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Ultrasound-Assisted Extraction of Phytochemicals from *Mangifera Indica* Leaves Using Natural Deep Eutectic Solvents

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Abstract

This study aimed to evaluate the effectiveness of natural deep eutectic solvent (NADES) as a green solvent compared to methanol in enhancing phytochemical extraction yield and phytochemical recovery from *Mangifera indica* leaves via ultrasound-assisted extraction (UAE). The prepared NADES solvent was obtained by mixing choline chloride (ChCl) with oxalic acid in a 1:1 molar ratio. Characterization was conducted using FTIR and SEM-EDX analyses, followed by qualitative phytochemical screening. The percentage yield of phytochemical compounds extracted with NADES (39.57 %) was higher than that of methanol (36.35 %). The surface morphology result indicated that NADES altered the *Mangifera indica* L. texture to a uniform size and pore structure. The phytochemical screening test showed positive results in the presence of alkaloids, flavonoids, terpenoids, tannins, and phenolics. This study demonstrated that NADES, functioning as an environmentally friendly solvent, exhibited the capacity to serve as a viable substitute for conventional organic solvents. To the best of our knowledge, this is the first study reporting the use of choline chloride–oxalic acid NADES combined with UAE for phytochemical extraction from Mangifera indica leaves.

1. Introduction

Mangifera indica L. is the scientific name for all mango species, encompassing many cultivars, including the Harum Manis mango, which is specifically cultivated in Perlis, Malaysia, and prized for its unique aroma and sweetness. The region's favorable climate and soil conditions help in the production of high-quality. From a scientific perspective, the leaves of *M. indica* mangoes are typically evergreen, organized in an alternating pattern, and have a simple structure, defined by an oval shape. Their exterior is smooth and has a glossy lustre. The leaves of *M. indica* exhibit typical features found in other mango kinds. They have an elliptical shape and are arranged alternately on the stem. The upper surface of the leaves is dark green, while the underside is lighter in color [1]. Although the details may differ, mango leaves, including *M. indica* leaves, are generally recognized for their possible health advantages. These substances possess antioxidant characteristics that can aid in the neutralization of free radicals within the body [2].

Phytochemicals are naturally occurring chemical compounds found in plants that have the potential to provide health advantages as well as play an important role in their biological processes. Phytochemical compounds present in plants that give the plants aroma and flavors, including terpenoids, alkaloids, flavonoids, steroids, and tannins. The leaves of *M. indica* are thought to contain a variety of phytochemical compounds, including polyphenols, such as flavonoids and phenolic acids, which have antioxidant qualities. These compounds can help reduce oxidative stress in the body. Studies have investigated the anti-inflammatory and anticancer properties of triterpenoids, which are chemicals found in *M. indica* leaves [2]. Mangiferin, a biologically active chemical found in mangoes, including their leaves, is renowned for its ability to combat oxidative stress and reduce inflammation [2]. Moreover, *M. indica* leaves have historically been employed to address digestive problems, indicating possible advantages for gastrointestinal well-being [2].

Natural-based energetic materials, known as natural deep eutectic solvents (NADES), often consist of naturally derived molecules such as organic acids, amino acids, or sugars, and have gained attention for their environmental benefits and ease of production as a green solvent [3].These solvents are typically biodegradable and align with green chemistry principles due to simple synthesis processes involving mild mixing and heating, which reduce harmful chemical use and energy consumption [4].NADES are increasingly used for extracting bioactive compounds, offering a sustainable alternative to traditional organic solvents [5]. NADES is a solvent formed by combining two or more natural substances in a specified molar ratio, often a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD). This combination forms a eutectic mixture with a melting point lower than that of each component [3, 6]. These solvents are classified as "deep" eutectic solvents since they exhibit a substantial decrease in melting points compared to the separate components. Choline chloride (ChCl) is a quaternary ammonium salt which is HBA type commonly used to combine with various HBD compounds, producing NADES solvent.

