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SHORT COMMUNICATION

Comparative Study of Deep Eutectic Solvents (des) Based Ultrasonic Assisted Extraction (UAE) and other Extraction Methods using *Acalypha indica*

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Abstract

Background: Ultrasonic aided extraction based deep eutectic solvent is an entire novel, efficient and eco-friendly method for phytochemical extraction. Flavonoids can be found in many areas of nuts, fruits, seeds, stems, flowers, vegetables and they are ubiquitous in photosynthetic cells. One of the main active ingredients that have been used to cure human illnesses is Flavonoids. Rutin and Naringenin are polyphenolic compounds having pharmacological activities. Naringenin has anti-inflammatory, anti-mutagenic and liver protective properties. Rutin has antioxidant, antidiabetic and anticancer properties. **Aim:** This study examined how deep eutectic solvents combined with ultrasonic assisted extraction influenced the rate at which flavonoids (rutin and naringenin) were extracted from the leaves of the edible and therapeutic herb *Acalypha indica* L. Despite being a weed, this plant widely distributed in this area. They eat sections of the plant for a variety of medicinal uses, including antibacterial, bronchitis, asthma, and wound healing, anthelmintic and antiulcer. **Method:** In *A. indica* L. leaves and the mixes were extracted using an ultrasonic device for 30 mins. Centrifugation was used for 30 mins 10,000 rpm, and subjected to High Performance Thin Layer Chromatography (HPTLC) to separate rutin and naringenin. **Results:** The HPTLC analysis result confirmed the presence of flavonoid components with high concentrations of rutin (Retention factor = 0.45) and naringenin (Retention factor = 0.61). The optimal mobile phase for rutin formation and quantitative analysis was determined to be a mixture of acetic acid, formic acid, water, and ethyl acetate under chamber saturation conditions. **Conclusion:** The hypothesis that the combination of ultrasonic energy and the solubilizing power of deep eutectic solvents improves efficiency and selectivity is strongly supported by the deep eutectic solvents ultrasonic assisted extraction samples consistent Retention factor value and distinct peak intensities.

Major Findings: This technique minimized the usage of hazardous organic solvents, which not only produced higher yields but also complied with environmental principles, environmentally friendly substitute for traditional extraction methods for the separation of rutin and naringenin from *A. indica* L.

Keywords: Deep Eutectic Solvent, Flavonoids, High Performance Thin Layer Chromatography, Naringenin, Rutin, Ultrasonic-assisted Extraction

Abbreviations: AUC - Area Under Curve, AR - Analytical Reagent, ChCl - Choline chloride, DES - Deep Eutectic Solvent, EG - Ethylene Glycol, GLY - Glycerol, HBA - Hydrogen Bond Acceptors, HBD - Hydrogen Bond Donors, HPTLC - High Performance Thin Layer Chromatography, PRO - L Proline, Rf - Retention Factor, RTIL - Room Temperature Ionic Liquid, SCF - Super Critical Fluids, UAE - Ultrasonic Assisted Extraction.

1. Introduction

Deep eutectic solvents, which are novel kinds of ionic liquid models, are generated through hydrogen bond interactions between hydrogen bond acceptors and hydrogen bond donors[1]. Since the beginning of "Green chemistry", a variety of chemical reactions have been conducted using carbohydrate substrates[2,3].

