, including HPTLC, HPLC, HPLC-MS/MS or LC-MS/MS, and GC-MS in various plant species and their formulations.
Keywords: *Lupeol, Pharmacology, Extraction Techniques, Analytical Methodology.*

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INTRODUCTION

Lupeol (3-beta-luo-20(29)3n-3-ol) is a pentacyclic triterpene that is natively present in various fruits, and vegetables, as well as in medicinal herbs. It was previously identified in olive, mango, fig, red grapes, strawberry, japanese pear, pepper, white cabbage, cucumber, carrot, tomato, Cucumis sativus, *Coccinia grandis, Capsicum annuum, American ginseng, Cajanus cajan,* Shea butter plant, American ginseng, *Himatanthus sucuuba, Zanthoxylum riedelianum, Tamarindus indica, Leptadenia hastata, Celastrus paniculatus, Bombax ceib, Sebastiania Adenophora* and *Crat aeva nurvala* [1,2]. Lupeol is primarily found on the surfaces of plant barks, stems, leaves, and fruit waxes [3]. Over the past 15 years, researchers worldwide have been making significant efforts to the pharmacological actions, and develop extraction and analytical methods for its isolation, identification, and quantification [4]. Lupeol was shown to have several therapeutic activities both in vitro as well as in vivo. This includes anti-inflammatory, antimicrobial, anti-angiogenic, anti-protozoal, antiproliferative, anti-invasive, antioxidant, cholesterol-lowering, and anticancer properties. Additionally, it has been utilized for treating a variety of conditions including kidney disorders, diabetes, arthritis, wound healing, and coronary artery disease [3,4]. Lupeol is identified and quantified using different analytical approaches from plant species and their combination including HPTLC, HPLC, HPLC-MS/MS or LC-MS/MS, and GC-MS in various plant species and their formulations.

Phytoconstituent Profile [5,6]

Profile of lupeol described in Table 1.

Plants Containing Lupeol

Plants such as *Aloe vera* (Aloe), *Apocynum cannabinum* (Bitter root), *Calendula officinalis* (Bull's Eyes), Cajanus cajan (Congo-pea), *Camellia sinensis* (Black Tea), *Capsicum annum* (African Pepper), *Cassia fistula* (Indian Laburnum), *Coccinia grandis* (Ivy gourd), *Cucumins sativus* (Cucumber), *Helianthus annuus* (Annual Sunflower), *Olea Europa* L .(Olive), *Pisum sativum* (Common pea), *Trilisa odoratissina* (Vanilla plant), *Vitis vinifera* (Common grapevine), *Vitellaria paradoxa* (Bombuk-buttertree Shea), *Juniperus communis* (Common Juniper), *Glycyrrhiza glabara* (Common Licorice), *Glycine max* (Soyabean), *Ficus carica* (Common

fig), *Daucus carota* (Carrot), *Pyrus pyrifolia* (Japanese pear), *Mangifera indica* L. (Mango fruit), *Lawsonia alba* (Henna), *Hemidesmus indicus* (Indian Sarsaparilla), *Lycopersicon esculentum* (Tomato), Morus alba (White mulberry), *Panax ginseng* (Asiatic ginseng), *Phoenix dactylifera* (Date Plan), *Psidium guajava* (Common guava), *Mangifera pajang Kosterm* (Mango sps (peel like banana) and *Ulmus* spp. (Elm plant) are among the plants found to have significant levels of lupeol [2,5]. The amount of lupeol found in various fruits and numerous medicinal plants such as olive fruits (3 g/g fruit), aloe leaves (280 g/g dried leaf), mango fruits (1.80 g/g mango pulp), and elm plant (880 g/g bark) have been reported [7].

EXTRACTION

Various methods are discovered for extracting the lupeol from various plant species, which include maceration, soxhlet, sonication, microwave-assisted, high hydrostatic pressure, exhaustive percolation, and cold percolation using solvents such as ethanol, methanol, petroleum ether, n-hexane, dichloromethane, ethyl acetate, chloroform, n-butanol, water, and acetone. Lupeol extraction from different species using the soxhlet extraction method and percolation method from various species including in Table 2 and Table 3. According to Santos et al., lupeol was extracted from *Zanthoxylum monogynum* stem bark using hexane via maceration technique, resulting in a yield of 9.8 g [15]. According to Doshi et al., lupeol was extracted from the shade-dried roots of *Carissa congesta* using the petroleum ether solvent by cold extraction method, resulting in a yield of 1.8% (w/w) [16]. Lupeol was extracted by exhaustive percolation method using solvent ethanol in *Vernonanthura ferruginea* (LESS.) H. Rob(leaves) [17].

