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ORIGINAL RESEARCH

Ethanolic extracts from mint (*Mentha arvensis*) and basil (*Ocimum basilicum)* leaves: Antioxidant, antimicrobial capacities and shelf-life extension of refrigerated squid mantle cut

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Abstract This research aimed to investigate antioxidant and antimicrobial activities of mint leaf ethanolic extract and basil leaf ethanolic extracts. The extract with highest bioactivities further applied as a preservative on squid mantle cut (SMC) to extend the shelf-life during refrigerated storage. Mint and basil leaves ethanolic extracts denoted as mint leaf ethanolic extract (ME) and basil leaf ethanolic extract (BE), respectively, were prepared using ethanol at varying concentrations as extracting media. To improve color, dechlorophyllization of all three mint leaf ethanolic extracts (MEs) and three basil leaf ethanolic extracts (BEs) was accomplished by sedimentation process, where chlorophyll contents were reduced. Among all extracts, total phenolic, flavonoid contents, antioxidant and antimicrobial activities were higher in ME-80 (ME using 80% ethanol with decholorophyllization) ($P < 0.05$). Linarin, kaempferol 3-O-rutinoside, quercetin and caffeic acid were the major phenolic compounds in ME-80. When ME-80 (0, 200 and 400 mg L-1) was applied by introducing the known amount of extracts dissolved in minimum volume of distilled water directly on squid mantle cut (SMC) and mixing thoroughly, SMC treated with 400 mg L^{-1} ME-80 (SMC-400) showed lower pH, total volatile base content (TVB), peroxide value (PV), thiobarbituric acid reactive substances (TBARS) and microbial load than other samples during refrigerated storage of 9 days ($P < 0.05$). Hence, SMC-400 had a shelf-life of 6 days, whilst the control could be stored for only 3 days.

Keywords Leaf extracts . Bioactivities . Preservation . Spoilage . Quality

Introduction

Synthetic additives have been used in preserving food to prevent microbial spoilage and reduced oxidation (Abdulmumeen et al. 2012). However, their hazardous effect negatively links to consumer health

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such as liver cirrhosis, kidney failure, and pancreatic disorders (Sultana et al. 2023). Thus, the use of natural preservatives for safe food production has gained significant attention. Therefore, natural additives from plant sources could serve as a great alternative to replace the synthetic counterparts and will lower the health risks (Ortega-Ramirez et al. 2014). To tackle such drawbacks of chemical additives, Thai plants such as mint and basil could be a promising alternative as they have several advantages, e.g. availability, no toxicity, and high bioactivities (Salguero 2003). Mint (*Mentha arvensis*) and basil (*Ocimum basilicum*) are the members of Lamiaceae family. They are popular herbs in Thailand used in diverse cuisines, recognized by their distinct flavor and taste (Tilebeni 2011). In both leaves, the chemical composition of phenolic compounds was identified, where salvianic, caffeic acid, ferulic, rosmarinic acid, luteolin, salvigenin, chrysoeriyol, carnosol and thymonin were the major components. For basil leaves, vanillic acid, benzoic acid, hydroxybenzoic acid, gentisic acid and flavonol-glycosides were dominated (Brewer 2011; Zheng and Wang 2001). Phenolic compounds in both leaves are responsible for antimicrobial and antioxidant, anticancer, and anti-inflammatory activities (Albayrak et al. 2013). Subsequently, the chemical structure, abundance, and types of phenolic compounds extracted from leaves determine their bioactivities (Shan et al. 2005). Furthermore, they also contain high alkaloids, steroids and tannins. Phenolic compounds, derived from the secondary metabolisms, having one or more benzene rings with hydroxyl ions can retard the formation of free radicals, thus delaying the oxidation process by acting as antioxidant (Maqsood et al. 2012). In addition, polyphenols and phenolic acids act upon the cell wall of bacteria, resulting in the rupture of peptidoglycan or lipoglycan layer associated with microbial cell death (Lee and Scagel 2010; Tarchoune et al. 2012; Chotphruethipong et al. 2017). Therefore, the extracts from both leaves could be used as natural preservatives to replace synthetic counterparts. The natural leaf extracts have become necessary for food preservation since synthetic preservatives have been of concern related with the health risk.

Plant chlorophyll is crucial for facilitating photosynthesis (Olatunde et al. 2020b). Chlorophyll, particularly chlorophyll-a (chl-a), is responsible for the green color of leaves. However, chlorophyll limits their uses in foods due to its green color and it acts as pro-oxidant, leading to accelerated oxidation process (Brown et al. 2019; Olatunde et al. 2018). To prevent such a negative effect, sedimentation process has been adopted to assist the reduction of green coloration (Sinlapapanya et al. 2022). The sedimentation method allowed chlorophyll to settle down and be separated from the extract. Dechlophyllized plant extracts have been used to extend the shelf-life of refrigerated seafoods such as noni leaf extracts for striped catfish slices (Olatunde et al. 2021), betel leaf extract for tilapia slices (Tagrida and Benjakul 2021) and chamuang leaf extract for Pacific white shrimp (Sheikh et al. 2019).

Based on the Department of Fisheries in Thailand annual report, the squid harvest in 2022 was estimated to be 380000 tons. This represents approximately 7% of the total commercial captured marine animals. Furthermore, the squid's share of artisanal fishery accountability was approximately 8% among all aquatic organisms (Department of Fisheries, Thailand 2022). After harvesting, vendors and retailers generally maintain the cold chain from fishing vessel to retail market to avoid quality loss using crushed ice. In addition, some portion has been processed into ready-to-cook such as refrigerated deskinned mantle or ring, etc., which are mainly available in supermarket. Squid muscles are composed of high protein, non-protein nitrogenous compounds, and unsaturated fatty acids, which are degraded and decomposed by microorganisms and oxidation can take place easily (Parlapani 2021; Shahidi 1994). In addition, squid is one of the popular seafoods due to its unique taste and texture but easily undergo spoilage (Singh et al. 2020). However, to maintain its quality, the use of natural preservatives from mint and basil extracts could retard the deterioration of the squid mantle by inhibiting microbial growth and lipid oxidation.

No information on using mint and basil leaves extracts for lengthening the shelf-life of squid mantle cut exists. Hence the focus of this study is to analyze antioxidant and antimicrobial activities of both leaves extracts as affected by extracting media. In addition, the chemical and microbial changes of squid mantle cut treated with mint the selected leaf extract at varying concentrations were monitored during refrigerated storage.

Materials and methods

Chemicals

All purchased chemicals were from Sigma-Aldrich Inc. (St. Louis, MO, USA) except ethanol, which was obtained from Loba chemie Pvt. Ltd. (Mumbai, Maharashtra, India). Plate count agar, triple sugar iron agar, *Pseudomonas* isolation agar, and Mueller-Hinton broth were acquired from Hi Media (Mumbai, Maharashtra, India). Mueller-Hinton Agar was procured from Merk (Chemie GmbH, Steinheim, Germany).

Collection and preparation of mint and basil leaves

Fresh mature leaves of mint *(Mentha arvensis)* and basil (*Ocimum basilicum)* were bought from the local market in Hat Yai between October and November 2022 and brought to the laboratory. Both leaves were separately washed using tap water and dried in a tray dry oven at 60 °C for 12 h to obtain a moisture content less than 10%. Then the dried leaves were blended (Panasonic, Model MX-898N, Berkshire, UK) and sieved (mesh size of 80 mm). Fine powder of both leaves was placed in zip-lock bags and stored in a desiccator at room temperature.

Preparation of mint and basil leaf ethanolic extracts with and without dechlorophyllization

The method for preparation of both mint and basil leaf ethanolic extracts was described by Tagrida et al. (2021) using a powder/solvent ratio of 1:15 (*w/v*). Ethanol (60, 80, and 100% *v/v*) was used as extraction solvent for both leaf powders. The mixtures were continuously stirred for 3 h, followed by centrifugation at 5,000×g for 30 min at 4 °C. The supernatants were passed through Whatman filter paper No. 1 for filtration and each filtrate was separated into 2 portions (200 mL each). Each portion of filtrate was evaporated at 40 °C using an Eyela rotary evaporator for solvent removal (Tokyo Rikakikai, Co. Ltd., Tokyo, Japan), followed by nitrogen purging. To remove chlorophylls from the extract, a sedimentation process was employed (Olatunde et al. 2018). A solution containing the extract and distilled water at the ratio of 1:1 (*v/v*) was prepared and chlorophyll was allowed to precipitate overnight (24 h) at 4 °C. Subsequently, the supernatant was collected by centrifugation at 10,000×g for 30 min at 4°C. The supernatant was then frozen at -50 °C and lyophilized. Finally, those dry extract powders were placed in a zip-lock bag and kept in the desiccator for further use. Dechlorophyllized extract powders were termed as ME-60, ME-80, ME-100 and BE-60, BE-80 and BE-100 for mint leaf ethanolic extract (ME) and basil leaf ethanolic extract (BE) using 60, 80 and 100 % ethanol as extraction solvents, respectively. ME-60-ND, ME-80-ND, ME-100-ND, BE-60-ND, BE-80-ND and BE-100-ND were the corresponding extracts without dechlorophyllization (non-dechlorophyllized termed as ND). All the extract powders were subjected to analysis.

