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Development and Evaluation of Microcapsules Containing Combined Extracts of Bay, Cherry, and Green Betel Leaves as Natural Antioxidants

Reza Pratama, Wempi Budiana, Diki Zaelani, Aiyi Asnawi

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Abstract: Bay leaf (*Syzygium polyanthum*), cherry leaf (*Muntingia calabura*), and green betel leaf (*Piper betle*) contain phenolic and flavonoid compounds with antioxidant potential, but their utilization is limited by physicochemical instability. This study aimed to develop microcapsules containing a combined extract of these three leaves and to evaluate their physicochemical properties and *in vitro* antioxidant activity as an initial formulation feasibility study. Each extract was prepared by maceration using 96% ethanol, yielding 11.42–15.86%, and combined in a 1:1:1 (w/w/w) ratio prior to microencapsulation. Microcapsules were produced using a fluidized bed dryer with lactose as the core material and polyvinyl alcohol (PVA) as the coating polymer. Physicochemical characterization included moisture content, flow rate, angle of repose, compressibility index, dissolution time, particle size, and surface morphology. Antioxidant activity was assessed using DPPH and CUPRAC assays, with IC_{50} values calculated from triplicate measurements. The coating process increased mean particle size from 636.2 µm to 728.0 µm and prolonged dissolution time from 2.14 to 3.55 minutes, indicating coating layer formation. Among the individual extracts, cherry leaf extract showed the strongest antioxidant activity. The microcapsules exhibited antioxidant activity within the same order of magnitude as the combined extract under initial, non-stressed testing conditions. These results demonstrate the feasibility of formulating combined plant extracts into microcapsules with acceptable physical properties, while further stability and comparative studies are required to support antioxidant preservation and potential applications.

Introduction

Oxidative stress is a condition characterized by an imbalance between the production of free radicals and the capacity of endogenous antioxidant defense systems, leading to oxidative damage of lipids, proteins, and DNA (1-3). This condition has been widely implicated in the pathogenesis of various chronic and degenerative diseases, including cardiovascular disorders, diabetes mellitus, cancer, and premature aging (2). Therefore, antioxidants play an essential role in maintaining redox homeostasis and preventing oxidative damage.

In recent years, increasing concerns regarding the safety and long-term use of synthetic antioxidants such as BHA and BHT have driven growing interest in natural antioxidants derived from plants (4, 5). Plant-based antioxidants are rich in phenolic acids and flavonoids, which are capable of scavenging free radicals through hydrogen or electron donation mechanisms and inhibiting chain oxidation reactions (6, 7). The presence of multiple phytochemicals in plant extracts may contribute to enhanced antioxidant capacity; however, evidence of synergistic effects is highly dependent on plant species, composition, and formulation conditions (8).

Bay leaf (Syzygium polyanthum), cherry leaf (Muntingia calabura), and green betel leaf (Piper betle) are medicinal plants commonly used in Indonesia and reported to possess antioxidant activity due to their phenolic and flavonoid content (9-11). Previous studies have demonstrated that cherry leaf extract exhibits relatively strong antioxidant activity, while bay leaf and green betel leaf show moderate to strong radical-scavenging capacity (9, 10, 12, 13). Despite these findings, most studies have focused on single-plant extracts, and investigations on the formulation feasibility of combining these extracts in a single delivery system remain limited. In this study, the combination approach was not intended to assume synergistic effects but rather to evaluate the feasibility of incorporating multiple antioxidant-rich extracts into one formulation.

A major limitation in the application of plant-derived antioxidants is their susceptibility to degradation caused by oxidative, thermal, and photolytic pathways during

processing and storage, which may reduce their effectiveness (11,14). To address this issue, microencapsulation has been widely applied to protect sensitive bioactive compounds, improve handling properties, and modulate release behavior (15). Among various encapsulation techniques, fluidized bed systems offer advantages such as uniform coating, good process control, and suitability for scale-up in solid dosage formulations (15-17).

Lactose was selected as the core material in this study due to its good flowability, compatibility with active compounds, and extensive use in pharmaceutical formulations (15). Polyvinyl alcohol (PVA) was chosen as the coating polymer because of its film-forming ability, water solubility, and reported effectiveness in improving physical stability and dissolution behavior of microencapsulated plant extracts (15, 18). Previous studies have shown that PVA-coated particles produced via fluidized bed techniques exhibit improved powder properties and protection of bioactive compounds (15, 18, 19).

