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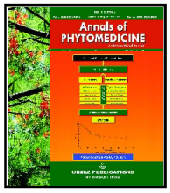

Original article

Quantification of lupeol in selected popular cultivars of mango (*Mangifera indica* L.) cultivated in Telangana state of India

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Abstract

Mango (*Mangifera indica* L.) is a fruit consumed worldwide, being one of the most commonly consumed fruits in the tropical countries, hence, called the 'king of fruits'. Lupeol (Lupa-21, 20 (29) dien 3 beta-ol) is a naturally occurring pentacyclictriterpene (also known as Fagarsterol) under the lupanetriterpene family, predominantly present in mango. High concentrations of lupeol are found in the peels of mango fruits, especially during the maturity stage (180 µg/100 g average); it is very potential triterpene which acts against many diseases, especially cancer which indicates a large scope in the pharmaceutical industries. To investigate the "quantification of lupeol in selected popular cultivars of mango (*M. indica*), cultivated in Telanagana State of India", through high performance liquid chromatography (HPLC) method, using completely randomized design with 2 factors. One of the factors is 12 cultivars and another factor is 3 storage days. This is the first report of lupeol in mango cultivars, grown in Telangana state. Significant differences were noted among the cultivars and storage days. The maximum amount of lupeol (67.22 ± 11.09 µg/100 g) was recorded in cultivar, Chinnarasam, whereas minimum lupeol was perceived in Himayath (8.3 ± 0.9 µg/100 g). Among the storage days, highest lupeol content was recorded on 8th (39.11 ± 7.63 µg/100 g) day of storage. The results consolidated that Chinnarasam is a juicy cultivar, grown in Telangana state and it had highest lupeol content in pulp rather than the rest of cultivars of mango.

Keywords: Lupeol, triterpine, pharmaceutical, mango cultivars, storage days

1. Introduction

The mango (*Mangifera indica* L.) is a juicy stone fruit (drupe) and also one of the most important climacteric tropical fruits in the world and currently ranked 5th in total world production among the major fruit crops (Ravani and Joshi, 2013). It belongs to the family *Anacardiaceae* (2n = 40) and originated in Southeast Asia. It is the national fruit of India and Philippines and the national tree of Bangladesh. Enormous genetic diversity in mango exists in India, which is the primary centre of domestication of this crop. There are 1000 mono embryonic and polyembryonic mango cultivars in India (Negi, 2000).

India, China, Indonesia and Mexico are the world leaders in mango production. The principal mango producing states are Uttar Pradesh, Karnataka, Andhra Pradesh, Telangana, Bihar, West Bengal and Gujarat. India is the largest producer and prominent exporter of mango with annual production of 19.68 million tonnes and share of more than one-third (which accounts 36%) of the world's mango production (FAOSTAT, 2017). In India, mango is cultivated in an area of 2263 thousand hectares with production of 196.87 lakh tonnes and 8.7 MT/ha productivity. Major mango growing states are Uttar Pradesh, Karnataka, Andhra Pradesh, Telangana, Bihar, West Bengal and Gujarat. Telangana state occupies 4th position in India. In Telangana area, production and productivity of mango to be 180.62 thousand ha, 1681.6 MT and 9.3 MT/ha, respectively. (NHB Database, 2017).

Mangoes can be considered as a good source of dietary antioxidants, such as ascorbic acid, carotenoids and phenolic compounds. Having been part of the indigenous medical systems for over 4000 years, mango fruits have been considered a good source of bioactive compounds that can be used towards preventing diseases and promoting overall good health in humans (Chaturvedi *et al*., 2008). Mango is naturally rich in fiber (1.6 g/100 g), antioxidant, vitamin A (54 µg/100 g) and vitamin C (36.4 mg/100 g) and vitamin B6 (0.119 mg/100 g) (Lemmens and Emmanuel, 2013; Rincon and Keer, 2010; Sogi and Ibrahim, 2012) present in mango peel and pulp, such as triterpene, lupeol which is under basic research for its potential biological effects. The fruit also contains substances called triterpene and lupeol, which inhibit skin and colon cancer.

