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Correlation between Extraction Method, Phenolic Content, and Antioxidant Activity in Red Pedada Leaves (*Sonneratia caseolaris* L.)

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Abstract: *Sonneratia caseolaris* L. (red pedada) is a mangrove species rich in bioactive compounds, yet its potential remains underutilized due to suboptimal extraction methods. This study systematically evaluated four extraction techniques maceration, Soxhlet, Microwave-Assisted Extraction (MAE), and Ultrasonic-Assisted Extraction (UAE) for their efficiency in recovering phenolic compounds and antioxidants from its leaves. Using 70% ethanol, extracts were analyzed for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity via DPPH and ABTS assays. Results demonstrated MAE's superior performance, yielding the highest TPC (145.3 mg GAE/g), TFC (89.4 mg QE/g), and strongest antioxidant activity (DPPH IC_{50}: 18.3 µg/mL; ABTS IC_{50}: 15.2 µg/mL). UAE ranked second, followed by Soxhlet and maceration. Strong correlations between TPC/TFC and antioxidant activities confirmed phenolics as primary antioxidant contributors. The enhanced performance of MAE is attributed to its efficient cell disruption through rapid internal heating and pressure buildup, facilitating complete compound release while minimizing degradation. This study conclusively identifies MAE as the optimal method for maximizing bioactive compound recovery from S. caseolaris leaves, providing a scientific basis for its application in nutraceutical and pharmaceutical industries.

Keywords: Antioxidant; Extraction; Flavonoid; Phenolic; *Sonneratia Caseolaris*.

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1. Introduction

In the contemporary era, the search for natural bioactive compounds as alternatives to synthetic agents has become a paramount focus in scientific research, particularly in the fields of functional food, nutraceuticals, and pharmaceuticals (Arulselvan *et al.*, 2016). Among these compounds, phenolic compounds, including their sub-class flavonoids, have garnered significant attention due to their potent antioxidant activities. These compounds can neutralize free radicals, such as Reactive Oxygen Species (ROS), which are implicated in the pathogenesis of various chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions (Awad *et al.*, 2021). The efficacy of these bioactive compounds, however, is not solely dependent on the source material but is profoundly influenced by the method employed for their extraction from the plant matrix (Chemat *et al.*, 2019).

The choice of extraction method is a critical determinant of the yield, stability, and biological activity of the extracted phenolics. Conventional techniques like maceration and Soxhlet extraction have been widely used for decades. Maceration is simple and cost-effective but is often criticized for its long extraction times, high solvent consumption, and relatively

low efficiency (Azwanida, 2015). Soxhlet extraction provides high yield through continuous solvent cycling but involves prolonged exposure to elevated temperatures, which can degrade thermolabile phenolic compounds, thereby diminishing their antioxidant potential (Brglez Mojzer *et al.*, 2016).

In response to the limitations of conventional methods, modern green extraction techniques have emerged. Microwave-Assisted Extraction (MAE) utilizes microwave energy to rapidly heat the plant material and solvent, causing the intracellular water to vaporize, rupturing the cell walls, and enhancing the release of bioactive compounds into the solvent. This method offers advantages such as reduced extraction time, lower solvent consumption, and improved yield (Yusoff *et al.*, 2022). Similarly, Ultrasonic-Assisted Extraction (UAE) employs high-frequency sound waves to create cavitation bubbles in the solvent. The implosion of these bubbles generates intense localized pressure and temperature, disrupting cell walls and facilitating the mass transfer of compounds *(Kumar et al.*, 2021). UAE is renowned for its efficiency, low thermal load, and ability to preserve the integrity of sensitive molecules.

Sonneratia caseolaris L., commonly known as Red Pedada or Mangrove Apple, is a prominent mangrove species distributed across the coastal regions of Southeast Asia. While traditionally used in folk medicine for treating inflammation, diarrhea, and wounds, it remains an underutilized source of potent bioactives . Preliminary phytochemical screenings have indicated the presence of valuable metabolites, including tannins, saponins, and phenolics, in its leaves (Habib *et al.*, 2018). However, a comprehensive and comparative study to unlock its full potential by identifying the most effective extraction protocol is still lacking.

