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A review on Lupeol: Superficial triterpenoid from horticulture crops

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Abstract
Traditional systems of medicine have been progressively gaining interest and acceptance all over the world, even among the practitioners of modern medicine. Consequently plant materials and herbal drugs derived from them represent a substantial proportion and all these are supplied by horticultural crops like Fruits, vegetables, spices, medicinal crops and plantation crops. Natural products and herbal remedies used in traditional folklore medicine have been the source of many medically beneficial drugs because they elicit fewer side effects, relatively cheap, affordable and claimed to be effective. Lupeol, a triterpene found in horticulture crops like mango, grapes, strawberry and olive, some vegetables white cabbage, green pepper was reported to possess beneficial effects as a therapeutic and preventive agent for a range of disorders. It is very potential triterpene which acts against many diseases especially cancer for this purpose a large scope in the pharmaceutical industries. Lupeol exhibited an array of biological activities like anti-inflammatory, anti-arthritic, anti-mutagenic and anti-malarial activity both in in vitro and in vivo systems. So, there is a growing interest in natural triterpenoids, also known as phytosterols, due to their wide spectrum of biological activities.

Keywords: Lupeol, triterpene, anti-inflammatory, anti-arthritic, anti-mutagenic

Introduction

According to an estimate of World Health Organisation (WHO), nearly eighty per cent of the population of developing countries relies on traditional medicine, mostly plant drugs for their primary health care needs (Arvind Kumar, 2006) [3]. Renewed interest in traditional plant-based medicines during last two decades in the developed world has opened enormous opportunities for megadiversity country like India. This revival of interest in the plant-based drugs is mainly due to the widespread belief that ‘green medicine’ is safe with no adverse side-effects. Also modern pharmacopocia contains at least 25 per cent drugs derived from plants or synthetic analogues built on prototype compounds isolated from plants. Transition from synthetic drugs and microbially produced antibiotics to plant-based drugs, is now rapidly gaining acceptance. This global resurgence in use of plant-based drugs is an opportunity for India to attain self-reliance and boost export of herbal drugs. India has one of the richest medicinal plant traditions in the world with remarkable contemporary relevance for ensuring health security to millions. Triterpenes are important structural components of plant membranes, and free triterpenes serve to stabilize phospholipid bilayers in plant cell membranes just as cholesterol does in animal cell membranes (Liby *et al*., 2007) [17]. Lupeol is found in many fruits such as olive, fig, mango, strawberry, red grapes (Mohammad Saleem., 2009) [20] and in medicinal plants such as American ginseng, Shea butter plant, *Tamarindus indica, Celastrus paniculatus, Zanthoxylum riedelianum, Leptadenia hastata, Crataeva nurvala, Bombax ceiba* and *Sebastiania adenophora* (Beveridge *et al*., 2002, Alander *et al*., 2005, Imam *et al*., 2007) [5, 1, 13]. In the fruits, numerous phytochemicals are present in mango peel and pulp, such as triterpene, lupeol which is under basic research for its potential biological effects. Mango peel pigments under study include carotenoids, such as provitamin A, beta carotene, lutein and polyphenols, such as quarcetin, kaemferol, gallic acid and catechin. The flavor of mango fruit is constituted by several volatile organic chemicals mainly belonging to terpene, furanone, lactone and ester classes. Lupeol is a pharmacologically active triterpenoid. It has several potential medicinal properties (Wal *et al*., 2011) [39]. There is a growing interest in natural triterpenoids, also known as phytosterols, due to their wide spectrum of biological activities.