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These include the use of natural carbohydrate polymers as renewable feedstocks, biocatalysed chemical transformations, Solvent-free reactions, the development of multistep reactions that preserve atom economy, and the use of water and biodegradable solvents, Room Temperature Ionic Liquids (RTILs), Deep eutectic solvents, supercritical fluids[4,5]. Furthermore, a variety of unconventional energy sources have been used to perform reactions, electrochemical reactions, ultrasonication, ball milling, microwave irradiation and reactions under high pressure[6]. In the words of Abbott *et al.*, DES is a recently developed non-toxic and ecologically friendly plasticiser. Based on Shamsuri , the plasticiser and biopolymer will form a further connection owing to this DES mixture. Considering the DES is acidic, the combination of hydrogen bond acceptor (choline chloride) and hydrogen bond donor (glycerol) will result in a mixture with a low pH value that may stop bacterial development[7]. These days, its common practice to extract bioactive natural chemicals from plant material using traditional organic solvents including methanol, ethanol, chloroform *etc*. Large-scale use of these volatile and dangerous organic solvents, however, may pollute the environment and leave undesirable solvent residues in extracts. A new kind environmentally friendly and sustainable solvent is already taking shape: Deep eutectic solvents. DESs are made by simply combining two or more inexpensive, biodegradable, naturally occurring substances to create a eutectic combination. Because of the components accessibility, affordability, biodegradability, and environmental friendliness, DESs are flexible substitutes for traditional organic solvents. DESs have recently been used to extract and separate flavonoids, saponins, and bioactive phenolic chemicals from plant sources. However, there aren't many publications on using DESs to extract natural products and it's still unclear how effective DESs are in extracting other kinds of active natural compounds like anthraquinones, alkaloids *etc*. In this study, the herbal medicine *A. indica* L. is chosen to thoroughly assess the effectiveness of DESs on the extraction of flavonoids respectively in order to expand the applications of DESs in the extraction of bioactive naturally occurring chemicals. To create the most efficient process, a number of DESs based on choline chloride, ethylene glycol, L-proline with varying mixing ratios were initially customized[8,9]. Plant-based medicinal ingredients are crucial to both conventional and western medicine. In China and India, plant-based medications are widely utilised. Plants are a rich source of various medications since they produce a wide variety of bioactive compounds. Alkaloids, tannins, flavonoids and phenolic compounds are the most significant of these bioactive plant components. These compounds are typically found in a variety of plant components, including the bark, root, leaves and shoots[10]. In addition to the overall process, economic viability and sustainability, the design of the extraction processes has a major impact on the yield and purity of the bioactive components. Extraction is a crucial stage in the production of functional beverages[11]. A unique kind of sound wave that is inaudible to humans is ultrasound. In chemistry, it is typically between 20 kHz and 100 MHz. It travels across a medium by causing compression and expansion, just like other waves. Cavitation or the creation, expansion and collapse of bubbles, is a phenomenon brought by this mechanism. The conversion of motion's kinetic energy into heat for the bubble's contents can provide a significant quantity of energy. Bubbles have a temperature of roughly 5000K, a pressure of 1000atm and a heating and cooling rate of more than 1010K/s, according to Suslick and Doktycz. The UAE was created on the basis of this idea. The cavitation effect is only present in liquids and liquids that contain solid components. Because ultrasonic radiation makes it easier for organic and inorganic components to leach from plant matrix, the primary advantage of UAE is evident in solid plant sample. For the extraction of bioactive compounds from herbal plants, UAE appears to be successful extraction method[12]. Ultrasound processing and extraction is one such system that has developed to keep the development wheel turning. When compared to conventional extraction, ultrasound uses physical and chemical processes that are fundamentally different. The overall benefits of ultrasound include increased productivity, yield, and selectivity, shorter processing times, improved quality, less physical and chemical risks and environmental friendliness. These drawbacks have prompted the adoption of new sustainable "green and innovative" methods for extraction, pasteurisation and processing that usually need less energy, water and time such as ultrasound-assisted processing[13]. DES based ultrasonic-assisted extraction may significantly

decrease solvent utilization, workforce and energy consumption when compared to other extraction technologies (conventional extraction methods) with organic solvents. Additionally, it can improve the recovery of bio-active constituents by using ultrasound-induced cavitation to breakdown the structure of plant cell walls[14]. Using HPTLC, the optimal extraction conditions and interactions between the DES and natural chemicals were investigated.

2. Materials and Methods

Rutin and Naringenin, the biomarkers, were purchased from Otto Chemie Pvt Ltd in Mumbai, Maharashtra. Choline Chloride, L Proline, Ethylene Glycol, Glycerol, Ethanol, Acetic acid, Formic acid, Ethyl acetate and Toluene - all of which are AR grade chemicals were purchased from Precision Scientific Co. Coimbatore. Merck (Coimbatore) provided the HPTLC Silica gel 60 F254 for the analysis of the samples and standards. The biomarkers (Standards) and various extracts were applied band-wise to the chromatographic plates using CAMAG and HST02. CAMAG VISION CATS software was then used to scan and document the produced HPTLC plates.

2.2 Plant Material

The Plant materials were gathered from the nearby area of Erode district, Tamil Nadu between November 2024. The gathered leaves were dried after being chopped into little bites. After drying the leaves are grounded into a coarse powder. The plant material *A. indica* L. locally known as Indian Nettle or Indian Copper leaf was authenticated by Dr. S. S. Hameed (BSI/SRC/5/23/2024-25/93) Scientist 'F' and Head of Office, Ministry of Environmental, Forest and Climate Change, Southern Regional Centre, T. N. A. C. Campus, Lawley Road, Coimbatore.

2.3 Ethanol and Water Extracts from *A. indica*

For ten days, the leaves were left at room temperature to dry in the shade. For seven days, the dried leaves (10g) were shaken occasionally while being ground into a fine powder using blenders and extracted with 100ml of 100% ethanol. Using new solvent, the extraction procedure was carried out twice. Extracts were then combined, filtered through Whatmann Filter paper No. 1, and examined. The water extract (100ml of 100% water) was prepared using the same procedure and cooked at a medium temperature (50 to 80° C)[15-19].