Chlorophyll content

To obtain a concentration of 2 mg mL^{-1}, dry extracts with and without chlorophyll removal were dissolved in ethanol and the spectrophotometer was used to estimate total chlorophyll content. Absorbance at 663 and 645 nm was read for the solutions of extracts with and without chlorophyll removal. Chlorophyll-a (chl-a) and chlorophyll-b (chl-b), and total chlorophyll contents (tot-chl) were measured (Olatunde et al. 2019). The following equations were used to calculate chlorophyll contents:

Chl-a ($\mu g\ mL^{-1}$) = 12.7 (A_{663}) – 2.69 (A_{645})
Chl-b ($\mu g\ mL^{-1}$) = 22.9 (A_{645}) - 4.68 (A_{663})
Tot-chl ($\mu g\ mL^{-1}$) = 20.2 (A_{645}) - 8.02 (A_{663})

Color

Colorimeter (Color Flex, Hunter Lab, Reston, VA, USA) was used for measuring lightness (L^*), redness/greenness (a^*), and yellowness/ blueness (b^*) and ΔE^* (total color differences) was computed relative to the

those of white standard plate (L^* = 90.77, a^* = -1.27 and b^* = 0.50) as follows:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Characterization of mint and basil leaf ethanolic extracts with dechlorophyllization

Since all the extracts from both leaves without chlorophyll removal were dark greenish in color, they could not be applied as food additive. Only dechlorophyllized extracts were further characterized.

Total phenolic content (TPC) and total flavonoid content (TFC)

Total phenolic content (TPC) was determined and reported as mg gallic acid equivalent (GAE) g^{-1} dry extract as tailored by Benjakul et al. (2014). The total flavonoid content was estimated as described by Tagrida et al. (2021). Total flavonoid content (TFC) was expressed as mg catechin equivalent (CE) g^{-1} dry extract.

Antioxidant activities

Antioxidant activities were assayed following the method of Mittal et al. (2022). DPPH• radical scavenging activity, ABTS•+ radical scavenging activity and ferric reducing activity power (FRAP) and metal chelation activity (MCA) were measured. The oxygen radical absorbance capacity (ORAC) was examined by using a microplate reader (FLUOstar Omega, model: 415-101, BMG LABTECH, Ooffenburg, Germany) as detailed by Ahmad et al. (2024). All antioxidant activities were expressed as µmol Trolox equivalent (TE) g^{-1} extract, except MCA, which was reported as µmol ethylenediaminetetraacetic acid (EDTA) g^{-1} extract.

DPPH• radical scavenging activity

The sample (1.5 mL) was added with 1.5 mL of 0.15 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH•) in 60 % ethanol. Then, the mixed solution was left in the dark at room temperature for 30 min. The absorbance was measured at a wavelength of 517 nm using a spectrophotometer. A sample blank was prepared using the same procedure, with the exception that the corresponding solvent was used instead of the DPPH• solution. For the standard curve, Trolox was used in a range of 10-60 Provide 'µM' as the unit of standard for DPPH• radical scavenging activity assays. . The activity was calculated after subtracting the sample blank.

ABTS•+ radical scavenging activity

Firstly, the stock solution of 7.4 mM ABTS and 2.6 mM potassium phosphate were prepared and mixed at a ratio of 1:1. The mixture was kept in dark for 12 h. To obtain an absorbance between 1.1 ± 0.2 at 734 nm, the solution was diluted by mixing 1 mL of ABTS•+ solution with 50 mL of methanol. A sample with a concentration of 0.2 mg mL^{-1} was prepared and 150 µL of the sample was mixed with 2850 µL of ABTS•+ solution. The mixture was incubated at room temperature for 2 h and measured at a wavelength of 734 nm. The standard curve of Trolox in a range of 50-600 Provide 'µM' as the unit of standard for ABTS•+ radical scavenging activity assays. was prepared. After subtracting the sample blank, the activity was calculated.

Ferric reducing antioxidant power (FRAP)

Stock solutions of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution were prepared. A fresh working solution was then created by combining 25 mL of acetate buffer with 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solution. A sample with a concentration of 0.25 mg mL^{-1} was prepared and 150 µL of the sample was mixed with 2850 µL of FRAP stock solution. The mixture was then incubated in dark at room temperature for 30 min. Following the incubation period, the final absorbance of the solution was measured at a wavelength of 593 nm. A standard curve was generated using Trolox in the concentration range of 50-600 µM. The activity was measured after subtracting the sample blank.

Metal chelating activity (MCA)

To determine MCA, a sample with a concentration of 0.2 mg mL^{-1} was prepared and 940 µL of the sample was mixed with 20 µL of $FeCl_2$ and 40 µL of ferrozine. The mixture was then incubated at room temperature for 20 min. Following the incubation period, the absorbance of the solution was measured at a wavelength of 562 nm. The standard curve ranging from 10 to 60 µM was prepared using EDTA.

Oxygen radical absorbance capacity (ORAC)

The sample solution at a concentration of 0.1 mg mL^{-1} was prepared. Next, 25 µL of the sample solution was transferred into a 96-well microplate, followed by the addition of 50 µL of 4 µM fluorescence solution. The microplate was then incubated at 37 °C for 20 min in a microplate reader. After the incubation period, 100 µL of a 221 mM AAPH solution was added to each well, and the excitation and emission were measured at 486 nm and 520 nm, respectively, using the microreader. The measurement was carried out at 5-min interval for a total duration of 170 min. To obtain the blank measurement, the same procedure was followed, but the sample was replaced with a 75 mmol L^{-1} phosphate buffer (pH 7.2).

Antimicrobial activity

Bacterial strains

Both Gram-positive bacteria and Gram-negative bacteria were used for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of all extracts. Four bacterial strains included *Escherichia coli* DMST 4122*,* and *Pseudomonas aeruginosa* PSU.SCB.16S.12 donated by the Food Safety Laboratory, Faculty of Agro-Industry, Prince of Songkla University (PSU), Thailand, *Shewanella* spp. TBRC 5775 from Thailand National Center for Genetic Engineering and Biotechnology and *Staphylococcus aureus* DMST 4547 obtained from the Department of Medical Sciences, Ministry of Health, Thailand.

MIC and MBC

Determination of MIC and MBC of all the extracts was done using the broth microdilution method (Olatunde et al. 2020b). The extracts were prepared at concentrations ranging from 0.5 to 256 mg mL^{-1}. Subsequently, the extracts were subjected to double-fold serial dilutions using sterile Mueller-Hinton broth in 96-well microplates. Standardized bacterial cell suspensions of 1×10^6 colony forming unit CFU mL^{-1} were inoculated into the extracts (50 µL) at each concentration. The microplates were incubated at 37 °C for 16-18 h. The lowest concentration of the extracts that inhibited the visible growth of the microorganisms was determined as MIC. The medium was used as positive control and the medium with the extract (without microorganisms) was used as a negative control. The MIC was defined as the lowest concentration of the extract that inhibited bacterial growth. For MBC of the extracts, 0.1 mL of inoculum from each of the broth wells was sub-cultured on Mueller-Hinton agar (MHA) plates. After incubation at 37 ºC for 24 h, the lowest concentration of the extracts yielded a survival rate of the inoculum less than 0.1% was considered as MBC.

LC-MS profiling and identification of compounds

Among all dechlorophyllized extracts, ME-80 having the highest antioxidant and antimicrobial activity was selected for Liquid Chromatography-Mass Spectrophotometric profiling and identification as detailed by Olatunde et al. (2020). The Liquid Chromatograph-Quadrupole Time-of Flight Mass Spectrometer- LC-QTOF MS (1290 Infinity II LC-6545 Q-TOF, Agilent Technologies, USA) was used for the qualitative identification of the compounds in extracts An Agilent 1100 series liquid chromatography system (Agilent Technologies, Waldbronn, Germany) equipped with a LiChroCART Purospher STAR RP-18e column (Merck, Branchburg, NJ, USA) having dimensions of 150 × 4.6 mm and a particle size of 5 µm for the initial sample separation was used. The mobile phases consisted of acetonitrile (phase A) and a 10 mM

ammonium formate buffer with a pH of 4, adjusted with formic acid (phase B). The flow rate was set at 1.0 mL min^{-1} and the column temperature was maintained at 40 °C. A gradient elution program was employed as follows: the mobile phase composition was held constant at 100% phase B for the first 5 min, followed by a linear increase from 0% to 20% phase A over the next 5 min. The composition of phase A was then held constant at 20% for the subsequent 10 min, and finally, a linear increase from 20% to 40% phase A was carried out over the next 40 min. The detection of analytes was performed using a multi-wavelength (270 nm, 330 nm, 350 nm and 370 nm). Both positive and negative ionization modes were employed for mass spectrometry (MS) detection, utilizing an electrospray ionization source with nitrogen gas as the drying gas. The MS parameters were set as follows: a capillary voltage of 4000 V, a gas temperature of 320 °C, a drying gas flow rate of 13 L min^{-1}, and a nebulizer pressure of 60 psi. MS detection was implemented in the Selected Ion Monitoring (SIM) mode for quantitative analysis (Sinlapapanya et al. 2022).

