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Research Article

Phytochemicals, Antioxidants and Anti-tyrosinase Analyses of Selected Ginger Plants

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ABSTRACT

Background: Some of Zingeberaceae are not widely used for medicine of food, although in Thailand have been used them for many reasons about health or the diet. This study evalued the phytochemicals and anti-tyrosinase activities of 16 plant species of *Alpinia*, *Amomum*, *Curcuma*, *Etlingera* and *Kaemferia* (Zingiberaceae). **Methods:** The extractions of dried powdered rhizomes were performed using n-hexane, ethylacetate and ethanol. Percentage extract yield of the samples varied among species and solvent extracts. Chemical groups (alkaloids, flavonoids, tannins, polyphenols, steroids and terpenoids) were identified using phytochemical screening. The total phenolic contents (TPC) were analyzed using the Folin-Ciocalteu's reagent, while antioxidant activities were detected using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the 2,2'-azino-bis (3-ethylbenzothizoline-6-sulphonic acid) ($ABTS^{\cdot+}$). The anti-tyrosinase was expressed to the half maximal inhibitory concentration (IC_{50}) value (mg/mL). **Results:** The ethyl acetate extract of *Amomum* showed the highest value of TPC. The strongest antioxidant activity were found in *Amomum* and *Kaemferia* extracts, while ethyl acetate and ethanol extracts of all samples have a better antioxidant properties than the *n*-hexane extracts. On the other hand, the *n*-hexane extracts have the highest anti-tyrosinase potential in all samples and of these, *Curcuma* extracts were the best group. **Conclusion:** Our research indicated that plants of the Zingiberaceae would be new sources of antioxidants and anti-tyrosinase for further natural product developments in cosmetics, food or nutraceuticals.
Key words: ABTS assay, Anti-tyrosinase, DPPH assay, Phenolic content, Phytochemicals, Zingiberaceae.

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INTRODUCTION

The phytochemicals are biological compounds that produced by plants throughout primary and secondary metabolisms. Many phytochemicals are widely known for nutritional, biological and pharmacological for health benefits.[1,2] Phenolic compounds are strong antioxidants and important parts in the human diet. The antioxidant property of phenolic compounds depends on the structure, the positions and number of the OH groups, in particular the aromatic substitutions.[3] Moreover, phenolics possess anti-inflammatory activities and potentially reduce the risks of cardiovascular diseases.[4-7] Other phytochemicals, such as flavonoids, tannins and terpenoids are also strong antioxidants that are known to reduce the risk of cardiovascular diseases, brain dysfunction neurodegenerative disorders and rheumatism.[8] Phenolic compounds could againt melanin synthesis and tyrosinase is the key enzyme, which present in plant, animal and human tissues that catalyses the production of melanin from tyrosinase by oxidation process as brown pigments on human skin, vegetables and fruits. The accumulation of melanin formation causes freckles, melisma, age spots, post-inflammatory hyperpigmentation and skin cancer.[9,10]

Zingiberaceae family is widely distributed in the tropical and subtropical regions of the world.[11,12] In Asia, Zingiberaceae can be found in South and South East Asia. Twenty six genera and three hundred species of ginger plants are found in Thailand. Rhizome parts of Zingiberaceae are used by human for medicinal purposes such as the rhizomes of *Curcuma longa*, *Boesenbergia rotunda*, *Alpinia galanga* and *Zingiber officinale* which are typically used to treat diarrhea, stomachache and flatulence that involved in their phytochemicals.[13-16] Zingiberaceae species have been previously reported about phytochemicals of several parts, especially in rhizome. The rhizome of *Alpinia nigra* contained total flavonoids (54.14 mg), total phenols (120.7 mg) and high level of total alkaloids (215.0 mg).[17] The aqueous and methanolic rhizome extracts of *Curcuma aromatica* and *C. xanthorrhiza* presented flavonoids, tannin, saponin, sterols, terpenoids. *Curcuma* extracts contained amount of Curcumin at 1.0863 g/100g for *C. xanthorrhiza* and 0.0175 g/100g for *C. longa*, moreover *C. xanthorrhiza* and *C. longa* extracts were demonstrated to inhibit the negative grams pathogenic bacteria, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, and *Salmonella typhi*, which are in nature.[18] While, ethanolic rhizome extract of turmeric (*C. longa*) had presented reducing sugars, alkaloids, coumarins, flavonoids, phenol, steroids and terpenoids at high concentration and the turmeric extract was found to affect against the bacteria that cause of diarrhoea (*Shigella flexnerri*, *Staphylococcus aureus*, *S. epidermidis*, *Klebsiella pneumoniae*, *E. coli*, *P. aeruginosa*, *Lactobacillus*, *Vibrio cholerae*, and *S.*