Therefore, this study aimed to develop microcapsules containing a combined extract of bay leaf, cherry leaf, and green betel leaf using a fluidized bed dryer and to evaluate their physicochemical properties and *in vitro* antioxidant activity. The working hypothesis was that microencapsulation would yield particles with acceptable physical characteristics while retaining antioxidant activity under initial testing conditions. This study was designed as an initial formulation feasibility investigation to provide a basis for further studies involving stability evaluation, comparative formulation strategies, and application-oriented assessments.

Methodology

Materials

Dried simplicia of bay leaves, cherry leaves, and green betel leaves were obtained from a local plantation in Manoko, Lembang, Bandung, Indonesia. Ethanol 96% and lactose were purchased from PT. Brataco, Indonesia. Polyvinyl alcohol (PVA), hydroxypropyl methylcellulose (HPMC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), CUPRAC reagents, and FRAP reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of Extracts and Yield Determination

Each dried leaf powder was extracted separately by maceration using 96% (v/v) ethanol for 4 days with solvent replacement every 24 h. The extracts were filtered and concentrated under reduced pressure at 40 °C using a rotary evaporator. Extraction yield was calculated as the percentage ratio of dried extract weight to the initial dry simplicia weight to allow evaluation of extraction efficiency and batch-to-batch reproducibility.

Preparation of Combined Extract

Before microencapsulation, the dried extracts of bay leaf, cherry leaf, and green betel leaf were combined in a fixed weight ratio (1:1:1, w/w/w). This ratio was selected to ensure equal contribution of each extract to the formulation and to facilitate interpretation of antioxidant activity results.

Phytochemical Screening

Phytochemical screening was performed to identify the presence of secondary metabolites in the extracts. The tests included qualitative identification of flavonoids, alkaloids, quinones, saponins, tannins, steroids, and triterpenoids (14).

Microcapsule Preparation

Microcapsules were prepared using a fluidized bed dryer with lactose as the core material. A total of 500 g of lactose was used as the solid loading. Before coating, the combined extract was prepared by dispersing the dried extracts in an aqueous HPMC binder solution (20% w/v) with a total volume of 500 mL, and the dispersion was sprayed onto the lactose cores. Subsequently, a polyvinyl alcohol (PVA) solution (15% w/v) was applied as the film-forming coating layer using a total spray volume of 500 mL. The fluidized bed process parameters were set as follows: inlet temperature 37.8 °C, outlet temperature 37.2 °C, product temperature 37.8 °C, spray interval 10 s, and spray speed 20.5 rpm. The concentrations of the spray solutions and solid loading were kept constant throughout the process to ensure coating uniformity. Coating efficiency was not quantitatively determined and is acknowledged as a limitation of the study (15, 16).

Characterization of Microcapsule

Moisture Content

Approximately 1 g of microcapsules was placed in a moisture analyzer (Moisture Analyzer MA 50. R, Radwag, Miami, FL, USA). The temperature was set at 105 °C, and the moisture loss was recorded when a constant weight was reached.

Flow Rate and Angle of Repose

A total of 25 g of microcapsules was placed into the funnel of a flowmeter (GTB Series, Erweka, Langen, Germany). The flow rate was determined by measuring the time required for the microcapsules to pass completely through the funnel. The angle of repose was obtained by measuring the height and diameter of the heap formed (17).

Compressibility Index

A total of 25 g of microcapsules was placed into a measuring cylinder of a tapped density tester (Tapped Density Tester, Erweka SVM 221, Erweka). The compressibility index was determined based on the final volume after 500 taps (15).

Solubility of Microcapsules

One gram of microcapsules was dispersed into 100 mL of distilled water in a beaker and stirred using a magnetic stirrer at 100 rpm, maintained at 37 ± 0.5 °C. The dissolution time was recorded from the moment the microcapsules came into contact with the water until complete dissolution with no visible solid residue.

Shape, Morphology, and Particle Size

The shape and surface morphology of the microcapsules were examined using a scanning electron microscope (SEM) (JSM-6360, Jeol, Tokyo, Japan) at 150× magnification. The particle size was determined using a particle size analyzer (Horiba SZ-100, Horiba Ltd., Kyoto, Japan) after dispersing the sample in phosphate buffer pH 6.8, followed by the collection of 1 mL of the dispersion for testing.

