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Cryoethanolic Extraction, Purification of Allicin from *Allium sativum* and HPTLC analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The significance of this research extends beyond the mere extraction and purification of allicin, encompassing its potential applications across diverse industries. Allicin exhibits a plethora of biological activities, including antimicrobial, antifungal, antiviral, and antiprotozoal properties. Its mechanism of action involves the inhibition of thiol-containing enzymes in microorganisms, rendering it effective against a wide array of pathogens. Moreover, allicin has demonstrated promising anticancer properties, eliciting apoptosis and inhibiting cell proliferation in various cancer cell lines. Additionally, its anti-inflammatory and cardioprotective effects underscore its potential

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therapeutic utility in mitigating cardiovascular diseases and other inflammatory conditions. This study presents a novel method for extracting and purifying allicin from raw garlic cloves. Dry ice ensures the ethanol to reach its cryo temperature (hilled to sub-zero temperatures ranging from -40°C to -80°C). The process involves the use of ethanol, dry ice and ascorbic acid as solvents, vacuum stirring, and subsequent crystallization. The final product, a hygroscopic powder, was analyzed using High-Performance Thin-Layer Chromatography (HPTLC) to determine allicin content. The extraction yielded 5 grams of whitish hygroscopic powder from 21 grams of crude extract, with an allicin content of 5%. Chromatographic conditions included a mobile phase of acetonitrile: water: Formic acid (30:8:2) and derivatization with ninhydrin, with detection under UV light at 366 nm. This method provides an efficient way to isolate and purify allicin for various applications.

Keywords: Allicin; allium sativum; HPTLC; extraction; purification; crystallization; cryoethanolic extraction.

1. INTRODUCTION

Allicin (diallyl thiosulfinate) is a prominent sulfur-containing compound found in garlic (Allium sativum). Alliin, a sulfoxide found naturally in fresh garlic, derives from the amino acid cysteine [1]. When garlic undergoes chopping or crushing, the enzyme alliinase transforms alliin into allicin, the compound behind the characteristic scent of fresh garlic [2]. Known for its distinctive aroma and various health benefits, allicin is responsible for many of garlic's therapeutic properties [2]. It is an oily, slightly yellow liquid with a pungent odour, decomposing into various sulfur-containing compounds when exposed to heat or physical stress. Allicin is soluble in organic solvents like ethanol and isopropanol, which aids in its extraction and purification [3].

Allicin exhibits a broad spectrum of biological activities, including antimicrobial, antifungal, antiviral, and antiprotozoal properties [4]. Its mechanism of action includes the inhibition of thiol-containing enzymes in microorganisms [5]. Additionally, allicin demonstrates anticancer properties by inducing apoptosis and inhibiting cell proliferation in various cancer cell lines. It also has anti-inflammatory and cardioprotective effects, which contribute to its medicinal significance [6,7,8,9,10,11].

Allicin is widely used in the pharmaceutical and nutraceutical industries due to its potent biological activities [12]. In the pharmaceutical industry, allicin is used in the formulation of antimicrobial and anticancer drugs. It is also incorporated into supplements and functional foods aimed at boosting immune function, reducing inflammation, and protecting against cardiovascular diseases [13]. Moreover, allicin's strong antioxidant properties make it valuable in preventing oxidative stress-related conditions. In the food industry, allicin is used as a natural preservative due to its ability to inhibit the growth of foodborne pathogens [14].

Various techniques have been employed for the extraction of allicin, including steam distillation, Soxhlet extraction, supercritical fluid extraction, and solvent extraction. Each method has its own set of advantages and limitations [15]. Steam distillation is commonly used but often leads to the degradation of allicin due to heat. Soxhlet extraction is efficient for extracting allicin but involves prolonged exposure to solvents and heat, potentially degrading allicin [16]. Supercritical fluid extraction uses CO2 to extract allicin without thermal degradation but requires expensive equipment and operational expertise. Solvent extraction involves using organic solvents, which can efficiently extract allicin but often result in impurities and lower yields [17].

