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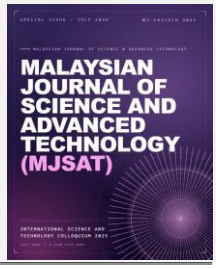

Extraction of *Senna alata* and Its Bioactive Potential: A Comparative Study of Organic and Deep Eutectic Solvents

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ABSTRACT

Senna alata is one of the Malaysian local medicinal plants with promising yet underexplored therapeutic value. Efficient extraction of plant compounds is essential to unlock its potential, with selection of solvent plays a critical role in bioactivity. This study aims to compare the extraction potential of organic solvent (ethanol) and green solvent (deep eutectic solvent, DES) using ultrasonic-assisted extraction (UAE). Phytochemical analysis indicated that *Senna alata*-ethanolic extract contained saponins, alkaloids and flavonoids, while *Senna alata*-DES extract exhibited additional compounds of tannins, phenolics and acids. The DPPH assay revealed antioxidant activity in all extracts at 0.2 mg/ml, with ethanolic extract showing the highest radical scavenging (84.08%), while DES extracts exhibited concentration-dependent RSA (50% DES: 29.1%, 60% DES: 47.7%, 70% DES: 51.2%). Conversely, *Senna alata*-DES extracts showed antibacterial activity with susceptible bacterial responses towards *Escherichia coli* and *Bacillus subtilis*, but none in *Senna alata*-ethanolic extract. *Senna alata*-50% DES extract exhibited potent antibacterial activity, with MIC and MBC values of 20 and 10 mg/mL for *E. coli* and 5 and 2.5 mg/mL for *B. subtilis*, outperforming ethanolic extracts (> 600 mg/mL). Overall, these results highlight that different extraction systems selectively enrich distinct classes of bioactive compounds, thereby influencing functional bioactivities. While ethanolic extraction appears more efficient for antioxidant enrichment, DES extraction may be more advantageous for antimicrobial applications.

1. INTRODUCTION

In recent years, interest in natural source such as plant-derived product has been increasing which offering safer and more effective options than synthetic drugs. The herbal approach had served an alternative therapeutic potential, that primarily used to eliminate the targeted diseases [23]. Local Malaysian infamous plant of Senna alata is a flowering shrub plants that is locally known as “pokok gelenggang” and similar name of Cassia alata [19]. Senna alata had been claimed to contain bioactive compounds [32], used for health benefits that functioning in various ailments, including malaria, asthma, ringworm, scabies, herpes, and eczema [9, 22]. However, the efficiency of plant extract largely depends on choice of solvent used in extraction process. The selection of solvent during the extraction process is essential, considering aspects such as selectivity, solubility, and safety [18].

Ethanol is widely employed as an organic solvent in plant xtraction because of its ability to efficiently solubilize and stabilize diverse bioactive constituents. Nevertheless, its application is associated with certain drawbacks, particularly its high flammability and volatility, which may pose safety and handling concerns [17]. In contrary, deep eutectic solvent (DES) had been synthesized from a homogenous mixture of citric acid and glycerol have emerged as promising green extraction solvent due to their favourable physicochemical properties and enhanced extraction performance. DES system offers tuneable solvent characteristics that enable selective and efficient recovery of targeted phytoconstituents [28]. Furthermore, their low toxicity, negligible volatility, and non-flammable nature position them as safer and more sustainable alternatives for plant-based extraction processes [16]. This study involved a comparative analysis between a conventional

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organic solvent of ethanol and a green solvent of DES in extracting bioactive compounds from Senna alata. The evaluation on both extracts in biological activities is also highlighted. DES introduced as an alternative extraction medium as it had additional interactions such as inter and intra-molecular interactions, Van der Waals, hydrogen bond, and electrostatic interactions, which helps in improving the extraction efficiency and biological activities [33]. This study expected to provide insights that may support the development of more sustainable extraction approaches within natural product research development.

2. METHODOLOGY

2.1 Preparation of Senna alata leaves

Fresh Senna alata leaves were obtained from a local market in Arau, Perlis (northern Peninsular Malaysia). The leaves were thoroughly washed with running tap water, rinsed with distilled water, and dried at room temperature (25–28 °C) for 7 days until constant weight. The dried material was pulverized using a mechanical grinder. The powdered leaves were stored in an airtight glass container with silica gel desiccant to minimize moisture absorption and kept in a cool, dry environment, protected from direct sunlight, until further use.

