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Optimization of Solvent Systems and Extraction Techniques for Enhanced Multifunctional Bioactivities *of Vernonia Cinerea* (L.) Less.: A Comprehensive Study on Antioxidant, Tyrosinase Inhibitory, Antibacterial, and Antidiabetic Enzyme Activities

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Abstract

The objective of the study was to identify the suitable condition of solvent and an effective extraction technique for improving the biological activity of the extracts of *Vernonia cinerea* plant, focusing on free radical scavenging, tyrosinase and inhibition, bacterium inhibition, α-amylase and α-glucosidase inhibition activity. The sample from leaves, flowers, and stems of the plants was extracted with 5 types of solvent in the present study. The efficacy of biological activity was evaluated in terms of total phenolic and flavonoid content, DPPH and FRAP scavenging activity, tyrosinase inhibition activity, bacterium inhibition, agar well diffusion assay, and inhibition of α-amylase and α-glucosidase. The results showed that the MAE method with methanol solvent was the most effective in all tests. The extracts from the leaf exhibited maximum biological activity with the highest TPC and TFC of 195.48 ± 4.18 mg GAE/g extract and 98.07 ± 0.33 mg QE/g extract, respectively, IC_{50} values for DPPH and FRAP was 45.02 ± 0.13 µg/mL and 169.91 ± 0.51 mg $FeSO_4$/g extract, respectively, IC_{50} value for tyrosinase inhibition was 18.48 ± 0.19 mg/mL, which was approximate inhibition as kojic acid, bacterial inhibition for *Staphylococcus aureus*, *Bacillus cereus*, and *Salmonella typhimurium* was maximum in 12.19 ± 0.01 mm, 13.03 ± 0.01 mm and 12.03 ± 0.02 mm, against α-amylase and α-glucosidase inhibition was 73.76% and 63.61% which was approximately 96.5% and 93.5% of market acarbose, respectively. The study evidenced that the MAE with methanol is an effective method for extracting multitasking active ingredient from the *V. cinerea* at once in the future when plants have the highest potential.

Keywords: *Vernonia cinerea* (L.) Less., Total phenolic content, Antioxidant, Tyrosinase inhibitory, Antibacterial, Anti-α-amylase Activity, Anti-α-glucosidase Activity

Introduction

At present, the incorporation of plant-derived compounds into wellness formulations, beauty preparations, and nutritional products demonstrates a steadily growing trajectory, with particular emphasis on naturally occurring substances exhibiting varied biological functionalities including radical scavenging agents, enzymatic modulators, and pathogen-resistant compounds. These bioactive constituents serve essential functions in chronic disease prevention, aging deceleration, and constitute fundamental elements in therapeutic cosmetic formulations [1,2]. Market demand for versatile botanical products delivering comprehensive therapeutic benefits within singular preparations continues expanding due to their wide-ranging advantages and manufacturing cost optimization potential.

Vernonia cinerea (L.) Less., vernacularly recognized as "white-flowered aster," represents an endemic therapeutic plant species extensively distributed across Thailand's geographical regions.

Traditional healing practices have employed this botanical resource for generations, encompassing applications in pyretic relief, urinary stimulation, inflammatory response modulation, glycemic regulation, bacterial proliferation control, and nicotine dependency management [3]. Contemporary scientific investigations have identified multiple phytochemical categories within this species, including flavonoid derivatives, phenolic constituents, terpenoid structures, and alkaloid compounds, which demonstrate remarkable and diverse biological potentials.

Radical scavenging compounds serve fundamental roles in cellular protection against oxidative deterioration, representing the principal causative mechanism underlying chronic pathological conditions and age-associated degenerative phenomena. Research findings indicate that white-flowered aster demonstrates substantial free radical neutralizing capabilities [4], establishing its applicability potential in nutritional supplementation and cosmetic preparations targeting age retardation and dermal protection.

The tyrosinase enzyme system functions critically in melanogenesis pathways within cutaneous tissues. Modulating this enzymatic activity provides cosmeceutical benefits, specifically in ameliorating hyperpigmentation disorders, age spots, skin discoloration, and melanotic lesions. These properties of tyrosinase inhibit use ingredient in the development of cosmetic [5].

Naturally derived antimicrobial compound attract in growing interest due to drug resistant of microbial. Previous studies have shown that *Vernonia cinerea* has antimicrobial activities [6] can be developed into antimicrobial product. Type 2 Diabetes Mellitus presents a major global public health challenge, primarily caused by insulin resistance and dysfunction of enzymes involved in the carbohydrate digestion process. α -amylase (α-amylase) and α-glucosidase (α-glucosidase) has an important in the degradation of starch and polysaccharides into glucose. When these enzymes are overactive resulting in (postprandial hyperglycemia) [7]. The inhibition of α-amylase and α-glucosidase enzyme activity is important in controlling blood glucose levels and is a fundamental in the treatment of diabetes. According to previous studies have shown that extract of *Vernonia cinerea* significantly inhibits α-amylase and α-glucosidase [8]. This gives it potential for development as dietary supplement to help control blood glucose levels for diabetic patients or at-risk groups.

Recovering bioactive constituents from therapeutic plants to achieve optimal efficiency while maintaining comprehensive activity profiles simultaneously represents a substantial technical challenge, given that individual active components possess distinct physicochemical characteristics. Parameters requiring systematic evaluation include solvent polarity specifications, thermal conditions, processing duration, material-to-solvent proportions, and extraction methodologies such as maceration, ultrasonic-assisted extraction (UAE), and microwave-assisted extraction (MAE). Each technique significantly influences the yield, chemical stability, and compositional diversity of recovered bioactive materials [9,10]. Effective solvent systems must accommodate bioactive compound recovery across polarity ranges, from moderately polar phenolic structures to non-polar terpenoid molecules. Implementing mixed solvent approaches or modifying proportions of solvents with varying polarity characteristics may constitute an efficient methodology.

While individual biological activity investigations of white-flowered aster exist in scientific literature, comprehensive research systematically comparing diverse extraction methodologies and solvent configurations remains limited. Such studies would optimize recovery efficiency for bioactive compounds encompassing radical scavenging capacity, tyrosinase modulation, pathogen suppression, α-amylase inhibition, and α-glucosidase suppression within unified processing protocols. Identifying optimal parameters for simultaneous multi-activity extraction would enhance economic viability and minimize production expenditures. Furthermore, comparative evaluations of contemporary extraction technologies including ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) versus conventional methodologies such as maceration regarding processing time, energy requirements, and extract quality characteristics provide essential information for commercial scale implementation.

Therefore, this research aims to determine optimal conditions of solvent systems and extraction techniques that can maximize yield and effectiveness of diverse

bioactive constituents from indigenous Thai *Vernonia cinerea*, particularly enhancing antioxidation activity, tyrosinase modulation, pathogen suppression, α-amylase inhibition, and α-glucosidase inhibition within a single extraction process. Currently, hyperpigmentation disorders, type 2 diabetes and antimicrobial resistance are creating significant global economic and health burdens, resulting in continuously increasing demand for highly effective and safe natural products. The exploration of extraction methods that can utilize multiple bioactive compounds from medicinal plants for these applications is therefore critically important in the contemporary context. This research will also contribute to establishing crucial new knowledge for designing highly efficient extraction processes, promoting effective and cost-efficient utilization of local herbal resources, and leading to the development of herbal product, cosmetics, or dietary supplements from local resources that possess multiple activities, are safe, environmentally friendly, and have potential for international commercial competitiveness. This will help promote the advancement of traditional wisdom through modern scientific processes and create added value for community and national economies.

Materials and methods

Chemicals

Chemical reagents utilized in this research were of analytical grade purity and were purchased from Fisher Chemical, Merck, Ajax Finechem, and Sigma-Aldrich.

Plant material preparation

The botanical specimen utilized comprised *Vernonia cinerea* obtained from Khlong Luang District, Pathum Thani Province, collected during April 2023. Plant identification verification was conducted by the Herbarium Office, Forest and Plant Conservation Research Bureau, Department of National Parks, Wildlife and Plant Conservation, Bangkok. The investigation employed leaf, flower, and stem portions. Plant materials underwent thorough cleansing with distilled water, air-drying, and sectioning into small fragments, followed by dehydration in a hot-air oven at 45 °C until achieving constant mass. Materials were pulverized using a grinding apparatus and sieved through 60-mesh screening. Precise weight measurements were recorded, and processed materials were stored in light-proof containers under sealed conditions at 4 °C [9].

Maceration methodology

Vernonia cinerea specimens from different plant sections (leaves, flowers, and stems) were individually processed at 5 g portions with 50 mL distilled water as the extraction medium. Samples underwent gentle agitation, sealed containment, and ambient temperature incubation for 48 h. Filtration proceeded through Whatman No.1 filter paper with solution collection. Concentrate recovery utilized rotary vacuum evaporation at 45 °C. The protocol was replicated with alternative solvents including 50% ethanol, 70%ethanol, methanol, and acetone. Crude extract masses were determined and extraction yield percentages calculated [9].

Crude extract yield calculation (%w/w) = (Crude extract mass×100)/Dry weight

Ultrasonic-assisted extraction (UAE)

Vernonia cinerea plant materials (leaves, flowers, and stems) were processed separately using 5 g in 50 mL distilled water. After brief mixing and sealing, specimens were subjected to ultrasonic bath treatment at 40 kHz frequency and 40 °C for 30 min. Post-treatment cooling preceded filtration through Whatman No.1 filter paper with solution collection. Concentrate recovery utilized rotary vacuum evaporation at 45 °C. The methodology was repeated with alternative solvents including 50% ethanol, 70% ethanol, methanol, and acetone. Crude extract masses were determined and extraction yield percentages [11,12].

Crude extract yield calculation (%w/w) = (Crude extract mass×100)/Dry weight

Microwave-assisted extraction (MAE)

Vernonia cinerea specimens from various components (leaves, flowers, and stems) were prepared 5 g portions with 50 mL distilled water. Following gentle agitation, microwave settings were configured at 400 W with 5-min extraction duration. Post treatment cooling preceded filtration through Whatman No.1 filter paper with solution collection. Concentrate recovery

utilized rotary vacuum evaporation at 45 °C. The procedure was replicated with alternative solvents including 50% ethanol, 70% ethanol, methanol, and acetone. Crude extract masses were determined and extraction yield percentages calculated [13,14].

