Analytical Method Development and Validation of Nelumbo Nucifera Ethanolic Seed Powder Extract by UV-Visible Spectrophotometry

***Elluru Naga Deepthi, P. Tejaswani, V. Sravani, S. Chaitanya, S. Vaishnavi, K. Bharath, R. Rakiul Islam**

Department of Pharmaceutical Analysis, Dr. K. V. Subba Reddy Institute of Pharmacy, Jawaharlal Nehru Technological University Anantapur, Dupadu, Kurnool 518218, India.

***Corresponding Author**

DOI: https://doi.org/10.51584/IJRIAS.2025.10060099

Received: 10 June 2025; Accepted: 14 June 2025; Published: 15 July 2025

ABSTRACT

A precise, accurate, sensitive, and simple UV-Spectrophotometric method was established and validated for the estimation of Nelumbo Nucifera ethanolic seed powder extract. Ethanol was employed as a solvent in Soxhlet extraction process. The ethanolic extract of Nelumbo nucifera seed Powder has been extensively worked out for their therapeutic value with antioxidant and anti-inflammatory activity. UV spectroscopyisa quick and effective technique for the phytochemical analysis and confirmation of the occurrence of bioactive constituents in such extracts. Method development and validation require optimization of parameters such as wavelength selection, solvent compatibility, and calibration curves to obtain accurate measurement of active constituents. Standard stock solutions and working standard solutions were prepared according to the given procedure and Ethanol was employed as a solvent to prepare the working standard solutions of the range of 10-60 µg/ml and the λ max was 215nm. The range was found to be 10-60µg/ml and the correlation coefficient was 0.01x+0.0288. The equation for regression was calculated as 0.9934. The recovery came out to be between the range of 100.6%. The sensitivity of the method was confirmed by the method of performing LOD and LOQ values 1.16 µg/ml &3.50 µg/ml respectively for the Nelumbo Nucifera Drug.

Keywords: Nelumbo nucifera, Method Development, Regression Coefficient, Calibration, Accuracy, Precision, Validation, LOD, LOQ

INTRODUCTION

Pharmaceutical Analysis Pharmaceutical analysis is a science that is designed to identify substances, purify them isolate them, measure them, identify the molecular structures of chemical compounds that constitute a pharmaceutical compound, and identify how these compounds are combined to constitute a pharmaceutical product.

UV-Visible Spectroscopy

Spectroscopy is the field of science concerning the interaction between electromagnetic radiation and matter i.e., the measurement of electromagnetic radiation absorbed or emitted by analyte UV-vis spectroscopy has broad detection range corresponding to material molecules absorption properties to electromagnetic waves ranging from 200-760 nm to electromagnetic waves ranging from 200-760 nm.

Plant Profile

Lotus seeds, or makhana or Kamal seeds, are the seeds of the sacred lotus flower (Nelumbo nucifera) and are rich source of nutrition and have been utilized in many cultures for thousands of years both in cooking and traditional medicine.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VI June 2025

Synonym: fox nuts, makhana, waterlily seeds

Biological Source

The two most well-known species are:

Nymphaea lotus

Nelumbo nucifera

Family: Nelumbonaceae

Description

Lotus seeds are from the lotus plant, from the Nelumbo nucifera species, commonly referred to as the Indian or frightened lotus.

Physical appearance:

Shape: Small, Round and somewhat Flattened.

Size: Each seed is about 1-2cm in diameter.

Colour: There are typically white, cream, or pale brown.

Chemical constituents [9]

Alkaloids: Nuciferine

Flavonoids: Isoquercitrin

Tannins: Indicates antioxidant properties.

Saponins: Present in various parts of lotus plant.

Polyphenols: They are responsible for its antioxidant activity.

Polysaccharides: Found in seeds, beneficial for immune modulation.

Amino Acids: glutamic acid and aspartic acid.

Volatile Oils: Contributing to the aroma, particularly in flowers.

Pharmacological Activities

Antioxidant activity

Anti-inflammatory effects

Anti-diabetic properties

Anti-microbial effect

Nutritional benefits

Rich in proteins, Fiber, Vitamins and Minerals

Low in fat

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VI June 2025

Traditional and medicinal uses:

In traditional Chinese and Ayurvedic medicine, lotus seeds provides various health benefits, including improving digestion, boosting heart health, and calming the mind.