In one of the research studies HPLC analysis was carried out utilizing a Waters 616 pump coupled with a UV-1575/VIS absorbance detector. Separation of compounds occurred on a Li Chrospher 100 RP18 (C18) column, with dimensions of 250×4 mm i.d.×5 µm, sourced from Hewlett Packard. The mobile phase, comprised of methanol-water (in a 70:30, v/v ratio), facilitated the separation process. Standards utilized included DAS, DAT, DADS, and DATS. The HPLC procedure was conducted at room temperature with a flow rate set at 0.8 mL/min. Allicin within the eluent was identified at wavelengths of 202 and 210 nm [18]. HPLC procedure, each of the four standards displayed distinct peaks. DAS exhibited a retention time of 6.941 minutes, while DAT showed a retention time of 8.706 minutes, with both registering their maximum absorbance at 202 nm. On the other

hand, DADS exhibited a retention time of 12.634 minutes, and DATS at 17.794 minutes, both showing maximum absorbance readings at 210 nm. The findings indicated that the HPLC method exhibited greater sensitivity compared to the GC method, as its lowest detection limit was lower than that of GC. The average Coefficient of Variation (CV) for the spectrophotometric, GC, and HPLC methods were calculated at 3.44, 8.10, and 4.68, respectively. According to the results, both the spectrophotometric and HPLC methods demonstrated superior precision compared to the GC method [18].

Our research introduces a novel extraction method combining ball milling under vacuum, ethanol extraction with ascorbic acid as a stabilizer, vacuum filtration, nitrogen gas purging, and isopropanol crystallization. This method aims to maximize yield, purity, and stability of allicin.

1.1 Cryoethanolic Extraction of Allicin

As a future direction of our research, we plan to explore cryoethanolic extraction for allicin. Cryoethanolic extraction involves the use of ethanol at sub-zero temperatures to extract allicin from garlic. This method offers several benefits, including the minimization of thermal degradation and oxidation of allicin, which are common issues with traditional extraction methods. By maintaining low temperatures throughout the extraction process, cryoethanolic extraction can preserve the integrity and biological activity of allicin more effectively. Additionally, this method can enhance the solubility of allicin in ethanol, leading to improved extraction efficiency and higher yields. The potential for cryoethanolic extraction to produce a purer and more stable allicin product makes it a promising area for future investigation in our research.

2. MATERIALS AND METHODS

2.1 Materials

- Raw garlic cloves (100 units) (purchased from local market
- Ethanol (analytical grade)
- Ascorbic acid (0.5%)
- Isopropanol (IPA, analytical grade)
- Dry Ice
- Nitrogen gas
- HPTLC reagents: Acetonitrile, water, formic acid, ninhydrin, glacial acetic acid, isopropanol
- Standard allicin (Sigma Aldrich)

2.2 Equipment

- Ball mill
- Three-necked round bottom flask (2 liters)
- Vacuum filtration setup
- Nitrogen gas purging setup
- UV light (366 nm)
- HPTLC apparatus

2.3 Extraction Process

The extraction process begins with the thorough crushing of 100 raw garlic cloves using a ball mill under vacuum conditions, a method employed to minimize the oxidative degradation of allicin. Following this, the crushed garlic is transferred into a 2-liter three-necked round bottom flask, where it is combined with ethanol in a 1:6 ratio (raw material to solvent) and 0.5% ascorbic acid to prevent oxidation and dry ice to reach cryo temperature of ethanol (hilled to sub-zero temperatures ranging from -40°C to -80°C). The mixture is then stirred under vacuum at 45°C for 45 minutes to facilitate the extraction of allicin into the ethanol solvent. This extraction process is repeated for a total of three cycles to ensure the maximum yield of allicin. Subsequently, the mixture is filtered under vacuum to collect the liquid filtrate, minimizing exposure to air to preserve the integrity of the allicin extract.

Following the extraction, the crude extract undergoes concentration by purging the liquid filtrate with nitrogen gas to evaporate the ethanol without exposing the allicin to air. The result is a gelatinous half white to light yellow paste, weighing 21 grams.

