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Glycerol Extraction of Bioactive Compounds from Thanaka (*Hesperethusa crenulata*) Bark through LCMS Profiling and Their Antioxidant Properties

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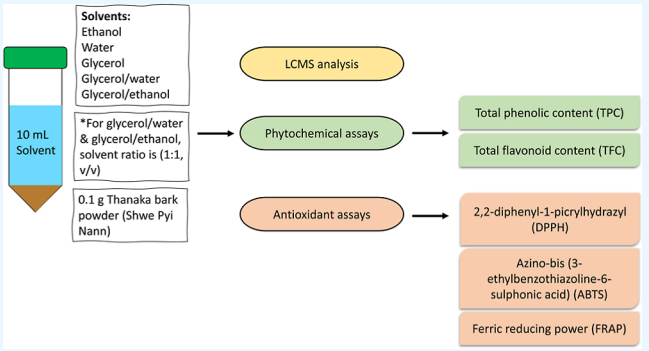
ABSTRACT: Organic solvents are hazardous to human and environmental health. The emergence of interest in finding greener solvents to replace organic solvents has sparked a series of studies in the use of glycerol for extracting bioactive compounds from natural products. In this study, we will first identify the bioactive compounds of glycerol- and nonglycerol-based Thanaka (*Hesperethusa crenulata*) bark extracts using liquid chromatography−mass spectrometry profiles; then, we will determine their antioxidant capacity, free radical scavenging activity, and total phenolic and flavonoid contents. Thanaka bark powder was extracted using solvents, namely, ethanol (TKE), water (TKW), glycerol (TKG), glycerol/water (1:1, v/v) (TKGW), and glycerol/ethanol (1:1, v/v) (TKGE). Among the five extracts, the extract of TKG has the highest number of bioactive compounds, as well as the highest total flavonoid content. TKGE possessed the highest total phenolic content and highest antioxidant activity shown in azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and ferric-reducing antioxidant power assays among the five extracts. Overall, glycerol has better efficiency in extracting bioactive compounds from Thanaka bark as compared to ethanol and water. Hence, from the phytochemical content and antioxidant properties of Thanaka extracts, we conclude that glycerol is a good green solvent alternative to replace organic solvents.



1. INTRODUCTION

Commercial organic solvents are used in great quantities, particularly in the manufacturing of active pharmaceutical ingredients comprising 80−90% of the total mass.[1] Furthermore, a substantial amount of waste is produced, impacting in terms of cost, safety, as well as health issues. Organic solvents such as benzene, *n*-hexane, petroleum ether, diethyl ether, dichloromethane, and chloroform are classified as hazardous or toxic substances, and the use of these solvents is severely restricted by legislation,[2] while solvents such as methanol, ethanol, methyl acetate, and ethyl acetate are considered more favorable to the environment with an environmental, health, and safety indicator score of less than 3.0 points and yet still bring some impact onto the environment including air hazard, water hazard, and persistency in the soil, water, or air as well as onto human health including chronic toxicity, irritation, and acute toxicity and safety issues such as fire or explosion risk and solvent release potential. Moreover, long-term contact with alcohol solvents can cause human health issues such as irritant contact dermatitis[3] and reported cases of immunological and nonimmunological contact urticaria.[4,5]

While there are various methods such as the distillation method, pressing, and sublimation method for the study and manufacture of natural bioactive compounds from natural products, the most widely used method is still solvent extraction. The selection of solvents in solvent extraction is crucial as based on the law of similarity and intermiscibility, solvents whose polarity is more similar to the polarity of the solutes are more likely to perform better and vice versa.[6] Therefore, despite the toxicity and environmental and health risks, alcohols were still used as universal solvents in solvent extraction for phytochemical investigation.

Currently, the idea of using "greener" alternatives to hazardous or toxic solvents is being accepted by people to minimize the impact of hazardous solvent use to the environment in chemical production. The emergence of "green" solvents such as ionic liquids, liquid polymers, and deep eutectic solvents clearly have the potential in substituting hazardous organic solvents. Among these green solvents, glycerol, which is a byproduct of biodiesel and petroleum production, has been reported to potentially accelerate the

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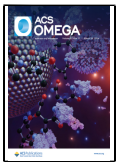

reaction rate of organic reactions when used as a solvent.[7] This attracted much interest due to its less hazardous property even though it is derived from petroleum that is often a volatile organic compound and its potential as a biorenewable solvent derived from renewable resources, which can be relatively cost-efficient.

Glycerol (1,2,3-propanetriol) is a colorless, odorless, and viscous liquid with a sweet flavor that is produced as a byproduct from saponification, hydrolysis, and transesterification reactions in oleochemical or biodiesel plants as well as fossil sources. Glycerol possesses three hydroxy groups, making it water soluble and provide hygroscopicity.[8] It has a high polarity that enables it to dissolve inorganic salts, enzymes, acids, bases, many transition metal complexes, and even organic compounds that cannot be dissolved in water.[9] Kong et al. (2016) also state that glycerol is miscible in water and slightly miscible in diethyl ether,[10] which allows glycerol to replace solvents of the polarity index ranging from 10 to 3 (between glycerol and diethyl ether). Based on the data provided in Pubchem and DrugBank, there is little to none in toxicity of glycerol on human skin, and glycerol was stated to have a published toxic dose (toxic dose low) of 1428 mg kg^{-1} when orally taken by humans.[11] In food products, glycerol is added as a sweetener and humectant. The pharmaceutical uses of glycerol include the application in drugs such as ophthalmic and dermal products as well as external analgesic. In cosmetics, it is a commonly used ingredient that functions as a skin protectant and humectant.

On the other hand, glycerol is considered a renewable material in the production of a series of glycerol-derived liquids such as 1,2,3-trialkoxy-propanes and 1,3-dialkoxy-2-propanols that have been tested as green solvents with potential as the alternatives to petroleum-derived solvents.[12] Hence, the use of glycerol and its derivative solvents as replacement media for organic reactions came to the fore as an auspicious new field of research, opening new ways in re-evaluation of the application of glycerol in synthetic organic chemistry, catalysis, and biocatalysis.[13] Correspondingly, the use of glycerol-based solvents such as glycerol carbonate and glycerol esters has been assessed to further emphasize the significance of glycerol as a versatile solvent.

In 2014, Apostolakis et al. proposed mixtures of water/glycerol as a distinctly efficient medium for the extraction of polyphenolic phytochemicals from olive leaves, which sparked off a series of investigations that demonstrated the potential of water/glycerol mixtures in the extraction of polyphenols from botanicals and various plant food processing byproducts, including olive leaves,[14,15] apple peels,[16] onion solid waste,[17,18] red grape pomace,[19,20] coffee brewing residue,[21,22] eggplant peels,[22,23] potato peels,[22,24] oak acorn husks,[25] rice bran,[26] grapefruit peels,[27] and mangosteen pericarp.[28] However, these investigations are limited to the extraction of polyphenolic phytochemicals and the corresponding antioxidant activity of the extracts, while the comparison of extraction efficiency of glycerol and other solvents in other aspects such as the compound profiles of the extracts was not thoroughly done. On that account, it is worth studying the extraction efficiency of glycerol and glycerol-based mixtures as extraction solvents as a comparison to the organic solvent extracts by re-evaluating the plant materials that have been studied before, such as the Thanaka bark, which is used as a traditional natural sunscreen through its antioxidant properties.

In our previous extensive review of Thanaka,[29] there is a mentioning of Wangthong et al. (2016) who studied the biological activities of Thanka using solvents such as hexane, dichloromethane, ethyl acetate, methanol, 85% aqueous ethanol, and distilled water to extract bioactive compounds from Thanaka barks.[30] Despite having the same concentration of extracts used, their results varied with each other. They commented that the polarity of solvents significantly affects the results of bioassays as polar buffers are commonly used in the bioassays. Furthermore, the extraction of the polar compounds such as polyphenols from plant materials depends on the polarity of solvents, while the toxicity of the solvents also significantly affects the results of cytotoxicity assays. Thus, a green solvent such as glycerol could be replaced with the organic solvent as it is nontoxic[11] and has a wide range of polarity indexes which may replace solvents between water (10.2) and diethyl ether (2.8). Thus, the objectives of this study are to identify and compare the bioactive compounds of glycerol- and nonglycerol-based Thanaka extracts through liquid chromatography−mass spectrometry (LCMS) profiling and to determine their antioxidant properties.

2. METHODOLOGY

2.1. Solvent Extraction of Thanaka Bark Powder. 0.1 g of Thanaka bark powder was added into 10 mL of ethanol, water, glycerol, glycerol/water (1:1, v/v), and glycerol/ethanol (1:1, v/v). The mixtures of glycerol/water (1:1, v/v) and glycerol/ethanol (1:1, v/v) were premixed prior the extraction. The extraction mixtures were vortexed and then sonicated in an ultrasonic bath for 30 min. After sonication, the mixtures were incubated at room temperature (28 °C) for 24 h. Sonication was repeated after 24 and 48 h of incubation, respectively. After 48 h, the extraction mixtures were centrifuged at 2000 rpm for 10 min. As a precaution step, all extracts were first filtered with 0.45 μm syringe filters, as centrifugation may be incomplete to separate Thanaka powder from supernatant (extract), followed by filtration using 0.2 μm syringe filters. It is worth mentioning that nylon membrane syringe filters were used to filter extracts of water and ethanol; meanwhile, cellulose acetate membrane syringe filters were used to filter glycerol-based extracts. The final concentration of all extracts was 0.01 g mL^{-1}. All extracts were stored in glass vials at 4 °C until further use.