Table 1. List of deep eutectic solvents

S. No.	Solvent Abbreviation	Hydrogen Bond Acceptor	Hydrogen Bond Donor	Molar Ratio
1	ChCl - EG	Choline chloride	Ethylene glycol	1:2
2	Pro - Gly	L-Proline	Glycerol	2:5

2.4 DESs Preparation and Screening

Different kinds of DESs are made for this study using a previously documented process[20]. Typically, the HBAs and HBDs were mixed in an appropriate ratio and then exposed to continuous stirring using magnetic stirring equipment resulting in the development of transparent and homogeneous liquids, which is indicated as DES. Table 1 included the compounds employed, the molar ratio, and the acronyms for the DESs that were synthesized.

2.5 Preparation of UAE Method

Prepared DESs 10ml were combined with 0.5g of powder dried *A. indica* L. leaves and the mixes were extracted using an ultrasonic device for 30 mins. Centrifugation was used for 30 mins 10,000 rpm to get the supernatant[21,22].

2.6 Developing Typical Stock Solutions

Carbinol was used to create standard stocks of rutin and naringenin (1mg/mL). Additionally, rutin and naringenin stocks were prepared in varying volumes, ranging from 1.0µl to 6.0µl. To calibrate the linearity range of the band for rutin and naringenin, all volumes were applied *via* a microlitre syringe that was connected to the injector on the HPTLC plate.

2.7 Conditions and HPTLC Instrumentation

The biomarkers were analyzed using HPTLC on 200 x 100mm HPTLC plates. The HPTLC plates have been coated with the samples at a flow rate of 50 nL/s. The plates were prepared using acetic acid, formic acid, water and ethyl acetate (1.5:1.5:3.5:13.5 v/v/v/v) for rutin analysis and formic acid, ethyl acetate and toluene (0.8:4:6 v/v/v) for naringenin analysis in a

pre-saturated twin-trough glass chamber (20 x 10cm) at room temperature (25±2° C) and humidity (60±5%). In absorbance mode, the generated and dried HPTLC plates were quantitatively examined at 333-362nm and 254-450nm, respectively.

3. Results and Discussions

3.1 Development of Methods

By experimenting with various solvent compositions, the mobile phase employed in HPTLC studies for rutin and naringenin was chosen. The optimal mobile phase for rutin formation and quantitative analysis was determined to be a mixture of acetic acid, formic acid, water, and ethyl acetate (1.5:1.5:3.5:13.5 v/v/v/v) under chamber saturation conditions. However, the combination of formic acid, ethyl acetate, and toluene (0.8:4:6 v/v/v) was the most effective mobile phase for the analysis of naringenin, allowing for their distinct separation. 20 minutes and 20 milliliters of mobile phase were the ideal saturation times and volumes, respectively. The rutin and naringenin bands were clearly separated, compact, crisp, symmetrical, and had great resolution, according to the densitometric examination of the biomarkers using HPTLC techniques. The bands of naringenin were recorded at (Rf 0.609 +/- 0.043), respectively Figure 1(a) here as the bands of rutin were acquired at (Rf 0.451 +/- 0.005), respectively Figure 1(b). As a result, the developed approaches were found to have a strong baseline resolution and to be highly selective.

3.2 Evaluation of Extraction Techniques in Comparison

The area under the curve for each peak in each sample shows the concentration of rutin and naringenin. Superior extraction efficiency was demonstrated by the DES-UAE approach, which showed noticeably larger peak intensity and area than the traditional extract. The polarity of solvents has a significant impact on the extraction of several phenolic or flavonoid chemicals from plant materials, in accordance with the "like-dissolve-like" principle[23]. Rutin's peak differences with Figure 1(c) PRO-GLY (Rf = 0.427; H = 0.0476), Figure 1(d) ChCl-EG (Rf = 0.497; H = 0.0288), Figure 1(e) Ethanol rutin extraction (Rf = 0.421; H = 0.0275) Figure 1(f) Water extraction of rutin (Rf = 0.419; H = 0.0234). The plant extract's peak difference with Figure 1(g) ChCl-EG (Rf = 0.618; H = 0.0445); Figure 1(h) PRO-GLY (Rf = 0.631; H = 0.0442); Figure 1(i) ethanol (Rf = 0.597; H = 0.0357) and Figure 1(j) water (Rf = 0.497; H = 0.0288) for Naringenin. *A. indica* extracts produced using the DES-based UAE approach had greater extraction efficiency, according to data from the current study (Figure2). This is because the polarity of the extraction solvents affected how well phenolic chemicals were extracted from plant matrices[24,25].