Crude extract yield calculation (%w/w) = (Crude extract mass×100)/Dry weight

Total phenolic content determination

Phenolic compound quantification in extracts employed gallic acid standard solution preparation at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL. Standard solutions (125 µL) were pipetted into test tubes, followed by addition of 0.5 mL distilled water and 125 µL Folin-Ciocalteu reagent. After mixing and 3-min ambient temperature incubation, 1.25 mL of 7% sodium carbonate (Na_2CO_3) solution and 1 mL distilled water were added. Following thorough mixing, samples underwent 90-min dark incubation. Absorbance measurements proceeded at 760 nm wavelength using UV-Visible spectrophotometry (Libra S70 model) with distilled water as blank. Crude extract analysis involved triplicate determinations. Total phenolic quantification utilized gallic acid standard curves, with results expressed as milligrams gallic acid equivalents per gram dry extract (mg GAE/g extract) [15].

Total flavonoid content determination

Flavonoid compound quantification in extracts utilized quercetin as the reference standard for calibration curve construction. Quercetin solutions were prepared at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL. Standard solutions (0.2 mL) were pipetted into test tubes, followed by addition of 1.8 mL of 1% aluminum chloride ($AlCl_3$) solution. After mixing and 10-min ambient temperature incubation, absorbance measurements proceeded at 415 nm wavelength using UV-Visible spectrophotometry (Libra S70 model or equivalent) with distilled water as blank. Crude extract analysis involved triplicate determinations. Total flavonoid quantification utilized quercetin standard curves, with results expressed as milligrams quercetin equivalents per gram dry extract (mg QE/g extract) [16].

DPPH radical scavenging assessment

Free radical neutralizing capacity of extracts was evaluated using reference standards including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol, alongside crude extract specimens prepared at concentrations of 50, 250, 500 and 1,000 µg/mL. Standard solutions or extracts at each concentration were pipetted (50 µL volumes) into test tubes, followed by addition of 5 mL DPPH (2,2-diphenyl-1-picrylhydrazyl) solution at 6×10^{-5} molar concentration. After mixing and 30-min dark incubation at ambient temperature, absorbance measurements proceeded at 516 nm wavelength using UV-Visible spectrophotometry (Libra S70 model) with distilled water as blank. Triplicate determinations were conducted for each specimen. Free radical neutralizing capacity was calculated as percentage radical elimination using the formula:

% Radical scavenging = [(Ac – As)/Ac] × 100

where: Ac = absorbance of DPPH solution; As = absorbance of sample-DPPH mixture

Results were analyzed to determine concentration achieving 50% DPPH radical reduction (IC_{50} values) from concentration-response curves plotting sample concentration versus % Radical Scavenging [17].

Ferric reducing antioxidant power (FRAP) assessment

FRAP reagent preparation involved combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6 tripyridyl-s-triazine) in 40 mM hydrochloric acid, and 20 mM ferric chloride hexahydrate ($FeCl_3\cdot6H_2O$) at 10:1:1 volumetric ratio, with pre-warming at 37 °C for 4 min. Ferrous ion standard solutions were prepared at 200, 400, 600, 800 and 1,000 µM concentrations. Standard solutions (50 µL) were combined with 950 µL prepared FRAP reagent, mixed via vortex, and incubated in darkness at ambient temperature for 30 min. Absorbance measurements proceeded at 593 nm wavelength using spectrophotometry. Standard curves were constructed plotting ferrous ion concentration versus 593 nm absorbance. Sample solutions were prepared at 2 mg/mL concentration. Testing involved combining 50 µL sample solution with 950 µL FRAP reagent, vortex mixing, 30-min dark incubation at

ambient temperature, and 593 nm absorbance measurement. Standard curves facilitated Fe^{2+} quantification from trolox or crude extract reactions. FRAP values represented Fe^{3+} to Fe^{2+} reduction capacity; elevated FRAP values indicated enhanced free radical neutralizing potential [18]. Triplicate analyses were conducted for each crude extract specimen.

Tyrosinase inhibition assessment

Sample solutions and kojic acid reference standards were prepared at 50, 250, 500 and 1,000 µg/mL concentrations dissolved in 99% ethanol. Each concentration underwent tyrosinase enzymatic inhibition testing using the Dopachrome methodology compared with kojic acid standards. Control solutions comprised 1,500 µL sodium phosphate buffer (0.02 M, pH 6.8), 500 µL tyrosinase enzyme solution (1,000 units/mL), and 500 µL 99% ethanol. Sample solutions contained 1,500 µL sodium phosphate buffer (0.02 M, pH 6.8), 500 µL tyrosinase enzyme solution, and 500 µL sample or standard solution in microplate reader cell culture plates (EnSpire 2300N model). Following mixing and 30-min incubation at 25 °C, absorbance measurements proceeded at 475 nm. Subsequently, 500 µL L-DOPA solution was added to each well, volume adjusted with 99% ethanol, mixed, incubated at 25 °C for 2 min, and re-measured at 475 nm. Triplicate experiments facilitated percentage tyrosinase inhibition calculation using:

$$\% \text{ Tyrosinase Inhibition} = (A_{control} - A_{sample})/A_{control} \times 100$$

Concentration-response curves plotting extract concentration versus % Tyrosinase Inhibition enabled IC_{50} determination [19].

Antibacterial activity assessment

Crude extract antimicrobial properties were evaluated using agar well diffusion methodology. Pathogenic bacterial cultures included gram-positive *Staphylococcus aureus* (TISTR746) and *Bacillus cereus* (TISTR1449), and gram-negative *Salmonella Typhimurium* (TISTR1472) cultivated in Mueller Hinton broth for 24 h with turbidity adjusted to McFarland #0.5 standard (≈10^8 CFU/mL). Bacterial suspensions were spread onto Mueller Hinton agar using sterile cotton swabs. Wells (5 mm diameter) were created using cork borers, filled with 100 µL crude extract specimens at 10 mg/mL concentration, and incubated at 37 °C for 18 - 24 h. Inhibition zone diameters were measured in millimeters and compared with DMSO (solvent control) and antibiotic controls including Streptomycin and Tetracycline at equivalent concentrations. Triplicate experiments were conducted [20].

Minimum inhibition concentration assessment

Three bacterial species were cultivated in nutrient broth and incubated at 37 °C for 18 - 24 h. Bacterial concentration was adjusted to 10^8 CFU/mL using McFarland standard #0.5, then diluted 100-fold with MHB to achieve 1.5×10^6 CFU/mL. Crude extract specimens from leaves, flowers, and stems were prepared at 1.562, 3.125, 6.25, 12.5, 25, and 50 mg/mL concentrations. 96-well microtiter plates received 100 µL MHB per well, followed by 100 µL bacterial suspension (1 %v/v) and 100 µL extract solution. Incubation proceeded at 37 °C for 24 h with negative control (DMSO ≤ 1%), positive controls (tetracycline and streptomycin antibiotics), and blank control (no bacterial addition). Post incubation, microtiter plate contents were streaked onto solid media (MHA) and incubated at 37 °C for 24 h. MIC values represented lowest extract concentrations preventing visible bacterial growth on culture plates. Triplicate experiments were conducted [20].

Minimal bactericidal concentration assessment

Bactericidal concentration determination (MBC) employed plate dilution methodology. Extract specimens underwent dilution in Mueller Hinton broth with bacterial cultivation and 37 °C incubation for 24 h. Sterilization efficacy was assessed by distributing 100 µL volumes onto Mueller Hinton agar surfaces, followed by 37 °C incubation for 24 h. Bacterial growth monitoring facilitated bactericidal activity evaluation. MBC values represented lowest extract concentrations preventing visible bacterial growth in test cultures. Triplicate experiments were conducted [21].

Anti-α-amylase activity assessment

α-Amylase inhibition testing was adapted from Gella *et al.* [22]. Acarbose standard or sample solutions (10 mg/mL, 20 µL) were combined with 100 µL sodium

phosphate buffer (pH 6.9) and 20 µL α-amylase enzyme solution in sodium phosphate buffer using 96-well microplate readers. Following 15-min ambient temperature incubation, 20 µL 2-chloro-4-nitrophenyl-α-D-maltatrioside solution was added to each well, mixed, and incubated for 30 min at ambient temperature. Sodium carbonate solution (40 µL) was added before absorbance measurement at 405 nm wavelength. Triplicate experiments facilitated percentage α-amylase inhibition calculation using:

% α-amylase inhibition = [(A-B)/A] × 100

where: A = absorbance without test substance; B = absorbance with test substance

Anti-α-glucosidase activity assessment

α-glucosidase inhibition testing was adapted from Matsui *et al.* [23]. Acarbose standard or sample solutions (10 mg/mL, 20 µL) were combined with 100 µL sodium phosphate buffer (pH 6.9) and 20 µL α-glucosidase enzyme solution in sodium phosphate buffer using 96-well microplate readers. Following 15-min ambient temperature incubation, 20 µL p-nitrophenyl-α-D-glucopyranoside solution was added, mixed, and incubated for 30 min. Sodium carbonate solution (40 µL) was added before absorbance measurement at 405 nm wavelength. Triplicate experiments facilitated percentage α-glucosidase inhibition calculation using:

% α-glucosidase inhibition = [(A – B)/A]×100

where: A = absorbance without test substance; B = absorbance with test substance

Statistical analysis

Experimental results underwent variance analysis using ANOVA methodology with mean comparisons via Duncan's multiple-range tests at $p < 0.05$ significance levels.

Results and discussion

Impact of extraction techniques and solvent types on crude extract yield

When white-flowered aster specimens from various plant sections including leaves, flowers, and stems underwent processing with 50 mL solvent systems comprising distilled water, 50% ethanol, 70% ethanol, methanol, and acetone, followed by filtration and concentrate recovery via rotary vacuum evaporation, crude extract specimens were obtained with calculated percentage yields as demonstrated in **Table 1**.