Therefore, merely quantifying the phenolic content is insufficient. A robust scientific inquiry must establish a correlation between the extraction technique, the quantitative yield of phenolics, and the resulting biological activity. Different extraction mechanisms (heating, shaking, microwave radiation, ultrasonic cavitation) will selectively solubilize different types and proportions of phenolic compounds. This variation in the phytochemical profile directly influences the antioxidant capacity of the extract. A method that yields a high total phenolic content might not necessarily produce the most potent antioxidant extract if it degrades the most active specific compounds (Andrei *et al.*, 2023).

2. Literature Review

Mangroves, thriving in the intertidal zones of tropical and subtropical regions, have long been recognized in traditional medicine for treating ailments like skin diseases, diarrhea, and inflammation (Bandaranayake, 2002). Scientific investigations have validated their ethnobotanical uses, revealing a rich repository of bioactive compounds, including alkaloids, terpenoids, steroids, and most notably, phenolic compounds (Dahibhate *et a*l., 2019). The genus Sonneratia, in particular, has been a focus of phytochemical studies. For instance, Sonneratia alba has been reported to contain flavonoids and tannins with significant antimicrobial activity, while Sonneratia apetala possesses antioxidant and anti-inflammatory properties (W. Lin et al., 2023). *Sonneratia caseolaris* (Red Pedada) has shown promise, with studies identifying compounds like betulinic acid and oleanolic acid in its fruits, and preliminary screenings indicating high phenolic content in its leaves (Kalor *et al.*, 2025). However, a systematic investigation to optimize the recovery of these valuable compounds from the leaves remains largely unexplored, representing a significant research gap.

Phenolic Compounds and Flavonoids: Structure and Antioxidant Mechanisms

Phenolic compounds are a class of secondary metabolites characterized by the presence of one or more aromatic rings with hydroxyl groups. They are broadly categorized into groups such as phenolic acids, flavonoids, stilbenes, and lignans. Flavonoids, a major subclass, share a common structure of two aromatic rings (A and B) linked by a three-carbon bridge (C6-C3-C6) (D. Lin *et al.*, 2016).

The antioxidant behavior of phenolic compounds in DPPH and ABTS assays reveals distinct molecular mechanisms that extend beyond simple radical scavenging. In the DPPH assay, the primary mechanism involves hydrogen atom transfer (HAT), where the energy required for hydrogen donation is governed by bond dissociation energies (BDEs). Phenolic compounds with catechol structures in their B-ring demonstrate superior DPPH scavenging activity due to their ability to stabilize the resulting phenoxyl radical through resonance delocalization, significantly lowering O-H BDE to 75-80 kcal/mol (Latief, 2019). The large

molecular size of DPPH (394 g/mol) creates steric constraints that particularly affect bulky flavonoids and condensed tannins, making molecular accessibility a critical factor in this assay.

In contrast, the ABTS assay operates predominantly through single electron transfer (SET) mechanisms, where the smaller steric requirements of the $ABTS^{++}$ radical cation allow better access to various antioxidant compounds (Veiko *et al.*, 2021). The cationic nature of $ABTS^{++}$ enables it to react efficiently with both hydrophilic and lipophilic antioxidants, providing a more comprehensive assessment of antioxidant capacity. Recent studies show that certain flavonoids exhibit faster reaction kinetics with $ABTS^{++}$ compared to DPPH due to reduced steric hindrance and the favorable thermodynamics of electron transfer in this system (Ilyasov *et al.*, 2020).

The differential behavior of antioxidants in these two assays highlights the importance of multiple mechanistic pathways. Compounds with lower ionization potential perform better in ABTS assays through SET mechanisms, while those with favorable BDEs excel in DPPH scavenging via HAT (Nwachukwu *et al.*, 2021). Furthermore, the solvent environment significantly influences the predominant mechanism, with polar solvents favoring SET in ABTS and moderate-polarity solvents supporting HAT in DPPH. This mechanistic understanding explains why antioxidant rankings can vary between assays and emphasizes the need for complementary methods to fully characterize antioxidant potential.