In recent years, ultrasonic-assisted extraction (UAE) has been recognized as an efficient technique to improve the extraction process by using ultrasonic waves to generate cavitation. Cavitation degrades plant cell walls, allowing the release of bioactive chemicals into the solvent. The UAE is very advantageous when utilized alongside NADES, as it decreases extraction time, enhances yields, and preserves heat-sensitive compounds, making it optimal for delicate phytochemicals [7,8]. Numerous studies showed the effective application of UAE with NADES for the extraction of phenolic and antioxidant compounds. In the study by [9], the UAE with NADES was employed to extract phenolic components from blueberry leaves, resulting in significantly better yields compared to methanol. According to [10], the UAE with NADES was utilised for the extraction of mangiferin from mango leaves, showing its effectiveness compared to traditional methods. These findings highlight the potential of combining UAE with NADES to improve extraction efficiency and sustainability.

To the best of our knowledge, the only research that has described the extraction of phytochemical components from *M. indica* leaves are [11] and [12]. However, their works are different from the present study, which [11] extracted *M. indica* leaves for dyeing silk clothes. Distilled water was used as a solvent for the extraction process using the microwave method. After the material was brought to a boil at pH 10, an aqueous extraction was produced. This was followed by centrifugation and decantation to get the *M. indica* leaf extract. While [12] researched phytochemicals derived from *M. indica* leaves. The approach used in the research is a decoction of *M. indica* leaves with distilled water at a ratio of 1:10 (w/v). Then, a vacuum concentrator was used to remove the solvents after the extraction was conducted twice. Fortunately, no investigation on the extraction of phytochemical compounds from *M. indica* leaves with NADES (ChCl+ oxalic acid) via UAE has been reported. Also, the characterization results for proving the influences of NADES during the extraction have not yet been discussed. Therefore, this study aims to explore the potential of NADES (combining ChCl + oxalic acid) based UAE method as a simple, green, and environmentally friendly method for extracting phytochemical compounds from *M. indica* leaves. The study was aided by characterization and phytochemical screening analyses. Methanol, being an organic solvent, was used as a reference.

2. Material and Method

2.1 Chemicals

Choline chloride, ChCl (99%), Oxalic acid (99.6%, organic acid food-grade enzyme), and chloroform (99.8%) were bought from Sigma Aldrich. Methanol, MeOH (99.9%) was supplied from LabSery, sodium hydroxide, NaOH (98%) was supplied from Emory, iron (III) chloride hexahydrate (99 %), sulfuric acid (98.3%), and concentrated hydrochloric acid, HCl (37 %), were bought from Supelco. Furthermore, Iodine was supplied by PC Laboratory Reagent. Potassium iodide (99%) was purchased from R&R Chemicals. All chemicals were used in the phytochemical screening testing, including terpenoids, phenolics, tannins, flavonoids, alkaloids, and saponin compounds.

2.2 Preparation of *M. Indica* Leaves

M. indica leaves were collected from a Harum Manis mango farm area in Perlis, Malaysia. The leaves were dried overnight at 80°C in a drying oven. Then the dried leaves were ground into powder by using a grinder and sieving the powder with a pore size of 80 µm. Dried and ground leaves were kept in a zipper bag coated in aluminium foil, kept away from light, and frozen at -15°C.

2.3 Preparation of Natural Deep Eutectic Solvents (NADES)

The ratio of hydrogen bond acceptor (HBA) and hydrogen bond donors (HBD), for the preparation of a natural deep eutectic solvent (NADESs) mixture is 1:1 (mmol). Oxalic acid was used as the HBD, and ChCl as the HBA. NADES mixtures were prepared using the heating-stirring method. HBA and HBD were weighed and heated at 80°C, then stirred at 600 rpm until a homogeneous, clear liquid formed [13].

2.4 Extraction of Phytochemical Compounds from *M. indica* Leaves Ultrasound-Assisted Extraction

The powder-to-solvent ratio was 1:10 g/mL, which involves 1 g of powder *M. indica* leaves per 10 millilitres of different NADES and methanol, extracted using an ultrasonic bath (Wiggens Ultrasonic Cleaner, UE03SFD model) for 90 minutes at 40 kHz [14]. The extracts were subsequently centrifuged for 15 minutes at 30°C at 5000 rpm, and the supernatant was decanted.

