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Original Research Paper

A Novel HPTLC Method Development For Simultaneous Estimation of Phytoconstituents in *Ficus Benghalensisi*Including QSAR Approach

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ABSTRACT

Background: *Ficus benghalensis* Linn., a member of the Moraceae family, is native to Bangladesh, India, and Sri Lanka. This study focused on developing a high-performance thin-layer chromatography (HPTLC) method to simultaneously estimate lupeol and gallic acid in *Ficus benghalensis*. **Methods**: A methanolic extract from the leaves of *Ficus benghalensis* was prepared through maceration. HPTLC analysis for gallic acid and lupeol was conducted at 276 nm. Method validation was performed according to ICH guidelines for accuracy, precision, and repeatability. **Results**: The linearity range for gallic acid and lupeol was determined to be 1000–6000 ng/spot. In the methanolic extract, gallic acid and lupeol content were found to be 0.164 ng and 1.753 ng, respectively. The limits of detection (LOD) were 4919.495 ng for gallic acid and 3315.416 ng for lupeol, with limits of quantification (LOQ) at 14907.791 ng and 1004.671 ng, respectively. The developed method was observed to be accurate, precise, and simple, offering enhanced resolution from other phytoconstituents in the extract. This method can be effectively applied to assess the quality of herbal materials and formulations containing *Ficus benghalensis*. **Conclusion**: According to ICH guidelines, the HPTLC method developed for simultaneous estimation of lupeol and gallic acid proved to be accurate, precise, and specific. This method is valuable for the phytochemical standardization of methanolic leaf extracts of *Ficus benghalensis*, linking it with potential anti-stress and anti-depression effects.

Keywords: *Ficus benghalensis,* HPTLC, Lupeol, Gallic acid, anti-stress and anti-depression activity, QSAR etc.

1. INTRODUCTION

The central nervous system (CNS) is made up of the brain and spinal cord, and abnormalities of this system have an impact on the CNS's structure or functionality. All of these ailments are referred to be diseases or disorders of the central nervous system[1]. These problems can be caused by a variety of reasons, including autoimmune illnesses, congenital defects, age-related degeneration, infection, trauma, blood clots, and malignancy. Treatments and symptoms are quite diverse. Disorders of the central nervous system (CNS) are among the most prevalent, incapacitating, and undertreated illnesses[2].However, there is a clear need for strategies to boost the productivity of this field's research and development, as evidenced by the dearth of truly new CNS medications that have been licensed recently[3,4].

Traditional medicine uses the plant to treat a number of diseases and health conditions[5]. According to Ayurveda, *Ficus benghalensis* is an astringent to the intestines and can help treat a variety of inflammations, leprosy, ulcers, vomiting, vaginal complaints, and fever. According to Unani medicine, this plant's latex has vulnerary, maturant, tonic, and aphrodisiac properties. Furthermore, the latex lowers inflammation, which helps with nose diseases, piles, and gonorrhea.[6]. Two drops of fresh latex in a lump of sugar are given once a day, early in the morning, on an empty stomach. The milky liquid is applied topically to rheumatism, pain, and bruises. Seeds can be used to treat spermatorrhoea as a cooling and

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tonic. The leaf buds of *Ficus benghalensis* has an astringent taste. Infusions of leaves are used to treat diarrhoea and dysentery, and a poultice of heated leaves is applied to abscesses. The bark is used to treat leucorrhoea, diabetes, ulcers, sores, and bruises. It is also tonic and astringent.[7, 8, 9]

Additionally, drugs with anti-asthmatic, anti-inflammatory, antidiarrheal, cholesterol-lowering, and anthelmintic qualities are made from the root and bark of *Ficus benghalensis* (*F.benghalensis*). Numerous therapeutic effects, such as antidiabetic, anti-tumour, analgesic, anti-pyretic, anti-helminthic, anti-microbial, hepatoprotective, immunomodulatory, hypoglycaemic, antioxidant, anti-stress and anti-allergic, wound healing, growth-promoting, and anti-cancer properties, have been demonstrated for *Ficus benghalensis* aerial root.[10,11]It has been discovered that the aerial root helps treat syphilis, dysentery, and spermatorrhea. The bark is used to treat blisters, ulcers, bruises, and diabetes because it has an astringent effect[12].