2.2 Preparation of DES solution

The deep eutectic solvent (DES) was synthesized according to Wu et al. [31]. Citric acid monohydrate was used as the hydrogen bond acceptor (HBA), while glycerol functioned as the hydrogen bond donor (HBD). The components were mixed at a mole ratio of 1:2 (1 mole of citric acid monohydrate : 2 mole of glycerol), calculated based on their respective molecular weights (210.14 g/mol for citric acid monohydrate and 92.09 g/mol for glycerol). The mixture was heated at 80 °C under continuous magnetic stirring until a clear, viscous, and homogeneous liquid was obtained, confirming the formation of DES.

2.3 Preparation of DES solutions at different concentrations

Working DES solutions at 50%, 60%, and 70% (v/v) were prepared by diluting the stock DES with deionised water to a final volume of 100 ml. Each solution was thoroughly homogenized, transferred into airtight containers, and stored at room temperature until further use.

2.4 Ultrasonic-Assisted Extraction (UAE) of Senna alata Leaves Using Different Solvent Systems

The powdered leaves were mixed with the respective solvent systems (ethanol or DES) at a solid-to-solvent ratio of 20 mg/ml. The mixtures were soaked for 1 hour. Based on Sanjaya et al. [26], the mixtures were subjected to UAE under controlled conditions: ultrasound frequency 20 kHz, power 144 W, and extraction time 30 minutes at 40ºC. Following extraction, the solutions were centrifuged at 4000 rpm for 10 minutes, and the supernatants were collected and stored at 4 °C until further analyses.

2.5 Rotary evaporation

The rotary evaporation was only performed on *Senna alata*-ethanolic extract to yield the crude extract. The initial weight of ethanolic extraction sample was weighed and recorded. Collected extracts were then evaporated using a rotary evaporator to remove the ethanol. The sample was vaporized until the formation of whey under 40ºC. After evaporation, the sample was weighed again, and the extracted yield were calculated based on equation 1

$$\%W = \frac{W_3}{W_4} \times 100 \quad (1)$$

Where W3 = Initial weight of sample,
W4 = Obtained weight of the sample.

After concentrating the crude extract using a rotary evaporator under reduced pressure, the dried residue was dissolved in 5% dimethyl sulfoxide (DMSO) to obtain stock solution at the desired concentration for downstream assays. In contrast to conventional organic solvent, the DES extracts were processed without rotary evaporation, leveraging their non-volatile and stable properties, thereby simplifying the workflow and minimizing solvent loss.

2.6 Phytochemical screening

Phytochemical screening was performed on both ethanolic and DES extracts of *Senna alata* to determine the presence of key bioactive compounds, including flavonoids, alkaloids, tannins, saponins, phenolics, and organic acids.

2.6.1 Test for flavonoid

Two millilitres of plant extract were treated with a few drops of sodium hydroxide (NaOH) solution, followed by hydrochloric acid (HCl). The appearance of an intense yellow colour that disappeared upon acid addition indicated the presence of flavonoids [24].

2.6.2 Test for alkaloid

Two millilitres of plant extract were mixed with a few drops of Wagner's reagent and gently swirled. The formation of a reddish-brown precipitate confirmed the presence of alkaloids [24].

2.6.3 *Test for tannins*

Two millilitres of plant extract were mixed with a few drops of ferric chloride ($FeCl_3$) solution and gently swirled. A brown or bluish colouration indicated the presence of tannins [24].

2.6.4 *Test for saponin*

One millilitre of plant extract was mixed with 2 mL of distilled water in a test tube, vigorously shaken, and allowed to stand for 5 min. The persistence of foam indicated the presence of saponins [10].

2.6.5 Test for phenolic

Five millilitres of plant extract were mixed with 3 mL of lead acetate solution. The formation of a dense white precipitate indicated the presence of phenolic compounds [28].

2.6.6 *Test for acids*

A mixture of 0.5 mL of plant extract and 1 mL of sodium bicarbonate solution was prepared. Effervescence confirmed the presence of organic acids [30].

2.7 *Antioxidant test*

The antioxidant activity of *Senna alata* extracts was evaluated using the DPPH assay, adapted from the method of [3] with minor modifications. A 0.1 mM DPPH stock solution was prepared by dissolving 1.97 mg of DPPH in 50 ml of absolute methanol. For the assay, 1 ml of DPPH solution was mixed with 1 ml of extract at various concentrations (0.2, 0.4, 0.6 and 0.8 mg/ml) in separate cuvettes. Ascorbic acid, prepared at equivalent concentrations, was used as the positive control. A control solution containing only DPPH and solvent (without extract) was also prepared. All mixtures were vortexed briefly, incubated in the dark at room temperature for 30 min, and absorbance was measured at 517 nm using a UV–Vis spectrophotometer. Methanol served as the blank. Each assay was performed in triplicate, and the percentage of radical scavenging activity was calculated using equation 2. The experiment was also repeated using 0.1 mM ethanolic DPPH solution with *Senna alata* extract.