There is a growing interest in natural triterpenoids, also known as phytosterols, due to their wide spectrum of biological activities (Ovesná *et al*., 2004). Lupeol is a pharmacologically active triterpenoid. It has several potential medicinal properties. Triterpenoids lupeol are compound with a carbon skeleton based on six isoprene units which are derived biosynthetically from the acyclic C30 hydrocarbon squalene. It is found in all vegetables, fruits and medicinal plants. Lupeol has a complex pharmacology, display antiprotozoal, antimicrobial, anti-inflammatory, antitumor and chemopreventive properties (Rahman and Saleem, 2011).

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Lupeol is a novel anti-inflammatory and anticancer dietary triterpine (Mahammod, 2009).

The objectives of present research work is to estimate/quantify the lupeol content in selected popular cultivars (12 Table and Juicy cultivars) of mango taking into consideration of 3 storage days (4th, 8th and 12th day) at ambient conditions. High performance liquid chromatography method is suitable method for estimation of lupeol content present in mango pulp, by using methanol:acetonitrile (30:70%) solvent as mobile phase and C18 column as stationary phase and DAD (diode array detector) at 210 nm, flow rate of 1 ml/1 min carried out at MFPI-Quality Control Laboratory, PJTSAU, Rajendranagar, Hyderabad, India.

2. Materials and Methods

12 (Table and Juicy) cultivars of mango (*M. indica*) fruits were collected from the Fruit Research Station, Sangareddy, India in the year of 2016. Cultivars were harvested at fully matured stage (Juicy varieties harvested at 7-9ºB and Table varieties harvested at 8-9ºB) subjected to ethylene treatment kept for storage at ambient conditions up to 12 days.

2.1 Chemicals and reagents

Pharmaceutical grade lupeol was purchased from Sigma Aldrich (Mumbai, India) and all other solvents (methanol and acetonitrile) were used for estimation of lupeol are HPLC grade and purchased from Merck Ltd., Mumbai, India). Solvents were filtered through a 0.45 µm membrane filter, C18 (250 × 4.6 mm) column was used for analysis.

2.2 Standard preparation

A stock solution of lupeol was prepared by dissolving 1 mg/ml of accurately weighed lupeol in methanol : acetonitrile (3:7 V/V), making up the volume to 10 ml. Six working solutions of respective compounds were prepared by dilution. 0.5 ppm, 1 ppm, 5 ppm, 10 ppm, 15 ppm and 20 ppm were prepared by the stock solution.

2.3 Sample preparation

Twelve cultivars of mango fruits were subjected to pulp extraction and extracted pulp was kept for drying in solar drier for 15 days at 60°C. The extraction efficiency of target compound was optimized by using solvent mixture of methanol and acetonitrile (3:7 V/V) was selected to extract the lupeol content in pulp of mango (250 mg) powder. Mango pulp powder (250 mg) was extracted through 10 ml of methanol:acetonitrile (3:7 V/V) and filtered through Watman No. 1 filter paper and vortex 5 min, after this kept for overnight at room temperature. Next day, solution was again subjected to vortex for 5 min and finally filtered through 0.45 µm membrane filter and used for further chromatographic analysis.

2.3 Chromatographic conditions

Quantification of lupeol was done by HPLC (High Performance Liquid Chromatography). Mobile phase was prepared by methanol and acetonitrile (30:70 V/V) solvents. Solvents degassed and filtered through 0.2 µm filters. C18 column (254 x 4.6 mm), column flow rate was 1 ml/min, column temperature was 25°C and wave length was 210 nm and injection volume was 20 µl (0.02 ml). DAD (diode array detector) was used for the detection of targeted compound at 210 nm. It was made with deuterium lamp. Lupeol standard showed its peak at 27.5 to 28.5 min of HPLC isocratic method.

2.4 Detection of lupeol

The identity of band of lupeol in dried mango powder samples (250 mg) was confirmed by DAD detector with standard lupeol at periodical intervals of 4th, 8th and 12th day of storage. Finally, lupeol (µg/100g) amount was calculated on the basis of following formula given by Anyakora *et al.* (2008).

$$\frac{\text{Sample peak area}}{\text{Std peak area}} \times \text{Conc. Of Std} \times \frac{\text{Vol. of dilution X 100}}{\text{Injection Volume X wt of Sample}}$$

Dil = dilution, Wt = weight, Conc = Concentration, Std = standard.

2.5 Statistical analysis

The design adopted was completely randomized design with 2 factors (storage days and varieties). Statistical analysis was performed in 2 replicates of samples and the results were presented as Mean ± standard deviation. Data were processed at the Computer Centre, Hyderabad, using (SAS version 9.1, Statistical Analysis System Institute, Inc. C).

