Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env.Pharmacol. Life Sci., Vol 11[1] December 2021 : 92-101

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Stability-indicating and analysis of Quercetin, Rutin and Lupeol in *Benincasa hispida* (Cucurbitaceae) fruit, leaves extracts and formulation by High Performance Thin layer Chromatographic methods.

Kakad Kalyani Shrinivas*[1], Kundan Chimanaram Choudhary[1], Mukesh Rajaram Choudhary[1]
1*Department of Pharmacognosy, Progressive Education Society's Modern College of Pharmacy,Sector - 21, Yamunanagar Nigdi, Pune-411044, Maharashtra.
*E Mail ID: kk_pharma20@rediffmail.com

ABSTRACT

In present study stability indicative and analysis of Quercetin, Rutin and Lupeol in Benincasa hispida (Cucurbitaceae) was developed and validated by using HPTLC. Analysis of samples were performed on TLC aluminium precoated plate (60_F 254) by using mobile phase toluene: ethyl acetate:formic acid: methanol: chloroform (3.5:3:1:0.5:2 v/v). The developed plate was scanned at 255nm. The developed method found to give compact spot for quercetin, rutin& lupeol at Rf 0.55±0.02, 0.6±0.005 and 0.70±0.006 accordingly. The method was validated using International Council for Harmonization (ICH) guidelines including linearity, precision, accuracy, and robustness. Quercetin, rutin and lupeol was found to be present in extract of fruit and leaves and Kushmanda Pak formulation of Benincasa hispida and in adequate amount. A good relationship was found to be (200-1200ng spot^{-1}, 200-1000ng spot^{-1} and 500-3000ng spot^{-1}) with correlation coefficient (r^2) value 0.9935, 0.991, 0.990 for quercetin, rutin and lupeol respectively.Limit of detection and limit of quantitation was found to be (10.23, 11.12, 16.31), and (41.44,33.71, 49.44) for quercetin, rutin and lupeol respectivly. The developed method was found to be accurate and precise with accurate %RSD for interday and intraday precision. Accuracy of method was performed by recovery studies at three different concentration level. The proposed method for the quantitation of quercetin, rutin and lupeol was found to be simple, specific, accurate and robust in Benincasa hispida and its polyherbal formulations.

Key Words: *Benincasa hispida, Cucurbitaceae, Quercetin, Rutin , Lupeol*

Received 11.09.2021 Revised 21.10.2021 Accepted 23.11.2021

INTRODUCTION

The Cucurbitaceae family is most wide spread family in plant kingdom. This family reported 825 species and 118 genera [1]. The members of this family are annual herbaceous climbers. Mostly all plant fruits of this family are eatable like Cucumber, Watermelon, Muskmelon, Pumpkin, Wax melon etc. *Benincasa hispida* is well known species of Cucurbitaceae family. The Plant is native to Southeast Asian countries, Java and Japan. It is commonly known as kushmanda in Ayurveda's and white melon, wax ground, ash ground and fuzzy ground according to local area etc. The plant fruit is mostly used as vegetable in India and other countries. The fruit is medicinally useful in Ayurveda to make different types of formulation like rasayanas and liquid formulations etc. The fruit of *Benincasa hispida* is highly nutritive and possesses alternative and styptic properties and popularly known as a valuable antimercurial, cooling, tonic, diuretic, antiepileptic, other nervous diseases and specifically applicable for haemoptysis, internal haemorrhages. The fresh juice of fruit possesses purgativeproperties.As per recommendations of Vrindamadhavathe fruit juice use as medicines in snake bites. It is one of the good antidote for many kind of vegetable, mercurial and alcoholic poisons.The seed has anthelmintic, vermifuge, tenia property [2-3]. *Benincasa hispida* have numerous therapeutic uses like healing of neuron defects, gastro-intestinal harms antioxidant, anti-inflammatory, pain reliever, anti-asthmatic, anti-diabetics and antimicrobial.

Benincasa hispida fruits have reported for a variety of active chemical constituent's like volatile oils, proteins, carotenes, ß-sitosterin, flavonoids, glycosides, vitamins, minerals and uronic acid [4-7].Fruit of this plant species yielded some new triterpenoids such as friedooleana-7,9(11)-diene, oleanolic acid etc together with 12 known compounds (multiflorenol, isomultiflorenyl acetate, stigmasterol, stigmasterol3-

O-β-d-glucopyranoside, α-spinasterol, α-spinasterol 3-O-β-d-glucopyranoside, β-sitosterol, daucosterol, arbutin, nicotinic acid, (+)-pinonesinol, and ethyl β-d-glucopyranoside [8].

Hardly any techniques stated the quantitation of flavonoids and triterpenoids in *Benincasa hispida* plant extract and in itsformulation by the HPTLC and HPLC, but no description was found about the simultaneous estimation and forced degradation study of quercetin, rutin and lupeol in *Benincasa hispida* containing formulations.

Quercetin(2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one) is a plant-originated aglycone form of flavonoid glycosides (Fig 1), has a dietary significance and may be valuable beside a diversity of disorders. A few of the helpful special effects consists of anticancer[9], antitumor, antihypertensive[10], gastroprotective, anti-allergy, anti-viral, anti-inflammatory[11], anti-diabetic, immunomodulatory and anti-infective.

Rutin (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside (Figure 2)is a flavonoid of the flavonol-nature that is general in the plant territory [12]. It is efficiently usefull as antidiabetic[13], anti-inflamatory [14-15], anticonvulsant [16], anticholesteremic[17] antihypertensive, antitumor [18]. Lupeol (Lup-20(29)-en-3β-ol) is a triterpenoid (Fig.3), majorly component originate in the plant, exhibits a wide variety of natural actions such as antiprotozoal [19], antimalarial, antitumor[20], anti-inflammatory [21], antimicrobial [22].