$$\% \text{ RSA} = \frac{Absorbance\ of\ DPPH - Abdorbance\ of\ sampe}{Absorbance\ of\ DPPH} \times 100 \quad (2)$$

2.8 *Antibacterial test*

The antibacterial activity of Senna alata extracts was evaluated using the disc diffusion method on Mueller–Hinton agar (MHA), following CLSI guidelines. Bacterial lawns were prepared by evenly swabbing overnight cultures of Escherichia coli and Bacillus subtilis onto the agar surface using sterile cotton swabs. The bacteria suspension was prepared at 0.5 MacFarland ≈ 1-2 x 10^8 CFU/ml and checked spectrophotometrically (A625 ≈ 0.08 – 0.13).

For DES extracts, *Senna alata*-50% DES, *Senna alata*-60% DES and *Senna alata*-70% DES were tested at same concentration from extraction process (20 mg/ml). Sterile blank discs were impregnated with 20 µL for each extract. Control discs containing only DES at corresponding concentrations (50%, 60%, and 70% v/v) were included, while gentamicin served as the positive control.

For ethanolic extracts, discs were impregnated with different concentrations of *Senna alata* extract (20, 200, 300, 400, and 600 mg/ml). Negative controls included discs loaded with 5% DMSO and 80% ethanol, whereas gentamicin served as the positive control. All discs were aseptically placed on inoculated MHA plates using sterile forceps. Plates were incubated in an inverted position at 37 °C for 18–24 h. The diameter of inhibition zones was measured in millimetres, and each assay was performed in triplicate.

2.9 *Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) test*

The MIC of *Senna alata*-DES extracts were determined using the two-fold serial dilution method in sterile test tubes. Each tube contained 1 ml of nutrient broth, to which 1 ml of extract at the initial concentration was added. Serial two-fold dilutions were performed by transferring 1 ml from one tube to the next, discarding 1 ml from the final tube to maintain equal volumes. Subsequently, 100 µL of a standardized bacterial suspension was inoculated into each tube.

Controls included: (i) positive control containing bacterial suspension with antibiotic to confirm bacterial susceptibility, (ii) sterility control containing only broth and extract, (iii) broth-only control to exclude contamination, and (iv) growth control containing only bacterial suspension in broth. Tubes were incubated at 37 °C for 24 h. Bacterial growth was assessed visually and spectrophotometrically at 625 nm. The MIC was defined as the lowest extract concentration showing no visible turbidity.

For the MBC assay, 20 µl from tubes without visible growth was spread on nutrient agar plates and incubated at 37 °C for 24 h. The MBC was defined as the lowest extract concentration at which no bacterial colonies were observed.

3. RESULT AND DISCUSSION

3.1 Phytochemical compounds of Senna alata

In this study, DES was not removed from the extracted compounds because of it is becoming an integral part of the DES system, making separation difficult without losing yield or activity. Therefore, rather than removing DES entirely, this study focused on maintain green chemistry principles while ensuring efficient extraction.

The phytochemical compounds found in *Senna alata* extracts revealed notable differences between both DES and ethanolic extractions. As summarized in Table 1, all *Senna alata*-DES extracts (50% DES, 60% DES, and 70% DES) contained flavonoids, alkaloids, tannins, saponins, phenolics, and organic acids, indicating a broader spectrum of detectable bioactive compounds compared to ethanolic extracts [10]. This enhanced extraction performance of DES reflects the combined influence of solvent composition, such as the present of organic acids due to the component of DES itself which consists of citric acid. Thus, the contribution of physicochemical properties and extraction conditions may become the influence factors for the recovery of plant metabolites.

On the other hands, the *Senna alata*-ethanolic extract showed present of flavonoids, saponins and alkaloids. However, under the assay condition, no phenolics, tannins and organic acids found within the extract. This result suggested that ethanol is less efficient at extracting certain polar metabolites. The used of DES in *Senna alata* extraction medium demonstrates greater selectivity and higher recovery potential due to hydrogen bonding interactions between DES and compounds [15]. These findings underscore the suitability of DES as a versatile and environmentally friendly alternative to conventional organic solvents for phytochemical extraction.

Table 1. The phytochemical compounds presence in *Senna alata*-ethanolic extract and *Senna alata*-DES extracts.