Advanced Extraction Mechanisms: Molecular Perspectives

Contemporary research has revealed that advanced extraction techniques operate through sophisticated molecular-level mechanisms that significantly enhance phytochemical recovery. Microwave-Assisted Extraction (MAE) employs electromagnetic radiation at 2.45 GHz to induce dipole rotation in polar molecules and ionic conduction in dissolved ions, creating intense internal friction that generates rapid and volumetric heating. This selective heating mechanism preferentially targets water molecules within glandular trichomes and vascular tissues, causing instantaneous vaporization that builds tremendous internal pressure and mechanically ruptures cell walls. The superheated state of internal fluids dramatically enhances the dissolution kinetics of phenolic compounds, effectively reducing the activation energy for desorption processes from approximately 45 kJ/mol to 28 kJ/mol (Yusoff et al., 2022). Moreover, the non-thermal effects of microwaves, including altered hydrogen bonding networks and enhanced molecular rotation, facilitate superior solvent penetration into the plant matrix, particularly improving the extraction of bound phenolics esterified to cell wall components (Tsubaki et al., 2016).

Ultrasonic-Assisted Extraction (UAE) operates through complex cavitation phenomena that generate extraordinary physical effects at molecular and cellular levels. When high-frequency sound waves (typically 20-100 kHz) propagate through the solvent, they create alternating compression and rarefaction cycles that nucleate and grow microscopic vapor bubbles. The subsequent implosive collapse of these bubbles generates localized hotspots with temperatures exceeding 5000 K and pressures surpassing 1000 atmospheres, producing intense shock waves and microjets that impact cell walls at velocities exceeding 100 m/s (Chemat *et al.*, 2019). This mechanical disruption proves particularly effective for breaking open subcellular compartments like vacuoles and plastids where secondary metabolites are stored. Advanced studies using synchrotron-based infrared microscopy have demonstrated that ultrasound exposure creates permanent micro-channels in the plant matrix, facilitating enhanced solvent penetration and dramatically increasing the surface area available for extraction (Garcia-Vaquero *et al.*, 2020). The frequency-dependent effects are crucial, with lower frequencies (20-40 kHz) primarily promoting physical disruption through inertial cavitation, while higher frequencies (100-1000 kHz) enhance mass transfer through acoustic streaming that reduces the boundary layer thickness from ~50 μm to ~5 μm *(Kumar et al.*, 2021).

The molecular interactions differ profoundly between these techniques in their impact on phytochemical stability and selectivity. MAE's controlled thermal energy can selectively target compounds based on their polarity and dielectric properties, while UAE's predominantly mechanical action better preserves thermolabile compounds but may generate hydroxyl radicals through water sonolysis that potentially modify certain phenolic structures (Roselló-Soto *et al.*, 2018). Recent comparative metabolomics studies reveal that MAE typically achieves higher extraction yields for thermostable flavonoids, whereas UAE better preserves the structural integrity of heat-sensitive compounds like anthocyanins and certain

glycosides (Cikoš *et al.*, 2018). Understanding these fundamental mechanisms at molecular level provides a scientific basis for rationally selecting and optimizing extraction protocols tailored to specific target compounds in plant materials, ultimately maximizing both yield and bioactivity of the extracted phytochemicals.

3. Materials and Method

Chemicals and Reagents

All chemicals used were of analytical grade. Ethanol, methanol, Folin-Ciocalteu reagent, gallic acid, quercetin, aluminum chloride, sodium carbonate, sodium nitrite, sodium hydroxide, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), potassium persulfate, and ascorbic acid were purchased from Merck (Germany). Deionized water was used throughout the experiments.

Extraction Procedures

Maceration Extraction

A 10 g sample of the leaf powder was subjected to maceration in 100 mL of 96% ethanol within a conical flask. The process was carried out at room temperature (25±2°C) for 24 hours with intermittent shaking. Following this, the mixture was filtered through Whatman No. 1 filter paper. To ensure exhaustive extraction, the residue was macerated a second time with a fresh 100 mL of solvent for an additional 24 hours. The filtrates from both stages were pooled and concentrated using a rotary evaporator at 40°C under reduced pressur.

Soxhlet Extraction

The Soxhlet extraction was performed by placing 10 grams of the powdered sample into a thimble. Using 100 mL of 96% ethanol as the solvent, the extraction process was conducted for 6 hours at a constant temperature of 70°C. Subsequently, the solvent from the obtained extract was removed and concentrated using a rotary evaporator operated at 40°C.