They are often used in teas, tonics, and herbal remedies to treat issues such as insomnia, anxiety, and digestive problems

METHODOLOGY

Methodology of extraction of the drug (Nelumbo Nucifera Ethanolic Seed Extract) [14]

Soxhlet Extraction Method was used for the extraction of lotus seed powder, by using Ethanol as solvent for the extraction process.

Extraction: It is the process of isolating and separating the active pharmaceutical ingredient (API) or other desired compounds from a complex matrix, such as plants & other Plant materials.

Extraction Method For Nelumbo Nucifera Seed Extract By Soxhlet Apparatus

Materials

Hot air oven was utilized for drying the lotus seeds. Analytical balance was utilized for weighing the seed powder. Sieve was utilized for separating fine particles from seed powder. Filter paper was utilized for filtration process. Heating Mantle was utilized for heating the solution during Soxhlet extraction period. Soxhlet chamber was utilized for the extraction process. 100 ml volumetric flasks and conical flasks were utilized for solution preparation and measurement.

Chemicals (Reagent)

Methanol, Ethanol, N-hexane, Distilled water, were employed as various solvents for the extraction of Nelumbo Nucifera seed extract in powder form. From the studies of solubility, Ethanol was selected to be the ultimate solvent for Method development and Validation of the drug.

Preparation of Nelumbo Nucifera Seed Powder

Seeds were washed many times to remove impurities and other dirt and then dried in oven at 50°C until it reached constant moisture content. The seeds were then powdered to obtain the particle sizes and made ready for extraction process, seeds was powdered, 10mg of lotus seed powder and 250ml of ethanol[solvent] was used to get the extract.

Procedure for extraction of Nelumbo Nucifera Seed Extract

10mg seed powder was inserted into the thimble and inserted in Soxhlet extractor. 250 ml of the chosen solvents were inserted into each of round bottom flask and set up for Soxhlet extractor then distillation process was carried out for the 6 hours. Upon completion of extraction process, solvent and extractor were set on water bath to drive off the solvent. Lastly, the seed extract is kept in the air tight container for further analysis.

Spectrophotometric Method:

The UV -visible spectrophotometer employed in the development was Analytical technologies ltd 2080N.

The pathlength was 1 cm matched quartz cells.

Data processing was carried out with Analytical technologies software. The development parameters were the following:

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VI June 2025

Wavelength selection

Fig. 1. Soxhlet Apparatus

UV method development:

The UV method was developed by scanning the drug in the wavelength range of 200-400nm. Initially different solvents were used namely distilled Water, Methanol, Ethanol. The solubility of drug was studied and from above solvents, finally highest solubility of the drug was achieved in drug: ethanol. The drug was solubilized in various solvents and the spectrum were recorded. The absorption spectrum of drug and ethanol shown the peak at 215nm, which was considered as a λ max.

Validation of proposed methods

The following are the parameters validated.

Linearity

Accuracy

Precision

Intraday precision and Inter day precision

Robustness (Change in Wavelength and Temperature)

Ruggedness (Change in Analyst)

Limit of Detection (LOD)

Limit of Quantification (LOQ)

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VI June 2025

Validation of the method:

The procedure wasvalidated for parameters such as linearity, accuracy, precision, ruggedness, limit of detection, limit of quantification and robustness.

Preparation of solvent

Drug extract and ethanol was taken in 1:1 ratio, that is 10ml extract and 10ml of ethanol was taken to prepare 100ml solvent.

Preparation of standard stock solution

Nelumbo Nucifera drug extract stock solution was made by dissolving 10 mg in 10 ml of ethanol to achieve a concentration of 1000 µg /ml (Stock-1). Stock-1 was further diluted with mobile phase (ethanol) to achieve 100 µg/ml (stock-2) solution. The prepared stock-2 solution was employed as a standard solution.

Determination of wavelength of maximum absorbance (λ max) Nelumbo Nucifera seed in ethanol

The standard solution prepared was available after scanned in a UV- Visible double beam spectrophotometer between 200 and 400 nm against the mobile phase as a blank. The λ_{max} was determined by taking the spectrum.