Phytochemical compound	Ethanolic extract	DES extracts
Saponin	+	+
Flavonoid	+	+
Alkaloid	+	+
Phenolic	-	+
Tannin	-	+
Acid	-	+

*Note; "+" presence; "-" absent

3.2 Analysis of antioxidant activity

Based on the result of phytochemical screening in *Senna alata* extracts, the antioxidant compounds such as flavonoids, phenolics and tannins are known to play an important role in protecting biological systems from oxidative damage caused by reactive oxygen species (ROS) [8, 14]. In this present study, DPPH is applied to determine the free radical scavenging ability of compounds that showed antioxidant potential in *Senna alata* extracts despite using different extraction methods (Table 2).

DPPH radical scavenging activity (RSA) which expressed as % inhibition was used to evaluate antioxidant activity of *Senna alata* extracts. In this experiment, ascorbic acid was used as controlling standard. The proportion of RSA values in the *Senna alata*-DES extracts (50% DES, 60% DES, and 70% DES v/v) along with that in the *Senna-alata* ethanolic extract were tabulated according to Table 2. Different concentrations of extract (0.2, 0.4, 0.6 and 0.8 mg/mL) were used in assay to evaluate concentration-dependent changes in antioxidant activity.

The present result indicates that ethanolic extract showed high RSA value ranging from 84.08 – 87.01% in all extracts concentration. This indicates that ethanol has a higher efficiency in solubilizing antioxidant compounds, particularly flavonoids which are well-recognized as the primary contributors to radical scavenging activity [13].

Table 2. Percentage RSA in different *Senna alata* extracts.

Concentration (mg/ml)	Ascorbic acid (control)	*Senna alata* extract			Ethanolic extract
		50% DES	60% DES	70% DES	
0.2	75.58	29.07	47.67	51.16	84.08
0.4	86.63	40.70	51.74	55.23	85.45
0.6	89.53	43.35	55.81	56.98	85.51
0.8	92.44	54.65	59.30	59.30	87.01

Among all the *Senna alata*-DES extracts, 70% DES extract previously showed the highest RSA value at 59.3 % in 0.8 mg/ml concentration of extract. The relatively lower RSA values observed in DES extracts may be attributed to the limited solvent selectivity and solubilization capacity for certain phytochemicals present in *Senna alata*. Despite this limitation, the RSA values obtained from the DES extracts indicate that these solvents were still capable of recovering multiple antioxidant constituents, although with lower efficiency compared to ethanol. The presence of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) in DES may have contributed to enhancing the radical scavenging capacity of the extracts, supporting their potential role as functional green solvents in phytochemical extraction [29].

A progressive increment in RSA values was observed as the concentration of the plant extract increased, indicating that antioxidant activity became more effective at higher concentrations [7]. This finding was supported by a previous study that also showed that the DPPH radical scavenging activity of *Senna alata* was concentration dependable [4].

3.3 Analysis of antibacterial activity

In this study, *Escherichia coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive) were selected as the test organisms for the disc diffusion assay. This method was employed because it is cost-effective, allows flexible selection of antimicrobial agents, and produces clear zones of growth inhibition that are easy to interpret [5, 12]. The antibacterial potential was evaluated onto *Senna alata*–DES extracts and *Senna alata*-ethanolic extract, while gentamicin used as control standard for the test. Antibacterial activity was assessed by measuring the diameter of inhibition zones, and the bacterial responses were classified as susceptible, intermediate, or resistant according to established interpretive criteria [1].

As shown in Table 3, *Senna alata*-DES extracts at all concentrations (50% DES, 60% DES, and 70% DES) produced larger inhibition zones against both *E. coli* and *B. subtilis* that indicated susceptible bacteria responds.

The ethanolic extract of *S. alata* failed to produce any inhibition zone under the tested conditions even though different concentrations were used to adjusted from 20 mg/ml up to 600 mg/ml. This highlights the influence of solvent extraction on the recovery of bioactive compounds. Phytochemicals such as alkaloids and flavonoids are well-documented contributors to the antimicrobial properties of *S. alata* [2, 22]. However, in ethanol-based extractions, their stability and concentration may be reduced, particularly during solvent removal by rotary evaporation, which can caused compound degradation.

DES may also enhance antibacterial efficacy through mechanistic pathways. The citric acid-based DES have pH values below 3 that capable to affect the bacteria through denaturing the cell wall [6]. Moreover, the ionic and polar nature of DES can increase the solubility of phenolic and acidic compounds while simultaneously facilitating better diffusion of bioactive metabolites into bacterial cells. The DES may disrupt microbial membranes or alter permeability, thereby synergizing with plant-derived metabolites to enhance antibacterial action [29].

Table 3. The zone of inhibition of *E. coli* and *B. subtilis* for different *Senna alata* extracts.

