Pak. J. Bot., 56(2): 697-702, 2024. DOI: http://dx.doi.org/10.30848/PJB2024-2(13)

GREEN AND EFFICIENT EXTRACTION OF 10-DEACETYLBACCATIN AND CEPHALOMANINE BY DEEP EUTECTIC SOLVENTS FROM *TAXUS CHINENSIS*

JAFAR KHAN[1], SHAKIR ULLAH[1], KHIZAR HAYAT[1], UZMA SALAM[1], SHAHID ALI[3] AND YU-JIE FU[1,2*]

[1]*Key Laboratory of Forest Plant Ecology, Northeast Forestry University, Harbin 150040, China*
[2]*Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing Forestry University, 100083 Beijing, PR China*
[3]*Key Laboratory of Optoelectronic Devices and Systems of Ministry of Education and Guangdong Province, College of Optoelectronic Engineering, Shenzhen University, Shenzhen 518060, China.*
**Corresponding author's email: yujie_fu@163.com*

Abstract

The green extraction of plant bioactive compounds is intensively needed at present. Many efforts have been practiced so far to seek greenish and environmentally benign substitutes to conventional solvents to avoid or reduce the hazardous effects of the extraction media. Microwave irradiation extraction in combination with deep eutectic solvents is used in the current study to extract 10-DAB III and cephalomanine from *Taxus chinensis.* Eight deep eutectic solvents were evaluated with betaine as a hydrogen bond acceptor and various hydrogen bond donors such as polyalcohol, organic acids, and sugars. As a controlled solvent, ethanol 80% (v/v) was used. Our findings revealed that Be-lactic acid had the maximum extraction yield of 10-DAB III (0.85 mg/g dry weight) and cephalomanine (0.80 mg/g dry weight), in contrast with conventional solvents (0.67 mg/g dry weight) and (0.30 mg/g dry weight), respectively. The extraction yield was increased significantly when the Be:LA molar ratio (mol:mol) was increased to 1:3 (0.94 mg 10-DAB III and 1.10 mg cephalomanine/g dw, respectively). Besides this, different extraction methods were also evaluated for comparative assessment, which showed the significant superiority of the DES-MAE (microwave-assisted extraction) method in extracting the target compounds compared to the DES-UAE (ultrasound-assisted extraction) and DES-HRE (heat reflux extraction) method. It is concluded that deep eutectic solvents can be an easy, reliable, safe, cheaper, and efficient extraction medium in *T. chinensis* plants compared to conventional solvents.

Key words: Cephalomanine, Deep eutectic solvents, Green extraction, *Taxus chinensis* and 10-deacetylbaccatin.

Introduction

Taxol (paclitaxel) is an alkaloid derivative that has proven effective in treating various cancers, particularly breast cancer. Paclitaxel was first reported from *Taxus brevifolia* in 1961 and later identified by (Wani *et al*., 1971; Schiff *et al*., 1979) based on its chemical structure and anti-cancerous potential (Lee & Kim, 2017). However, studies have also showni its key role on Alzheimer's and neuro-degeneration diseases, including front temporal diseses (Lee & Kim, 2019). Interestingly, paclitaxel is an inhibitor of the apoptotic cell division to speed up microtubules' polymerization and restrict the de-polymerization of microtubules (Li *et al*., 2009). Paclitaxel quantity in plant biomass is very minute, and its clinical demand exceeds the supply through other taxanes, i.e., 10-deacetylbaccatin III and cephalomanine in *Taxus* species (Fu *et al*., 2009). 10-deacetylbaccatin III has a similar skeletal structure to paclitaxel, which was identified in 1980 as a semi-synthetic material for paclitaxel (Chemat *et al*., 2019). It is a diterpene precursor of paclitaxel, isolated from *T. baccata* and *T. yunnanensis* with satisfactory quality and quantity. Furthermore, studies revealed that 10-DAB III is ten times greater than paclitaxel, which has long been used as a precursor of paclitaxel and docetaxel (Zhu *et al*., 2017). Like other *taxanes*, 10-deacetybaccatin III also plays a vital role as an anti-neoplasmic agent due to its four-member ring structure (Yang *et al*., 2016; Wang *et al*., 2018). Usually, *T. chinensis* called the Yew plant in China, is a conserved and rapidly growing species. Different studies concerning traditional phytochemicals and pharmacological approaches have shown significant anti-cancer effects (Liu *et al*., 2015). Currently, the isolation of taxanes from *T. chinensis* has been done through conventional extraction approaches (Fu *et al*., 2009; Li *et al*., 2009).