All in the course of before a few decades, therapeutic plants and their products have mostly been customized for curative reason. Recently various normal goods have been established to be a potential supply for action of a lot of disease however usually for these data medicinal flora requires procedural authentication and report towards it. The current study includes detailed phytochemical analysis of *Benincasa hispida* plant species with its parts like fruit, leaf and its formulation by HPTLC method. The marketed formulation Kushmanda Pak (Vatsalayurvedic products Pvt. Ltd.) was purchased from local market of Pune. Each 10g of Kushmanda Pakcontain *Benincasa hispida*(kushmanda) fruit juice (9ml), Cow milk (9ml), *Embilica officinale* fruit (amalaki) (0.8g) and Cane sugar stem (sharkara) (4.4g) respectively.

MATERIAL AND METHODS

Plant materials

The plant material *Benincasa hispida* (Cucurbitaceae) was collected from the western part of Pune,India and authentication of plant material was done by Botanical survey of India with reference number BSI/WRC/100-1/Tech./2017/17.

Extraction process and preparation of sample

The fruit and leaf of *Benincasa hispida* (Cucurbitaceae) were air-dried and pulverized. The fruit and leaf were individually collected and dried and treated with petroleum ether for 24 hrs for defatting. 1gm of defatted plant material was kept for 24hr in methanol for maceration. Thereafter, methanolic extracts were filtered through Whatman paper No. 42 and use for analysis.

Chemicals and reference standard

Standards of Quercetin, Rutin and Lupeol were obtained from Yucca enterprises Pvt. Ltd Mumbai. The analytical grade solvents and reagents (toluene, ethyl acetate, formic acid, methanol, and chloroform) were purchased from Merck (Germany). Alluminium precoated silica gel $60F_{254}$HPTLC plates (20 cm×20 cm) were purchased from Merck (Germany).

HPTLC instrumentation and Chromatography conditions

Analysis was carried out on (10 cm × 20 cm)$60F_{254}$HPTLC plates. Extract and standard samples were applied as bands, (6mm) wide and (8-mm) apart, by Linomat-IV sample applicator. Themicrosyringe (Linomat, Hamilton-Bonaduzschweiz, Camag, Switzerland) is used to apply the sample as well as standard. The sample applied on plate at rate of 150 nl/s. The plate was developed in pre-saturated (saturation time 15 min at 25ºC with mobile phase vapour)Twin Trough Chamber in linear ascending modewith toluene: ethyl acetate: formic acid: methanol: chloroform (3.5:3:1:0.5:2 v/v) as a mobile phase. The plate was dried after development, at room temperature. The plate was activated at 100ºC for 10 minutes in hot air oven. The qualitative analysis was performed at wavelength 254 nm by CAMAG TLC Scanner 3 operated by WINCATS software.

HPTLC study of *Benincasa hispida*

The optimized method was applied for identification of rutin, quercetin and lupeol in *Benincasa hispida*species and in herbal formulation. In present method volume 15µl of *Benincasa hispida*fruit and leaf extract were use for analysis. Also three volumes 2, 4 and 6 µl of extracted fraction of formulation (Kusmandapak) were applied on HPTLC plate for quantitative analysis. The presence of rutin, quercetin and lupeol in specimen extract and formulation were confirmed by the 3D display of all tracks and overlay spectra of standards as well as samples.

Method validation

Method Validation was performed out as per the, the International Council for Harmonization (ICH, 2005) guidelines for precision, linearity, accuracy, robustness, recovery, limit of detection (LOD), and limit of quantification (LOQ).

Forced degradation study

In acid and base degradation study, stock solution was prepared by dissolving 10 mg of quercetin, rutin and lupeol separately in 100ml of ethanol, methanol and chloroform respectively. 10 ml from individual stock solution was withdraw and mixed with 10ml of methanolic solution of 1M HCL and 0.2M NaOH. These solutions were refluxed for 30 min at 70^0C in the dark to exclude the possible light degradation. The resultant solution was applied on HPTLC plates.

In oxidative degradation study,10 ml from individual stock solution was mixed with 10ml of methanolic solution of hydrogen peroxide (6% w/v). These solutions were refluxed for 30 min at 70^0C in the dark to exclude the possible light degradation. The resultant solution was applied on TLC plates.

In Photo stability study, quercetin, rutin and lupeol solution (100µl/ml) was directly exposed to sunlight of 24hr and the resultant solution was applied on TLC plates.All the TLC plates was developed and scanned in TLC scanner 3.

RESULT AND DISCUSSION

Solvent system optimization

After trying various solvent systems, the selected solvent system toluene: ethyl acetate: formic acid: methanol: chloroform (3.5:3:1:0.5:2 v/v) bring the excellent resolution and gives compact peak of standards in samples and formulations. Observation shows the same Rf value (Fig. 7) for Quercetin, Rutin & Lupeol in samples as compare with stadard. The similar solvent system has been in worked for the separation of methanol fraction of the formulation applied in the three volumes 2, 4 and 6 µl followed by 15 minutes saturation time. The analysis was done at wavelength 255nm in absorbance mode (Fig 7)

Method validation

Linearity:

For determining the linearity range of standard Quercetin, Rutin & Lupeol, a series of spots of different volumes were applied so as to get specific quantity of standard per band, respectively (Table 1). Linearity was evaluated in triplicate. The plate was scanned at 255nm and curve was prepared with respect to area *vs.* amount per spot (Fig. 4,5 & 6). A good linearity relationship was found to be with correlation coefficient (r^2) value of 0.9935, 0.991 & 0.990 for Quercetin, Rutin and Lupeol respectively (Table 1 and Fig. 4,5 & 6)

Quantitation of quercetin, rutin and lupeol in the plant extract and Kushmanda Pak formulation of *Benincasa hispida:*