Preparation of working standard solution and construction of standard curve

Working standard solution and standard curve preparation A working standard solution containing concentrations from 10 µg/ml to 60 µg/ml was obtained by pipetting 1ml, 2ml, 3ml, 4ml, 5ml, and 6ml, from the stock solution into 6 different 10ml volumetric flasks, making the volume up with ethanol. These solutions were scanned at λ max (270nm), and the data are given below. Considering the obtained data, a standard curve between concentration on X-axis and absorbance on Y-axis was drawn.

METHODOLOGY

Linearity

For testing the linearity, serial dilution of analyte prepared from standard working solution was diluted with solvent to obtain a series of concentration ranging from 10 -60 µg/ml. The solutions prepared were filtered through Whatman filter paper (NO41). Calibration curve was drawn by plotting the absorbance on Y-axis against the concentration on X-axis.

Precision

The precision of the analysed method was studied by analysis of multiple sampling of homogeneous ample. The precision is expressed as standard deviation (or) relative standard deviation. The precision of the method was demonstrated by intra-day and inter-day variation studies.

Intraday precision

In the intraday studies, the standard solutions of 60µg/ml was analysed for six times in different time interval within a day. %RSD was calculated.

Inter day precision

In the study of inter-day variation, the standard solution 60 µg/ml was analyzed six times on different days. The % RSD was computed.

Accuracy

Recovery studies were conducted by the standard addition method with the aim of justifying the accuracy of the

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VI June 2025

suggested method. Spiked samples of already analyzed drug extract were taken with 80, 100, 120% of drug standard and mixture were analyzed by the suggested method. Experiment was done in triplicate and pure drug recovery, %RSD were found as 1.082, 1.465 and 1.5618% respectively.

Sensitivity

Sensitivity of the presented method was determined by measurement of drug by use in terms of limit of detection (LOD) and the limit of quantitation (LOQ), LOD, LOQ were calculated and the values are expressed as 1.16 and 3.50[µg/ml].

Ruggedness

Ruggedness is an assessment of the reproducibility of a test result under expected, normal operating condition from instrument to instrument and analyst to analyst. Ruggedness test results are reported as 1.380%.

Robustness

Robustness is an assessment of capacity of a procedure to be unaffected by small, but intentional change in the procedure condition, variation of wavelength (205nm and 215 nm) had remarkable impact on the absorbance of 60µg/ml solution, which reflects that the procedure was robust, %RSD was determined as 0.767%. %RSD of all parameters must be less than 2%.

RESULTS AND DISCUSSION

UV SPECTROSCOPY METHOD

Table No 1. Characteristic parameters of Nelumbo Nucifera for the proped UV Spectroscopy Method

Parameters	UV spectroscopic method
Calibration concentration (µg/ml)	10 – 60(µg/ml)
Wavelength (λ max)	210nm
Regression equation (y*)	0.01x + 0.0288
Slope	0.0288
Correlation co efficient (r2)	0.9934