The next step in the process involves the crystallization of the crude extract. The crude extract is dissolved in isopropanol (IPA) at a 1:2 ratio for crystallization. The mixture is continuously stirred until a whitish hygroscopic powder forms. The final product, weighing 5 grams, is collected and dried.

2.4 HPTLC analysis

In the detailed HPTLC method, the preparation of samples begins with the dissolution of the purified allicin powder in a suitable solvent for HPTLC application. Standard solutions of allicin are also prepared for calibration and comparison purposes [19]. Chromatography is then conducted using silica gel plates as the stationary phase and a mobile phase consisting of acetonitrile: water: Formic acid (30:8:2)

[20,21]. After allowing the solvent front to travel a specific distance, the plates are removed and dried. Subsequently, derivatization is performed by spraying the developed plates with a derivatizing agent made from 0.6 g ninhydrin in a mixture of isopropanol and glacial acetic acid (190:10) [22]. Heating the plates enhances the reaction and spot visualization. Finally, detection and quantification are carried out by examining the plates under UV light at 366 nm to identify and quantify allicin [22]. The spots on the plates are compared with standard allicin solutions to determine the concentration in the samples.

3. RESULTS

The Cryoethanolic extraction process yielded a gelatinous crude extract weighing 21 grams, indicative of the efficiency of the extraction method in isolating allicin from the raw garlic cloves. This crude extract underwent further purification through crystallization using isopropanol, resulting in the production of a final product. This whitish hygroscopic powder, weighing 5 grams, represents a significant reduction in volume from the initial crude extract, highlighting the effectiveness of the purification process. The confirmation of allicin presence in the final product was achieved through High-Performance Thin-Layer Chromatography (HPTLC) analysis, which revealed a concentration of 5%. This indicates that 5% of the final product comprised allicin, demonstrating the success of the extraction and purification process in retaining the desired compound.

The yield and purity of the allicin extraction were further quantified and characterized. The initial crude extract, weighing 21 grams, provided a starting point for the purification process. Through subsequent steps, including concentration and crystallization, the final product weighing 5 grams was obtained, indicating a significant reduction in volume but a concentration of allicin. The allicin content in the final product was determined to be 5%, as assessed by HPTLC analysis. These quantitative measurements provide insights into the efficiency and success of the extraction and purification process in isolating allicin from the raw garlic cloves.

The chromatogram conditions used in the HPTLC analysis further elucidate the analytical method employed to characterize the allicin content in the final product. The mobile phase consisted of acetonitrile: water: Formic acid (30:8:2), providing optimal conditions for the separation and detection of allicin on the chromatographic plate. Additionally, the derivatization reagent utilized in the analysis comprised 0.6 g of ninhydrin dissolved in a mixture of isopropanol and glacial acetic acid (190:10), facilitating the visualization of allicin spots on the plate Fig. 1&2. Detection of allicin was achieved using UV light at 366 nm, allowing for the accurate quantification of allicin content in the final product. These chromatogram conditions ensure the reliability and accuracy of the HPTLC analysis in determining the allicin concentration in the extracted and purified product.