3. Results

Lupeol is a non-polar compound, estimated by RP-HPLC (reverse phase high performance liquid chromatography) and detected by DAD (diode array detector).

On the basis of results obtained as the storage days increased, there was significant increase in lupeol content from 4th day to 8th day and later decreased on 12th day of storage. Among the 12 cultivars, Table and Juicy cultivars significantly (p<0.05), high amount of lupeol was recorded in the cultivar Chinnarasm (67.22 ± 11.09 µg/ 100 g), followed by Baneshan (50.88 ± 12.25 µg/100 g), Allampur Baneshan (49.88 ± 28.56 µg/100 g) and Suvarnarekha (47.22 ± 17.92 µg/100 g). While, lowest amount was noticed in cultivar Himayath (8.27 ± 0.94 µg/100 g) and, followed by Pandurivari Mamidi (8.40 ± 0.11 µg/100 g). Quantity of lupeol varied from 8.27 ± 0.94 µg/100 g to 67.22 ± 11.09 µg/100 g among the 12 cultivars whereas Table and Juicy cultivars showed highest lupeol content whereas recorded in Juicy variety, Chinnarasam and lowest noticed in Himayath, indicating that lupeol content was highest in almost all cultivars Table and Juicy cultivars at fully ripened stage.

Significant differences were noted in relation to storage days (4th, 8th and 12th day of storage), the highest amount of lupeol was noticed on 8th day of storage (39.11 ± 7.63 µg/100 g), followed by 12th day of storage (38.95 ± 7.22 µg/100 g) and lowest was noticed on 4th day of storage (27.11 ± 6.92 µg/100 g). It is indicating that lupeol content maximum at ripened stage rather than the fully ripened stage when stored at ambient conditions.

The interaction between 12 cultivars (Table and Juicy cultivars) and 3 storage days (4th, 8th and 12th day of storage) showed significant variation (Table I). Significantly highest amount of lupeol was recorded in Allampur Baneshan (140.19 ± 0.04 µg/100 g) on 8th day, followed by Mahamooda Vikarabad (117.34 ± 0.01 µg/100 g) on 12th day of storage and Chinnarasam (102.31 ± 0.02 µg/100 g) on 4th day of storage, while significantly lowest amount of lupeol

was recorded in Mahamooda Vikarabad (1.27 ± 0.02 µg/100 g) on 8th day of storage, followed by Suvarnarekha (1.81 ± 0.03 µg/100 g) and Manjeera (2.66 ± 0.00 µg/100g) on 4th day of storage.

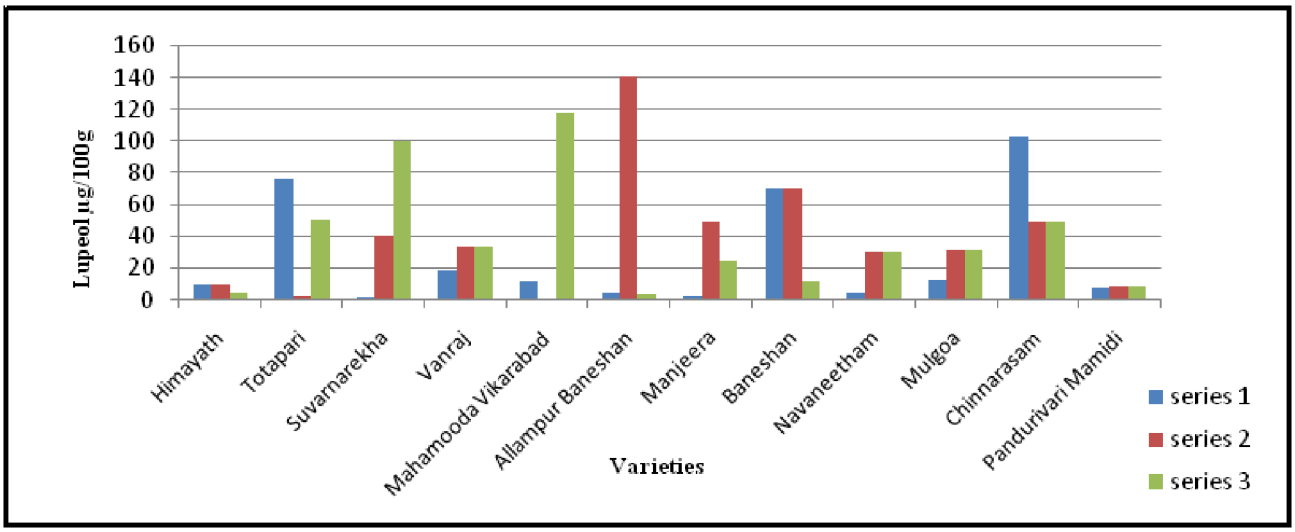
Luepol content in Table and Juicy cultivars of mango represented in Table 1 and Figure 1. Some of the chromatograms are presented in Figure 2 to Figure 5 which are related to the lupeol content in samples of 12 mango cultivars on 3 different storage days at ambient conditions.