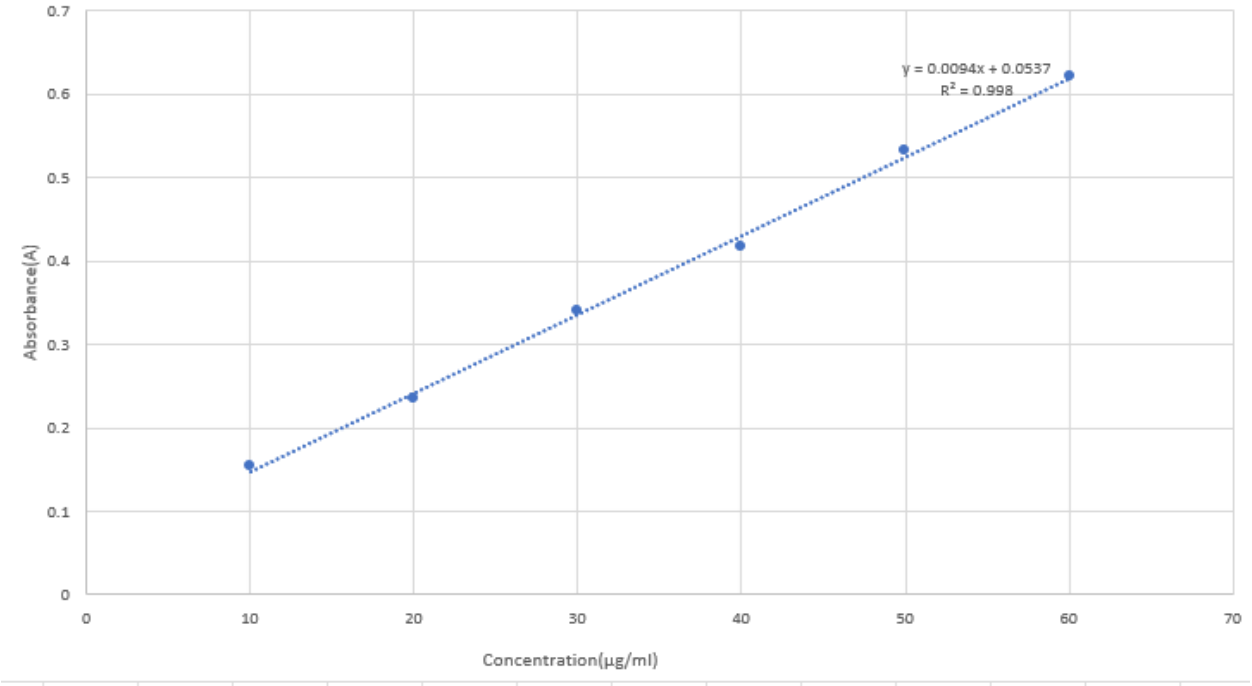

Fig: 2 Calibration Curve of Nelumbo Nucifera

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VI June 2025

Linearity

Table No 2. Calibration Table for Nelumbo Nucifera

SNO	Concentration (µg/ml)	Absorbance
1	10	0.154
2	20	0.236
3	30	0.341
4	40	0.418
5	50	0.532
6	60	0.621

Precision

Table No 3. Intraday precision for Nelumbo Nucifera Absorbance

S.no	Conc. µg/ml	1	2	3	4	5	6	AVG	SD	%RSD
1	10	0.150	0.149	0.146	0.148	0.145	0.147	0.1475	0.0019	1.288
2	20	0.219	0.215	0.217	0.211	0.213	0.214	0.2148	0.0029	1.312
3	30	0.335	0.331	0.333	0.337	0.339	0.340	0.3358	0.0035	0.751
4	40	0.425	0.423	0.427	0.426	0.422	0.424	0.4245	0.0019	0.448
5	50	0.522	0.519	0.517	0.515	0.520	0.513	0.5177	0.0033	0.637
6	60	0.612	0.615	0.609	0.618	0.610	0.613	0.6128	0.0033	0.539

Table No 4. Inter day precision for Nelumbo Nucifera Absorbance

S.no	Conc. µg/ml	1	2	3	4	5	6	AVG	SD	%RSD
1	10	0.149	0.147	0.148	0.145	0.143	0.150	0.1470	0.0026	1.7680
2	20	0.215	0.212	0.214	0.211	0.218	0.216	0.2143	0.0026	1.2132
3	30	0.325	0.327	0.322	0.323	0.326	0.329	0.3253	0.0026	0.7992
4	40	0.411	0.415	0.408	0.405	0.417	0.410	0.4110	0.0044	1.0700
5	50	0.528	0.525	0.523	0.529	0.530	0.532	0.5278	0.0033	0.6252
6	60	0.618	0.615	0.614	0.613	0.620	0.625	0.6175	0.0045	0.7287

Accuracy

Table No 5. Observation for Accuracy standard (30µg/ml)

S. No.	Concentration (µg/ml)	Absorbance
1	Set-1	0.342
2	Set-2	0.340
3	Set-3	0.335
4	AVG	0.339
5	SD	0.003606
6	%RSD	1.0634

Table No.6. Observation for Accuracy standard 80%(24µg/ml)

S. No	Concentration(µg/ml)	Absorbance
1	Set-1	0.229
2	Set-2	0.231
3	Set-3	0.234
4	AVG	0.231

5	Result	20.44
6	%Rec	99.7
7	SD	0.0025
8	%RSD	1.082

Table No 7. Observation for Accuracy standard 100% (30µg/ml)