The optimized method was applied for identification of rutin, quercetin and lupeol in *Benincasa hispida*species and in herbal formulation (Fig.7). In present method volume 15µl of *Benincasa hispida*fruit and leaf extract were use for analysis. Also three volumes 2, 4 and 6 µl of extracted fraction of formulation (Kusmandapak) were applied on HPTLC plate for quantitative analysis. The presence of rutin, quercetin and lupeol in specimen extract and formulation were confirmed by the 3D display of all tracks and overlay spectra of standards as well as samples (Fig.7 & 8). Quercetin, rutin and lupeol was found to be present in fruit and leaves of *Benincasa hispida*(0.29%, 0.4%, 0.7%) in fruit, (0.23%, 0.2%, 0.06%) in leaves, (0.13%,0.60% & 1%) in formulations respectively. The proposed method for the quantitation of quercetin, rutin and lupeol was found to be simple, specific, accurate and robust in *Benincasa hispida* and its polyherbal formulations.

Limit of detection and quantitation:

In order to determine limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to formula {LOD= 3.3(SD/S) and LOQ= 10 (SD/S)} and noted in (Table 1).

Precision:

Precision studies were carried out to show the reproducibility of the proposed developed method. Intraday precision study was carried out by applying six times 1000ng per band of same concentration. It can be analyzing at three different times in a day for intraday precision and the same procedure was followed for three different days to determine interday precision. The results were reported as SD (%RSD) (Table 2). The low %RSD indicated the method is precise for the analysis (Table 2).

Specificity:

The specificity of the method was determined by analysing standard drug and sample. The presence of Quercetin, Rutin and Lupeol in plant species and formulations were confirmed by comparing R_f and ultraviolet–visible spectra of sample with standard. Purity of sample spot corresponding to standard in

sample and both formulations were analysed by superimposing the spectrum of standard and sample peaks (Fig.4)

Recovery studies (Accuracy):

Accuracy of method was studied for fruit extract of *Benincasa hispida* by performing recovery studies at 3 levels for all standards. The pre-analyzed samples were spiked with 80% of the standard Quercetin, Rutin & Lupeol and analyzed by the proposed HPTLC method. The experiment was conducted six times the percentage recovery at three different levels of standards was found to be 98.59, 98.24, 98.82% respectively (Table 3).

Robustness:

Robustness was studied by making small change in composition of mobile phase and change in duration of saturation time. Mobile phase prepared from toluene: ethyl acetate: formic acid: methanol: chloroform (3.5:3:1:0.5:2 v/v) in different proportion. The mobile phase variation was consider as ±20% for study (3.3:3:1:0.5:2 v/v, 3.7:3:1:0.5:1.8 v/v) respectively. The duration of saturation time was applying as 15± 5 min (10, 15 and 20 min). The results were evaluated in terms of relative standard deviation (RSD %) and standard error of peak areas (Table 4)

Forced degradation study

The result from the stress testing indicated the method was highly specific for Quercetin, Rutin and Lupeol. The degradation products were completely distinguishable from the parent compound.(Fig. 9, 10 & 11)