ANALYTICAL METHODS FOR DETERMINATION OF LUPEOL AND THEIR COMBINATION

This review focuses on the importance of analytical methods in determining medication and biomarker quality. This review covers a variety of analytical methods, including HPTLC, HPLC, HPLC-MS/MS or LC-MS/MS, and GC-MS. Overview of analytical methods described in Table 4.

HPTLC Methods

High-performance thin-layer chromatography (HPTLC) serves as a modified version of thin-layer chromatography (TLC), This is frequently employed as an inexpensive technique for quickly investigating sample mixtures. HPTLC is frequently used on pre-coated plates using an unaltered silica layer as the stationary phase with slit-scanning densitometry as the UV-visible light detection method. HPTLC is utilized to develop qualitative and quantitative evaluation techniques for any sample components [18,19]. The HPTLC method for separating and quantifying lupeol in *Hygrophila schulli* root and aerial parts has been published by Ghule et al. At an absorbance wavelength of 540 nm, the examined utilizing precoated aluminium-backed silica gel $60F_{254}$ plates using benzene, chloroform, and methanol in the proportions of 93:5.75:1.25, v/v. The R_f value was discovered to be 0.43 ± 0.02. During the study, it was observed that both roots and aerial portion extracts of *Hygrophila schulli* contained 5.02 ± 0.23 and 0.39 ± 0.11% w/w percent lupeol, respectively [8].

Pandya et al. described the HPTLC method for estimating lupeol from a petroleum ether extract of *Oxystelma esculentum*. The investigation was conducted on precoated 20×10 cm silica gel $60F_{254}$ plates (0.2 mm thickness) with a mobile phase of toluene and methanol in 9:1 v/v ratios. Lupeol has an R_f value of 0.65 when detected and measured at 254 nm. Lupeol concentration in O. esculentum was determined to be 0.829 0.09% w/w [20].

Khan et al. established a novel HPTLC technique for quantifying lupeol from the ethyl acetate extract of *Betula alnoides*. They investigated using stationary phase 20×10 cm silica gel G $60F_{254}$ plates and solvents of n-hexane and ethyl acetate in 8:2 v/v ratios at absorbance wavelength 254 nm, and the R_f value was found 0.61. In the study concentration of lupeol was found to be 0.0168 % [9].

In the study of Venkatachal Apathi et al., lupeol was isolated and quantified from aerial parts of Strobilanthes ciliates petroleum ether extract. For the analysis, they used the 4×10 cm silica gel $60F_{254}$ TLC plate with 8:2 v/v petroleum ether and ethyl acetate mobile phase. At the scanning wavelength of 388nm, the R_f value of lupeol was found to be 0.67. In the study content of lupeol was found 0.16 ± 0.02% w/w [13].

G.P. Ganu et al. established the validated HPTLC densitometric technique for analyzing lupeol from *Mimosoups elengi* bark. In their study, they used stationary phase 20×10 cm silica gel 60 F_{254} (Merck, 0.2 mm thickness) with a mobile phase consisting of toluene, ethyl acetate, and formic acid in a 12:2:1 v/v ratio. At a densiometric wavelength of 220 nm, the lupeol R_f value was determined to be 0.64 ± 0.02 [11].

Rout et al. developed a validated HPTLC technique for simultaneously quantifying betulin and lupeol in the *Crataeva nurvala* Buch-Ham stem bark. The estimation was performed using precoated silica gel 60 F_{254} (20×10 cm, 0.2 mm thickness) aluminium-backed plates as a stationary phase, with ethyl acetate and hexane in 1.8:8.2 v/v ratios as the mobile phase. Betulin has an absorbance wavelength of 510 nm, and lupeol has an absorbance wavelength of 560 nm. Betulin and lupeol had R_f values of 0.25 and 0.46, respectively. The content of betulin and lupeol was found 0.08 % and 0.13%, respectively [21].

Modi et al. described a validated HPTLC technique for simultaneously quantifying lupeol and oleanolic acid from *Mollugo oppositifolia* Linn. The experiment was carried out on precoated 10 × 10 cm silica gel 60 F_{254} stationary phase and a solvent of toluene, and methanol in 9.4: 0.6 v/v ratios. At 550 nm, the R_f values for lupeol and oleanolic acid were obtained as 0.51 and 0.16 respectively. Lupeol and oleanolic acid were determined to have % w/w contents of 0.015-0.016 and 0.027-0.02, respectively [22].