2.5 Phytochemical Compounds Extraction Yield

The percentage yields of the resulting extracts were determined in triplicate and calculated by using Equation (1).

$$Yield\ (\%) = \frac{Weight\ of\ extract\ (g)}{Weight\ of\ dried\ ground\ M.indica\ \text{leaves}\ used\ (g)} \times 100\% \tag{1}$$

2.6 Characterization

2.6.1 Fourier Transform Infrared Spectroscopy

NADESs and M. indica leaves extraction were characterized using a Fourier Transform Infrared Spectroscopy (FTIR) Spectrophotometer (Perkin Elmer UATR Two) in the wavenumber range of 4000-1000 cm-1. The surface morphology of M. indica before and after extraction with NADES and methanol was observed using SEM-EDX (Model EM-30N COXEM).

2.6.2 Phytochemical Screening

Phytochemical screening was used for the qualitative analysis of *M. indica* leaves in terms of alkaloids, phenolics, tannins, flavonoids, and terpenoids.

2.6.3 Iodine Test

An iodine test reagent or alkaloid reagent was prepared by mixing 10% potassium iodide and 5% iodine. The test was performed by adding 2 to 3 drops of sample to 1mL of reagent and boiling it in a water bath until it was colorless. Once the mixture cools, the alkaloid content of the sample is shown by its formation of a blue-black color [15].

2.6.4 Ferric Chloride Test

Ferric chloride reagent or phenolic reagent was prepared by mixing 5 % $FeCl_3$ and water. The sample was then dropped a few drops into 2mL of reagent, and the formation of a blue colour indicates a positive result [10].

2.6.5 Braymer's Test

Braymer's test reagent or tannins reagent was prepared by mixing 1 % $FeCl_3$ and water. The sample was then dropped a few drops of 5mL distilled water and then boiled in a water bath. After that, the cooled mixer was mixed with 5mL reagent, formation of a blue colour indicates a positive result [14].

2.6.6 Alkaline Reagent Test

Alkaline reagent or flavonoid reagent was used at 2 mL 2% of NaOH with a few drops of the sample, and an intense yellow colour was formed. After that, a few drops of HCl will result in the solution forming colourless [14].

2.6.7 Salkowski Test

1 mL sample was mixed with 2 mL chloroform and thoroughly agitated before adding a few drops of concentrated H_2SO_4. When a reddish-brown coloring of the interface or a golden yellow precipitate was generated, the Salkowski test yielded a good result [16].

3. Results and Discussion

3.1 Percentage Yield of *M. Indica* Leaves Extract

Table 1 shows the % yield of *M. indica* leaves extract obtained during the extraction method utilizing methanol and NADES solvents. The mass and percentage yield of the *M. indica* leaves extract obtained through NADES extraction (0.3957 g, 39.57%) exceeds that obtained through methanol extraction (0.3635 g, 36.35%). Similar to [9], the blueberry leaves were extracted using the UAE method using different solvents, which are NADES and methanol. They reported that the extraction with NADES resulted in a larger amount of total phenol content compared to methanol extraction. Specifically, NADES extraction yielded 109 g of total phenol content, whereas methanol extraction yielded 86.9 g.

The increased extraction yield achieved with NADES is due to the distinctive characteristics of NADES, including their capacity to efficiently break cell structures, boost mass transfer, and improve the solubility of diverse bioactive chemicals. The precise formulation of the NADES used, which involves the amalgamation of choline chloride and oxalic acid, might potentially enhance its efficacy in extracting phytochemicals from the *M. indica* leaves ([10] [17] [18]. The good productivity achieved with NADES indicates its capacity as a viable and enduring substitute for conventional solvents such as methanol in the process of extracting bioactive components from *M. indica* leaves. The increased extraction yield with NADES aligns with recent findings that show NADES effectiveness in maximizing phenolic compound yields, as shown in blueberry extractions [9].

Ultrasonic-assisted extraction (UAE) further enhanced extraction efficiency by using ultrasonic waves to induce cavitation, a process that disrupts plant cell walls and promotes the release of bioactive compounds. According to [10], the power of UAE in extracting mangiferin from *M. indica* leaves was demonstrated, where UAE significantly boosted the extraction yield through mechanical disruption. Their study highlighted that ultrasound waves enable greater solvent penetration into plant tissues and faster solute diffusion, thereby enhancing extraction efficiency. This process not only improves yield but also reduces extraction time and preserves heat-sensitive compounds, making it ideal for delicate phytochemicals.

