Trop J Nat Prod Res, July 2025; 9(7): 3035 - 3039 **ISSN 2616-0684 (Print)**
**ISSN 2616-0692 (Electronic)**

# Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

*Original Research Article*

# Triterpenoid Extraction from *Ficus racemosa* Leaves: Impact of Key Processing Factors

Thuan N. Nguyen[1], Mai S. Dam[1], Luong T. Nguyen[2], Phuong V. Do[1]*

[1]*Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City, 71410, Vietnam*
[2]*Nguyen Dinh Chieu High School, Thu Dau Mot City, Binh Duong Province, 75111, Vietnam*

ARTICLE INFO

*Article history:*
Received 29 March 2025
Revised 01 June 2025
Accepted 09 June 2025
Published online 01 August 2025



ABSTRACT

*Ficus racemosa* leaves are a rich source of triterpenoids with anti-inflammatory, antibacterial, and antioxidant properties. These compounds have potential applications in pharmaceuticals, cosmetics, and functional foods. This study aimed to optimize triterpenoid extraction from *Ficus racemosa* leaves by evaluating the effects of raw material-to-solvent ratio, temperature, and extraction time. The presence of secondary metabolites, including phenolics, flavonoids, tannins, saponins, and terpenoids, was analyzed. Extraction efficiency was optimized based on yield and compound stability under different conditions. *Ficus racemosa* leaves contain various bioactive compounds, with phenolics and saponins being the most abundant. The extraction conditions were a drying temperature of 50°C, a raw material-to-solvent ratio of 1:150, an extraction temperature of 50°C, and an extraction time of 60 minutes, ensuring maximum triterpenoid yield without degradation. This study provides a basis for efficient triterpenoid extraction from *Ficus racemosa* leaves, supporting their potential applications in pharmaceuticals, cosmetics, and functional foods.

***Keywords:*** Biological activity, Extract, *Ficus racemosa*, Triterpenoid

## Introduction

*Ficus racemosa* (*F. racemosa*) leaves are a rich source of natural compounds, among which the triterpene group has attracted significant attention due to its diverse biological activities.[1] Triterpenes are a class of organic compounds belonging to the terpenoid family, consisting of 30 carbon atoms formed from six isoprene units. These compounds commonly occur in either aglycone or glycoside (saponin) forms and play a crucial role in plant defense, as well as in medical applications.[2] Numerous studies have demonstrated that triterpenes possess valuable biological properties, including anti-inflammatory, antimicrobial, antioxidant, and immunomodulatory effects. In traditional medicine, *F. racemosa* leaves have been used to treat various ailments, such as infections, digestive disorders, and skin conditions, suggesting the presence of potent bioactive compounds.[3] Investigating the extraction of triterpenes from *F. racemosa* leaves not only helps elucidate their pharmacological potential, but also contributes to investigate extraction methods to enhance compound recovery and purity.By investigating the extraction processes, this study aims to facilitate the application of triterpenes from *F. racemosa* leaves in the pharmaceutical, cosmetic, and functional food industries, unlocking significant potential for natural product-based formulations. While the broader applications of *F. racemosa* are recognized, comprehensive studies specifically focusing on the triterpene profile and extraction optimization from *F. racemosa* leaves within the Vietnamese context remain limited. Further investigation into local varieties could reveal unique chemotypes and optimize extraction methods tailored to Vietnamese resources.

*Corresponding author. Email: dovietphuong@iuh.edu.vn
Tel: +84967092094

Citation: Thuan N.N, Mai S.D, Luong T.N, Phuong V.D*. Triterpenoid extraction from *Ficus racemosa* leaves: Impact of key processing factors. Trop J Nat Prod Res. 2025; 9(7): 3035 – 3039 https://doi.org/10.26538/tjnpr/v9i7.12

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

## Materials and Methods

*Chemicals*

The chemicals used in the experiment included ethyl acetate (99.8% purity, China), perchloric acid ($HClO_4$) (70% purity, China), acetic acid ($CH_3COOH$) (99.7% purity, China), and other analytical-grade reagents.