Hussain et al. used a validated HPTLC-UV technique to simultaneously estimate the stigmasterol and lupeol in *Hygrophila auriculata* (K. Schum) methanolic extract. The estimation was performed using 20 × 10 cm precoated aluminium lichrosphere silica gel 60F_{254} stationary phase and a mobile phase consisting of toluene, methanol, and formic acid (7.0:2.7:0.3 v/v/v). Stigmasterol and lupeol had R_f values of 0.52 ± 0.02 and 0.28 ± 0.05, respectively, at a detection wavelength of 530 nm. Linearities for lupeol and stigmasterol were 0.9994 and 0.9941, respectively. Lupeol and stigmasterol were found to have % contents of 0.19 ± 0.1% and 0.47 ± 0.1 % respectively [23].

Maurya et al. established a simple and reliable validated HPTLC to simultaneously quantify four biomarkers lupeol, ursolic acid, β-sitosterol, and betulinic acid from *Alstonia scholaris* stem and root barks using silica gel 60F_{254} plates (10 × 10 cm or 20 × 10 cm) and solvent system of chloroform and methanol in 99:1 v/v proportions. At 680 nm wavelength, lupeol, β-sitosterol, ursolic acid, and betulinic acid had R_f values of 0.77 ± 0.02, 0.57 ± 0.02, 0.18 ± 0.01, and 0.27 ± 0.02, respectively. Lupeol, β-sitosterol, ursolic acid, and betulinic acid obtained %w/w contents of 0.156, 0.076, 0.103, and 0.092 in stem bark, and 0.141, 0.103, 0.104, and 0.013 in root bark, respectively [24].

Adhyapak et al. established a validated normal-phase HPTLC technique to simultaneously quantify beta-amyrin and lupeol by different species: *Caesalpinia bonducella* Linn. seed kernel powder; *Coccinia indica* Wight & Arn. fruit powder; and root powder. The investigation was done on stationary phase silica gel 60F_{254} (10 × 10 cm) using a mobile phase consisting of n-pentane and ethyl acetate in an 8.0:2.0 v/v ratio. The R_f values for beta-amayrin and lupeol were obtained as 0.69 and 0.60, respectively, with a maximum wavelength of 580 nm [25].

Khatoon et al. investigated the HPTLC densitometric technique in *Tephrosia purpurea* L. extracts to simultaneously detect β-sitosterol, lupeol, and rotenone**.** The experiment was carried out by coating 20 × 10 cm silica gel plates (0.2 mm thickness) with a mobile phase containing toluene, ethyl acetate, and formic acid in a 9:1:1 v/v/v ratio. The R_f values for lupeol, rotenone, and β-sitosterol, were found as 0.52, 0.45, and 0.38, respectively, when scanned at a wavelength of 600 nm for β-sitosterol and lupeol and 320 nm for rotenone [26].

In the study of A. Gupta et al., ursolic acid and lupeol were simultaneously determined using HPTLC in a methanolic fraction of four different species, namely *Bauhinia purpurea* **L,** *Bauhinia acuminata* **L**, *Bauhinia variegata L***,** and *Bauhinia tomentosa* L leaves on the 20 × 10 cm silica gel 60 F_{254} (25 mm thickness) and mobile phase containing toluene, ethyl acetate, and formic acid in proportions of 8:2:0.1, v/v. Ursolic acid and lupeol were densitometrically determined at 520 nm and 520 nm, respectively, and their R_f values were 0.68 ± 0.01 and 0.46 ± 0.01, respectively. The greatest amounts of ursolic acid (0.11%) and lupeol (0.15%) were detected in B. acuminata leaves [27].

Lupeol and β-sitosterol were simultaneously quantified using a validated TLC-densiometric method in an unsaponifiable matter of Hygrophila spinosa seeds by Niraj Vyas et al**.** The experiment was done using a silica gel 60 F_{254} HPTLC plate with a solvent system of toluene, ethyl acetate, and formic acid in a 15:3:0.1 v/v/v ratio. At 520 nm wavelength, lupeol and β-sitosterol had R_f values of 0.64 and 0.54, respectively. Lupeol and β-sitosterol quantities were discovered to be 0.040 ± 1.21 gm% and 0.133 ± 0.97%, respectively [28].

K. Modi et al**.** developed an HPTLC technique to determine lupeol, ursolic acid, stigmasterol, and oleanolic acid from the entire plant of *Oldenlandia corymbosa* Linn utilizing precoated 10 cm × 10 cm silica gel 60 F_{254} plates with 540 nm wavelength. The mobile phase for oleanolic and ursolic acid is hexane, ethyl acetate, and methanol in a proportion of 8.2:1.8:0.5 v/v, while for the lupeol and stigmasterol, it is toluene and methanol in a proportion of 9.4:0.6, v/v. The R_f values of oleanolic and ursolic acid were 0.41 and 0.28, respectively, and 0.51 and 0.39 for lupeol and stigmasterol, respectively. In the study, the concentrations of lupeol, ursolic acid, stigmasterol, and oleanolic acid in the whole plant were 0.026 ± 0.008, 0.053 ± 0.009, 1.19 ± 0.04 and 0.012 ± 0.006 % w/w, % w/w and respectively [29].