2.2. LCMS Analysis. The secondary and primary metabolite analysis was done by using an Agilent 1290 Infinity LC system coupled with an Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with a dual-electron spray ionization (ESI) source which involves positive and negative modes using the settings of Lim et al. (2023)[31] and Thiyagarasaiyar et al. (2021)[98] with slight modifications.[31,97] The mass range (m/z) for both positive and negative modes is from 100 (minimum) to 3200 (maximum). The column used is the Agilent Zorbax Eclipse XDB-C18, Narrow-Bore column with a diameter of 2.1 mm, length of 150 mm, and pore size of 3.5 mm The column temperature and autosampler temperature were set at 25 and 4 °C, respectively, using water and acetonitrile (60:40) as the mobile phase with a flow rate of 0.5 mL min^{-1}. The Thanaka samples were injected 3.0 μL into the system and run in positive and negative polarity with an applied fragmentor voltage at 125 V and use of nitrogen as the collision gas. The extracts were analyzed in the positive-ion ESI mode with the scanning mass range set to 100−3200 m/z, with an acquisition rate of 1.03 s. For the peak integration, Agilent MassHunter

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Table 1. Total Number of Compounds Detected by the LCMS and the Total Number of Compounds Interpreted from Each Thanaka Extract

extracts	positive (P)/negative (N)	total compounds	identified by METLIN	not available METLIN	duplicated compound in P and N	total interpreted
TKW	P	46	33	13	1	55
	N	42	25	17	0	
	total	88	58	30	1	overlap: 2
TKE	P	35	28	7	3	55
	N	71	36	35	5	
	total	106	64	42	8	overlap: 1
TKG	P	131	64	35	3	107
	N	93	52	37	2	
	total	224	116	72	5	overlap: 4
TKGW	P	89	63	26	1	83
	N	38	22	16	1	
	total	127	85	42	2	overlap: 0
TKGE	P	71	23	48	0	41
	N	46	18	28	0	
	total	117	41	76	0	overlap: 0

Qualitative Analysis (version B.05.00) software was used. Bioactive compound identification was achieved using the METLIN database by comparing LCMS peaks in the mass spectra (Tables S1−S5).

2.3. Determination of Total Phenolic Content Using Folin−Ciocalteu Assay. The total phenolic content (TPC) of the Thanaka extracts was determined according to the method of Lim et al. (2023) with slight modifications.[31] Briefly, gallic acid (GA) with concentration ranging from 0 to 1000 μg mL^{-1} was used as the standard to generate the calibration curve. In each reaction tube, 5 μL of sample (10 mg mL^{-1}) or GA standard was added and mixed with 25 μL of the Folin-Ciocalteu reagent, 350 μL of ddH_2O, 75 μL of 20% sodium carbonate, and another 45 μL of ddH_2O within 10 min and incubated in the dark for 60 min. Absorbance was measured at 750 nm with a UV−vis spectrophotometer microplate reader. A standard curve was plotted for the absorbance reading at 750 nm against the concentration of GA standard to obtain the linear regression. TPC of extracts was calculated against a GA calibration curve linear regression and expressed as milligram (mg) of gallic acid equivalents (GAE) per gram (g) of extract (mg GAE g^{-1}).

2.4. Total Flavonoid Content Assay. Total flavonoid content (TFC) of the Thanaka extracts was determined through the method of Lim et al. (2023) with slight modifications.[31] Quercetin with concentration ranging from 0 to 1000 μg mL^{-1} was used as the standard for calibration curve generation. In the reaction tube, 0.01 mL of Thanaka extracts (10 mg mL^{-1}) or standard was added and mixed with 0.25 mL of 2% $AlCl_3$, 0.25 mL of 1 M CH_3COONa, and 0.49 mL of ddH_2O. The reaction tube was incubated for 15 min prior to measuring the absorbance at 425 nm using a UV−vis spectrophotometer microplate reader (TECAN, infinite M200 PRO). A standard curve was plotted to plot the absorbance reading at 425 nm against the concentration of quercetin to obtain the linear regression. TFC of extracts was calculated against the quercetin calibration curve linear regression and expressed as milligram of quercetin equivalent (QE) per gram of extract (mg QE g^{-1}).

2.5. 2,2-Diphenyl-1-picrylhydrazyl Assay. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity assay followed the method of Lim et al. (2023) with slight modifications.[31] The positive control used is ascorbic acid (AA) with a concentration ranging from 0 to 25 μg. In 1.5 mL microcentrifuge tubes, 50 μL of the positive control or the extracts was allowed to react with 1 mL of 0.1 mM DPPH in the dark for 30 min. The blank set (negative control) is prepared by adding 1 mL of methanol instead of DPPH into the 50 μL extract samples and positive control. Absorbance of the reaction and blank sets was measured at 518 nm using a UV−vis spectrophotometer microplate reader (TECAN, infinite M200 PRO). The half-maximal effective concentration (EC_{50}) is calculated from the linear regression equation of the DPPH scavenging activity scatter plot against the sample concentration.

2.6. Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Assay. The azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activity followed the method of Lim et al. (2023) with slight modifications.[31] The positive control used is AA ranging from 0 to 25 μg in concentration. Stock solution for the reaction was prepared by mixing 15 μL of 7 mM of ABTS with 15 mL of 2.45 mM potassium persulfate and allowed to react in the dark for 12−16 h prior to assay. The activated stock solution was then diluted with ethanol at an approximate 1:10 (v/v) ratio to achieve ABTS working solution with an absorbance of 0.7 ± 0.02, read at 734 nm. Initial absorbance reading of the ABTS working solution was recorded to calculate for true absorbance reading. Extract samples and positive control at 100 μL each were allowed to react with 1 mL of ABTS working solution in the dark for 6 min. The absorbance was then measured at 734 nm with a UV−vis spectrophotometer microplate reader (TECAN, infinite M200 PRO). The EC_{50} is then calculated from the linear regression equation of the scatter plot of ABTS scavenging activity % against the concentration of extracts.

2.7. Ferric-Reducing Antioxidant Power Assay. The ferric-reducing antioxidant power (FRAP) assay was conducted following the methods of Lim et al. (2023) with slight modifications.[31] Positive control used was AA with concentrations ranging from 0 to 25 μg. The reaction was done by first mixing 250 μL of 0.2 M phosphate buffer and 250 μL of 1% potassium ferricyanide with 100 μL each of extract samples and positive control, followed by 20 min of incubation at 50 °C. After incubation, 250 μL of 10% trichloroacetic acid was added into each reaction tube and centrifuged at 3000 rpm for 10 min. The supernatant of the centrifuged reaction tubes was

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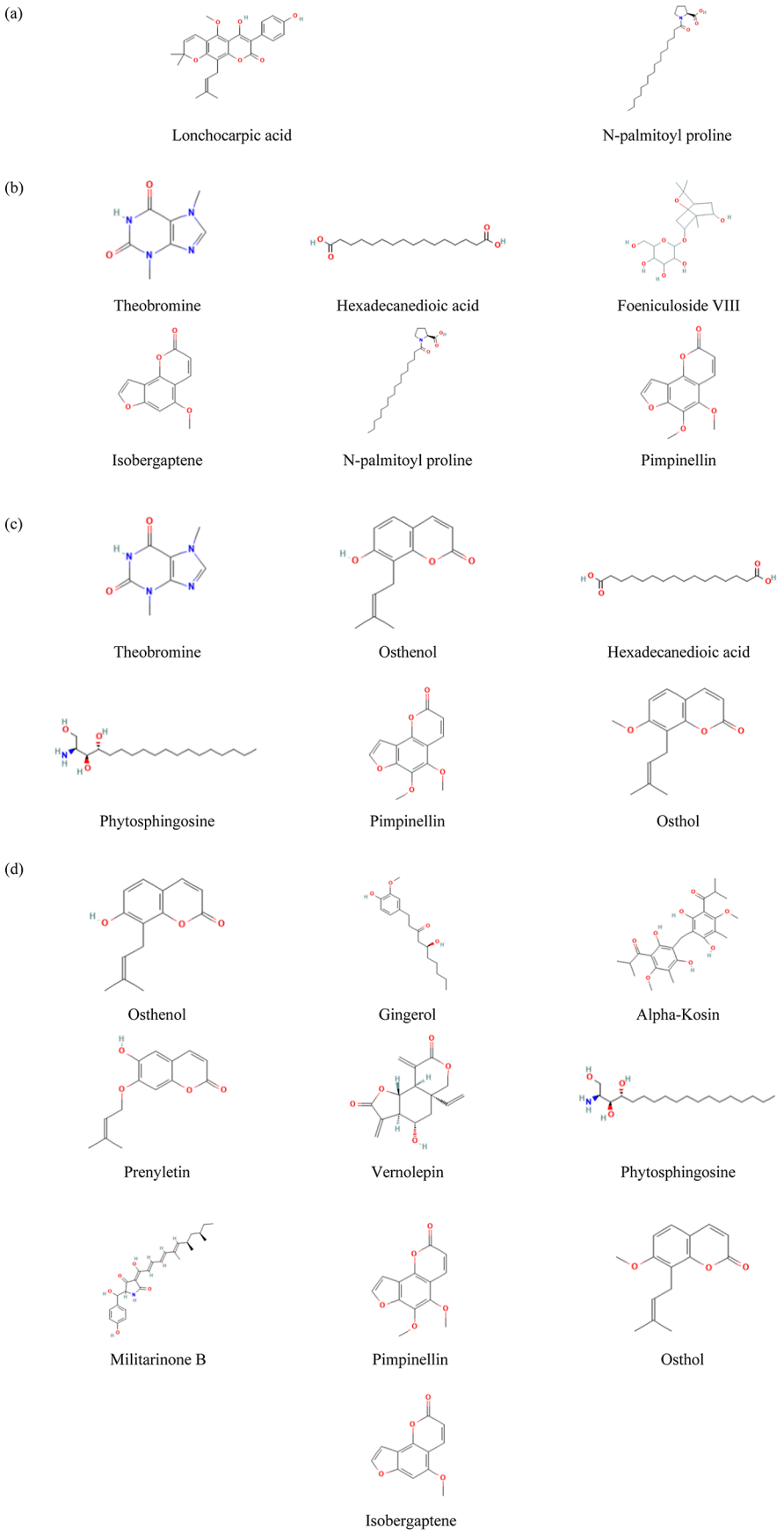