Metabolites are the basic constituents of the cells of the organisms that combine to form deep eutectic solvents (DESs), which act as hydrogen-bond acceptors (HBAs) or hydrogen-bond donors (HBDs). Recently, green chemistry is an emerging field of using green solvents as an alternative to commercial solvents, which are dangerous to the environment. Green chemistry of using solvents in industrial uses, such as cosmetics, food, and pharmaceutics, to avoid the use of toxic and hazardous solvents that is not eco-friendly to the environment. Enormous efforts have been made to overcome the problem of protecting human health and the environment from the risk of hazardous and volatile solvents (Khandelwal *et al*., 2016; Li *et al*., 2019). The number of methods were supposed to include efficient solvents that are labile for various kinds of solutes with no side effects. These solvents are currently classified as ionic liquids (ILs) and eutectic solvents and are used in academia and industry. Deep eutectic solvents (DESs) are a new class of (ILs) analogues that consist of mixtures of at least one hydrogen bond acceptor (HBA), such as choline chloride and betaine, and one (HBD) such as renewable carboxylic acids, polyols, and saccharides at room temperature (Cai *et al*., 2019). Concerning ILs, their market availability has evaluated a large amount of information related to their hazardous, transportive, and thermo-physical characteristics, as well

as phase equilibrium with subsequent and ultimate use (Choi *et al*., 2011). In general, DESs rely on forming hydrogen bonding among the binding compounds, with one having the capability of a hydrogen donator and another with a hydrogen acceptor (Nam *et al*., 2015). Although, as seen previously in fluids where hydrogen bonding is the primary concentration, showing a huge disadvantage of the higher viscous nature of these combinations in contrast with organic solvents and, in some cases, ionic liquids or even their solid and gel-like states (Radosevic *et al*., 2016; Ribeiro *et al*., 2015). DESs can be used as a substituent for organic solvents, which has fascinated widespread consideration as a new collection of green solvents.

Moreover, DESs are frequently composed of two or more non-hazardous, decomposable, inexpensive, and non-inflammable materials that can be joined together via hydrogen bonding (Chanioti & Tzia 2018). DESs are considered exclusive combinations of the spacious limit of HBAs and HBDs (Song *et al*., 2020). Furthermore, DESs are a promising solvent due to their ease of preparation, biodegradability, novel properties, and low toxicity (Qin *et al*., 2019; Ma *et al*., 2020; Wu *et al*., 2020). Although, one major drawback of the conventional extraction method is the use of huge volumes of expensive, dangerous, non-degradable and toxic organic solvents (Armenta *et al*., 2008). To address these issues, a promising new generation in the form of green media of green solvents has emerged in the last decades (Pan *et al*., 2021, 2022). As a result, the creation and better comprehension of new and environmentally friendly solvents and extraction techniques has increased the efficiency of biomass and bioactive compounds extraction (Pan *et al*., 2023). Therefore, the present work was conducted to determine the possibility of DESs to enhance the extraction of the desired compounds from *T. chinensis*, under different conditions.

Material and Methods

Plant materials and experimentation: Fresh twigs of the plant were possessed from the botanical garden of the Key Laboratory of Forest Plant Ecology, Department of Botany, Northeast Forestry University, Harbin, China. Plant samples (twigs) were separated into needles and dried for 24 hr in an electric oven set to 40°C. The dried samples were crushed into a fine powder (using an electric grinder), sieved (40 mesh) and reserved.

Different sources provided the equipment and chemical used in the present study. The lactic acid, oxalic acid, D-Sorbitol, DL-Malic acid, ethylene glycol, glycerol, glucose, and sucroseare supplied by Tokyo Chemical Industry Co. Ltd. Betaine (>98.0%) were obtained from Aladin Chemical Industry Co. Ltd (Shanghai, China). 10-deacetylbaccatin III (95%, 10-DAB III), and cephalomanine (95%, CE) were obtained from Sigma Chemical Industry Germany. The J&K Chemical Industryin Beijing, China, provided Acetonitrile. A water filtration (Milli-Q water system made in the USA) was used for the deionized water.

Preparation and screening of DESs: Binary combinations of betaine-based DES were prepared by combining the specific molar concentration of HBD with HBA resulting in translucent homogenate at 80˚C by vigorous shaking. In the same way, eight types of DES were assembled with cheap and common constituents having easy availability and biodegradation (Table 1). DESs were kept in the dark in glass bottles at room temperature, for further use (Abbott *et al*., 2004).

Screening of DESs for extraction of target compounds: A total of 1 g of pulverized powder of *T. chinens* leaves was extracted with 10 mL of DES using a 30 mL microwave irradiation flask in combination with microwave-assisted extraction (DES-MAE) apparatus, MAS-II plus (2450 Hz) normal pressure microwave synthesis/extraction response workstation (Sineo Microwave Chemistry Technology Co. LTD, Shanghai, China. The selection of solvent for the extraction of any compounds is of immense importance. Hence, to select the most suitable DES to extract the target compound from *T. chinensis* leaves, the following initial conditions were set up for the extraction:

The DESs were assessed with 30% water, and extractions were accomplished for a treatment time of 25 min at 45°C. Extracts were then placed in screw-cup tubes and centrifuged for 20 min at 6000 r/m; the supernatant was decanted and stored at 4°C until further HPLC analyses were performed. A similar method was conceded with EtOH purity, 95%, where its extraction efficiency was compared with the extraction competence exhibited by the DESs using water (80:20 v/vEtOH/ Water) as a conventional organic solvent. Overall, extractions were achieved in triplicate, and the results were articulated as milligrams of taxanes per gram of dry plant material (mg/g dw).