Jyotshna et al**.** have simultaneously investigated the lupeol and mangiferin from the peel and pulp parts of Mangifera indica using the uni-dimensional double development HPTLC (UDDD-HPTLC) technique. The analysis was done by silica gel 60F_{254} plate (10 ×20 cm) with two different solvent systems compositions toluene, ethyl acetate, and methanol in a 7:2:1 v/v/v ratio, and second ethyl acetate and methanol in a 6:4 v/v ratio, for the optimal extraction and detection of lupeol and mangiferin, respectively. Lupeol had an R_f value of 0.88 at 610 nm, while mangiferin had an Rf value of 0.60 at 390 nm [30].

Saxena et al. established the validated HPTLC technique to simultaneously quantify lupeol, β-sitosterol, and betulinic acid from *Dillenia pentagyna* Roxb fruits, leaves, root, and stem bark using a 10 × 20 cm aluminium-packed TLC plate precoated with silica gel $60F_{254}$ (0.2 mm thickness) and solvents of petroleum ether, ethyl acetate, and acetonitrile in a proportion of 8.2:1.8:0.1, v/v. Lupeol, β-sitosterol, and betulinic acid had R_f values of 0.36, 0.23, and 0.17, respectively, plates were scanned at 580 nm wavelength. In the study, they found that the stem bark has the highest content of lupeol (0.369 ± 0.01%) and betulinic acid (0.920 ± 0.02%), while the leaves contain the highest amount of β-sitosterol (1.555 ± 0.07%) [31].

Upadhye et al. developed a novel validated HPTLC technique to simultaneously estimate lupeol and quercetin in *Ficus glomerata* root ethanolic extract. The experiment was carried out using an aluminium 20 ×10 cm pre-coated silica gel $60F_{254}$ plate with a solvent of toluene and methanol in a 9:1% v/v ratio. Lupeol and quercetin obtained the R_f values 0.65 ± 0.02 and 0.14 ± 0.02 at detection wavelengths 525 nm and 250 nm respectively. In the study content of lupeol and quercetin was obtained at 1400 ng and 2531.8 ng respectively [32].

HPLC Methods

High-performance liquid chromatography (HPLC) is among the most extensively utilized methods for analysis. HPLC is a separation module that consists basically of the stationary phase and mobile phase with opposite polarities, both of which are equipped with high-pressure pumps, and the separation is achieved through their interaction with the solvent of the mobile phase and the solid particles of a tightly packed column. In a single step, HPLC may perform both quantitative and qualitative analysis [18,33].

Techaoie et al. reported the HPLC technique employing a DAD detector to quantify lupeol in mango (*Mangifera indica* L.) cultivars. The estimation was conducted using stationary phase C18 column (254 ×4.6 mm) and a mobile phase consisting of methanol and acetonitrile in a 30:70 v/v ratio, at a 1 ml/min of flow rate. Lupeol retention time was discovered to be 27.5-28.5 min at 210 nm wavelength [34].

Oliveira et al**.** developed the HPLC-PDA technique to isolate and quantify the lupeol in *Vernonanthura ferruginea* (Less.) H. Rob species using reverse-phase Luna C8 column (250 × 4.6 mm, 5 µm) with a solvent system of acetonitrile and acetic acid in a 99.99:0.01 v/v proportion at a flow rate of 0.8 mL/min. At 210 nm wavelength, lupeol run time was determined to be 38 minutes [17].

A validated HPLC method utilizing a UV-SPD-20A detector for simultaneously quantifying the stigmasterol and lupeol from *Butea monosperma* (Lam) bark has been evaluated by Modh et al**.** The experiment was conducted by employing Phenomenex, Luna C18 column (150×4.6, 5µ) with a mobile phase consisting of isocratic methanol and water in a 98:2% v/v ratio using a flow rate of 1mL/min. At 220 nm wavelength, stigmasterol and lupeol retention time was found 15 min [35].

A validated reverse-phase HPLC method for simultaneously quantifying lupeol, stigmasterol, and betulin in *Asteracantha longifolia* Nees ethanol extract was evaluated by Maji et al. The estimation was conducted on Luna C18 column (5 µm, 250 × 4.6 mm) with a mobile phase containing isocratic acetonitrile and 0.1% acetic acid in water in a 94: 6 v/v ratio, at a flow rate of 1 mL/min. Lupeol, stigmasterol, and betulin retention time were determined to be 15.84, 26.24, and 8.56, respectively at 215 nm wavelength [36].