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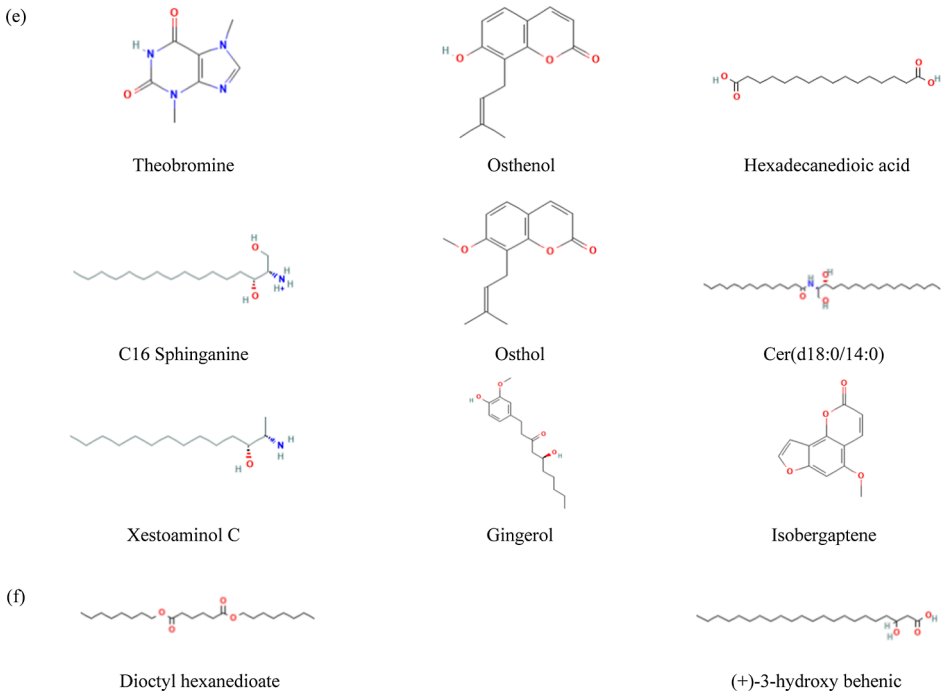

Figure 1. Chemical structure of compounds that exhibited (a) antipigmentation, (b) antioxidant, (c) anti-inflammatory, (d) antimicrobial, and (e) anticancer properties and (f) cosmetic preparation compounds found in TKE.

aliquoted 250 μL each and mixed with 250 μL of 0.1% iron(III) chloride and 250 μL of ddH_2O. The absorbance of the extract samples and positive control was read at 700 nm with a UV−vis spectrophotometer microplate reader (TECAN, infinite M200 PRO). The EC_{50} values were calculated based on the linear regression equation from the scatter plot of absorbance reading against the concentration of samples.

2.8. Statistical Analysis. Statistical analysis was done using SPSS ver. 27. The means of data were subjected to one-way analysis of variance with post hoc tests for significant difference between extracts. Turkey's test was done for results of TPC, Tamhane's T2 test for results of TFC, DPPH, ABTS, and FRAP.

3. RESULTS AND DISCUSSION

3.1. LCMS Interpretation of Thanaka Extracts. The profiling of bioactive compounds in Thanaka extracts was analyzed using LCMS and identified by METLIN database. An overview of the number of detected, identified, unidentified, and overlapping compounds in the respective Thanaka extracts, namely, Thanaka water extract (TKW), Thanaka ethanol extract (TKE), Thanaka glycerol extract (TKG), Thanaka glycerol/water extract (TKGW), and Thanaka glycerol/ethanol extract (TKGE), is tabulated in Table 1.

TKG possessed the highest number of compounds (224), followed by TKGW with 127 compounds, TKGE with 117 compounds, TKE with 106 compounds, and last TKW with 88 compounds. The glycerol and glycerol-based extracts exhibited a better ability to extract compounds from Thanaka, compared with ethanol and water. It also shows that when mixing ethanol or water with glycerol, it helps to enhance the efficacy of both solvents in Thanaka bark powder extraction, where TKE only extracted 106 compounds, while TKGE extracted 117 compounds, and TKW only extracted 88 compounds, while TKGW extracted 127 compounds. Another point to notice is that there are lists of unidentified compounds in every Thanaka extract that are not available in the METLIN database, and it is most obvious in TKGE where a total of 117 detected compounds resulted in only 41 compounds identified by the METLIN database. The list of interpreted compounds are tabulated in Tables S1−S5. The lists of unidentified compounds are tabulated in Tables S8−S12.

3.2. Biological Properties of Compounds Identified in Thanaka Extracts. In order to identify whether the glycerol-based solvents have the potential to replace ethanol and water in compound extraction from Thanaka and also to know the difference in the extraction ability of the three glycerol-based solvents, the LCMS-profiled compounds in the five extracts were compared among each other in groups of three to determine and compare the common compounds between Thanaka extracts.

Glycerol was able to extract 28 of the same compounds as ethanol from Thanaka bark powder, followed by the glycerol/water (1:1, v/v) mixture being able to extract 15 same compounds as ethanol, the glycerol/water (1:1, v/v) mixture being able to extract 12 of the same compounds as water, and glycerol being able to extract 7 of the same compounds as water (Figure S1). The LCMS compound reports of all Thanaka extracts showed that compounds exhibit biological properties including UV protection, antipigmentation, antioxidant, anti-inflammatory, and anticancer, as well as compounds used in cosmetic preparations, which will be discussed in detail in the following sections.

3.2.1. Thanaka Ethanol Extract. Compounds exhibited biological activities including antipigmentation, antioxidant, anti-inflammatory, antimicrobial, and anticancer, as well as cosmetic preparation compounds were found in TKE and interpreted with their biological functions (Figure 1).

Lonchocarpic acid was identified to prevent hyperpigmentation on the skin.[32] *N*-Palmitoyl proline is already an ingredient used in skin and hair conditioning cosmetics, with functions of

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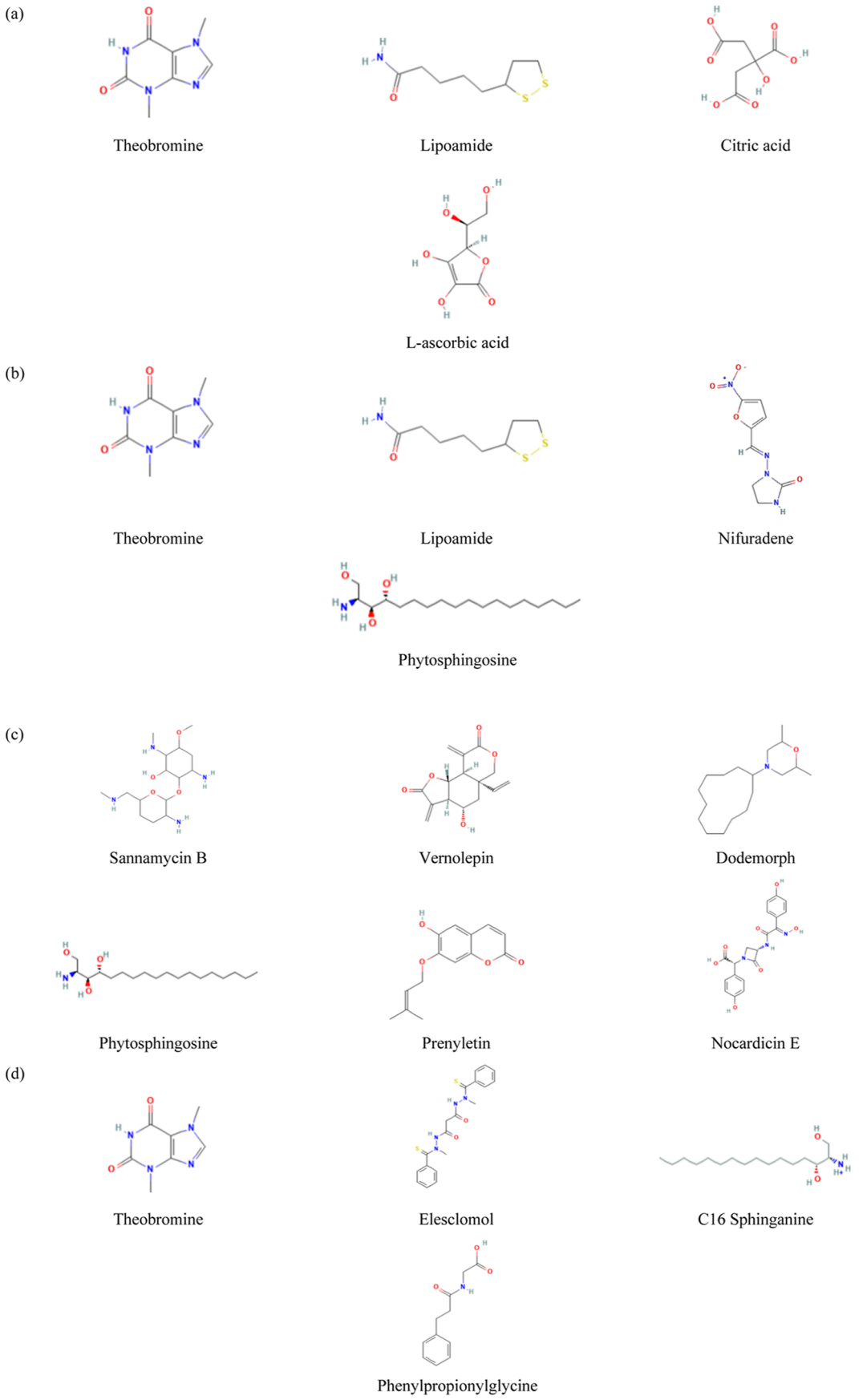