Table1. Different combinations of DESs ranging from 1 to 8 represent various compositions, especially solvents with low-cost, easy, and cheap availability.

Solvents	HBA	HBDs	Molar ratio	Appearance
DES-1	Betaine	D-Sorbitol	1:1	Transparent
DES-2	Betaine	Oxalic acid	1:1	Transparent
DES-3	Betaine	DL-Malic acid	1:1	Transparent
DES-4	Betaine	Glycerol	1:1	Transparent
DES-5	Betaine	Ethylene glycol	1:1	Transparent
DES-6	Betaine	Sucrose	1:1	Transparent
DES-7	Betaine	Glucose	1:1	Transparent
DES-8	Betaine	Lactic acid	1:1, 1:2, 1:3, 1:4, 1:5	Transparent

Chromatographic conformations: An Agilent 1200 HPLC system coupled with an Agilent 1200 multiple wavelength detector was used to determine the compounds. A Curosil-PFP C18 column (250mm × 4.6mm, i.d. 5 µm, Phenomenex Inc., USA) was used to separate the chromatograph of the target compounds. The mobilephase, made up of acetonitrile (A) and double-distilled water (B), was vacuumed, filtered through a 0.45 m membrane, and degassed in an ultrasonic bath for one hr. Gradient elution was used to separate 10-DAB III and CE. The crude extract was examined at 227 nm UV wavelength. Injection volume was selected as 20 µL and flow rate 1 µL/min, respectively. The temperature was retained at 25°C (Mroczek & Glowniak, 2001).

Traditional Methods

DES-Ultraviolet Assisted Extraction (DES-UAE) and DES- Heat Reflux Extraction (DES-HRE) were next applied to compare the extraction of targeted compounds using an ultrasonic bath and reflux device. The steps used for these methods were the same as those used for DES-Microwave Assisted Extraction (DES-AE).

Data analysis: Experiments were repeated three times, and the average of the three replicates was taken. The statistical analysis of data was subjected to Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) (Duncan, 1955) at $p \leq 0.05$ using COSTAT package ver. 6.4 (CoHort software Monterey, USA) according to Snedecor& Cochran, 1980). Findings were manifested as mean ± standard deviation (±SD) in tables, while bars in figures indicated SD.

Results and Discussion

Preparation of various types of DESs: In the current work prevailing conditions for DESs component selection were accessible findings, low prices, assurance and good biodegradation. Similarly, 8 different types of betaine based DESs were significantly formed as a distinct and steady combinations at normal temperatures with no solidification (Table 1). Betaine and lactic acid composition revealed significant yield, compared to control, i.e., ethanol 80% (Fig. 1). The extracted quantity of 10-DAB III and CE was noted with the evaluated DESs (except for sugar-based) for 10-DAB III, while glucose and glycerol-based DESs for CE were compared with ethanol. It is worth noting that these DESs and DL-MA-based DES for 10-DAB III, compared with other types of HBDs, showed a lower extraction capability for the target compounds. Furthermore, Be-LA accomplished the higher extractability of 10-DAB III and CE. In contrast, the least significant yield was found for the betaine and glucose composition.

The results in (Fig. 1) indicated a great variation in the extraction capacity of tested DESs due to the types of HBDs used in DESs. Be-Glu (DES-7) and Be-Suc (DES-6) DESs revealed comparatively subordinate extraction potential of 10-DAB III compared with ethanol. As shown in (Fig. 1), the extraction competence of DESs diversified well using various sorts of HBD. DESs based on sugar, i.e., Be-Sor (DES-1), Be-Glu and Be-Suc entrusted comparatively low competence for the extraction, while organic acids, amides and alcohols based showed haughty extraction ability for target taxanes. Evident from the data, 3 DESs for 10-DAB III and 6 DESs for CE, the extraction yield was comparable with ethanol, predominantly Be-LA (DES-8), Be-OA (DES-2) and Be- Gly (DES-4) for 10-DAB III, while all tested DESs exhibited higher extraction amounts of CE except Be-Gly and Be-Suc with a significant difference. Usually, the extraction capability of DESs to extract natural bio-components varies greatly due to the nature of the DESs and target compounds (Dai *et al*., 2016). In this study, the extraction capability of sugar-based DESs was the lowest. 10-DAB III and CE are non-polar molecules; consequently, the different extraction competence amongst different DESs due to their different polar nature. i.e., the organic acid-based DESs showed better extraction amongst the tested DESs because of high polarity, while sugar-based DESs showed lower results due to the least polarity (Jiang *et al*., 2019). Viscosity is another principal variable of DESs for target compound extraction because the addition of water brings a reduction in nature of the extraction source, which can enhance the extraction power of the media (Dai *et al*., 2013).