*Plant collection and identification*

*Ficus racemosa* leaves were collected in June 2024, from Ho Chi Minh City, Vietnam (coordinates: 10°51'41.386"N, 106°40'48.760"E). The plant material was identified at the Faculty of Biology - Biotechnology, VNU-HCM University of Science, Ho Chi Minh City, Vietnam. An herbarium specimen with voucher number VNMN2024006 (Figure 1) was deposited in the herbarium unit.

*Qualitative analysis of components in Ficusracemosa leaves*

Powdered *F. racemosa* leaves (0.5 g) was weighed and extracted with ethanol at a 1:30 ratio (w/v) by maceration at room temperature for 8 hours under continuous magnetic stirring at 200 rpm). After extraction, the first extract (solution 1) was collected by vacuum filtration through Whatman No. 1 filter paper to separate the liquid from the remaining solid. The extraction process was repeated twice under the same conditions, each lasting 8 hours, yielding solutions 2 and 3. Finally, all three extracts were combined, and used for the qualitative analysis of bioactive components, as presented in Table 1.

**Table 1:** Qualitative analysis of phytoconstituents of *Ficus racemosa* leaves

| Phytochemical | Test procedure | Observation | Reference |
|---|---|---|---|
| Phenolics and tannins | 2 mL extract + 2 mL $H_2O$ + 2- | Blue-black or brownish-green colour | [19] |

| | | | |
|---|---|---|---|
| Alkaloids | 3 drops of $FeCl_3$ (5%)<br>2 mL extract + 3-4 drops of Wagner's reagent | Reddish-brown precipitate | [20] |
| Flavonoids | 2 mL extract + 2 mL $Pb(COOH)_2$ (10%) | Yellow precipitate | [21] |
| Saponins | 2 mL extract + 10 mL distilled water + heat for 2 minutes | Foam formation | [22] |
| Terpenoids and steroids | 5 mL extract + 2 mL chloroform + 3 mL concentrated $H_2SO_4$ | Reddish-brown colour | [23] |
| Coumarin | 2 mL extract + 3 mL NaOH (10%) | Yellow color | [21] |

*Investigation of factors affecting triterpenoid content*
In this study, the effects of drying temperature (40°C, 50°C, 60°C, and 70°C) and extraction factors, including material-to-solvent ratio (1:50, 1:100, 1:150, 1:200, 1:250), extraction temperature (40, 50, 60, 70, and 80°C), and extraction time (30, 60, 90, 120, and 150 minutes), on triterpenoid content were investigated. In each experiment, one variable was altered while the others were kept constant.

*Investigation of drying temperature*
The effect of drying temperature was examined at 40°C, 50°C, 60°C, and 70°C, with the moisture content maintained at 6-7% as per Sarkar *et al*. (2023).[4]

*Investigation of material-to-solvent ratio*
The effect of the material-to-solvent ratio was evaluated at 1:100, 1:150, 1:200, and 1:250, with the extraction temperature set at 70°C and the extraction time fixed at 120 minutes.[5]

*Investigation of extraction temperature*
The selected temperature ranges for the extraction experiment were 40, 50, 60, 70, and 80°C, using the optimal material-to-solvent ratio determined above. The extraction time was fixed at 120 minutes.

*Investigation of extraction time*
The extraction time was varied within the range of 30, 60, 90, 120, and 150 minutes, with increments of 30 minutes. The material-to-solvent ratio and extraction temperature were set based on the optimal conditions determined above.