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typhi.[19] From previous research were suggested that turmeric have anti-inflammatory, anti-fungal, anti-tumor, against arthritis, anti-wrinkles and antityriosinase.[20-22] Phytochemicals of methanolic leaves and stems extracts of *Etlingera coccinea* presented cardiac glycosides, steroids, saponins, anthraquinones, while rhizome of *E. coccinea* was found cardiac glycosides and steroids, leaves of *E. coccinea* had total antioxidant capacity, antibacterial, and antifungal higher than stem and rhizome parts.[23] Another, stigmast-4-en-3-one and stigmast-4-en-6b-ol-3-one were isolated from the rhizome extracts of *E. elatior*, which were displayed high anti-tumour activity.[24] Methanolic extract of leaves of *E. elatior*, *E. flugens*, and *E. maingayi* displayed the tyrosinase inhibition activity at 55.2%, 49.3%, and 42.6% respectively.[25] Finally, the chloroform rhizome extract of *Kaempferia rotunda* showed inhibition against DPPH radical scavenging.[26] Ethyl acetate rhizome extract of *K. rotunda* showed antibacterial activity and contained alkaloids, steroids, terpenoids, flavonoids and saponins.[27] The extract of *K. rotunda* have tyrosinase inhibition activity that safe and effective ingredient, which use in skin lightenning cosmetics.[28] Rhizome of *K. rotunda* are used for traditionally medicine as diarrhea, cold, cancer diseases, skin diseases and abdominal pain that used in food flavoring and cosmetics.[29] However, although some species of the Zingiberaece are commonly used as medicinal or food ingredients. In contrast, many species of ginger plants are not widely used, while the local people in Thailand have been used them for many reasons such as anthelmintic, antifungal, antibacteria, anti-inflammatory, anti-diabets, anti-cancer or the diet.[17,33]

Therefore, the purpose of this research was to study phytochemicals, antioxidants and anti-tyrosinase activities of some selected ginger plants collected in Thailand. These findings would be considered as new knowledge about natural ingredients for future pharmaceutical, food or cosmetic products.

MATERIALS AND METHODS

Plant materials and sample preparation

The rhizomes of 16 species of Zingiberaceae, *Alpinia* sp., 4 *Amomum* spp., 7 *Curcuma* spp., 3 *Etlingera* spp. and *Kaempferia* sp. were used for our experiments. The samples were collected from different locations in Thailand and voucher specimens were taxonimically identified according to Zingiberaceae experts' comments and literatures from the Queen Sirikit Botanic Garden (QSBG).[13] The plant specimens were then deposited at the Queen Sirikit Botanic Garden herbarium (QBG). The rhizomes were weighted and thoroughly washed, after that rhizomes were sliced into small pieces by using a slicer. The sliced rhizomes were dried by hot air oven (Memmert UF750, Germany) at 45°C for 48 h or moisture content in samples were reduced below 5% for sample preservation of microbial while storage and moisture or microbial in sample may cause a certain amount of error in the reserch and then grounded to a fine powder by using an electrical grinder (DMF – 6A, Japan) at high speed.[34-35]

Sample extraction

A dried powder was macerated with solvent (powder:solvent, 1:5 w/v). First the sample was extracted with *n*-hexane. The mixture was shaken for 24 hours at ambient temperature, and then the mixture was filtered by using vacuum filtration with filter paper (Whatman, No.1). The supernatant was collected and evaporated with rotary evaporator (BUCHI R-3, Switzerland). The residue powder was repeated processing with ethyl acetate and 95% ethanol respectively. Crude extract from each solvent was kept at 4°C that used for antioxidant and anti-tyrosinase analyses. The extraction of each solvent was performed in tripicate replication.[36-37]

Phytochemical screening of the extracts

Qualitative phytochemical screening of the extracts were evaluted phytochemical constituents including alkaloids, flavonoids, anthocyanins, tannin and polyphenol, saponin, steriods and terpenoids that follwing the standard method.

Alkaloids

0.025 g of crude extracts were dissolved in 20 mL of 5% hydrochloric acid (HCl) and sonicated to improve solubitity. The mixture was used to determine with the following test;[38]

Dragendorff's test

A drop of extract was plated on microscope slide and added a drop of Dragendorff's reagent. The presence of reddish-brown precipitates indicated the presence of alkaloids.

Wagner's test

A drop of extract was plated on microscope slide, a drop of Wagner's reagent was added. The formation of reddish-brown precipitates indicated the presence of alkaloids in the extract.

5% Tannic acid test

A drop of extract was plated on microscope slide, and then a drop of 5% tannic acid was added. The formation of white precipitates indicated the presence of alkaloids in the extract.

Flavonoids test

0.5 g of 0.01 g/mL crude extracts in reverse osmosis-deionization (RO-DI) water was transfered into test tube and a few drops of ammonia T.S. reagent were added. The solution which show yellow, orange red, orangish-brown or immediately redish-purple coloranation indicated the presence of flavonoids.[39]

Anthocyanins test

0.5 g of 0.01 g/mL crude extract in RO-DI water was transfered into test tube. One drop of 2N HCl and ammonia T.S. reagent were added. The appearance of red color after dropped 2N HCl and turned to blue color when dropped ammonia T.S. reagent indicated presence of anthocyanins.[39]

Tannin and polyphenol

0.1 g of crude extracts were dissolved with 10 mL of RO-DI water. The mixture was used to perform the following test;[39]

1% gelatin solution test

0.5 mL of extracts were pipetted into test tube and a few drops of 1% gelatin solution were added. The formation of white precipitates indicated the presence of tannin and polyphenol.

Gelatin salt solution test

0.5 mL of extracts were pipetted into test tube and a few drops of gelatin salt solution were added. A white precipitates indicated the presence of tannin and polyphenol.

1% Ferric chloride test

0.5 mL of extracts were pipetted into test tube. a few drops of 1% Ferric chloride were added. A blue-green or black colour indicated the presence of tannin and polyphenol.