An overview of lupeol in horticulture plants

Lupeol, is found in fruits such as olive, fig, mango, strawberry, red grapes, Japanese Pear, vegetables such as white cabbage, pepper, cucumber, tomato, carrot, *Cajanus cajan, Capsicum annuum, Coccinia grandis, Cucumis sativus* and in medicinal plants such as American ginseng, Shea butter plant, *Tamarindus indica, Allanblackia monticola, Himatanthus sucuuba, Celastrus paniculatus, Zanthoxylum riedelianum, Leptadenia hastata, Crataeva nurvala, Bombax ceiba* and *Sebastiania adenophora* used by native people in North America, Latin America, Japan, China, Africa and Caribbean islands (Mohammad Saleem, 2009) [20]. Papireddy *et al*. (2009) reported that isolation of triterpenoid lupeol from the leaves extract of *Aegle marmelos* naturally occurred lupeol and screened for their anti-hyper glycemic activity and antidyslipidemic activity reduction in the circulating triglyceroids and other fatty acid by ester derivative played major role to improve the hyperglycemia and strengthen the insulin response.

Soujanya *et al*., (2017) [33] reported that the Quantification of lupeol from selected juicy cultivars of mango (*Mangifera indica* L.) by High performance Liquid Chromatography (HPLC) method and showed that Chinnarasam variety recorded highest amount of lupeol (67.24±8.77 µg/100g) and lowest amount of lupeol was recorded in Pandurivari Mamidi (8.45±0.10 µg/100g). Wal *et al*., (2011) [39] studied that lupane principally present in common fruit plants such as olive, mango, strawberry, grapes etc. were reported to possess beneficial effects as a therapeutic and preventive agent for a range of disorders and also exhibited an array of biological activities like anti-inflammatory and anti-arthritic activities both in invitro and invivo synthesis. The studies also provided the mechanism of action of lupeol and suggested that it is a multi-target agent with immense anti-inflammatory potential targeting key molecular pathways which involve nuclear factor Kappa B, CFLIP, Far, Krass, phosphotidyl inositol-3-Kinase in a variety of cells. Han and Bakovic (2015) [10] found that fruits like apple, grape berry, tomato and mango all contain triterpenoid compounds with cardio protective and anti-oxidant activities. Dietary triterpenoids, a group of plant secondary metabolites. Lupeol was known to have properties that significantly influence anti-inflammatory activities. It is naturally occurring pentacyclic triterpene. High concentration of lupeol was found in the peels of mango fruits, especially during maturity stage.

Caesalpinia bonducella Linn. is used as a folklore medicine for treatment of diabetes. Commonly known as Grey Nicker, is a species of flowering plant in the senna tribe of family Caesalpinieae. The tribal people of India use it for controlling x blood sugar. The seeds are reported to possess anti-diabetic or hypoglycemic activity (Rahmatullah *et al*., 2010) [28]. (*Coccinia indica* Wight & Arn.) commonly known as 'Tondali' is used as a vegetable. The parts like fruits, leaves, roots of (*Coccinia indica* Wight & Arn) have been widely used in treatment of diabetes mellitus. The various extract of fruit and root juice has been reported to cure dysentery, vomiting, mouth ulcers and bronchitis, asthma and gastrointestinal disturbances (Gunjan *et al*., 2010) [9]. Both the plants (Grey Nicker and Tondali) have high medicinal value with important phytochemicals present in them. The two common triterpenoids present in the selected parts of both the plants are lupeol and beta-amyrin (Prabhakar *et al*., 2008 and Khare *et al*., 2004) [25, 15]. Jyotshna *et al*. (2015) [14] estimated mangiferin and lupeol content in 4 mango cultivars and reported that highest amount of lupeol was found in Dashehari (1082 µg/100g) as compared to Bombay green (505 µg/100g), Langra (167 µg/100g) and Chausa (65 µg/100g) in pulp and peel during storage period. Lupeol was quantified by HPTLC densitometry method. The mangiferin content in Bombay green pulp was significantly correlated with titrable acidity and lupeol remained unaffected in the pulp of all four varieties. Total soluble solid content had no effect on lupeol content in Bombay green and Dashehari pulp. Contrary to this, total soluble solid content and lupeol concentration in Langra and Chausa were highly correlated. Content of lupeol in fruits and in plants (lg/g). Hemant and Gaurav (2014) [11] reported that the *Benincasa hispida* (Thunb.) Cogn. is an extensive climbing annual herb in an agricultural country like India. Lupeol, a constituent of this species, has been reported to possess good amount of pharmacological potential. Soujanya *et al*., (2017) [33] reported that the Quantification of Lupeol among different colored varieties of mango (*Mangifera indica* L.) highest lupeol content was recorded in Suvarnareka (47.26±12.09 µg/100g) than Vanraj (28.86±2.09 µg/100g).