Table 1 Efficiency of extraction in different methods and solvents from *Vernonia cinerea.*

Methods	Solvent	Extraction yield (%)		
		Leaf	Flower	Stem
Maceration	DI water	7.6 ± 0.06^{e}	7.0 ± 0.00^{f}	8.2 ± 0.08^{e}
	EtOH 50%	9.2 ± 0.15^{d}	8.4 ± 0.08^{e}	9.8 ± 0.10^{d}
	EtOH 70%	11.0 ± 0.00^{c}	10.8 ± 0.10^{d}	11.6 ± 0.08^{c}
	MeOH	13.0 ± 0.00^{b}	12.2 ± 0.10^{c}	13.2 ± 0.00^{b}
	Acetone	15.0 ± 0.00^{a}	14.2 ± 0.10^{b}	15.7 ± 0.10^{a}
UAE	DI water	9.0 ± 0.00^{d}	8.4 ± 0.15^{e}	9.4 ± 0.15^{d}
	EtOH 50%	10.6 ± 0.31^{c}	10.2 ± 0.00^{d}	11.0 ± 1.00^{c}
	EtOH 70%	12.6 ± 0.15^{b}	12.0 ± 0.06^{c}	13.4 ± 0.10^{b}
	MeOH	15.0 ± 0.00^{a}	14.4 ± 0.40^{b}	15.4 ± 0.20^{a}
	Acetone	15.8 ± 0.00^{a}	16.2 ± 0.10^{a}	16.8 ± 0.10^{a}
MAE	DI water	8.6 ± 0.00^{d}	8.8 ± 0.00^{e}	9.2 ± 0.15^{d}
	EtOH 50%	11.4 ± 0.10^{c}	10.6 ± 0.25^{d}	11.6 ± 0.00^{c}
	EtOH 70%	13.4 ± 0.20^{b}	13.0 ± 0.03^{c}	13.8 ± 0.15^{b}

Methods	Solvent	Extraction yield (%)		
		Leaf	Flower	Stem
	MeOH	14.6 ± 0.35[a]	14.4 ± 0.20[b]	15.2 ± 0.19[a]
	Acetone	16.6 ± 0.10[a]	16.2 ± 0.10[a]	17.5 ± 0.10[a]

[a], [b], [c], [d], [e] the mean different is significantly at the 0.05 level using Ducan.

DI water = Distilled Water, EtOH = ethanol, MeOH = Methanol.

UAE = Ultrasound-Assisted Extraction, MAE = Microwave-Assisted Extraction.

Comparative evaluation of crude material recovery efficiency from leaves, flowers, and stems of *Vernonia cinerea* utilizing 3 extraction methodologies Maceration, Ultrasound-Assisted Extraction (UAE), and Microwave-Assisted Extraction (MAE) with 5 solvent systems including distilled water, 50% ethanol, 70% ethanol, methanol, and acetone, demonstrated statistically significant variations ($p < 0.05$) in recovered extract quantities. Traditional maceration methodology yielded the lowest recovery rates across all solvent systems, while acetone achieved maximum yields across all plant segments: leaves (15.0% ± 0.00%), flowers (14.2% ± 0.10%), and stems (15.7% ± 0.10%), followed by methanol. The least effective solvent was distilled water (7.0% - 8.2%). UAE methodology, employing high-frequency acoustic waves to accelerate extraction processes, demonstrated enhanced efficiency with acetone maintaining superior yields across all plant segments (15.8% - 16.8%) and methanol producing comparable results. Ethanol concentrations (70% and 50%) achieved intermediate performance levels, while distilled water maintained the lowest efficiency. MAE technique, utilizing microwave energy for extraction acceleration, exhibited the highest overall efficiency with acetone producing maximum yields from all plant segments (leaves 16.6% ± 0.10%, flowers 16.2% ± 0.10%, stems 17.5% ± 0.10%), followed by methanol (14.6% - 15.2%) and 70% ethanol with similar performance. Across all techniques, solvent performance patterns remained consistent with acetone demonstrating superior efficiency (14.2% - 17.6%) due to its moderate polarity characteristics optimal for dissolving phenolic and flavonoid compounds from Asteraceae family plants [24]. Methanol exhibited secondary performance (12.2% - 15.4%) attributed to its excellent polar and semi-polar compound solubility and small molecular size facilitating cellular penetration [25]. Ethanol 70% (10.8% - 13.8%) significantly outperformed 50% ethanol due to water proportions enhancing simultaneous dissolution of polar and non-polar bioactive constituents [26]. Distilled water demonstrated minimal efficiency (7.0% - 9.4%) due to limitations in non-polar compound solubility [27]. Comparative technique evaluation revealed MAE superiority (8.6% - 17.6%) through microwave energy transfer mechanisms capable of rapid cell wall disruption and enhanced mass transfer [28,29]. UAE demonstrated intermediate efficiency (8.4% - 16.8%) via cavitation mechanisms facilitating cell wall breakdown and increased surface contact between solvents and plant tissues [10]. Maceration, relying on natural diffusion processes, exhibited lowest efficiency (7.0% - 15.6%) while maintaining advantages in simplicity and cost-effectiveness [9]. Plant segment comparisons revealed stems producing highest extract yields (8.2% - 17.6%), consistent with Sonar and Rathod *et al.* [30] findings indicating Asteraceae family stem tissues typically accumulate elevated phenolic and terpenoid levels. Leaves demonstrated secondary yields (7.6% - 16.6%) with high bioactive accumulation but reduced tissue mass compared to stems [31]. Flowers produced lowest yields (7.0% - 16.2%), potentially attributed to reduced dry weight and thinner structural characteristics [32]. In conclusion, acetone and methanol represent the most efficient solvents for *Vernonia cinerea* extraction, particularly when combined with MAE technique demonstrating optimal performance across all conditions, emphasizing the significant potential of modern extraction technologies for enhancing bioactive constituent recovery from Thai medicinal plants.

Total phenolic content and total flavonoid content

When crude extract specimens from various *Vernonia cinerea* aster segments, obtained through

different extraction methodologies and solvent systems, underwent Total Phenolic Content and Total Flavonoid Content analysis, results are presented in **Table 2**.

Table 2 Total phenolic content of *Vernonia cinerea* extract in different methods and solvents.

Methods	Solvent	Total phenolic content (mg GAE/g extract)			Total flavonoid content (mg QE/g extract)		
		Leaf	Flower	Stem	Leaf	Flower	Stem
Maceration	DI water	67.78 ± 1.18^{l}	42.09 ± 1.89^{p}	55.81 ± 2.08^{n}	34.76 ± 0.51^{d}	29.02 ± 0.40^{d}	30.85 ± 0.38^{d}
	EtOH 50%	81.72 ± 1.42^{j}	56.99 ± 3.15^{m}	66.37 ± 1.83^{l}	38.16 ± 0.61^{d}	30.88 ± 0.29^{d}	35.20 ± 0.67^{d}
	EtOH 70%	125.52 ± 2.79^{f}	100.35 ± 0.87^{i}	125.06 ±1.50^{f}	40.72 ± 0.36^{d}	34.22 ± 0.68^{d}	38.55 ± 0.43^{d}
	MeOH	146.93 ± 3.76^{d}	112.49 ± 2.17^{h}	136.12 ± 3.85^{e}	56.29 ± 1.41^{d}	37.98 ± 0.53^{d}	48.68 ± 0.21^{d}
	Acetone	135.86 ± 1.45^{e}	116.24 ± 2.84^{h}	126.46 ± 4.00^{f}	47.01 ± 0.32^{d}	33.48 ± 1.21^{d}	39.18 ± 0.48^{d}
UAE	DI water	77.84 ± 1.47^{k}	46.22 ± 0.84^{o}	69.82 ± 1.62^{l}	37.01 ± 0.48^{d}	27.43 ± 0.82^{d}	31.89 ± 1.44^{d}
	EtOH 50%	125.10 ± 0.58^{f}	96.85 ± 2.06^{j}	111.63 ± 0.93^{g}	46.03 ± 0.54^{d}	28.69 ± 0.34^{d}	34.59 ± 0.65^{d}
	EtOH 70%	147.03 ± 1.51^{d}	127.14 ± 2.61^{f}	134.00 ± 0.81^{e}	51.50 ± 0.63^{d}	38.81 ± 0.42^{d}	46.59 ± 0.93^{d}
	MeOH	168.18 ± 3.08^{b}	126.88 ± 1.83^{f}	138.10 ± 1.64^{e}	78.88 ± 0.22^{a}	63.01 ± 1.59^{c}	66.30 ± 0.59^{c}
	Acetone	155.71 ± 2.68^{c}	136.57 ± 4.02^{e}	145.05 ± 3.25^{d}	66.71 ± 2.89^{b}	47.88 ± 3.03^{d}	53.40 ± 2.00^{d}
MAE	DI water	88.34 ± 1.22^{i}	65.39 ± 0.93^{m}	73.34 ± 2.92^{k}	40.67 ± 0.87^{d}	28.67 ± 0.48^{d}	35.22 ± 0.79^{d}
	EtOH 50%	140.56 ± 0.94^{d}	114.77 ± 3.66^{h}	124.83 ± 3.48^{f}	44.63 ± 0.76^{d}	31.17 ± 0.38^{d}	39.20 ± 0.34^{d}
	EtOH 70%	157.54 ± 2.71^{c}	134.08 ± 1.05^{e}	141.56 ± 0.55^{d}	61.91 ± 0.35^{d}	42.61 ± 0.67^{d}	53.14 ± 1.72^{d}
	MeOH	195.48 ± 4.18^{a}	168.24 ± 0.31^{a}	174.01 ± 4.09^{a}	98.07 ± 0.33^{a}	71.39 ± 0.74^{b}	86.97 ± 1.50^{a}
	Acetone	166.14 ± 1.78^{b}	132.97 ± 1.41^{e}	147.51 ± 2.23^{d}	71.53 ± 0.62^{b}	51.39 ± 1.72^{d}	64.11 ± 0.47^{c}

$^{a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p}$ the mean different is significantly at the 0.05 level using Ducan.

DI water = Distilled Water, EtOH = ethanol, MeOH = Methanol.

UAE = Ultrasound-Assisted Extraction, MAE = Microwave-Assisted Extraction.

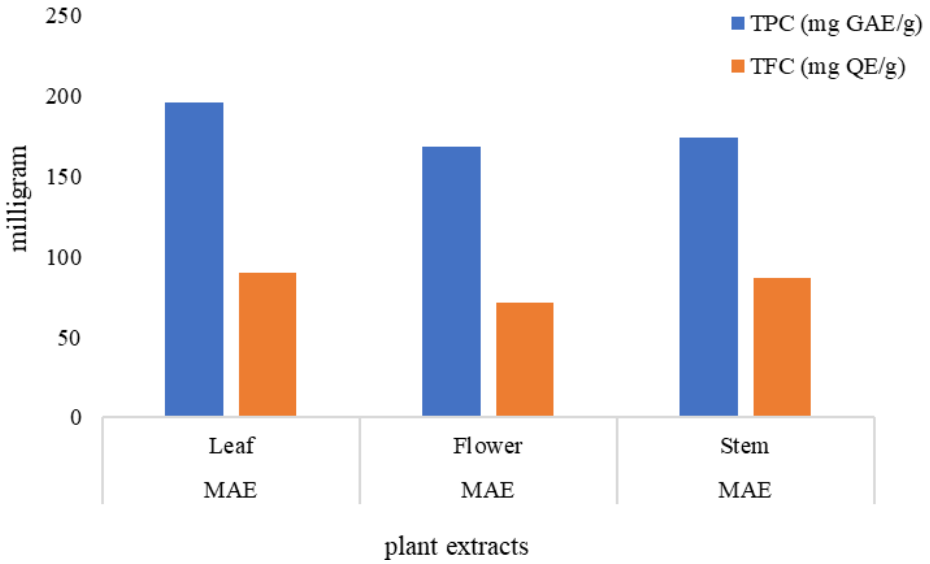

Figure 1 The highest Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in leaves, flowers, and stems of *Vernonia cinerea* methanolic extracts obtained via microwave-assisted extraction (MAE) methodology.