Saponins

3 mL of 0.025 g/mL extracts in RO-DI water were transfered into test tube and shaken with vortex (Fine vortex, FINEPCR) at level 5 for 1 min. The formation of stable bubble (1-10 cm) indicated the presence saponins.[39]

Terpenoids and steroids

0.5 mL of 0.002 g/mL of extracts in chloroform were pipetted into test tube. 0.5 mL of sulfuric acid) were slowly added. The formation of reddish-brown coloranation of the interface indicated the presence of terpenoids while the appearance of red coloranation in $CHCl_3$ layer and yellow fluorescent coloranation in H_2SO_4 layer indicated the presence of steroids.[40-41]

Total phenolics content

Total phenolics content of the samples was evaluted following a modified procedure.[42] 20 µL of 1 mg/mL extract in methanol were transferred into a 96-well plate and 100 µL of 10% of Folin-Ciocalteu's reagent was added. After incubation (1 min), 80 µL of 7.5% of sodium carbonate was added, and then incubated for 30 min in the dark at ambient temperature. Finally, the absorbance of the solution was measured at 765 nm by using a microplate spectrophotometer (EZ Read 2000, England). Total phenolics content was reported as mg of Gallic Acid Equivalents per g of extracted sample (mg GAE/g extract). Gallic acid was obtained from Sigma-Aldrich (Hong Kong, China).

DPPH radical scavenging activity

2,2-diphenyl-1-picrylhydrazyl or DPPH radical scavenging activity is an antioxidant assay based on electron transfer reaction. DPPH is a stable free radical that generates the deep purple colour in solution. The presence of antioxidant, the DPPH will become colourless or pale yellow when neutralised this processing can be measured spectrophotometrically. Although DPPH assay is easy and quick method, it has some limitation about the structure of DPPH. Nitrogen atom is located at the centre of structure and while this location is accessible to small or large molecules may have limited enter to the radical portion or nitrogen atom due to the steric hindrances.[43-44] DPPH assay was determined following the modified method.[45] 40 mg of crude extract was dissolved in 1 mL of methanol, then diluted the methanol extract wtih methanol (0.01 mg/mL upto 4 mg/mL). 67 µL of methonol extracts were transferred to a 96-well plate and 133 µL of methanolic DPPH solution were added. The mixtures were then incubated for 30 min in the dark and at ambient temperature. After incubation, the absorbance was measured at 517 nm with a microplate spectrophotometer (EZ Read 2000, England). Trolox, 3-tert-Butyl-4-hydroxyanisole (BHA), L-ascorbic acid, and *alpha*-Tocophenol were used as positive control. The DPPH radical scavenging activity of each sample was expressed as the IC_{50} value for DPPH (mg/mL) and the percent inhibition (%), which was calculated using the following equation: DPPH scavenging activity (%) = [1-(absorbance of sample / absorbance of control)] x 100, where the control sample was contained of 67 µL of methanol and 133 µl of methanolic DPPH solution. Trolox, BHA and L-ascorbic acid were obtained from Sigma-Aldrich (MO, USA) and *alpha*-Tocophenol was obtained from Fluka (Buchs, Switzerland)

$ABTS^{\cdot+}$ radical scavenging activity

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$) radical scavenging activity is used a coloured stable radical compound. $ABTS^{\cdot+}$ radical cation has a dark blue colour. In the presence of antioxidant, colour of $ABTS^{\cdot+}$ will charged to colourless because antioxidant can be reduce $ABTS^{\cdot+}$ into ABTS. $ABTS^{\cdot+}$ method is similar to DPPH method that based on electron transfer reaction. In contrast, the radical form of ABTS must be oxidised into the radical cation form at the beginning of method, while radical form of DPPH is already generated. The advantage of ABTS is soluble in organic and aqueous solvent that can be used to determinate antioxidant capacity of both hydrophilic and lipophilic compounds.[43-44] $ABTS^{\cdot+}$ radical scavenging activity was determined following the slightly modification procedure.[46] $ABTS^{\cdot+}$ was dissolved in RO-DI water to the concentration of 7 mM, and potassium sulfate was added up to the concentration of 2.45 mM. The mixture was incubated in the dark at room temperature for 16-18 hours before use. $ABTS^{\cdot+}$ stock solution was diluted with absolute ethanol to obtain an absorbance value of 0.70–0.90 at 734 nm with a microplate spectrophotometer (EZ Read 2000, England), this solution was the $ABTS^{\cdot+}$ working solution. 20 mg of crude extract of each sample was dissolved with 1 mL of absolute ethanol. 1.9 µL of the ethanol extract and transferred into a 96-well plate, followed by 7.5 µL of absolute ethanol and 190.6 µL of $ABTS^{\cdot+}$ working solution. The mixture was softly shaken and incubated in the dark at room temperature for 5 min. After incucation, the absorbance was measured at 734 nm with a microplate spectrophotometer (EZ Read 2000, England). The ABTS results were expressed as mg of Trolox Equivalent Antioxidant Capacity per g of extracted sample (mg TEAC/g extract). $ABTS^{\cdot+}$ radical and Trolox were obtained from Sigma-Aldrich (MO, USA)