Application of the selected plant leaf extract for shelf-life extension of squid mantle cut

Preparation of squid mantle cut (SMC) treated with ME-80 extract at varying levels.

The freshly caught (12 h after capture) squids (*Loligo vulgaris*) were bought from the Hat Yai fresh market, placed in a polystyrene box with flake ice (ice: squid ratio of 2:1) and immediately brought to the laboratory within 30 min. The squids were then washed, beheaded and eviscerated. The mantle obtained was deskinned manually. The deskinned mantle was washed with clean water and cut with a sharp stainless-steel knife. The yield of cleaned squid was 32 %. All prepared mantle cuts were washed with 3% NaCl solution for 2 min to remove some microorganisms and to reduce nutrient loss and shrinkage (Kolodziejska et al. 1999). For each treatment, 25 g of squid mantle cut (SMC) sample (5×5 cm^2) were mixed with 500 µL of distilled water containing 5 mg and 10 mg of ME-80, yielding the final concentrations of 200 mg kg^{-1} and 400 mg kg^{-1}, respectively. The mixture was mixed manually for 1 min to ensure the uniform distribution of extract in SMC. Samples without treatment were considered as the control. The control and ME-80 treated SMC were named "SMC-C", "SMC-200" and "SMC-400", respectively. All samples were placed in a polystyrene tray and wrapped with shrink film and then kept at 4 ºC for 9 days. Microbiological and biochemical analyses were carried out every 3 days.

Microbiological load

All samples including SMC-C, SMC-200, and SMC-400 were subjected to microbiological analyses, where the sample preparation method was adopted from Sheikh et al. (2019). Total viable count (TVC), psychrophilic bacterial count (PBC), *Pseudomonas* spp. count and H_2S-producing bacterial count (HSPBC) were determined as reported by Ahmad et al. (2024). Precisely 5.0 g of the sample was weighed and transferred into a sterile stomacher bag containing 45 mL of 0.85% saline water. The contents of the bag were then homogenized using a stomacher blender at 230 rpm for 3 min (M400, Seward, West Sussex, UK) to ensure uniform mixing of the sample and the saline solution. Consecutive dilutions of the samples were prepared using 0.85% saline water where 100 µL of aliquot of each dilution was then transferred onto a sterile petri dish for plating. The plates were then left to dry under aseptic conditions. For total viable count and psychrophilic bacterial count, the plate count agar (PCA) was used where the optimum incubation temperature and time were at 37 ºC for 48 h and 4 ºC for 10 days, respectively. For H_2S-producing bacterial count and *Pseudomonas* spp count, triple sugar iron (TSI) and *Pseudomonas* Isolation agar base (PIA) were used at 25 °C for 3 and 2 days, respectively.

Texture profile

According to Gani et al. (2018), the texture profiles analysis was performed for each SMC by using a texture analyzer TA-XT2i (Stable Micro Systems, Surrey, England). Warner Bratzler blade probe was used. The pre-test and test compression for each SMC were 2.0 mm sec^{-1} and post-test speed was 10.0 mm s^{-1}. Distance was set at 25 mm with a target force of 20.0 g.

Chemical analyzes

pH

Sample (2 g) was mixed with 20 mL of distilled water and homogenized at 13,000 rpm for 1 min (IKA-Werke GmbH & Co. KG, Staufen, Germany). The mixture was stood for 5 min at room temperature. The homogenate pH was analyzed by using a pH meter (Sartorious North America, Edgewood, NY, USA) (Nirmal and Benjakul 2012).

Total volatile base content (TVB)

TVB was determined using the Conway method (Sae-leaw and Benjakul 2019). TVB content was reported as mg N 100 g^{-1} of SMC.

Peroxide value (PV) and thiobarbituric acid reactive substances (TBARS)

PV and TBARS value determinations were done, following the procedure of Sheikh et al. (2019) and reported as mg cumene hydroperoxide kg^{-1} SMC and mg malonaldehyde (MDA) kg^{-1} SMC, respectively.

Statistical analysis

A completely randomized design (CRD) was employed for the entire study. One-way analysis of variance (ANOVA) was performed and Duncan's multiple range test was conducted to compare the means with the aid of IBM SPSS Statistics 26.0 software (SPSS Inc, Chicago, IL, USA).

Results and discussion

Chlorophyll content and color of various mint and basil leaf ethanolic extracts without and with dechlorophyllization

Chl-a, chl-b and tot-chl of different MEs and BEs were varied (Table 1) ($P < 0.05$). Non-dechlorophyllized samples, namely ME-60-ND, ME-80-ND and ME-100-ND, contained chl-a, b and tot-chl with the range of 3.20-6.31, 0.37-1.05 and 3.57-7.36 mg 100g^{-1} sample, respectively. After dechlorophyllization, the tot-chl in ME-60, ME-80 and ME-100 were reduced by 47, 87 and 34% compared to those without chlorophyll removed. For BEs, chl-a, b and tot-chl were 4.50-9.78, 0.39-0.76 and 4.89-10.54 mg 100g^{-1} samples, respectively. After dechlorophyllization tot-chl in BE-60, BE-80 and BE-100 were decreased by 35, 73 and 27%, respectively. For both extracts, total chlorophyll was removed by 27-87% through the sedimentation process. Chlorophyll is composed of several smaller molecules, having both a hydrophobic tail and a hydrophilic head. When ethanol is used as extracting solvent, chlorophyll from plant material including the hydrophobic tail and polyphenolic ring interacted more strongly with ethanol, especially at higher concentration as witnessed by higher chl-a, b and tot-chl in the extracts using 100% ethanol as the extracting medium ($P < 0.05$). Chlorophyll soluble in ethanol was related to more green color (Ma et al. 2013). In contrast, when water (high polarity) is used to remove chlorophyll, chlorophyll becomes less soluble in the water and more likely to precipitate as sediment (Lefebvre et al. 2021). Overall, the solubility of chlorophyll in different solvents depends on the relative strength of the intermolecular forces between the different components of the system and can be manipulated by changing the polarity of the solvent used (Mahmoudi et al. 2019). Tagrida and Benjakul (2020) reported that the ethanolic extracts of betel *(Piper betle L.)* and chaphlu (*Piper sarmentosum* Roxb.) leaves had lower chl-a, b and tot-chl after sedimentation process than the crude extract without chlorophyll removal. A similar trend was observed for guava and noni leaf extract documented by Olatunde et al. (2021), in which a remarkable reduction of tot-chl was observed after sedimentation ($P < 0.05$).

L^*, a^*, b^* and ΔE^* values of MEs and BEs without and with chlorophyll removal are presented in Table

Table 1 Chlorophyll content and color of different mint (*Mentha arvensis*) and basil (*Ocimum basilicum*) leaf ethanolic extracts without and with dechlorophyllization

Samples	Chlorophyll a	Chlorophyll b	Total Chlorophyll	L^*	a^*	b^*	ΔE^*
ME-60-ND*	4.81 ± 0.12^{B}	0.88 ± 0.41^{D}	5.69 ± 0.88^{C}	0.21 ± 0.31^{B}	-2.36 ± 0.51^{C}	-2.02 ± 0.85^{B}	90.31 ± 0.01^{A}
ME-80-ND*	3.20 ± 0.01^{A}	0.37 ± 0.12^{A}	3.57 ± 0.13^{A}	0.98 ± 0.52^{E}	-0.52 ± 0.18^{E}	-0.24 ± 0.54^{E}	89.30 ± 0.20^{B}
ME-100-ND*	6.31 ± 0.34^{D}	1.05 ± 0.61^{E}	7.36 ± 0.35^{D}	0.38 ± 0.15^{C}	-3.61 ± 0.62^{B}	-3.06 ± 0.33^{A}	90.43 ± 0.10^{A}
BE-60-ND*	5.39 ± 0.22^{C}	0.57 ± 0.38^{B}	5.96 ± 0.67^{C}	0.27 ± 0.67^{B}	-2.91 ± 0.14^{C}	-0.28 ± 0.17^{E}	90.25 ± 0.72^{A}
BE-80-ND*	4.50 ± 0.58^{B}	0.39 ± 0.28^{A}	4.89 ± 0.90^{B}	0.81 ± 0.35^{D}	-1.62 ± 0.17^{D}	-0.93 ± 0.20^{D}	89.85 ± 0.81^{B}
BE-100-ND*	9.78 ± 0.32^{E}	0.76 ± 0.50^{C}	10.54 ± 0.05^{E}	0.12 ± 0.15^{A}	-4.49 ± 0.38^{A}	-1.00 ± 0.16^{C}	90.98 ± 0.42^{A}
ME-60*	0.95 ± 0.03^{b}	0.71 ± 0.16^{c}	1.66 ± 0.59^{c}	0.69 ± 0.75^{c}	-0.49 ± 0.16^{b}	-1.02 ± 0.65^{c}	83.27 ± 0.76^{c}
ME-80*	0.70 ± 0.21^{a}	0.24 ± 0.53^{a}	0.94 ± 0.86^{a}	2.81 ± 0.79^{a}	0.58 ± 0.32^{d}	-0.50 ± 0.09^{a}	81.21 ± 0.91^{d}
ME-100*	1.35 ± 0.07^{d}	0.78 ± 0.67^{c}	2.13 ± 0.76^{d}	0.51 ± 0.32^{e}	-0.75 ± 0.97^{c}	-1.52 ± 0.07^{e}	84.05 ± 0.12^{b}
BE-60*	1.05 ± 0.08^{c}	0.57 ± 0.83^{b}	1.62 ± 0.43^{b}	0.63 ± 0.35^{d}	-0.55 ± 0.36^{c}	-0.97 ± 0.34^{b}	82.19 ± 1.32^{d}
BE-80*	1.02 ± 0.19^{c}	0.52 ± 0.54^{b}	1.54 ± 0.25^{b}	1.56 ± 0.18^{b}	-0.43 ± 0.46^{b}	-0.88 ± 0.76^{b}	82.01 ± 0.51^{d}
BE-100*	2.08 ± 0.25^{e}	0.90 ± 0.62^{d}	2.98 ± 0.65^{e}	0.42 ± 0.37^{f}	-1.69 ± 0.29^{e}	-1.25 ± 0.36^{d}	85.23 ± 0.29^{a}

