

Bioactivity of Zwitterion Green Solvent Antioxidants With The Mae Method of Senduduk Leaf (*Melastoma malabathricum* L.) and Identification Of Bioactive Compounds With LC-MS/MS

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Abstract

Background: Senduduk (*Melastoma malabathricum* L.) is a medicinal plant commonly found in Indonesia and traditionally used as an antibacterial, antidiarrheal, anti-inflammatory, and wound healing agent. This potential encourages the development of more innovative and environmentally friendly natural-based pharmaceutical preparations.

Aims: This study aims to determine the antioxidant activity of NaDES zwitterion extract of senduduk leaves using the Microwave Assisted Extraction (MAE) method and to identify bioactive compounds using LC-MS/MS analysis.

Methods: Extraction was carried out using Natural Deep Eutectic Solvent (NaDES) consisting of betaine as Hydrogen Bond Acceptor (HBA) and lactic acid as Hydrogen Bond Donor (HBD) with a mole ratio of 1:1, 1:3, 1:5. The extraction process was carried out using the Microwave Assisted Extraction (MAE) method with variations in power and extraction time. The antioxidant activity test was carried out using the DPPH method with the determination of IC₅₀ values, while compound identification was carried out using LC-MS/MS.

Result: The results showed that the extract was liquid, yellowish brown, reddish, to blackish in color with a distinctive aroma. The pH value of the extract was 3.44 (1:1), 2.85 (1:3), and 2.59 (1:5) which were classified as acidic. The antioxidant activity showed a very strong category with an IC₅₀ value of (2.35 µg/mL), approaching ascorbic acid of (2.21 µg/mL). LC-MS/MS analysis identified 20 chemical compounds consisting of carbohydrates, alkaloids, as well as amino acids and peptides which also play a role as antioxidants.

Conclusion: NaDES zwitterion extract of senduduk leaves using the MAE method has very strong antioxidant activity and contains potential bioactive compounds that can be utilized in the food, nutraceutical, and pharmaceutical industries.

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INTRODUCTION

Plants in Indonesia are very diverse, one of which is sedunia (*Melastoma malabathricum* L.) Indonesia has a plentiful population and has long been used as a traditional medicine. Various studies report that it has pharmacological activities, including antibacterial, antidiarrheal, antiseptic, anti-inflammatory, and wound healing (Awang-Jamil et al., 2021). These activities are related to its secondary metabolite content, such as flavonoids, tannins, phenylpropanoids, organic acids (esters), terpenoids, and other polyphenolic compounds (He Rui et al., 2022). In line with this enormous potential, the development of natural-based pharmaceutical preparations continues to experience innovation, so it is hoped that the utilization of Indonesia's natural resources, especially in the pharmaceutical sector, can develop and keep up with the times. This effort is in line with the direction of national development as outlined in Asta Cita Indonesia Emas 2045, which emphasizes

strengthening natural resources and human resources through downstreaming of natural resources and the use of environmentally friendly technologies (Hermawan et al., 2025).

Extracts obtained through appropriate extraction methods are expected to maintain the biological activity of active compounds, one of which is antioxidant activity. Antioxidants play a crucial role in counteracting free radicals that can originate from hazardous chemicals such as pesticide use, air pollution, cigarette smoke, alcohol, and synthetic chemicals in medicines and food, which can trigger various diseases (Hasanah et al., 2017). According to research by Kumaradewi et al., (2021), the use of synthetic antioxidants carries the risk of being cancer-causing agents. Therefore, antioxidants obtained from natural sources are needed. One potential source of natural antioxidants is senduduk leaves (*Melastoma malabathricum* L.). In the study of Hainil et al., (2023) showed that the ethanol extract of senduduk leaves (*Melastoma malabathricum* L.) has quite high antioxidant activity based on the DPPH test. In this study, the DPPH method was chosen because it is simple, fast and efficient, with this test parameter in the form of an IC₅₀ value obtained by the relationship between the percentage of inhibition and the concentration of the test solution (Hasanah et al., 2017).