Nandhini Ilango et al. established a validated reverse phase HPLC technique to simultaneously quantify β-sitosterol, stigmasterol and lupeol from *Adhatoda vasica* Nees leaves extracts and its marketed formulations using reverse phase Phenomenex C18 column (250mm × 4.6mm; 5µ) and a solvent system of 0.1% v/v formic acid in water and methanol in a 28:82 % v/v ratio with a flow rate of 0.8 mL/min. Lupeol, stigmasterol, and β-sitosterol were shown to have retention times of 20.72, 18.26, and 16.89, respectively. In the study, they discovered that hexane extract contained 0.952, 3.126, and 0.255 %w/w, chloroform extract contained 0.548, 8.649, and 0.285% w/w, ethyl acetate extract contained 0.487, 1.472, and 0.105 %w/w, and methanolic extract contained 0.105, 5.062, and 0.113 %w/w lupeol, β-sitosterol and stigmasterol w/w respectively [37].

LC-MS/MS Methods

High-pressure liquid chromatography-mass spectrometry (HPLC-MS/MS), also known as liquid chromatography-tandem mass spectrometry (LC-MS/MS), is a type of analytical method combining sensitive mass spectral detection with high-resolution chromatographic separation. In laboratories, LC-MS/MS is frequently used to analyze biological materials, drug molecules, and drug products on a qualitative and quantitative level [39-40].

Patel et al. reported the LC-APCI-MS/MS technique to simultaneously quantify betulinic acid, lupeol, and β-sitosterol in a methanolic *Madhuca longifolia* bark extract. The analysis was done on the Gemini C18 column (50 × 2.0mm, 3µm id) using a 0.4 mL/min flow rate. In gradient mode, a mobile phase was composed of water and 0.1% formic acid as solvent A and acetonitrile: methanol (50:50, v/v) and 0.1% formic acid as solvent B. The retention time of betulinic acid, lupeol, and β-sitosterol was discovered to be 1.25, 3.08, and 3.53 respectively. The [M-H] transition for betulinic acid, lupeol, and β-sitosterol was at m/z 457.2→ 81.0, 427.4 →67.0 and 397.3→55.0 respectively, and the nebulizer gas flow was kept at 3.0 L/min [41].

Voronov *et al***.** reported the HPLC-MS/MS method with a triple quadrupole mass analyzer to detect the lupeol, α-amyrin, 3β-taraxerol, β-amyrin, betulin, uvaol, erythrodiol, oleanolic, ursolic and betulinic acids in plant biomass using column Hypercarb, 30 × 3.0 mm, 3.0 µm particle size, and porous graphitic carbon stationary phase. The mobile phase was made up of A methanol and acetonitrile in a 1:1 v/v ratio, and B ethyl acetate and isopropanol in a 1:1 v/v ratio, both of which contained 0.5% formic acid using interface temperature - 350 ◦C; heat block temperature - 250 ◦C; desolvation line temperature - 250 ◦C, nebulizing gas flow rate - 4 L min^{-1} and drying gas flow rate - 15 L min^{-1} respectively. The retention times of lupeol, α-amyrin, 3β-taraxerol, β-amyrin, betulin, uvaol, erythrodiol, oleanolic, ursolic, and betulinic acids were determined to be 2.81, 3.38, 1.93, 4.74, 4.96, 3.97, 6.38, 7.69, 4.18 and 5.36 min, respectively [42].

GC-MS Methods

Gas Chromatography-Mass Spectrometry (GC-MS) is a hyphenated analytical method that can be used on solid, liquid, or gaseous materials. The materials are first converted to a gaseous condition before being analyzed using the mass-to-charge ratio. GC-MS can be used for determining several phytochemicals in a test sample [18,43].

Chache et al**.** reported the GC-MS method for simultaneous estimation of β-sitosterol and lupeol from Terminalia tomentosa extract and eugenol and kaempferol from *Syzygium cumini* extract. The analysis was done on column Rtx- 1ms (100% dimethylpolysiloxane) as stationary phase and helium gas as mobile phase with interphase temperature 280°C. For T. tomentosa flow rate, ion source temperature and scan speed were kept at 1.5 ml/min, 200˚C, and 1111 while for *S. cumini* flow rate, ion source temperature and scan speed were kept at 0.9 ml/min, 250˚C and 909 respectively [44].