Note: * Numbers indicate ethanol concentrations (60-100%); ND: Non-dechlorophyllized samples; ME: mint leaf ethanolic extract; BE: basil leaf ethanolic extract. Values are mean ± standard deviation (n=3). Different uppercase superscripts within the same column for non-dechlorophyllized samples indicate significant differences and ($P < 0.05$). Different lowercase superscripts within the same column for dechlorophyllized samples indicate significant differences ($P < 0.05$).

1. Dechlorophyllized samples had significantly reduced green color, compared to non-dechlorophyllized counterparts ($P < 0.05$). Among all samples, ME-80 and BE-80 showed the highest reduction in color than others after dechlorophyllization ($P < 0.05$), whilst L^*, a^* and b^* values were highest than other extracts. It might be due to the presence of less chl-a, compared to others. The greenish color is generally attributed to chl-a in plant leaves, which is the primary pigment responsible for photosynthesis (Björn et al. 2009). Therefore, dechlorophyllization could remove chl-a after sedimentation process, resulting in less green color (Sheikh and Benjakul 2020). Dechlorophyllization of neem, peppermint, and basil resulted in a remarkable reduction in tot-chl, leading to a noticeable lighter color (Singh et al. 2015). Sedimentation method was also effective to remove tot-chl for betel leaf as observed by Tagrida et al. (2021). Overall, the tot-chl of mint and basil leaves decreased notably after dechlorophyllization.

Therefore, the sedimentation method could be adopted as an effective method for green color reduction, where further application did not cause discoloration in the treated product. Only dechlorophyllized extracts were further characterized.

Yield, TPC, and TFC of dechlorophyllized mint and basil leaf ethanolic extracts

ME-80 and BE-80 samples had higher yields than those extracted using 60% ethanol. Yields of both extracts ranged from 15.89 to 16.04% (Table 2). ME-60, and BE-60 showed higher yields than ME-100, and BE-100. This indicated that ethanol concentration had a marked impact on extraction yield. Compounds having different polarities could be extracted at varying degrees using ethanol at various concentrations. Ethanolic extraction enhanced the yield of cashew and guava leaf, however, the extraction efficacy varied with plant species, composition and age of leaves (Sheikh et al. 2019; Olatunde et al. 2020b).

The variations in TPC were noticeable among the samples ($P < 0.05$). ME-80 had the highest TPC (127.89 mg GAE g^{-1} extract) and ME-100 showed the lowest TPC (113.54 mg GAE g^{-1} extract). BE generally showed similar trends in TPC with ME ($P < 0.05$) but the lower TPC was found in BE at all ethanol concentrations used. According to Oh et al. (2013), TPC of water and ethanolic peppermint extracts were 75.31 ± 3.58 and 33. 68 ± 0.44 mg GAE g^{-1} sample, respectively. In another study, ethanolic extract of Algerian mint (*Mentha spicata* L) and ethanolic *Mentha longifolia* extract had TPC of 39. 47 ± 1.81 and 67.05 ± 0.85 mg GAE g^{-1} dry extract, respectively (Fatiha et al. 2012; Bahadori et al. 2018). Van der Sluis et al. (2001) reported that the phenolic compounds in leaves can be varied because of their composition, protective mechanism, geographical variation, and seasonal changes, extraction conditions (time, temperature, extracting media, percent of solvent used).

In general, TFC was higher in MEs than BEs, regardless of ethanol concentrations. The highest TFC was recorded for ME-80, 97.12 mg CE g^{-1} dry extract ($P < 0.05$). At the same ethanol concentration used for extraction, flavonoids in both leaf powders were extracted differently. TFC showed a similar trend to TPC as influenced by ethanol concentrations. The significant variations of all extracts are possibly governed by species variations, composition, age of leaf, seasonal variation and genotype (Atanassova and Georgieva 2011; Alfaro et al. 2013). TPC and TFC in lemon leaf extract were 48.06 mg GAE 100 g^{-1} extract and 27.54 mg CE 100 g^{-1} samples, respectively. The positive relationship between TPC and antioxidant activities in the extracts of betel leaf was reported by Tagrida et al. (2021). Overall, ME-80 had the highest TPC and TFC, suggesting the remarkable antioxidant activity of the extract.

Antioxidant activities of dechlorophyllized mint and basil leaf ethanolic extracts

All extracts for both leaves showed varying antioxidant activities ($P < 0.05$). These were dependent on the concentration of ethanol used, which determined the polyphenols or other compounds present in both plant extracts. In addition, varying solubilization of active compounds in extracting solvent might be different. The MEs had DPPH• and ABTS•+ radical scavenging activities ranging from 1634.78 to 3111.08 µmol TE g^{-1} extract and from 1822.87 to 4468.88 µmol TE g^{-1} extract, respectively. ME-80 had highest DPPH• and ABTS•+ radical scavenging activities ($P < 0.05$). BE-80 also exhibited the highest for DPPH• and ABTS•+ radical scavenging activities, which were 1725.97-2298.23 µmol TE g^{-1} extract and 1869.34-4547.23 µmol TE g^{-1} extract, respectively. The differences in activities were more likely governed by the concentration of

solvent used for extraction (Olatunde et al. 2019). DPPH• radical scavenging activity was commonly used in a lipophilic system. In contrast, ABTS•+ radical scavenging activity was evaluated for the amphiphilic system (Chotphruethipong et al. 2017). Ethanolic extract of *Mentha longifolia* had the DPPH• and ABTS•+ radical scavenging activities of 162.08 ± 3.90 and 242.06 ± 1.53 mg TE g^{-1} dry extract, respectively (Bahadori et al. 2018). Peppermint extract showed DPPH• and ABTS•+ radical scavenging activities of 98.43 ± 2.39 and 136.81 ± 1.78 mg TEg^{-1}, respectively (Pavlić et al. 2021). IC_{50} values of lemon leaf extract and saga leaf extracts for DPPH• radical scavenging activity was 10.23 and 12.64 mL L^{-1}, respectively (Atanassova et al. 2011).

FRAP and MCA were performed by ferric reduction (Fe^{3+}-TPTZ to Fe^{2+}-TPTZ by single electron transfer) and iron chelation, respectively. Maqsood et al. (2012) reported that metal ions bind with a chelator to form a stable complex. As a consequence, lipid oxidation induced by pro-oxidation ions could be retarded. Chelators have two or more donor atoms that form a ring-like structure around the metal ion (Garcia-Mier et al. 2021). FRAP values of MEs and BEs ranged from 518.10 to 3230.17 µmol TE g^{-1} extract and MCA ranged from 11.23 to 107.75 µmol EDTA g^{-1} extract. ME-80 showed higher FRAP and MCA than the remaining MEs ($P < 0.05$). Olatunde and Benjakul (2021) substantiated that FRAP and MCA were higher in non-dechlorophyllized noni leaf extract because some polyphenols could be lost during sedimentation. Furthermore, phenolic compounds in the extract might have high potential in metal chelation (Hider et al. 2001). In general, ME had higher FRAP and MCA than BE, irrespective of ethanol concentrations used for extraction.