Anti-tyrosinase activity

Tyrosinase inhibitory test of ginger plants were investigated following the modified procedure.[47-49] Crude extract of each sample was dissolved in 10% DMSO and then diluted the extract (0.39 mg/mL upto 12.5 mg/mL). 30 µL of 50 mM phosphate buffer (pH = 6.8) and 30 µL of the extract of each sample were pipetted into a 96-well plate. Then, 30 µL of 5 mM L-DOPA was added into each well. After incubation in the dark at room temperature for 10 minutes, 30 µL of 500 units/mL mushroom tyrosine (substrate) was added and incubated the plate in the dark at room temperature for 30 minutes. An absorbance was measured at 475 nm with a microplate spectrophotometer (EZ Read 2000, England). This determination, Kojic acid (1 mg/mL) was used as the positive control. A percent inhibition of anti-tyrosinase activity (% inhibition) was calculated as follows: Tyrosinase inhibition (%) = [((A-B) – (C-D)) x 100] / (A-B), where A was the absorbance of control, B was the absorbance of blank of control, while C was the absorbance of sample, and D was absorbance of blank of sample. Anti-tyrosinase activity was expressed as the half maximal inhibitory concentration (IC_{50}) value (mg/mL) Mushroom tyrosinase was obtained from Sigma-Aldrich (MO, USA), Kojic acid was obtanied from Sigma-Aldrich (Prague, Czech Republic) and L-DOPA was obtained from Sigma-Aldrich (Hong Kong, China)

Statistical analysis

All results were performed in triplicate and reported as means ± SD. Differences between samples were determined by Duncan's multiple range tests in SPSS statistical program ver.17 (SPSS Inc, Chicago, IL, USA). A probability level of 99% was used in testing the statistical significance of all experimental data.

RESULTS AND DISCUSSION

Percentage yield of ginger plant extracts

Rhizomes were extracted with non-polar solvent, and then gradually increase the polarity to most polar by *n*-hexane, ethyl acetate and ethanol respectively. The difference in polarity of the solvents that effects the solubility of the components in solvent,[50] from this reason that may be affects to the variation of %yield in each sample extract (Figure 1). The *n*-hexane extraction, rhizome of *Curcuma aromatica* showed the highest % yield of 9.30% (w/w) and followed by *C. longa* [9.02% (w/w)], *C. latifolia* [7.675% (w/w)] and *C. areruginasa* [7.22% (w/w)]. The highest %yield of ethyl acetate extraction was found in rhizome of

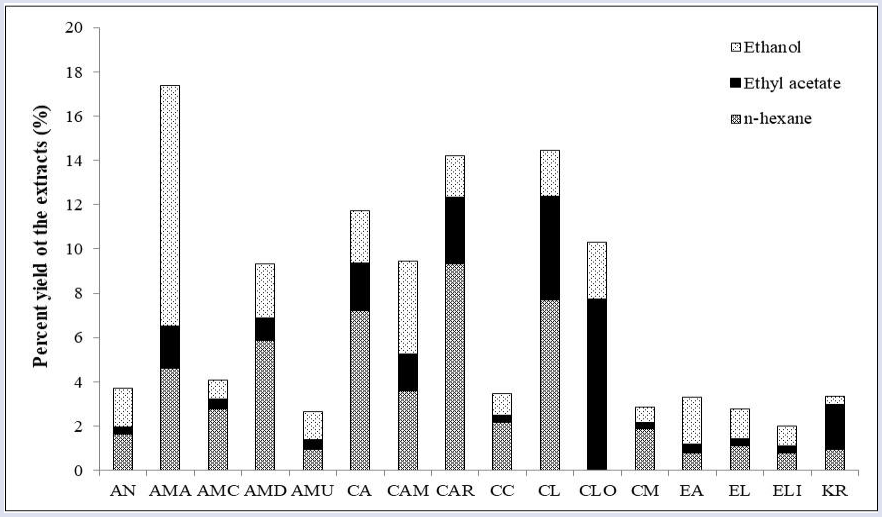

Figure 1: The percentage yield of of selected 16 ginger plants which extracted with *n*-hexane, ethy acetate and ethanol respectively; AN =*Alpinia nigra*, AMA=*Amomum aculeatum*, AMC=*Amomum coriandriodorum*, AMD=*Amomum dealbatum*, AMU=*Amomum uliginosum*, CA=*Curcuma aeruginosa*, CAM=*Curcuma amada*, CAR=*Curcuma aromatica*, CC=*Curcuma candida*, CL=*Curcuma latifolia*, CLO=*Curcuma longa*, CM=*Curcuma mangga*, EA=*Etlingera araneosa*, EL=*Etlingera elatior*, ELI=*Etlingera linguiformis*, KR=*Kaempferia rotunda*.

C. longa [7.73% (w/w)] followed by rhizome parts of *C. latifolia* [4.69% (w/w)] and *C. aromatica* [3.04% (w/w)], while the highest %yield of ethanol extraction was found in rhizome of *Amomum aculeatum* [10.84% (w/w)] followed by *C. amada* rhizome [4.19% (w/w)].