S. No	Concentration (µg/ml)	Absorbance
1	Set-1	0.342
2	Set-2	0.349
3	Set-3	0.352
4	AVG	0.348
5	Result	30.79
6	%Rec	100.60
7	SD	0.0051
8	%RSD	1.465

Table No 8. Observation for Accuracy standard120 %(36µg/ml)

S. No	Concentration (µg/ml)	Absorbance
1	Set-1	0.456
2	Set-2	0.469
3	Set-3	0.457
4	AVG	0.461
5	Result	40.79
6	%Rec	100.7
7	SD	0.0072
8	%RSD	1.5618

Table No. 9. For Accuracy Summary

Sample (%)	Initial Amount (µg/ml)	Amount added (µg/ml)	Amount Recovered (µg/ml)	% Recovery ± SD*	% RSD
80	24	0.5	99.7	99.7±0.0025	1.08
100	30	0.5	100.60	100.6±0.0051	1.46
120	36	0.5	100.7	100.7±0.0072	1.56

Sensitivity

Table No 10. Observation of Limit of Detection

S. No	Slope	SD of Precision	LOD(µg/ml)
1	0,01	0.0035	1.16

Table No 11. Observation of Limit of Quantitation

S. No	Slope	SD of Precision	LOD(µg/ml)
1	0.01	0.0035	3.50

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VI June 2025

Ruggedness

Table No 12. For Ruggedness (Analyst to Analyst)

Analyst -1 Analyst-2

S. No	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	Set-1	0.336	Set-1	0.325
2	Set-2	0.332	Set-2	0.328
3	Set-3	0.339	Set-3	0.320
4	Set-4	0.341	Set-4	0.322
5	Set-5	0.343	Set-5	0.329
6	Set-6	0.338	Set-6	0.332
7	AVG	0.3382	AVG	0.3260
8	SD	0.0039	SD	0.0045
9	%RSD	1.153	%RSD	1.380

Robustness:

Table No 13. For Robustness 205 and 215nm wavelengths

S. No	Concentration	Absorbance (at 205nm)	Absorbance (at 215nm)
1	Set-1	0.337	0.341
2	Set-2	0.333	0.339
3	Set-3	0.335	0.335
4	Set-4	0.339	0.338
5	Set-5	0.341	0.342
6	Set-6	0.336	0.337
7	AVG	0.3368	0.3387
8	SD	0.0029	0.0026
9	%RSD	0.8610	0.7676

Table No 14. For Robustness Summary

S. No	Concentration	Absorbance (at +5 °C)	Absorbance (at -5 °C)
1	Set-1	0.345	0.340
2	Set=2	0.348	0.338
3	Set-3	0.350	0.342
4	Set-4	0.347	0.335
5	Set-5	0.343	0.337
6	Set-6	0.352	0.345
7	AVG	0.3475	0.3395
8	SD	0.00327	0.00362
9	%RSD	0.9410	1.066

Table No 15. For Robustness +5 °C and -5 °C Temperature

S. No	Condition	Modification	Mean absorbance ± SD*	%RSD for absorbance
1	Wavelength(nm)	205	0.3368±0.0029	0.8610
2	Wavelength(nm)	215	0.3387±0.0026	0.7676

Table No 16. For Robustness Summary

S. No	Condition	Modification	Mean absorbance ± SD*	%RSD for absorbance
1	Wavelength(nm)	5°C	0.3475±0.00327	0.9410
2	Wavelength(nm)	_5°C	0.3395±0.00362	1.066

CONCLUSION

In the present work an attempt was made to provide a Unique Method for Estimation of Nelumbo Nucifera Ethanolic Seed Powder Extract. A Simple, Accurate, Precise, sensitive and low-cost UV-Spectrophotometric method for the estimation of Nelumbo Nucifera in powder (standard) Form the optimum wavelength of detection was found to be 215nm at which better drug response was obtained. The calibration curve was linear in the concentration range of 10- 60µg/ml in the table no: 2 of Nelumbo Nucifera respectively. The sensitivity of the drug has been calculated and the LOD and LOQ values of Nelumbo Nucifera was found to be 1.16& 3.50(µg/ml. The mean recoveries were found to be range of 98- 102%. Ruggedness %RSD was found to be less than 2% in the table no: 12. Robustness %RSD was found to be 1.066%.