Table 1: List of selected plants containing lupeol

S. no	Plants	Scientific name	Content	Reference
	Fruits			
1	Guava	*Psidium guajava*	-	Imam S, Azhar.,2007 [13]
2	Common fig	*Ficus carica*	-	Imam S, Azhar.,2007 [13]
3	Japanese pear	*Pyrus pyrifolia*	175 lg/g twig bar	Nguemfo *et al*., 2007
3	Ginseng oil		15.2 mg/100g	Nguemfo *et al*., 2007
4	Olive fruit	*Olea europa L.*	3 lg/g of fruit	Nguemfo *et al*., 2007
5	Mango sps (peel like banana)	*Mangifera pajang Kosterm*	>300 (µg/ml)	Sadikah Ahmad *et al*., 2015
6	Date palm	*Poenix dactlylifera*	-	Imam S, Azhar.,2007 [13]
7	Mango fruit	*Mangifera indica L.*	180 lg/g	Nguemfo *et al*., 2007 [13]
8	Grapes	*Vitis vinifera*		Imam S, Azhar.,2007 [13]
9	White mulberry	*Morus alba*		Imam S, Azhar.,2007 [13]
	Vegetables			
10	Cucumber	*Cucumis sativus*	-	Imam S, Azhar.,2007 [13]
11	Tea	*Camellia sinensis*	-	Imam S, Azhar.,2007 [13]
12	Ivy gourd	*Coccinia grandis*	-	Imam S, Azhar.,2007 [13]
13	Carrot	*Daucus carota*	-	Imam S, Azhar.,2007 [13]

14	Capsicum	*Capsicum annum*	-	Imam S, Azhar.,2007 [13]
15	Tomato	*Lycopersicon esculentum*	-	Imam S, Azhar.,2007 [13]
16	Common pea	*Pisum sativum*	-	Imam S, Azhar.,2007 [13]
	Medicinal plants			
17	Aloe leaves	*Aloe vera L.*	280 lg/g dry leaf	Nguemfo *et al*., 2007
18	Elm plant	*Ulmus spp.*	880 lg/g bark	Nguemfo *et al*., 2007
19	Bitter root	*Apocynum cannabinum*	-	Imam S, Azhar.,2007 [13]

Chemical structure and analysis

The chemical formula of Lupeol is $C_{30}H_{50}O$ and its melting point is 215–216 °C. Properties computed from the structure of Lupeol show that it has a molecular weight of 426.7174 [g/mol], H-Bond donor 1, H-Bond acceptor 1, rotatable bond count 1, exact mass 426.386166, mono isotopic mass 426.386166, topological polar surface area 20.2, heavy atom count 31, formal charge 0, complexity 766, isotope atom count 0, defined atom stereo center count 10, and bonded unit count 1 (PubChem, NIH library, Compound ID 259846). The infra-red spectrum of Lupeol shows the presence of a hydroxyl function and an olefinic moiety which show their presence in the spectrum at 3235 and 1640 cm-1, respectively (Imam *et al*., 2007) [13]. Study conducted by Martelanc *et al*. (2007) [19] using high performance liquid chromatographic (HPLC) method with UV and mass spectrometric [MS] showed that Lupeol exhibits a parent ion peak at m/z 409 [M+H-18][+].