Phytochemical screening

Results of phytochemical screening of ginger plant extracts were demonstrated in Table 1, the different solvent that using for extraction and different species of sample showed variation in their chemical compounds. Alkaloids, which are found in roots, stems, leaves or seeds of plants and expressed various biological properties such as anti-inflammatory, antioxidants, antitumoral and antibacterial.[51-53] Flavonoids are phenolic compounds and widely distributed in plants. Flavonoids are important for plant pigments that composes flavonone, flavone, isoflavone, flavonol, catechin, naringin and anthocyanins, which are found in vegetables, fruits and some beverages such as tea and wine. Moreover, flavonoids provides health benefits and have biological activities including antioxidants, anti-inflammatory, antitumoral and antimicrobial.[54-55] While anthocyanin are a subclass of flavonoids and can occur in any tissues of higher plants. Many researches about anthocyanins have shown to have anti-cancer, antioxidants and anti-aging.[56-57] Antioxidation capacity of anthocyanins that depends on its structure. Anthocyanins have the glycosylated B-ring, orthohydroxylation and methoxylation in the structure.[58] Moreover, in previous research mentioned that anthocyanin isolated from *Hibicus sabdariffa* can inhibit melanin synthesis in human A375 melanocytes, the inhibition of melanin synthesis was evaluted through two different regulatory mechanisms, one is the direct inhibition of tyrosinase activity was measured with cellular tyrosinase assay, the result found anthocyanin could inhibited tyrosinase actvity in 30% of control (A375 cells) at concentration 50 mg/mL and the other is suppression of protein expression of tyrosinase that was measured using Western blot analysis.[59]. Anthocyanin (cyanidin-3-O-glucoside, delphinidin-3-O-glucoside and peonidin-3-O-glucoside) from the seed coat of black soybean possed antihuman tyrosinase activity, the % inhibition rate showed high antihuman tyrosinase activity (94.8%).[60] While anthocyanins contents from red rice bran had the IC_{50} value of mushroom tyrosinase activity were 4.26 µg/mL that showed anthocyanin has a good inhibitory of tyrosinase although these anthocyanins is slightly weaker tyrosinase inhibition than ascorbic acid (IC_{50}=2.18 µg/mL), which was used the positive control. Ascorbic acid was a strong tyrosinase inhibitor refered to reducing melanin by binding to copper in tyrosinase.[61] The classification of tyrosinase inhibitor strenge or the IC_{50} value based on the structure and mechanism of tyrosinase action is not easy due to the results of the kinetics presented with different testing system from mushroom tyrosinase, mammaliam tyrosinase, melanocytic cultures, cocultures of keralinocytes and melanocytes and *in vivo* application to animal skin.[62] Saponin can be found in vegetables, herbs and beans such as peas, soybeans, saponin have foaming propterties that caused by the combination between a hydrophobic and a hydrophillic parts. Health benefits of saponins are shown in many from research including protective bone loss, cholesterol reduction, anti-cancer and antibacterial.[63-64] Antioxidants, anti-cancer, antimicrobial and antibiotic are biological properties of tannins that used in various fileds of food and beverage, animal nutrition and agriculture.[65] Terpenoids are organic compound that found in all tissues of higher plants and terpenoids are widely use for colourant and fragrance in food industry, raw materials of resin manufacturer and printing ink manufacturer.[66-67] About steroids, which are used widely used in pharmaceutical to anti-inflammatory, anti-cancer, antioxidants and contraception.[68-69]*Alpinia* extract was found alkaloids in *n*-hexane and ethanolic extracts, while flavonoids, tannin and polyphenol were found in ethanolic extracts only. Terpenoids were found in *n*-hexane and ethyl acetate *Alpinia* extracts. Our results were related with previous research that discoverd chemical groups of alkaloids, flavonoids and phenol in the water extract of *A. nigra* shoot.[70]

Alkaloids and terpenoids were found in all solvent extracts of *Amomum*, except for *A. aculeatum* extract that found alkaloids in ethyl acetate extract alone. There were absent of alkaloids in all solvents extraction of *A. dealbatum*, while the ethyl acetate and ethanolic extracts of *Amomum* were found to contain flavonoids, tannin and polyphenol. For *Curcuma* extracts, alkaloids were found in all solvents extraction of *C. amada*, *C. candida*, *C. latifolia*, and *C. mangga*. Then flavonoids were found in all solvent extraction of *C. amada*, *C. aromatica*, *C. longa*, and *C. mangga*, except for *C.candida* extract which showed flavonoids in ethyl acetate and ethanolic extracts. Saponin was found in the *n*-hexane extract of

Table 1A: Phytochemical screening results of the rhizome extracts by different solvent extraction.

Plants	Solvent Extraction	Alkaloids	Flavonoids	Anthocyanins	Saponins	Tanins and polyphenol	Steroids	Terpenoids
A. nigra	Hexane	✓						✓
	Ethyl acetate							✓
	Ethanol	✓	✓			✓		
A. aculeatum	Hexane							✓
	Ethyl acetate	✓	✓			✓		✓
	Ethanol		✓			✓		✓
A. coriandriodorum	Hexane	✓						✓
	Ethyl acetate	✓	✓					✓
	Ethanol	✓	✓			✓		✓
A. dealbatum	Hexane							✓
	Ethyl acetate		✓					✓
	Ethanol		✓					✓
A. uliginosum	Hexane	✓						✓
	Ethyl acetate	✓	✓			✓		✓
	Ethanol	✓	✓			✓		
C. aeruginasa	Hexane			✓		✓		✓
	Ethyl acetate			✓		✓	✓	✓
	Ethanol			✓		✓		✓
C. amada	Hexane	✓	✓					✓
	Ethyl acetate	✓	✓					✓
	Ethanol	✓	✓					✓
C. aromatica	Hexane		✓		✓	✓		✓
	Ethyl acetate		✓			✓	✓	✓
	Ethanol		✓			✓		✓
C. candida	Hexane	✓						✓
	Ethyl acetate	✓	✓					✓
	Ethanol	✓	✓			✓	✓	✓
C. latifolia	Hexane	✓				✓		✓
	Ethyl acetate	✓				✓	✓	✓
	Ethanol	✓				✓		✓

Table 1B: Phytochemical screening results of the rhizome extracts by different solvent extraction (continued).