Data presented in **Table 2** and **Figure 1**. demonstrates phenolic and flavonoid constituent quantification from *Vernonia cinerea* extracts utilizing diverse methodologies and solvent configurations. MAE (Microwave-Assisted Extraction) exhibited superior efficiency for both compound categories, particularly when employing methanol as the extraction medium, yielding maximum total phenolic concentrations in leaves (195.48 ± 4.18 mg GAE/g extract), flowers (168.24 ± 0.31 mg GAE/g extract), and stems (174.01 ± 4.09 mg GAE/g extract). Peak total flavonoid concentrations were similarly observed in leaf tissues (98.07 ± 0.33 mg QE/g extract). Comparative evaluation with UAE and Maceration revealed statistically significant superiority ($p < 0.05$) for MAE methodology. Plant segment comparisons demonstrated leaves containing the highest bioactive constituent concentrations, followed sequentially by stems and flowers.

Investigation outcomes indicate *Vernonia cinerea* leaves possess maximum total phenolic and flavonoid concentrations compared to flowers and stems, particularly when utilizing MAE methodology combined with methanol solvent, achieving total phenolic levels of 195.48 ± 4.18 mg GAE/g extract and total flavonoid concentrations of 98.07 ± 0.33 mg QE/g extract in leaf tissues. Such secondary metabolite distribution patterns align with Tungmunnithum *et al.* [31] findings indicating Asteraceae family leaves typically accumulate elevated phenolic concentrations compared to other plant segments due to UV radiation protection functions and free radical neutralization during photosynthetic processes. Additionally, Pandey and Rizvi [25] specify that leaves serve crucial protective roles against herbivore damage and pathogen attacks, necessitating elevated defensive compound production. Stem tissues demonstrated secondary phenolic and flavonoid concentrations following leaves (174.01 ± 4.09 mg GAE/g extract and 86.97 ± 1.50 mg QE/g extract respectively) attributed to their nutrient storage and transport functions, consistent with Ghasemzadeh and Ghasemzadeh [33] findings indicating medicinal plant stems function as secondary metabolite reservoirs for distribution to other plant components. Flowers exhibited minimum concentrations (168.24 ± 0.31 mg GAE/g extract and 71.39 ± 0.74 mg QE/g extract) potentially due to shortened functional lifespan and presence of alternative pigments including carotenoids and anthocyanins facilitating pollinator attraction rather than exclusive flavonoid dependence [34].

Extraction methodology efficiency comparisons revealed MAE demonstrating superior performance for phenolic and flavonoid recovery from all *Vernonia cinerea* segments, consistent with Proestos and Komaitis [35] research indicating microwave energy significantly enhances phenolic extraction efficiency through water molecule vibrations within plant cells, causing cell wall disruption and desired compound liberation. Mandal *et al.* [13] further emphasize MAE reduced processing time (2 - 5 min) compared to conventional methods, minimizing heat-sensitive compound degradation. UAE demonstrated secondary efficiency following MAE, particularly for leaf extraction, producing results comparable to Maceration. This aligns with Chemat *et al.* [10] explanations that ultrasonic waves effectively disrupt cell walls in robust tissue structures such as thick cuticle leaves, but may demonstrate reduced efficiency compared to microwave energy in softer tissues like flowers and stems. Maceration's effectiveness for leaf extraction may result from extended processing duration (24 - 48 h) facilitating complete solvent cellular penetration, as Azwanida [9] describes prolonged immersion enhancing cellular compound diffusion. Solvent influence analysis revealed methanol demonstrating maximum efficiency for phenolic and flavonoid compound extraction from all *Vernonia cinerea* segments across all extraction methodologies, consistent with Do *et al.* [36] findings indicating methanol's capability for diverse phenolic structure dissolution due to moderate polarity characteristics suitable for both flavonoid aglycones and glycosides. Dai and Mumper [37] additionally emphasize methanol's capacity for disrupting hydrogen bonds between phenolic compounds and cellular proteins and carbohydrates, enhancing extraction efficiency. Acetone demonstrated secondary performance following methanol, particularly for leaf extraction (155.60 ± 1.45 mg GAE/g extract), consistent with Naczk and Shahidi [27] explanations that acetone possesses lower polarity than methanol, making it suitable for flavonoid compound extraction. Ethanol 50% achieving intermediate results can be explained through Spigno and Faveri [28]

research indicating ethanol-water mixtures at appropriate ratios effectively extract phenolic compounds, though efficiency remains below pure methanol. Distilled water demonstrated minimum efficiency for phenolic and flavonoid compound extraction from all white-flowered aster segments, consistent with Dai and Mumper [37] explanations that most phenolic compounds prefer organic solvents over water due to non-polar or low-polarity structural characteristics. However, water's capacity for extracting phenolic compounds at certain levels (67.78 ± 1.18 mg GAE/g extract in leaves) indicates presence of water-soluble flavonoid glycosides within the plant.

Regarding relationships between total phenolic and flavonoid constituents, investigation results demonstrate positive correlations between total phenolic compound and total flavonoid concentrations across all *Vernonia cinerea* aster segments, consistent with Wojdyło *et al.* [38] research identifying similar relationships in Asteraceae family plants. Flavonoid proportions of approximately 40% - 50% of total phenolics in this investigation align with Pietta [39] reports indicating flavonoids as primary phenolic compound groups in this plant family. Comparative analysis with Lim [40] studies on other *Vernonia* species revealed this investigation yielding higher phenolic concentrations, potentially attributed to varietal differences, cultivation areas, and sample preparation methodologies. Additionally, Zakaria *et al.* [41] research on *V. amygdalina* found MAE combined with methanol producing optimal results, supporting this investigation's findings. Constituent concentration variations between plant segments observed in this study align with Surveswaran *et al.* [42] reports investigating secondary metabolite distribution in tropical medicinal plants, finding similar trends with leaves containing maximum bioactive concentrations, followed sequentially by stems and flowers. This investigation clearly demonstrates *Vernonia cinerea* as a valuable source of total phenolic compounds and flavonoids, particularly in leaf tissues, with MAE methodology combined with methanol providing maximum extraction efficiency, holding significant importance for future dietary supplements and herbal medicine product development.

Antioxidant activity

DPPH radical scavenging assessment

Free radical neutralizing capacity evaluation of crude white-flowered aster extracts using DPPH radical scavenging methodology revealed all plant segments demonstrating antioxidant potential with IC_{50} values ranging 45.02 - 76.09 µg/mL. Extraction methodology comparisons indicated MAE achieving superior performance with minimum IC_{50} values (45.02 - 76.09 µg/mL), followed by UAE (48.27 - 75.43 µg/mL) and Maceration (49.32 - 75.73 µg/mL) sequentially. Regarding solvent systems, methanol demonstrated optimal extraction efficiency with IC_{50} values spanning 45.02 - 56.89 µg/mL compared to acetone (48.27 - 62.08 µg/mL) and 70% ethanol (60.81 - 66.42 µg/mL). Plant segment comparisons revealed leaves exhibiting maximum radical scavenging capacity (IC_{50} = 45.02 - 72.62 µg/mL), followed by stems (49.62 - 74.17 µg/mL) and flowers (51.54 - 76.09 µg/mL). Comparative analysis with reference standards BHA (74.66 ± 0.58 µg/mL), BHT (59.07 ± 0.33 µg/mL), and α-tocopherol (46.53 ± 0.81 µg/mL) demonstrated that *Vernonia cinerea* extracts obtained via MAE methodology using methanol solvent exhibited radical scavenging capacity comparable to all 3 standard compounds, highlighting *Vernonia cinerea* potential as an effective natural antioxidant source, as presented in **Table 3**.

Ferric Reducing Antioxidant Power (FRAP) Assessment Antioxidant capacity evaluation using FRAP methodology measures Fe^{3+}-TPTZ complex reduction capability to Fe^{2+}-TPTZ by compounds possessing antioxidant characteristics, where elevated FRAP values indicate enhanced radical neutralizing capacity. Investigation findings revealed crude white flowered aster extracts demonstrating FRAP values ranging 70.91 - 169.91 mg $FeSO_4$/g extract. Extraction methodology comparisons indicated MAE achieving superior performance (130.77 - 169.91 mg $FeSO_4$/g extract), followed by UAE (104.93 - 141.98 mg $FeSO_4$/g extract) and Maceration (70.91 - 101.92 mg $FeSO_4$/g extract) sequentially. Solvent system comparisons revealed methanol producing maximum FRAP values (93.32-169.91 mg $FeSO_4$/g extract), followed by acetone (87.73 - 158.59 mg $FeSO_4$/g extract) and 70% ethanol (84.74 - 151.82 mg $FeSO_4$/g extract). Plant segment analysis demonstrated leaves exhibiting maximum antioxidant capacity (80.64 - 169.91 mg

$FeSO_4$/g extract), followed by stems (72.31 - 161.31 mg $FeSO_4$/g extract) and flowers (70.91 - 158.62 mg $FeSO_4$/g extract). These findings align with DPPH methodology results, confirming that white-flowered aster leaf extraction via MAE methodology using methanol solvent produces maximum antioxidant capacity, as detailed in **Table 3**.

Table 3 Total Phenolic Content of *Vernonia cinerea* extract in different methods and solvents.