Table 1 *Percentage of phytochemical compounds yield in M. indica leaves*

Solvent	Methanol	NADES
Mass of dried ground leaves (g)	1.0000	1.0000
Mass of mangiferin (g)	0.3635	0.3957
Percentage yield (%)	36.35	39.57

3.2 Morphology Analysis

Scanning electron microscopy was used to characterize the surface morphology of M. indica leaves both before and after extraction. The micrograph of *M. indica* leaves before extraction (Figure 1a) shows a significant surface area with a rough, porous, and uneven texture. After the extraction process, the *M. indica* leaves exhibit surface morphological alterations. The surface morphology of *M. indica* leaf extraction by NADES (Figure 1b) is seen to be greater alteration compared to the extraction by methanol (Figure 1c). The holes obtained on the *M. indica* leaves affected by the NADES solvent showed uniform size and pores. The significant morphological alterations observed with NADES-treated leaves correspond with recent studies that document NADES' enhanced capacity to break down plant cell walls more effectively than conventional solvents, leading to uniform pore sizes and increased cellular disruption [19]. Whilst the *M. indica* leaves influenced by the methanol solvent indicated a smooth surface [20].

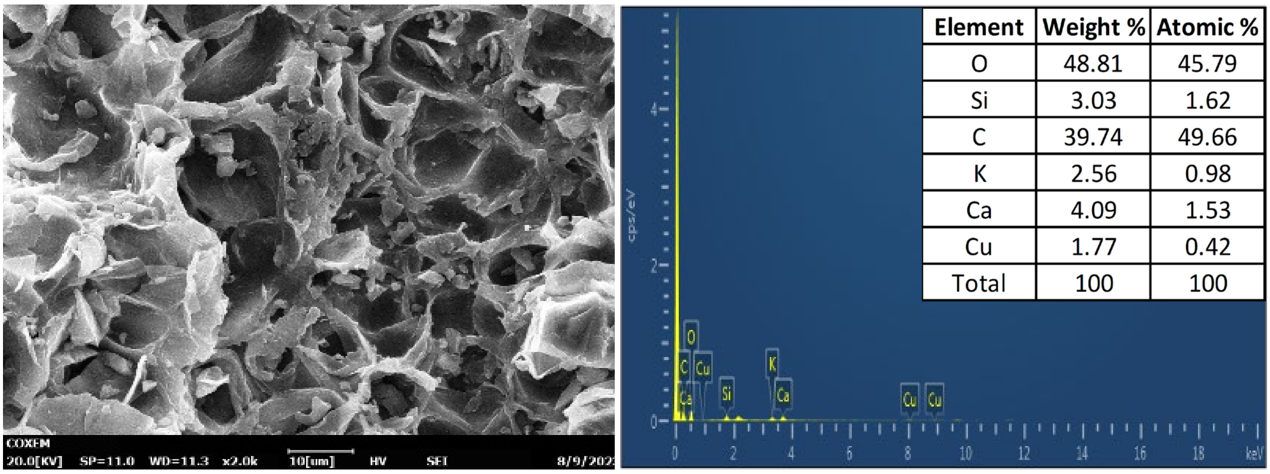
This difference in morphology between NADES and methanol-extracted leaves can be attributed to the unique properties of NADES, particularly the choline chloride and oxalic acid mixture used. NADES solvents are highly polar with extensive hydrogen-bonding capacity, allowing them to penetrate and disrupt cell walls effectively, leading to a more porous structure with uniform pores, as seen in Figure 1(b) [3] [21]. Methanol, although effective in dissolving polar compounds, lacks the same level of interaction with cell walls, which results in a smoother, less altered surface, as shown in Figure 1(c).