Ficus benghalensis has been found to contain a number of bioactive substances, such as flavonoids, leucocyanidin, benghalenosides, and heartwood's quercetin-3-galactoside. A review of the literature reveals a large number of compounds that have been isolated from the plant *Ficus benghalensis* Linn. Examples include leucocyanidin's 3,5,7-trimethyl ether, beta-sitosterol, leucocyanaidin-3-O-beta-D-galactosylcellobioside, and quarcetin-3-galactoside. 20-tetraiacontene-2-one[13], pentatriacontane-5-one, 6-heptatriacontene-10-one, and meso-inositol. Significant amounts of phytocomponents, such as rutin, β-amyrin, leuco-pelargonin, Benghalenosides, psoralen, β-sitosterol, and bergapten[14], are found in the leaves of *F.benghalensis*. The leaves seem to boost the immune system and are used to treat leucorrhea.
Numerous components that are responsible for the plant's wide range of pharmacological effects were identified through phytochemistry research on *F. benghalensis.*

Major components investigated are tiglic acid ester and different esters, ketones, flavonoids, flavanols, sterols, and triterpenoids[15] among those flavanols are rutin and quercetin-3-galactoside. *F. benghalensis* stem bark additionally includes benghalenosides, which are flavonoids or glycosides. The leaves of *F. benghalensis* incorporate pentacyclic triterpenes and triterpenoids, such as friedelin, beta sitosterol, and lupeol. some other psoralen from *F.benghalensis* that become investigated is bergapten (five-methoxy psoralen). This plant's various phytochemical ingredients, which consist of terpenoids, coumarins, ketones, flavonols, flavonoids, esters, and carbohydrates, have been connected to its wonderful medicinal value. Anion exchange chromatography turned into utilized in a unmarried step to purify a serine protease from the latex of the medicinal plant to homogeneity[16.]

Because of its many advantages, including dependability, high performance thin layer chromatography (HPTLC) is regarded because the most applicable and famous analytical method. This method is concept to be least expensive and is based totally on the quantitative estimation of different phytoconstituents.
Ficus benghalensis was investigated for isolation of bergapten (five-methoxy psoralen). This plant's various phytochemical parts, which consist of terpenoids, coumarins, ketones, flavanols, flavonoids, esters, and carbohydrates, had been linked to its brilliant medicinal cost. Anion trade chromatography was utilized in a single step to purify a serine protease from the latex of the medicinal plant *F. benghalensis* to homogeneity[16].

The past few years have seen a substantial boom in interest in the observe of plant-primarily based medicinal drugs from the conventional clinical machine which might be more secure and extra environmentally friendly. greater than 21,000 flora with big medicinal features have been indexed by using the WHO. India is regarded as a mine wealthy in medicinal vegetation and is called the world's botanical lawn. In India, there are approximately 2500 medicinal species with a number of pharmacological moves. of these, over one hundred fifty species are used commercially inside the advent of novel drugs[17].

Due to its many advantages, including dependability, high performance thin layer chromatography (HPTLC) is appeared as the maximum applicable and popular analytical method. This approach is concept to be reasonable and is based totally on the quantitative estimation of different phytoconstituents. it is stated to be a very successful method[18].

the use of the HPTLC approach, phytoconstituents of *F. benghalensis*, such as gallic acid and luteol, were remoted and quantified. using the HPTLC technique, researchers have performed each qualitative and quantitative analyses of phytoconstituents together with stigmasterol, quercetin, gallic acid, and lupeol, either one after the other or simultaneously[19].

2. MATERIALS AND METHODS

1.1 Chemicals

All of the analytical research (AR) grade chemicals used were acquired from different producers, such

as Yucca Pharmaceuticals Pvt. Ltd. and Abbott Healthcare Pvt. Ltd.

1.2 Collection of plant material, preparation and fractionation of extract

The plant material of *F.benghalensis* was collected from rural parts of Pune. The plant was authenticated by the Botanical Survey of India, which is based in Pune, Maharashtra, India. Before being further processed for extraction, the medication was ground into a coarse powder and left to air dry at room temperature under a shed. In the Soxhlet apparatus extraction procedure, methanol was employed as a solvent. Following the evaporation of the methanolic extract using an electric water bath, a percentage yield was calculated[20].