Methods	Solvent	DPPH (IC_{50} µg/mL)			FRAP (mg of $FeSO_4$ /g extract)		
		Leaf	Flower	Stem	Leaf	Flower	Stem
Maceration	DI water	68.68 ± 0.33^{d}	75.73 ± 0.44^{e}	73.01 ± 0.94^{e}	80.64 ± 0.48^{e}	70.91 ± 0.87^{e}	72.31 ± 0.40^{e}
	EtOH 50%	67.46 ± 0.68^{d}	68.46 ± 0.78^{d}	68.01 ± 0.85^{d}	86.28 ± 0.33^{e}	81.15 ± 0.43^{e}	82.24 ± 0.79^{e}
	EtOH 70%	60.81 ± 0.48^{c}	66.00 ± 1.70^{c}	62.03 ± 0.43^{c}	91.05 ± 0.65^{e}	84.74 ± 0.35^{e}	86.97 ± 0.77^{e}
	MeOH	49.32 ± 0.05^{a}	56.89 ± 1.77^{b}	53.36 ± 0.78^{b}	101.92 ± 3.27^{d}	93.32 ± 1.57^{e}	97.06 ± 0.71^{e}
	Acetone	55.63 ± 0.90^{b}	60.89 ± 0.36^{b}	59.00 ± 0.32^{b}	95.08 ± 0.52^{e}	87.73 ± 2.49^{e}	92.16 ± 0.41^{e}
UAE	DI water	72.27 ± 1.66^{e}	75.43 ± 0.90^{e}	74.08 ± 0.55^{e}	110.52 ± 1.11^{d}	104.93 ± 0.91^{d}	107.42 ± 0.47^{d}
	EtOH 50%	64.89 ± 0.42^{c}	68.83 ± 1.02^{d}	66.69 ± 0.51^{d}	127.62 ± 0.45^{c}	117.18 ± 1.16^{d}	114.89 ± 0.85^{d}
	EtOH 70%	60.99 ± 0.54^{c}	65.17 ± 0.56^{c}	62.37 ± 1.73^{c}	135.13 ± 0.39^{c}	128.38 ± 1.01^{c}	131.40 ± 0.82^{c}
	MeOH	48.57 ± 0.85^{a}	53.17 ± 0.86^{b}	51.40 ± 1.41^{b}	141.98 ± 0.82^{b}	133.87 ± 0.61^{c}	138.73 ± 0.88^{c}
	Acetone	48.27 ± 0.80^{a}	53.06 ± 0.42^{b}	51.92 ± 1.69^{b}	137.50 ± 1.46^{c}	130.93 ± 1.01^{c}	133.99 ± 1.15^{c}
MAE	DI water	72.62 ± 1.65^{e}	76.09 ± 0.69^{e}	74.17 ± 0.59^{e}	136.60 ± 0.87^{c}	130.77 ± 0.97^{c}	135.62 ± 0.41^{c}
	EtOH 50%	70.18 ± 0.58^{e}	72.32 ± 1.55^{e}	71.84 ± 1.11^{e}	147.40 ± 2.12^{b}	140.99 ± 0.02^{b}	145.29 ± 0.63^{b}
	EtOH 70%	63.99 ± 0.53^{c}	66.42 ± 1.73^{d}	65.62 ± 0.25^{c}	151.82 ± 0.44^{b}	147.79 ± 1.06^{b}	149.30 ± 0.50^{b}
	MeOH	45.02 ± 0.13^{a}	51.54 ± 0.58^{b}	49.62 ± 0.46^{a}	169.91 ± 0.51^{a}	158.62 ± 0.74^{b}	161.31 ± 0.78^{a}
	Acetone	52.33 ± 0.77^{b}	59.83 ± 0.31^{b}	62.08 ± 0.25^{c}	158.59 ± 1.94^{b}	151.18 ± 0.75^{b}	153.81 ± 0.33^{b}

^{a}, ^{b}, ^{c}, ^{d}, ^{e}, ^{f}, ^{g}, ^{h}, ^{i}, ^{j}, ^{k}, ^{l} the mean different is significantly at the 0.05 level using Ducan.

DI water = Distilled Water, EtOH = ethanol, MeOH = Methanol.

UAE = Ultrasound-Assisted Extraction, MAE = Microwave-Assisted Extraction.

Experimental outcomes demonstrate Microwave-Assisted Extraction (MAE) achieving superior efficiency for antioxidant compound recovery from *Vernonia cinerea*, producing minimum radical inhibition IC_{50} values and maximum FRAP values compared to alternative extraction methodologies. These findings correspond with Belwal *et al.* [43] research developing multi-component analysis approaches for nutritional supplement extraction from *Berberis jaeschkeana* roots, discovering that MAE under optimized conditions significantly enhanced alkaloid and polyphenolic compound yields compared to conventional extraction techniques. Additionally, Alara *et al.* [44] confirmed MAE advantages when combined with natural deep eutectic solvents for antioxidant compound extraction from hazelnut pomace, emphasizing MAE's capacity for reduced processing time, decreased solvent consumption, and enhanced environmental compatibility compared to conventional techniques. Kaur *et al.* [45] investigations provide additional supporting evidence regarding MAE development for Ginkgo biloba leaf extraction, reporting that 60% ethanol-water mixtures with 120-watt microwave energy for 20 min achieved maximum antioxidant capacity in DPPH, ABTS, and FRAP assessments, demonstrating MAE potential for efficient antioxidant compound recovery. Methanol selection as extraction solvent demonstrates considerable

appropriateness, with González-Palma *et al.* [46] research indicating that methanolic *Pleurotus ostreatus* extracts exhibited superior radical scavenging capacity compared to aqueous extracts across multiple testing systems, particularly in DPPH and ABTS evaluations. Methanol's efficiency for bioactive compound extraction potentially results from enhanced polyphenolic compound solubility characteristics, which serve crucial roles in demonstrating antioxidant capacity, consistent with Rajurkar and Hande *et al.* [47] findings identifying statistically significant correlations between iron reduction capacity (FRAP values) and total phenolic concentrations. Furthermore, current experimental results revealed significant consistency between DPPH and FRAP testing value trends, aligning with Clarke *et al.* [48] investigations testing 92 Malaysian rainforest plant extract specimens from 27 species, discovering high correlations between DPPH and FRAP results despite different underlying principles. DPPH measures electron or hydrogen donation capacity to DPPH• radicals, while FRAP measures Fe^{3+} to Fe^{2+} reduction capacity. However, consistent testing results indicate *Vernonia cinerea* extracts possess diverse antioxidant mechanisms, representing crucial characteristics of valuable bioactive compounds for future dietary supplement or cosmeceutical product development.

Tyrosinase inhibition assessment

Tyrosinase enzymatic inhibition capacity investigation of crude *Vernonia cinerea* extracts using Dopachrome methodology revealed all plant segments demonstrating tyrosinase suppression capability with IC_{50} values ranging 18.48 - 37.30 mg/mL. Extraction methodology comparisons indicated MAE achieving superior performance with minimum IC_{50} values (18.48 - 28.32 mg/mL), followed by UAE (19.93 - 30.63 mg/mL) and Maceration (24.76 - 37.30 mg/mL) sequentially. Regarding solvent systems, methanol demonstrated optimal performance (IC_{50} = 18.48 - 27.30 mg/mL) compared to acetone (20.00 - 28.83 mg/mL) and 70% ethanol (20.69 - 31.94 mg/mL). Plant segment comparisons revealed leaves exhibiting maximum inhibitory capacity (IC_{50} = 18.48 - 35.59 mg/mL), followed by stems (19.57 - 36.27 mg/mL) and flowers (20.68 - 37.30 mg/mL). Comparative analysis with kojic acid reference standard (IC_{50} = 9.43 ± 0.33 mg/mL) demonstrated that *Vernonia cinerea* extracts obtained via MAE methodology using methanol solvent exhibited tyrosinase inhibitory capacity comparable to kojic acid, as presented in **Table 4**.

Table 4 Tyrosinase inhibitory activity of *Vernonia cinerea* extract in different methods and solvents.

Methods	Solvent	Tyrosinase Inhibition Assay (IC_{50} mg/mL)		
		Leaf	Flower	Stem
Maceration	DI water	35.59 ± 0.18^{k}	37.30 ± 0.38^{l}	36.27 ± 0.34^{l}
	EtOH 50%	32.27 ± 0.23^{j}	36.53 ± 0.41^{l}	35.84 ± 0.14^{k}
	EtOH 70%	29.89 ± 0.12^{h}	31.94 ± 0.08^{i}	30.63 ± 0.27^{i}
	MeOH	24.76 ± 0.70^{e}	27.30 ± 0.46^{g}	25.47 ± 0.12^{f}
	Acetone	26.75 ± 0.28^{g}	28.83 ± 0.58^{h}	27.93 ± 0.18^{g}
UAE	DI water	28.50 ± 0.09^{h}	30.63 ± 0.45^{i}	29.52 ± 0.35^{h}
	EtOH 50%	25.90 ± 0.17^{f}	28.20 ± 0.83^{h}	27.10 ± 0.67^{g}
	EtOH 70%	24.62 ± 0.97^{e}	26.97 ± 0.25^{g}	26.03 ± 0.33^{f}
	MeOH	19.93 ± 0.06^{b}	22.48 ± 0.40^{d}	21.01 ± 0.43^{c}
	Acetone	22.71 ± 0.87^{d}	25.45 ± 0.71^{f}	24.46 ± 0.66^{e}
MAE	DI water	23.21 ± 0.86^{d}	28.32 ± 0.27^{h}	26.31 ± 0.08^{f}
	EtOH 50%	22.98 ± 0.03^{d}	24.62 ± 0.21^{e}	23.27 ± 0.34^{d}

Methods	Solvent	Tyrosinase Inhibition Assay (IC_{50} mg/mL)		
		Leaf	Flower	Stem
	EtOH 70%	20.69 ± 0.25[c]	22.67 ± 0.29[d]	21.52 ± 0.47[c]
	MeOH	18.48 ± 0.19[a]	20.68 ± 0.26[c]	19.57 ± 0.18[b]
	Acetone	20.00 ± 0.16[b]	22.91 ± 0.60[d]	20.92 ± 0.65[c]

[a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p] the mean different is significantly at the 0.05 level using Ducan.

DI water = Distilled Water, EtOH = ethanol, MeOH = Methanol.

UAE = Ultrasound-Assisted Extraction, MAE = Microwave-Assisted Extraction.

