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Phytochemical Screening of Deep Eutectic Solvent Extract of Butterfly Pea (*Clitoria ternatea L.*)

Indah Puspita Sari[1*], Sinta Bella Triyanti[1], Annisaa Siti Zulaicha[1]
1. Cosmetic Engineering Study Programme, Institut Teknologi Sumatera, South Lampung, 35365, Indonesia
***Corresponding Email:** indah.sari@km.itera.ac.id

ABSTRACT
This study aims to evaluate the phytochemical content of butterfly pea (*Clitoria ternatea L.*) extract using the ultrasound-assisted extraction (UAE) method with a deep eutectic solvent (DES) composed of choline chloride and citric acid in a 1:1 molar ratio, with the addition of 10% water. The extraction process was carried out for 30 minutes at a temperature of 50°C. Phytochemical screening results showed that the DES extract of butterfly pea contains various bioactive compounds, including alkaloids, flavonoids, tannins, terpenoids, and saponins, each exhibiting distinct color reactions or precipitates during testing. Flavonoids, particularly anthocyanins, produced a stable red color under acidic conditions and upon interaction with metal ions, indicating potential as natural colorants and antioxidants. Tannins formed dark green complexes with $FeCl_3$, while terpenoids produced a reddish-brown color as a result of the reaction with Salkowski reagent. Saponins generated stable foam due to their amphiphilic nature, indicating surfactant activity beneficial for cosmetic formulations. Overall, these findings suggest that the DES-based butterfly pea extract holds strong potential as a natural bioactive source for cosmetic applications, particularly due to its prominent flavonoid content and antioxidant activity.
Key words: *Clitoria ternatea*, deep eutectic solvent, ultrasound-assisted extraction, phytochemical screening, cosmetic bioactive.

INTRODUCTION
The growing global interest in natural-based cosmetic and therapeutic ingredients has driven a surge in research on botanicals with rich phytochemical profiles. *Clitoria ternatea L.*, commonly known as butterfly pea, is a leguminous plant widely cultivated in Southeast Asia and known for its vibrant blue flowers and high content of bioactive constituents, including anthocyanins, flavonoids, tannins, and other polyphenolic compounds (Putri et al). These metabolites have been reported to possess potent antioxidant, anti-inflammatory, antimicrobial, and neuroprotective properties, making C. ternatea a promising candidate for applications in pharmaceutical, nutraceutical, and especially cosmetic industries (Jeyaraj et al., 2021; Oguis

et al., 2019; Shen et al., 2022). Notably, the flower's abundant anthocyanin content contributes not only to its biological activity but also to its potential as a natural colorant with functional benefits (Marpaung et al., 2020).

Efficient extraction of these compounds is essential to realizing their commercial value. Conventional solvents such as ethanol and methanol are commonly used but raise concerns over toxicity, flammability, and environmental sustainability (Chemat et al., 2019). As a response, deep eutectic solvents (DES) have emerged as a new class of green solvents composed of biodegradable and low-toxicity components, offering tunable polarity and high solubilization capacity for a wide range of natural products (Smith et al., 2014). Among the various DES systems, those based on choline chloride and organic acids—such as citric acid—have shown promising results due to their low viscosity, hydrogen-bonding capacity, and biocompatibility (Zainal-Abidin et al., 2017). Simultaneously, ultrasound-assisted extraction (UAE) has gained attention as a non-thermal intensification method that enhances mass transfer and cell wall disruption, enabling higher yields in shorter times while preserving thermolabile compounds (Vilkhu et al., 2008).

Despite the promising potential of UAE-DES systems, there is a notable research gap in the comprehensive phytochemical profiling of Clitoria ternatea extracts obtained using choline chloride–citric acid-based DES. Most existing studies have focused on conventional solvents or alternative DES formulations, with limited insights into how these greener systems affect the solubility and stability of phytoconstituents, especially anthocyanins and other flavonoids relevant to cosmetic functionality (Benvenutti et al., 2019; Dai et al., 2013). Furthermore, there is a lack of comparative evidence regarding the qualitative behavior of key phytochemical groups in DES extracts, which is essential to support their use in product development for skincare, haircare, or dermatological formulations.

The urgency of this investigation lies in the cosmetic industry's increasing demand for sustainable and effective plant-based actives, alongside a strong regulatory and consumer shift toward green chemistry-based ingredients. Developing eco-friendly, high-efficiency extraction methods is not only aligned with sustainable development goals but also crucial for enabling cleaner production chains in cosmetic formulation science.