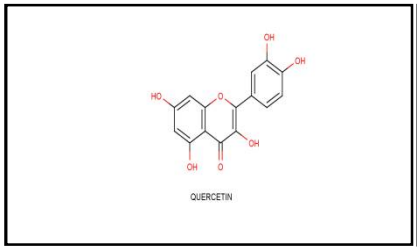


Fig. 1. Structure of Quercetin

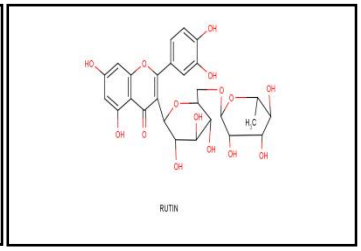


Fig. 2. Structure of Rutin

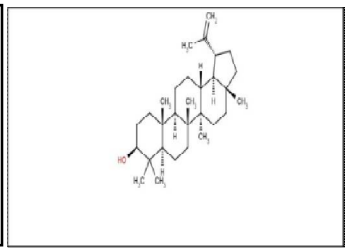


Fig. 3. Structure of Lupeol

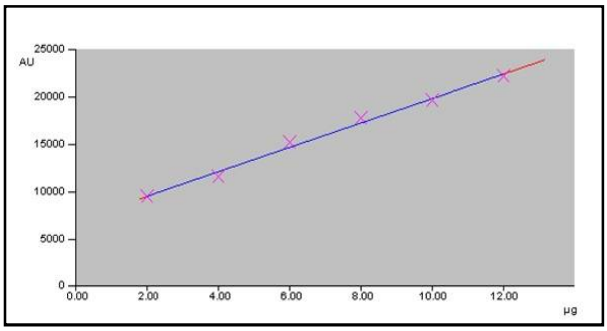


Fig. 4. Calibration curve of Quercetin

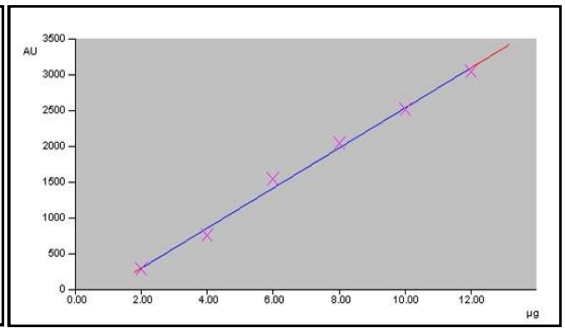


Fig. 5. Calibration curve of Rutin

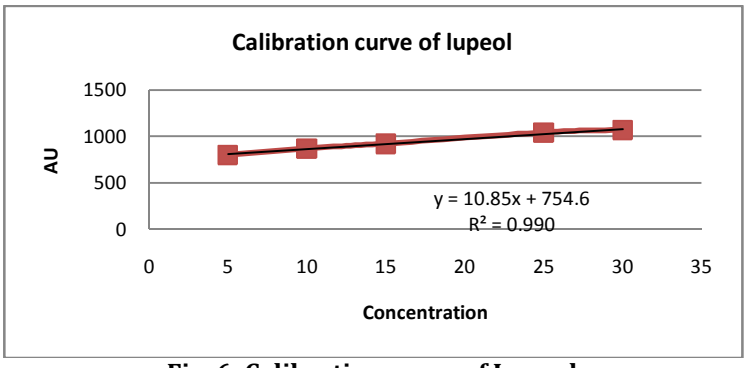


Fig. 6. Calibration curve of Lupeol

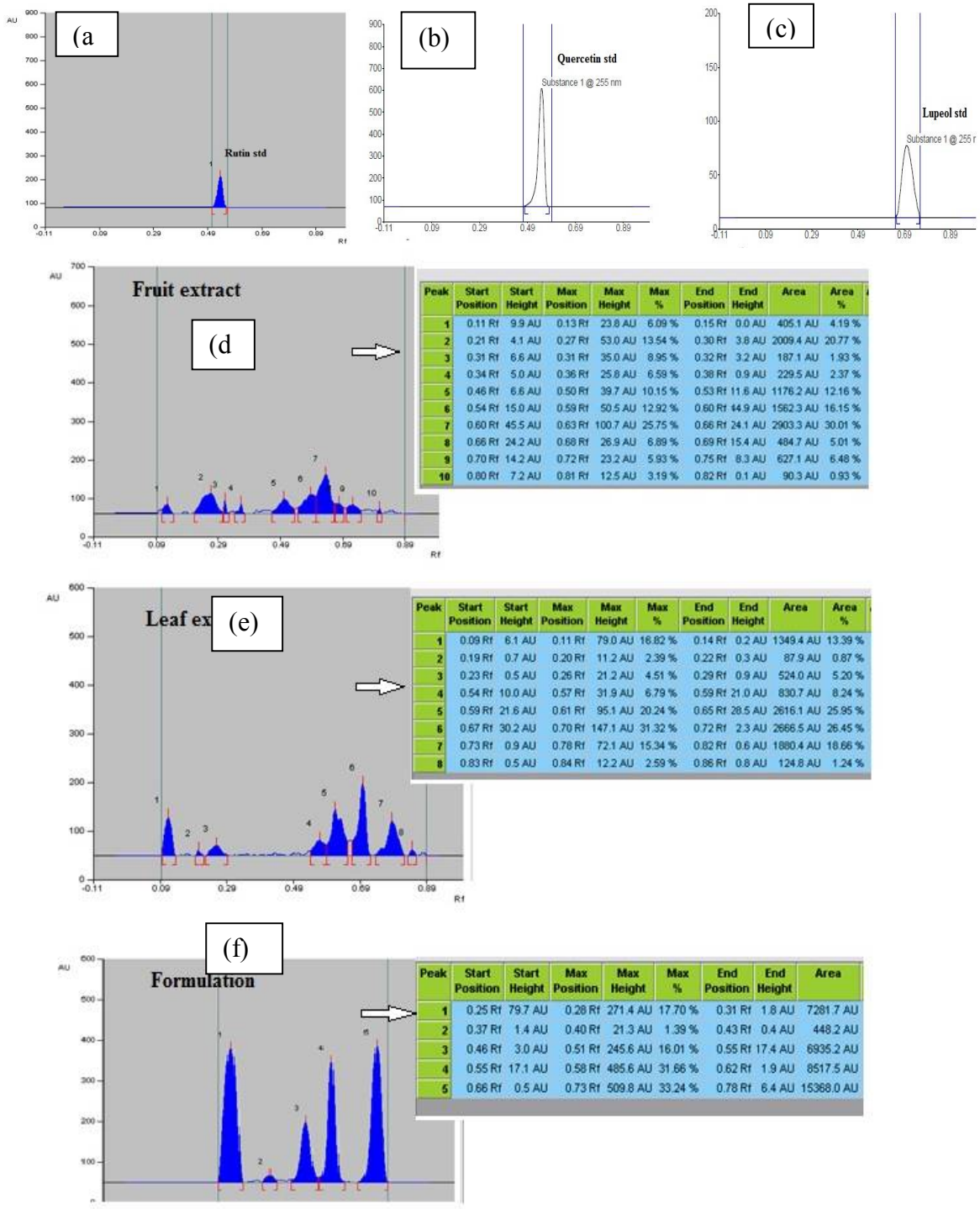


Figure. 7
(a) Standard Rutin (b) standard Quercetin (c) standard Lupeol
(d) Chromatogram of fruit extract of *Benincasa hispida*(e) Chromatogram of leaf extract of *Benincasa hispida*(f) Chromatogram of fruit extract of *Benincasa hispida*