Table 1: Lupeol content in dried mango powder (µg/100 g) as influenced by storage days (4th, 8th and 12th day) at ambient conditions in 12 mango cultivars

Varieties	Storage days			
	4th day	8th day	12th day	Mean
Baneshan	70.18 ± 0.00	70.32 ± 0.03	12.13 ± 0.01	50.88 ± 12.25^{h}
Himayath	9.85 ± 0.02	9.67 ± 0.02	5.32 ± 0.01	8.27 ± 0.94^{a}
Totapari	75.84 ± 0.03	3.31 ± 0.01	50.55 ± 0.07	43.23 ± 13.44^{e}
Suvarnarekha	1.81 ± 0.03	40.59 ± 0.01	99.27 ± 0.04	47.22 ± 17.92^{f}
MahamoodaVikarabad	11.55 ± 0.04	1.27 ± 0.02	117.34 ± 0.01	43.39 ± 23.46^{e}
Vanraj	19.07 ± 0.04	33.71 ± 0.04	33.83 ± 0.02	28.87 ± 3.10^{d}
AllampurBaneshan	5.34 ± 0.01	140.19 ± 0.04	4.13 ± 0.01	49.89 ± 28.56^{g}
Manjeera	2.66 ± 0.00	49.67 ± 0.01	24.70 ± 0.03	25.68 ± 8.59^{c}
Mulgoa	13.20 ± 0.00	31.53 ± 0.01	31.55 ± 0.01	25.43 ± 3.87^{b}
Navaneetham	5.37 ± 0.01	30.75 ± 0.00	30.47 ± 0.06	22.20 ± 5.32^{b}
Chinnarasam	102.31 ± 0.01	49.71 ± 0.01	49.65 ± 0.01	67.22 ± 11.09^{i}
PandurivariMamidi	8.20 ± 0.02	8.60 ± 0.06	8.40 ± 0.02	8.40 ± 0.11^{a}
Mean	27.11 ± 6.92^{A}	39.11 ± 7.63^{C}	38.95 ± 7.22^{B}	

Factors	SEM	CD at 5%
Varieties (A)	0.075	0.215
Storage period (B)	0.038	0.108
A x B	0.130	0.373

Note: All the values are expressed as Mean ± SD. Values with similar superscripts are statistically similar at 5% level.



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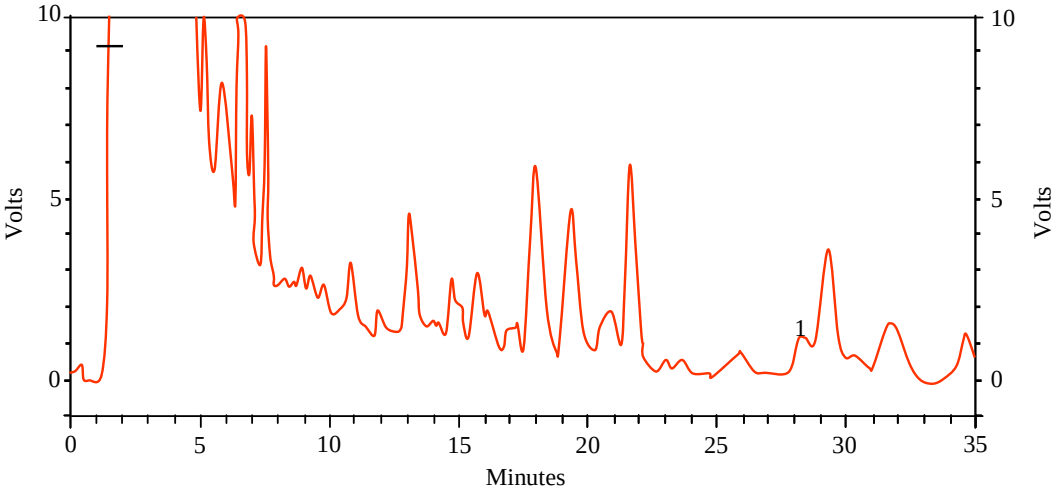

Figure 2: Chromatogram of lupeol in variety of mango Baneshan on 4th day obtained by HPLC Agilent 1260.