Different ORAC were attained among all extracts ($P < 0.05$). ORAC varied from 194.33 to 298.52 µmol TE g^{-1} extract. MEs generally showed higher ORAC than BEs. ME-80 had the highest ORAC than others ($P < 0.05$). High ORAC of ME-80 indicated the ability to scavenge peroxyl radicals, R-O-O• (Tagrida et al. 2021). Plant extracts containing different polyphenols such as flavonoids and phenolic acids can neutralize and stabilize the peroxyl radicals to terminate the chain reactions by donating hydrogen atoms or electrons (Park 2011). ORAC had a strong correlation with TPC and TFC of the extracts. Phenolics or flavonoids could serve as antioxidants in the extract, but the amount might vary with extraction time, concentrations and solvent used. The correlation between total phenolic and flavonoid contents in mint and tea with mint extract was also documented by Efenberger-Scmechtyk et al. (2021).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of dechlorophyllized mint and basil leaf ethanolic extracts

All decholorphyllized extracts were tested against Gram-positive and Gram-negative bacterial strains (Table 3). Among all the extracts tested, ME-80 had the MIC with the range of 32-256 mg mL^{-1} and MBC values were 64-256 mg mL^{-1}. The BE-80 exhibited MIC ranging from 128 to 256 mg mL^{-1} and MBC was 256 mg mL^{-1}. The remaining leaf extracts using 60% or 100% ethanol showed lower antibacterial activity as indicated by higher MIC and MBC values. This might be attributed to the difference in polyphenols present in both extracts, which were higher in ME-80 than others. Brown et al. (2019) documented that Spearmint, Medina, and Hasawi mint extracts containing varying distinct polyphenols exhibited potent antimicrobial activity by causing bacterial cell lysis, disruption of osmoregulation, and induction of leakage of intracellular material. These phenomena resulted in either cell death or impaired growth. Tagrida and Benjakul (2021) observed that the susceptibility of Gram-negative bacteria was more intense than Gram-positive due to the thinner peptidoglycan layer than the thicker peptidoglycan layer in Gram-positive when treated with betel leaf extract. Among all phenolic compounds, the catechins and flavonoids had potent antibacterial effects against Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Shewanella* spp. (Papuc et al. 2017). These compounds can destabilize the cell membrane and caused bacterial cell death (Olatunde et al. 2018). Oxidative stress can be initiated due to prooxidant formation, resulting in the leakage of the cell or the release of cytoplasmic constituents, such as nucleotides and small cellular molecules (Padmini et al. 2008). The inhibitory effect of ME-80 on Gram-positive bacteria was quite low. This was due to the thick layer of peptidoglycan (Olatunde et al. 2019). For all tested bacterial strains, potassium sorbate (PS), a synthetic antimicrobial agent, was used as positive control, where its MIC was 8 mg mL^{-1} and MBC was 8-16 mg mL^{-1} for all bacteria tested, respectively. The effectiveness of leaf

Table 2 Extraction yield, total phenolic content, total flavonoid content, antioxidant activity of different dechlorophyllized mint and basil leaf ethanolic extracts

Samples	Extraction yield (%)	TPC (mg GAE g^{-1} extract)	TFC (mg CE g^{-1} extract)	DPPH• radical scavenging activity (µmol TE g^{-1} extract)	ABTS•+ radical scavenging activity (µmol TE g^{-1} extract)	FRAP (µmol TE g^{-1} extract)	ORAC (µmol TE g^{-1} extract)	MCA (µmol EDTA g^{-1} extract)
ME-60	13.67±1.09^{b}	113.54±1.72^{d}	65.11±3.64^{d}	1755.53±54.55^{c}	1822.87±190.09^{d}	518.10 ±21.52^{f}	267.73±16.02c	78.44±10.88^{c}
ME-80	16.04±1.19^{a}	127.89±0.99^{a}	97.12±1.34^{a}	3111.08±302.16^{a}	4468.88±614.92^{a}	3230.17±120.81^{a}	298.52±34.38a	107.75±30.59^{a}
ME-100	6.12±0.62^{d}	120.60±0.64^{c}	88.54±0.75^{b}	1634.78±290.57^{c}	3108.45±286.58^{b}	1086.43±103.16^{d}	194.33±11.90f	81.49±5.61^{b}
BE-60	11.09±0.98^{c}	110.95±0.51^{e}	59.57±3.27^{e}	2248.23±156.94^{b}	1869.34±97.26^{d}	836.56±60.65^{e}	214.74±34.76e	11.23±5.15^{e}
BE-80	15.89±1.21^{a}	124.34±0.74^{b}	81.39±5.19^{c}	2891.79±345.49^{a}	4547.23±267.714^{a}	2423.79±231.13^{b}	282.82±37.58b	35.83±5.06^{d}
BE-100	3.17±0.64^{e}	115.59±0.45^{d}	57.95±2.71^{e}	1725.96±141.41^{c}	2911.75±46.34^{c}	1836.27±143.45^{c}	275.57±54.10c	14.34±3.95^{e}

Note: ME: Mint leaf ethanolic extract; BE: Basil leaf ethanolic extract. The values are mean ± standard deviation *(n=3)*. Different lowercase superscripts in the same column indicate significant differences ($P < 0.05$). TPC: total phenolic content; GAE: gallic acid equivalent; CE: catechin equivalent; TE: Trolox equivalent; TFC: total flavonoid content; FRAP: ferric reducing antioxidant activity; ORAC: oxygen radical absorbance capacity, MCA: metal chelation activity.

Table 3 Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of different dechlorophyllized mint (*Mentha arvensis*) and basil (*Ocimum basilicum*) leaf ethanolic extracts

Samples	MIC (mg mL^{-1})				MBC (mg mL^{-1})			
	PA	SS	EC	SA	PA	SS	EC	SA
ME-60	128	64	64	>256	256	128	256	>256
ME-80	64	32	32	256	128	64	64	>256
ME-100	128	>256	128	256	256	256	256	>256
BE-60	>256	>256	>256	>256	>256	>256	>256	>256
BE-80	128	128	128	256	256	256	256	>256
BE-100	>256	>256	>256	>256	>256	>256	>256	>256
PS	8	8	8	8	8	16	16	16

Note: PA*: Pseudomonas aeruginosa*; SS, S*hewanella* spp.; EC: *Escherichia coli*; SA: *Staphylococcus aureus*, and PS: Potassium Sorbate (used as standard).

extracts was lower than PS as shown by the lower MIC and MBC value of the latter. MBC is the lowest concentration required from an antimicrobial agent to kill 0.99 % of bacteria. The lowest MBC value of the corresponding MIC ranged from 64 to 128 mg mL^{-1} for ME-80 extract toward all bacteria, except for SA. When the MBC/MIC ratio is >2, a compound is classified as bacteriostatic, whereas it is categorized as bactericidal when the MBC/MIC ratio is ≤ 2 (Olatunde et al. 2019). Since the ratio was higher than 2 when tested against *Pseudomonas aeruginosa*, *Shewanella* spp. and *Escherichia coli*, it could be inferred that ME-80 was bacteriostatic.

Among all the extracts, ME-80 had the highest antioxidant and antibacterial activities, palest color, and lowest chlorophyll content. ME-80 was further selected for the identification of phenolic compounds and shelf-life study of refrigerated squid mantle cut (SMC).

LC-MS profile for ME-80

Various phenolic compounds in ME-80 identified using LC-MS are presented in Table 4. The positive mode generally exhibited higher sensitivity compared to the negative mode for compound identification (Olatunde et al. 2021). Among all phenolic compounds, flavonoids, phenolic acids, their glycosides were identified including linarin, kaempferol 3-O-rutinoside, quercetin and caffeic acid were dominant, followed by kaempferol-7-O-neohesperidoside, luteolin, lysinotin, quinolin-7-ol, quercetin, quercetin 3, 4'-di-O-glucoside, luteolin-7,3'-di-O-glucoside, bisdemethoxycurcumin, thymoquinone, apigenin-7-glucoside and rutin. Furthermore, cinnamic acid, caffeic acid, shikimic acid, salvianolic acid, salicylic acid and ferulic acid were also found in ME-80. Among all identified compounds, linarin, rutin, kaempferol and quercetin were reported to possess antioxidant and antimicrobial activities, dominantly found in mint leaf extracts as documented from several studies (Dorman et al. 2003; Brewer 2011; Park 2011). The variation of those activities depends on the structure of polyphenolic compounds such as benzene rings, the number of hydroxyl ions and their position. Brown et al. (2019) also found that two mint species contained caffeiyolnic acid, salvianic, rosmarinic, luteolin, salvigenin, chrysiol, thymosin and carnosol. Those compounds acted as strong antioxidant agents by delocalizing their electrons, chelating metal ions or donating hydrogen. Olatunde et al. (2020) revealed that kaempferol and quercetin were the predominant phenolic compounds found in noni leaf extract and were known for their antioxidant and antimicrobial activities. The Chamuang leaf extract contained two dominant polyphenols involving 2-feruloyl-Lsinapoylgentiobiose and chrysoberyl 6-C-glucoside-8-C-arabinopyranoside, as identified by Sheikh et al. (2019). Dominant phenolic compounds in guava leaf ethanolic extract were Isovitexin, vitexin 4′-O-galactoside, quercetin 3-(2′'-galloyl-alpha-L-arabinopyranoside) and aclurin 3-C-(6''-p-hydroxybenzoyl-glucoside) identified by Olatunde et al. (2020). Betel leaf ethanolic extract had piceatannol 4′-galloylglucoside, 8-hydroxyluteolin 8-sulfate and epicatechin as the dominant components (Tagrida and Benjakul 2020). Those exhibited antioxidant and antimicrobial activities. The phenolic compounds in the same or different species might be varied with seasons, age of leaf, genotypes, and geographical position (Maqsood et al. 2014). Based on LC-MS profile of ME-80, linarin, kaempferol 3-O-rutinoside, salvianolic acid, salicylic acid, ferulic acid and caffeic acid were identified. The same aforementioned compounds were found as reported by Zheng and Wang (2001). Those compounds are responsible for the leakage of intracellular content from Gram-negative bacteria by lysis of a thin peptidoglycan layer. Mint contains non-polar and low molecular weight compounds, which penetrate and retard bacterial cell growth through cell rupture (Park 2011).