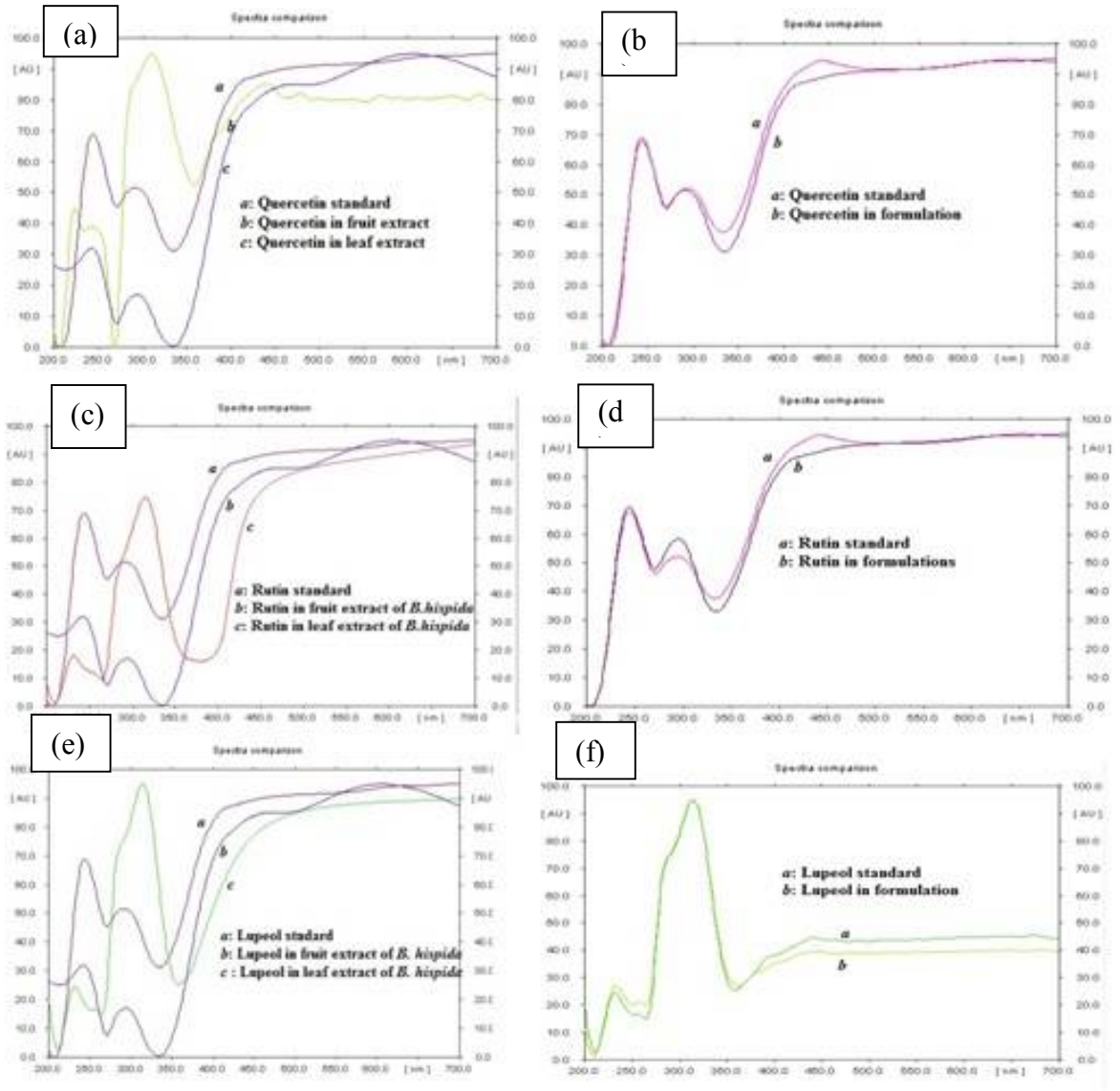


Figure 8
(a) Overlay UV absorption spectra of Quercetin in peaks of standard and extracts,
(b) Quercetin in peaks of standard and formulations (c) overlay UV absorption spectra of Rutin in peaks of standard and extracts (d) overlay UV absorption spectra of Rutin in peaks of standard and formulation (e) overlay UV absorption spectra of Lupeol in peaks of standard and extracts (f) overlay UV absorption spectra of Lupeol in peaks of standard and formulation at 255nm.

Table 1. Method validation parameters for the quantitation of lupeol, rutin and quercetin by HPTLC.

Parameters	Results		
	Quercetin	Rutin	Lupeol
Range of linearity (ng/band)	200-1200	200-1000	500-3000
Regression of equation	y=6994.80+1243.21x	y=261.63x+148.13	y=10.85x+754.6
Slope	1243.21	261.63	10.85
Correlation coefficient (r^2)	0.9935	0.991	0.990
LOD (ng/band)	10.23	11.12	16.31
LOQ (ng/band)	41.44	33.71	49.44

Table 2. Interday and intraday precision of HPTLC (n=6)

Amount (ng/band)	Interday precision			Intraday precision		
	Mean[a] area	SD	%RSD	Mean[a] area	SD	%RSD
Quercetin 1000	18151.5	329.89	1.81	19280.4	322.46	1.67
Rutin 1000	2560.3	32.65	1.2	2491.16	25.05	1
Lupeol 1000	643.43	7.35	1.14	758.99	7.34	0.96

[a] Mean of six determinations.

Table 3. Results of accuracy study (Fruit)

Level of recovery (80 %)	Theoretical content (µg/band)	Experimental content (µg/band)	% RSD	% mean[a] recovery
Quercetin	0.71	0.70±0.005	0.7	98.59
Rutin	0.57	0.56±0.005	0.89	98.24
Lupeol	0.85	0.84±0.005	0.58	98.82

[a] Mean of three determinations.