Furthermore, the acidic and basic nature of the DESs may also affect the extraction capacity. Due to acidic-basic neutralization reactions,the extraction of the targeted compounds seems higher than sugar-based DESs. The pH also plays a vital role in the stable equilibrium of taxanes. Among all the screened DESs, acid-based DESs such as Be-LA and Be-OA achieved satisfactory workouts. It is trustworthy to see that acidic DESs, i.e., Be-LA or Be-OA enabled elevated concentrations of the analyzed taxanes. On opposing, with neutral solvents, the extraction competence of taxanes decreased. HPLC outline of taxanes extracted revealed DESs as a better extraction protocol for the targeted compounds than aqueous ethanol (Fig. 2).

Variabilityassurance of the extracted DESs compounds: The DESs with different Be: LA ratios were evaluated for the extraction yields of two target compounds. With the increased molar ratio of Be: LA from 1:1 to 1:3, the derivated production of two selected compounds escalated remarkably (Fig. 3). The extraction yields of the two target compounds increased significantly when the Be: LA molar ratio was increased from 1:1 to 1:3 (mol: mol) (Fig. 3). While the Be: LA molar ratio reached 1:4 and 1:5, the extracted production of the target compounds was reduced. Previous research has shown that increasing the HBD ratio in DESs reduces viscosity and surface tension, which may enhance the transformation and diffusion (Qi *et al*., 2015; Cao *et al*., 2017a; Lamei *et al*., 2017). On the other hand, a sustained elevation in the HBD ratio in DES would reduce interactions between the target compounds and betaine (Huang *et al*., 2017). As a result, the DES-8 of Be-LA (1:3, mol:mol) with appropriate physicochemical characteristics and polarity was chosen for the following experimentations.

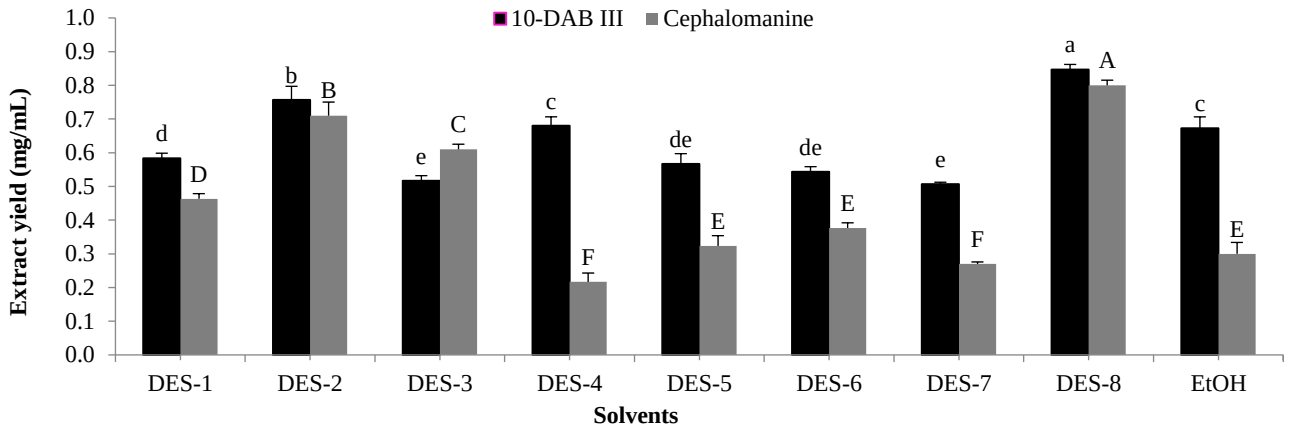

Fig. 1. Different solvent compositions, i.e DESs ranging from DES1 to DES8, while ethanol was assessed as a control medium. The mean of triplicates is presented as columns, while bars represent SD, n=3. Different letters above the columns indicate statistical differences ($p \leq 0.05$) according to DMRT.