*Determination of triterpenoid content*
A calibration curve was constructed using oleanolic acid as the standard. The triterpenoid content was determined by UV-Vis spectrophotometry (Genesys 20, Thermo Scientific, USA), following the method described by Yang *et al*. (2020) with modifications.[6]

A 1 mL sample (prepared by dissolving 0.5 g of the extract in 15 mL of ethanol) was placed in a test tube, followed by the addition of 0.2 mL of 5% acetic acid and 1.2 mL of perchloric acid ($HClO_4$). The mixture was stirred and incubated at 70°C for 15 minutes, then rapidly cooled for 2 minutes. The solution was diluted to 5 mL with ethyl acetate and measured at a wavelength of 550 nm. The total triterpenoid content was calculated using the following formula:

$$\text{TTC (mg OAE/g DW)} = C_x \times \frac{V_{dm}}{10^3} \times \frac{100}{a(100 - W)} \times K$$

Where; $C_x$: Measured concentration of oleanolic acid (ppm); Vdm: Sample dilution volume (mL); a: Sample weight (g); W: Moisture content (%); K: Dilution factor; $10^3$: Conversion factor

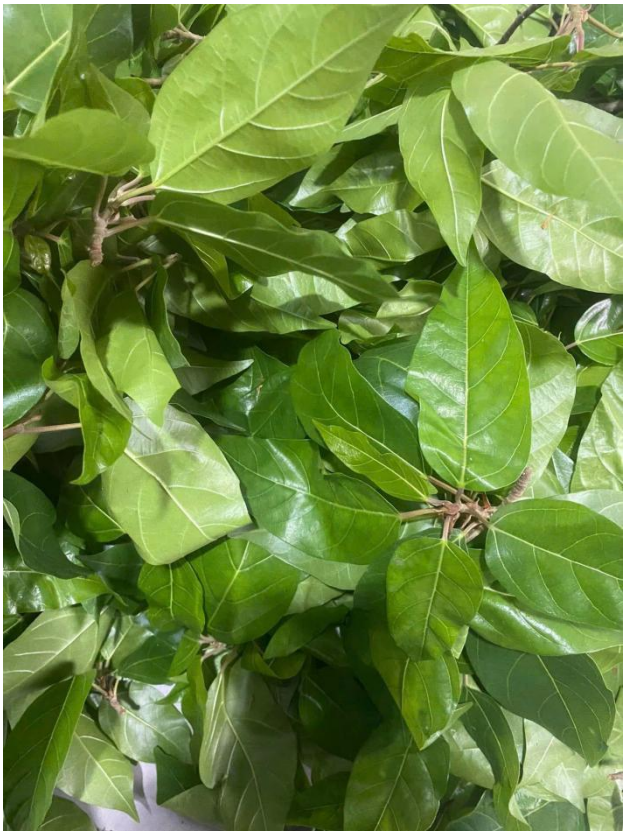

**Figure 1:** *Ficus racemosa* leaves

*Statistical analysis*
All experiments were performed in triplicate, and the results are expressed as the mean ± standard deviation (SD). A one-way analysis of variance (ANOVA), followed by Tukey's honest significant difference (HSD) was used to determine statistically significant differences between the means ($P < 0.05$). Data analysis was carried out using Statgraphics Centurion XX software (Statgraphics Technologies, Inc., USA).

## Results and Discussion

*Phytoconstituents of Ficus racemosa leaves*
The qualitative analysis results as presented in Table 2 indicate that *Ficus racemosa* leaves contain various secondary metabolites at different concentrations. Phenolics, tannins, and saponins were detected in the highest amounts (+++), suggesting strong antioxidant potential as well as significant antibacterial, and immune-supporting properties.[7] Flavonoids, terpenoids, and steroids were present at moderate levels (++), contributing to anti-inflammatory, cytoprotective, and biological regulatory effects.[8] In contrast, coumarins and alkaloids were found in lower concentrations (+), indicating a more limited role in the medicinal properties of *F. racemosa* leaves.

These findings help explain the widespread traditional medicinal use of *F. racemosa* leaves, particularly in treating infections, aiding digestion, and promoting overall health. The results align with the study by Hidayanti *et al*. (2023),[9] which identified the presence of steroids, flavonoids, saponins, and tannins in *F. racemosa* leaves in Indonesia and highlighted their antioxidant potential.