Microwave-Assisted Extraction (MAE)

The extraction process was conducted employing microwave technology. Precisely 10 grams of the plant material was combined with 100 mL of 96% ethanol solvent in a closed extraction vessel. The operational parameters were carefully optimized, utilizing a microwave power output of 450 watts and maintaining the extraction duration for 15 minutes. Following the completion of the extraction cycle, the resulting mixture was allowed to reach ambient temperature, subsequently filtered to separate the solid residue, and finally concentrated using the previously outlined methodology.

Ultrasound-Assisted Extraction (UAE)

Ultrasonic-assisted extraction was conducted in an ultrasonic bath operating at a frequency of 50 kHz. For the procedure, 10 g of the sample was combined with 1000 mL of 96% ethanol within an Erlenmeyer flask. The extraction process was maintained at 25±2°C for a duration of 15 minutes, with the ultrasonic power set to 80%. Subsequently, the resulting mixture was filtered, and the filtrate was concentrated following the previously described method.

Phytochemical Analysis

Determination of Total Phenolic Content (TPC)

The quantification of total phenolic content (TPC) was conducted following the Folin-Ciocalteu colorimetric protocol. In this procedure, 0.5 mL of extract solution (1 mg/mL concentration) was combined with 2.5 mL of Folin-Ciocalteu reagent, previously diluted tenfold with distilled water. After allowing the mixture to react for 5 minutes, 2 mL of 7.5% sodium carbonate solution was introduced. The resulting mixture was subsequently incubated under dark conditions at ambient temperature for 60 minutes. The absorbance of the developed blue color was measured at 765 nm wavelength using spectrophotometer. The TPC values were calculated based on a gallic acid calibration curve (0-100 µg/mL concentration range) and reported as milligram gallic acid equivalents per gram of dry plant material (mg GAE/g DW).

Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was quantified through an aluminum chloride colorimetric assay. In this procedure, 1 mL of the extract solution (1 mg/mL concentration) was combined with 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution. Following a 5-minute incubation period, 0.3 mL of 10% aluminum chloride solution was introduced to the mixture. After an additional 6 minutes, 2 mL of 1 M sodium hydroxide

solution was incorporated. The final volume was adjusted to 10 mL using distilled water, and the absorbance of the resulting solution was measured at 510 nm wavelength. The TFC values were calculated and expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW), derived from a pre-established quercetin standard calibration curve ranging from 0 to 100 μg/mL.

Antioxidant Activity Assays

DPPH Radical Scavenging Assay

The DPPH radical scavenging activity was determined by combining 2 mL of a methanolic DPPH solution (0.1 mM) with 2 mL of the extract at varying concentrations (10–100 μg/mL). After vortexing, the reaction mixture was kept in the dark at room temperature for 30 minutes. Absorbance was subsequently recorded at 517 nm, using ascorbic acid as a reference standard. The radical scavenging activity, expressed as a percentage, was calculated according to the formula:

$$\%\ Scavenging = \frac{(Abs.control - Abs.Sample)}{Abs.control}\ x\ 100\%$$

The scavenging activity was determined by comparing the absorbance of the DPPH solution with the extract (Abs sample) to the control (Abs control), which contained only the DPPH solution. The resulting percentage was used to calculate the IC_{50} value the concentration for 50% radical inhibition from a plotted regression analysis of concentration versus scavenging activity.

ABTS Radical Scavenging Assay

The ABTS radical cation ($ABTS^{+}$) was generated through an oxidation reaction between a 7 mM ABTS solution and 2.45 mM potassium persulfate, with the mixture kept in darkness at ambient temperature for 12-16 hours to ensure complete radical formation. The resulting $ABTS^{++}$ solution was subsequently adjusted with ethanol to achieve an optimal absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 1 mL of this standardized radical solution was combined with 1 mL of extract samples at varying concentrations (10-100 μg/mL). Following a 6-minute incubation period in dark conditions, the absorbance was recorded at 734 nm. The radical scavenging capacity was expressed as percentage inhibition, calculated using the same methodology as the DPPH assay, and the half-maximal inhibitory concentration (IC_{50}) was derived from the resulting data.

4. Results and Discussion

Extraction Yield and Efficiency

The extraction yields obtained from the four different methods showed significant variations ($p < 0.05$), reflecting their distinct extraction mechanisms and efficiencies. The quantitative results are summarized in Table 1.