1.3 Preparation of standard solutions

Gallic acid (1 mg/ml) and lupeol (1 mg/ml) standard solutions were produced. A precise 1 mg dosage of lupeol and 1 mg of gallic acid were dissolved in 1 ml of methanol. To create standard solutions with concentrations of 1-6 µg/ml for gallic acid and 1-6 µg/ml for lupeol, respectively, stock solutions were further prepared by diluting them with methanol[21].

1.4 Instrument and chromatographic parameters for method development

For the HPTLC study, pre-coated silica gel 60F254, a 20 by 10 centimetre aluminum sheet from Merck, was used. All of the sample and standard solution bands were applied to the plate using the Linomat V automatic sample applicator (Camag, Switzerland). The optimal resolution was achieved by using a mobile phase and a chamber saturation duration of 15 minutes. The plate was created at a distance of mm in the Camag twintrough chamber. The densitometer analysis was carried out in the TLC chamber. The scanning was done with the TLC scanner 4 (Camag, Switzerland) and WinCATS software. It was found that the wavelengths at which lupeol and gallic acid could be detected were 276 nm[22].

1.5 HPTLC method validation

Validation of this HPTLC technique was accomplished according with global convention on Harmonization (ICH) guidelines (IUPAC, 2002). The parameters that had been evaluated had been accuracy, precision, linearity, LOQ, LOD, specificity, repeatability, and percent recovery.At wavelength 276 nm, which corresponds to concentrations of a thousand ng–6000 ng, the linearity check become performed at extraordinary concentrations of the standard inventory answer of gallic acid and lupeol.

The graphs that show the peak vicinity versus concentration of each preferred had been used to estimate the linearity of the calibration curves for the requirements. The Rf values of trendy gallic acid and lupeol were in comparison that allows you to estimate the specificity of this technique. There have been six repetitions of the system. The aim of the experiment become to check the usual's repeatability and restoration percentage. The restriction of detection (LOD) and limit of quantitation (LOQ) have been used to check and validate the sensitivity of this method. Gallic acid and lupeol were observed to have limits of detection (LOD) of 3.three /S and bounds of quantification (LOQ) of 10 /S. Repeatability and percentage drug recuperation were used to verify accuracy and precision for methanol[23]

1.6. Validation of the HPTLC Method:

There was no evidence of drug degradation during the method's development and validation. Good peaks of lupeol and gallic acid are produced by the applied condition. The following parameters were used in the development and approval of the HPTLC technology.

1.6.1. Linearity

The counselled approach's linearity became assessed in accordance with ICH guidelines. To attain an answer inside the awareness variety of 10-60µL (1000-6000 ng) for Gallic acid and Lupeol, the same old stock answer of 1000µg/ml of every turned into quantitatively diluted. For lupeol and gallic acid, a peak region as opposed to awareness graph became plotted. It was hooked up what the regression line's slope and intercept had been.

1.6.2. Precision

The degree of repeatability or reproducibility of test results to an analytical method is measured by precision. There are two varieties: intermediate precision (between-day precision) and repeatability (within-day precision). For both intra-day and inter-day precision studies, the method's precision was assessed six times on identical replicates at a concentration level of 10 µL (1000 ng). The measured peak area is expressed as a percentage of the relative standard deviation (% RSD), and the intra-day precision was measured at 1000 ng for both gallic acid and lupeol on the same day. The aforementioned concentrations were used in triplicate for the three separate days of the inter-day precision investigation.

1.6.3. Accuracy study

In triplicate, the method's accuracy was assessed at three concentration levels: 80%, 100%, and 120 %. Gallic acid and lupeol were recovered using the standard addition method, which involved adding three different amounts at different concentrations. Thus, in a solution prepared using the commercial biomarker, 80, 100, and 120 ng of gallic acid and lupeol were added following sample dilution.

1.6.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Standard formulas were used to investigate the developed method's detection and quantitation limits. Using the slope (S) and standard deviation (α) of the calibration curve with y-intercept, the values of LOD and LOQ for gallic acid and lupeol were determined. The limits of detection and quantification can be found using the formulas 3α/S and 10α/S, respectively.