**Table 2:** Phytochemical constituents of *Ficus racemosa* leaves

| Phytochemical | Result |
|---|---|
| Phenolics | + |
| Tannins | + |
| Flavonoids | + |
| Coumarin | + |
| Alkaloids | + |
| Terpenoids | + |
| Steroids | + |
| Saponins | + |

**Key:** (+) Detected, (-) Not detected

*Drying temperature*

The results presented as presented in Figure 2 indicate that the total triterpenoid content (TTC, mg/g DW) gradually decreases as drying temperature increases, with values of 0.1314 (40°C), 0.1242 (50°C), 0.1083 (60°C), and 0.074 (70°C), respectively. At 40°C and 50°C, the triterpenoid content did not differ significantly, suggesting that these compounds are relatively well preserved at lower temperature conditions. However, at 60°C and especially at 70°C, a substantial decline in TTC indicates that higher temperatures may accelerate triterpenoid degradation.

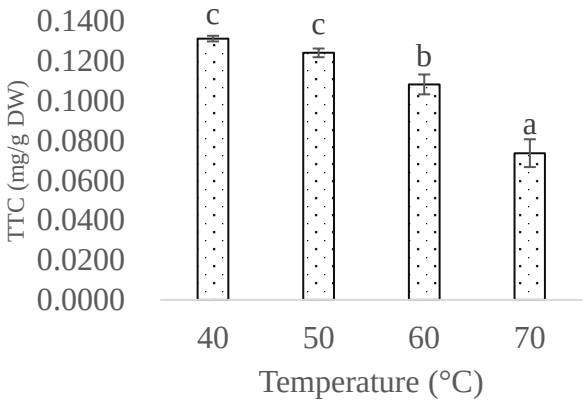


**Figure 2:** Effect of temperature on total triterpenoid content Different letters in the same column indicate significant differences between samples ($p \leq 0.05$).

The present findings revealed a significant reduction in TTC as drying temperature increases, which aligns with the conclusion that high temperatures can substantially reduce total triterpenoid saponin content due to increased oxidation, chemical bond disruption, and structural degradation of bioactive compounds.[10] This trend can be attributed to the impact of elevated temperatures on the decomposition or volatilization of phenolic compounds, flavonoids, and other antioxidants present in the sample.[11] The selection of drying temperature should balance TTC preservation and process efficiency. Drying at 40°C retains the highest TTC content but prolongs the drying time, and increases costs. In contrast, 50°C maintains a TTC level comparable to 40°C while reducing drying time and improving efficiency. However, temperatures of 60°C and 70°C significantly decrease TTC content and may also affect other bioactive compounds. Therefore, 50°C is considered the most suitable drying temperature, ensuring a balance between bioactive compound preservation and drying efficiency.

*Material-to-solvent ratio*

The material-to-solvent ratio is a critical factor influencing triterpenoid extraction efficiency (TTC). The results indicated that varying this ratio significantly impacted the obtained TTC content (Figure 3). As the ratio increased from 1:100 to 1:150, TTC content increased from 0.224 mg/g to a peak of 0.247 mg/g. However, further increasing the solvent ratio to 1:200 and 1:250 led to a sharp decline in TTC content, dropping to 0.174 mg/g and 0.170 mg/g, respectively.

Solvent-to-material ratio significantly affects the extracted compound content. As this ratio increases, the concentration difference between the inside and outside of the material also increases, facilitating mass transfer and allowing the solvent to easily extract compounds from the material.[12] A low solvent-to-material ratio limits the solubility of triterpenoids, leading to low extraction efficiency. Conversely, when the amount of solvent is high, the diffusion of triterpenoids into the solvent occurs more intensively until saturation is reached.[5] However, at a solvent-to-material ratio up to 1:200, the obtained triterpenoid content decreased, possibly due to the presence of other dissolved compounds, as saturation of the solvent with other dissolved compounds as been shown to decrease phenolic compounds content.[13] Therefore, a ratio of 1:150 was determined to be suitable for further experiments.