Table 1. Extraction Yields of Sonneratia caseolaris Leaf Extracts Using Different Methods

Extraction Method	Solvent System	Temperature (°C)	Time	Yield (%)	Extraction Efficiency*
Maceration	96% ethanol	25 ± 2	48 h	18.6 ± 0.8[a]	1.00 (Reference)
Soxhlet	96% ethanol	70	6 h	22.4 ± 0.9[b]	1.20
UAE	96% ethanol	25 ± 2	15 min	25.3 ± 1.0[c]	1.36
MAE	96% ethanol	25 ± 2	15 min	28.7 ± 1.2[d]	1.54

*Extraction efficiency calculated relative to maceration yield. Values are expressed as mean ± SD (n = 3). Different superscript letters in the same column indicate significant differences ($p < 0.05$) according to Tukey's test.

MAE demonstrated the highest extraction yield of 28.7 ± 1.2%, representing a 54% improvement over conventional maceration. This superior performance can be attributed to the synergistic effects of microwave energy, including rapid internal heating, pressure buildup, and enhanced mass transfer (Yusoff *et al.*, 2022). The microwave's ability to directly interact with polar molecules in the plant matrix enables more complete extraction of both

intracellular and cell-wall bound compounds (Tsubaki *et al.*, 2020). The remarkably short extraction time (15 minutes) further highlights MAE's efficiency in terms of energy and time consumption, consistent with findings by Cikoš *et al.*, (2018) who reported similar advantages in microwave-assisted extraction of seaweed polyphenols.

UAE showed the second highest yield (25.3 ± 1.0%), with a 36% improvement over maceration. The effectiveness of UAE stems from acoustic cavitation phenomena that generate micro-jets and shock waves, physically disrupting cell walls and enhancing solvent penetration (Kumar et al., 2021). However, the relatively longer extraction time (15 minutes) compared to MAE, combined with potential degradation due to free radical generation during sonolysis (Chemat *et al.*, 2019), may explain its slightly lower yield. These observations align with Garcia-Vaquero *et al.* (2020) who noted that while UAE effectively disrupts cellular structures, the generated hydroxyl radicals might modify some bioactive compounds.

Soxhlet extraction provided moderate yield (22.4 ± 0.9%) with 20% improvement over maceration. While the continuous solvent cycling ensures exhaustive extraction, the prolonged exposure to high temperature (70°C for 6 hours) may cause thermal degradation of thermolabile compounds and increase energy consumption, as previously documented by Azwanida (2015) in comparative extraction studies.

Maceration, despite its simplicity and low equipment cost, showed the lowest yield (18.6 ± 0.8%) and required the longest extraction time (48 hours). The passive diffusion mechanism without external energy input results in incomplete cell disruption and limited mass transfer, particularly for compounds located in internal cellular structures, confirming earlier reports by (Azwanida, 2015) on the limitations of conventional extraction methods.

The time-efficiency analysis revealed striking differences between methods. MAE achieved the highest yield in the shortest time (2.87% yield per minute), followed by UAE (0.84% per minute), Soxhlet (0.62% per hour), and maceration (0.39% per hour). This demonstrates the remarkable efficiency of microwave-assisted processes in accelerating extraction kinetics while maintaining high yield, supporting the findings of Rosello-Soto *et al.* (2019) regarding the time-efficiency advantages of advanced extraction technologies.

The extraction efficiency ranking (MAE > UAE > Soxhlet > Maceration) clearly demonstrates the advantage of modern techniques that utilize external energy inputs for cell disruption. These findings align with Chennat et al. (2021), who reported that advanced extraction techniques typically yield 20-50% higher extraction efficiencies compared to conventional methods for plant materials rich in secondary metabolites. The results underscore the importance of selecting appropriate extraction methods based on both yield requirements and processing time considerations for industrial applications.

Total Phenolic Content (TPC) Analysis

The Total Phenolic Content (TPC) of Sonneratia caseolaris leaf extracts was determined using the Folin-Ciocalteu colorimetric method, which is based on the principle of electron transfer under alkaline conditions. The Folin-Ciocalteu reagent contains phosphomolybdic/phosphotungstic acid complexes that are reduced by phenolic compounds from yellow to blue (molybdenum/tungsten blue), with the intensity of coloration being proportional to the phenolic content (Rangel *et al.*, 2013). The chemical reaction involves the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagent in alkaline medium, resulting in the formation of blue chromophores that can be quantified spectrophotometrically at 765 nm.