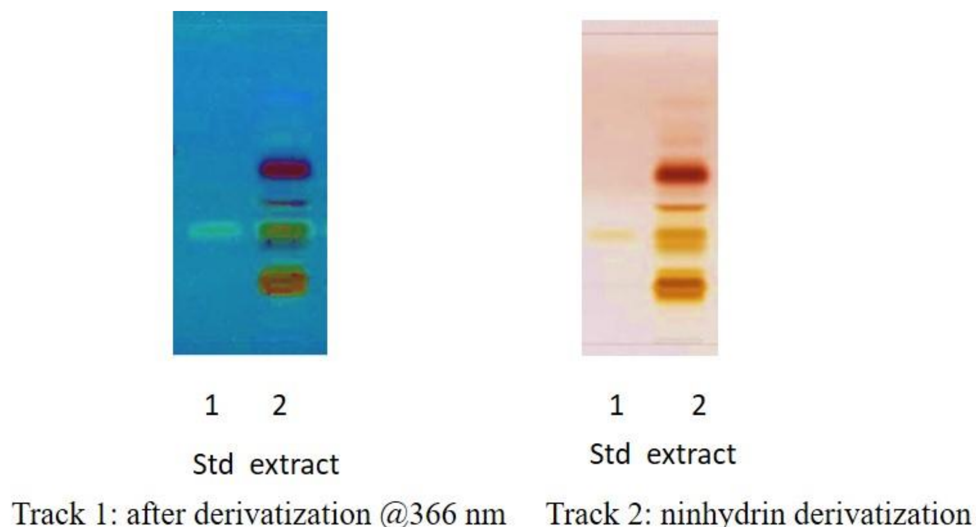
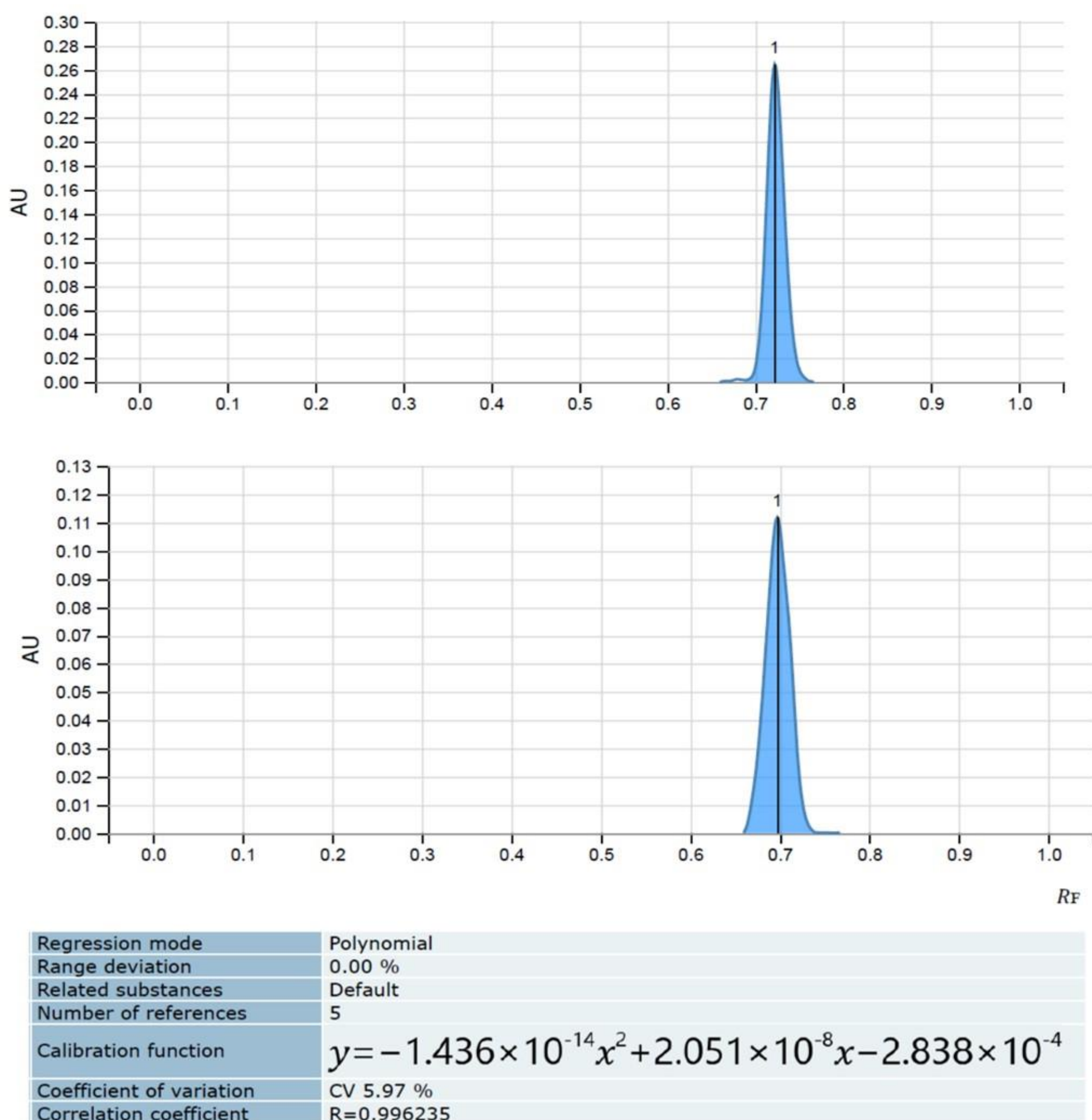