The increased cell wall disruption and morphological changes seen with NADES are further enhanced when combined with ultrasonic-assisted extraction (UAE). UAE's cavitation effect creates microbubbles that collapse near cell walls, intensifying the breakdown of plant tissue. This synergy with NADES leads to improved extraction yield and more visible structural alterations [7, 10]. Therefore, the combination of NADES and UAE in this study proved to be highly effective in altering *M. indica* leaf morphology, supporting the increased yield of bioactive compounds compared to methanol extraction. The larger and more uniform pores observed after NADES-UAE extraction indicate stronger cavitation-assisted disruption of plant cell walls, which enhanced solvent penetration and mass transfer efficiency.

The energy-dispersive X-ray (EDX) spectra of *M. indica* leaves exhibited distinct signals corresponding to different elements (carbon, oxygen, silicon, potassium, and calcium). The peak intensities of each element in *M. indica* leaves exhibited distinct changes in their characteristics due to the extraction solvent effect [22]. The biosorbents derived from *M. indica* leaves (Figure 1d) show weight percentages of 39.74%, 48.81%, 2.56%, 4.09%, 1.77%, and 3.03% for the elements C, O, K, Ca, Cu, and Si, respectively. Additionally, the weight percentages of these elements are 49.66%, 45.79%, 0.98%, 1.53%, 0.42%, and 1.62%, respectively. The *M. indica* leaves contain larger concentrations of the elements C and O, making them biosorbents. The extraction of *M. indica* leaves results in a decrease in element O and an increase in element C.

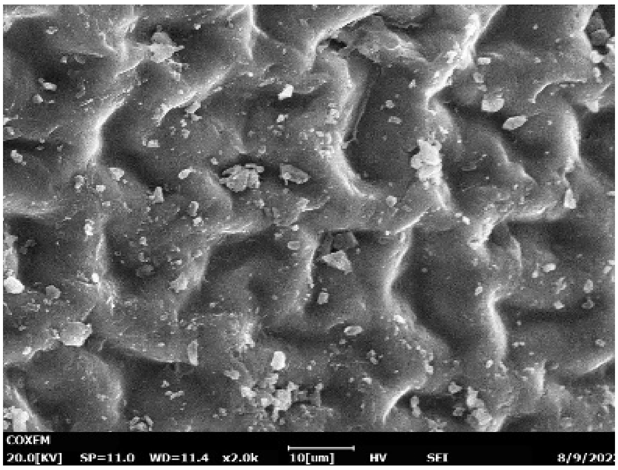
Specifically, for methanol (Figure 1e), element O represents 42.84% of the weight percentages and 40.84% of the atomic percentages, while element C represents 41.05% of the weight percentages and 52.12% of the atomic percentages. In comparison, NADES (Figure 1f) has element O representing 13.33% and 13.46% of the weight and atomic percentages, and element C represents 55.29% and 74.34% of the weight and atomic percentages. The decrease in oxygen and increase in carbon content after methanol extraction might be due to the removal of polar compounds such as carbohydrates, which are rich in oxygen, and the retention or enrichment of non-polar compounds like lipids and waxes, which contain more carbon. Like methanol extraction, the decrease in oxygen and increase in carbon after NADES extraction may indicate the removal of certain polar compounds and the retention or enrichment of non-polar compounds [22, 23].

However, the substantial increase in carbon might suggest a different selectivity of NADES in extracting carbon-rich compounds compared to methanol. Furthermore, the carbon signals exhibit increased intensity and enhanced resolution due to the utilization of an organic solvent containing carbon (Figure 1e) [22]. The addition of the appearance of elements Cl and Al after NADES extraction could be attributed to the specific components of the NADES solvent system, such as choline chloride. Choline chloride, being a salt, could introduce Cl and Al into the extracted material.