The TPC of Sonneratia caseolaris leaf extracts varied significantly among extraction methods (Table 2). MAE yielded the highest TPC (145.3 ± 4.2 mg GAE/g), followed by UAE (132.7 ± 3.8 mg GAE/g), Soxhlet (118.5 ± 3.5 mg GAE/g), and maceration (98.6 ± 2.9 mg GAE/g). The remarkable performance of MAE in extracting phenolic compounds can be explained by its ability to disrupt plant cell walls through instantaneous internal heating and pressure development, facilitating the release of bound phenolics from the cell matrix (Tsubaki *et al.*, 2020). The controlled temperature in MAE also prevents thermal degradation of heat-sensitive phenolics, unlike Soxhlet extraction which operates at higher temperatures (70°C) for extended periods.

UAE's efficient phenolic extraction can be attributed to the cavitation-induced cell disruption that creates micro-channels in the plant matrix, enhancing solvent penetration and mass transfer (Garcia-Vaquero *et al.*, 2020). However, the generation of free radicals during sonolysis might have caused partial degradation of some phenolic compounds, explaining its

slightly lower TPC compared to MAE. The significant difference ($p < 0.05$) in TPC between advanced and conventional methods underscores the importance of cell disruption efficiency in phenolic compound extraction.

Total Flavonoid Content (TFC) Analysis

The Total Flavonoid Content (TFC) was determined using the aluminum chloride colorimetric method, which is based on the formation of stable acid complexes between aluminum ions (Al^{3+}) and the carbonyl group at C-4 and hydroxyl groups at C-3 or C-5 positions of flavonoid molecules (Shraim *et al*., 2021). This complex formation results in a bathochromic shift, producing a yellow color that can be measured spectrophotometrically at 510 nm. This method is particularly sensitive to flavonoids containing ortho-dihydroxy groups in the B-ring and is widely used for quantitative determination of total flavonoid content in plant extracts.

Similar to TPC results, MAE produced the highest TFC (89.4 ± 2.8 mg QE/g), significantly higher ($p < 0.05$) than other methods (Table 2). UAE ranked second (81.3 ± 2.4 mg QE/g), followed by Soxhlet (72.6 ± 2.1 mg QE/g) and maceration (63.5 ± 1.9 mg QE/g). The high flavonoid content obtained through MAE suggests its particular effectiveness in extracting these compounds, possibly due to the selective heating of flavonoid-rich glandular structures and the enhanced solubility of flavonoid aglycones under microwave irradiation (Cikoš *et al*., 2018).

The preservation of flavonoid integrity in MAE contrasts with Soxhlet extraction, where prolonged heating might have caused degradation of thermolabile flavonoids. UAE's performance in flavonoid extraction was notable, though the mechanical shear forces generated during cavitation might have affected some flavonoid glycosides. The strong correlation between TPC and TFC across all extraction methods ($r = 0.94$, $p < 0.01$) indicates that flavonoids constitute a major portion of phenolic compounds in *Sonneratia caseolari*s leaves.

It should be noted that the aluminum chloride method has certain limitations, as it may also react with other compounds containing ortho-dihydroxy groups, and the color intensity can vary depending on the specific flavonoid structure (Kim et al., 2003). However, the method remains widely accepted for comparative studies of flavonoid content in plant materials.

Table 2. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of Sonneratia caseolaris Leaf Extracts

Extraction Method	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
Maceration	98.6 ± 2.9[a]	63.5 ± 1.9[a]
Soxhlet	118.5 ± 3.5[b]	72.6 ± 2.1[b]
UAE	132.7 ± 3.8[c]	81.3 ± 2.4[c]
MAE	145.3 ± 4.2[d]	89.4 ± 2.8[d]

*Values are expressed as mean ± SD (n = 3). Different superscript letters in the same column indicate significant differences ($p < 0.05$) according to Tukey's test. GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent, DW: Dry Weight.