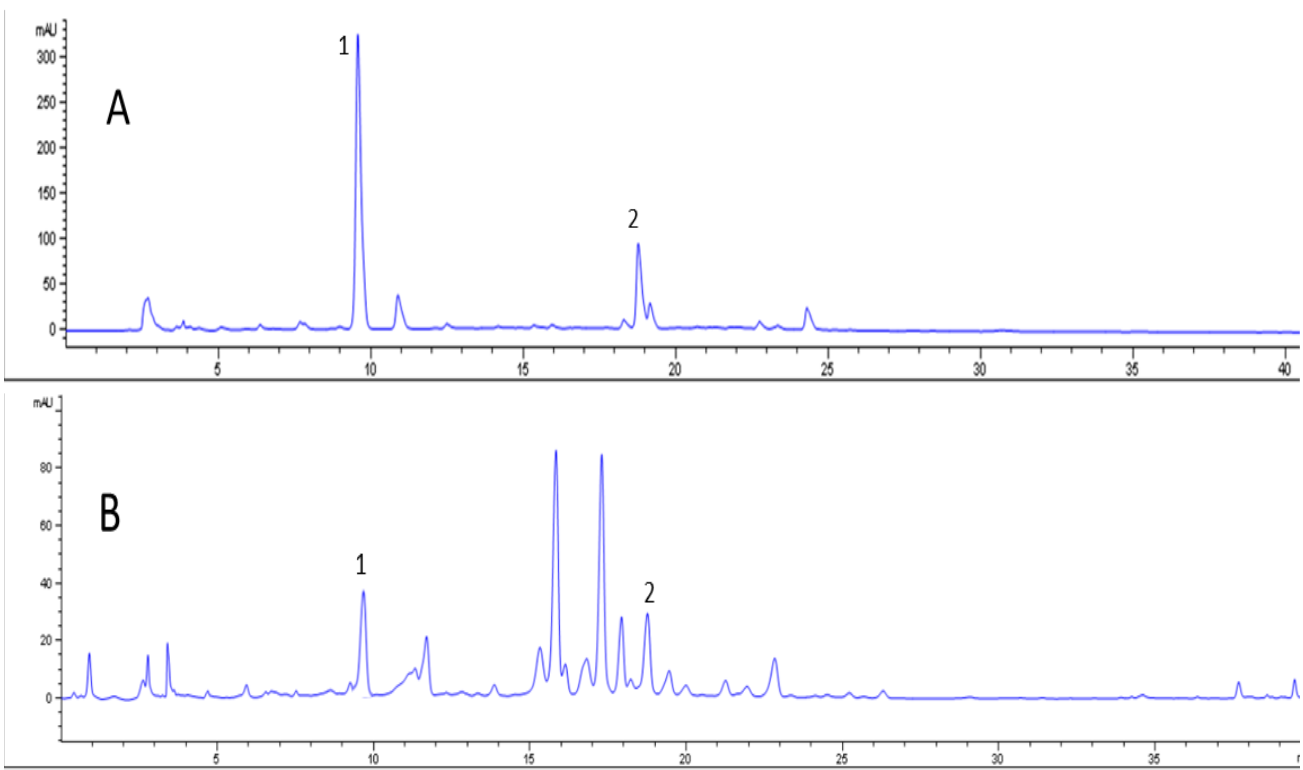

Fig. 2. HPLC chromatograms of reference compounds (A) and *T.chinensis* leaves (B) by 227 nm: (1) 10-DAB III, (2) Cephalomanine.

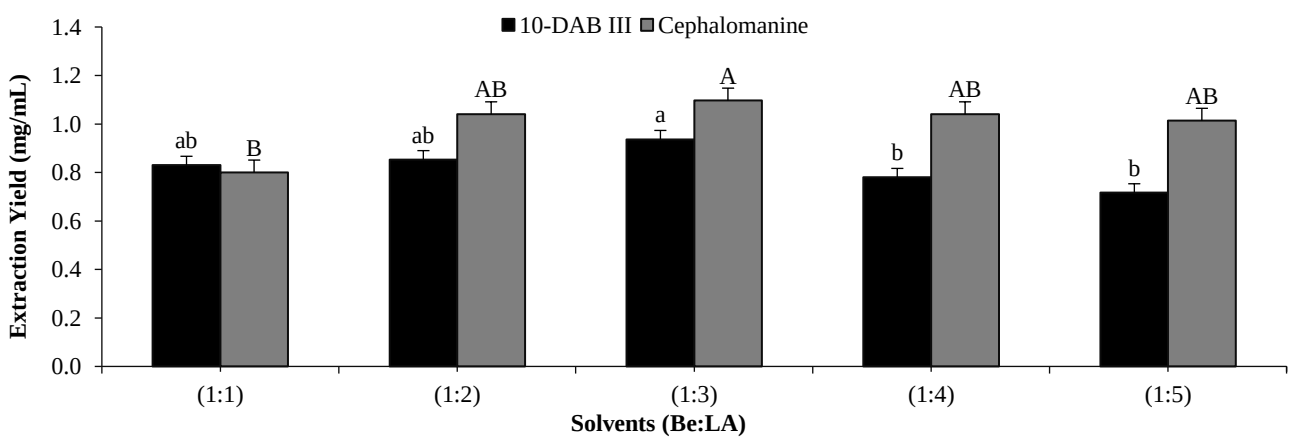

Fig. 3. DESs with different Be:LA ratios range from 1:1 to 1:5 solvents. Columns represent the mean, while bars represent SD, n=3.Different letters above the columns indicate statistical differences ($p \leq 0.05$) according to DMRT.