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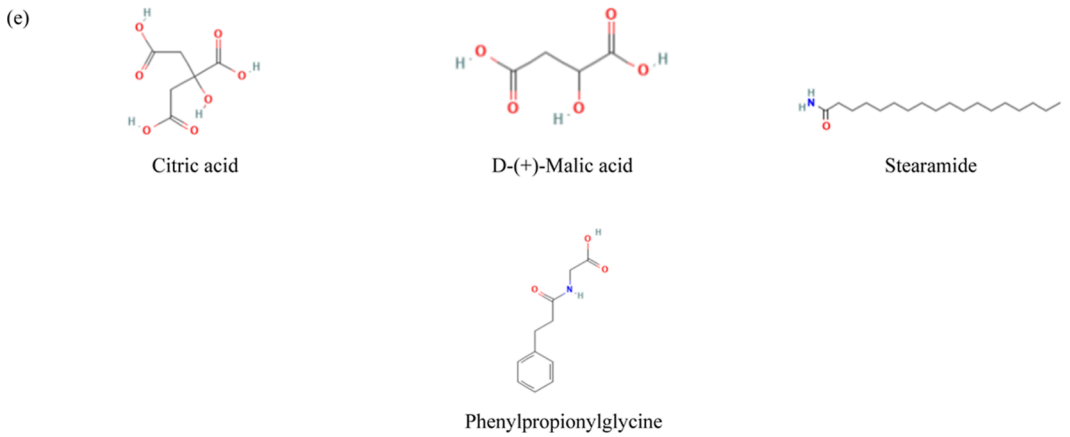

Figure 2. Chemical structure of compounds that exhibited (a) antioxidant, (b) anti-inflammatory, (c) antimicrobial, and (d) anticancer properties and (e) cosmetic preparation compounds found in TKW.

skin lightning and antiaging effects.[33] Isobergapten is a furanocoumarin that possesses antioxidant,[34] antimicrobial,[35] and antitumor[36] effects. Lipoamide is an antioxidant with anti-inflammatory effects.[37] Theobromine is an antioxidant compound that can be used in the treatments of respiratory tract problems such as coughing and asthma where no definitive drug has been developed.[38,39] It also exhibits anti-inflammatory and anticancer properties. Hexadecanedioic acid possesses antioxidant, anti-inflammatory, and anticancer properties. Foeniculoside VIII is an antioxidant that has already been used as a skin conditioning ingredient in cosmetics.[40] Pimpinellin is a coumarin with antioxidant,[41] anti-inflammatory,[42] and antimicrobial[43] activities.

Osthenol is a furanocoumarin with anti-inflammatory, anticancer, and antifungal properties. It also has antiviral activity.[44] Phytosphingosine is an anti-inflammatory compound with antimicrobial activity used in the treatment of acne vulgaris.[45] Osthol possesses anti-inflammatory, anticancer, antihypertensive, antiarrhythmic, antiosteoporosis, and anti-fungal properties.[6] Gingerol is an antimicrobial that can be used against periodontal bacteria;[46] it also has anticancer activity. Alpha-Kosin is an antibacterial agent used in the treatment of *Propionibacterium acnes*.[47] Militarinone B is an antibiotic.[48] Prenyletin is a coumarin with antifungal activity.[49] Vernolepin is an antibacterial agent with immunomodulatory, hepatoprotective, antiulcerogenic, and antihistaminic effects.[50]

C16 sphinganine and Cer(d18:0/14:0) are anticancer compounds with an important role in cellular signals for inducing apoptosis.[51,52] Xestoaminol C is an antitumor compound that acts as an antiproliferative agent in human glioblastoma cell line SHG-44.[53] Dioctyl hexanedioate can be used as a lubricant liquid emollient for cosmetic application (PubChem), while (+)-3-hydroxy behenic acid is used in cosmetic product preparations (PubChem).

3.2.2. Thanaka Water Extract. Compounds exhibited biological activities including antioxidant, anti-inflammatory, antimicrobial, and anticancer, as well as cosmetic preparation compounds were found in TKW and interpreted with their biological functions (Figure 2).

Theobromine is an antioxidant compound that can be used in the treatment of respiratory tract problems such as coughing and asthma where no definitive drug has been developed.[38,39] It also exhibits anti-inflammatory and anticancer properties. Citric acid is an antioxidant that can also be used as a preservative, an acidulant to control pH, as well as an anticoagulant for chelating calcium in blood (PubChem). Lipoamide is an antioxidant with anti-inflammatory effects.[37] L-ascorbic acid is a naturally occurring vitamin C that is both a potent reducing and antioxidant agent that functions in fighting bacterial infections, detoxification, and in the formation of collagen in fibrous tissue, teeth, bones, connective tissue, skin, and capillaries (PubChem).

Phytosphingosine is an anti-inflammatory compound with antimicrobial activity used in the treatment of acne vulgaris.[45] Nifuradene is an anti-inflammatory drug for the eye.[54] Prenyletin is a coumarin with antifungal activity.[49] Dodemorph is an antifungal (Chemical Entities of Biological Interest (ChEBI)). Sannamycin B is an antibiotic (PubChem). Nocardicin E is an antimicrobial agent.[55] Vernolepin is an antibacterial agent with immunomodulatory, hepatoprotective, antiulcerogenic, and antihistaminic effects.[50]

C16 sphinganine is an anticancer compound with an important role in cellular signals for inducing apoptosis.[51] Elesclomol is a small molecule with potential antineoplastic activities.[56] Phenylpropionylglycine is used as a precursor for fragrance, an antineoplastic agent, a bronchodilator, as well as a drug for skeletal disorders (The Human Metabolome Database (HMDB)).

Stearamide is a naturally occurring surfactant that can be used in cosmetic preparations.[57] D-(+)-Malic acid is used as a food flavoring agent and an acidity adjuster in cosmetics manufacturing and also used as a supplement to boost energy (ChEBI).

3.2.3. Thanaka Glycerol Extract. Compounds exhibit biological activities including antioxidant, anti-inflammatory, antimicrobial, and anticancer, as well as cosmetic preparation compounds were found in TKG and interpreted with their biological functions (Figure 3).

Isobergapten is a furanocoumarin that possesses antioxidant,[34] antimicrobial,[35] and antitumor[36] effects. Lipoamide is an antioxidant with anti-inflammatory effects.[37] Perseitol that can also be found in avocado possesses antioxidant, antiaging, and anti-inflammatory activities and is used in the treatment and prevention of skin disorders.[58] Citric acid is an antioxidant that can also be used as a preservative, an acidulant to control pH, as well as an anticoagulant for chelating calcium in blood (PubChem). Beta-D-glucosyl crocetin is an antioxidant with anticancer activity.[59] Pimpinellin is a coumarin with anti-oxidant,[41] anti-inflammatory,[42] and antimicrobial[43] activities.

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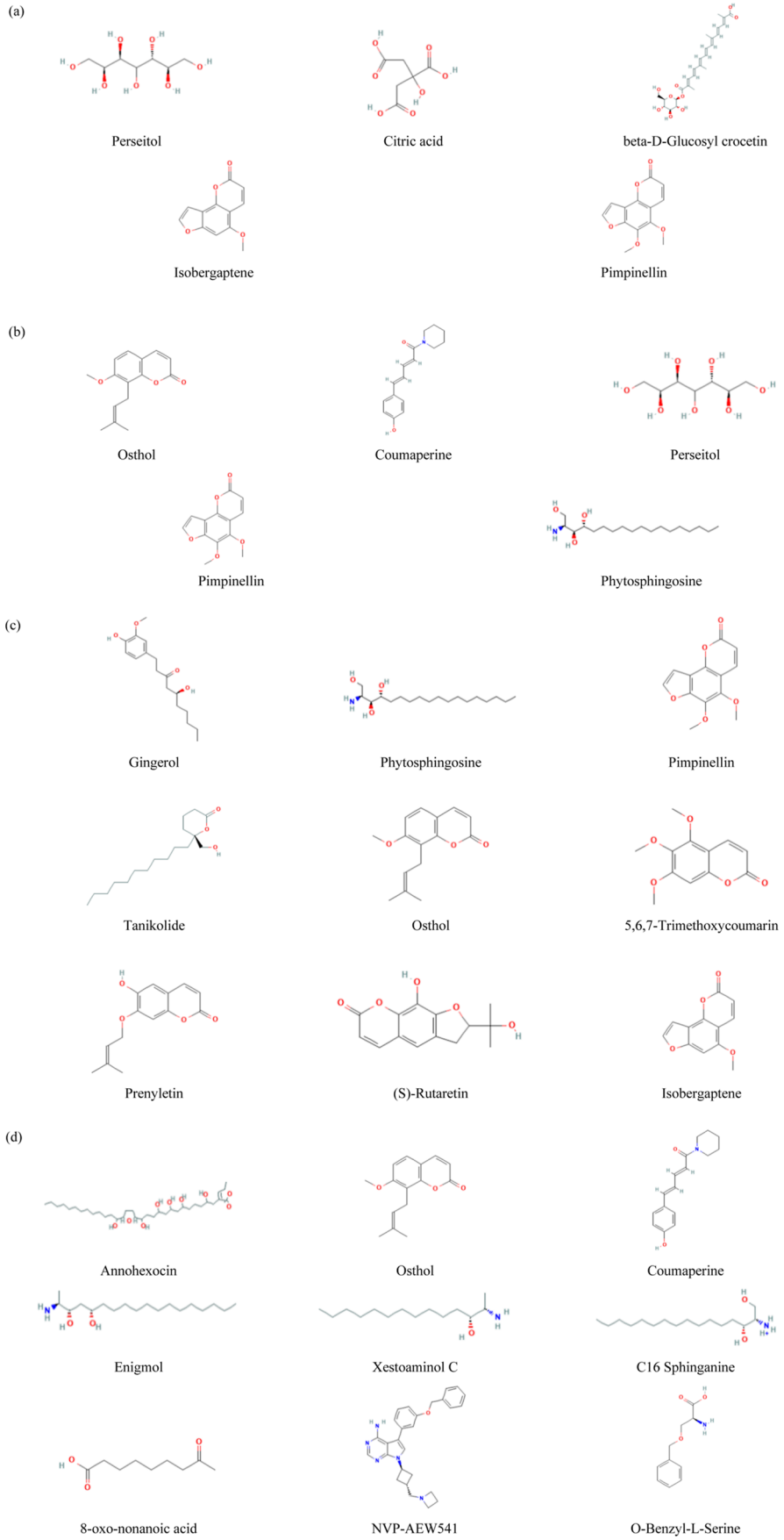