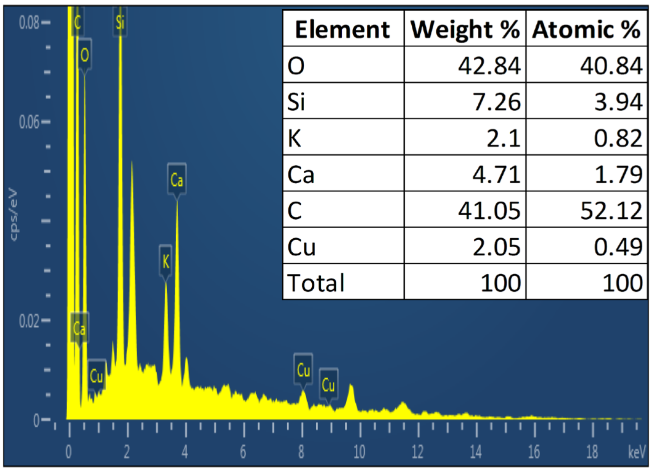


Element	Weight %	Atomic %
O	48.81	45.79
Si	3.03	1.62
C	39.74	49.66
K	2.56	0.98
Ca	4.09	1.53
Cu	1.77	0.42
Total	100	100

(a) **(d)**

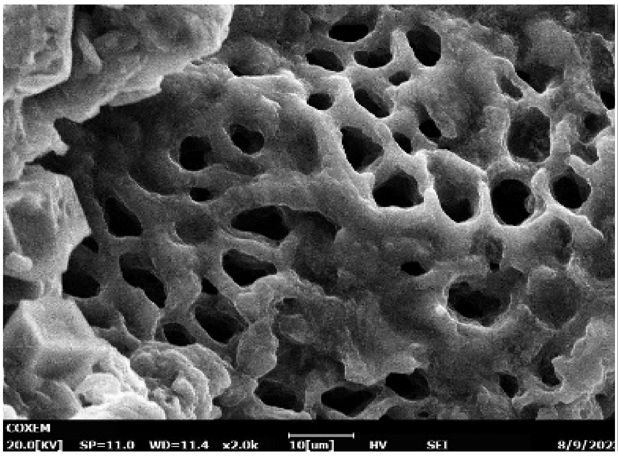


(b)

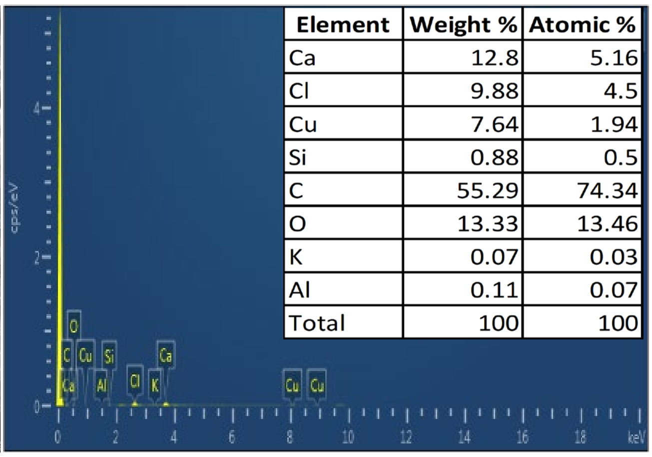


Element	Weight %	Atomic %
O	42.84	40.84
Si	7.26	3.94
K	2.1	0.82
Ca	4.71	1.79
C	41.05	52.12
Cu	2.05	0.49
Total	100	100

(e)



(c)



Element	Weight %	Atomic %
Ca	12.8	5.16
Cl	9.88	4.5
Cu	7.64	1.94
Si	0.88	0.5
C	55.29	74.34
O	13.33	13.46
K	0.07	0.03
Al	0.11	0.07
Total	100	100

(f)

Fig. 1 *SEM images of M. indica leaves (a) Before extraction; (b) After extraction with methanol; (c) After extraction with NADES; (d) Corresponding EDX spectra of M. indica leaves; (e) After extraction with methanol; and (f) After extraction with NADES*

3.3 Functional Group Analysis

In Figure 2, the synthesis mechanism of [ChCl + oxalic acid] NADES is shown. Figure 3 shows the use of FTIR spectra to identify the functional groups that are present on the [ChCl+ oxalic acid] NADES (Figure 3b), *M. indica* leaves extracted with methanol (Figure 3a), and NADES (Figure 3c). The [ChCl + oxalic acid] NADES showed a shift in the broad OH stretching peak at a lower frequency of 3330 cm^{-1}, indicating the possibility of new hydrogen bond formation [24]. Furthermore, it is shown that the C=O stretching frequency of DES showed a decrease at a frequency of 1720 cm^{-1}, indicating the possibility of new hydrogen bonding interactions [24].