Plant extract	**Bacteria**	**Type of disc / Extract conc.**	**Mean ± SD (mm)**	**Bacterial response**
Senna alata-50% DES	*E. coli*	Gentamicin	14.67 ± 0.58	Intermediate
		50% DES	12.33 ± 0.45	Intermediate
		20 mg/mL	16.67 ± 1.15	Susceptible
Senna alata-60% DES	*E. coli*	Gentamicin	16.67 ± 0.58	Susceptible
		60% DES	15.33 ± 4.51	Susceptible
		20 mg/mL	16.67 ± 1.15	Susceptible
Senna alata-70% DES	*E. coli*	Gentamicin	14.33 ± 4.04	Intermediate
		70% DES	17.00 ± 3.00	Susceptible
		20 mg/ml	19.67 ± 2.08	Susceptible
Senna alata-ethanolic extract	*E. coli*	Gentamicin	20.0 ± 0.00	Susceptible
		20 mg/ml	6.0 ± 0.00	Resistant
		200 mg/mL	6.0 ± 0.00	Resistant

		300 mg/mL	6.0 ± 0.00	Resistant
		400 mg/mL	6.0 ± 0.00	Resistant
		600 mg/mL	6.0 ± 0.00	Resistant
Senna alata-50% DES	*B. subtilis*	Gentamicin	32.00 ± 1.73	Susceptible
		50% DES	38.33 ± 3.06	Susceptible
		20 mg/mL	44.67 ± 2.89	Susceptible
Senna alata-60% DES	*B. subtilis*	Gentamicin	29.67 ± 1.53	Susceptible
		60% DES	41.00 ± 3.61	Susceptible
		20 mg/mL	45.33 ± 3.51	Susceptible
Senna alata-70% DES	*B. subtilis*	Gentamicin	29.67 ±1.53	Susceptible
		70% DES	41.00 ±3.61	Susceptible
		20 mg/ml	45.33 ±3.51	Susceptible
Senna alata-ethanolic extract	*B. subtilis*	Gentamicin	20.0 ± 0.00	Susceptible
		20 mg/mL	6.0 ± 0.00	Resistant
		200 mg/mL	6.0 ± 0.00	Resistant
		300 mg/ml	6.0 ± 0.00	Resistant
		400 mg/mL	6.0 ± 0.00	Resistant
		600 mg/mL	6.0 ± 0.00	Resistant

The antibacterial potential of DES has been reported in earlier study [20], where DES-based composites inhibited the growth of clinically relevant bacterial strains. This effect is often related to the physicochemical properties of DES, which may disrupt bacterial cell membranes bacterial cell membranes while improving the solubility and stability of bioactive compounds. When DES interacts with plant-derived metabolites, a synergistic effect may occur, resulting in stronger antibacterial activity compared with the action of DES or the phytochemicals individually. This interaction may explain the larger inhibition zones observed for the extract of *Senna alata* obtained using DES compared with the ethanolic extract.

However, the ethanolic extract of *Senna alata* did not show measurable inhibition zones against *E. coli* or *B. subtilis*, indicating that the bacteria were resistant response under the tested condition. Before the assay, ethanol was removed and replaced with 5% DMSO to maintain extract solubility, which also served as the negative control. DMSO is widely used to dissolve hydrophobic plant extracts, isolated metabolites, and synthetic derivatives [21]. The present of phytocompounds in the extracts may not strong to inhibit the bacteria growth. This may be related to the relatively low concentration of these compounds in the ethanolic extract, which could limit their diffusion from the discs into the agar medium [11].

These results indicate that DES may provide a more suitable extraction medium than ethanol for recovering antibacterial compounds from *Senna alata*. Besides improving extraction efficiency, DES may help stabilize sensitive phytochemicals and facilitate interactions with plant metabolites, which could contribute to the stronger antibacterial activity observed [25].

3.4 The minimum inhibitory concentration (MIC) & minimum bactericidal concentration (MBC) evaluation

The *Senna alata*-50% DES at 20 mg/ml concentration was selected as it showed the largest inhibition zone in disc diffusion test. The antibacterial activity of *Senna alata* extracts was evaluated through a two-fold serial dilution method ranging from 20 to 1.25 mg/ml. The concentration-dependent exhibited a trend of increasing turbidity as the extract concentration decreased. This observation indicated bacterial growth in both *E. coli* and *B. subtilis*. Antibacterial activity was considered when the absorbance value was below 0.05, corresponding to bacterial elimination or reduced cell viability. Visual observation also supported these findings, as the presence of turbidity reflected viable microbial cells in the broth.

In MIC determination, turbidity of *E. coli* was observed at minimum concentration of 20 mg/ml of *Senna alata*-DES extract, whereas turbidity of *B. subtilis* was observed up to 5 mg/ml of the *Senna alata*-DES extract.