Antioxidant Activity Assays (DPPH and CUPRAC)

The DPPH assay was conducted to evaluate the free radical scavenging activity of the extracts and microcapsules. A 0.1

mM DPPH solution was prepared in methanol. Samples and ascorbic acid (positive control) were prepared at various concentrations. For each concentration, all measurements were performed in triplicate, in independent experiments. A total of 1 mL of sample was mixed with 1 mL of DPPH solution and incubated for 30 min in the dark at room temperature. Absorbance was measured at 517 nm using a UV–Visible spectrophotometer, and the percentage of inhibition was calculated. IC_{50} values were determined from the concentration–inhibition curves generated from triplicate measurements and expressed as mean values (20).

Similarly, the CUPRAC assay was performed to determine the cupric ion-reducing antioxidant capacity. One milliliter of the sample was added to the CUPRAC reagent mixture and incubated for 30 min at room temperature. All CUPRAC measurements were carried out in triplicate, and absorbance was measured at 450 nm (21). Antioxidant activity was calculated and expressed as mean ± standard deviation (SD).

Result and Discussion

Extraction Yield of Bay Leaf, Cherry Leaf, and Green Betel Leaf Extracts

The extraction yield of bay leaf, cherry leaf, and green betel leaf obtained by ethanolic maceration is presented in **Table 1**. The yields ranged from 11.42% to 15.86%, indicating that the extraction method was effective and reproducible for all three plant materials.

Cherry leaf extract showed the highest extraction yield (15.86%), followed by green betel leaf (13.27%) and bay leaf (11.42%). Variations in yield among the extracts may be attributed to differences in plant matrix composition and the content of ethanol-soluble secondary metabolites, particularly phenolic and flavonoid compounds. Similar yield ranges have been reported for ethanolic maceration of medicinal plant leaves in previous studies (9, 12, 22).

The extraction yield data provide quantitative support for the extraction procedure used in this study and confirm its suitability for subsequent formulation and antioxidant activity evaluation. It should be emphasized that extraction yield reflects extraction efficiency rather than antioxidant potency, as biological activity depends on the qualitative and quantitative composition of bioactive constituents rather than extract mass alone.

Phytochemical Screening of Bay Leaf, Green Betel Leaf, and Cherry Leaf Extracts

Qualitative phytochemical screening confirmed the presence of major secondary metabolite groups, including flavonoids, alkaloids, quinones, tannins, saponins, and steroids/triterpenoids in the extracts **Table 2**. These findings are consistent with previous reports describing bay leaf, cherry leaf, and green betel leaf as rich sources of phenolic and flavonoid compounds (9, 22, 23).

However, it should be noted that the phytochemical screening performed in this study was qualitative in nature. Therefore, the results are presented to indicate the presence of antioxidant-related compound classes rather than to establish a quantitative correlation between specific phytochemical content and antioxidant activity. Quantitative determination of total phenolic or flavonoid content would be required to support such correlations statistically and is recommended for future studies, particularly to strengthen data interpretation.

Table 1. Extraction yield of bay leaf, cherry leaf, and green betel leaf extracts.

Extract	Dry Weigt (g)	Extract Weight (g)	Yield (%)
Bay leaf	5000	571.1	11.42
Cherry leaf	5000	793.0	15.86
Green betel leaf	5000	663.5	13.27

Table 2. Phytochemical screening of bay leaf, green betel leaf, and cherry leaf extracts.

Phytochemicals	Extract Bay Leaf	Extract Green Betel Leaf	Extract Cherry Leaf
Flavonoids	+	+	+
Alkaloids	+	+	+
Quinones	+	+	+
Saponins	+	-	-
Tannins	-	+	+
Steroids/Triterpenoids	+	+	+

Description: (+) detected, (−) not detected.

Physical Properties of Microcapsules

The physical characteristics of the microcapsules before and after coating are summarized in **Table 3.** The moisture content of both formulations remained below 3%, which is generally acceptable for solid pharmaceutical preparations and indicates efficient drying. The angle of repose values (<30°) and compressibility index values (<15%) for both coated and uncoated microcapsules fall within ranges associated with good to excellent flowability according to pharmacopeial powder classification criteria. These results indicate that the coating process did not adversely affect powder handling properties and, in some parameters, slightly improved flow behavior, likely due to increased particle size and surface smoothing following coating. Overall, these physical characteristics support the suitability of the microcapsules for further processing and formulation development, particularly in solid dosage form manufacturing. Such properties are particularly relevant for ensuring uniform dosing and reproducible performance in solid dosage forms. The macroscopic appearance of the microcapsules is shown in **Figure 1**.