Table 2. Effect of comparison of extraction techniques on the extraction yield of 10-DAB III and Cephalomanine from *T. chinensis* leaves.

Extraction methods	Extraction yield (mean ± SD, mg/g DW)		Extraction conditions		
	10-DAB III	Cephalomanine	Temperature	Power	Time
DES-MAE	0.93 ± 0.032 a	1.09 ± 0.023 a	45	500	25 min
DES-UAE	0.75 ± 0.024 b	0.75 ± 0.029 b	40	250	1 hr
DES-HRE	0.68 ± 0.012 b	0.68 ± 0.009 b	80	--	2 hr
EtOH-MAE	0.67 ± 0.034 b	0.60 ± 0.004 b	45	500	25 min

Mean values with the same letter in the column are not statistically different according to DMRT ($p \leq 0.05$)

Differentiation of various extraction techniques: Various extraction techniques and media applied for the extraction of 10-DAB III and CE from *T.chinensis* were assessed and compared. The results obtained evidenced that DES-MAE significantly increased the extracted amount of the target compounds, followed by DES-UAE and DES-HRE, respectively. The ascendency in extracted yield by DES-MAE is due to the microwave irradiation effective mass transfer in terms of increasing extraction process and decreasing extraction time (Cao *et al*., 2017b). Compared to MAE extraction followed by UAE, the result was good as it extracted a high quantity of the compounds due to the easy breakage of the vegetal cells releasing effective cell contents in the extraction medium. Ultrasound-assisted extraction is based on the principle of acoustic cavitations, which can damage the cell walls of the plant matrix, thereby favoring the release of bioactive compounds from the matrix into the solvent (Huang *et al*., 2017;Yoo & Kim, 2018). The lower extraction was seen applying DES-HRE, which was incompatible with DESs due to its high viscosities and low volatilities (Table 2) (Abbott *et al*., 2004). However, no statistical differences were recorded between DES-UAE, DES-HRE, and EtOH-MAE. As a result, the present extraction way based DES-MAE was an environmentally safe, effective extraction method with improved efficiency that could be used to extract natural compounds from plant biomass (Qi *et al*., 2015).

Conclusions

The findings of the current study revealed that combining DESs with different extraction approaches, microwave-assisted extraction (MAE), was the most effective medium to extract taxanes of *T. chinensis.* Approximately all DESs methods improved the extraction of taxanes compared with the traditional solvents (ethanol 80%). The organic acid-based DESs, Betaine/lactic acid (Be-LA), were selected as the best favorable solvents and manifested to be more operational in extracting *T. chinensis* taxanes compounds, in contrast with traditional solvents. Similarly, the Be-LA influenced the main taxane content (10-DAB III and CE). Additionally, the Be-LA was found to be the finest DES for the MAE at 45 °C in extracted compounds.

Moreover, MAE was a practical approach with better extraction efficiency than UAE and HRE. Besides, the HPLC outline of taxane extracts showed that DESs were better extraction solvents for the target compounds than aqueous ethanol. The DESs could be used as a potential green solvent for the extraction of taxanes from *T. chinensis*. Our findings suggest a method for large-scale reclamation of taxanes from *T. chinensis*, with green DESs joined with innovative extraction approaches.

Acknowledgments

The authors would like to thank the China Scholarship Council (CSC) for their financial support, as well as the Key Laboratory of Forest Plant Ecology at Northeast Forestry University in Harbin, China, for providing an excellent research environment.

References

Abbott, A.P., D. Boothby, G. Capper, D.L. Davies and R.K. Rasheed. 2004. Deep eutectic solvents formed between Choline chloride and carboxylic acids: versatile alternatives to ionic liquids. *J. Amer. Chem. Soc.*, 126: 9142-9147.

Armenta, Sergio, Salvador Garrigues and Miguel de la Guardia. 2008. Green analytical chemistry. *Trends Anal. Chem.*, 27(6): 497-511.

Cai, C., W. Yu, C. Wang, L. Liu, F.Li and Z. Tan. 2019. Green extraction of cannabidiol from industrial hemp (*Cannabis sativa* L.) using deep eutectic solvents coupled with further enrichment and recovery by macroporous resin. *J. Mol. Liq*., 287: 110957.

Cao, J., M. Yang, F.L. Cao, J.H. Wang and E.Z. Su. 2017a. Well-designed hydrophobic deep eutectic solvents as green and efficient media for the extraction of Artemisinin from *Artemisia annua* leaves. *ACS Sustain. Chem. Eng*., 5: 3270-3278.

Cao, J., M. Yang, F.L. Cao, J.H. Wang and E.Z. Su. 2017b. Tailor-made hydrophobic deep eutectic solvents for cleaner extraction of polyprenyl acetates from *Ginkgo biloba* leaves. *J. Clean. Prod*., 152: 399-405.