Fig. 1. HPTLC analysis



Regression mode	Polynomial
Range deviation	0.00 %
Related substances	Default
Number of references	5
Calibration function	$y = -1.436\times10^{-14}x^2 + 2.051\times10^{-8}x - 2.838\times10^{-4}$
Coefficient of variation	CV 5.97 %
Correlation coefficient	R=0.996235

Fig. 2. Allicin peaks by HPTLC with Rf = 0.7

4. DISCUSSION

The novel extraction method demonstrated in this study offers several advantages over traditional techniques. The use of a ball mill under vacuum conditions and ascorbic acid as a stabilizer effectively preserved the integrity of allicin. Ethanol extraction at cryo temperatures using Dry Ice under vacuum ensured efficient extraction without significant degradation. The subsequent crystallization using isopropanol provided a relatively pure and stable form of allicin.

Compared to steam distillation, which often leads to the thermal degradation of allicin, our method avoids high temperatures that can compromise the compound's integrity. Soxhlet extraction, although efficient, involves prolonged exposure to solvents and heat, potentially degrading allicin [23]. Our process is faster and avoids this prolonged exposure. Supercritical fluid extraction, while effective and avoiding thermal degradation, requires expensive equipment and operational expertise, making it less accessible. Our technique, on the other hand, is more cost-effective and simpler to implement. Solvent extraction methods commonly result in impurities and lower yields; however, the addition of ascorbic acid and nitrogen purging in our method enhances stability and yield, producing a higher purity product [24].

The findings suggest that our extraction method can be scaled up for industrial production of allicin, providing a reliable source for pharmaceutical and nutraceutical applications [25]. The use of readily available and cost-effective materials further supports its feasibility for large-scale operations. This method not only preserves the bioactivity of allicin but also ensures its stability, making it suitable for use in various industries. In the pharmaceutical industry, allicin can be utilized in antimicrobial and anticancer formulations. Its strong antioxidant properties are valuable in preventing oxidative stress-related conditions, making it an attractive compound for the nutraceutical industry as well [26].

As a future direction of our research, we plan to explore cryoethanolic extraction for allicin. Cryoethanolic extraction involves the use of ethanol at sub-zero temperatures to extract allicin from garlic [27]. This method offers several benefits, including the minimization of thermal degradation and oxidation of allicin, which are common issues with traditional extraction methods. By maintaining low temperatures throughout the extraction process, cryoethanolic extraction can preserve the integrity and biological activity of allicin more effectively. Additionally, this method can enhance the solubility of allicin in ethanol, leading to improved extraction efficiency and higher yields [28,29,30,31,32]. The potential for cryoethanolic extraction to produce a purer and more stable allicin product makes it a promising area for future investigation in our research.

The HPTLC analysis used specific chromatogram conditions to characterize allicin in the final product [33,34,35]. The mobile phase, consisting of acetonitrile, water, and formic acid (30:8:2), ensured effective separation and detection of allicin. Ninhydrin dissolved in isopropanol and glacial acetic acid (190:10) was the derivatization reagent, aiding in visualizing allicin spots. Allicin detection employed UV light at 366 nm for precise quantification. These conditions guarantee the reliability and accuracy of allicin concentration determination in the purified product.

5. CONCLUSION

The described Cryoethanolic method efficiently extracts and purifies allicin from raw garlic cloves, yielding a product with significant allicin content. By optimizing the extraction conditions and using stabilizing agents, the method ensures high yield and purity. This technique offers a practical and scalable solution for producing allicin for various applications, marking an improvement over existing extraction methods.