Table 4. Robustness study for the HPTLC method

	Quercetin			Rutin			Lupeol		
	Peak area	SD	%RSD	Peak area	SD	%RSD	Peak area	SD	%RSD
Mobile phase composition	15409.25	1930.18	12.52	3080.0	49.07	1.59	511.1	87.25	17.07
3.5:3:1:0.5:2	15411.75	1362.35	8.83	3080.0	49.07	1.59	510.6	87.96	17.22
3.3:3:1:0.5:2 3.7:3:1:0.5:1.8	17553.55	1569.89	8.9	3381.15	50.27	1.48	496.3	67.74	13.64
Saturation time									
10	17743.85	600.82	3.3	2292.9	294.29	12.83	432.45	42.21	9.76
15	20611.1	2236.01	10.84	2492.29	41.29	1.6	396	18.95	4.7
20	21738.7	64.91	0.2	2781.05	454.03	16.32	516.4	45.82	8.8

[a] Mean of three determinations

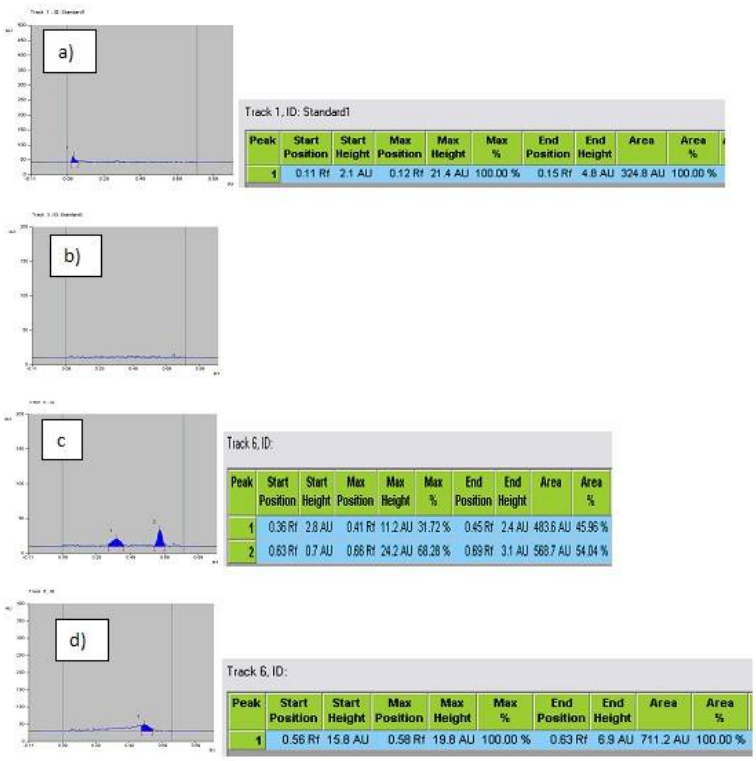
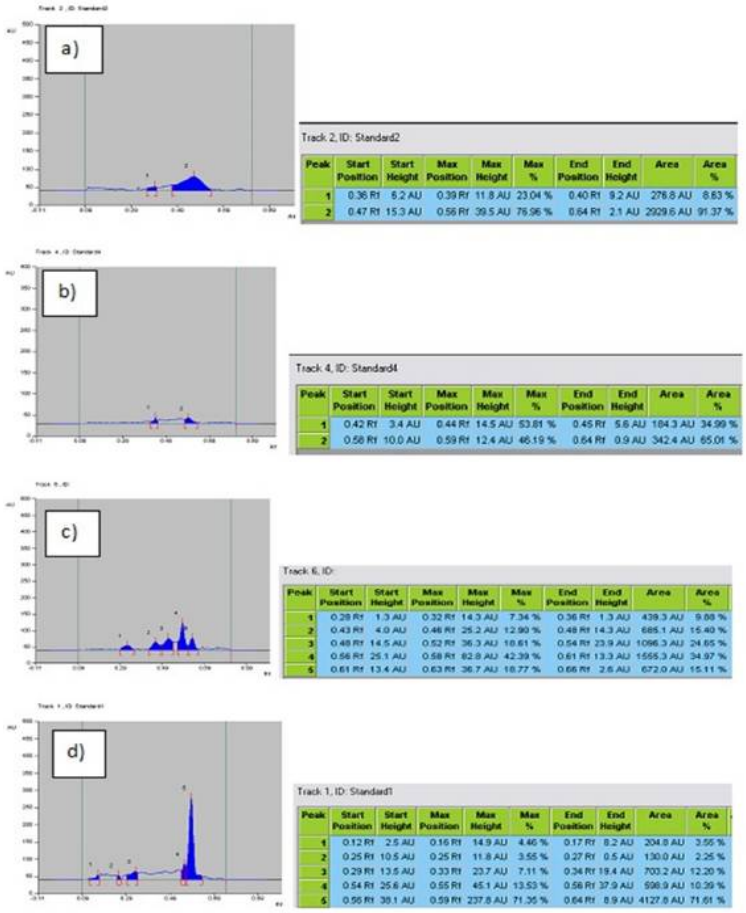


Fig. 9. Forced degradation study of Lupeol a) With HCL b) NAOH c) H_2O_2 d) Photostability



Track 2, ID: Standard2

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.36 Rf	6.2 AU	0.39 Rf	11.8 AU	23.04 %	0.40 Rf	9.2 AU	276.8 AU	8.63 %
2	0.47 Rf	15.3 AU	0.56 Rf	39.5 AU	76.96 %	0.64 Rf	2.1 AU	2929.6 AU	91.37 %

Track 4, ID: Standard4

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.42 Rf	3.4 AU	0.44 Rf	14.5 AU	53.81 %	0.45 Rf	5.6 AU	184.3 AU	34.99 %
2	0.58 Rf	10.0 AU	0.59 Rf	12.4 AU	46.19 %	0.64 Rf	0.9 AU	342.4 AU	65.01 %