In supporting the sustainable use of natural resources, environmentally friendly technologies and approaches are needed, one of which is through the use of alternative solvents. Natural Deep Eutectic Solvent (NaDES) is a green solvent that can help avoid potential environmental hazards compared to conventional organic solvents such as ethanol or ionic liquids because it is biodegradable and has a lower risk of environmental pollution (Liu et al., 2018). In this study, the NaDES component used as HBA (Hydrogen Bond Acceptor) is Betaine, a zwitterionic compound that is non-toxic, stable, and efficient in extracting bioactive compounds. According to Abranches et al., (2020) betaine is able to act as a good hydrogen bond acceptor, increasing the solubility of polar and non-polar compounds, and functions as a natural osmolyte that supports the stability of active compounds during the extraction process. In addition, its amphoteric properties make it suitable for the extraction of active compounds with weak acidic characteristics and help maintain a neutral pH system, thus potentially supporting the development of stable and safe drug raw materials.

Apart from the choice of solvent, the extraction method also plays an important role in determining the quality of the resulting extract. One method that can be used in NaDES-based extraction is MAE (Microwave Assisted Extraction), which is known to increase extraction efficiency. However, research on the extraction of senduduk leaves using NaDES with the MAE method is still limited. Therefore, researchers chose the combination of NaDES solvent and the MAE method as an innovative approach that is expected to open up new opportunities in environmentally sustainable technology (Grujic et al., 2025).

However, biological activity testing alone is insufficient without the identification of active compounds contained in the extract. In utilizing natural ingredients as alternative raw materials for medicines and cosmetics, active ingredient analysis is a crucial step. The Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) method can provide information on the molecular weight, structure, and specific identity of compounds contained in sample extracts, thus supporting the scientific characterization and standardization of natural ingredients (Yulion et al., 2023).

METHOD

Study Design and Research Framework

This study used a laboratory experimental design. The research was conducted at the Research Laboratory and Chemistry Laboratory of Harapan Ibu Jambi College of Health Sciences from February to April 2026. Sample determination was carried out at the Plant Taxonomy Laboratory of the Faculty of Mathematics and Natural Sciences, Padjadjaran University, while LC-MS/MS analysis was carried out at the Bogor Police Forensic Laboratory Center.

Material

The materials used include fresh senduduk leaves (*Melastoma malabathricum* L.), betaine (ActiBiso™ BA) as Hydrogen Bond Acceptor (HBA), lactic acid (Emprove) as Hydrogen Bond Donor (HBD), DPPH (Tokyo Chemical Industry CO., LDT), methanol (Emsure), ascorbic acid (Emsure), distilled water (Amidis), and pH buffer.

Instrument

The tools used include a microwave (Sharp R-753GX®), hotplate, magnetic stirrer (Ika C MAG MS 10®), UV-Vis spectrophotometer (Shimadzu U-1800®), pH meter (Hanna H1285-6®), beaker glass (Pyrex®), measuring flask (Pyrex®), measuring cylinder (Iwaki®), analytical balance (Fujitsu FSR – A 220®), pipette pump, filter paper (Whatman), blender (welhome), cuvette (Shimazu), aluminum foil (Klinpak), and LC-MS/MS instrument.

Sampling

Fresh Senduduk (*Melastoma malabathricum*) leaf samples were obtained from Mendalo Darat Village, Jambi Luar Kota District, Muaro Jambi Regency, Jambi Province with coordinates 1.62540° S, 103.54193° E and the samples were determined in the Plant Taxonomy Laboratory of the Biology Department, FMIPA UNPAD to ensure the accuracy of the plants. The leaves used for extraction are fresh leaves taken before sunrise. The leaf characteristics selected are mature leaves in the top 10 of one stem, with no defects or holes. The leaves are then washed and dried from water, then chopped into small pieces and crushed using a blender until smooth. The mashed senduduk leaves are then weighed as much as 1.5 grams. The extraction was performed using a sample to solvent ratio of 10:100 (w/v), where in one variation it is made in 15 mL.