Experimental investigations demonstrate Microwave-Assisted Extraction (MAE) achieving superior efficiency for tyrosinase inhibitory compound recovery from white-flowered aster, producing yields surpassing conventional extraction methodologies including Maceration and Soxhlet extraction. These outcomes align with Sonar and Rathod *et al.* [30] reports indicating MAE capability for marmelosin extraction from *Aegle marmelos* fruits within 30 s, while Soxhlet methodology required 7 h yet achieved significantly lower yields. MAE mechanisms utilizing microwave energy facilitate rapid cellular structure disruption, enhancing bioactive compound diffusion into solvents effectively. Methanol selection as extraction solvent demonstrated favorable outcomes, based on Belwal *et al.* [43] research achieving elevated berberine and palmatine recovery from Berberis roots using 100% methanol at pH 2.0 with 598 W for 2 min, consistent with methanol's capacity for dissolving polyphenolic compounds possessing tyrosinase inhibitory potential. *Vernonia cinerea* extract IC_{50} values ranging 18.48 - 37.30 mg/mL compared to kojic acid (19.43 ± 0.33 mg/mL) demonstrated comparable levels, with kojic acid confirmed for stable inhibitory activity across diverse systems [49-51]. Although certain natural compounds including 6,7,4'-trihydroxyisoflavone and quercetin-4'-O-β-D-glucoside exhibit superior inhibitory activity compared to kojic acid, white-flowered aster extracts maintain compelling commercial potential. Additionally, white-flowered aster leaf segments demonstrated maximum tyrosinase inhibitory activity, consistent with Petrillo *et al.* [52] findings indicating *Asphodelus microcarpus* flower segments achieving minimum IC_{50} values among all plant segments with non-competitive inhibition characteristics similar to glabridin, reported by Wang *et al*. [53] for efficient enzyme binding through static quenching processes and molecular binding complex formation. Comparative evaluation between MAE, UAE, and Maceration techniques revealed MAE maintaining distinct advantages, consistent with López-Salazar *et al.* [11] research demonstrating MAE significantly enhancing polyphenolic compound extraction efficiency from sage leaves using Box Behnken experimental design. Experimental outcomes indicate MAE represents a promising technique for tyrosinase inhibitory compound extraction from *Vernonia cinerea*, providing feasible potential for future cosmetic or pharmaceutical product development.

Antibacterial activity

Antimicrobial activity investigation of white-flowered aster extracts utilizing diverse extraction techniques and solvent systems against bacterial strains *Staphylococcus aureus* (TISTR746), *Bacillus cereus* (TISTR1449), and *Salmonella typhimurium* (TISTR1472) using Disk Diffusion methodology revealed that extracts obtained via MAE technique using methanol solvent from *Vernonia cinerea* leaves demonstrated maximum inhibitory activity, with inhibition zone values against *S. aureus, B. cereus*, and *S. typhimurium* measuring 12.19 ± 0.01, 13.03 ± 0.01, and 12.03 ± 0.02 mm respectively. Comparative factor analysis demonstrated MAE technique achieving superior efficiency, followed sequentially by UAE and Maceration methodologies. Regarding solvent systems, methanol demonstrated optimal performance, followed by acetone, 70% ethanol, 50% ethanol, and distilled water sequentially. Plant segment considerations revealed leaves exhibiting maximum activity, followed by stems and flowers respectively. Bacterial strain sensitivity comparisons indicated *B. cereus*

demonstrating highest susceptibility, followed by *S. aureus* and *S. typhimurium* respectively. These differences result from varying cell wall structures and defensive mechanisms, particularly *S. typhimurium* (gram-negative bacteria) exhibiting superior resistance. The group demonstrating minimum activity comprised Maceration extraction using distilled water from flowers against *S. typhimurium*, producing inhibition zones measuring only 1.06 ± 0.03 mm. Comparative analysis with reference standards Streptomycin and Tetracycline revealed *Vernonia cinerea* extracts maintaining antimicrobial activity below synthetic antimicrobial agents yet demonstrating potential for natural antimicrobial agent development, as detailed in **Table 5**.

Table 5 Antibacterial activity of *Vernonia cinerea* extract in different methods and solvents.

Methods	Solvent	Inhibition Zone (mm ± SD)								
		***S. aureus* (TISTR746)**			***B. cereus* (TISTR1449)**			***S. typhimurium* (TISTR1472)**		
		Leaf	**Flower**	**Stem**	**Leaf**	**Flower**	**Stem**	**Leaf**	**Flower**	**Stem**
Maceration	DI water	3.21 ± 0.18^{j}	2.01 ± 0.01^{l}	3.02 ± 0.02^{j}	3.32 ± 0.01^{j}	2.14 ± 0.02^{l}	3.13 ± 0.01^{j}	1.43 ± 0.01^{l}	1.06 ± 0.03^{l}	1.38 ± 0.01^{l}
	EtOH 50%	4.88 ± 0.57^{i}	3.14 ± 0.22^{j}	3.22 ± 0.02^{j}	4.52 ± 0.02^{i}	3.13 ± 0.01^{j}	3.42 ± 0.01^{j}	1.91 ± 0.02^{l}	1.49 ± 0.02^{l}	1.64 ± 0.01^{l}
	EtOH 70%	5.31 ± 0.01^{h}	3.86 ± 0.01^{i}	4.43 ± 0.02^{i}	5.22 ± 0.01^{h}	4.88 ± 1.16^{i}	4.01 ± 0.01^{i}	2.03 ± 0.02^{k}	1.82 ± 0.02^{l}	1.96 ± 0.03^{l}
	MeOH	6.04 ± 0.01^{g}	4.85 ± 0.06^{i}	5.45 ± 0.01^{h}	6.12 ± 0.01^{g}	4.22 ± 0.01^{i}	5.98 ± 0.01^{g}	3.02 ± 0.02^{j}	2.62 ± 0.02^{k}	2.94 ± 0.01^{k}
	Acetone	5.01 ± 0.01^{h}	3.05 ± 0.02^{j}	4.74 ± 0.03^{i}	4.94 ± 0.04^{i}	3.13 ± 0.02^{j}	4.22 ± 0.02^{i}	2.61 ± 0.02^{k}	2.04 ± 0.02^{k}	2.45 ± 0.01^{k}
UAE	DI water	6.22 ± 0.02^{g}	4.32 ± 0.02^{i}	5.95 ± 0.04^{g}	6.34 ± 0.02^{g}	4.03 ± 0.01^{i}	6.03 ± 0.01^{g}	3.63 ± 0.02^{j}	2.93 ± 0.02^{k}	3.04 ± 0.02^{j}
	EtOH 50%	7.31 ± 0.02^{f}	4.98 ± 0.02^{h}	6.02 ± 0.02^{g}	7.01 ± 0.01^{f}	4.12 ± 0.01^{i}	5.98 ± 0.01^{g}	4.23 ± 0.02^{i}	3.94 ± 0.01^{i}	4.01 ± 0.01^{i}
	EtOH 70%	9.02 ± 0.02^{d}	7.10 ± 0.01^{f}	8.95 ± 0.06^{d}	8.02 ± 0.02^{e}	6.05 ± 0.01^{g}	7.03 ± 0.01^{f}	5.04 ± 0.01^{h}	4.03 ± 0.01^{i}	4.73 ± 0.03^{h}
	MeOH	10.21 ± 0.01^{c}	8.01 ± 0.01^{e}	9.01 ± 0.01^{d}	10.10 ± 0.10^{c}	7.22 ± 0.01^{f}	9.26 ± 0.04^{d}	5.95 ± 0.04^{g}	5.03 ± 0.01^{h}	5.52 ± 0.02^{g}
	Acetone	9.12 ± 0.02^{d}	7.02 ± 0.02^{f}	8.28 ± 0.02^{e}	9.01 ± 0.01^{d}	6.04 ± 0.02^{g}	8.01 ± 0.00^{e}	7.05 ± 0.01^{f}	6.03 ± 0.02^{g}	6.95 ± 0.03^{f}
MAE	DI water	8.64 ± 0.03^{e}	6.09 ± 0.01^{g}	7.10 ± 0.01^{f}	8.57 ± 0.06^{e}	6.11 ± 0.02^{g}	7.32 ± 0.02^{f}	8.23 ± 0.02^{e}	8.50 ± 0.57^{e}	8.02 ± 0.02^{e}
	EtOH 50%	10.12 ± 0.02^{c}	8.11 ± 0.01^{e}	9.00 ± 0.01^{d}	10.87 ± 0.03^{c}	8.64 ± 0.01^{e}	9.07 ± 0.02^{d}	9.70 ± 0.07^{d}	8.75 ± 0.03^{e}	9.02 ± 0.02^{d}
	EtOH 70%	11.31 ± 0.01^{b}	9.03 ± 0.02^{d}	10.72 ± 0.03^{c}	11.32 ± 0.01^{b}	9.03 ± 0.01^{d}	10.34 ± 0.01^{c}	10.66 ± 0.02^{c}	9.86 ± 0.01^{d}	10.02 ± 0.01^{c}
	MeOH	12.19 ± 0.01^{a}	10.31 ± 0.01^{c}	11.67 ± 0.06^{b}	13.03 ± 0.01^{a}	10.23 ± 0.02^{c}	11.32 ± 0.02^{b}	12.03 ± 0.02^{a}	10.66 ± 0.02^{c}	11.83 ± 0.03^{b}
	Acetone	11.81 ± 0.02^{b}	10.21 ± 0.02^{c}	11.01 ± 0.01^{b}	12.87 ± 0.03^{a}	10.17 ± 0.15^{c}	11.51 ± 0.01^{b}	11.22 ± 0.02^{b}	10.30 ± 0.02^{c}	10.97 ± 0.02^{c}

^{a}, ^{b}, ^{c}, ^{d}, ^{e}, ^{f}, ^{g}, ^{h}, ^{i}, ^{j}, ^{k}, l the mean different is significantly at the 0.05 level using Ducan.

DI water = Distilled Water, EtOH = ethanol, MeOH = Methanol.

UAE = Ultrasound-Assisted Extraction, MAE = Microwave-Assisted Extraction.

Minimum inhibitory concentration (MIC) assessment

Minimum bacterial growth inhibitory concentration testing results demonstrated that extracts obtained using distilled water solvent exhibited maximum MIC values at 6.25 mg/mL against all bacterial species, while extracts obtained from organic solvents demonstrated superior performance. Leaf and stem extracts utilizing 70% ethanol, methanol, and acetone achieved minimum MIC values of 1.562 mg/mL against *S. aureus* and *B. cereus* but demonstrated reduced efficacy against *S. typhimurium* with MIC values at 3.125 mg/mL. Flower extracts exhibited lower performance compared to other plant segments, demonstrating MIC values at 3.125 mg/mL against all bacterial species, as presented in **Table 6**.