MBC determination was carried out at concentration where no turbidity was detected. The extract exhibited bactericidal activity at 10 mg/mL against *E. coli* and at 2.5 mg/mL against *B. subtilis*. These values were in agreement with the MIC results, supporting the consistency of the assay. Based on these findings indicate that *Senna alata* extracted using 50% DES exhibits bactericidal activity at relatively low concentrations.

Table 4. The MIC and MBC value of *Senna alata* extracts (DES vs ethanol)

Extract type	Bacteria strain	MIC (mg/ml)	MBC (mg/ml)
50% DES	*E. coli*	20	10
50% DES	*B. subtilis*	5	2.5
Ethanolic	*E. coli*	> 600	> 600
Ethanolic	*B. subtilis*	> 600	> 600

Taken together, *Senna alata*-ethanol extract reported in previous studies [5,16] showed relatively higher MIC (31.25–250 mg/ml) and MBC (62.5–500 mg/mL) values, although MIC values as low as 0.97–1.17 mg/mL against *E. coli* have also been reported in certain cases. In the present study, the ethanolic extract was tested up to the highest concentration of 600 mg/ml. Since no inhibition was observed, the MIC and MBC values were considered to be greater than 600 mg/mL under the tested conditions. The differences observed may be related to variations in extraction conditions, and the phytochemical composition of the extract. Taken together, these findings suggest that DES-based extraction may enhance the recovery of antibacterial compounds from Senna alata and improve their antibacterial effectiveness compared with conventional ethanol extraction.

4. CONCLUSION

In conclusion, leaf extracts of *Senna alata* had showed strong potential as natural sources of antioxidant and antibacterial agent when using different types of solvent (DES and ethanol). DES was found to be a favourable solvent that demonstrating an efficient ability to extract phytocompounds from *Senna alata*. In antioxidant activity, the *Senna alata*-ethanol extract showed higher RSA than *Senna alata*-DES extracts. This is due to dose-dependent activity found in DES extracts. In addition, DES extracts found to be effective in antibacterial activity, particularly against *E. coli* and *B. subtilis*. However, no antibacterial activity was found for ethanolic extract even at highest concentration of 600 mg/ml. These outcomes indicate the potential of *Senna alata* as a source of bioactive compounds through combination of DES-UAE that

found to be an efficient method of extraction that environmentally friendly. Future work should emphasize different types of DES to improve the potential of plant extraction in bioactivities and useful in aspect of drug-delivery system.

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REFERENCES

[1] Aziz, A. R., Mohamed, S. M. & Nawi, M. N. F. (2023). Phytochemical screening and antibacterial activity of Pimeleodendron griffithianum. Malaysian Journal of Chemistry, 25 (3), 10-18. doi: https://doi.org/10.55373/mjchem.v25i3.10

[2] Anibijuwon, I. I., GBala, I. D., Nnadozie, B. I. & Ifayefumi, O. (2018). Susceptibility of selected multi-drug resistant clinical isolates to different leaf extracts of *Senna alata*. *Notulae Scientia Biologicae*, 10 (1), 26–32. doi: https://doi.org/10.15835/nsb10110176

[3] Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P. & Chang, C. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules, 27(4), 1326. doi: https://doi.org/10.3390/molecules27041326

[4] Banso, A., Dachi, S., Usman, J. I., Ajewole, A. E. & Etsu-Musa, N. (2024). Exploring the antioxidant performance and identification of bioactive phytochemicals of leaf extracts of *Senna alata* (L.) Roxb. *Journal of Current Opinion in Crop Science*, 5(2), 70–77. doi: https://doi.org/10.62773/jcocs.v5i2.237

[5] Bassey, E. E., Alaribe, A. A. A., Ikpi, E. E. & Thomas, P. S. (2022). Studies on the antibacterial susceptibility of uropathogens to *Senna alata* extracts in Calabar, Cross River State, Nigeria, *Annual Research & Review in Biology*, 37 (12), 39–60. doi: https://doi.org/10.9734/arrb/2022/v37i1230556

[6] Bedair, H., Samir, T., & Mansour, F. (2024). Antibacterial and antifungal activities of natural deep eutectic solvents. *Applied Microbiology and Biotechnology*, 108, 1-16. doi: https://doi.org/10.1007/s00253-024-13044-2.