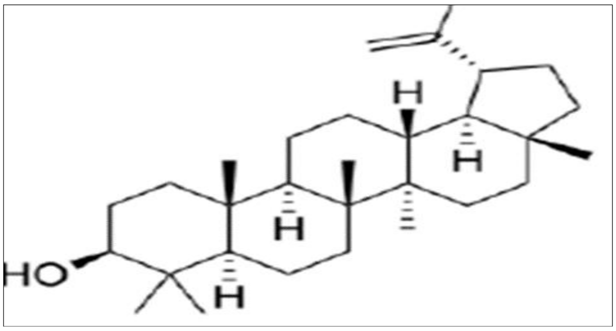

Fig 1: Structure of lupeol

Estimation of lupeol from horticulture crops

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 (GS) and High Performance Thin Layer Chromatography (HPTLC) techniques are the most employed methods to quantitate lupeol in horticulture plants. HPTLC is cost efficient, flexible and quick. Silica gel 60 F254 is used as the stationary phase; the plate development can be carried out with a variety of solvents system like toluene/methanol (9:1), n-hexane/ ethyl acetate (5:1) and lupeol is detected and quantified by densitometry after reaction with anisaldehyde-sulfuric acid, Lieberman- Burchard reagent or antimony trichloride (Padashetty and Mishra, 2007; Shailajan S, Menon SN, 2009) [36]. Lupeol content was quantified by High Performance Liquid Chromatography (HPLC) grade Agilent 1260. Saratha *et al*. (2011) [31] developed this method of quantification of lupeol. Fruit pulp was dried in solar drier for 15 days and made into powder of 50-100gm from each fruit. That powder was useful for the extraction of lupeol in fruits pulp. Measured exact 250 mg of dried powder and extracted through 10ml of Methanol and Acetonitrile (V:V) 30:70% extracted sample allowed to vortex for 5 minutes and kept it for overnight and it was filtered through whatman no.1 paper and again filtered through 0.45µm membrane (syringe filters 0.45µm and 0.25 diameter). Final filtrate was taken in to small vials and used for HPLC analysis for identification and quantification of lupeol in each mango variety at 4 days intervals. Results obtained in this method were compared with the standard retention time was between 26.5 to 28 min and also calculation of lupeol in mango dried pulp was determined by the following formula according to Anyakora *et al*.(2008). Sunita Shailajan *et al*., 2013 [36] reported that the impact of regional variation on lupeol content in *Carissa carandas* Linn. Here the fruits collected from different geographical regions of India using a validated HPTLC method. Chromatographic separation was achieved on TLC plates pre-coated with silica gel 60 F254 using toluene: methanol (8:1, v/v) as a mobile phase. Detection of lupeol was carried out by derivatizing the plates with 10 % methanolic sulphuric acid reagent followed by its densitometric scanning using CAMAG TLC scanner 4 at 366 nm.

A HPTLC method (Hernandez *et al*., 2010) [12] for identification of an isomer of lupeol i.e. epi-lupeol and beta amyrin from resins and various valuable medicinal plants was done on silica gel 60 F254 plates coated with 10 % aqueous solution of silver nitrate. The mobile phase used was nhexane: dicholoromethane: methanol in volume ratio of 10: 10:1. The post derivatization was done by spraying anisaldehydesulphuric acid reagent on to the developed plate. This method has not been able to resolve isomer of lupeol i.e. epi-lupeol and beta amyrin. However, the present developed method uses a simple prederivatization technique using iodine vapours prior to development of TLC plate with mobile phase to separate lupeol and beta-amyrin. In another reported method (Martelanc *et al*., 2009) C18 RP-HPTLC plates prewashed with acetone were used for separation of common plant triterpenoids. The mobile phases used were ethyl acetate: acetonitrile (3.0:2.0 v/v) and acetone: acetonitrile (5.0:1.0 v/v). Both the methods separated lupeol and beta-amyrin and other triterpenoids with different functional groups. The plates were further derivatized with anisaldehyde-sulphuric acid reagent. However, in the present research work, the separation of lupeol and beta-amyrin was done on normal phase TLC Silica gel 60 F254 plates.