Therefore, this study aims to evaluate the phytochemical composition of Clitoria ternatea flower extract obtained through ultrasound-assisted extraction using a choline chloride–citric acid DES system. Qualitative phytochemical screening was performed to identify major bioactive groups including alkaloids, flavonoids, tannins, terpenoids, and saponins. The outcomes of this study are expected to contribute to the growing body of knowledge supporting the application of DES in green extraction technologies and provide a scientific foundation for the development of sustainable bioactive ingredients in cosmetics.

MATERIAL AND METHODS

This section of the report gives a detailed account of the procedure that was followed in completing the experiment(s) discussed in the report. Such an account is very important, not only so that the reader has a clear understanding of the experiment, but a well written Materials and Methods section also serves as a set of instructions for anyone desiring to replicate the study in the future.

Material

Materials

Fresh butterfly pea (*Clitoria ternatea L.*) flowers were collected from a local cultivation site, authenticated, and used immediately for extraction. Technical-grade chemicals including choline chloride and citric acid were sourced from local chemical suppliers. Other technical-grade reagents for qualitative phytochemical screening, such as hydrochloric acid (HCl), ferric chloride ($FeCl_3$), magnesium ribbon, chloroform ($CHCl_3$), sulfuric acid (H_2SO_4), and Mayer's reagent, were obtained commercially.

Methods

Preparation of Deep Eutectic Solvent (DES)

The DES was prepared by mixing choline chloride and citric acid at a molar ratio of 1:1. The mixture was stirred and gently heated at approximately 60°C until a clear homogeneous liquid formed. Afterwards, distilled water (10% v/v) was added to lower the viscosity and enhance the extraction capability.

Extraction Procedure

Approximately 10 g of fresh butterfly pea flowers were weighed, finely chopped, and mixed with 100 mL of prepared DES solution in an Erlenmeyer flask. The extraction was performed using ultrasound-assisted extraction (UAE) in an ultrasonic bath (Elmasonic S60H, Elma, Germany) at a frequency of 40 kHz for 30 minutes with the temperature maintained at 50°C. Following sonication, the extract was filtered through Whatman No. 1 filter paper, and the filtrate was stored at 4°C until further phytochemical screening.

Qualitative Phytochemical Screening

The phytochemical screening of the butterfly pea (*Clitoria ternatea L.*) DES extract was performed following standard procedures as described below:

Alkaloid Identification

In a test tube, 1 mL of concentrated extract was mixed with 5 drops of reagent A (NH_3), then filtered if any precipitate formed. To the filtrate, 2 mL of reagent B (2 N H_2SO_4) was added, and the mixture was vigorously shaken until two distinct layers were visible. The upper layer was then tested by adding one drop of Mayer's reagent. The formation of a white to yellowish precipitate indicated the presence of alkaloids.

Flavonoid Identification

In a test tube, 1 mL of concentrated extract was mixed with 5 drops of reagent F1 (absolute ethanol) and shaken until homogeneous. A small piece of magnesium ribbon (reagent F2) was then added, followed by 5 drops of concentrated hydrochloric acid (reagent F3). The presence of flavonoids was confirmed by the appearance of a yellow, orange, or red coloration.

Tannin Identification

In a test tube, 1 mL of concentrated extract was mixed with 3 drops of reagent T1 ($FeCl_3$ solution). The presence of tannins was indicated by a color change to bluish-green or dark green.

Saponin Identification

In a test tube, 1 mL of concentrated extract was combined with 2 mL distilled water and shaken until homogeneous. The mixture was then heated gently for 2–3 minutes and allowed to cool. After cooling, the test tube was shaken vigorously. The formation of stable foam lasting for at least 30 seconds indicated the presence of saponins.

Terpenoid Identification

In a test tube, 1 mL of concentrated extract was mixed with 1 mL reagent T2 (chloroform, $CHCl_3$). Subsequently, Salkowski's reagent was added dropwise, forming a distinct layer. The appearance of a reddish-brown color indicated the presence of terpenoids.

RESULT AND DISCUSSION

Qualitative Phytochemical Screening of *Clitoria ternatea* DES Extract

The phytochemical screening of *Clitoria ternatea* flowers extracted using a choline chloride–citric acid-based deep eutectic solvent (DES) confirmed the presence of diverse bioactive compounds including alkaloids, flavonoids, tannins, terpenoids, and saponins (Table 1). Each class of compounds demonstrated distinct qualitative reactions, reflecting their structural and chemical uniqueness. The result is showed in Table 1.