The increase in dissolution time after coating (from 2.14 to 3.55 min) suggests the formation of a polymeric coating layer that moderately retarded water penetration. This behavior is consistent with previous fluidized-bed coating studies using hydrophilic polymers such as PVA, which have been reported to modulate dissolution profiles without compromising overall dispersibility (15, 18). The physical property trends observed in this study are comparable to those reported for other plant-extract microcapsules prepared using fluidized bed systems (15-17).

Morphology and Particle Size

SEM analysis at 150× magnification revealed irregular, aggregated particles with rough and porous surfaces. Although this magnification allowed general observation of

Table 3. Physical properties of microcapsules of bay leaf, cherry leaf, and green betel leaf extracts (*n* = 3).

Parameter	Microcapsule	
	Before Coating	After Coating
Loss on Drying (%)	2.33 ± 0.10	2.45 ± 0.11
Flow Rate (g/s)	8.06 ± 0.05	7.03 ± 0.05
Angle of Repose (°)	29.36 ± 0.10	28.53 ± 0.43

Figure 1. Microcapsules of bay leaf, cherry leaf, and green betel leaf extracts.

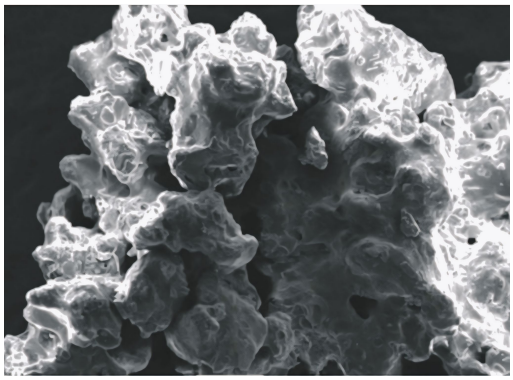

Figure 2. Microcapsule morphology observed by SEM at 150× magnification.

particle morphology and aggregation, it was insufficient to assess coating uniformity or film integrity at the microscale. Therefore, SEM images are interpreted cautiously and used primarily to confirm particle formation rather than coating quality as shown in **Figure 2**. Higher magnification imaging is recommended in future work to evaluate coating continuity and encapsulation quality better.

Particle size analysis showed an increase in mean particle size from 636.2 µm before coating to 728.0 µm after coating, indicating successful deposition of the coating layer. Similar increases in particle size following polymer coating have been reported in previous microencapsulation studies using fluidized bed techniques (17, 24).

Antioxidant Properties

The antioxidant activity results obtained using DPPH and CUPRAC assays are presented in **Table 4**. Among the individual extracts, cherry leaf extract exhibited the strongest antioxidant activity, consistent with previous studies reporting high phenolic and flavonoid content in *Muntingia calabura* leaves (9).

Table 4. Antioxidant activity of ascorbic acid, extracts, and microcapsules (n = 3).

Sampel	IC_{50} DPPH (µg/mL)	IC_{50} CUPRAC (µg/mL)
Ascorbic Acid	7.47 ± 0.03	6.42 ± 0.04
Cherry Leaf Extract	32.67 ± 0.01	28.84 ± 0.09
Bay Leaf Extract	35.90 ± 0.12	31.48 ± 0.08
Green Betel Leaf Extract	38.75 ± 0.21	34.85 ± 0.10
Microcapsules	37.82 ± 0.17	33.76 ± 0.12

The IC_{50} values of the microcapsules were higher than those of the cherry leaf extract alone, indicating a slight reduction in apparent antioxidant activity after microencapsulation. This reduction may be attributed to diffusion limitations caused by the polymeric coating layer, which can restrict immediate interaction between antioxidant compounds and assay reagents. Similar trends have been reported in other encapsulation studies, where encapsulated antioxidants showed delayed or reduced apparent activity in direct *in vitro* assays despite retained bioactivity (15, 19).