Plants	Solvent Extraction	Alkaloids	Flavonoids	Anthocyanins	Saponins	Tanins and polyphenol	Steroids	Terpenoids
Curmuma longa	Hexane		✓			✓		✓
	Ethyl acetate		✓			✓	✓	✓
	Ethanol		✓			✓		✓
C. mangga	Hexane	✓	✓					
	Ethyl acetate	✓	✓					
	Ethanol	✓	✓			✓		✓
Etlingera araneosa	Hexane							✓
	Ethyl acetate	✓	✓				✓	✓
	Ethanol	✓	✓					
E. elatior	Hexane	✓						
	Ethyl acetate	✓					✓	✓
	Ethanol	✓	✓			✓		
E. linguiformis	Hexane	✓						
	Ethyl acetate	✓					✓	✓
	Ethanol	✓	✓				✓	✓
Kaemferia rotunda	Hexane	✓	✓					✓
	Ethyl acetate	✓	✓					✓
	Ethanol	✓	✓					✓

C. aromatica. In addition, anthocyanins were found in three solvents extraction of *C. aeruginasa*, while tannin and polyphenol were found in all solvent extracts of *C. aeruginasa*, *C. aromatica*, *C. latifolia* and *C. longa*. Steroids were found in the ethyl acetate extracts of *C. aeruginasa*, *C. aromatica*, *C. latifolia* and *C. longa*, On the other hand, the ethanol extract of *C. candida* was showed steriods. Terpenoids were found in all solvents extraction of *Curcuma* extracts in this investigation except *C. mangga* that found terpenoids in the ethyl acetate extract. In previous studies, terpenoids, flavonoids, tannin and alkaloids were detected in the *n*-hexane extract, while the ethanolic extract was expressed steroids and alkaloids.[71-72] For *Etlingera* extracts alkaloids were found in all solvent extraction of *E. elatior* and *E. linguiformis*, except ethyl acetate and ethanolic extracts of *E. araneosa*. While flavonoids were found in ethyl acetate and ethanolic extracts of *E. araneosa* and ethanolic extracts of *E. elatior* and *E. linguiformis*. Tannin and polyphenol of *Etlingera* extracts were found in the ethanolic *E. elatior* extract. Moreover steroids and terpenoids evaluation, steroids were showed in the ethyl acetate extracts of *E. araneosa* and *E. elatior*, ethyl acetate and ethanolic extracts of *E. linguiformis*, while terpenoids were found in the *n*-hexane and ethyl acetate extracts of *E. araneosa*, the ethyl acetate extract of *E. elatior*, ethyl acetate and ethanolic extracts of *E. linguiformis*. The results in this study are related with previous research, which was reported that ethanolic rhizome extract of *E. linguiformis* was presented alkaloids, steroids and tannins.[73] Finally, alkaloids, flavonoids and terpenoids were found in all extracts of *Kaempferia rotunda*.

Total phenolic compounds

Phenolic compounds have antioxidant properties that depend on the presence of OH groups in their chemical structure. In addition the total phenolic concentration has been used to screen for antioxidant activity of plants.[74-75] Total phenolic compounds (TPC) of the sample extracts are ranged from 0.94 to 587.15 mg GAE/g extract. As presented in Figure 2, the comparison among different solvent extracts showed that ethyl acetate and ethanol extracts contained more TPC than the *n*-hexane extract due to the the difference in polarity of the solvents and chemical compounds in plants.[50] The *n*-hexane extracts were ranged 1.55 to 77.72 mg GAE/g extract, *A. aculeatum* contained the highest levels of TPC at 77.92 mg GAE/g extract, followed by *C. latifolia*, *C. amada* and *C. longa* at 60.96 mg GAE/g extract, 55.18 mg GAE/g extract and 43.92 mg GAE/g extract respectively, while the extract of *E. linguiformis* had the lowest TPC content at 1.55 mg GAE/g extract. About the ethyl acetate extracts had TPC levels between 16.37 to 587.15 mg GAE/g extract, the highest TPC was expressed in the ethyl acetate extract of *C. longa* at 587.15 mg GAE/g extract, followed by *A. uliginosum* (536.67 mg GAE/g extract), and *C. amada* (508.42 mg GAE/g extract). While TPC in the ethanolic extracts are ranged from 0.94 to 232.29 mg GAE/g extract. *A. nigra* had the highest value of TPC at 232.29 mg GAE/g extract, followed by *A. uliginosum* (128.71 mg GAE/g extract) and *C. amada* (86.50 mg GAE/g extract).