Table 6 Minimal inhibitory concentration (MIC) of *Vernonia cinerea* extract in different methods and solvents.

Methods	Solvent	Minimal inhibitory concentration (MIC) (mg/mL)								
		***S. aureus* (TISTR746)**			***B. cereus* (TISTR1449)**			***S. typhimurium* (TISTR1472)**		
		Leaf	**Flower**	**Stem**	**Leaf**	**Flower**	**Stem**	**Leaf**	**Flower**	**Stem**
Maceration	DI water	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
	EtOH 50%	3.125	3.125	3.125	3.125	3.125	3.125	3.125	3.125	3.125
	EtOH 70%	1.562	3.125	1.562	1.562	3.125	1.562	3.125	3.125	3.125
	MeOH	1.562	3.125	1.562	1.562	3.125	1.562	3.125	3.125	3.125
	Acetone	1.562	3.125	1.562	1.562	3.125	1.562	3.125	3.125	3.125
UAE	DI water	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
	EtOH 50%	3.125	3.125	3.125	3.125	3.125	3.125	3.125	3.125	3.125
	EtOH 70%	1.562	1.562	1.562	1.562	3.125	1.562	3.125	3.125	3.125
	MeOH	1.562	1.562	1.562	1.562	3.125	1.562	3.125	3.125	3.125
	Acetone	1.562	1.562	1.562	1.562	3.125	1.562	3.125	3.125	3.125
MAE	DI water	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
	EtOH 50%	3.125	3.125	3.125	3.125	3.125	3.125	3.125	3.125	3.125
	EtOH 70%	1.562	1.562	1.562	1.562	3.125	1.562	3.125	3.125	3.125
	MeOH	1.562	1.562	1.562	1.562	3.125	1.562	3.125	3.125	3.125
	Acetone	1.562	1.562	1.562	1.562	3.125	1.562	3.125	3.125	3.125

DI water = Distilled Water, EtOH = ethanol, MeOH = Methanol.
UAE = Ultrasound-Assisted Extraction, MAE = Microwave-Assisted Extraction.

Minimum bactericidal concentration (MBC) assessment

Minimum bacterial killing concentration testing revealed most extracts demonstrating MBC values at 50 mg/mL. However, high-performance extracts, particularly those obtained via MAE and UAE methodologies using methanol and acetone solvents from leaf and stem segments, successfully eliminated *S. aureus* and *B. cereus* at 25 mg/mL concentrations, similar to leaf extracts using methanol in Maceration methodology, which demonstrated MBC values at 25 mg/mL against both bacterial species. Nevertheless, *S. typhimurium* exhibited superior resistance, requiring 50 mg/mL concentrations for bacterial elimination. Investigation outcomes demonstrate white-flowered aster extracts possessing antibacterial potential, particularly against gram-positive bacteria (*S. aureus* and *B. cereus*) more than gram-negative bacteria (*S. typhimurium*), with MAE technique using methanol solvent from *Vernonia cinerea* leaf segments exhibiting maximum antibacterial performance, as presented in **Table 7**.

Table 7 Minimal bactericidal concentration (MBC) of *Vernonia cinerea* extract in different methods and solvents.

Methods	Solvent	Minimal bactericidal concentration (MBC) mg/ml)								
		***S. aureus* (TISTR746)**			***B. cereus* (TISTR1449)**			***S. typhimurium* (TISTR1472)**		
		Leaf	**Flower**	**Stem**	**Leaf**	**Flower**	**Stem**	**Leaf**	**Flower**	**Stem**
Maceration	DI water	50	50	50	50	50	50	50	50	50
	EtOH 50%	50	50	50	50	50	50	50	50	50
	EtOH 70%	50	50	50	50	50	50	50	50	50

Methods	Solvent	Minimal bactericidal concentration (MBC) mg/ml)								
		***S. aureus* (TISTR746)**			***B. cereus* (TISTR1449)**			***S. typhimurium* (TISTR1472)**		
		Leaf	**Flower**	**Stem**	**Leaf**	**Flower**	**Stem**	**Leaf**	**Flower**	**Stem**
	MeOH	25	50	50	25	50	50	50	50	50
	Acetone	50	50	50	50	50	50	50	50	50
UAE	DI water	50	50	50	50	50	50	50	50	50
	EtOH 50%	50	50	50	50	50	50	50	50	50
	EtOH 70%	50	50	50	50	50	50	50	50	50
	MeOH	25	50	25	25	50	25	25	50	50
	Acetone	25	50	25	25	50	25	25	50	50
MAE	DI water	50	50	50	50	50	50	50	50	50
	EtOH 50%	50	50	50	50	50	50	50	50	50
	EtOH 70%	25	50	50	50	50	50	50	50	50
	MeOH	25	50	25	25	50	25	25	50	50
	Acetone	25	50	25	25	50	25	25	50	50

[a], [b], [c], [d], [e], [f], [g], [h], [i], [j], [k], [l] the mean different is significantly at the 0.05 level using Ducan.

DI water = Distilled Water, EtOH = ethanol, MeOH = Methanol.

UAE = Ultrasound-Assisted Extraction, MAE = Microwave-Assisted Extraction.

The superior performance demonstrated by Microwave-Assisted Extraction (MAE) technique for antibacterial compound recovery from *Vernonia cinerea* aligns with Gowda *et al.* [54] research reporting that MAE utilization for *Lantana camara* flower extraction at 150 µL concentration significantly inhibited *E. coli*, *Salmonella*, and *S. aureus.* GC-MS analysis revealed hexadecanoic acid as the principal bioactive component in extracts from MAE, UAE, and Soxhlet methodologies, demonstrating this fatty acid's role in bacterial suppression. This data corresponds with Nisca *et al.* [55] investigations utilizing MAE under optimized conditions (30 min at 850 W for aqueous extracts and 18 min at 650 W for hydroalcoholic extracts) for Quercus cerris bark extraction, discovering elevated polyphenolic yields with effective antibacterial activity against Gram-positive strains and *Klebsiella pneumoniae*. Methanol selection as extraction solvent receives support from Ibrahim and Kebede [56] research indicating methanolic extracts from medicinal plants (*Moringa oleifera, Azadirachta indica,* and *Lepidium sativum*) demonstrated significantly superior bacterial suppression compared to aqueous extracts, similar to Chaisawangwong *et al.* [57] findings showing methanol producing maximum *E. coli* inhibition while ethanol demonstrated pronounced effects against *S. aureus*, suggesting ethanol's potentially superior safety profile compared to methanol. Bacterial sensitivity level variations between Gram-positive and Gram-negative species receive support from multiple research investigations, including Gonelimali *et al.* [58] reporting *Bacillus cereus* demonstrating highest susceptibility to clove extracts with MIC values as low as 0.315%, while Mogana *et al.* [59] reported *Canarium patentinervium* extracts exhibiting moderate to good activity against both bacterial groups with MIC values ranging 0.25 - 16.00 mg/mL. Gram-negative strains typically demonstrate superior resistance compared to Gram-positive organisms, attributable to complex cell wall structures and outer membrane layers, as Al-Mariri and Safi [60] reported essential oils from plants including *Origanum syriacum* and *Syzygium aromaticum* achieving Gram-negative bacterial elimination including *Proteus spp.* and *K. pneumoniae* with MIC values spanning 1.5 - 25 µL/mL. Regarding inhibitory and bactericidal concentration values (MIC and MBC), Hemeg *et al.* [61] research investigating extracts from 5 medicinal plant leaf species discovered

MIC and MBC values ranging 625 - 5,000 µg/mL and 625 - 1250 µg/mL respectively, demonstrating clear effects against *S. aureus*, while Al-Mostafa *et al.* [62] found ethanolic extracts from *Punica granatum* and *S. aromaticum* achieving MIC values of only 2.5 - 5.0 mg/mL and MBC 5.0 - 10 mg/mL against *S. aureus* and *P. aeruginosa*. This investigation employed disk diffusion methodology, representing a standard approach for preliminary activity assessment. Although Klancnik *et al.* [63] indicated this method's suitability for screening prior to broth dilution MIC testing, it remains popular for preliminary antimicrobial activity evaluation. Balouiri *et al.* [20] research supports appropriate bacterial strains for antibacterial activity assessment including *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*, which were utilized in this investigation. Additionally, *Vernonia cinerea* leaf segments demonstrated maximum antibacterial activity, consistent with Balouiri *et al.* [20] findings indicating leaf extracts from certain plant species including *Cremaspora triflora* and *Maesa lanceolata* achieving MIC values as low as 0.07 - 0.09 mg/mL against Gram-positive and Gram-negative strains, while Oracz *et al.* [64] reported baicalein and baicalin compounds in *Scutellaria* genus plants effectively suppressing *E. coli*, *B. cereus*, *S. aureus*, and *Salmonella anatum*, corresponding with flavonoid compound mechanisms present in *Vernonia cinerea* leaves. Therefore, the aforementioned research demonstrates *Vernonia cinerea* leaf extracts possessing substantial potential as natural antibacterial compound sources, particularly when utilizing MAE techniques combined with methanol, consistent with contemporary research trends emphasizing natural alternative discovery for addressing current antibiotic resistance challenges.

α-Amylase and α-glucosidase enzymatic inhibition activities

α-Amylase enzymatic inhibition assessment of crude extracts from *Vernonia cinerea* aster segments utilizing different extraction methodologies and solvent systems, compared with acarbose reference standard (76.40% ± 1.57%), revealed extract inhibition efficiency ranging 42.86% - 73.76%. Extraction methodology comparisons indicated MAE achieving superior performance (66.84% ± 6.42%), followed by UAE (59.67% ± 5.98%) and Maceration (54.63% ± 7.86%) sequentially. Solvent system comparisons revealed methanol demonstrating maximum efficiency (66.78% ± 4.85%), followed by acetone (64.79% ± 4.71%) and 70% ethanol (61.56% ± 5.84%) respectively. Plant segment comparisons indicated leaves exhibiting superior performance (61.97% ± 8.42%), followed by stems (60.33% ± 7.95%) and flowers (57.89% ± 8.21%) respectively. Alpha-glucosidase enzymatic inhibition testing, compared with acarbose reference standard (68.06% ± 1.32%), revealed extract inhibition efficiency ranging 30.70% - 63.61%. Extraction methodology comparisons indicated MAE achieving superior performance (55.30% ± 5.08%), followed by UAE (44.14% ± 5.45%) and Maceration (37.62% ± 4.72%) sequentially. Solvent system comparisons revealed methanol demonstrating maximum efficiency (52.01% ± 7.51%), followed by acetone (48.87% ± 8.16%) and 70% ethanol (45.85% ± 8.72%) respectively. Plant segment comparisons indicated leaves exhibiting superior performance (47.74% ± 9.32%), followed by stems (45.73% ± 8.84%) and flowers (43.60% ± 8.65%) respectively. Investigation findings demonstrate MAE methodology utilizing methanol solvent for *Vernonia cinerea* aster leaf extraction exhibiting maximum inhibition efficiency for both enzymatic systems, achieving α-amylase inhibition of 73.76% (representing 96.5% relative to acarbose) and α-glucosidase inhibition of 63.61% (representing 93.5% relative to acarbose), as detailed in **Table 8**.