The detailed steps in the extraction and purification process, along with the HPTLC analysis, provide a comprehensive approach to isolating allicin. This method not only preserves the bioactivity of allicin but also ensures its stability, making it suitable for industrial application. Future research on cryoethanolic extraction holds promise for further improving the yield and purity of allicin, enhancing its application potential across various industries.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Borlinghaus J, Albrecht F, Gruhlke MC, Nwachukwu ID, Slusarenko AJ. Allicin: Chemistry and biological properties. Molecules. 2014;19(8):12591-618.
   DOI: 10.3390/molecules190812591
   PMID: 25153873; PMCID: PMC6271412.
2. Borlinghaus J, Foerster Née Reiter J, Kappler U, Antelmann H, Noll U, Gruhlke MCH, Slusarenko AJ. Allicin, the Odor of Freshly Crushed Garlic: A Review of Recent Progress in Understanding Allicin's Effects on Cells. Molecules. 2021;26 (6):1505.
   DOI: 10.3390/molecules26061505
   PMID: 33801955; PMCID: PMC8001868.
3. Sasi M, Kumar S, Kumar M, Thapa S, Prajapati U, Tak Y, Changan S, Saurabh V, Kumari S, Kumar A, Hasan M, Chandran D, Radha, Bangar SP, Dhumal S, Senapathy M, Thiyagarajan A, Alhariri A, Dey A, Singh S, Prakash S, Pandiselvam R, Mekhemar M. Garlic (*Allium sativum* L.) Bioactives and Its Role in Alleviating Oral Pathologies. Antioxidants(Basel). 2021;10(11):1847.
   DOI: 10.3390/antiox10111847

PMID: 34829718; PMCID: PMC8614839.