Solvent Preparation and Extraction Process

NaDES solvent was made by mixing betaine as HBA and lactic acid as HBD with a mole ratio of 1:1, 1:3, and 1:5. Each mixture was added with 25% distilled water and heated at 80°C for one hour using a hot plate magnetic stirrer at speed no. 3 until a homogeneous and clear solution was obtained (Putra et al., 2024).

The extraction process begins with fresh senduduk leaves, which are ground and then mixed with NaDES solvent. Extraction was carried out using the Microwave Assisted Extraction (MAE) method with extraction times varying from 1, 2, and 3 minutes and power variations of 100, 200, and 300 watts, resulting in 27 extract variations. These can be seen in Table 1.

Table 1. Variation of NaDES Extract

No	HBA : HBD (mol)	Time (Minutes)	Power (Watts)
1.	1 : 1	1,2,3	100,200,300
2.	1 : 3	1,2,3	100,200,300
3.	1 : 5	1,2,3	100,200,300

NaDES Extract Testing

Organoleptic testing was conducted using the five senses of NaDES extract in the form of color, aroma, and texture (Yulion et al., 2023). The pH measurement of the extract was carried out using a pH meter that had been calibrated at room temperature with triplicate repetitions. Antioxidant activity was tested using the DPPH method (Safitri et al., 2024). Absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 515 to 517. The IC₅₀ value was obtained from a linear regression equation between concentration and percentage inhibition.

LC-MS/MS analysis of extracts with the best antioxidant activity was analyzed using LC-MS/MS to identify bioactive compounds based on molecular mass and fragmentation patterns. The results of LC/MS-MS data analysis will display a chromatogram in the form of a peak-height plot, where each peak indicates the presence of a particular compound in the extract. From this chromatogram, the molecular weight of each compound can be obtained, allowing for a more precise understanding of the amount of compound contained in the extract (Mangurana et al., 2019). The data obtained were analyzed descriptively. IC₅₀ values were calculated using linear regression analysis.

RESULTS AND DISCUSSION

Result

The extraction results in a liquid extract with the distinctive aroma of NaDES solvent. The color varies from yellowish brown, reddish brown, to blackish brown depending on the extraction conditions. The results of the pH test from the NaDES extract of senduduk leaves (*Melastoma malabathricum* L.) can be seen in **table 1**. The pH results obtained for the NaDES extract of senduduk leaves were 1:1, 3.44, 1:3, 2.85, and 1:5, 2.59, all three of which were classified as acidic.

Table 2. pH Analysis of NaDES Extract

No	Sample	pH	Category
1.	Extract 1:1	3.44	Acidic
2.	Extract 1:3	2.85	Acidic
3.	Extract 1:5	2.59	Acidic

The results of antioxidant activity can be seen in **table 2**. researchers used ascorbic acid in the same way and with the same treatment. The IC₅₀ value of the NaDES extract of senduduk leaves was found to be 2.35µg/mL which is classified as very strong and the antioxidant activity of ascorbic acid was 2.215µg/mL which is classified as very strong.