Shelf-life of SMC treated with ME-80 at different levels stored at 4 ºC

Microbiological changes

Total viable count (TVC), psychrophilic bacterial count (PBC), H_2S-producing bacterial count (HSPBC), and *Pseudomonas* spp. count (PDC) increased throughout the storage of 9 days (Fig. 1A-D). TVC at day 0 ranged from 3.33 to 3.79 $\log_{10}$ CFU g^{-1}. The control had higher TVC than that treated with 400 mg L^{-1} ME-80 (Fig. 1A) ($P < 0.05$). However, both treated samples (SMC-200 and SMC-400) had no differences in TVC ($P > 0.05$). A similar result was found at day 3. However, at day 6, the control sample exceeded

Table 4 Phenolic compounds in dechlorophyllized mint leaf ethanolic extract (ME-80) as determined by liquid chromatography-mass spectrometry (LC-MS)

Phenolic compounds	m/z	Molecular Formula	Abundance ($\times 10^6$)
Linarin	593.18	$C_{28}H_{32}O_{14}$	0.97
Kaempferol-3-gentiobioside	611.16	$C_{27}H_{30}O_{16}$	0.97
1,11-Undecanedicarboxylic acid	226.12	$C_{13}H_{24}O_4$	0.93
(+)-trans-Chrysanthemic acid	169.12	$C_{10}H_{16}O_2$	0.94
5,8,11-Eicosatriynoic acid	301.21	$C_{20}H_{34}O_2$	0.9
Thymoquinone	165.09	$C_{10}H_{12}O_2$	0.91
Caffeic acid	179.03	$C_9H_8O_4$	0.91
L-Tartaric acid	149.01	$H_2C_4H_4O_6$	0.92
Kaempferol 3-O-rutinoside	595.16	$C_{12}H_{30}O_{15}$	0.94
2-Isopropylmalic acid	175.06	$C_7H_{17}O_5$	0.92
Methyl perillate	181.12	$C_{11}H_{16}O_2$	0.89
Isoleucine	132.1	$C_6H_{13}NO_2$	0.84
Shikimic acid	173.04	$C_7H_{10}O_5$	0.85
Neochlorogenic acid	353.08	$C_{16}H_{18}O_9$	0.83
1,4-Dihydroxy-2-naphthoic acid	203.03	$C_{11}H_8O_4$	0.85
Bisdemethoxycurcumin	309.09	$C_{19}H_{16}O$	0.74
8-Acetyl-7-hydroxycoumarin	205.05	$C_{11}H_8O_4$	0.77
trans-Anethole	149.09	$C_{10}H_{12}O$	0.75
Glycitin	447.12	$C_{22}H_{22}O_{10}$	0.76
Salvianolic acid	717.14	$C_{26}H_{22}O_{10}$	0.76
Luteolin-7,3'-di-O-glucoside	609.14	$C_{27}H_{30}O_{26}$	0.71
Scutellarin	461.07	$C_{21}H_{18}O_{12}$	0.7
Quinolin-7-ol	144.04	C_9H_7NO	0.77
2-Methoxycinnamic acid	177.05	$C_{10}H_{10}O_3$	0.75
2-Methoxycinnamaldehyde	163.112	$C_{10}H_{10}O_2$	0.61
13-Keto-9Z,11E-octadecadienoic acid	295.15	$C_{18}H_{30}O_3$	0.63
9-Oxo-10E,12Z,15Z-octadecatrienoic acid	293.21	$C_{18}H_{28}O_3$	0.64
Nicotinamide	123.05	$C_6H_6N_2O$	0.69
D- (+)-Trehalose	341.16	$C_{12}H_{22}O_{11}$	0.65
Apigenin 7-O-beta-D-glucuronide	121.03	$C_7H_6O_2$	0.69
Chlorogenic acid	377.08	$C_{12}H_{22}O_{11}$	0.67
(-)-Homoeriodictyol	137.02	$C_7H_6O_3$	0.6
2,4-Decadienal,	197.04	$C_9H_{10}O_5$	0.63
Isophorone	139.11	$C_{21}H_{18}O_{11}$	0.59
Quercetin	303.04	$C_{16}H_{18}O_9$	0.57
Complanatoside	625.14	$C_{19}H_{14}O_6$	0.58
Pectolinarigenin	153.12	$C_{10}H_{16}O$	0.54
6-Methylchromanone	139.11	$C_9H_{14}O$	0.53
epsilon caprolactam	303.04	$C_{15}H_{10}O_7$	0.5
Pectolinarigenin	315.08	$C_{17}H_{14}O_6$	0.53
6-Methylchromanone	163.03	$C_{10}H_8O_2$	0.52
epsilon caprolactam	114.09	$C_6H_{11}NO$	0.51
Cannabidiolic acid	359.17	$C_{22}H_{30}O_4$	0.52
Ferulic Acid	193.05	$C_{10}H_{10}O_4$	0.57
Phenylalanin	164.07	$C_9H_{11}NO_2$	0.52

Table 4 Continued

Phenolic compounds	m/z	Molecular Formula	Abundance (×10^6)
Apigenin-7-glucoside	433.11	$C_{21}H_{20}O_{10}$	0.46
Diosmetin	301.07	$C_{16}H_{12}O_6$	0.45
Quercetin 3,4'-di-O-glucoside	627.15	$C_{27}H_{30}O_{17}$	0.4
Pipecolinic acid	147.07	$C_6H_{11}NO_2$	0.48
Piperitone	153.12	$C_{10}H_{16}O$	0.46
2-(3-Hydroxyphenyl) ethanol	121.06	$C_8H_{10}O_2$ |	0.44
cis-5,8,11-Eicosatrienoic acid	307.08	$C_{21}H_{36}O_2$	0.41
Cuminaldehyde	149.09	$C_{10}H_{12}O$	0.4
L-Tryptophan	593.15	$C_{27}H_{30}O_{15}$	0.42
Rutin	203.08	$C_{11}H_{12}N_2O_2$	0.41

the TVC limit (6 $\log_{10}$ CFU g^{-1}) whereas both treated samples showed TVC at 4.75 and 4.95 $\log_{10}$ CFU g^{-1}, respectively. SMC-400 had a lower load than SMC-200. After 9 days of storage, SMC-400 had TVC of 6.88 $\log_{10}$ CFU g^{-1}, signifying rejection for consumption, while the count was 7.02 $\log_{10}$ CFU g^{-1} in SMC-200. Treatment of SMC with ME-80 at 200 and 400 mg L^{-1} could prolong shelf-life up to 6 days. The ME-80 was found to be effective at higher concentrations by limiting the growth of most mesophilic bacteria. This indicated that ME-80 at higher concentration effectively impeded bacterial proliferation and assisted to increase the shelf-life due to its high antimicrobial activity. In addition, proper handling, and minimum cross-contamination might also be remarkably associated with keeping good quality and enhancing the storage time of SMC-treated samples. According to Tagrida and Benjakul (2021), tilapia slices treated with betel leaf ethanolic extract (BLEE-SED) at lower concentrations (200 mg L^{-1}) could be kept for 6 days but those treated with higher concentrations (400 and 600 mg L^{-1}) had lower TVC and the shelf-life could be extended up to 9 days, while the control exceeded TVC limit at day 3. A similar result was also documented by Olatunde et al. (2019) where ethanolic coconut husk extract treated with Asian seabass slices had an extended shelf-life of up to 9 days. Therefore, the treatment of SMC with ME-80 retarded bacterial growth during refrigerated storage.

Psychrophilic bacterial count (PBC) in SMC (with and without ME-80 treatment) upsurged with increasing storage time ($P < 0.05$). PBC for the control and treated samples with ME-80 at different levels increased after day 3 ($P < 0.05$) (Fig. 1B). The PBC of the control was 6.85 $\log_{10}$ CFU g^{-1} at day 6. SMC-400 treated sample still had PBC lower than 6 $\log_{10}$ CFU g^{-1} after 9 days of refrigerated storage. During iced storage of squid, trimethylamine N-oxide (TMAO) was broken to trimethylamine (TMA) by psychrophilic bacteria enzymes (Masniyom et al. 2005). The higher concentrations (400 and 600 mg L^{-1}) of betel leaf ethanolic extract applied to tilapia slices effectively retarded psychrophilic bacterial growth during refrigerated storage (Tagrida and Benjakul 2021). Generally, psychrophilic bacteria played a main role in spoilage of fish or shellfish when stored at refrigerated condition. Overall, the effectiveness of ME-80 in reducing proliferation of psychrophilic bacteria depended on the concentrations of ME-80 used.