Tabel 1. Phytochemical Screening Result

Phytochemical Test	Observation	Result
Alkaloid	White to yellowish precipitate formed	Positive (+)
Flavonoid	Formation of deep red color	Positive (+)
Tannin	Dark green color observed	Positive (+)
Terpenoid	Reddish-brown layer formed	Positive (+)
Saponin	Stable foam (>30 sec)	Positive (+)

Alkaloids are recognized by their nitrogen-containing, weakly basic structure. Their identification involved a reaction with Mayer's reagent resulting in white to yellowish precipitates, indicative of alkaloid-metal complex formation (Muawanah, Febrina, & Sunarti, 2023). This reaction aligns with previous studies that confirm alkaloids typically form visible precipitates due to their ability to complex with metal ions (Ningsih, Syahrani, Septama, & Sukardiman, 2023).

Flavonoids were positively identified by a distinct deep red coloration following the Shinoda test (using magnesium and concentrated hydrochloric acid). This reaction occurs because flavonoids, particularly anthocyanins, change color significantly under acidic conditions and through metal complexation, which stabilizes the anthocyanin structure (Li et al., 2015; Liang et al., 2022). Such stability highlights potential applications as antioxidant-rich natural colorants in cosmetic formulations.

Tannins reacted positively with $FeCl_3$, yielding a dark green coloration due to stable complexes formed between the phenolic hydroxyl groups and iron ions (Fe^{3+}). The intensity and darkness of the green coloration are consistent with the presence of complex tannin structures, which enhance their metal-binding capacity (Kurniati, Baharuddin, & Zahra, 2022). These polyphenolic compounds are well documented for their antioxidant activities and their ability to form stable complexes with proteins, offering potential for skincare applications (Faradilla & Rizal, 2023).

Terpenoids produced a distinctive reddish-brown layer when reacted with Salkowski's reagent. This observation suggests that DES extraction successfully isolated non-polar terpenoids. The Salkowski reaction mechanism involves interaction with sulfuric acid and chloroform, triggering structural changes and producing colored terpenoid complexes (Hidayat, Riyadi, Gustiani, & Dwicahya, 2022). These terpenoid compounds hold considerable promise in cosmetic applications due to their fragrance, antimicrobial, and anti-inflammatory properties (Silvani, Riga, & Agustini, 2023).

Saponins demonstrated the formation of stable foam persisting for more than 30 seconds. This reaction arises due to their amphiphilic structure, containing hydrophilic (glycone) and hydrophobic (aglycone) regions, enabling them to act effectively as natural surfactants (Qaisar et al., 2023). The high foam stability observed indicates the potential for cosmetic formulation as emulsifiers or foaming agents (Kręgiel et al., 2017; Ribeiro, Barreto, & Coelho, 2013).

Overall, the extraction of *Clitoria ternatea* flowers using a choline chloride–citric acid DES system via ultrasound-assisted extraction significantly enhanced the recovery of bioactive compounds. Compared to conventional solvent extraction methods, DES provides a safer, sustainable, and efficient alternative, aligning well with contemporary principles of green chemistry. These findings underscore the viability of DES as an innovative solvent system for obtaining functional ingredients for cosmetic formulations.

CONCLUSION

The present study demonstrated the effective use of ultrasound-assisted extraction (UAE) combined with a deep eutectic solvent (DES) composed of choline chloride and citric acid for extracting bioactive compounds from fresh butterfly pea (*Clitoria ternatea L.*) flowers. Phytochemical screening revealed that the DES extract successfully contained a diverse array of secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids, and saponins. The observed qualitative phytochemical profiles indicate that the use of DES not only serves as a sustainable and environmentally friendly solvent but also effectively preserves and stabilizes the structural integrity of bioactive components, particularly flavonoids and anthocyanins, known for their antioxidative properties. These findings support the potential of DES-based extracts of butterfly pea flowers as valuable bioactive ingredients for future cosmetic formulations and natural antioxidant applications. Further quantitative analysis and biological activity assays are recommended to substantiate these initial findings and facilitate industrial-scale applications.