Table 8 % α-amylase inhibition and % α-glucosidase inhibition of *Vernonia cinerea* extract in different methods and solvents.

Methods	Solvent	% α-amylase inhibition			% α-glucosidase inhibition		
		Leaf	Flower	Stem	Leaf	Flower	Stem
Maceration	DI water	48.10 ± 0.64^{n}	42.86 ± 0.65^{p}	45.47 ± 0.26°	33.77 ± 0.43^{g}	30.70 ± 0.41^{s}	32.09 ± 0.26^{r}
	EtOH 50%	54.73 ± 0.34^{k}	51.16 ± 0.63^{m}	53.20 ± 0.20^{l}	36.78 ± 0.41°	32.84 ± 0.30^{g}	34.88 ± 0.48^{p}
	EtOH 70%	58.49 ± 0.47^{i}	55.44 ± 0.29^{k}	57.13 ± 0.25^{j}	38.91 ± 0.12^{n}	33.54 ± 0.30^{g}	36.80 ± 0.75°
	MeOH	65.21 ± 0.64^{f}	60.54 ± 0.24^{h}	63.62 ± 0.15^{g}	45.91 ± 0.23^{j}	42.73 ± 0.53^{l}	44.66 ± 0.48^{k}
	Acetone	64.06 ± 0.81^{f}	57.43 ± 0.44^{i}	60.96 ± 0.09^{h}	41.46 ± 0.42^{m}	38.78 ± 0.24^{n}	40.47 ± 0.19^{m}
UAE	DI water	53.48 ± 0.22^{l}	50.56 ± 0.34^{m}	52.78 ± 0.26^{l}	37.38 ± 0.27°	35.45 ± 0.39^{p}	36.72 ± 0.17°
	EtOH 50%	58.23 ± 0.15^{i}	53.92 ± 0.38^{l}	55.67 ± 1.10^{j}	42.40 ± 0.43^{l}	38.44 ± 0.30^{n}	40.96 ± 0.05^{m}
	EtOH 70%	62.61 ± 0.38^{g}	57.74 ± 0.35^{i}	60.49 ± 0.32^{h}	47.77 ± 0.32^{i}	43.21 ± 0.06^{l}	44.71 ± 0.31^{k}
	MeOH	68.04 ± 0.77^{e}	63.72 ± 0.27^{g}	65.33 ± 0.22^{f}	52.70 ± 0.29^{f}	47.95 ± 0.25^{i}	51.08 ± 0.54^{g}
	Acetone	67.52 ± 0.28^{e}	62.60 ± 0.29^{g}	64.84 ± 0.19^{f}	49.08 ± 0.10^{h}	46.37 ± 0.48^{j}	47.94 ± 0.07^{i}
MAE	DI water	60.52 ± 0.37^{h}	54.73 ± 0.25^{k}	58.79 ± 0.09^{i}	51.12 ± 0.66^{g}	46.13 ± 0.08^{j}	48.50 ± 0.38^{i}
	EtOH 50%	65.10 ± 0.05^{f}	60.78 ± 0.22^{h}	62.65 ± 0.18^{g}	55.79 ± 0.39^{e}	49.76 ± 0.38^{h}	51.62 ± 0.31^{g}
	EtOH 70%	70.99 ± 0.38^{c}	67.45 ± 0.34^{e}	69.16 ± 0.60^{d}	58.09 ± 0.77^{d}	53.59 ± 0.34^{f}	56.07 ± 0.05^{e}
	MeOH	73.76 ± 0.80^{b}	70.55 ± 0.22^{d}	72.23 ± 0.18^{c}	63.61 ± 0.35^{b}	58.53 ± 0.41^{d}	60.89 ± 0.45^{c}
	Acetone	71.42 ± 0.40^{c}	68.51 ± 0.33^{e}	70.31 ± 0.13^{d}	61.29 ± 0.44^{c}	55.96 ± 0.55^{e}	58.52 ± 0.33^{d}

$^{a, b, c, d, e, f, g, h, i, j, k, l, m, o, p, q, r, s}$ the mean different is significantly at the 0.05 level using Ducan.

DI water = Distilled Water, EtOH = ethanol, MeOH = Methanol.

UAE = Ultrasound-Assisted Extraction, MAE = Microwave-Assisted Extraction.

Experimental outcomes revealed Microwave-Assisted Extraction (MAE) efficiency for diabetes related enzymatic inhibitory compound extraction receiving support from Yuan *et al.* [65] research comparing phenolic compound extraction from brown algae using MAE at 110 °C for 15 min versus conventional ambient temperature extraction for 4 h. Findings indicated MAE producing superior yields and total phenolic concentrations, with *Lessonia trabeculate* extracts demonstrating α-glucosidase inhibitory activity exceeding acarbose performance. Additionally, Maaiden *et al.* [66] revealed MAE achieving maximum α-amylase inhibition values reaching 0.424 mmol acarbose equivalent/g extract compared to alternative methodologies, demonstrating this technique's efficiency. Methanol solvent selection for enzymatic inhibitor extraction receives confirmation from multiple research investigations, including Abdul Mousavi *et al.* [67] findings indicating methanolic *Ocimum tenuiflorum* extracts, particularly ethyl acetate-butanol fractions, demonstrated effective α-glucosidase and α-amylase inhibition compared to acarbose, and Bhatia *et al.* [68] reporting methanolic *Cornus capitata* extracts achieving maximum α-glucosidase inhibitory activity through competitive inhibition mechanisms. Acarbose comparative results align with Poovitha and Parani [69] discoveries indicating protein extracts from bitter melon (*Momordica charantia*) inhibited both enzymatic systems comparably to acarbose with IC_{50} values at 0.26 - 0.29 mg/mL, and Nguelefack *et al.* [70] investigations reporting methanolic *Ceiba pentandra* extracts achieving α-amylase inhibitory efficiency 3 times superior to acarbose. Furthermore, Magaji *et al.* [71] demonstrated methanolic and hexane extracts from *Moringa oleifera* leaves achieving IC_{50} values for α-amylase at 8.217 and 9.397 mg/mL respectively, while root extracts produced α-glucosidase IC_{50} values at 0.382 mg/mL, comparable to acarbose, similar to Shah *et al.* [72] findings indicating *Molineria capitulata* producing optimal α-amylase inhibition within the group with IC_{50} = 300.9 ± 3.38 µg/mL. Leaf segments demonstrating maximum enzymatic inhibitory activity

aligns with Thengyai *et al.* [73] research evaluating 37 Thai medicinal plant species in traditional antidiabetic formulations, discovering leaf extracts from *Vitex glabrata*, *Salacia chinensis*, *Senna siamea*, *Terminalia catappa*, and *Phyllanthus amarus* exhibiting elevated α-glucosidase inhibitory activity, with lupeol and β-amyrin compounds demonstrating superior performance compared to acarbose. This corresponds with Jhong *et al.* [74] studies identifying curcumin, berberine, catechin, and quercetin as compounds achieving α-amylase inhibition superior to acarbose, with curcumin demonstrating maximum activity 7.7 times higher, and Attaallah *et al.* [75] utilizing glucometer biosensor identification indicating *Cinnamomum cassia* achieving IC_{50} values for α-amylase and α-glucosidase at 1.9 and 1.42 mg/mL respectively, possessing elevated potential for natural bioactive compound development. Kumar *et al.* [76] reviews indicate that although acarbose, miglitol, and voglibose represent clinically utilized enzymatic inhibitors, they demonstrate gastrointestinal side effects, directing contemporary research toward discovering safer natural alternatives. Khadayat *et al.* [77] reported *Acacia catechu*, *Dioscorea bulbifera*, and *Swertia chirata* achieving α-amylase IC_{50} values at 49.9, 296.1 and 413.5 μg/mL respectively, demonstrating mixed-type inhibition patterns. Therefore, investigation outcomes demonstrate *Vernonia cinerea* leaf extracts possessing substantial potential for effective α-amylase and α-glucosidase enzymatic inhibition, particularly when utilizing MAE techniques combined with methanol, producing results comparable or equivalent to acarbose while indicating promising development prospects as natural antidiabetic bioactive compounds offering enhanced safety profiles and reduced costs compared to current synthetic pharmaceuticals.

Conclusions

This investigation aimed to determine optimal solvent system conditions and extraction techniques for enhancing biological activity efficiency of crude extracts from indigenous Thai *Vernonia cinerea* focusing on obtaining extracts demonstrating antioxidant capacity, tyrosinase enzymatic inhibition, antibacterial properties, and α-amylase and α-glucosidase enzymatic suppression related to Type 2 diabetes development, efficiently within unified extraction procedures. Experimental outcomes revealed microwave-assisted extraction (MAE) techniques combined with methanol (MeOH) solvent achieving maximum efficiency across all testing protocols, particularly for *Vernonia cinerea* leaf extraction, which demonstrated maximum total phenolic and flavonoid concentrations alongside superior biological activities compared to other plant segments, including antioxidant capacity (minimum IC_{50} in DPPH and maximum FRAP values), tyrosinase inhibitory activity (IC_{50} values comparable to kojic acid), antibacterial properties (inhibition zones comparable to reference standards), and sugar-metabolizing enzymatic inhibition (efficiency comparable to acarbose). From obtained results, conclusions indicate that MAE utilization combined with methanol for white flowered leaf extraction represents optimal conditions for simultaneous diverse bioactive compound recovery, leading toward development of high-quality, safe, and internationally competitive dietary supplements, cosmetics, or herbal pharmaceuticals from local medicinal resources.

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Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of generative AI tools (e.g., ChatGPT by OpenAI) in preparing this manuscript, limited to language refinement and grammar correction. No AI was involved in content creation or data interpretation. The authors assume full responsibility for all content and conclusions presented.

CRediT Author Statement

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