GC-MS analysis of key compounds including n-hexadecanoic acid,1,2-benzene dicarboxylic acid, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, mono(2-ethylhexyl) ester, 2-ethyl-2-methyl-, tridecanol, Squalene, campesterol, beta. -Sitosterol, dl-. alpha. -tocopherol, stigmasterol, lupeol and betulin in medicinal plant, *'Muntingia calabura'* acetate extract has been reported by Perumal *et al***.,** using Column DB5 MS (30 mm × 0.25 mm ID ×0.25 µm, composed of phenyl: methylpolysiloxane in the ratio of 5%: 95% and having carrier gas helium of 99.999% purity at a steady flow of 1 ml/min. In the study, the ion source and auxiliary temperatures were adjusted at 280°C and 290°C, respectively. The overall GC running time was found to be 32.02 minutes [45].

PHARMACOLOGY

Lupeol has potential to cure the wide range of pharmacological effects including anti-inflammatory, antiprotozoal, anti-cancer, anti-diabetic, hyperlipidaemic, cardioprotective Agent, antimicrobial and nephroprotective effects.

Anti-Inflammatory Agent

Inflammatory diseases are now a major global problem, categorized by swelling, redness, pain, heat, and loss of function. The most important nuclear factor is kappa B (NF- κB), which triggers inflammatory reactions. Lupeol lowers IL-4 (interleukin 4) synthesis by Th2 cells (T-helper type-2) and has an anti-inflammatory effect, which is demonstrated by a significant decrease in eosinophils in an allergic airway inflammation model. [2,5,7].

Antiprotozoal Agent

Lupeol is helpful against a wide range of pathogenic protozoa, including those responsible for leishmaniasis, trypanosomiasis, and malaria. Lupeol has been shown to reduce the growth of the malaria parasite (Plasmodium falciparum). Lupeol increases NO generation in *L.donovani*-infected macrophages and lowers parasite levels in the liver and spleen. The Lupeol was tested for antihelmintic efficacy against *H. contortus*, C. elegans, and *T. colubriformis* in *Curtisia dentate* extracts [5,46].

Anti-Cancer Agent

Lupeol has shown significant action in preventing various cancers, including skin, human prostate, liver, breast, lung, colorectal, bladder, osteosarcoma, and blood cancer, by altering essential molecular pathways associated with proliferation, survival, and apoptosis. Different cancers possess multiple cell lines, including the MCF-10A healthy human breast and the MCF-7 cancer cell lines. Lupeol is produced in the cell line and has an effect on the cell MCF-7 cell viability with an IC50 value of 80 µM. [5,46,47].

Anti-Diabetic Agent

Diabetes is a set of metabolic conditions characterized by higher levels of glucose in the body. That is categorized into type 1 and type 2 diabetes. Lupeol inhibits diabetes by altering the insulin receptors and the GLUT 4 protein. The impact of superoxide dismutase (SOD) and catalase enzymes (CAT), as well as non-enzyme antioxidants (Vitamin-C), along with lowered antioxidant (Vitamin-C, CAT, and SOD) thresholds, were examined in type 2 diabetic male rodents. Lupeol lowers glycated haemoglobin, blood glucose, and nitric oxide levels. Lupeol can also be used to treat diabetes by inhibiting the action of alpha-glucosidase. [5,47].

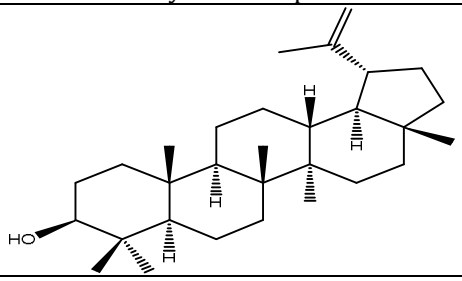
Hyperlipidaemic and Cardioprotective Agent

Hyperlipidaemia is a significant risk factor associated with high cholesterol, heart conditions, and other cardiac problems. Lupeol, with its ester, lupeol linoleate, is utilized to treat hypercholesterolemia in rats while also lowering the activity of enzymes including Na+, K+-ATPase, Ca2+-ATPase, and Mg2+. In HepG2-Lipo human hepatoma cells, lupeol lowers triglyceride and cholesterol secretion and suppresses the expression of fatty acid synthase, 3-hydroxy-3-methylglutaryl-Coenzyme A synthetase-1, and farnesyl-diphosphate farnesyl transferase-1 [5].

Antimicrobial Agent

Lupeol has antibacterial properties towards Gram-positive and Gram-negative pathogens, including *Candida albicans*. The antibacterial activity of *Visnea mocanera* leaf extract containing lupeol was also anti-viral. Lupeol inhibits the activity of the Herpes simplex virus-1 reverse transcriptase (HIV-1 RT) associated RNA-dependent DNA polymerase (RDDP) and also exhibits antiviral effects against – glucosidase [5].