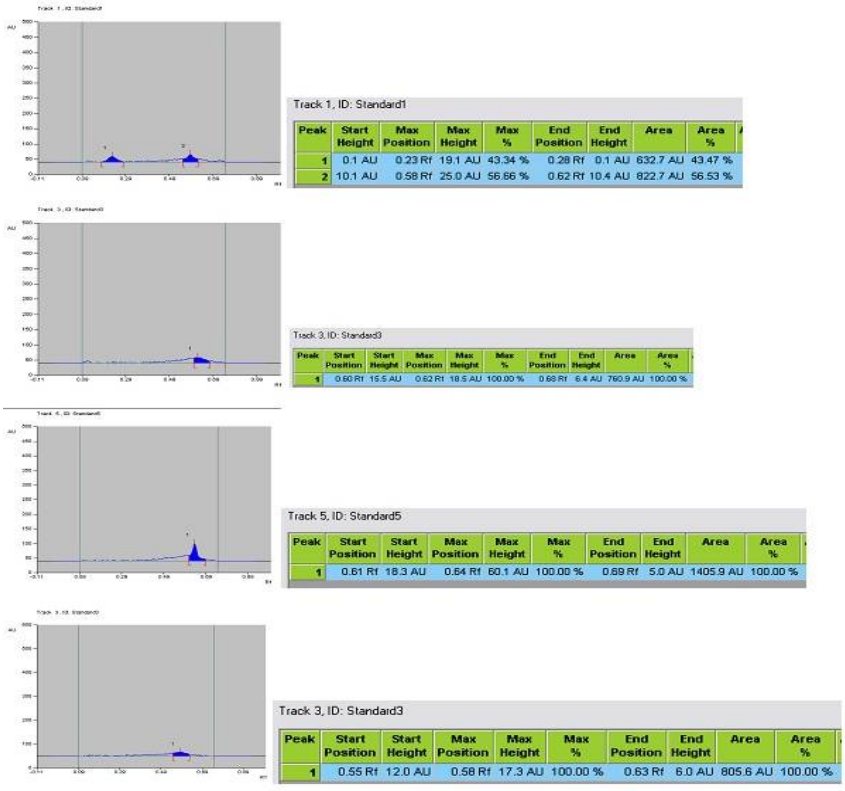
Track 6, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.28 Rf	1.3 AU	0.32 Rf	14.3 AU	7.34 %	0.36 Rf	1.3 AU	439.3 AU	9.68 %
2	0.43 Rf	4.0 AU	0.46 Rf	25.2 AU	12.90 %	0.48 Rf	14.3 AU	685.1 AU	15.40 %
3	0.48 Rf	14.5 AU	0.52 Rf	36.3 AU	18.61 %	0.54 Rf	23.9 AU	1096.3 AU	24.65 %
4	0.56 Rf	25.1 AU	0.58 Rf	82.8 AU	42.39 %	0.61 Rf	13.3 AU	1555.3 AU	34.97 %
5	0.61 Rf	13.4 AU	0.63 Rf	36.7 AU	18.77 %	0.66 Rf	2.6 AU	672.0 AU	15.11 %

Track 1, ID: Standard1

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.12 Rf	2.5 AU	0.16 Rf	14.9 AU	4.46 %	0.17 Rf	8.2 AU	204.8 AU	3.55 %
2	0.25 Rf	10.5 AU	0.25 Rf	11.8 AU	3.55 %	0.27 Rf	0.5 AU	130.0 AU	2.25 %
3	0.29 Rf	13.5 AU	0.33 Rf	23.7 AU	7.11 %	0.34 Rf	19.4 AU	703.2 AU	12.20 %
4	0.54 Rf	25.6 AU	0.55 Rf	45.1 AU	13.53 %	0.58 Rf	37.9 AU	596.9 AU	10.39 %
5	0.56 Rf	39.1 AU	0.59 Rf	237.8 AU	71.36 %	0.64 Rf	8.9 AU	4127.8 AU	71.61 %

Fig. 10. Forced degradation study of Quercetin a) With HCL b) NAOH c) H_20_2 d) Photostability



Track 1, ID: Standard1

Peak	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.1 AU	0.23 Rf	19.1 AU	43.34 %	0.28 Rf	0.1 AU	632.7 AU	43.47 %
2	10.1 AU	0.58 Rf	25.0 AU	56.66 %	0.62 Rf	10.4 AU	822.7 AU	56.53 %

Track 3, ID: Standard3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.60 Rf	15.5 AU	0.62 Rf	18.5 AU	100.00 %	0.68 Rf	6.4 AU	760.9 AU	100.00 %

Track 5, ID: Standard5

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.61 Rf	18.3 AU	0.64 Rf	60.1 AU	100.00 %	0.69 Rf	5.0 AU	1405.9 AU	100.00 %

Track 3, ID: Standard3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.55 Rf	12.0 AU	0.58 Rf	17.3 AU	100.00 %	0.63 Rf	6.0 AU	805.6 AU	100.00 %

Fig. 11. Forced degradation study of Rutin a) With HCL b) NaOH c) H_20_2 d) Photostability

CONCLUSION

In present study HPTLC method was developed and validated for the determination of Quercetin, rutin and lupeol in *Benincasa hispida* species and in its formulation. Quercetin, rutin and lupeol was found to be present in *Benincasa hispida* (0.29%, 0.4%, 0.7%) in fruit, (0.23%, 0.2%, 0.06%) in leaves, (0.13%,0.60% & 1%) in formulations respectively. The proposed method for the quantitation of quercetin, rutin and lupeol was found to be simple, specific, accurate and robust in *Benincasa hispida* and its polyherbal formulations. Based on these results fruit of this plant contain higher Quercetin, rutin and lupeol, so concentrated fractions or extract of fruit of *Benincasa hispida*is a rich source of quercetin, rutin and lupeol and may be more useful for formulations development.

ACKNOWLEDGMENTS

Authors are thankful to Botanical Survey of India (BSI), Pune, PES Modern college of Pharmacy Nigdi Pune and Dr. D. Y. Patil Institute of Pharmaceutical Sciences & Research, Pimpri, Pune for providing instrumental facility.