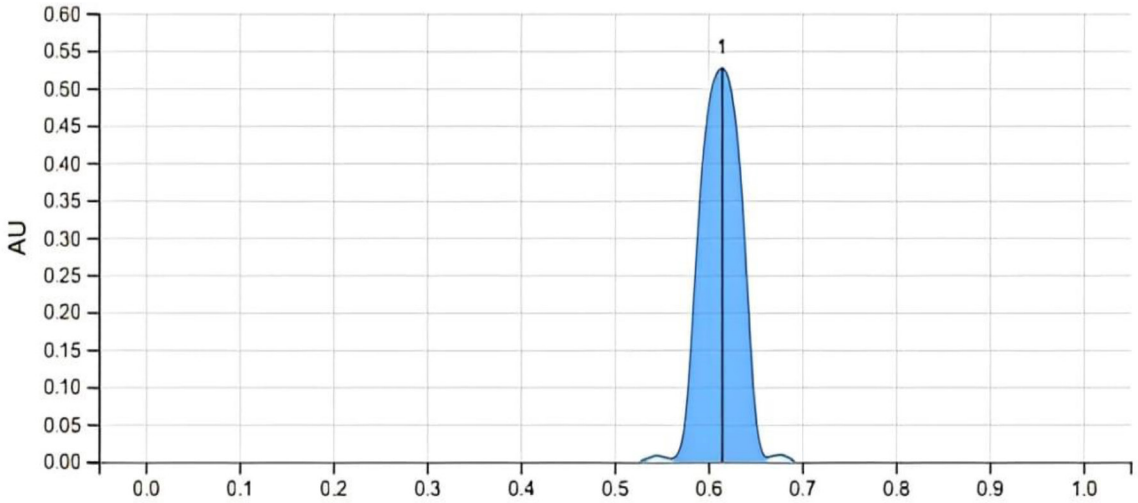


Figure 1(a). Standard - Naringenin (Rf = 0.61) chromatogram.

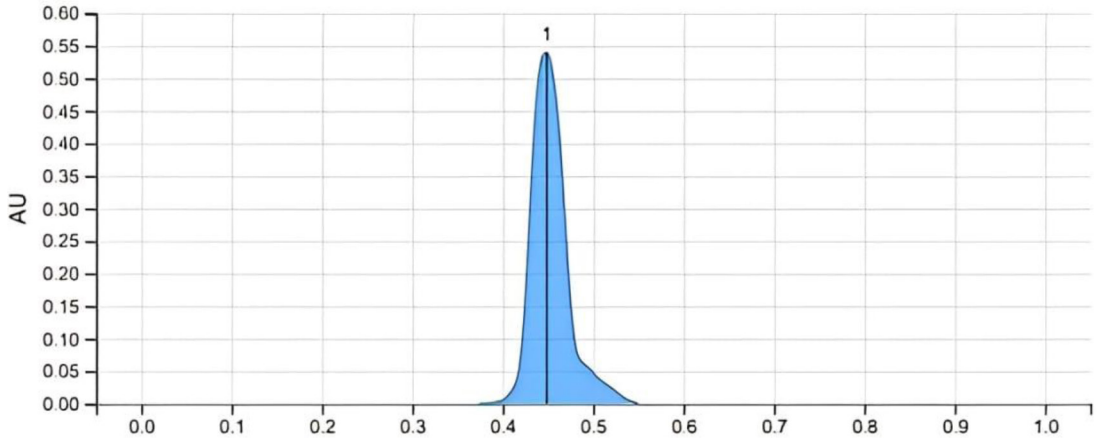


Figure 1(b). Standard - rutin (Rf = 0.45) chromatogram.