Importantly, the antioxidant activity of the microcapsules remained comparable to that of the combined extract at the time of testing. This observation suggests that the microencapsulation process using a fluidized bed system, lactose as the core material, and polyvinyl alcohol (PVA) as the coating polymer did not result in a substantial immediate loss of antioxidant capacity. Under the non-stressed experimental conditions applied in this study, the encapsulated formulation was therefore able to retain measurable free radical scavenging and reducing activities as evaluated by the DPPH and CUPRAC assays.

Nevertheless, it is important to emphasize that no claims regarding antioxidant “protection,” controlled release behavior, or long-term stability can be made based on the present data. The study did not include accelerated stability testing, storage studies, or exposure to environmental stressors such as heat, light, or humidity. Consequently, the ability of the coating layer to preserve antioxidant compounds over time or to prevent degradation cannot be inferred from the current results.

Accordingly, the antioxidant data should be interpreted as preliminary evidence of retained activity immediately following formulation, rather than as confirmation of enhanced stability or functional improvement. Within this context, the primary role of antioxidant testing in the present work was to support formulation feasibility and to verify that the microencapsulation process itself did not adversely affect antioxidant performance under initial testing conditions.

Furthermore, although the combined extract formulation was evaluated as a single microcapsulated system, potential synergistic or antagonistic interactions among the individual plant extracts were not investigated. No comparison was performed with a simple physical mixture of the extracts or with individually encapsulated components. Therefore, conclusions regarding synergistic enhancement of antioxidant activity cannot be drawn. The combination

strategy employed in this study is discussed strictly from a formulation perspective, aiming to demonstrate process compatibility and baseline functional retention, while providing a foundation for future studies involving comparative evaluations, mechanistic assessments, and stability analyses, as well as optimization of extract ratios to better elucidate interaction effects.

Conclusion

This study addressed the formulation challenge associated with the physicochemical instability of plant-derived antioxidants by developing microcapsules containing combined extracts of bay leaf (*Syzygium polyanthum*), cherry leaf (*Muntingia calabura*), and green betel leaf (*Piper betle*). The extracts exhibited measurable *in vitro* antioxidant activity, with cherry leaf showing the strongest activity among the individual extracts. Microencapsulation using a fluidized bed system with lactose as the core and polyvinyl alcohol (PVA) as the coating polymer produced particles with acceptable physical properties, including good flowability, low moisture content, and increased particle size, indicating successful coating formation. The microcapsules retained antioxidant activity within the same order of magnitude as the combined extract under initial testing conditions; however, the higher IC_{50} values compared to at least one free extract suggest diffusion limitations imposed by the coating layer. This study does not claim long-term stability or antioxidant protection, as no stress or storage studies were conducted. Overall, the results demonstrate formulation feasibility, while further studies involving stability evaluation, quantitative phytochemical analysis, and safety assessment are required to support potential pharmaceutical or nutraceutical applications.

Abbreviations

DPPH:1,1-Difenil-2-Pikrilhidrazil, FRAP: Ferric Reducing Antioxidant Power, CUPRAC: Cupric Reducing Antioxidant Capacity, IC_{50}: Inhibitory Concentration 50%, HPMC: Hydroxypropyl Methylcellulose, PVA: Polyvinyl Alcohol, SEM: Scanning Electron Microscope, PSA: Particle Size Analyzer.

Declarations

Author Informations

Reza Pratama
Corresponding Author
Affiliation: Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Bhakti Kencana University, Bandung - 40614, Indonesia..
Contribution: Conceptualization, Data Curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing - Original Draft.

Wempi Budiana
Affiliation: Department of Biological Pharmacy, Faculty of Pharmacy, Bhakti Kencana University, Bandung - 40614, Indonesia..
Contribution: Data Curation, Investigation, Resources, Validation, Writing - Review & Editing.

Diki Zaelani
Affiliation: Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Bhakti Kencana University, Bandung - 40614, Indonesia..
Contribution: Data Curation, Formal analysis, Methodology, Validation, Writing - Review & Editing.

Aiyi Asnawi
Affiliation: Department of Pharmacochemistry, Faculty of Pharmacy, Bhakti Kencana University, Bandung - 40614, Indonesia..
Contribution: Conceptualization, Methodology, Supervision, Validation, Writing - Original Draft.

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The data generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Statement

Ethical approval was not required for this study.

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Additional Information

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