Figures 3a and 3c show the FTIR spectra of *M. indica* leaves extracted from methanol and NADES, respectively. The FTIR spectra of *M. indica* leaves extracted show a broad peak at 3329 cm^{-1} for methanol extraction and 3355 cm^{-1} for NADES extraction; these are attributable to the O-H bond stretching, indicating the existence of phenolic compounds such as flavonoids and phenolic [25]. The methanol extract shows peaks at 2939 cm^{-1} and 2832 cm^{-1}, which are indicative of aliphatic C-H stretching. This observation implies the existence of organic compounds containing methyl and methylene groups. Conversely, the absence of these peaks in the NADES extract implies a possible lower concentration of aliphatic hydrocarbons in comparison to the methanol extraction [24].

The prominent peak observed at 1720 cm^{-1} indicates the stretching of C=O bonds, which is commonly associated with carbonyl groups in compounds such as esters or ketones. The observation of a comparable signal in the NADES extract suggests the presence of carbonyl groups among the extracted constituents [24, 25]. The presence of C=C stretching (aromatic compounds) and C-O stretching (alcohols or ethers) is indicated by the peaks observed at 1630 cm^{-1} and 1482 cm^{-1} in the NADES extract [24]. The presence of similar O-H stretching peaks shows that both solvents show efficacy in the extraction of phenolic chemicals, which are frequently linked to diverse health advantages. The lack of aliphatic C-H stretching peaks observed in NADES extract indicates a reduced extraction of aliphatic hydrocarbons, which may lead to a more refined extract [9]. Both extracts show carbonyl peaks, indicating the extraction of chemicals such as esters or ketones; the specific molecules may vary. The NADES spectrum shows supplementary peaks, namely those associated with aromatic chemicals, indicating a greater variety of extracted molecules in comparison to methanol [9].