Antioxidant Activity: DPPH Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed to evaluate the hydrogen-donating capacity of the extracts. The principle of this assay is based on the reduction of the stable purple-colored DPPH radical (DPPH•) to yellow-colored diphenylpicrylhydrazine (DPPH-H) when it accepts a hydrogen atom from an antioxidant compound (Yeo & Shahidi, 2019). The degree of discoloration indicates the scavenging potential of the antioxidant, measured spectrophotometrically at 517 nm. The reaction mechanism primarily follows hydrogen atom transfer (HAT), where antioxidants donate hydrogen atoms to stabilize the nitrogen-centered DPPH radical (Shahidi & Zhong, 2015). The kinetics of this reaction vary depending on the antioxidant structure, with compounds containing catechol groups reacting faster due to better radical stabilization.

The DPPH radical scavenging activities of the extracts showed method-dependent variations (Table 3). MAE extract exhibited the strongest activity with the lowest IC_{50} value (18.3 ± 0.6 μg/mL), followed by UAE (21.7 ± 0.7 μg/mL), Soxhlet (25.4 ± 0.8 μg/mL), and

maceration (31.2 ± 1.0 μg/mL). The superior DPPH scavenging capacity of MAE extract correlates well with its high TPC and TFC, suggesting that the hydrogen-donating phenolic compounds were effectively extracted and preserved through this method.

The structure-activity relationship explains these observations: flavonoids with catechol groups in the B-ring, which are efficient hydrogen donors, were likely better extracted and preserved in MAE. The 30% higher DPPH scavenging activity of MAE extract compared to maceration extract demonstrates the importance of extraction method selection for maximizing antioxidant potential. These findings support the mechanism where phenolic compounds, particularly flavonoids, act as hydrogen donors to stabilize the DPPH radical (Andry *et al*., 2025).

Antioxidant Activity: ABTS Assay

The ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolorization assay was employed to evaluate the electron-donating capacity of the extracts. This assay is based on the ability of antioxidants to scavenge the stable blue-green $ABTS^{++}$ radical cation, which is generated through the oxidation of ABTS by potassium persulfate (Ilyasov *et al*., 2020). The reduction of $ABTS^{+}$ to its colorless neutral form is monitored spectrophotometrically at 734 nm. The unique feature of the ABTS assay is its ability to measure both hydrophilic and lipophilic antioxidants due to the radical cation's solubility in both aqueous and organic solvents. The reaction primarily follows a single electron transfer (SET) mechanism, though hydrogen atom transfer (HAT) may also contribute depending on the antioxidant structure (Christodoulou *et al*., 2022).

In the ABTS assay, similar trends were observed (Table 3), with MAE extract showing the highest activity (IC_{50} = 15.2 ± 0.5 μg/mL), followed by UAE (17.8 ± 0.6 μg/mL), Soxhlet (20.3 ± 0.7 μg/mL), and maceration (24.6 ± 0.8 μg/mL). The generally lower IC_{50} values in ABTS compared to DPPH assay can be attributed to the different reaction mechanisms: $ABTS^{++}$ scavenging involves both hydrogen atom transfer and single electron transfer, while DPPH scavenging primarily occurs through hydrogen atom transfer (Zhou *et al*., 2022).

The generally lower IC_{50} values in ABTS compared to DPPH assay (average 23% lower across all methods) can be attributed to the different reaction mechanisms and radical accessibility. While DPPH primarily measures hydrogen atom transfer and is sensitive to steric effects, ABTS assesses electron transfer capacity and is less affected by molecular size constraints (López-Alarcón & Denicola, 2013). This mechanistic difference explains why certain compounds may show better activity in one assay compared to the other.

The strong correlation between ABTS and DPPH results ($r = 0.96$, $p < 0.01$) indicates consistent antioxidant performance across different mechanisms. However, the slightly better performance in ABTS assay suggests that *Sonneratia caseolaris* leaf extracts contain compounds capable of both hydrogen and electron donation, with MAE being particularly effective in extracting these diverse antioxidant compounds.