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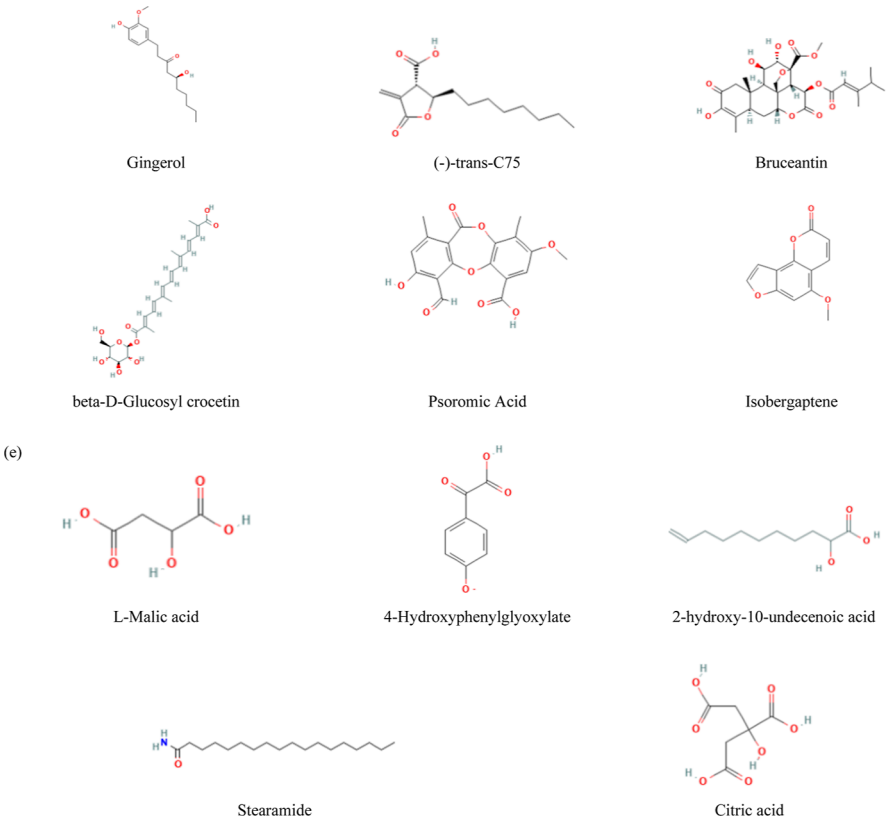

Figure 3. Chemical structure of compounds that exhibited (a) antioxidant, (b) anti-inflammatory, (c) antimicrobial, and (d) anticancer properties and (e) cosmetic preparation compounds found in TKG.

Phytosphingosine has anti-inflammatory and antimicrobial effects used for treating acne vulgaris.[45] Osthol possesses anti-inflammatory, anticancer, antihypertensive, antiarrhythmic, antiosteoporosis, and antifungal properties.[60] Coumaperine possesses anti-inflammatory and anticancer properties.[61]

Prenyletin is a coumarin with antifungal activity.[49] Gingerol has antitumor and antimicrobial activities.[46] (*S*)-Rutaretin is an antibacterial agent with antituberculosis activity.[62] 5,6,7-Trimethoxycoumarin is an antibacterial agent.[63] Tanikolide possesses antifungal properties.[64]

8-Oxo-nonanoic acid is an anticancer compound with neuroprotective effects (The Human Metabolome Database (HMDB)). Xestoaminol C is an antitumor compound that acts as an antiproliferative agent in human glioblastoma cell line SHG-44.[53] C16 sphinganine plays a role in cellular signals, which induce apoptosis in cancer cells. Enig mol has anticancer properties.[65] O-Benzyl-L-serine has anticancer properties that can also act as an antiasthmatic agent.[66] Annohexocin, (−)-*trans*-C75, NVP-AEW541, bruceantin, and psoromic acid possess anticancer properties.[67−71]

Stearamide is a naturally occurring surfactant that can be used in cosmetic preparations,[57] while 2-hydroxy-10-undecenoic acid can be used as a natural preservative in topical cosmetic preparations.[72] L-Malic acid can be used as an acidity adjuster in cosmetics, as a food flavoring agent, and as an energy boosting supplement (ChEBI). 4-Hydroxyphenylglyoxylate can be used as a moisturizing agent in cosmetics formulation (Pubchem).

3.2.4. Thanaka Glycerol/Water Extract. Compounds exhibited biological activities including UV protection, antioxidant, anti-inflammatory, antimicrobial, anticancer, as well as cosmetic preparation compounds were found in TKGW and interpreted with their biological functions (Figure 4).

One compound is found to have UV protective effect. D-Threonic acid is a derivative from AA degradation, with photoprotective effects that can be used in the treatment of skin disorders as well as improves the renovation and clarity of skin.[73]

Isobergapten is a furanocoumarin that possesses antioxidant,[34] antimicrobial,[35] and antitumor[36] effects. Lipoamide is an antioxidant with anti-inflammatory effects.[37] Perseitol that can also be found in avocado possesses antioxidant, antiaging, and anti-inflammatory activities and is used in the treatment and prevention of skin disorders.[58] 6-Paradol is an antioxidant and anti-inflammatory compound that leads to its anticancer properties.[74−76] Nifuradene is an anti-inflammatory drug for the eye.[54] Phytosphingosine has anti-inflammatory and antimicrobial effects used for treating acne vulgaris.[45]

S-Methylmethanesulfinothioate has antimicrobial activity.[77] Tetrahydrodipicolinate is an antibacterial agent, while vidarabine is an antiviral agent. Prenyletin is a coumarin with antifungal activity.[49] Gingerol has antitumor and antimicrobial properties.[46] Dodemorph is an antifungal agent (ChEBI). (*S*)-Rutaretin is an antibacterial agent with antituberculosis activity.[62] 8-Oxo-nonanoic acid is an anticancer compound with neuroprotective effects (HMDB). Moprolol is an anticancer and antiasthmatic agent (HMDB). Xestoaminol C is an antitumor compound that acts as an antiproliferative agent in human glioblastoma cell line SHG-44.[53] C16 sphinganine plays a role in cellular signals, which induce apoptosis in cancer cells.[51]

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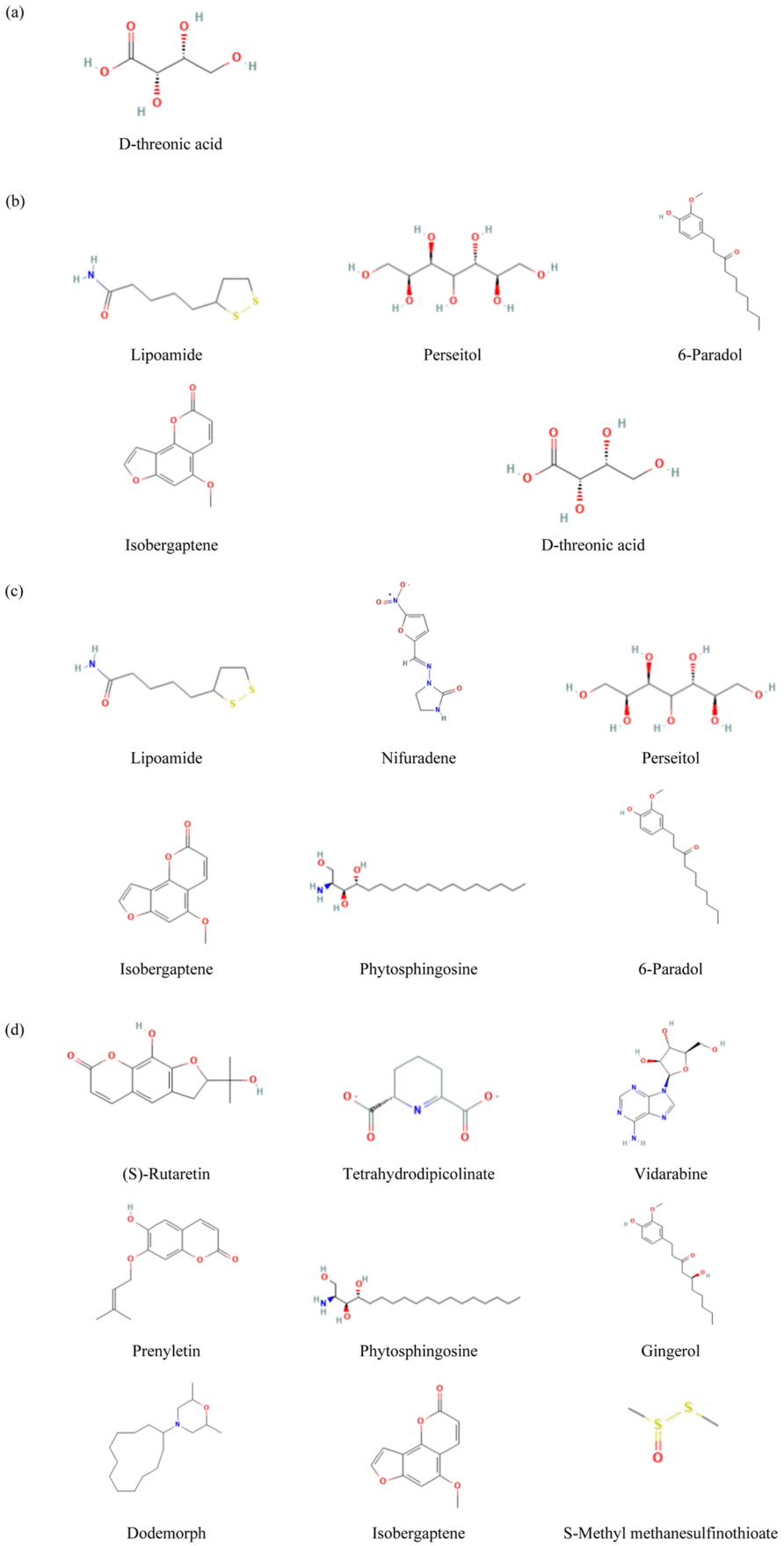