Conflict of interest

The authors have no conflicts of interest regarding this investigation.

ACKNOWLEDGEMENTS

First of all, I take this opportunity to express my profound gratitude towards my esteemed guide Elluru Nagadeepthi, M. Pharm, (ph.d.) of Pharmaceutical analysis, Dr. K. V. Subba Reddy Institute of Pharmacy, Dupadu, Kurnool. We express our deep sense of gratitude to Dr. K. V. Subba Reddy, Chairman of Dr. K.V. Subba Reddy Group of institutions and Smt. K. Vijayalakshmi Correspondent of Dr. K.V. Subba Reddy Group of institutions supporting us in providing all equipment's and required things. Finally, we would like to thank to Principal Dr. B.V. Ramana sir, all department HODS and all the teaching staff for their support during the project work.

BIBLIOGRAPHY

1. K. B. Dhyey Bhikadiya1*, "“Pharmaceutical Quality Management Systems: A Comprehensive Review,”," African Journal of Biomedical Research, pp. 27[55]:644-653, 2024.
2. Z. A. A. a. Masoom Raza Siddiqui, " “Analytical techniques in pharmaceutical analysis: A review," ” Arabian Journal of Chemistry, , vol. 10, pp. 1409-S1421, 2017.
3. S. A. K. A. S. Alaboodi1, " “Ultraviolet-Visible Spectroscopy, Importance, Principle, Structure and Most Important Applications: A Study Review,," International Journal of Novel Research in Physics Chemistry & Mathematics,., vol. 12, no. 1, pp. 53-60, 2005.
4. J. M. a. R. M. Mandru, " A Review on UV-visible spectroscopy.," ” Journal of Pharma Insights and Research,,, vol. 1, no. 2, pp. 091-096, 2023.
5. Q. W. Zhang, "“Techniques for extraction and isolation of natural products: a comprehensive review," Chinese Medicine, , vol. 13, 2018.
6. M. S. S. a. D. S. Divya Raj, " “A review: Extraction, isolation and determination of secondary metabolite (Phenols) from medicinal plants and its benefits,," Journal of Medicinal Plants Studies,, vol. 2, pp. 10(4): 181-193, , 2022.
7. P. D. I. Pal1, "A Review on Lotus (Nelumbo nucifera) Seed,”," International Journal of Science and Research (IJSR) , vol. 4, no. 7, pp. 2319-7064, 2013.
8. S. I. 1. M. I. 1. M. S. R. R. 2. M. A. 1, "Lotus seeds (Nelumbinis semen) as an emerging therapeutic seed: A comprehensive review,," Food Science & Nutrition, , vol. 9 , no. 7, pp. 3971-3987,, 2021.
9. K. D. Y. Taewoong Rho, "Chemical Constituents of Nelumbo nucifera Seed," Korean Association of Medical Journal, pp. 23(4):253-257., 2017.
10. Z.-C. S. J. L. T. L.-F. L. Y.-P. L. W. Z. Q. Z. Yi-Fei Wang1, "Phytochemicals, biological activity, and industrial application of lotus seedpod (Receptaculum Nelumbinis): A review," Front. Nutrion , vol. , pp.

9:1022794, , 2022.
11. M. Y. Yoshikai Kouzuma, "Lotus – A Source of Food and Medicine: Current Status and Future Perspectives in Context of the Seed Proteomics,," International Jounal of Life sciences,, vol. 7, no. 1, 2013.
12. C. 1. a. G. K. G. 2. Shipra Thapar 1, " "A concise review on method development and validation parameters,," GSC Biological and Pharmaceutical sciences, , vol. 19, no. 1, pp. 066-077, 2022.
13. D. C. 1. a. D. M. D. 2. Sushila Daga, " "Analytical method validation: A brief review,," World Journal of Advanced Research and Reviews, , vol. 16, no. 2, pp. 389-402, 2022.
14. Q. W. Zhang, "Techniques for extraction and isolation of natural products: a comprehensive review,," Chinese Medicine, , vol. 13, 2018.