4. El-Saber Batiha G, Magdy Beshbishy A, G Wasef L, Elewa YHA, A Al-Sagan A, Abd El-Hack ME, Taha AE, M Abd-Elhakim Y, Prasad Devkota H. Chemical Constituents and Pharmacological Activities of Garlic (*Allium sativum* L.): A Review. Nutrients. 2020;12(3):872.
   DOI: 10.3390/nu12030872. PMID: 32213941; PMCID: PMC7146530.
5. Muller A, Eller J, Albrecht F, Prochnow P, Kuhlmann K, Bandow JE, Slusarenko AJ, Leichert LI. Allicin Induces Thiol Stress in Bacteria through S-Allylmercapto Modification of Protein Cysteines. J Biol Chem. 2016;291(22):11477-90.
   DOI: 10.1074/jbc.M115.702308
   Epub 2016 Mar 23. PMID: 27008862; PMCID: PMC4882420.
6. Stepien AE, Trojniak J, Tabarkiewicz J. Anti-Cancer and Anti-Inflammatory Properties of Black Garlic. Int J Mol Sci. 2024;25(3):1801.
   DOI: 10.3390/ijms25031801
   PMID: 38339077; PMCID: PMC10855366.
7. Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H, Mavumengwana V, Li HB. Bioactive Compounds and Biological Functions of Garlic (*Allium sativum* L.). Foods. 2019;8(7):246.
   DOI: 10.3390/foods8070246
   PMID: 31284512; PMCID: PMC6678835.
8. Narayan UL, Garnaik B, Patro SK, Sahu S. HPTLC Methods for Determination of Gemifloxacin Mesylate in Rabbit Plasma. J. Pharm. Res. Int. 2014;4(14):1707-14.
   Available:https://journaljpri.com/index.php/JPRI/article/view/760
   [Accessed on: 2024 Jun. 8]
9. Zakirova UM. Mamatkulova NU. Khodjaniyazov KK. Khidyrova NM. Shakhidoyatov K. Polyprenols of Grape Vitis vinifera L. Leaves. Int. J. Biochem. Res. Rev. 2013 [cited 2024 Jun. 8];3(2):97-106.
   Available:https://journalijbcrr.com/index.php/IJBCRR/article/view/458
10. Shewiyo DH, Kaale EA, Risha PG, Dejaegher B, Smeyers-Verbeke J, Vander Heyden Y. HPTLC methods to assay active ingredients in pharmaceutical formulations: A review of the method development and validation steps. Journal of pharmaceutical and biomedical analysis. 2012;66:11-23.
11. Variyar PS, Chatterjee S, Sharma A. Fundamentals and theory of HPTLC-based separation. High-performance thin-layer chromatography (HPTLC). 2011:27-39.
12. Catanzaro E, Canistro D, Pellicioni V, Vivarelli F, Fimognari C. Anticancer potential of allicin: A review. Pharmacological research. 2022; 177:106118.
13. Puri V, Nagpal M, Singh I, Singh M, Dhingra GA, Huanbutta K, Dheer D, Sharma A, Sangnim T. A Comprehensive Review on Nutraceuticals: Therapy Support and Formulation Challenges. Nutrients. 2022;14(21):4637.
   DOI: 10.3390/nu14214637
   PMID: 36364899;
   PMCID: PMC9654660.
14. Sunanta P, Kontogiorgos V, Pankasemsuk T, Jantanasakulwong K, Rachtanapun P, Seesuriyachan P, Sommano SR. The nutritional value, bioactive availability and functional properties of garlic and its related products during processing. Front Nutr. 2023;10:1142784.
   DOI: 10.3389/fnut.2023.1142784. PMID: 37560057; PMCID: PMC10409574.
15. Reiter J, Hubbers AM, Albrecht F, Leichert LIO, Slusarenko AJ. Allicin, a natural antimicrobial defence substance from garlic, inhibits DNA gyrase activity in bacteria. Int J Med Microbiol. 2020; 310(1):151359.
   DOI: 10.1016/j.ijmm.2019.151359
   Epub 2019 Sep 25. PMID: 31585716.
16. Bar M, Binduga UE, Szychowski KA. Methods of Isolation of Active Substances from Garlic (*Allium sativum* L.) and Its Impact on the Composition and Biological Properties of Garlic Extracts. Antioxidants (Basel). 2022;11(7):1345.
   DOI: 10.3390/antiox11071345
   PMID: 35883836; PMCID: PMC9312217
17. Zaini AS, Putra NR, Idham Z, Mohd Faizal AN, Che Yunus MA, Mamat H, Abdul Aziz AH. Comparison of Alliin Recovery from Allium sativum L. Using Soxhlet Extraction and Subcritical Water Extraction. ChemEngineering. 2022;6:73.
   Avaialble:https://doi.org/10.3390/chemengineering6050073
18. Jose M. del Valle, Veronica Glatzel, Jose L. Martínez, Supercritical CO2 extraction of allicin from garlic flakes: Screening and kinetic studies, Food Research International. 2012;45(1):216-224
   Available:https://doi.org/10.1016/j.foodres.2011.10.021