Table 3. Antioxidant Activity Analysis Using the DPPH Method

No	Sample	IC ₅₀	Category
1.	Ascorbic Acid	2.21	Very strong
2.	NaDES Extract	2.35	Very strong

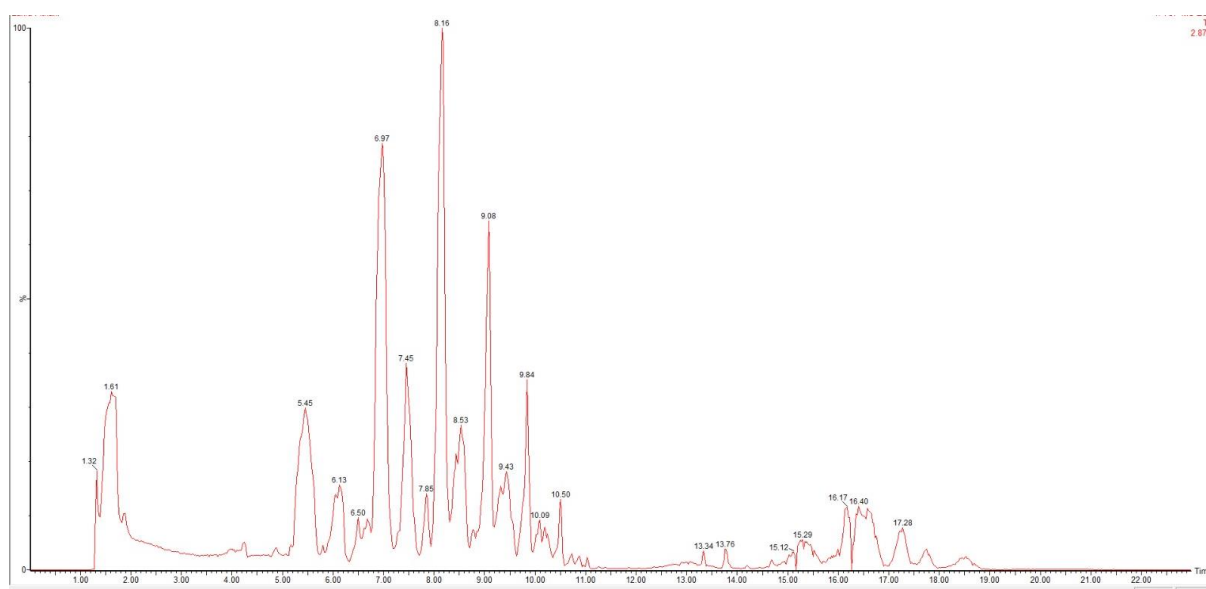


Figure 1. Spectrum Total Ion Chromatogram (TIC)

The results of the LC-MS/MS analysis showed that there were 20 complex chemical compounds contained in the NaDES zwitterion extract of senduduk leaves. The classes of compounds contained include 4 carbohydrates, 3 alkaloids, and 13 amino acid and peptide compounds.

Discussion

In this study, the extraction of senduduk leaves (*Melastoma malabathricum* L.) using the green solvent NaDES (Natural Deep Eutectic Solvent) using the MAE (Microwave Assisted Extraction) method. NaDES has biodegradable properties, is non-volatile, and can reduce the need for toxic organic solvents, which is in line with the principles of green chemistry (Jalalzai et al., 2025). The NaDES solvents used by the researchers were betaine as HBA (Hydrogen Bond Acceptor) and lactic acid as HBD (Hydrogen Bond Donor) and 25% aquadest was added to reduce the solvent viscosity. The concentrations of each HBA and HBD solvent component were 1:1, 1:3, and 1:5. FTIR analysis confirmed that the formation of hydrogen bonds between HBA and HBD indicating the successful preparation of the NaDES system (Jalalzai et al., 2025).

Making NADES solvent, as much as 117.15 grams of Betaine and 90.08 grams of Lactic Acid and 25% distilled water are then mixed on a hotplate stirrer at a temperature of 80°C and speed No. 3 for up to 3 hours to obtain a homogeneous and clear NaDES solution.