Beneficial effects of lupeol

Antiprotozoals are used to treat protozoal infections, which include amebiasis, giardiasis, cryptosporidiosis, microsporidiosis, Malaria, babesiosis, trypanosomiasis, chaga's disease, leishmaniasis, and toxoplasmosis (Khaw *et al*., 1995) [95]. Plumbagin as the main active constituent (IC 50 5.0µg/ml) alongside a weak action displayed by lupeol against varied strains of leishmania and *trypanosome sps*. Furthermore, the bioassay guided research of a plant used in the treatment of malaria symptom by a pygmy tribe from Cameroon led to the isolation of an alkaloid rich fraction along with lupeol and derivatives 13, 14 and 20 (Fournet *et al*., 1992) [7].

Cancer chemo preventive: The word chemo preventive 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 (Sultana *et al*., 2003) [35]. Lupeol is reported to reduce blood glucose by reducing the activity of alpha-amylase (Gallo and Sarachine, 2009) [8]. Yogeshwar Shukla (1988) [40] reported that mango derives its anti-carcinogenic property from lupeol. Arresting the growth of cancer cells, this compound was fight well against other health disorders. Specific vitamins, organic acids, carbohydrates, and polyphenols present in lupeol give it the strength to fight several health disorders. Mahammad Saleem *et al*. (2005) [30] revealed that human pancreatic adenocarcinoma cells ASpc-1 were used to investigate the effect of lupeol on cell growth and its effects on the modulation of multiple Ras-iduced signaling pathways. Lupeol is a potent multi target anticancer cells via modulation of Ras-induced protein kinase Cα (PK Cα)/ornithine decarboxylase, p13K/AKt, MAPKs and NFKB signalling pathways. Lupeol is an effective agent that tested for its invivo efficiency against pancreatic cancer. Chaturvedi *et al*. (2008) [6] reported that pentacyclic lupane type triterpenes exemplified by lupeol were principally found in common fruit plants such as olive, mango, fig etc. Lupeol exhibited an array of biological activities like anti-inflammatory, anti-arthritic, anti-mutagenic and anti-malarial activity both in in-vitro and in-vivo systems. Cancer is a disease recognized by seven hallmarks: unlimited growth of abnormal cells self-sufficiency in growth signals, insensitivity to growth inhibitors, invasion of apoptosis, sustained. Angiogenesis, inflammatory micro environment and eventually tissue invasion and metastasis (Mantorani, 2009).

Hepatoprotective: Preetha *et al*. (2006) [26] studied that lupeol and analogues also displayed hepatoprotective effects, and lupeol showed some effectiveness in lessening the action of aflotoxin B1. Zhang *et al*. (2009) [41] reported that lupeol treatment induced growth inhibition and apoptosis in hepatocellular carcinoma receptor 3 (DR3) expressions, lupeol was revealed as a promising chemopreventive agent for hepato cellular of cancer.

Anti-inflammatory activity: Lupeol decreases the IL-4 (interleukin 4) production by Th2 cells (T-helper type-2) and also lupeol is a potent anti-inflammatory activity and this activity of lupeol in an allergic airway inflammation model as evidenced by a significant reduction in eosinophills. Infiltration and in Th2-associated cytokines (IL-4, IL-5, IL-13) levels triggers the immense responses in asthma (Bani *et al*., 2006, Vasconcelos *et al*., 2008) [4, 38].

Cardio protective: Andrikopulos *et al*. (2003) reported that lupeol investigated for its cardioprotective effects and was demonstrated to provide 34.4% protection against invitro Low Density Lipo protein oxidation. In 2011, researchers from Oklahoma State University found that mango consumption helps lower insulin resistance and improve glucose tolerance. The same study also found that mangoes help normalize lipid levels throughout the blood, which in turn can help to prevent the development of cardiovascular disease.

Conclusion

As evident from these studies, horticulture crops were rich in lupeol content. Hence, consumption of fresh fruits and vegetables would serve as a good source of lupeol content thereby providing good health for human beings. As this review demonstrates, lupeol and some analogues have been shown to possess a range of folk and proven biological activities, and 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 horticulture plants.

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