[7] Bibi, N., Shah, M. H., Khan, N., Al-Hashimi, A., Elshikh, M. S., Iqbal, A., Ahmad, S. & Abbasi, A. M. (2022). Variations in total phenolic, total flavonoid contents, and free radicals' scavenging potential of onion varieties planted under diverse environmental conditions. *Plants*, 11(7), 950. doi: https://doi.org/10.3390/plants11070950

[8] Bunya, S. R. & Lihan, S. (2022). Effect of extraction techniques on yield, total phenolic content, total flavonoid content and its antioxidant activity of leaves, stems and roots from *Cassia alata* Linn. *Asian Journal of Medicine and Biomedicine*, 6(S1), 81–82. doi: https://doi.org/10.37231/ajmb.2022.6.s1.536

[9] Chávez, H., Alvarado, A. T., asayco-Yataco, N. T., Pérez, M. P., García, J. A., Bendezú, M. R., Surco-Laos, F., Palomino-Jhong, J. J., Laos-Anchante, D., Melgar-Merino, E. J., Vega-Ramos, N., Ferreyra-Paredes, C., YarascaCarlos, P. E., Fermín, D., Bolarte-Arteaga, M. & Loja-Herrera, B. (2024). Anti-inflammatory effect of the total flavonoid content of the hydroalcoholic extract of the leaves of *Senna alata* (L.) Roxb. in an experimental model of acute inflammation. *Journal of Pharmacy & Pharmacognosy Research*, 13(2), 444 – 458. doi: https://doi.org/10.56499/jppres24.2100_13.2.444

[10] Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N. & Suleman, S. (2023). Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. *Journal of Experimental Pharmacology*. 15, 51–62. doi: https://doi.org/10.2147/jep.s379805

[11] Donkor, M.N., Donkor, AM. & Mosobil, R. (2023). Combination therapy: synergism among three plant extracts against selected pathogens. BMC Research Notes, 16(83), 1-5. doi: https://doi.org/10.1186/s13104-023-06354-7

[12] Edet, U. O., Nwaokorie, F. O., Mbim, E. N., Asanga, E. E., Agbor, Y. O., Okoroiwu, H. U., Edet, B. O., Umoafia, N. & Nkang, A. (2022). Evaluation of *Annona muricata* extract against *Staphylococcus aureus* isolate and *in-silico* activity of bioactive compounds against Capsular protein (Cap5O). *BMC Complementary Medicine and Therapies*, 22(192), 1-9. doi: https://doi.org/10.1186/s12906-022-03672-4

[13] Fri, R., Prihantini, N. N. & Hady, B. C. (2022). *In vitro* bioactivity test of *Senna alata* (L.) Roxb leaves extract. *International Journal of Health Sciences and Research*, 12(2), 304–317. doi: https://doi.org/10.52403/ijhsr.20220241

[14] Godlewska, K., Pacyga, P., Najda, A., Michalak, I. (2023). Investigation of chemical constituents and antioxidant activity of biologically active plant-derived natural products. *Molecules*, 28(14), 5572. doi: https://doi.org/10.3390/molecules28145572

[15] Ivanović, M., Razboršek, M. I. & Kolar, M. (2020). Innovative extraction techniques using deep eutectic solvents and analytical methods for the isolation and characterization of natural bioactive compounds from plant material. *Plants*, 9(11), 1428, 1-29. doi: https://doi.org/10.3390/plants9111428

[16] Kavela, E. T. A., Szaloki-Dorko, L. & Mate, M. (2023). The efficiency of selected green solvents and parameters for polyphenol extraction from Chokerry (Arona melanocarpa (Michx)) pomace. Foods, 12, 3639, 1 – 13 doi: https://doi.org/10.3390/foods12193639

[17] Kumar, R, Methven, L. & Oruna-Concha, M. J. (2023). A comparative study of ethanol and citric acid solutions for extracting betalains and total phenolic content from freeze-dried beetroot powder. Molecules, 28, 6405, 1- 22 doi: https://doi.org/10.3390/molecules28176405

[18] Lee, J.-E., Jayakody, J. T. M., Kim, J.-I., Jeong, J.-W., Choi, K.-M., Kim, T.-S., Seo, C., Azimi, I., Hyun, J. & Ryu, B. (2024). The influence of solvent choice on the extraction of bioactive compounds from Asteraceae: a comparative review. Foods, 13(19), 3151. doi: https://doi.org/10.3390/foods13193151

[19] Miwonouko, K. F., Dermane, A., Kombate, B., Gbekley, E. H., Anani, K., Metowogo, K. & Karou, D. S. (2024). Antioxidant and antimicrobial activities of Cassia alata (L.) Roxb. leaves. *International Journal of Biological and Chemical Sciences*, 18(1), 236–243. doi: https://doi.org/10.4314/ijbcs.v18i1.19