Chanioti, S. and C. Tzia. 2018. Extraction of phenolic compounds from olive pomace by using natural deep eutectic solvents and innovative extraction techniques. *Innov. Food Sci. Emerg. Technol*., 48(July): 228-39.

Chemat, F., M.A. Vian, A.S.F. Tixier, J. Strube, L. Uhlenbrock, V. Gunjevic and G. Cravotto. 2019. Green extraction of natural products. Origins, current status, and future challenges. *Trend. Anal. Chem*., 118: 248-263.

Choi, Y.H., S.J. Van, Y.T. Dai, M. Verberne, F. Hollmann, I.W.C.E. Arends and R. Verpoorte. 2011. Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology? *Plant Physiol*., 156: 1701-1705.

Dai, Y., E. Rozema, R.Verpoorte and Y.H. Choi. 2016. Application of natural deep eutectic solvents to the extraction of anthocyanins from *Catharanthus roseus* with high extractability and stability replacing conventional organic solvents. *J. Chromatogr. A*., 1434: 50-56.

Dai, Y., J.V. Spronsen, G.J. Witkamp, R.Verpoorte and Y.H. Choi. 2013. Natural deep eutectic solvents as new potential media for green technology. *Anal. Chim. Acta*., 766: 61-68.

Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics*, 11(1): 1-42.

Fu, Y.J., R. Sun, Y.G. Zu, M. Li, W. Liu, T. Efferth, C.B. Gu, Z. Lin and H. Luo. 2009. Simultaneous determination of main taxoids in *Taxus* needles extracts by solid-phase extraction-high-performance liquid chromatography with pentafluorophenyl column. *Biomed.Chromatog*, 23: 63-70.

Huang, Y., F. Feng, L. Jiang, Y. Qiao, T. Wu, J. Voglmeir and Z.G. Chen. 2017. Green and efficient extraction of rutin from tartary buckwheat hull by using natural deep eutectic solvents. *Food Chem*., 221: 1400-1405.

Jiang, Z.M., L.J. Wang, Z. Gao, B. Zhuang, Q. Yin and E.H. Liu. 2019. Green and efficient extraction of different types of bioactive alkaloids using deep eutectic solvents. *Microchem. J*., 145: 345-353.

Khandelwal, S., Y.K. Tailor and M. Kumar. 2016. Deep eutectic solvents (DESs) as eco-friendly and sustainable solvent/catalyst systems in organic transformations. *J. Mol. Liq.*, 215: 345-386.

Lamei, N., M. Ezoddin and K. Abdi. 2017. Air assisted emulsification liquid-liquid microextraction based on deep eutectic solvent for preconcentration of methadone in water and biological samples. *Talanta*, 165: 176-181.

Lee, C.G. and J.H. Kim. 2017. A kinetic and thermodynamic study of fractional precipitation of paclitaxel from *Taxus chinensis*. *Proc. Biochem*., 59: 216-222.

Lee, S.H. and J.H. Kim. 2019. Kinetic and thermodynamic characteristics of microwave assisted extraction for the recovery of paclitaxel from *Taxus chinensis*. *Proc. Biochem*., 76: 187-193.

Li, L., K. Liu, H. Xing, X. Li, Q. Zhang, D. Han, H. He, H. Yan and B. Tang. 2019. Deep eutectic solvents functionalized polymers for easily and efficiently promoting biocatalysis. *J. Catal.*, 374: 306-319.

Li, S., Y.J. Fu, Y. Zu, R. Sun, Y. Wang, L. Zhang, H. Luo, C. Gu and T. Efferth. 2009. Determination of paclitaxel and other six taxoids in *Taxus* species by high-performance liquid chromatography–tandem mass spectrometry. *J. Pharm. Biomed. Anal.*, 49: 81-89.

Liu, Z., X. Zheng, J. Lv, X. Zhou, Q. Wang, X. Wen, H. Liu, J. Jianng and L. Wang. 2015. Pharmacokinetic synergy from the taxanes extract of *Taxus chinensis* improves the bioavailability of paclitaxel. *Phytomedicine*, 22: 573-578.

Ma, S., F. Li, L. Liu, L. Liao, L. Chang and Z. Tan. 2020. Deep-eutectic solvents simultaneously used as the phase-forming components and chiral selectors for enantioselective liquid-liquid extraction of tryptophan enantiomers. *J. Mol. Liq*., 319: 114106.

Mroczek, T. and K. Glowniak. 2001.Solid-phase extraction and simplified high-performance liquid chromatographic determination of 10-deacetylbaccatin III and related taxoids in yew species. *J. Pharm. Biomed. Anal*., 26: 89-102.