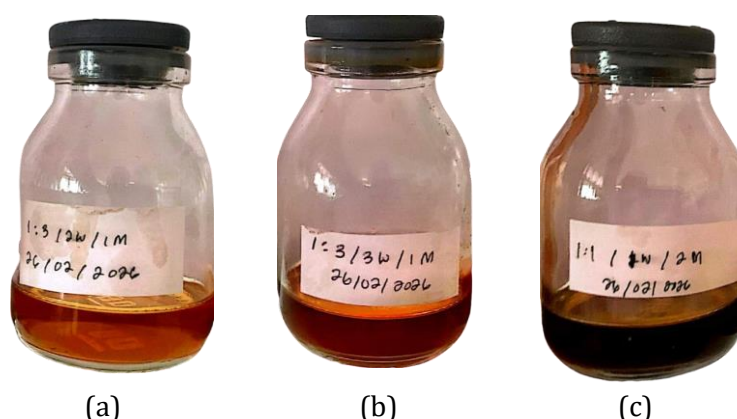


Figure 2. Example NaDES extract (a) yellowish-brown, (b) reddish-brown, and (c) blackish-brown

The final NaDES extract is a liquid, has a distinctive aroma, and is yellowish-brown, reddish-brown, or even blackish-brown in color. There are differences in the color of the NaDES extract from senduduk leaves. This is due to differences in the mole composition of the NaDES solvent, time, and temperature, which can affect the color and bioactive compounds contained ([Purwanti, 2024](#)). The average color difference was obtained in the extract variation with 300 watt power. This is because the longer the extraction time, the redder the resulting extract, as in the 300-watt extract variation in 3 minutes. This is because the temperature produced by the microwave increases with increasing power and duration. The change in red color can be related to the use of high temperatures and duration in the extraction process ([Azouz & Hayyan, 2026](#)). From differences in solvent composition, results can also be obtained, the higher the concentration of lactic acid in the solvent content, the redder the color produced by the extract. This is due to the pH contained by the extract with a ratio of 1:5 is the most acidic. As in previous research where in acidic environments the extract tends to be red ([Fu et al., 2021](#)).

The pH test was carried out on each variation of the NaDES extract of sedunia leaves. The test was conducted at room temperature and repeated triplicate. The pH results obtained in the NaDES extract of senduduk leaves 1:1 were 3.44, 1:3 was 2.85, and 1:5 was 2.59, all of which were classified as acidic. This is in accordance with previous research which obtained a pH value of 3.3 at a ratio of betaine: lactic acid (1:1) ([Carbonell-rozas et al., 2026](#)). The factor of this is the composition of the solvent used, namely lactic acid, which is proven by the higher the concentration of the lactic acid ratio, the more acidic the pH value.

In this test, researchers used a spectrophotometric tool to measure the absorbance value in the antioxidant test using the DPPH method. The wavelength used was 515 nm to 517 nm with the DPPH absorbance obtained at 0.944, this is in accordance with the literature where the absorbance value of the DPPH stock solution was 0.876 ([Amabel et al., 2024](#)). The absorbance range of the DPPH blank absorbance was confirmed to be around 0.7-0.9 as an accuracy value.

The best IC₅₀ value chosen is in the 1:3 3 minute 100 watt series. This is based on ideal linear regression measurement data, namely absorbance values that meet standard criteria. The standards used are absorbance values in the range of 0.2 to 0.8, inhibition values $\pm 10\%$ -90%, and R values >0.95 or >0.99 , which means the absorbance value obtained is linear ([Rozi et al., 2023](#)).

To compare the antioxidant activity of extracts, researchers used ascorbic acid in the same way and with the same treatment. The IC₅₀ value of the NaDES extract of senduduk leaves was found to be 2.35 $\mu\text{g/mL}$ which is classified as very strong and the antioxidant activity of ascorbic acid was 2.215 $\mu\text{g/mL}$ which is classified as very strong. The IC₅₀ value is the concentration of antioxidant that inhibits 50% of radicals ([Nurhaliza, 2023](#)). The lower the IC₅₀ value, the greater the antioxidant activity ([Yulion et al., 2023](#)). At 100 watts, the average IC₅₀ value was 1.9 $\mu\text{g/mL}$, while at 300 watts, the IC₅₀ value decreased to 4.25 $\mu\text{g/mL}$, indicating that increasing power does not necessarily

produce a more optimal extract. This finding aligns with research by [Hasdar & Pangeran \(2021\)](#), who reported that increasing MAE power can increase yield, but under certain conditions causes degradation of active compounds.