Figure 4. continued

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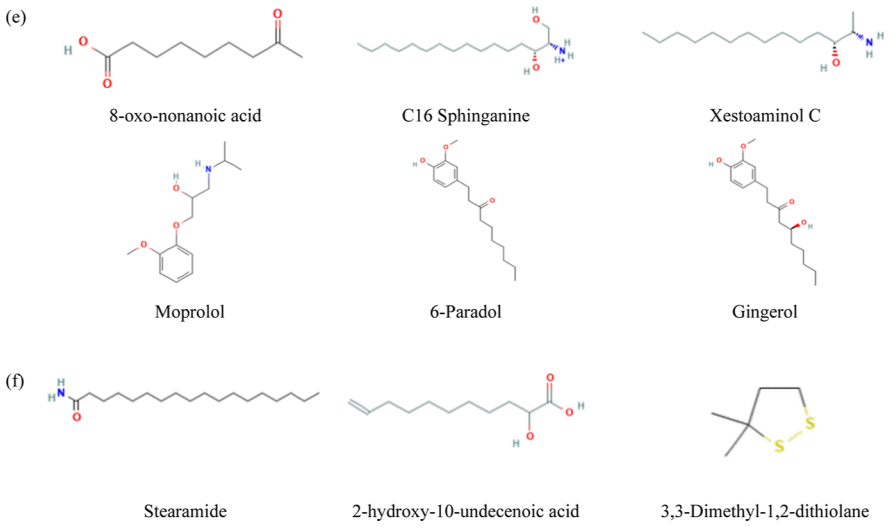

Figure 4. Chemical structure of compounds that exhibited (a) UV protection, (b) antioxidant, (c) anti-inflammatory, (d) antimicrobial, and (e) anticancer properties and (f) cosmetic preparation compounds found in TKGW.

Stearamide is a naturally occurring surfactant that can be used in cosmetic preparations,[57] while 2-hydroxy-10-undecenoic acid can be used as a natural preservative in topical cosmetic preparations.[72] 3,3-Dimethyl-1,2-dithiolane is a topical gelling agent (HMDB).

3.2.5. Thanaka Glycerol/Ethanol Extract. Compounds exhibited biological activities including UV protection, antioxidant, antimicrobial, and anticancer, as well as cosmetic preparation compounds were found in TKGE and interpreted with their biological functions (Figure 5).

Undecylprodigiosin is a compound possessing UV protective properties, along with antibacterial and antioxidative effects.[78] 2,3′,4,6-Tetrahydroxybenzophenone and loroxanthin ester/loroxanthin dodecenoate are compounds with significant antioxidant activity.[79,80]

Myriocin is an antibiotic (ChEBI). Prenyletin is a coumarin class compound with antifungal activity.[49] Sphagnum acid is a compound with potent antibacterial activity.[81] SQ 26180 is an antibiotic that is weakly active against Gram-positive and Gram-negative bacteria.[82] Farnesyl thiosalicylic acid is a compound possessing anticancer properties.[83] 4,4′-Thiobis-2-butanone is a flavoring agent (Pubchem).

3.3. Total Phenolic and Flavonoid Contents of Thanaka Extracts. The phytochemical content of the Thanaka extracts is shown in Table 2. The TPC and TFC of the water extract were the lowest with 0.1150 ± 0.0044 mg GAE g^{-1} and 0.0918 ± 0.0243 mg QE g^{-1}, respectively. The glycerol/ethanol extract had the highest amount of TPC among the five extracts (0.1778 ± 0.0229 mg (GAE g^{-1}), while the glycerol extract was the highest in TFC amount (0.1185 ± 0.0229 mg QE g^{-1}).

The TPC of the Thanaka extracts ranked from highest to lowest was glycerol/ethanol extract (TKGE) > ethanol extract (TKE) (0.1320 ± 0.0060 mg of GAE g^{-1}) > glycerol/water extract (TKGW) (0.1298 ± 0.0017 mg of GAE g^{-1}) > glycerol extract (TKG) (0.1245 ± 0.0014 mg of GAE g^{-1}) > water extract (TKW). On the other hand, the TFC of the Thanaka extracts potentially ranked from highest to lowest was TKG > TKE (0.0971 ± 0.0241 mg QE g^{-1}) > TKGE (0.0968 ± 0.0180 mg QE g^{-1}) > TKGW (0.0902 ± 0.0140 mg QE g^{-1}) > TKW.

Out of the three base solvents (ethanol, water, and glycerol), ethanol extract had the highest TPC amount extracted since ethanol is known to be good in polyphenol extractions. Surprisingly, glycerol comes in second, which indicates that glycerol has the ability to extract phenolic compounds better than water but slightly weaker than ethanol. Water is the weakest in the phenolic content extraction among the three solvents. However, glycerol/water extract had a higher TPC amount than both glycerol-only and water-only extracts, which was expected since in previous studies, the TPC results of glycerol-based natural product extracts, and especially glycerol/water extracts, are generally better than ethanol, water, and methanol extracts,[23,84,85] while the glycerol/ethanol extract exhibits TPC that is gradually higher than not only ethanol and glycerol but also showed the highest TPC results among the five Thanaka extracts. However, there are very few studies that can give rise to why glycerol/ethanol solvent has better ability in phenolic compound extraction. One theory may be that since phenolic compounds are sparingly water soluble, therefore ethanol with a lower polarity than water favors the solubilization of phenolics,[86] while the miscibility of glycerol that is able to solubilize in water and slightly soluble in diethyl ether makes it able to replace solvents within the polarity index range of 10−3, including ethanol in the list,[10] which contrastingly makes glycerol also favorable for the solubilization of phenolics. Therefore, the mixture of glycerol and ethanol enhances the solubilization of phenolic compounds in Thanaka. In the study by Kaplan et al. (2020), they incorporated glycerol into a solvent mixture of ethanol/water (50:50, v/v) for the extraction of *Origanum onites* L. and found that there was significant increase in the TPC and antioxidant activity compared to the ethanol/water-only solvent system.[87]

However, in the TFC results, glycerol showed a better capability in flavonoid extraction than ethanol. There are no reports in extraction of flavonoids using only glycerol as the solvent but can be explained by the LCMS analysis where TKG possesses the highest number of compounds compared to the other extracts (Table 1). Notwithstanding, there are studies on the extraction of flavonoids using glycerol-based solvents, stating that the glycerol-based solvent system is an efficient flavonoid extractant. Makris (2016) reported that the solvent system that consists of 90% (w/v) aqueous glycerol is