[20] Muhammad, S. L., Wada, Y., Mohammed, M., Ibrahim, S., Musa, K.Y., Olonitola, O. S., Ahmad, M. H., Mustapha, S., Abdul Rahman, Z., Sha'aban, A. (2021). Bioassay-guided identification of bioactive compounds from *Senna alata* L. against methicillin-resistant *Staphylococcus aureus*. *Applied Microbiology,* 1(3), 520-536. doi: https://doi.org/10.3390/applmicrobiol1030034

[21] Nyalo, P., Omwenga, G., & Ngugi, M. (2023). Antibacterial properties and GC-MS analysis of ethyl acetate extracts of *Xerophyta spekei* (Baker) and *Grewia tembensis* (Fresen). *Heliyon,* 9(e14461), 1-12. doi: https://doi.org/10.1016/j.heliyon.2023.e14461

[22] Oladeji, O. S., Adelowo, F. E., Oluyori, A. P. & Bankole, D. T. (2020). Ethnobotanical description and biological activities of *Senna alata*. *Hindawi, Evidence-based Complementary and Alternative Medicine*, 2020(1), 1-12. doi: https://doi.org/10.1155/2020/2580259

[23] Pachorkar, P. Y., & Patil, S. H. (2021). Therapeutic potential and characterization of senna alata: an ethano-medicinal plant. *International Journal of Pharmaceutical Sciences and Research*,12(9), 4985.doi: https://doi.org/10.13040/IJPSR.0975-8232.12(9).4985-92

[24] Rocha, P. P. V., Gargabite, B. F. L., Jacob, J. K. S., & Abucay, Jr. J. B. (2022). Phytochemical screening of indigenous plants utilized by agta community in Aurora. *International Journal of Agricultural Technology*, 18(2),771-778. url: https://www.researchgate.net/publication/389256475_Phytochemical_screening_of_indigenous_plants_utilized_by_agta_community_in_Aurora

[25] Radošević, K., Čanak, I., Panić, M., Markov, K., Bubalo, M. C., Frece, J., Srček, V. G., & Redovniković, I. R. (2018). Antimicrobial, cytotoxic and antioxidative evaluation of natural deep eutectic solvents. *Environmental Science and Pollution Research*, 25(14), 14188–14196. doi: https://doi.org/10.1007/s11356-018-1669-z

[26] Sanjaya, Y. A., Tola, P. S., & Rahmawati, R. (2022). Ultrasound-assisted extraction as a potential method to enhanced extraction of bioactive compound. *Nusantara Science and Technology Proceedings*, 191–198. doi: https://doi.org/10.11594/nstp.2022.2729

[27] Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 8(2), 603–608. doi: https://doi.org/10.22271/chemi.2020.v8.i2i.8834

[28] Shi, L., Wu, Y., Zhu, Y., Yu, T., Zhou, Y., Xie, M. & Pang, H. (2025). The rise of green solvents: application and efficiency of deep eutectic solvents in the extraction of flavonoids. *Journal of Food Science*, 90: e70381. doi: https://doi.org/10.1111/1750-3841.70381

[29] Sivaraj, R. S., Hanaphi, R. M., & Yusof, R. (2024). Unravelling The Bioactivities of Acmella paniculata Extract-Mediated Green Deep Eutectic Solvent of Citric Acid Monohydrate and Glycerol. Malaysian Applied Biology, 53(4), 139–152. doi: https://doi.org/10.55230/mabjournal.v53i4.3039

[30] Velavan, S. (2015). Phytochemical techniques - a review. *World Journal of Science and Research*, 1(2), 80–91. http://www.harmanpublications.com

[31] Wu, L., Chen, Z., Li, S., Wang, L., & Zhang, J. (2021). Eco-friendly and high-efficient extraction of natural antioxidants from *Polygonum aviculare* leaves using tailor-made deep eutectic solvents as extractants. *Separation and Purification Technology*, 262, 118339. doi: https://doi.org/10.1016/j.seppur.2021.118339

[32] Yadav, S., Singh, R. B., Mishra, M. K., Singh, A. K., Yadav, P. & Sharma, P. C. (2024). Exploring *Senna alata* bark as a potential therapeutic agent for wound healing: a comprehensive pharmacognostic, physicochemical, phytochemical & pharmacological study. *Journal of Chemical Health Risks*, 14(5), 1992-2003. http://www.jchr.org

[33] Yusoff, M. H. M., & Shafie, M. H. (2024). Microwave-assisted extraction of polysaccharides from *Micromelum minutum* leaves using citric acid monohydrate-glycerol based deep eutectic solvents and evaluation of biological activities. *Analytica Chimica Acta*, 1331:343351. doi: https://doi.org/10.1016/j.aca.2024.343351