1.6.5. Robustness specificity

The robustness analysis was carried out by assessing deliberate variations in the method parameters, such as the comparison of the mobile phase, the time of saturation, and the total changes in the mobile phase. Gallic acid and lupeol Rf value and peak area were assessed when the parameter was changed. By computing the relative standard deviations (RSD) for every parameter, the value and peak area were assessed.

1.6.6. Specificity

The specificity study evaluated the peak purity study of Gallic acid and Lupeol. Sample band and standard band were scanned at three distinct levels: peak start, peak apex, and peak end. In this study, a standard stock solution containing 1000 µg of luteol and gallic acid was prepared and utilized.[24]

1.7. QSAR Study

1.7.1 Data Set

A total of 11 compounds were chosen for QSAR analysis in the current work. Given the significant differences in biological activity and structural variations between the compounds in the series, it was thought to be the perfect series for QSAR analysis.

1.7.2 Selection of training and test sets

Given the discovery that q2 seems to be a necessary but now not sufficient situation for a version to have excessive predictive energy, the cutting-edge have a look at locations a strong emphasis on using take a look at units to validate the developed model. The thirteen compounds total have been cut up into corporations: the take a look at set (7 compounds) and the education set (4 compounds). To unfold the interest range, the maximum robust, moderately energetic, and low lively compounds have been blanketed in the education set. in an effort to select as a minimum one structural analog of the training set for the take a look at set, the check set compounds had been chosen carefully.[25]

1.7.3 Computational Details

2D structures of all molecules were drawn by using Marvin sketch, and then these structures were converted into 3D structures by using same software. Then all compounds were optimized by using Avogadro software. Commands are given to Open-3D align software for the alignment of structures. From all compounds data set result test data set and training data was chosen.[26]

1.7.4 Selection of Template and Molecular Alignment

The selection of the template conformation is the maximum essential step in growing a straightforward pharmacophore version for 3D-QSAR fashions. due to this, one of the most essential and sensitive variables in growing a dependable and considerable version is the spatial alignment of the molecules being studied. A template for the most active compound in the collection become its power minimized docked conformation. The atom-based totally rms suit and centroid have been used to perform the molecular alignment. by using defining the centroid and pairwise superimposing those centroids and atoms, these options appoint shape alignment to place all of the structure within the database within the equal frame of reference as the template compound.[27]

3. Results

1.6 HPTLC

Methanol solvent was used to extract the leaves of *F. benghalensis*. Methanol had the highest yield, at 4% w/w. Various mobile phase systems were tested for the simultaneous measurement of lupeol and gallic acid. The system consisting of Toluene, Ethyl Acetate, Formic acid, and Methanol was confirmed. The peaks for gallic acid and Lupeol were produced in this mobile phase. Table 1 shows the concentrations of lupeol and gallic acid found in the extract. Linearity ranged from 1000 to 6000 ng/spot for lupeol and from 1000 to 6000 ng/spot for gallic acid. The term "limit of quantitation" refers to the lowest amount of the standard that can be quantified with acceptable precision and accuracy, and the term "limit of detection" refers to the lowest amount of analyte that can be detected. The limits of detection and quantification for gallic acid and lupeol were found to be ng/spot and ng/spot, respectively. Table 3 shows the inter-day and inter-day precision.

Since Table 4 indicates higher concentrations of gallic acid and lupeol, recovery studies and repeatability—which are among the most crucial validation parameters—were carried out for methanolic extract at 80%, 100%, and 120% levels.

Table 1: Characteristics of alcoholic extract of *Ficus benghalensis* leaves and percent drug content of gallic acid and lupeol

Solvent	Colour and consistency	Percen w/w yield	Gallic acid (ng)	Lupeol (ng)
Methanol	Brown, Solid	4.466	0.164	1.753354

Table 2: HPTLC analysis data of gallic acid and lupeol

Extract	Gallic acid	Lupeol
Rf values	0.23±0.02	0.70±0.02
λ max	276 nm	276 nm
Linearity range	1000-6000 ng	1000-6000 ng
Regression equation	Y=2.6221x + 8062.7	Y= 0.8712x + 2684.5
Regression (r^2)	0.977	0.9748
LOD	4919.495	3315.416
LOQ	14907.791	1004.671

Table 3: Validation parameters for methanolic extract

Biomarker	Interday Precision % RSD	Interday Precision % RSD
Gallic acid	5.7 %	5.54%
Lupeol	1.32%	0.991%

Table 4: Percentage drug recovery from methanolic extract.