DECLARATION OF INTERESTS

There are no known competing financial interests

REFERENCES

1. Gill, N.S., Bali, M.(2011). Isolation of Anti UlcerCucurbitane Type Triterpenoid from the Seeds of *Cucurbita pepo. Research J. of Phytochemistry*., 2011:70-79.
2. Kirtikar, K.R.,Basu, B.D.(1999).Indian Medicinal plants, Second edition,Vol-III International Book distributors Dehradun, p.1838-1839.
3. Nadkarni, K.M.(2002). Indian Materia Medica. Volume II, Bombay popular prakashan,p. 274-313.
4. Nimbal, S.K.,Venkatrao, N.,Ladde, S.,Pujar, B. (2011).Anxiolytic evaluation of Benincasahispida (Thunb) Cogn. fruit extracts. *International J. of Pharmacy and Pharmaceutical Science Research*., 93-97.
5. Wu, C.M.,Liou, S.E.,Chang,Y.H.,Chiang W. (1987).Volatile compounds of the wax gourd (*Benincasa hispida*, Cogn) and a wax guard beverage. *J. Food Sci*.,132-134.
6. Yoshikawa, M.,(1998). Medicinal foodstuffs, XI, Histamine release inhibitors from wax gourd, the fruits of *Benincasa hispida*Cogn. YakugakuZasshi.,188–92.
7. Siddhant, R.,Ashish, S. (2012). Phytochemical Investigation and Evaluation of Free Radical Scavenging Potential of *Benincasa hispida*Peel Extracts, *International J. of Current Pharmaceutical Review and Research.*, 43-46.
8. Xiao-na,H.,Chun-yu, L., Yan-li, L.,Qiong-ming, X., Xiao-ran, L., Shi-lin, Y. (2013). New Triterpenoids and Other Constituents from the Fruits of *Benincasa hispida* (Thunb.) Cogn., *J. Agric. Food Chem.*, 12692–12699.
9. Lamson,D.W., Brignall,M.S. (2000). Antioxidants and cancer, part 3: quercetin. *Altern Med Rev.*,196-208.
10. Choi, G.N., Kim, J.H., Kwak, J.H.,Jeong, C.H.,Jeong, H.R., Lee, U. (2012). Effect of quercetin on learning and memory performance in ICR mice under neurotoxic trimethyltin exposure. *Food Chem*.,1019–1024.
11. Xiao, X.,Shi, D.,Liu, L.,Wang, J.,Xie, X.,Kang, T. (2011). Quercetin suppresses cyclooxygenase-2 expression and angiogenesis through inactivation of P300 signaling. *PLoS One*.,6(8):e22934. doi: 10.1371/journal.pone.0022934.
12. Vahideh, S. M., Sara, A.,Nassiri, A.M.,Hosseinzadeh, H. (2014). Effects of rutin on acrylamide-induced neurotoxicity, *Daru-Journal of faculty of pharmacy*., 22(1): 27. doi: 10.1186/2008-2231-22-27.
13. Niture, N.T., Ansar, A.A., R.N.R. Suresh, N. (2014).Anti-hyperglycemic activity of Rutin in streptozotocin-induced diabetic rats: An effect mediated through cytokines, antioxidants and lipid biomarkers, *Indian Journal of Experimental Biology*.,720-7.
14. Choi, K.S., Kundu, J.K.,Chun, K.S., HK, N.A.,Surh, Y.J. (2014). Rutin inhibits UVB radiation-induced expression of COX-2 and iNOS in hairless mouse skin: p38 MAP kinase and JNK as potential targets. *Arch BiochemBiophys*., 38–45.
15. Aruna, R., Geetha, A.,Suguna, P. (2013). Expression of caspase activation recruitment and pyrin domain levels of apoptosis-associated speck-like protein complex in the pancreas of rats subjected to experimental pancreatitis: Influence of rutin administration. *Hum Exp Toxicol*., 940–948.
16. Nieoczym, D.,Socała, K.,Raszewski, G.,Wlaź, P. (2014). Effect of quercetin and rutin in some acute seizure models in mice; *Prog Neuropsychopharmacol Biol Psychiatry*., 50-58. doi: 10.1016/j.pnpbp.2014.05.007
17. Sikder,K.,Kesh, S.B., Das, N.,Manna, K., Dey, S. (2014). The high antioxidative power of quercetin (aglycone flavonoid) and its glycone (rutin) avert high cholesterol diet induced hepatotoxicity and inflammation in Swiss albino mice, *Food Funct.,*1294-303. doi: 10.1039/c3fo60526d.
18. Ganga, R.M., Goud, P.P.,Nvl, S.R. (2019). Antihypertensive effect of rutin: pharmacological and computational approach.,*Asian Journal of Pharmaceutical and clinical research.,* 12(8): 87-92.
19. Fournet, A.,Angelo, A., Munoz, V.,Roblot, F. Hocquemiller, R.,Cave, A. (1992)*J.Ethnopharmacol*., 159–164.
20. Sheth, U.K.,Dadkar, N.K., Kamat, U.G. (1972). Selected Topics in Experimental Pharmacology, Kothari Book Depot, Bombay., 164–166.

21. Steven,J.B.et al. (2008). LXR signaling couples sterol metabolism to proliferation in the acquired immune response, Cell., 97-111. doi: 10.1016/j.cell.2008.04.052.
22. Shai, L.J.,McGaw, L.J.,Aderogba, M.A.,Mdee, L.K.,Eloff, J.N. (2008). Four pentacyclic triterpenoids with antifungal and antibacterial activity from Curtisia dentate (Burm.f) C.A. Sm. leaves.*J. Ethnopharmacol*.,238–44.

CITATION OF THIS ARTICLE

K K Shrinivas, K C Choudhary, M R Choudhary. Stability-indicating and analysis of Quercetin, Rutin and Lupeol in *Benincasa hispida* (Cucurbitaceae) fruit, leaves extracts and formulation by High Performance Thin layer Chromatographic methods.. Bull. Env. Pharmacol. Life Sci., Vol 11[1] December 2021 : 92-101.