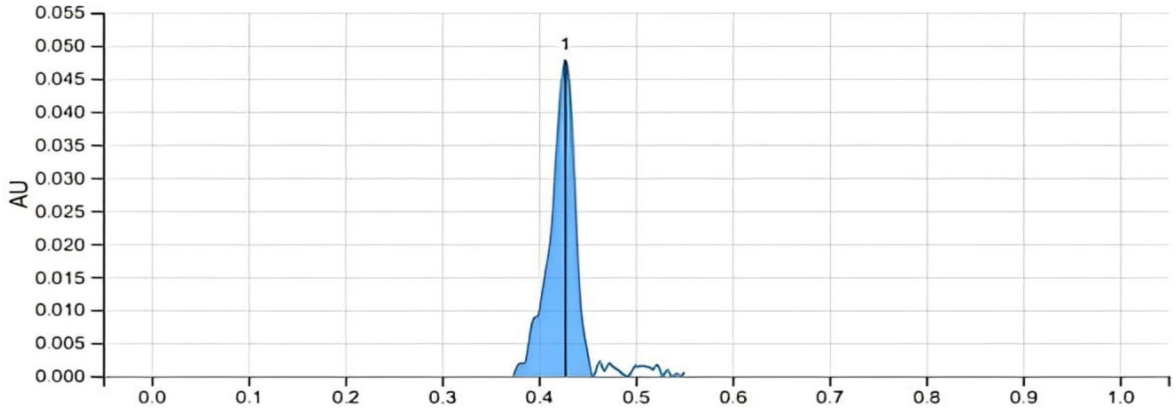


Figure 1(c). Chromatogram of PRO - GLY of rutin

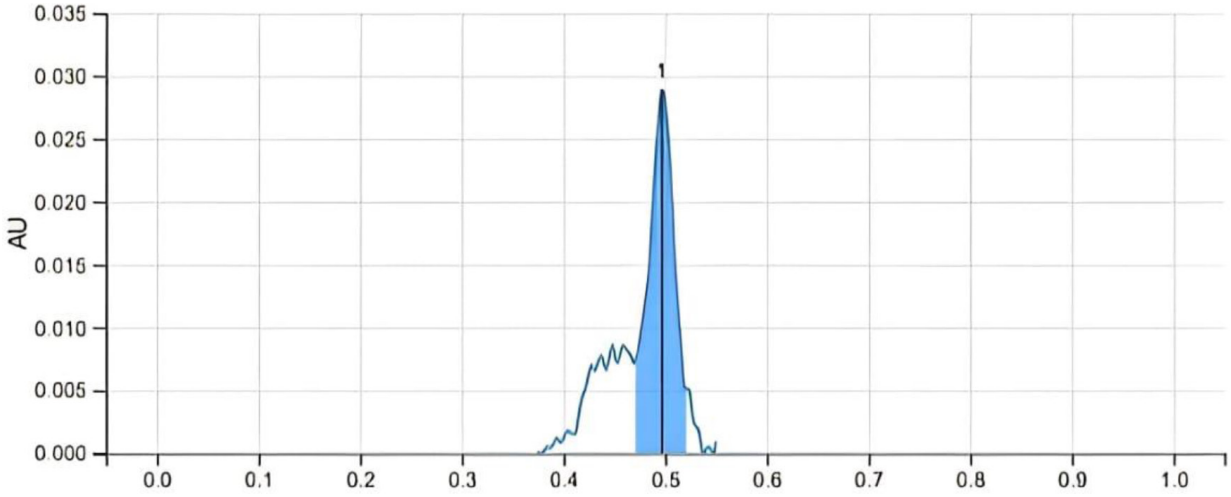


Figure 1(d). Chromatogram of ChCl - EG of rutin.

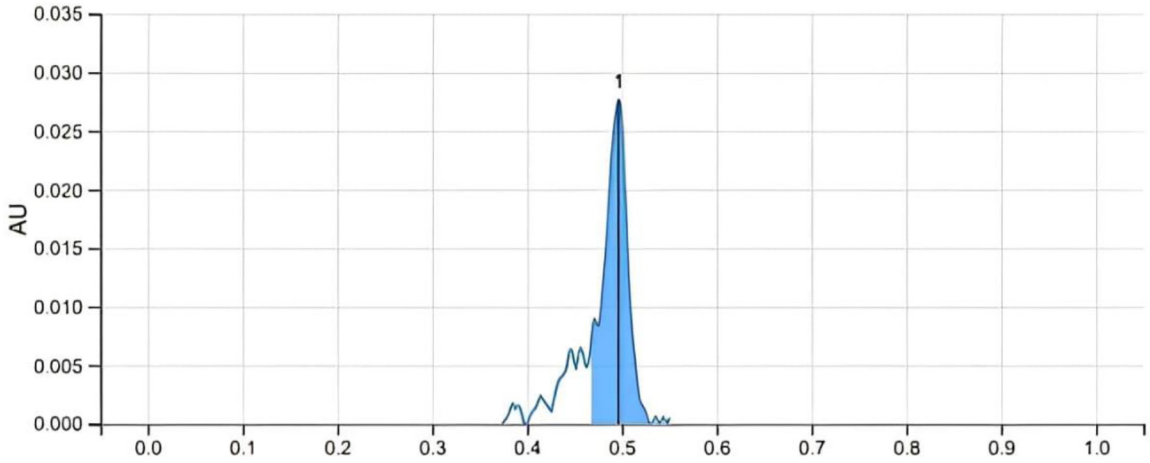


Figure 1(e). Chromatogram of ethanol extract of rutin.