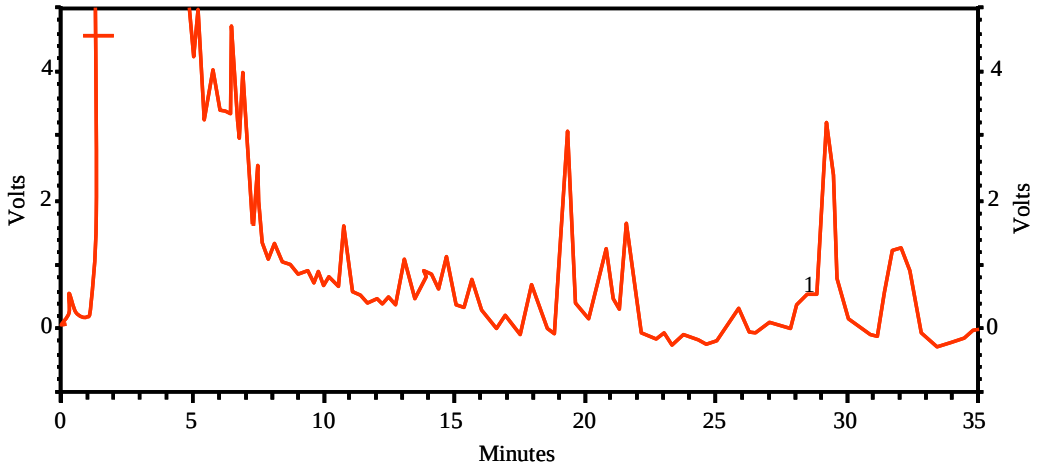

Figure 3: Chromatogram of lupeol in variety of mango Chinnarasam on 8th day obtained by HPLC Agilent 1260.

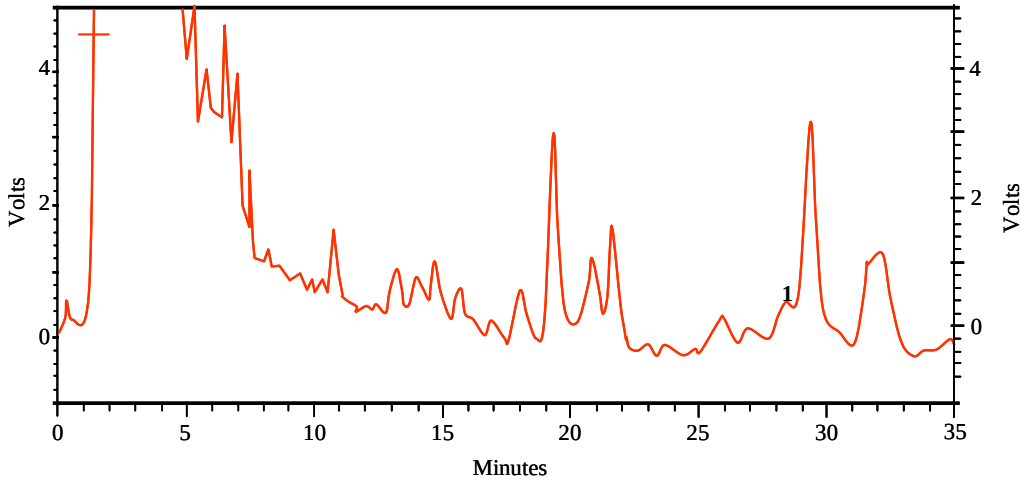

Figure 4: Chromatogram of lupeol in variety of mango Baneshan on 12th day obtained by HPLC Agilent 1260.