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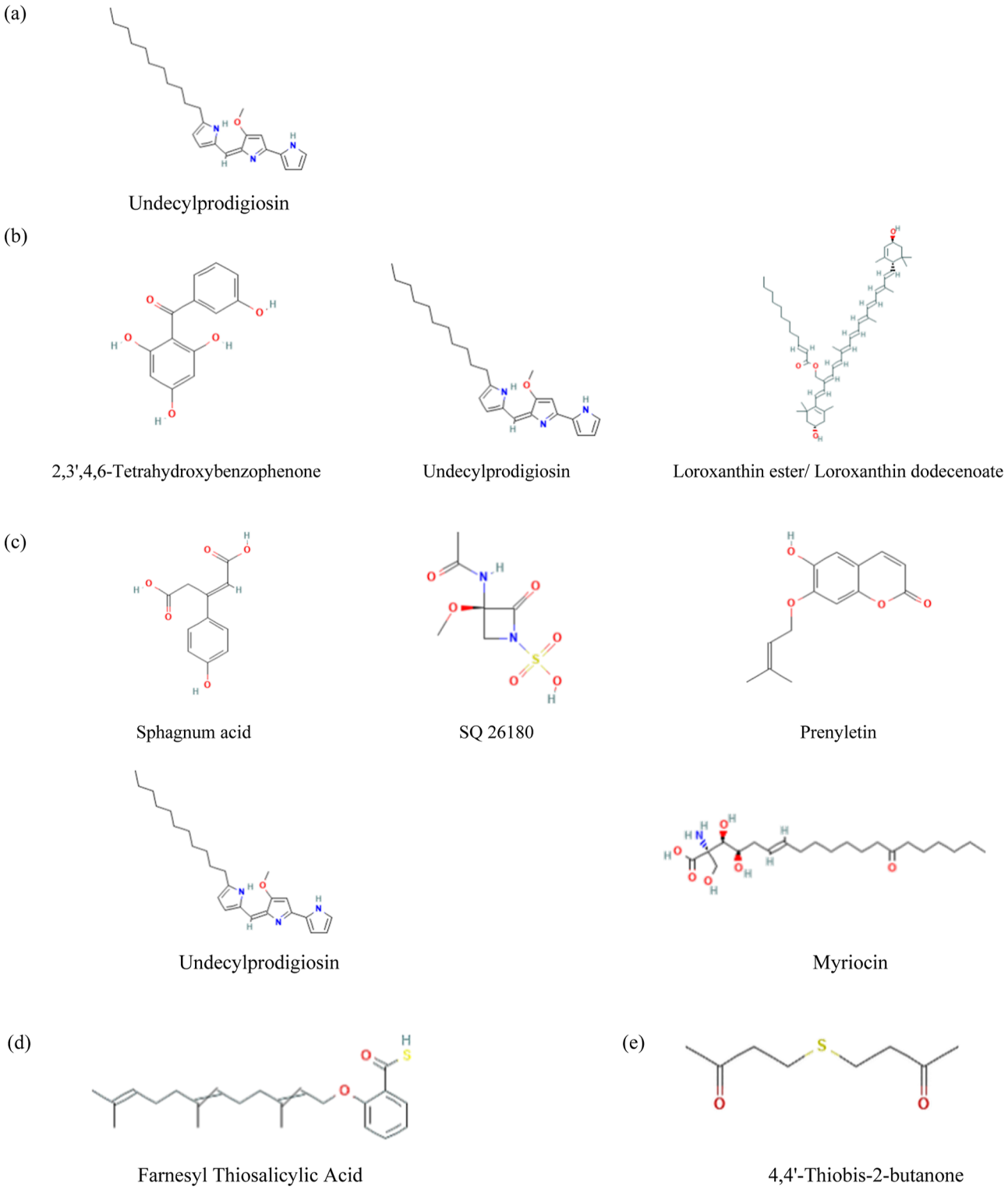

Figure 5. Chemical structure of compounds that exhibited (a) UV protection, (b) antioxidant, (c) antimicrobial, and (d) anticancer properties and (e) cosmetic preparation compounds found in TKGE.

efficient in extraction of flavonoids with the assistance of ultrasonication.[88] Philippi et al. (2016) reported that in consideration of the yield of total flavonoids, water/glycerol extract solvent is more effective than water/ethanol solvent.[23] Lakka et al. (2019) stated that the yield in total flavonoids of glycerol/alanine (5:1) was not as good as 60% ethanol and 60% methanol but had a higher yield in total flavanols than 60% methanol while still not as efficient as 60% ethanol.[85] Moreover, in previous studies of natural product extraction using glycerol mixtures, the TPC was generally higher than ethanol and methanol extracts, while the TFC of glycerol-based extracts was lower compared to ethanol and methanol extracts. The polarity of solvent extraction may influence the extraction of bioactive compounds.[89] In this case, the use of

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Table 2. Phytochemical Contents of the Thanaka Extracts (0.01 g mL^{-1})[a]

extract	TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})
TKE	0.1320 ± 0.0060[a,b]	0.0971 ± 0.0241
TKW	0.1151 ± 0.0044[a,b]	0.0918 ± 0.0243
TKG	0.1245 ± 0.0014[a]	0.1185 ± 0.0229
TKGW	0.1298 ± 0.0017[a,b]	0.0902 ± 0.0140
TKGE	0.1778 ± 0.0229[a]	0.0968 ± 0.0180

[a]Data presented as mean ± standard deviation of three independent experiments performed in triplicate. Different superficial lowercase letters ([a,b]) report a significant difference between extracts at $p < 0.05$.

cosolvents, such as, glycerol/water and glycerol/ethanol, respectively, has a wider range of polarity index due to the number of carbon atoms and hydrogen atoms as well as hydroxyl groups present in the cosolvents, which may extract more bioactive compounds with different polarity as shown in the LCMS profiles of TKGW and TKGE as compared to the single solvent extracts TKE and TKW.

There are reports showing the better efficacy of glycerol-based solvents in the extraction of phenolic compounds and flavonoids from plant extracts than ethanol and water to provide evidence to the TPC and TFC results of Thanaka extracts. However, it is also considerable to look at the LCMS profiling of phenolic compounds in Thanaka extracts since it was mentioned before that the F−C reagent may be interfered by AAs and reducing sugars.

The phenolic compounds of all the five Thanaka extracts in their respective LCMS profiles were identified by the METLIN database (Table S6). From the table, it can be seen that TKG possesses the highest number of phenolic compounds of 17, followed by TKE of 14 compounds, TKGW of 10 compounds, then TKGE with 9 compounds, and last TKW with 7 phenolic compounds. As mentioned earlier, phenolic compounds are sparingly soluble in water; hence, the number of phenolics identified in TKW are less than the other four Thanaka extracts. The TPC test results of TKW are also the least among the list with only 0.1151 mg of GAE g^{-1} phenolic content supporting this statement. Interestingly, TKG with TPC of only slightly higher than TKW possessed the greatest number of phenolic compounds identified in its LCMS profile, while TKGE resulted in the highest TPC when it only showed to possess only nine identified phenolic compounds in its LCMS profile. The same situation is also found in the TFC results of Thanaka extracts where the results do not match with the number of identified flavonoid compounds in the LCMS profiles of Thanaka extracts. Reason to this result could be that in the list of unidentified compounds of Thanaka extracts (Appendices F−J), there might be phenolic and flavonoid compounds that are not available in the METLIN database. The concentration of the identified phenolic compounds in the Thanaka extracts may also affect the results; however, the concentration was not quantified in this study. In future studies, multiple databases can be used to further identify the list of unknown compounds (Appendix F−J) or apply the use of a LCMS/MS to analyze the Thanaka extracts for the unknown compounds. Application of high-performance liquid chromatography is also suggested to quantify the concentration of the phenolics in each Thanaka extracts.

3.4. Antioxidant Activity of Thanaka Extracts. TKE possesses the highest number of identified antioxidant compounds, while TKGE possesses the least number of identified antioxidant compounds with only two compounds. TKG and TKGW possess the same number of antioxidant compounds, while isobergapten and perseitol are common antioxidant compounds between the two. TKW possessed the second least number of antioxidants among the five Thanaka extracts (Table S7).

AA was used as the positive control in comparison to the antioxidant activities of the Thanaka extracts. The TKW extract possesses a higher antioxidant activity in DPPH assay with the lowest EC_{50} at 9.7091 ± 0.3559 mg mL^{-1}, while the TKGE extract possesses the highest antioxidant activity in both ABTS and FRAP assays, with EC_{50} at 2.9841 ± 0.0833 and 6266.4561 ± 73.5386 mg mL^{-1}, respectively. The TKG extract showed the lowest antioxidant activity in DPPH and FRAP assay, with EC_{50} at 21.1527 ± 0.5808 and 11220.38 ± 178.3241 mg mL^{-1}, respectively. The glycerol extract also had the second lowest antioxidant activity in the ABTS assay, with EC_{50} at 8.4233 ± 0.2885 mg mL^{-1} (Table 3).

3.4.1. DPPH Assay. The extract with the highest DPPH scavenging activity is TKW with an EC_{50} value of 9.7097 ± 0.3559 mg mL^{-1}, followed by TKGE with an EC_{50} value of 11.8125 ± 0.0906 mg mL^{-1}, TKE with an EC_{50} value of 12.1778 ± 0.9502 mg mL^{-1}, TKGW with an EC_{50} value of 19.1951 ± 5.0764 mg mL^{-1}, and last TKG with an EC_{50} value of 22.3839 ± 2.1718 mg mL^{-1} (Table 3).

It can be seen from the results that they do not match the results of TPC and TFC presented earlier. Generally, flavonoids and phenolics contribute to antioxidant activities due to the presence of OH groups directly linking the carbon atoms of benzene ring in their chemical structures;[90−92] hence, it was assumed that the TKGE with the highest TPC would also have the highest DPPH scavenging activity. However, from the statistic of DPPH scavenging activity, TKW was shown to have the highest DPPH scavenging activity against the other Thanaka extracts. This may be due to the mechanism

Table 3. Antioxidant Activities of Different Solvent Extracts of Thanaka (0.01 g mL^{-1})[a]

	EC_{50} (mg mL^{-1})		
extract	DPPH	ABTS	FRAP
TKE	12.1778 ± 0.9502[a]	4.8503 ± 0.0624[a]	10547.6033 ± 127.7157[a]
TKW	9.7097 ± 0.3559[a]	8.9226 ± 0.1900[a]	9492.6777 ± 458.3212[a]
TKG	22.3839 ± 2.1718[a]	8.4233 ± 0.3533[a,b]	11539.19 ± 566.4008[a,b]
TKGW	19.1951 ± 5.0764	7.6753 ± 0.2324[a]	8920.2553 ± 271.0481[a]
TKGE	11.8125 ± 0.0906[a]	2.9841 ± 0.1020[a,b]	6266.4560 ± 90.0658[a,b]
AA	0.0141 ± 0.00015[a]	0.0686 ± 0.0016[a]	0.1146 ± 0.0010[a]

[a]Data are presented as mean ± standard deviation of three independent experiments performed in triplicate. Different lowercase letters ([a,b]) report significant differences between extracts at $p < 0.05$.

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of DPPH scavenging assay which is based on the theory that hydrogen donors are assumed to be antioxidants. The hydrogen donors such as citric acid and malic acid that may affect the scavenging activity of DPPH radicals are present in TKW. The extract solvent water itself is also a hydrogen donor. Nonetheless, L-ascorbic acid that is used as the positive control in this assay was also found in the LCMS profile of TKW. Therefore, the DPPH scavenging activity of TKW is higher than those of the other Thanaka extracts. The pale yellowish color of all Thanaka extracts may also affect the absorbance reading; however, apart from TKW, the DPPH scavenging activity results of the other four Thanaka extracts matched with the results of their TPC.