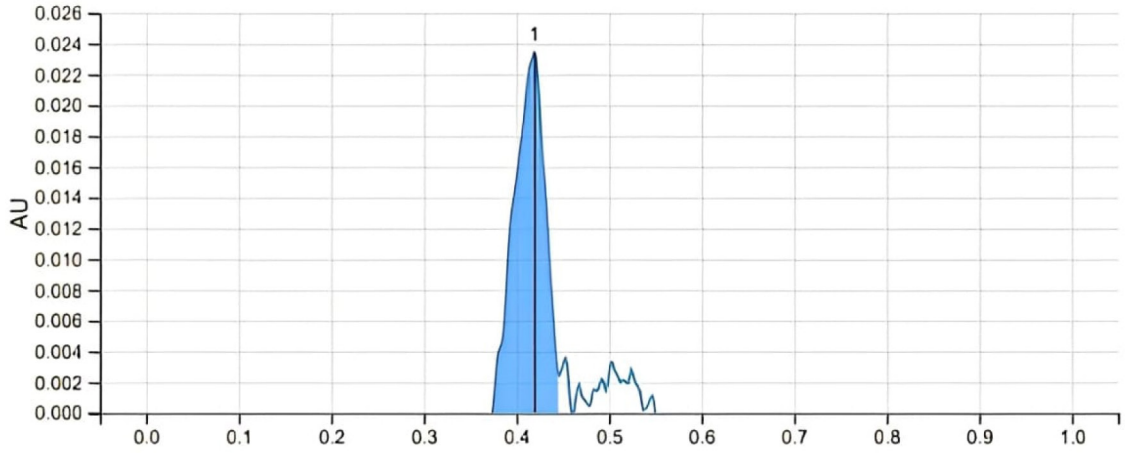


Figure 1(f). Chromatogram of decoction extract.

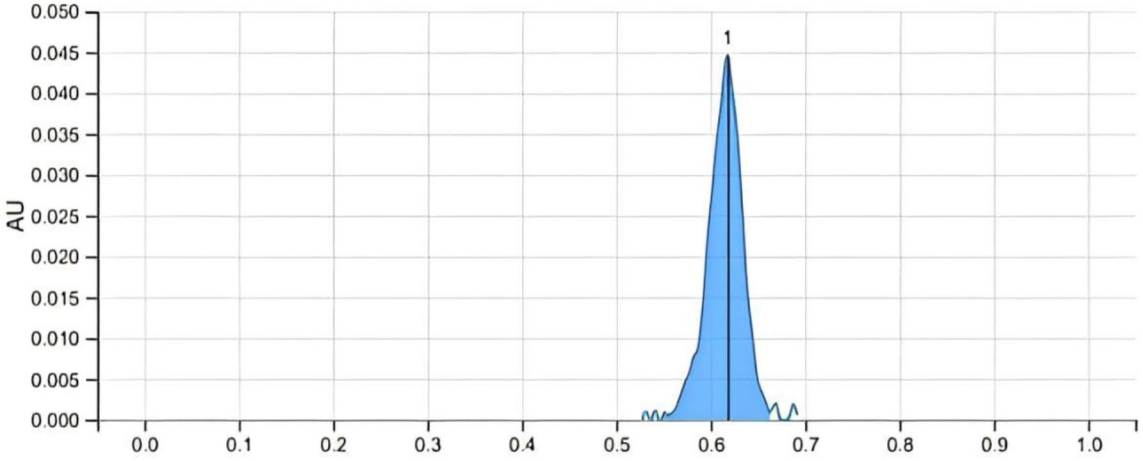


Figure 1(g). Chromatogram of ChCl - EG of naringenin.

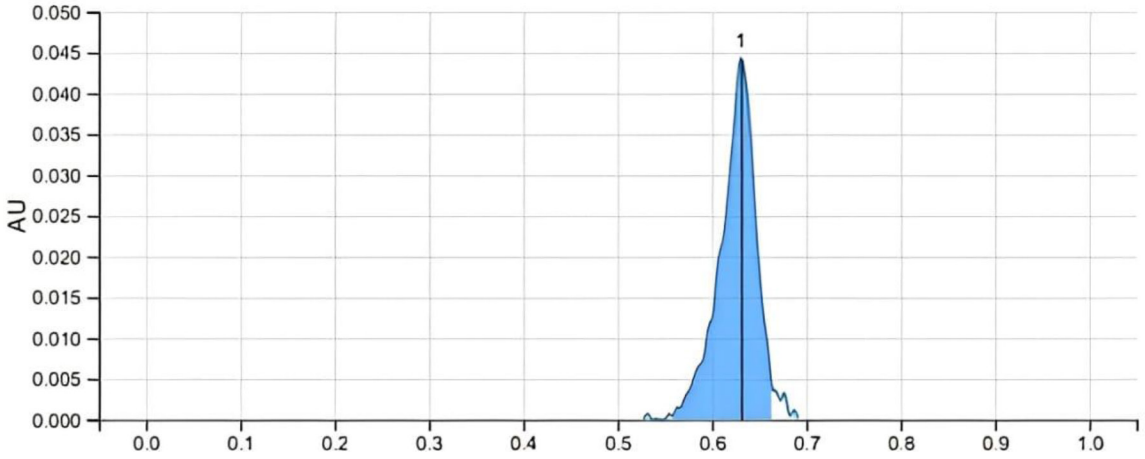


Figure 1(h). Chromatogram of PRO - GLY of naringenin.