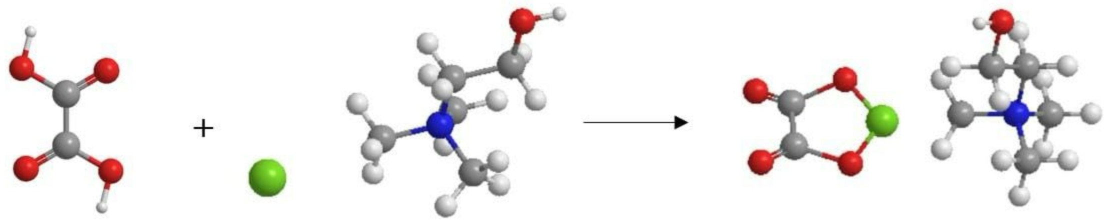


Fig. 2 *Synthesis of [ChCl+ oxalic acid]*

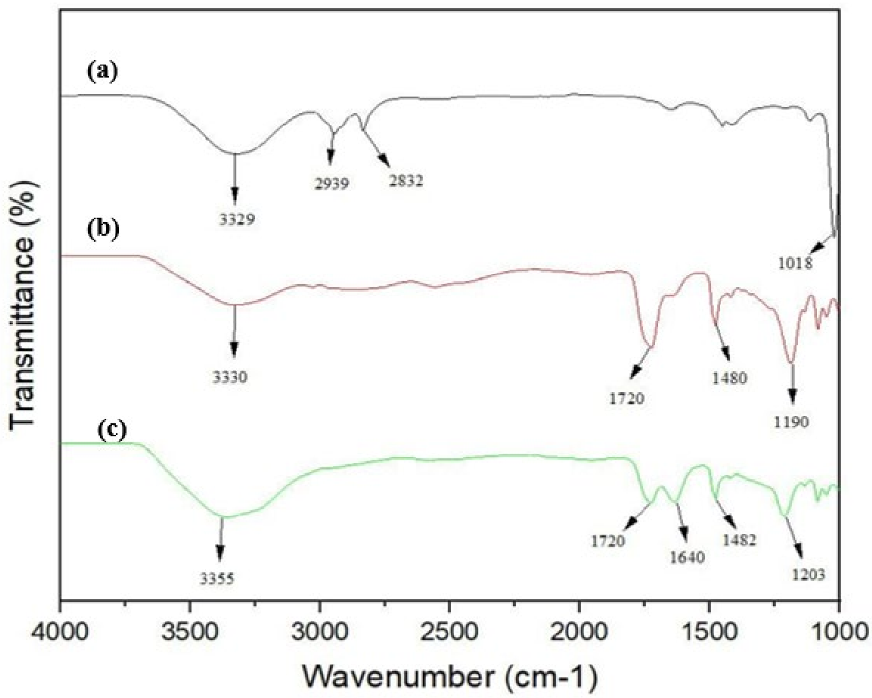


Fig. 3 *FTIR of M. indica leaves extract with (a) Methanol; (b) NADES solvent; (c) M. indica leaves extract with NADES*

3.4 Phytochemical Qualitative Analysis

Table 3 shows the results of *M. indica* leaves extract conducted a qualitative phytochemical test, which identified various physiologically significant secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, and phenolic compounds. The presence of phytochemicals was denoted by a "+" symbol, whereas the lack of phytochemicals was denoted by a "-" symbol. A similar report by [18] identified various phytochemical components, including flavonoids, phenols, steroids, glycosides, saponins, and terpenoids, in the aqueous extract of *M. indica*. Additionally, phytochemical analysis of several components of the *M. indica* plant has revealed the existence of phenolic compounds, triterpenes, flavonoids, phytosterols, and polyphenols [18].

The use of NADES in this study is effective for extracting these compounds, as its strong hydrogen bonding capacity and polarity enhance the solubility of a diverse range of phytochemicals [3]. The choline chloride-oxalic acid NADES formulation specifically aids in dissolving polar compounds such as phenolic acids and flavonoids, which have antioxidant properties that combat oxidative stress and inflammation [15]. Moreover, the acidic nature of the NADES used enhances the extraction of alkaloids and tannins, which are known to be more soluble in slightly acidic environments. This property of NADES has been shown to increase both yield and purity of these compounds compared to traditional solvents like methanol [21].

These classes are likely composed of unique chemical components that have significant health advantages. For example, alkaloids, such as caffeine or morphine, could have a role in providing pain relief and exhibiting antibacterial characteristics [9]. Flavonoids, such as quercetin or catechins, possess antioxidant and anti-inflammatory properties, which might promote cardiovascular health and enhance immunological function. Terpenoids, such as limonene or artemisinin, have potential anti-inflammatory and anti-cancer characteristics. Tannins, such as tannic acid or ellagic acid, can provide astringency and perhaps aid in wound healing. Phenolic substances, such as resveratrol or curcumin, are believed to offer antioxidant defense against chronic illnesses [18, 26].

Table 2 *Result of phytochemical qualitative analysis*

Solvent	Alkaloid	Flavonoids	Terpenoids	Tannins	Phenolic
Methanol	+	+	+	+	+
NADES	+	+	+	+	+

4. Conclusion

In summary, this study highlights the effectiveness of NADES combined with UAE in enhancing the yield and quality of phytochemical extraction from *Mangifera indica L*. NADES exhibited comparable extraction performance with a slightly higher extraction yield than methanol, achieving 39.57% compared to methanol's 36.35% and resulting in significant structural changes to the leaf morphology, favouring pore formation that facilitated compound release. Phytochemical analysis confirmed the presence of beneficial compounds, such as alkaloids, flavonoids, terpenoids, tannins, and phenolic acids, underscoring the potential of NADES as an environmentally friendly alternative to conventional organic solvents for sustainable extraction applications. Further quantitative identification using HPLC or GC-MS analyses as well as antioxidant activity, is recommended to confirm the extracted bioactive compounds.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

*The authors confirm contribution to the paper as follows: **study conception and design**: Vyranath Ko Mon, Saliza Asman; **data collection**: Vyranath Ko Mon; analysis and interpretation of results: Vyranath Ko Mon; **draft manuscript preparation**: Vyranath Ko Mon; **supervision, writing-reviewing**: Saliza Asman. All authors reviewed the results and approved the final version of the manuscript.*

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