Table 1: Physicochemical Profile of Lupeol

Category	Pentacyclic Triterpenoid
Structure	
IUPAC Name	1R,3aR,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-3a,5a,5b,8,8,11a hexamethyl-1-prop-1-en-2-yl 1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b hexadecahydrocyclopenta[a]chrysen-9-ol
Mol. Formula	$C_{30}H_{50}O$
Mol. Weight	426.7 g/mol
Melting Point	215–216 °C
Solubility	Very soluble in ethanol, chloroform, petroleum ether, benzene, acetone, and warm alcohol.

Tabel: 2 Lupeol Extraction by Soxhlet Extraction from different plant species

Sr.No	**Species**	**Solvent**	**% Yield**	**Reference**
1.	*Hygrophila schulli* (roots and aerial parts)	Petroleum ether	Root parts-6.23% and aerial parts- 11.17% w/w.	[8]
2.	*Betula alnoides* (bark)	petroleum ether	3.45% w/w	[9]
3.	*Calotropis gigantea latex* (aerial parts)	Petroleum ether	32.16% w/w	[10]
4.	*Mimosoups elengi* (bark)	Methanol	8.6 % w/w	[11]
5.	Different *Genus Ficus* (*Ficus nitida, Ficus vest, Ficus carica, Ficus ingens* and *Ficus palmata*) (leaves part)	Methanol	*Ficus nitida*- 5.1% w/w, *Ficus vest*- 6.5% w/w, *Ficus carica*- 4.0% w/w, *Ficus ingens*- 5.6% w/w, *Ficus palmata*-4.9% w/w	[12]

Table 3: Lupeol Extraction by Percolation Method from different plant species

Sr. No	**Species**	**Solvent**	**% Yield**	**Reference**

1.	*Strobilanthes ciliatus* Nees (aerial parts)	Petroleum ether	1.8g (0.18%)	[13]
2.	*Diospyros ehretioides* Wall (stem bark)	Ethyl acetate, Petroleum ether	Ethyl acetate crude - 1.87g, Petroleum ether extract- 1.41g	[14]

Table 4: Summary of HPLC and HPTLC Methods chromatographic description for Detection of Lupeol and their combinations

Method	Biomarker	Stationary Phase	Mobile Phase	Retention Factor (R_f)	Reference
HPTLC	Lupeol	Silica gel $60F_{254}$	Benzene: chloroform: methanol (93:5.75:1.25, v/v)	0.43 ± 0.02.	[8]
		Silica gel plates $60F_{254}$ plate (thickness 0.2 mm, 20×10cm)	Toluene: methanol (9:1v/v)	0.65	[20]
		Silica gel G ($60F_{254}$) plates (20×10 cm)	n-Hexane: ethyl acetate (8:2 v/v)	0.61	[9]
		Silica gel $60F_{254}$ TLC plate (4×10)	Petroleum ether: ethyl acetate (8:2 v/v)	0.67	[13]
		Silica gel 60 F_{254} (Merck, 20 cm × 10 cm, 0.2 mm thickness)	Toluene: ethyl acetate: formic acid (12:2, v/v)	0.64 ± 0.02	[11]
	Betulin, Lupeol	Silica gel 60 F_{254} (20 × 10 cm, thickness 0.2 mm)	Ethyl acetate: hexane (1.8:8.2, v/v)	Betulin- 0.25, Lupeol-0.46	[21]
	Lupeol, Oleanolic acid	Precoated silica gel 60 F_{254} (10 cm × 10)	Toluene: methanol (9.4: 0.6, v/v)	Lupeol- 0.51, Oleanolic acid-0.16	[22]
	Stigmasterol, Lupeol	Silica gel $60F_{254}$ (20×10cm, 200 µm thickness)	Toluene: methanol: formic acid (7.0:2.7:0.3 v/v/v)	Stigmasterol-0.52±0.02, Lupeol- 0.52±0.02	[23]
	Lupeol, Ursolic acid, β-sitosterol, Betulinic acid	Silica gel $60F_{254}$ plates (10 cm × 10 cm or 20 cm × 10 cm)	Chloroform: methanol (99:1 v/v).	Lupeol- 0.77 ± 0.02, Ursolic acid-0.18 ± 0.01, β-sitosterol-0.57 ± 0.02, Betulinic acid-0.27 ± 0.02	[24]
	Beta-amyrin, Lupeol	Silica gel $60F_{254}$ (10.0 cm × 10.0 cm)	n-Pentane: ethyl acetate (8.0: 2.0, v/v)	Beta-amyrin-0.69, Lupeol-0.60	[25]
	β-Sitosterol, Lupeol, Rotenone,	Silica gel plates, (20 × 10 cm ,0.2-mm thickness)	Toluene: ethyl acetate: formic acid (9:1:1 v/v/v)	β-Sitosterol-0.38, Lupeol-0.52, Rotenone-0.45	[26]
	Ursolic acid, Lupeol	Silica gel 60 F_{254} plates (20 cm × 10 cm,0.25 mm thickness)	Toluene: ethyl acetate: formic acid (8:2:0.1, v/v)	Ursolic acid-0.68 ± 0.01, Lupeol-0.46 ± 0.01	[27]
	Lupeol β-sitosterol	Silica gel 60 F_{254} plate	Toluene: ethyl acetate: formic acid (15:3:0.1 v/v/v	Lupeol-0.64 β-sitosterol-0.54	[28]
	Lupeol, Ursolic acid, Stigmasterol, Oleanolic acid	Precoated silica gel 60 F_{254} plates (10 cm × 10 cm)	Hexane: ethyl acetate: methanol (8.2:1.8:0.5, v/v)	Lupeol-0.51, Ursolic acid-0.28,	[29]