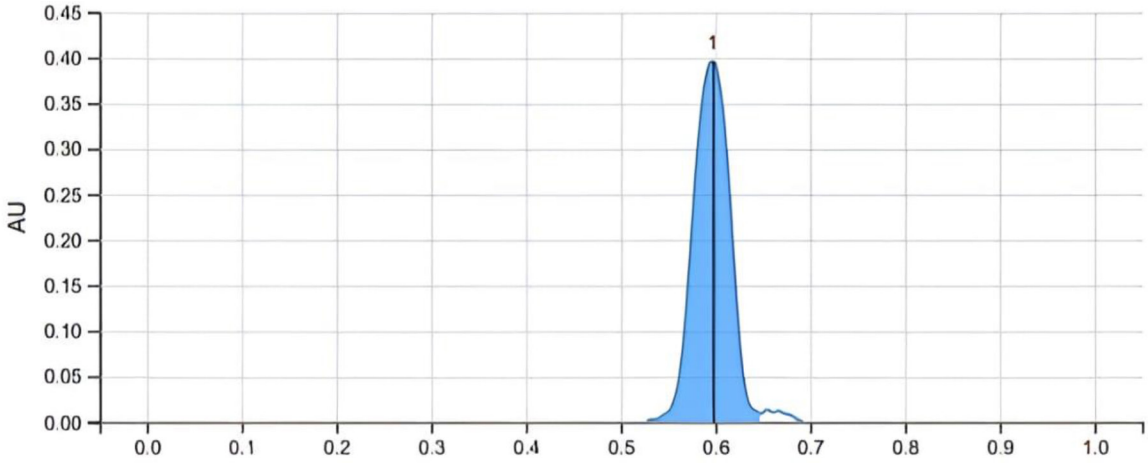


Figure 1(i). Chromatogram of ethanol extract of naringenin.

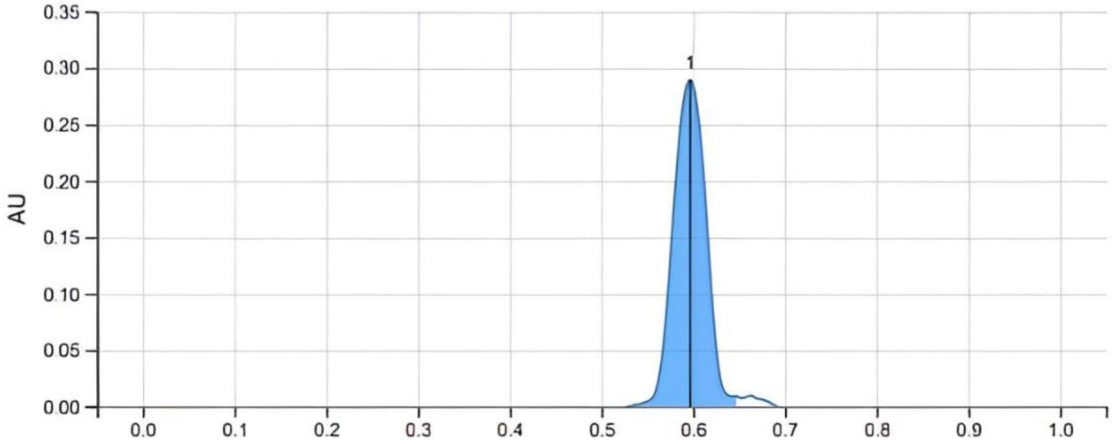


Figure 1(j). Chromatogram of decoction extract of naringenin. (H- Height, Rf- Retention factor).