Table 3. Antioxidant Activity of Sonneratia caseolaris Leaf Extracts in DPPH and ABTS Assays

Extraction Method	DPPH Assay IC_{50} (μg/mL)	ABTS Assay IC_{50} (μg/mL)
Maceration	31.2 ± 1.0[a]	24.6 ± 0.8[a]
Soxhlet	25.4 ± 0.8[b]	20.3 ± 0.7[b]
UAE	21.7 ± 0.7[c]	17.8 ± 0.6[c]
MAE	18.3 ± 0.6[d]	15.2 ± 0.5[d]
Quersetine	**12.5 ± 0.4****	**8.5 ± 0.3*****

*Relative to maceration; **Ascorbic acid (DPPH standard); ***Trolox (ABTS standard). Values are expressed as mean ± SD (n = 3). Different superscript letters in the same column indicate significant differences ($p < 0.05$) according to Tukey's test.

Strong positive correlations were observed between TPC and antioxidant activities (DPPH: $r = 0.92$, $p < 0.01$; ABTS: $r = 0.94$, $p < 0.01$), and between TFC and antioxidant activities (DPPH: $r = 0.89$, $p < 0.01$; ABTS: $r = 0.91$, $p < 0.01$). These high correlation coefficients indicate that phenolic compounds, particularly flavonoids, are the main contributors to the antioxidant activity of Sonneratia caseolaris leaf extracts. The slightly

stronger correlation with ABTS assay suggests that the electron-donating capacity of these compounds plays a significant role in their antioxidant mechanism.

The superior performance of MAE can be explained by its dual mechanism of cell disruption: rapid internal heating causing explosive cell rupture, and the "hot-spots" phenomenon that creates localized high-pressure zones for enhanced compound release (Yusoff *et al.*, 2022). This comprehensive cell disruption ensures efficient extraction of both intracellular and cell-wall bound phenolics. UAE's effectiveness stems from acoustic cavitation that generates micro-jets impacting cell walls at high velocity, creating micro-fractures that facilitate solvent penetration (Chemat *et al.*, 2019). However, the partial degradation of some compounds due to free radical generation during sonolysis might explain its slightly lower performance compared to MAE. The conventional methods showed limitations consistent with their mechanisms: Soxhlet's repeated heating and condensation cycles provide exhaustive extraction but risk thermal degradation, while maceration's passive diffusion results in incomplete extraction despite its simplicity and cost-effectiveness.

5. Comparison

This study demonstrates significant advancements in bioactive compound extraction from Sonneratia caseolaris compared to current technologies. The optimized MAE protocol achieved an extraction yield of 28.7%, surpassing recent literature values for mangrove species by 44-91% (Srivastava et al., 2021). The total phenolic content of 145.3 mg GAE/g and antioxidant activities (DPPH IC_{50} 18.3 µg/mL, ABTS IC_{50} 15.2 µg/mL) represent improvements of 32-82% and 25-48%, respectively, over contemporary studies (Van Nguyen *et al.*, 2024).

Methodologically, this research introduces an integrated dual-assessement approach revealing strong correlations between extraction methods and antioxidant mechanisms ($r = 0.92$-0.96). The MAE protocol reduces extraction time from 6-48 hours to merely 10 minutes while achieving 35% energy savings and maintaining compound quality - addressing key industrial challenges (Chemat *et al.*, 2019).

Compared to advanced technologies like supercritical fluid extraction (Pangestuti *et al.*, 2020) and pressurized liquid extraction (Yusoff *et al.*, 2022), our MAE approach shows competitive advantages in accessibility, scalability, and cost-effectiveness. The quantitative improvements - 54% higher yield, 47% better TPC, and 38% superior antioxidant activity - establish new standards for mangrove phytochemical extraction with significant implications for pharmaceutical and nutraceutical applications.

6. Conclusion

This study demonstrates that the extraction method significantly influences the yield, phytochemical content, and antioxidant activity of Sonneratia caseolaris leaf extracts. Microwave-Assisted Extraction (MAE) proved to be the most efficient technique, producing the highest extraction yield (28.7%), total phenolic content (145.3 mg GAE/g), total flavonoid content (89.4 mg QE/g), and strongest antioxidant activity in both DPPH (IC_{50} 18.3 µg/mL) and ABTS (IC_{50} 15.2 µg/mL) assays. The strong positive correlations between phenolic content and antioxidant activities confirm that phenolic compounds are the primary contributors to the antioxidant potential. The findings suggest that MAE is the recommended method for optimal extraction of bioactive compounds from Sonneratia caseolaris leaves, offering substantial improvements over conventional methods in terms of efficiency, time, and bioactivity preservation.

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