% Drug recovery	Gallic acid	Lupeol
80 %	101.64 %	99.900 %
100 %	100 %	100.007 %
120 %	105.83 %	103.33 %

Linearity plots of reference standards and estimation of methanolic extract of *F. benghalensis* leaves extract is shown in Figure 1 and 2 where, Densitogram of drug content in methanolic extract of *Ficus benghalensis* given Figure 3.

Figure 1: Linearity plots of Gallic acid

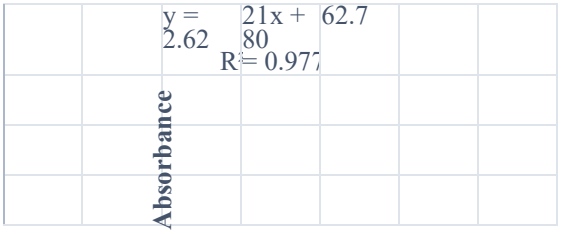


Figure 2: Linearity plots of Lupeol

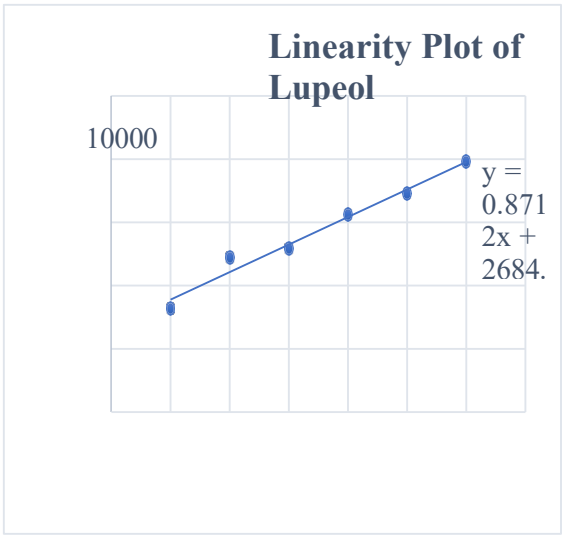
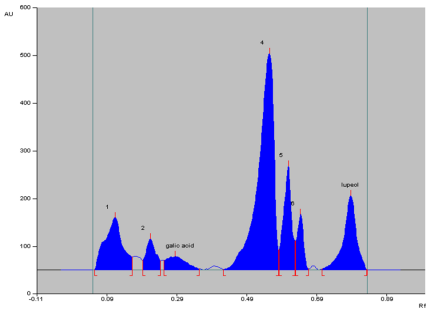


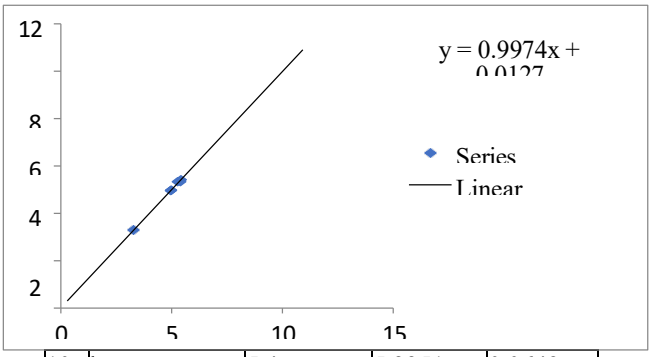
Figure 3: Densitogram of drug content in methanolic extract of *Ficus benghalensis.*



1.7 QSAR Study

Table 5: All Compounds Data

Set 1	Name of the compound	Actual	Predicted	Residual
1*	Quercetin	5.6	4.4868	1.1132
2*	Gallic acid	4.53	4.5749	-0.0449
3*	Lupeol	5.25	4.7892	0.4608
4*	B- sitosterol	5.9	4.8524	1.0476
5	Psoralen	5.4	5.42	-0.02
6	Catechin	3.29	3.29	0
7	Leucocyanidin	4.96	4.96	0
8	B- friedelin	4.97	4.97	0
9	B- amyrin	4.98	4.98	0



10	bergapten	5.4	5.3351	0.0649
11	5 methoxy psoralen	5.27	5.3348	-0.0648

* Indicates training set.