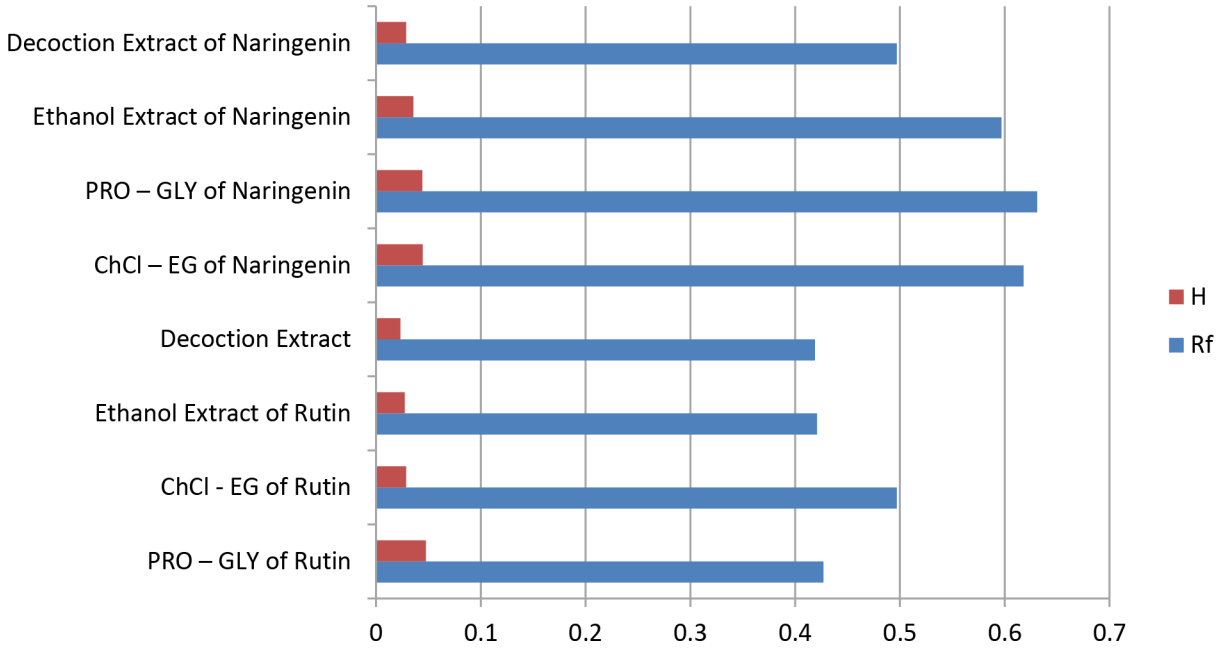


Figure 2. Graphical representation of plant extracts.

3.3 Discussion

The outcomes show how successful the DES-UAE approach is at extracting rutin and naringenin. The compound's identity is confirmed by the matching Rf values between the standard and extracts. Additionally, the DES-UAE extract's noticeably larger peak area suggests a higher yield and improved extraction efficiency. These findings are consistent with earlier studies: Because DESs may create hydrogen bonds with polar groups, it has been demonstrated that they increase the solubility of phenolic and flavonoid molecules[26]. Higher yields and less degradation of sensitive flavonoids and phenolic compounds were found in earlier DES-UAE investigations[27]. It is also important to emphasise DES-UAE's environmental advantages. Despite their effectiveness, traditional solvents like methanol and ethanol are hazardous to the environment and human health because of their toxicity and instability. DESs complement the ideas of green analytical chemistry by providing a more environmentally friendly option because they are non-toxic, biodegradable, and made from natural materials. The extraction kinetics is further enhanced by the combination of DES and ultrasound. Plant cell structures are broken down by the mechanical action of cavitation produced by ultrasonic waves, which improves solvent penetration and increases the mass transfer rate. This mechanistic benefit minimizes the thermal degradation of heat-sensitive flavonoids like rutin and naringenin by increasing the extraction yield at lower temperatures and in shorter extraction durations. Furthermore, the HPTLC method's specificity and selectivity attest to its applicability for herbal extract quality control and standardization. Flavonoids may be quickly, reliably, and economically screened using this approach, which can also be applied to other bioactive substances found in plant matrices.

4. Conclusion

To sum up, the current work effectively illustrates the potential of deep eutectic solvent-based ultrasonic-assisted extraction as a sustainable, effective, and environmentally friendly substitute for traditional extraction methods for the separation of rutin and naringenin from *A. indica* L. Superior selectivity and extraction efficiency were demonstrated by the use of HPTLC analysis, which validated increased extraction yields and improved peak characteristics in DES-UAE samples. These advancements were largely attributed to the combined action of ultrasonic energy and the special solubilizing properties of deep eutectic solvents. Additionally, DESs low toxicity and decreased need on hazardous organic solvents make them environmentally

benign, which fit in nicely with recent developments in sustainable analytical chemistry. This work opens the door for more investigation into natural DESs and their wider use in phytochemical and analytical investigations, in addition to contributing to the increasing amount of data in favor of green extraction techniques.

5. Author Contribution

Karthikeyan Sankar: Supervision, project administration, validation. Dharani and Kavin: Investigation, writing original draft and editing. All authors have read and agreed to the published version of the manuscript.

6. References

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