H_2S-producing bacterial count (HSPBC) in all treated samples increased continuously during the storage (Fig. 1C). Hence the sample treated with higher concentrations of ME-80 had a lower increasing rate of HSPBC. At day 6, the control and SMC-200 samples showed a remarkable increase in HSPBC ($P < 0.05$). The H_2S-producing bacteria are believed to degrade L-cysteine and Sulphur-containing amino acids (methionine) along with other proteins in fish and seafood and these are responsible for off-odor in deteriorated fish (Tagrida et al. 2021). HSPB during storage could degrade the squid mantle muscle and decompose muscle proteins into biogenic amines, leading to unpleasant flavor and odor, as documented by Sivertsvik et al. (2002). Cuttlefish and Indian squid treated with sage, mint and rosemary aqueous extracts at 400 and 600 mg L^{-1} had an extended shelf-life for 9 days, whereas mint extract showed higher inhibition activity against bacteria (Park 2011). Those extracts might be involved in interference with bacterial metabolism, disrupting key enzymes or pathways and destroying cellular integrity (Brown et al. 2019). Hence, ME-80

could be effective in extending shelf-life by reducing the growth of sulfur-containing amino acid degradation bacteria.

Pseudomonas spp. count (PDC) of all the samples upsurged as storage time increased. Among all the treated samples, the rate of bacterial proliferation was less when higher levels of ME-80 were used at all storage times (Fig. 1D). *Pseudomonas* spp. is directly related to the spoilage of seafood and can grow well at 4 °C (Miller et al. 1973; Ogidi et al. 2021). The initial lower PDC might be due to acclimatization under the storage condition of *Pseudomonas* spp. Sae-leaw and Benjakul (2019) also documented that an increased concentration of cashew leaf extract might slow down the formation of glucose and amino acids in Pacific white shrimp caused by microorganisms. Those compounds could serve as nutrients for spoilage microorganisms. In addition, *Pseudomonas* spp. was also detected in tilapia slices (Maqsood et al. 2012), Asian seabass slices (Olatunde et al. 2019) and Pacific white shrimp (Sheikh et al. 2019) during the refrigerated storage. Based on next-generation sequencing, *Pseudomonas* spp. was the dominant spoilage bacteria in Asian seabass slices (Olatunde et al. 2020a). Therefore, ME-80 could effectively delay the growth of *Pseudomonas* spp. especially when ME-80 at higher concentrations was used, thus prolonging the shelf-life of the treated SMC samples.

Changes in firmness and toughness

Firmness and toughness in SMC-C, SMC-200, and SMC-400 gradually decreased throughout the storage of 9 days (Fig. 2A and 2B). A drastic decrease was found on day 9. In general, the lowest decrease in firmness and toughness was found when treated with ME-80 at 400 mg L^{-1}. This might be associated with a lower

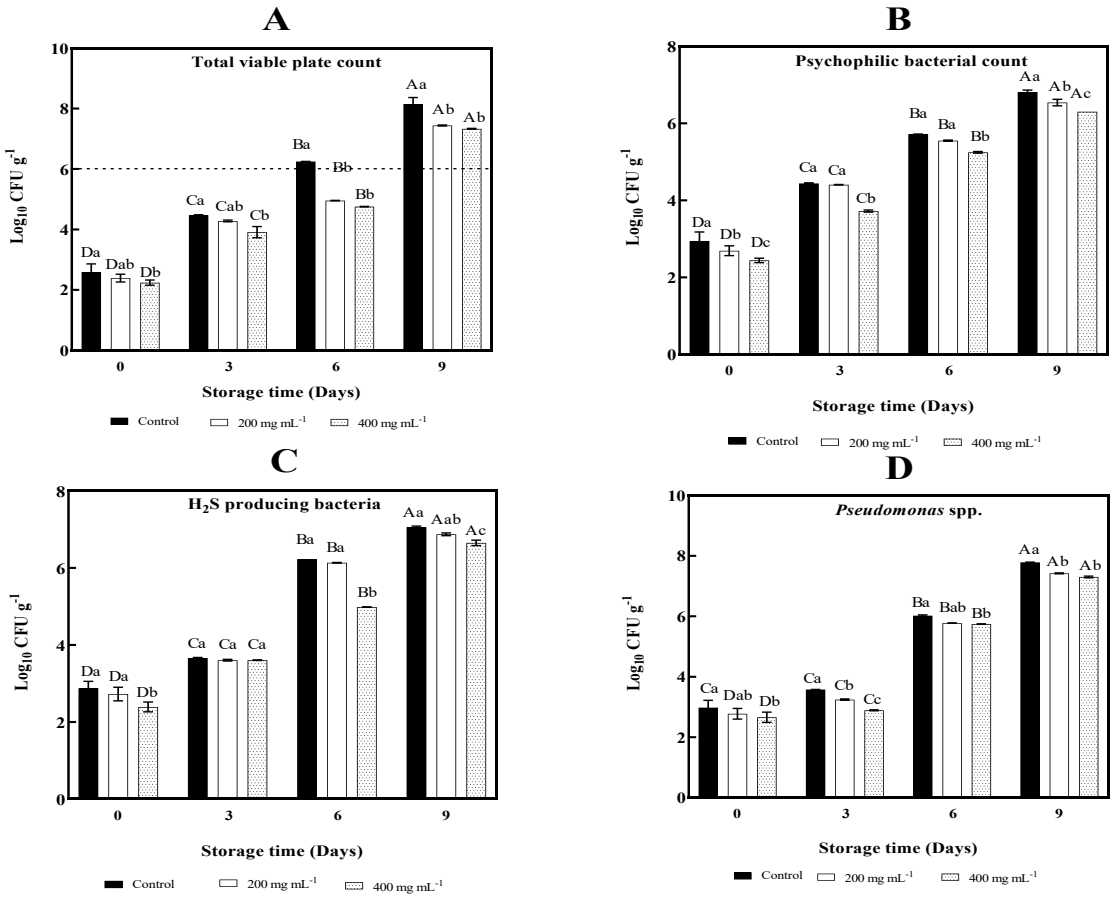

Fig. 1 Total viable count (A), psychrophilic bacterial count (B), H_2S-producing bacteria (C), and *Pseudomonas* spp. count (D) of squid mantle cut (SMC) treated without and with 200 and 400 mg L^{-1} ME-80 during 9 days of storage at 4°C. Different uppercase letters on the bars within the same storage time indicate significant differences ($P < 0.05$). Different lowercase letters on the bars within the same level of ME-80 indicate significant differences ($P < 0.05$). Bars represent the standard deviation (n=3). ME-80: Extract from mint leaves using 80% ethanol as medium.