				Stigmasterol-0.39Oleanolic acid-0.41	
	Lupeol, Mangiferin	Silica gel 60F254 plate (10 × 20 cm)	Two different solvent systems for optimization: Toluene: ethyl acetate: methanol (7:2:1, v/v/v), Ethyl acetate: methanol, (6:4, v/v)	Lupeol-0.88, Mangiferin-0.60	[30]
	Lupeol, β-sitosterol Betulinic acid	Silica gel $60F_{254}$ (10 × 20 cm, 0.2 mm thickness)	Petroleum ether: ethyl acetate: acetonitrile (8.2:1.8:0.1, v/v)	Lupeol-0.36 β-sitosterol-0.23, Betulinic acid-0.17	[31]
	Lupeol, Quercetin	Silica gel $60F_{254}$ (20 × 10 cm, Merck)	Toluene: methanol (9:1 % v/v)	Lupeol-0.65 ± 0.02, Quercetin-0.14 ± 0.02	[32]
Method	**Biomarker**	**Stationary Phase**	**Solvent System**	**Retention Time (R_t)**	**Reference**
HPLC	Lupeol	C18 column (254 × 4.6 mm	Methanol: acetonitrile (30:70 V/V)	27.5-28.5 min	[34]
		Luna C8 reverse-phase column (250 × 4.6 mm, 5 μm)	Acetonitrile: acetic acid (99.99:0.01, v/v)	38 min	[17]
	Stigmasterol, Lupeol	Phenomenex, Luna C18 column (150 × 4.6, 5μ)	Isocratic methanol: water (98:2% v/v)	15 minutes for both	[35]
	Lupeol, Stigmasterol, Betulin,	Luna C18 column (5 μm, 250 × 4.6 mm)	Isocratic elution of acetonitrile and 0.1% acetic acid in water (94:6, v/v)	Lupeol-15.84 min, Stigmasterol-26.24 min Betulin-8.56 min,	[36]
	β-sitosterol, Stigmasterol, Lupeol	Reverse phase-Phenomenex C18 (250mm × 4.6mm; 5μ) column	0.1%v/v formic acid in water and methanol (28:82%v/v)	β-sitosterol-20.72 min, Stigmasterol-18.26 min, Lupeol-16.89 min	[37]

CONCLUSION

Lupeol is a pentacyclic triterpenoid that is present in several medicinal plant species and has the potential to cure a wide range of diseases due to its diverse pharmacological properties. This review discusses various lupeol extraction methods such as maceration, soxhlet extraction, percolation, and cold extraction. Methanol and petroleum ether are the most commonly used solvents in lupeol extraction. This review preliminary focus on several analytical methods including HPTLC, HPLC-MS/MS or LC-MS-MS, GC-MS, and HPLC coupled to different detectors such as PDA, DAD, and UV-SPD-20A for the determination and quantification of lupeol and its combination in herbal formulation, ayurvedic formulation, and different plant species. This review reports 18 HPTLC methods, 5 HPLC methods, 2 HPLC-MS/MS or LC-MS/MS methods, and 2 GC-MS methods for the estimation of lupeol. Based on the literature, this review discovers that HPTLC is the most frequently used method for the identification of lupeol because of its simplicity, speed, accuracy, precision, sensitivity, and cost-effectiveness. As the outcome, it can be assumed that recent trends and existing analytical methods show that the data is useful for developing and validating analytical methods.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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