LC-MS/MS testing was conducted on one senduduk leaf extract with the best antioxidant value, namely 1:3 3 minutes 100 watts . The LC-MS test used SPE C18 with LC-QTOF (Quadrupole Time-of-Flight) which provided high mass accuracy ($m/z < 5$ ppm) ([Tomei et al., 2025](#)) . Preparation began with the extract sample into the syringe. The C18 cartridge is activated using methanol and then distilled water to optimally bind the target compound. The sample is then inserted into the C18 cartridge, where non-polar or semi-polar compounds are retained in the C18 phase, leaving impurities behind. The cartridge is then washed with distilled water to remove impurities without releasing the target compound. The target compound is then eluted with 1.5 to 2 mL of methanol and injected into the LC-MS.

The results are peak vs. time chromatograms, where each peak represents a different compound and the peak area indicates quantification. Samples will be separated based on polarity in the column, characterized by retention time: polar exits quickly, nonpolar ones slow ([Abbasi & Todeschini, 2025](#)) . Sample compounds were detected approximately at time points from 5 minutes 12 seconds to 17 minutes 48 seconds. This indicates that the compounds detected in the NaDES extract of senduduk leaves are semi-polar to nonpolar.

The overall chromatogram results that can be read by Sirius software are 91 organic compounds. And then sorted based on the researcher's criteria is data completeness regarding IUPAC names, SMILES codes, InChI, confidence scores, and feature quality to obtain 20 complex chemical compounds contained in the NaDES zwitterion extract of senduduk leaves. The groups of compounds contained include 4 carbohydrates, 3 alkaloids, and 13 amino acid and peptide compounds. The extract compounds are dominated by primary metabolites (carbohydrates, amino acids, and peptides) and secondary metabolites (alkaloids). These compounds are not included in the phenolic or flavonoid groups, but some of them still have the potential to contribute to antioxidant activity ([Hana et al., 2024](#)) .

Implications

The results of the study indicate that NaDES-based extraction using the MAE method has the potential to be developed as an environmentally friendly extraction technology in the pharmaceutical and nutraceutical fields.

Research contribution

This study provides information on the application of NaDES zwitterion combined with the MAE method to obtain antioxidant-rich senduduk leaf extract.

Limitations

This study focused solely on antioxidant activity and compound identification without isolating pure compounds. The power consumption in the extraction process was too high. Further research is recommended to optimize extraction conditions and isolate specific active compounds responsible for antioxidant activity, using a lower microwave power.

Suggestions

It is recommended that future research employ extraction methods other than MAE, such as UAE. This is because the high temperatures associated with MAE have the potential to cause the degradation of bioactive compounds within the senduduk leaf extract as evidenced by LC-MS/MS results that failed to detect the target flavonoid compounds. Alternatively, that microwave-assisted extraction be conducted using lower power settings. Furthermore, future studies should assess antioxidant activity and characterize the compound profile of NaDES senduduk leaf extracts using alternative analytical methods.

CONCLUSION

NaDES zwitterion extract of senduduk leaves (*Melastoma malabatricum* L.) using the MAE (Microwave Assisted Extraction) method has very strong antioxidant activity with an IC₅₀ value of 2.35 µg/ml. LC-MS/MS analysis identified 20 compounds consisting of carbohydrates, alkaloids, amino acids and peptides.

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AUTHOR CONTRIBUTION STATEMENT

ZF conducted the research, collected data, drafted the manuscript and supervised the research process, methodology, and data analysis. DS contributed to the data analysis and drafting.

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