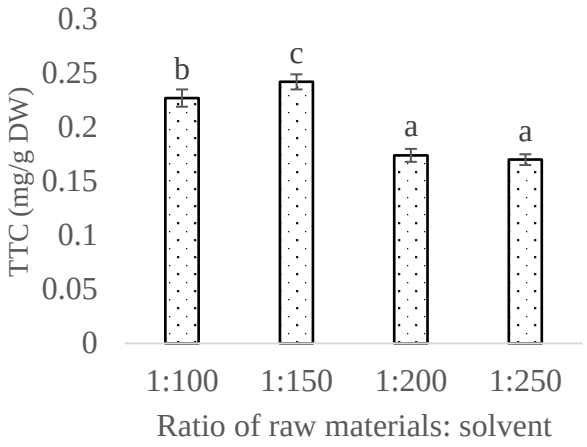


**Figure 3:** Effect of extraction material-to-solvent ratio on triterpenoid content. Different letters in the same column indicate significant differences between samples ($p \leq 0.05$).

*Extraction temperature*

Temperature is a key factor that plays a crucial role in the extraction process of triterpenoids, directly influencing yield. Figure 4 illustrates the variation in TTC content with temperature. Specifically, at 40°C, the TTC content was 0.242 mg/g, which slightly increased to 0.254 mg/g at 50°C. However, when the temperature was further increased to 60–80°C, the TTC content significantly decreased, reaching its lowest level of 0.166 mg/g at 80°C.

An increase in temperature enhances the solubility of triterpenoids, promotes diffusion and mass transfer, and reduces the viscosity of the solvent, thereby improving extraction efficiency.[14] However, when the temperature exceeds 60°C, significant thermal degradation occurs, leading to a decrease in triterpenoid content. Therefore, 50°C was identified as the optimal temperature, ensuring the highest TTC concentration while preventing compound degradation.[15] This trend is consistent with the study by Muzafri and Karno (2022),[16] which reported that the extraction of *Panax notoginseng* also yielded the highest triterpenoid content at 50°C. Thus, 50°C is considered the optimal extraction tempurature for triterpenoid extraction, balancing efficiency and compound stability.[16]

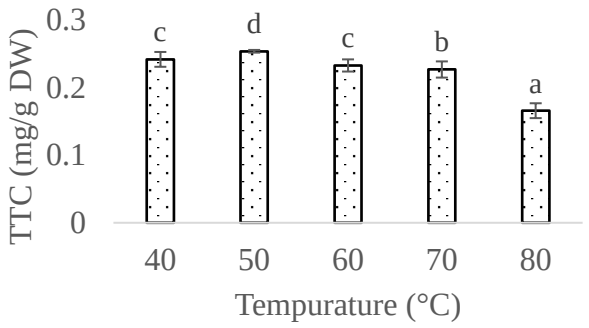


**Figure 4:** Effect of extraction tempurature on triterpenoid content. Different letters in the same column indicate significant differences between samples ($p \leq 0.05$).

*Extraction time*

As illustrated in Figure 5, extraction time was shown to have varying effects on triterpenoid content. Extraction time plays a crucial role in solvent diffusion into the material and the release of triterpenoids. The results show that TTC content gradually increased from 30 to 60 minutes, reaching its peak (0.458 mg/g) at 60 minutes. However, when the extraction time was extended further, TTC content gradually decreased due to compound degradation under thermal effects.[17]

The extraction process is influenced by the contact between the solvent and the material, as well as the interaction time between the solid and liquid phases. Extraction efficiency depends on solvent-material contact time and solid-liquid interaction duration.[18] When the extraction time is only 30 minutes, the solvent does not fully penetrate the material, leading to a lower yield of triterpenoids. However, excessive extraction time may cause compound degradation due to heat or oxidation. If the extraction time is excessively prolonged, the compounds may degrade due to thermal effects or oxidation. Therefore, 60 minutes was identified as the optimal extraction time, ensuring the highest extraction efficiency while maintaining triterpenoid stability.