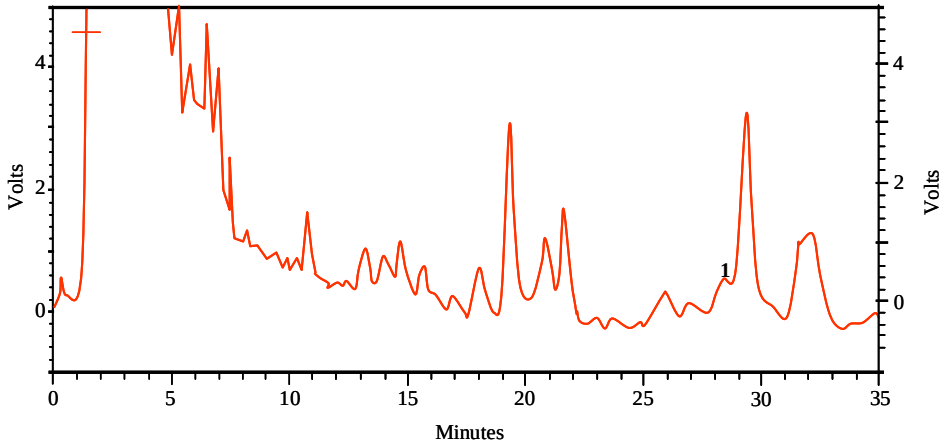

Figure 5: Chromatogram of lupeol in variety of mango Chinnarasam on 12th day obtained by HPLC Agilent 1260.

4. Discussion

Above results are also in line with Soujanya *et al.* (2017a) who have reported that significantly highest lupeol content in colored cultivar of Suvarnarekha (47.26 ± 12.09 µg/100 g) than Vanraj (28.86 ± 2.09 µg/100 g). Among the storage days, significantly highest lupeol content recorded on 12th day of storage (66.60 ± 12.33 µg/100 g) while lowest was noticed on 4th day of storage (10.44 ± 3.26 µg/100 g) and also same line of research reported in Juicy varieties of mango by Soujanya *et al.* (2017b), suggested that significantly Chinnarasam recorded highest amount of lupeol (67.24 ± 8.77 µg/100 g). While lowest amount of lupeol was recorded in Pandurivari Mamidi (8.45 ± 0.10 µg/100 g). Among the storage days, significantly highest amount of lupeol was recorded in 4th day of storage (38.63 ± 15.93 µg/100 g). While 8th and 12th day of storage were recorded similar amount of lupeol content 29.73 ± 5.93 µg/100 g and 29.53 ± 5.94 µg/100 g, respectively.

The results are in agreement with Jyotshna *et al.* (2015) who have estimated luepol and mangiferin content in 4 mango cultivars and results showed that highest amount of lupeol was noticed in Dashehari (1082 µg/100 g) as compared to Bombay green (505 µg/100 g), Langra (167 µg/100 g) and Chausa (65 µg/100 g) in pulp and peel during storage period. Similar results were reported by Saratha *et al.* (2011).

Ruiz *et al.* (2014) noticed that bioactive compounds, mangiferin and lupeol in higher concentrations at physiological maturity stage in Atulfo mango fruit peel.

As seen from above discussed results by different researchers on lupeol, there is a huge scope on lupeol quantification from available sources of plants which can be helpful for the preparation of many pharmaceuticals and fight against different diseases, especially against cancer. Present study can be useful to quantify essential triterpenes (lupeol) from all available cultivars of mango because there is a large number of mango varieties which are available in our country.

5. Conclusion

Mango varieties (12 commercial/popular cultivars) were tested for their lupeol content in pulp. Good results have been noticed regarding the lupeol content in the selected mango cultivars (Table and Juicy cultivars), however, there was a lot of variation observed among the cultivars and lupeol content ranged from 8.27 ± 0.94 µg/100 g (Himayath) to 67.22 ± 11.09 µg/100 g (Chinnarasam). Highest lupeol content was recorded in Chinnarasam (67.22 ± 11.09 µg/100 g) which is a Juicy cultivar, majorly grown in Telangana state. Among the 3 storage days, lupeol content varied from 27.11 ± 6.92 to 39.11 ± 7.63 µg/100 g (4th and 8th day of storage, respectively). Among the storage days, lupeol content was highest on 8th day of storage (39.11 ± 7.63 µg/100 g). As evident from the study promising, lupeol content (µg /100 g) was noticed among the cultivars and there is a lot of variation in the content level of lupeol among cultivars. This study can be helpful in estimation lupeol content from all available cultivars of mango in the country.

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Conflict of interest

The authors declare that no conflict of interest exists in the course of conducting this research. Both the authors had final decision regarding the manuscript and the decision to submit the findings for publication.

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