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REFERENCE

Benvenutti, L., Zielinski, A. A. F., & Ferreira, S. R. S. (2019). Which is the best food emerging solvent: IL, DES or NADES? Trends in Food Science & Technology, 90, 133–146. https://doi.org/10.1016/j.tifs.2019.06.003

Chemat, F., Abert Vian, M., Ravi, H. K., Khadhraoui, B., Hilali, S., Perino, S., ... & Fabiano-Tixier, A. S. (2019). Review of alternative solvents for green extraction of food and natural products: Panorama, principles, applications and prospects. Molecules, 24(16), 3007. https://doi.org/10.3390/molecules24163007

Dai, Y., van Spronsen, J., Witkamp, G.-J., Verpoorte, R., & Choi, Y. H. (2013). Ionic liquids and deep eutectic solvents in natural products research: Mixtures of solids as extraction solvents. Journal of Natural Products, 76(11), 2162–2173. https://doi.org/10.1021/np400051w

Faradilla, M., & Rizal, K. (2023). Phytochemical screening analysis of guava leaf extract (*Psidium guajava* L.) against the content of saponins, tannins, and flavonoids. *Journal of Natural Sciences and Mathematics Research, 9*(2), 117–126. https://doi.org/10.21580/jnsmr.2023.9.2.17835

Handayani, T. (2022). Aktivitas antioksidan, total fenolik, dan total flavonoid madu *Apis mellifera* dari hutan akasia (*Accacia crassicarpa*) Riau, Indonesia dengan beberapa perlakuan pengeringan. *Jurnal Biologi Indonesia, 18*(2), 231–243. https://doi.org/10.47349/jbi/18022022/231

Hidayat, L., Riyadi, S., Gustiani, S., & Dwicahya, A. (2022). Aplikasi ekstrak jintan hitam (*Nigella sativa* L.) sebagai zat antibakteri pada kain kapas dengan variasi metode. *Arena Tekstil, 37*(1). https://doi.org/10.31266/at.v37i1.7730

Jeyaraj, E. J., Lim, Y. Y., & Choo, W. S. (2021). Extraction methods of butterfly pea (Clitoria ternatea) flower and biological activities of its phytochemicals. Journal of Food Science and Technology, 58(6), 2054–2067. https://doi.org/10.1007/s13197-020-04707-2

Kręgiel, D., Berłowska, J., Witońska, I., Antolak, H., Proestos, C., Babić, M., et al. (2017). Saponin-based, biologically active surfactants from plants. In *Application and Characterization of Surfactants*. IntechOpen. https://doi.org/10.5772/68062

Kurniati, N., Baharuddin, M., & Zahra, U. (2022). Study of secondary metabolites of *Jatropha gossypifolia* leaf extract and its activity against *Propionibacterium acne*. *Teknosains Media Informasi Sains dan Teknologi, 16*(3), 324–334. https://doi.org/10.24252/teknosains.v16i3.29721

Li, C., Yao, M., Ma, C., Ma, J., Jin, J., & Chen, L. (2015). Differential metabolic profiles during the albescent stages of 'Anji Baicha' (*Camellia sinensis*). *PLOS ONE, 10*(10), e0139996. https://doi.org/10.1371/journal.pone.0139996

Liang, J., Zhang, G., Song, Y., He, C., & Zhang, J. (2022). Targeted metabolome and transcriptome analyses reveal the pigmentation mechanism of *Hippophae* (sea buckthorn) fruit. *Foods, 11*(20), 3278. https://doi.org/10.3390/foods11203278

Ma, Q., Hu, Y., Dong, X., Zhou, G., Liu, X., Gu, Q., et al. (2021). Metabolic profiling and gene expression analysis unveil differences in flavonoid and lipid metabolisms between 'Huapi' kumquat (*Fortunella crassifolia* Swingle) and its wild type. *Frontiers in Plant Science, 12*, 759968. https://doi.org/10.3389/fpls.2021.759968

Marpaung, A. M., Andarwulan, N., Hariyadi, P., & Faridah, D. N. (2020). The colour stability of butterfly pea (*Clitoria ternatea L.*) anthocyanin extract against pH and temperature. Journal of Nutritional Science and Vitaminology, 66(1), 1–8. https://doi.org/10.3177/jnsv.66.1

Muawanah, S., Febrina, D., & Sunarti, S. (2023). Identifikasi senyawa metabolit sekunder pada hasil ekstraksi bertingkat bunga telang (*Clitoria ternatea L.*). *Pharmacy Genius, 2*(3), 189–197. https://doi.org/10.56359/pharmgen.v2i3.296

Ningsih, A., Syahrani, A., Septama, A., & Sukardiman, S. (2023). Metabolite profiling and antibacterial activity of ethanol extract of the unripe fruit peel of kayu banana (*Musa paradisiaca* L. var. kayu). *Trends in Sciences, 21*(2), 7154. https://doi.org/10.48048/tis.2024.7154

Oguis, G. K., Gilding, E. K., Jackson, M. A., & Craik, D. J. (2019). Butterfly pea (Clitoria ternatea), a cyclotide-bearing plant with applications in agriculture and medicine. Frontiers in Plant Science, 10, 645. https://doi.org/10.3389/fpls.2019.00645