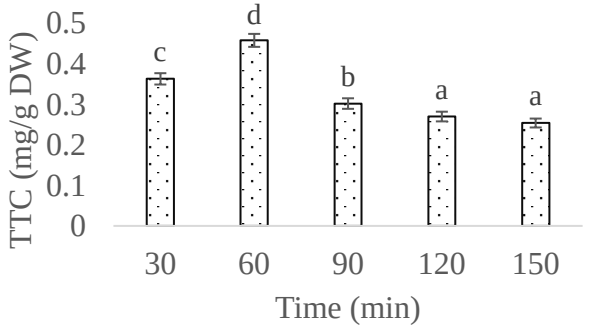


**Figure 5:** Effect of extraction time on triterpenoid content. Different letters in the same column indicate significant differences between samples ($p \leq 0.05$).

## Conclusion

This study determined the optimal conditions for the efficient extraction of triterpenoids from *Ficus racemosa* leaves, with a drying temperature of 50°C, a material-to-solvent ratio of 1:150, an extraction temperature of 50°C, and an extraction time of 60 minutes. Qualitative analysis confirmed that *Ficus racemosa* leaves contain important secondary metabolites, such as phenolics, flavonoids, tannins, saponins, and terpenoids, contributing to their biological activities. These findings highlight the potential applications of triterpenoids from *Ficus racemosa* leaves in pharmaceuticals, cosmetics, and functional foods. Identifying suitable extraction conditions enhances recovery efficiency, reduces costs, and improves feasibility for large-scale production. Future studies should focus on compound isolation, biological activity evaluation, and *in vitro* and *in vivo* experiments to validate the efficacy of these bioactive compounds.

## Conflict of Interest

The Authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

## Acknowledgements

The authors would like to express their gratitude to the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City for supporting this research. The authors also thank Le Huynh Tuoi, Le Thi Hoa Thom for their helpful advice on various technical issues examined in this paper.

## References

1. Sharma BR, Kumar V, Kumar S, Panesar PS. Microwave Assisted Extraction of Phytochemicals from *Ficusracemosa*. Curr Res Green Sustain Chem. 2020; 3:100020.
2. Liu J, Yin X, Kou C, Thimmappa R, Hua X, Xue Z. Classification, Biosynthesis, and Biological Functions of Triterpene Esters in Plants. Plant Commun. 2024; 5(4):100845.
3. Cavazos P, Gonzalez D, Lanorio J, Ynalvez R. Secondary Metabolites, Antibacterial and Antioxidant Properties of the Leaf Extracts of *Acacia rigidula* Benth and *Acacia berlandieri* Benth. SN Appl Sci. 2021; 3:522.
4. Sarkar T, Salauddin M, Roy S, Chakraborty R, Rebezov M, Shariati MA, Thiruvengadam M, Rengasamy KRR. Underutilized Green Leafy Vegetables: Frontier in Fortified Food Development and Nutrition. Crit Rev Food Sci Nutr. 2023; 63(33):11679-11733.
5. Egüés I, Hernandez-Ramos F, Rivilla I, Labidi J. Optimization of Ultrasound Assisted Extraction of Bioactive Compounds from Apple Pomace. Molecules. 2021; 26(13):3783.
6. Yang YP, Hussain N, Zhang L, Jia YZ, Jian YQ, Li B, Choudhary MI, Rahman A, Wang W. *Kadsura coccinea*: A Rich Source of Structurally Diverse and Biologically Important Compounds. Chin Herb Med. 2020; 12(3):214-223.
7. Dhawale PV, Vineeth SK, Gadhave RV, MJ JF, Supekar MV, Thakur VK, Raghavan P. Tannin as a Renewable Raw Material for Adhesive Applications: A Review. Mater Adv. 2022; 3(8):3365-3388.
8. Aboelez MO, Ezelarab HA, Alotaibi G, Abouzed DEE. Inflammatory Setting, Therapeutic Strategies Targeting Some Pro-Inflammatory Cytokines and Pathways in Mitigating Ischemia/Reperfusion-Induced Hepatic Injury: A Comprehensive Review.Naunyn-Schmiedeberg's Arch Pharmacol. 2024; 397(9):6299-6315.
9. Hidayanti BR, Suryani N, Dewi YK. Phytochemical Screening and Antioxidant Activity Test of Ara Fruit Extract (*Ficus racemosa* Linn.) Using DPPH Method. SPIN J Kim Pend. Kim. 2023; 5(2):177-191.
10. Mohapatra P, Ray A, Jena S, Nayak S, Mohanty S. Influence of Various Drying Methods on Physicochemical Characteristics, Antioxidant Activity, and Bioactive Compounds in *Centellaasiatica* L. Leaves: A Comparative Study. Biotechnologia. 2022; 103(3):235-247.
11. Wu Q and Zhou J. The Application of Polyphenols in Food Preservation. Adv Food Nutr Res. 2021; 98:35-99.
12. Tsevdou M, Ntzimani A, Katsouli M, Dimopoulos G, Tsimogiannis D, Taoukis P. Comparative Study of Microwave, Pulsed Electric Fields, and High Pressure Processing on the Extraction of Antioxidants From Olive Pomace. Molecules. 2024; 29(10):2303.
13. Ozdemir M, Gungor V, Melikoglu M, Aydiner C. Solvent Selection and Effect of Extraction Conditions on Ultrasound-Assisted Extraction of Phenolic Compounds from Galangal