DPPH radical scavenging activity

DPPH assay was reported as a percentage of inhibition against DPPH radical (% inhibition) at 4.0 mg/mL and as the IC_{50} value (Figure 3). Percentage inhibition of the *n*-hexane extracts were ranged from 23.34 to 94.04%, while the IC_{50} values were ranged from 0.23 to more than 4.00 mg/mL. *A. aculeatum* extract had the highest % inhibition and lowest IC_{50} values, which were 94.04% and 0.23 mg/mL respectively which congruent with the highest level of TPC contents and highest % inhibition at 93.81% (IC_{50}: 0.04 mg/mL) in ethyl acetate extract. These results can support the potential of *A. aculeatum* for its antioxidant property. While the rest ethyl acetate extracts of *C. latifolia* showed % inhibition at 93.07% (IC_{50}=0.04 mg/mL) followed by *C. aromatica* (% inhibition=93.04%, IC_{50}=0.25 mg/mL) and *C. candida* (% inhibition =30.55%; IC_{50} > 4.00 mg/mL) respectively. Finally, ethanolic extract of *A. aculeatum* presented the highest % inhibition at 93.89%, on the other hand *C. longa* had the lowest of IC_{50} value at 0.12 mg/mL, followed by *C. latifolia* at 0.13 mg/mL, and *A. aculeatum* at 0.32 mg/mL. From this research, ethyl acetate and ethanolic extracts had stronger DPPH radical scavenging activity than *n*-hexane extracts.

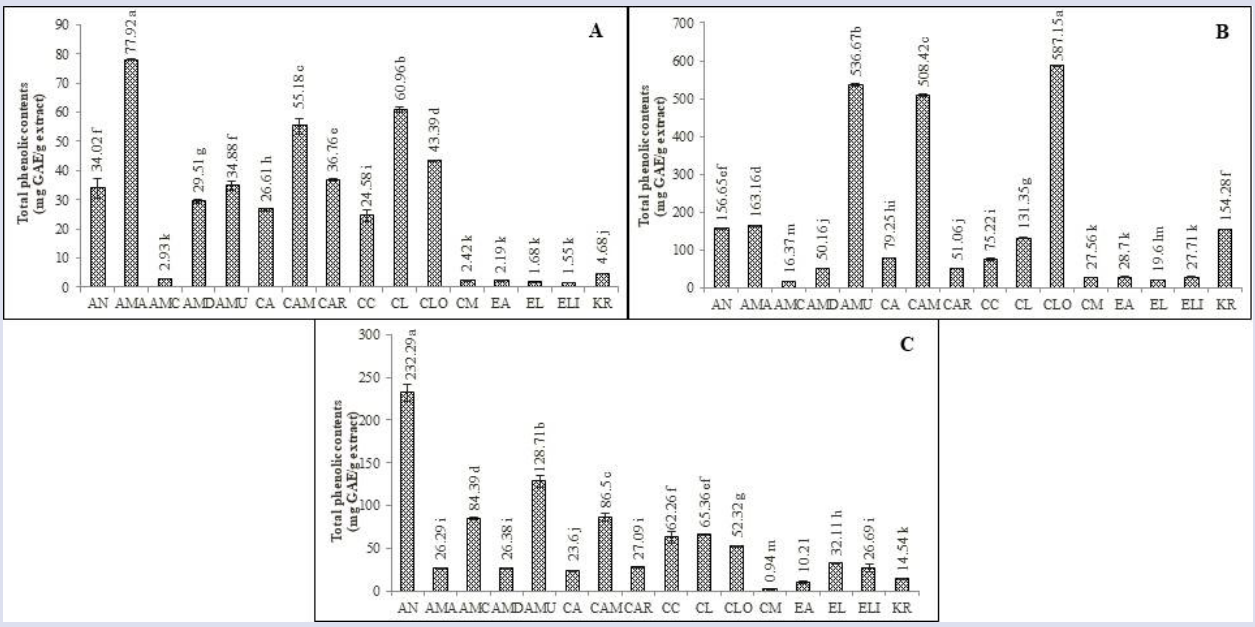

Figure 2: Total phenolic compounds of selected 16 ginger plants which extracted with n-hexane (A), ethyl acetate (B) and ethanol (C). Mean value with different letters of each peak are significantly different at *P<0.01*; AN =*Alpinia nigra*, AMA=*Amomum aculeatum*, AMC=*Amomum coriandriodorum*, AMD=*Amomum dealbatum*, AMU=*Amomum uliginosum*, CA=*Curcuma aeruginosa*, CAM=*Curcuma amada*, CAR=*Curcuma aromatica*, CC=*Curcuma candida*, CL=*Curcuma latifolia*, CLO=*Curcuma longa*, CM=*Curcuma mangga*, EA=*Etlingera araneosa*, EL=*Etlingera elatior*, ELI=*Etlingera linguiformis*, KR=*Kaempferia rotunda*.