Nam, M.W., J. Zhao, M.S. Lee, J.H. Jeong and J. Lee. 2015. Enhanced extraction of bioactive natural products using tailor-made deep eutectic solvents: Application to flavonoid extraction from *Flossophorae*. *Green Chem.*, 17: 1718-1727.

Pan, H., S. Nie, C. Li, L. Zang, X. Sun, A. A. Elateeq, S. Yan and Y. Fu. 2023. A green and novelstrategy based on CO_2-responsive surfactant-functionalized multi-walled carbonnanotubes with microwave-ultrasound assistant to improve extraction andenrichment of phytochemicals from plant waste. *Ind. Crops Prod*., (in press).

Pan, H., S. Nie, Z. Wang, L. Yu, Z. Liu, J. Xu and Y. Fu. 2022. Ultrasound-assisted extraction of phytochemicals from Cili leaves with a novel CO_2-responsive surfactant aqueous and extraction mechanism. *Ind. Crops Prod*., 175: 114241.

Pan, H.Y., Z. Wang, S. Nie, L. Yu, Y. Chang, Z. Liu, J. Xu and Y. Fu. 2021. Novel green three-constituent natural deep eutectic solvent enhances biomass extraction from *Acanthopanax senticosus* and the extraction mechanism. *ACS Sustain. Chem. Eng*., 9(26): 8835-8847.

Qi, X.L., X. Peng, Y.Y. Huang, L. Li, Z.F. Wei, Y.G. Zu and Y.J. Fu. 2015. Green and efficient extraction of bioactive flavonoids from *Equisetum palustre* L. by deep eutectic solvents-based negative pressure cavitation method combined with macroporous resin enrichment. *Ind. Crops Prod*., 70: 142-148.

Qin, H., Z. Song, Q. Zeng, H. Cheng, L. Chen and Z. Qi. 2019. Bifunctional imidazole-PTSA deep eutectic solvent for synthesizing long-chain ester IBIBE in reactive extraction. *AI Ch E J.*, 65(2): 675-683.

Radosevic, K., N. Curko, V.G. Srcek, M. Tomasevic, G.K. Kovacevic and R.I. Radojcic. 2016. Natural deep eutectic solvents as beneficial extractants for enhancement of plant extracts bioactivity. *LWT - Food Sci. Tech*., 73: 45-51.

Ribeiro, B.D., C. Florindo, L.C. Iff, M.A.Z. Coelho and I.M. Marrucho. 2015. Menthol based eutectic mixtures: hydrophobic low viscosity solvents. *ACS Sustain. Chem. Eng*., 3: 2469-2477.

Snedecor, G.W. and W.G. Cochran. 1980. *Statistical Methods*. 7th ed. Iowa State University Press, Ames, Iowa, USA.

Song, Z., X. Hu, H. Wu, M. Mei, S. Linke, T. Zhou and K. Sundmacher. 2020. Systematic screening of deep eutectic solvents as sustainable separation media exemplified by the CO_2 capture process. *ACS Sustainable Chemistry & Engineering*, 8(23): 8741-8751.

Schiff, P.B., J. Fant and S.B. Horwitz. 1979. Promotion of microtubule assembly *In vitro* bytaxol. *Nature*, 277: 655-67.

Wani, M.C., H.L. Taylor, M.E. Wall, P. Coggon and A.T. McPhail. 1971. Plant antitumor agents:VI. The isolation and structure of taxol, a novel antileukemic and antitumoragent from *Taxus brevifolia*. *J. Am. Chem. Soc*., 93: 2325-7.

Wang, B., S. Xu, Y. Cao, F. Liu, X. Zhao and X. Feng. 2018. Fungicidal activity of 10 deacetylbacatin III against *Phytophthoracapsici via* inhibiting lysine biosynthesis. *Pestic. Biochem. Physiol*., 152: 114- 121.

Wu, Z., Q. Zeng, H. Cheng, L. Chen and Z. Qi. 2020. Extractive separation of tetralin-dodecane mixture using tetrabutylphosphonium bromide-based deep eutectic solvent. *Chem. Eng. Process.: Process Intensification*., 149, p. 107822.

Yang, L., Z.S. Zheng, F. Cheng, X. Ruan, D.A. Jiang, C.D. Pan and Q. Wang. 2016. Seasonal dynamics of metabolites in needles of *Taxus wallichiana* var. *mairei. Molecules*, 21: 1403.

Yoo, K.W. and J.H. Kim. 2018. Kinetics and mechanism of ultrasound-assisted extraction of paclitaxel from *Taxuschinensis*. *Biotecnol. Bioproc. Eng*., 23: 532-540.

Zhu, F.Z., C. Cheng, X.S. Liu, K. Zhang, L.S. WANG, M. Wang and J.M. Luo. 2017. Optimization of biotransformation technology for 10-DAB production from baccatin III using response surface methodology. *Biotechnol. Bull*., 33(4): 238-246.

(Received for publication 15 November 2022)