Figure 4: Training set plot

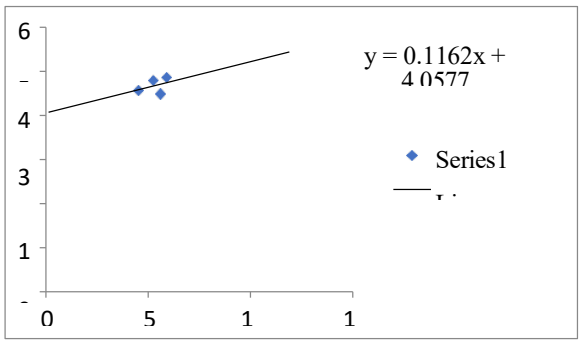


Figure 5: Test sets plot

4. DISCUSSION

Chromatographic fingerprint estimations have proven to be a practical and beneficial realistic approach for validating the identity and satisfactory of sizable traditional drug treatments. To decide unique drug identity parameters, the HPTLC approach was used. The consequences of the fingerprint evaluation can then be used to determine whether markers of interest are gift or absent[28]. Estimating the concentration and excellent of the primary chemical additives is crucial due to the fact even a tiny change in a primary ingredient has a massive impact at the product's effectiveness.[29]

The findings showed that the peaks of gallic acid and lupeol have been no longer interfered with in any manner by using other phytoconstituents found inside the plant extract. therefore, a specific approach became determined upon. The corresponding spots located inside the alcoholic extract of *Ficus benghalensis* leaves were located to be precisely much like the spectra of popular compounds gallic acid and lupeol, indicating the absence of interference from every other phytoconstituents of the plant. A comparison of the peaks at 3 unique stages—the peak start, peak apex, and peak give up positions—confirmed the purity of the peaks acquired. The developed method become found to be unique and repeatable, as evidenced via the low relative preferred deviation values received. it could be showed that the approach is interference-unfastened primarily based on properly healing values. This approach become developed and is useful for concurrently detecting lupeol and gallic acid in a methanolic extract of *F. benghalensis* leaves. thus, we have now disclosed the unconventional, clean-to-use, and sensitive HPTLC approach for identifying good sized phytoconstituents found in *F. benghalensis* leaves. The worldwide conference on Harmonization (ICH) tips had been followed in determining the validation parameters for this technique.[30,31]

The most efficient and useful technique for the simultaneous estimation of gallic acid and lupeol from the methanolic extract of *F. benghalensis* leaves is this advanced and demonstrated technique. it's been mounted that the HPTLC technique is straightforward, sensitive, precise, specific, and accurate for identifying and measuring lupeol and gallic acid. As a result, a developed and permitted technique can be used for *Ficus benghalensis* leaf standardization, great manage, analysis, and quantification of gallic acid and lupeol.

Moreover, as previously cited, *Ficus benghalensis* leaves have a better attention of tremendous phytoconstituents and offer great healing capability inside the management and remedy of a selection of medical situations. thus, the pharmacologically massive phytoconstituents can be concurrently predicted and isolated using this method. because anti-strain and anti-melancholy biomarkers were gift, the anti-pressure and anti-despair interest within the methanolic extract wealthy in gallic acid and lupeol turned into greater extensive when as compared to the usual and control.
The quantitative shape-hobby courting (QSAR) technique, that's notion of as a method to link a molecule's chemical association with its outcomes in biochemistry, physics, remedy, biology, etc., is used in this take a look at.

Create processes for drug discovery, chemo computing, and determining the organic hobby of chemical substances. in addition, it could be used for danger control in pharmacological medicine and ecotoxicological critiques of particular chemical compounds.

for the reason that the received R2 value, anticipated R2 cost, and Q2 value from the QSAR observe are all within the widespread variety, it's far viable to structurally regulate these molecules to give them

antidepressant motion.

5. CONCLUSION

The main therapeutic activities of the leaves of *F. benghalensis* are shown by the higher amounts of the compounds. The most possible fraction of *Ficus benghalensis* leaves extract was studied. The method for simultaneous estimation of gallic acid and lupeol from the extract of *F. benghalensis* was successfully developed. The amount of lupeol and gallic acid was estimated. Different validation parameters were assessed to determine the reliability method.

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