(*Alpiniaofficinarum*). J Appl Res Med Aromat Plants. 2024; 38:100525.
14. Zhang G, Ma Y, Huang M, Jia K, Ma T, Dai Z, Wang Q. Reprograming the Carbon Metabolism of Yeast for Hyperproducing Mevalonate, a Building Precursor of the Terpenoid Backbone. J Agric Food Chem. 2024; 73(1):606-616.
15. Celli GB, Ghanem A, and Brooks MS-L. Optimization of Ultrasound-Assisted Extraction of Anthocyanins from Haskap Berries (*Lonicera caerulea* L.) Using Response Surface Methodology. Ultrasonics Sonochemistry. 2015; 27:449-455.
16. Muzafri A and Karno R. Testing of Andaliman Extract (*Zanthoxylum Acanthopodium* Dc) With 4 Types of Solutions (Ethyl Acetate, Aquades, Methanol, and Hexane) on Growth of Bacteria *Escherichia Coli*. KESANS: Int J Health Sci. 2022; 1(4):337-343.
17. Dietemann P, Kälin M, Zumbühl S, Knochenmuss R, Wülfert S, and Zenobi R. A Mass Spectrometry and Electron Paramagnetic Resonance Study of Photochemical and Thermal Aging of Triterpenoid Varnishes. Anal Chem. 2001; 73(9):2087-2096.
18. Fontanals N, Borrull F, Marcé RM. Ionic Liquids in Solid-Phase Extraction. TrAC - Trends Anal Chem. 2012; 41:15-26.
19. Deka A, Sarma I, Dey S, and Sarma T. Antimicrobial Properties and Phytochemical Screening of Some Wild Macrofungi of Rani-Garbhanga Reserve Forest Area of Assam,India. Adv Appl Sci Res. 2017; 8(3):17-22.
20. Bodi D, Ronczka S, Gottschalk C, Behr N, Skibba A, Wagner M, Lahrssen-Wiederholt M, Preiss-Weigert A, and These A. Determination of Pyrrolizidine Alkaloids in Tea, Herbal Drugs and Honey. Food Addit Contam, Part A. 2014; 31(11):1886-1895.
21. Tailor G and Lawal A. Phytochemical Screening; Green Synthesis, Characterization and Biological Significance of Lead Oxide Nanoparticles from *Eucalyptus globulus*Labill.(Leaves). Nanotechnol Environ Eng. 2021; 6(3):48.
22. Odebiyi O and Sofowora E. Phytochemical Screening of Nigerian Medicinal Plants II. Lloydia. 1978; 41(3):234-246.
23. Llauradó G, Morris HJ, Lebeque Y, Gutiérrez A, Fontaine R, Bermúdez RC, and Perraud-Gaime I. Phytochemical Screening and Effects on Cell-Mediated Immune Response of *Pleurotus* Fruiting Bodies Powder. Food Agric Immunol. 2013; 24(3):295-304.