3.4.2. ABTS Assay. The extract with the highest ABTS scavenging activity is TKGE with an EC_{50} value of 2.9841 ± 0.1020 mg mL^{-1}, followed by TKE with an EC_{50} value of 4.8503 ± 0.0624 mg mL^{-1}, TKGW with an EC_{50} value of 7.6753 ± 0.2324 mg mL^{-1}, TKG with an EC_{50} value of 8.4233 ± 0.3533 mg mL^{-1}, and last TKW with an EC_{50} value of 8.9226 ± 0.1900 mg mL^{-1} (Table 3).

The results of ABTS scavenging activity of Thanaka extracts are consistent with the results of their TPC, with TKGE possessing the highest ABTS scavenging activity, while TKW possessing the lowest ABTS scavenging activity. ABTS scavenging activity assay has been applied in investigations of antioxidant activities of natural products. However, the ABTS assay is not commonly used to access the antioxidant activity to determine the polyphenol content of glycerol-based extracts in some published works. It is worth noting that the ABTS assay is capable of determining the presence of phenolics in natural products as well as their antioxidant activity while not being influenced by colors in the extracts of similar wavelength (λ). These factors could be the reason that the results of ABTS scavenging activity of Thanaka extracts match with their TPC results, which gives support to the validity of the results of both ABTS assay and TPC assay. From both results of TPC and ABTS, glycerol/water solvent-extracted TKGW possesses a higher antioxidant activity and phenolic content than the water-extracted TKW, and glycerol-extracted TKG, which supported the theory that glycerol with a lower dielectric constant of 44.38 is able to lower the polarity of water to enable the solubilization of more polyphenols.[13] However, another interesting find is that glycerol and ethanol of lower polarity when mixed are able to produce a solvent that is more favorable to the solubilization of polyphenols, as supported by the results where TKGE possesses the highest ABTS scavenging activity as well as the highest amount of TPC.

3.4.3. FRAP Assay. The FRAP assay is different from both DPPH and ABTS assays, and also a colorimetric assay but does not use free radicals; instead, it uses a redox-active compound, ferric iron (Fe^{3+}), that can be reduced to ferrous iron (Fe^{2+}) under reaction conditions, hence the change in absorbance that is directly related to the total reducing power of the electron-donating antioxidants present in the reaction mixture.[93,94] However, the reaction is nonspecific, and any compound that meets the suitable redox potential can drive the Fe^{3+} reduction.[95] Moreover, the test is time-consuming, as the result is expected to arbitrarily depend on the reaction time as different antioxidants take different amounts of time to reduce Fe^{3+}. For example, the reaction of AA with FRAP is instantaneous, while uric acid (UA) takes about 2 min. Therefore, after 4 min, both AA and UA will have the same FRAP power.[95]

TKGE possesses the highest FRAP activity with an EC_{50} value of 6266.4560 ± 90.0658 mg mL^{-1}, followed by TKGW with an EC_{50} value of 8920.2553 ± 271.0481 mg mL^{-1}, TKW with an EC_{50} value of 9492.6777 ± 458.3212 mg mL^{-1}, TKE with an EC_{50} value of 10547.6033 ± 127.7157 mg mL^{-1}, and last TKG with an EC_{50} value of 11539.19 ± 566.4008 mg mL^{-1} (Table 3).

The results of FRAP activity of Thanaka extracts do not match with any of the other assays or phytochemical content results. The FRAP assay does not use the principle of scavenging a specified free radical; instead, it is based on the redox reaction between the antioxidants and the ferric ion (Fe^{3+}); therefore, it is acceptable that the result trend of FRAP assay does not match with the TPC results. In this case, TKGW was shown to have higher FRAP activity than TKE, where in TPC, DPPH and ABTS assay results show that TKE has better results than TKGE. As mentioned earlier, any compounds that meet the redox potential are able to drive the reduction of Fe^{3+}; hence, there is a different trend in FRAP assay compared to the DPPH and ABTS assay, where the result trends do have some matchings with the TPC results, also in the case of TKW, which possesses a higher FRAP activity than the other Thanaka extracts. This can be due to the fact that the FRAP assay was first developed for the quantitation of AA in the serum or plasma;[96,97] therefore, TKW with L-ascorbic acid found in its LCMS profile presented a higher FRAP activity, which is similar in the case of DPPH scavenging activity result. One concern is that the EC_{50} values of the Thanaka extract FRAP activity are relatively high. The reason may be that the concentration of 0.01 g mL^{-1} is too low for the FRAP assay and that the antioxidant within the Thanaka extracts needed a longer time consumption to complete the reduction of Fe^{3+} ions. Nevertheless, the trend still suggests that the glycerol-based Thanaka extracts of TKGW and TKGE have the better FRAP activity than TKE and TKW.

4. CONCLUSIONS

The LCMS-based untargeted analysis showed that the bioactive compounds of the Thanaka bark powder with different biological activities were annotated. For example, compounds responsible for UV protection, including D-threonic acid and undecylprodigiosin, were found in TKGW and TKGE, respectively. Compounds responsible for anti-pigmentation including lonchocarpic acid and *N*-palmitoyl proline were found in TKE. Other compounds with biological properties such as antioxidant, anti-inflammatory, antimicrobial, and anticancer were also found in all the five Thanaka extracts (Figures 1−5). Isobergapten was the common antioxidant compound between TKW, TKG, and TKGW. With the UV protection, antipigmentation, antioxidant, anti-inflammatory, and antimicrobial compounds found in Thanaka extracts, it may evidentially prove to why Thanaka bark was used as the traditional skin conditioner among the Burmese for sun protection and acne prevention.

Thanaka bark powder has high potential as a natural antioxidant due to various antioxidant compounds found in the LCMS profiles. The antioxidants found in Thanaka extracts include isobergapten, theobromine, perseitol, citric acid, L-ascorbic acid, foeniculoside VIII, hexadecanedioic acid, beta-D-glycosyl crocetin, 6-paradol, D-threonic acid, *N*-palmitoyl proline, 2,3′,4,6-tetrahydroxybenzophenone, loroxanthin ester/loroxanthin dodecenoate, and undecylprodigiosin.

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Among the five Thanaka extracts, TKGW possessed the highest number of antioxidants identified in its LCMS profile, while TKGE appeared to be the least in the number of antioxidants identified in the LCMS profile. TKE and TKG possessed the same number of identified antioxidants. TKW was only one less antioxidant than TKE and TKG.

Despite having the least number of antioxidants, TKGE exhibited a higher TPC, ABTS scavenging activity, and higher ferric-reducing power, along with a moderate amount of TFC and second in DPPH scavenging activity. TKG showed the highest TFC and can be proved with it possessing the greatest number of flavonoids in its LCMS profile. TKW exhibited the highest DPPH scavenging activity due to its higher ability as a hydrogen donor and possessed L-ascorbic acid and citric acid in its LCMS profile. The result trends of TPC, ABTS matched with each another, with TKGE > TKE > TKGW > TKG > TKW. The result of DPPH assay was slightly different with TKW > TKGE > TKE > TKGW > TKG. Nonetheless, the trend of DPPH assay after TKW still matched with TPC and ABTS assay. The FRAP assay presented a different trend with the other assays with TKGE > TKGW > TKW > TKE > TKG. Overall, it can be said that TKGE performed the best antioxidant activity results among the five Thanaka extracts. One thing to take note is that there might be unidentified compounds in TKGE exhibiting antioxidant activity that contributed to its performance in the assays. Since this study only uses METLIN database in the identification of compounds in Thanaka extracts, it is suggested that future studies employ more database in the analysis and may also perform other mass spectrometry methods in identifying the unidentified compounds.

In conclusion, glycerol-based solvents are able to extract a large number of similar compounds and even extract more compounds than ethanol and water solvents from Thanaka bark powder. Moreover, the TKGE outperformed the other four Thanaka extracts in TPC, ABTS, and FRAP assay, and in particular, TKGE exhibited a better result than TKE where ethanol was commonly used for natural product extractions, while previous studies in Thanaka also mostly used ethanol as the solvent. Thus, glycerol-based solvents can be a good green solvent alternative to replace organic solvents in Thanaka extractions. For future studies, it is suggested that glycerol-based extracts of Thanaka can be used in the formulation of skincare products, as glycerol is commonly used as a moisturizing agent in skincare. It is also suggested that since Thanaka is commonly used as natural sunscreens by the Burmese, and there are UV protection compounds found in the LCMS profiles of the Thanaka extracts, future projects may investigate the antimelanogenesis activity of Thanaka extracts.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c00041.

Compounds identified in TKE and their reported activities; compounds identified in TKW and their reported activities; compounds identified in TKG and their reported activities; compounds identified in TKGW and their reported activities; compounds identified in TKGE and their reported activities; polyphenol compounds in Thanaka extracts; antioxidant compounds in Thanaka extracts; list of unidentified compounds found in TKE; list of unidentified compounds found in TKW; list of unidentified compounds found in TKG; list of unidentified compounds found in TKGW; list of unidentified compounds found in TKGE; comparison of identified compounds between TKE, TKW, and TKG; TKW, TKG, and TKGW; TKE, TKG, and TKGE; and TKE, TKGW, and TKGE; LCMS chromatograms [intensity (counts $\times 10^5$) versus retention time (in min)] of Thanaka ethanol (TKE) extract; chromatograms were produced using an Agilent 1290 Infinity LC system coupled with an Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with a dual-ESI source involved positive and negative modes (PDF)

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Notes

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