Okstaviyani, E., Lestari, P., Kawiji, K., Anandito, R., Yulviatun, A., Sefrienda, A., et al. (2024). Antioxidant, physicochemical and rheological properties of white and milk chocolate compounds supplemented with plant-based functional ingredients. *Foods, 13*(22), 3694. https://doi.org/10.3390/foods13223694

Putra, T., Zainol, M., Isa, N., & Maidin, N. (2021). Chemical characterization of ethanolic extract of butterfly pea flower (*Clitoria ternatea*). *Food Research, 5*(4), 127–134. https://doi.org/10.26656/fr.2017.5(4).744

Putri, T. F., Wasita, B., & Indarto, D. (2022). The effects of ethanol extract of asian pigeon wings (clitoria ternatea l.) flower on body weight and malondialdehyde level in diabetes rat model. Proceedings of the 4th International Conference on Life Sciences and Biotechnology (ICOLIB 2021), 303-311. https://doi.org/10.2991/978-94-6463-062-6_30

Qaisar, M., Kanwal, F., Siddique, M., Akram, M., Khan, Y., Inayat, H., et al. (2023). Detailed characterization of saponins isolated from *Zygophyllum propinquum* Decne. *International Journal of Biosciences, 22*(1), 70–76. https://doi.org/10.12692/ijb/22.1.70-76

Ribeiro, B., Barreto, D., & Coelho, M. (2013). Application of foam column as green technology for concentration of saponins from sisal (*Agave sisalana*) and juá (*Ziziphus joazeiro*). *Brazilian Journal of Chemical Engineering, 30*(4), 701–709. https://doi.org/10.1590/s0104-66322013000400002

Riga, R., Silvani, M., Oktria, W., Nasra, E., Kurniawati, D., & Agustini, D. (2023). Jamur endofitik BJS-3 asosiasi sambiloto (*Andrographis paniculata*): Skrining fitokimia dan aktivitas antioksidan. *Jurnal Sains dan Kesehatan, 5*(5), 695–700. https://doi.org/10.25026/jsk.v5i5.2034

Setiawan, E., Setyaningtyas, T., Kartika, D., & Ningsih, D. (2017). Potensi ekstrak metanol daun mangga bacang (*Mangifera foetida* L.) sebagai antibakteri terhadap *Enterobacter aerogenes* dan identifikasi golongan senyawa aktifnya. *Jurnal Kimia Riset, 2*(2), 108. https://doi.org/10.20473/jkr.v2i2.5753

Shen, K.-H., Hung, J.-H., Chang, C.-W., Weng, Y.-T., & Wu, M.-J. (2022). Antioxidant and anti-inflammatory activities of anthocyanin-rich extracts from butterfly pea flower (*Clitoria ternatea L.*). Molecules, 27(9), 2850. https://doi.org/10.3390/molecules27092850

Silvani, M., Riga, R., & Agustini, D. (2023). Aktivitas antioksidan jamur endofitik BS-1 yang diisolasi dari bunga sambiloto menggunakan beras putih sebagai media pertumbuhan. *Jurnal Sains dan Kesehatan, 5*(2), 149–156. https://doi.org/10.25026/jsk.v5i2.1734

Smith, E. L., Abbott, A. P., & Ryder, K. S. (2014). Deep eutectic solvents (DESs) and their applications. Chemical Reviews, 114(21), 11060–11082. https://doi.org/10.1021/cr300162p

Tsibranska, S., Ivanova, A., Tcholakova, S., & Denkov, N. (2019). Structure of dense adsorption layers of escin at the air–water interface studied by molecular dynamics simulations. *Langmuir, 35*(39), 12876–12887. https://doi.org/10.1021/acs.langmuir.9b02260

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound-assisted extraction in the food industry—A review. Innovative Food Science & Emerging Technologies, 9(2), 161–169. https://doi.org/10.1016/j.ifset.2007.04.014

Wan, Z., Sun, Y., Ma, L., Zhou, F., Guo, J., Hu, S., et al. (2018). Long-lived and thermoresponsive emulsion foams stabilized by self-assembled saponin nanofibrils and fibrillar network. *Langmuir, 34*(13), 3971–3980. https://doi.org/10.1021/acs.langmuir.8b00128

Zainal-Abidin, M. H., Hayyan, M., Hayyan, A., & Jayakumar, N. S. (2017). New horizons in the extraction of bioactive compounds using deep eutectic solvents: A review. Analytica Chimica Acta, 979, 1–23. https://doi.org/10.1016/j.aca.2017.05.012