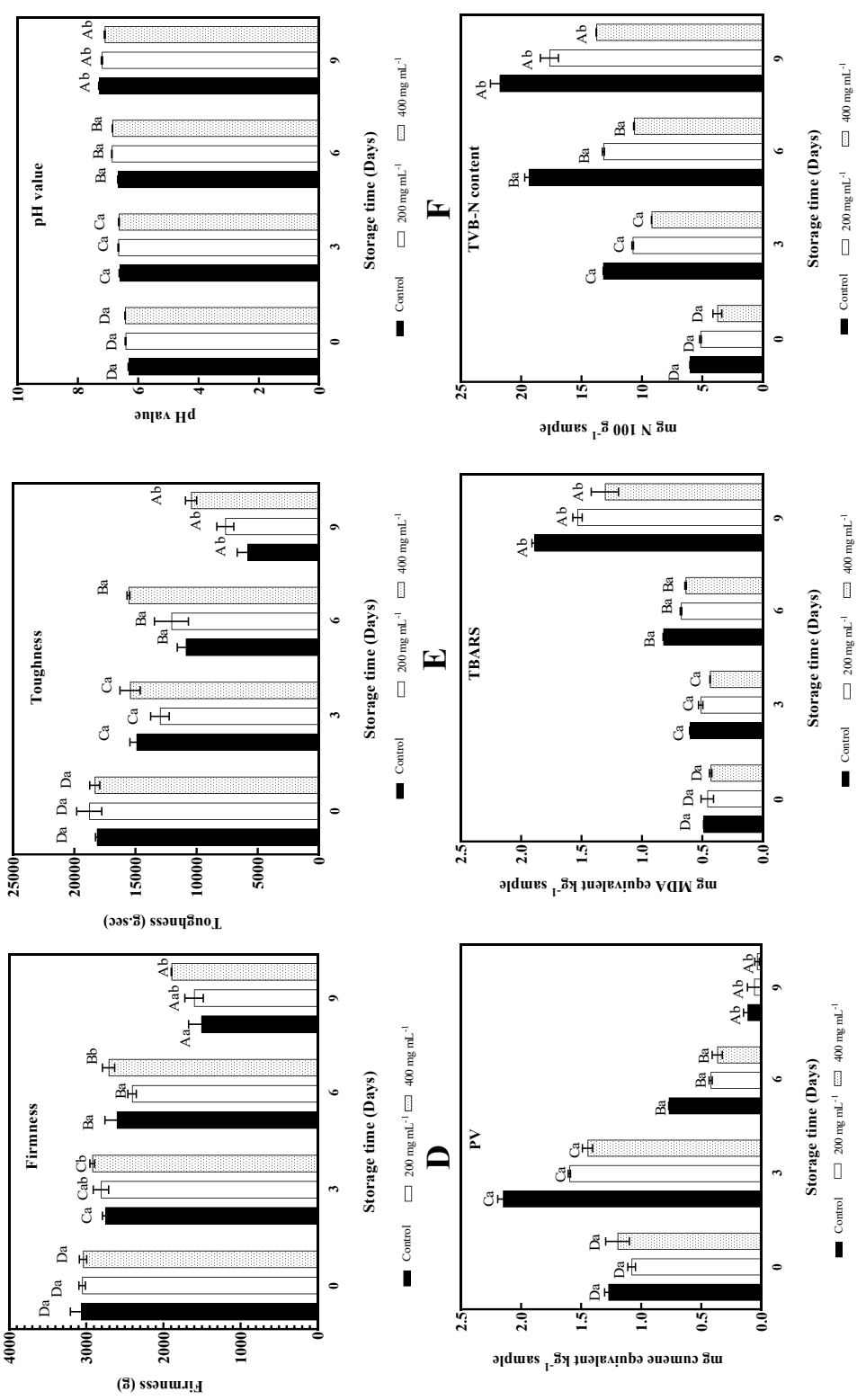

Fig. 2 Firmness (A), toughness (B), pH (C), peroxide value (D), thiobarbituric acid reactive substances (E), and total volatile base content (F) of squid mantle cut (SMC) treated without and with 200 and 400 mg L^{-1} ME-80 during 9 days of storage at 4 °C. Different uppercase letters on the bars within the same storage time indicate significant differences ($P < 0.05$). Different lowercase letters on the bars within the same level of ME-80 indicate significant differences ($P < 0.05$). Bars represent the standard deviation (n=3). ME-80: Extract from mint leaves using 80% ethanol as medium.

microbial load in this sample. Therefore, the degradation of proteins caused by proteases from microorganisms could be lowered. This led to the higher firmness and toughness of samples treated with ME-80 at 400 mg L^{-1} than others. Tantasuttikul et al. (2011) reported that connective tissue proteins (collagen) contributed to tough texture of squid mantle. After treatment with ME-80, the softening took place at a lower degree, suggesting lowered muscle protein degradation and fewer changes in the muscle fiber network (Rodger et al. 1984; Nagashima et al. 1992).

Chemical changes

There were no differences in pH of all the SMC samples on day 0, which ranged from 6.36 to 6.47 ($P > 0.05$). Application of ME-80 at 200 and 400 mg L^{-1} had therefore no impact on pH of SMC (Fig. 2C). Throughout 9 days of storage, pH slightly increased from 6.36 to 7.45. Such increases were more likely owing to the formation of volatile basic compounds generated from microorganisms and gradual increase of squid mantle decomposition by endogenous enzymes and microorganisms (Sungsri-in et al. 2011). There was no marked difference in pH among all the samples on the same day of storage. This might be mediated by the buffering capacity of muscle protein (Masniyom et al. 2005). TMAO also served as the buffering agent in muscle. (Tantasuttikul et al. 2011).

During the storage, PV increased up to day 3 (Fig. 2D). PV of SMC-200 and SMC-400 was lower than that of the control on day 3 and SMC-C showed the maximum value ($P < 0.05$). Subsequently, PV decreased in all samples. At the initial stage of storage, unsaturated fatty acids could form hydrogen peroxide after reacting with oxygen molecules (Sheikh and Benjakul 2020). Overall, PV for all samples was reduced with the reduction of lipid content (Feng and Xu 2014). The decreases in PV at day 6 and 9 were likely attributed to the decomposition of hydroperoxide to other secondary oxidation products. The SMC-400 showed less formation of PV because the ME-80 at higher concentration exhibited higher antioxidant activity and lipid oxidation was effectively retarded. Ethanolic kiam wood extract retarded the lipid oxidation of washed seabass mince added with hemolysate due to high antioxidant activity (Maqsood et al. 2013). Olatunde et al. (2019) found that Asian seabass slices treated with coconut husk ethanolic extract had lower PV than that of the control during 9 days of storage at 4 °C.

At the initial stage of storage, TBARS values of all the samples were in the range of 0.43-0.49 mg MDA equivalent kg^{-1} sample (Fig. 2E). The unsaturated fatty acids of all samples underwent rapid oxidation, which led to the formation of TBARS. Enzymatic degradation of microorganisms liberates free fatty acids from squid mantle and oxidation could take place at the faster rate. TBARS values were lower in the samples treated with ME-80 at both levels throughout the storage. A drastic increase in TBARS was found in all samples after 9 days. TBARS represent secondary oxidation products (Chaijan et al. 2006) and the values could be lowered by treatment SMC with ME-80 at higher level. Tagrida and Benjakul (2021) found that tilapia slices treated with ethanolic extract of betel leaf at 200 mg L^{-1} and the control exceeded the TBARS limit (2.38 mg MDA equivalent kg^{-1} sample) on day 6, whereas 400 and 600 mg L^{-1} treated samples still retained acceptable levels until 12 days. TBARS value of squid increased from 0.30 to 1.14 mg kg^{1} after the refrigerated storage for 16 days (Sungsri-in et al. 2011). Thus, treatment of SMC with ME-80 at higher concentration was effective in preventing lipid oxidation.

Total volatile base content (TVB) is a crucial indicator for detecting the spoilage in seafood. The formation of these volatile bases is primarily due to the breakdown of proteins by microbial activity, resulting in the generation of unpleasant flavors and odors (Capillas et al. 2002). The lesser the TVB value, the better the quality of squid mantle was obtained. Acceptable levels in seafood (20-40 mg N 100 g^{-1} sample) and fish flesh (30-35 mg N 100 g^{-1}) were established (Tagrida et al. 2021). The initial TVB contents of all treated samples (Fig. 2F) ranged from 3.25 to 6.55 mg N 100 g^{-1} sample. This might suggest low microbial load, and proper handling of raw materials as indicated by low TVC. TVB content augmented progressively with increasing storage time and the control and SMC-200 exceeded their acceptable limit on day 6, whereas the 400 mg L^{-1} treated sample still had TVB within the acceptable range (13.24 mg N 100 g^{-1} sample). TVB-N value reached 6.50 mg N 100 g^{-1} on day 10. Similarly, TVB-N value of squid reached 8.50 mg N 100 g^{-1} after12 days of refrigerated storage (Sungsri-in et

al. 2011). This confirmed that higher concentration of ME-80 could retard bacterial growth and retard degradation or decomposition of proteins into smaller units such as ammonia took place (Olatunde et al. 2020b). Nevertheless, TVB content of all the samples exceeded the limit after 9 days ($P < 0.05$) during iced storage. TVB content of tilapia slices increased after day 6 and reached an unacceptable limit at day 12 (45 mg N 100 g^{-1} sample) (Tagrida et al. 2021; Ke et al. 1984). In Japan, the acceptable value of TVB is considered below 15 mg N 100 g^{-1} squid meat, indicating that acceptability limit varies with raw materials (Capillas et al. 2002).

Conclusions

Ethanolic extracts of both mint and basil leaf extracts exhibited antioxidant activity after dechlorophyllization. Mint leaf ethanolic extract (ME-80) with the highest antioxidant contained linarin, kaempferol 3-O-rutinoside, quercetin, caffeic acid and ferulic acid as dominant compounds. Additionally, it showed the highest antimicrobial activity, particularly against Gram-negative bacteria. Furthermore, squid mantle cut (SMC) showed an extended shelf-life at 4 °C up to 6 days when treated with ME-80 at 400 mg L^{-1}. A remarkable reduction in bacterial proliferation and biochemical changes was achieved, while the control could be kept for 3 days. Therefore, ME-80 could be used as a natural preservative for seafood to inhibit microbial growth and retard lipid oxidation.

Conflict of interest No conflict of interest was declared by the authors.

Author's contributions Jasmin Naher: Writing original draft, methodology, investigation, formal data analysis; Krisana Nilsuwan: Editing and review; Suriya Palamae: Investigation and review; Hui Hong: Review and editing; Bin Zhang: Review and editing; Kazufumi Osako: Review and editing; Soottawat Benjakul; Conceptualization, project administration, supervision, funding acquisition, validation, data curation, review and editing. All authors have read and agreed to the published version of the manuscript.

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