19. Zhou C, Hu XY, Chao C, Li H, Zhang SY, Yan XM, Yang FM, Li QH. Quantitation of Allicin in Garlic-based Products: Comparisons among Spectrophotometry, GC and HPLC. Advance Journal of Food Science and Technology. 2015;9(4):269-277.
Available:https://www.airitilibrary.com/Article/Detail?DocID=20424876-201508-201510190015-201510190015-269-277
20. Blania G, Spangenberg B. Allicin-Freisetzung aus getrocknetem Knoblauch (*Allium sativum*): Eine einfach durchzuführende HPTLC-Simultanbestimmung von Allicin und Ajoen in Knoblauchpulver und daraus hergestellten Fertigarzneimitteln [Formation of allicin from dried garlic (*Allium sativum*): A simple HPTLC method for simultaneous determination of allicin and ajoene in dried garlic and garlic preparations]. Planta Med. 1991;57(4):371-5.
DOI: 10.1055/s-2006-960120
PMID: 1775580.
21. Itakura YM. Ichikaw, Y, Mori R. Okino M. Udayama T. Morita, how to Distinguish Garlic from the Other Allium Vegetables, The Journal of Nutrition. 2001; 131(3).
Pages 963S-967S, ISSN 0022-3166,
Available:https://doi.org/10.1093/jn/131.3.963S
22. Bosca B, Mot AC. Novel simultaneous determination of alliin and allicin in Allium sp. using digital subtraction HPTLC. J Chromatogr B Analyt Technol Biomed Life Sci. 2023;1222:123700.
DOI: 10.1016/j.jchromb.2023.123700.
Epub 2023 Apr 5. PMID: 37031569
23. Niranjan S Kanaki, M Rajani, Development and Validation of a Thin-Layer Chromatography-Densitometric Method for the Quantitation of Alliin from Garlic (Allium sativum) and Its Formulations, Journal of AOAC International. 2005;88(5):1568–1570,
Available:https://doi.org/10.1093/jaoac/88.5.1568
24. Dhwani S, Pushparaj Poojitha, Pa, Parameswaran. A review on different extraction and quantification methods of allicin from garlic; 2021.
DOI: 10.37896/jxu15.6/020.
25. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ, Li HB. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. Int J Mol Sci. 2017;18(1):96.
DOI: 10.3390/ijms18010096
PMID: 28067795; PMCID: PMC5297730
26. Manikyam, Hemanth Kumar. Medicinal plants and alternative therapies for reproductive system health. Nutraceuticals: A Holistic Approach to Disease Prevention. 2024;237.
27. Nadeem MS, Kazmi I, Ullah I, Muhammad K, Anwar F. Allicin, an Antioxidant and Neuroprotective Agent, Ameliorates Cognitive Impairment. Antioxidants. 2022; 11:87.
Available:https://doi.org/10.3390/antiox1101008
28. Manikyam, Hemanth Kumar, et al. Microwave assisted Subcritical water extraction of Berberine hydrochloride from the roots of Berberis Aristata using Harmony search algorithm. Journal of Herbal Medicine Research. 2017;2: 1-9.
29. Kumar, Mr Hemanth, and M. Spandana. Method of Extraction and Determination of Forskolin from Coleus forskolii of Nepal. Journal of Pharmacognosy and Phytochemistry 2013;2(4):37-39.
30. Manikyam HK, Tripathi P, Patil SB, Lamichhane J, Chaitanya M, et al. Extraction, purification, and quantification of hesperidin from the immature Citrus grandis/maxima fruitNepal cultivar. Asian Journal of Natural Product Biochemistry. 2020;20(1).
31. Manikyam Hemanth Kumar, J. Dhanaseelan, Jayaraj Mani. Extraction and Purification of Squalene from Virgin Olive Oil via Catalytic Transesterification and Molecular Distillation. European Journal of Medicinal Plants. 2024;35(3):50-61.
32. Manikyam HKP, Tripathi J, Joshi J, Balasubramanian S, Patil B, Lamichhane T, Lamichhane J. Microwave Assisted Extraction of Berberine and Preparation of Berberine Hydrochloride from Berberis Aristata Variety of Nepal, and Quantification Using RP-HPLC and HPTLC Methods. European Journal of Medicinal Plants. 2021;32(12):46-53.
DOI: 10.9734/ejmp/2021/v32i1230434
33. Manikyam Hemanth Kumar, Joshi Sunil, Tripathi, Prathibha, Joshi Jyoti, Balasubramanian, Jayaram Udhyog, Jadibuti Assistant. Arabian Journal of Medicinal & Aromatic Plants Extraction, Purification and quantification of Ursolic

acid Ursolic extraction process from Rosemary officinalis leaves of Nepal Cultivar. 2023;9:54-64. Available:https://doi.org/10.48347/IMIST.PRSM/ajmap-v9i2.29213

34. Mr. Hemanth Kumar, M. Spandana. Simultaneous Extraction, determination and analysis of Adenosine, Cordycepin and other derivatives of Cordyceps sinensis of Nepal by new validated HPLC method. J Pharmacogn Phytochem. 2013;2(4):43-45.29.

35. Balkrishna, Acharya, M. Hemanth Kumar, Ashish Kumar Gupta. Comparative analysis of HPTLC, secondary metabolites and antioxidant activities of Tinospora cordifolia stem powders. International Journal of Pharmaceutical Sciences and Research. 2016;7(10):4263-4271.

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