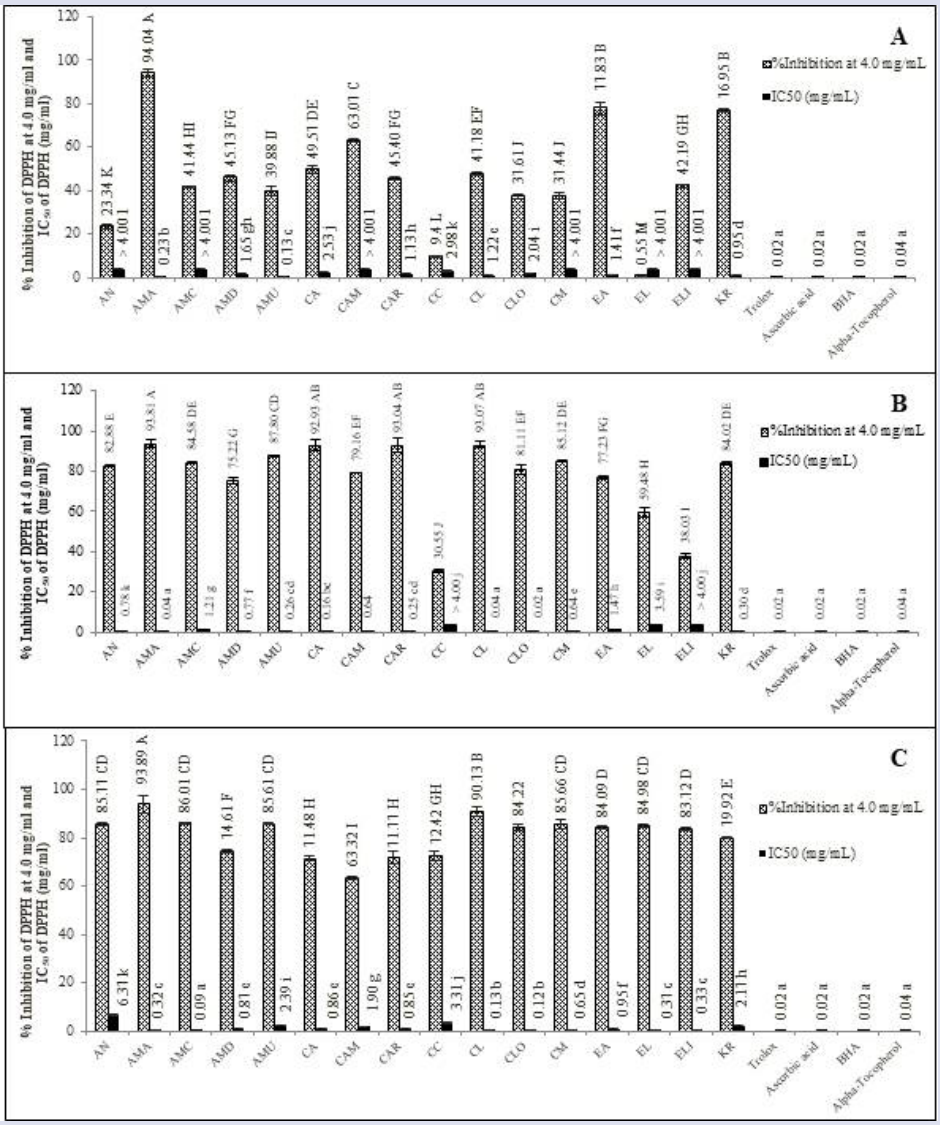

Figure 3: Percentage inhibition of DPPH at 4.0 mg/mL and IC_{50} of DPPH radical scavenging (mg/mL) of selected 16 ginger plants which extracted with *n*-hexane (A), ethyl acetate (B) and ethanol (C). Mean value with different letters of each peak are significantly different at *P<0.01*. The capital letters are significantly different data of % inhibition of DPPH, and another are significantly different data of IC_{50} (mg/mL) value; AN =*Alpinia nigra*, AM=*Amomum aculeatum*, AMC=*Amomum coriandriodorum*, AMD=*Amomum dealbatum*, AMU=*Amomum uliginosum*, CA=*Curcuma aeruginosa*, CAM=*Curcuma amada*, CAR=*Curcuma aromatica*, CC=*Curcuma candida*, CL=*Curcuma latifolia*, CLO=*Curcuma longa*, CM=*Curcuma mangga*, EA=*Etlingera araneosa*, EL=*Etlingera elatior*, ELI=*Etlingera linguiformis*, KR=*Kaempferia rotunda*.

ABTS^{+} radical scavenging activity

The ABTS^{+} radical scavenging activity of ginger plant extracts were ranged from 1.08 to 521.42 mg TEAC/g extract. According to different solvent extractions, *n*-hexane extracts were ranged from 1.08 to 133.52 mg TEAC/g extract (Figure 4). *A. aculeatum* had the highest ABTS^{+} value at 133.52 mg TEAC/g extract followed by *E. araneosa* at 71.00 mg TEAC/g extract, *K. rotunda* at 67.24 mg TEAC/g extract and *C. latifolia* at 61.15 mg TEAC/g extract. For the ethyl acetate extracts were ranged from 2.88 to 528.42 mg TEAC/g extract, *C. longa* had the highest ABTS value at 481.96 mg TEAC/g extract followed by *C. latifolia* (372.48 mg TEAC/g extract) and *K. rotunda* (307.18 mg TEAC/g extract). While *A. coriandriodorum* was presented the highest ABTS value (359.90 mg TEAC/g extract) for the ethanolic extract and followed by *K. rotunda* (280.18 mg TEAC/g extract), *C. longa* (278.34 mg TEAC/g extract) and *C. latifolia* (255.68 mg TEAC/g extract). ABTS results were exhibited similar trend to TPC and DPPH results except the sample that using ethanol as the extracting solvent. The anti-oxidant analyses of ethanolic extracts evaluated by DPPH were not correlated with ABTS^{+} assay probably due to the difference of chemical structure or the location of hydroxylation, glycosylation and methoxylation in the plant extracts.[76]

Anti-tyrosinase activity

Tyrosinase is a key enzyme that can catalyse enzyme browing and melanin synthesis that obtained from bacteria, fungal, plants, insects and UV radiations. In human, tyrosinase can lead melanin on the skin, freckles and age spots, while free radicals are important role for biosynthesis of melanin.[77-78] The effects of sample extract on anti-tyrosinase activity are reported as IC_{50} value that presented in Figure 5. The IC_{50} value were ranged from 0.01 to more than 12.50 mg/mL, while kojic acid was used as a effective control that had

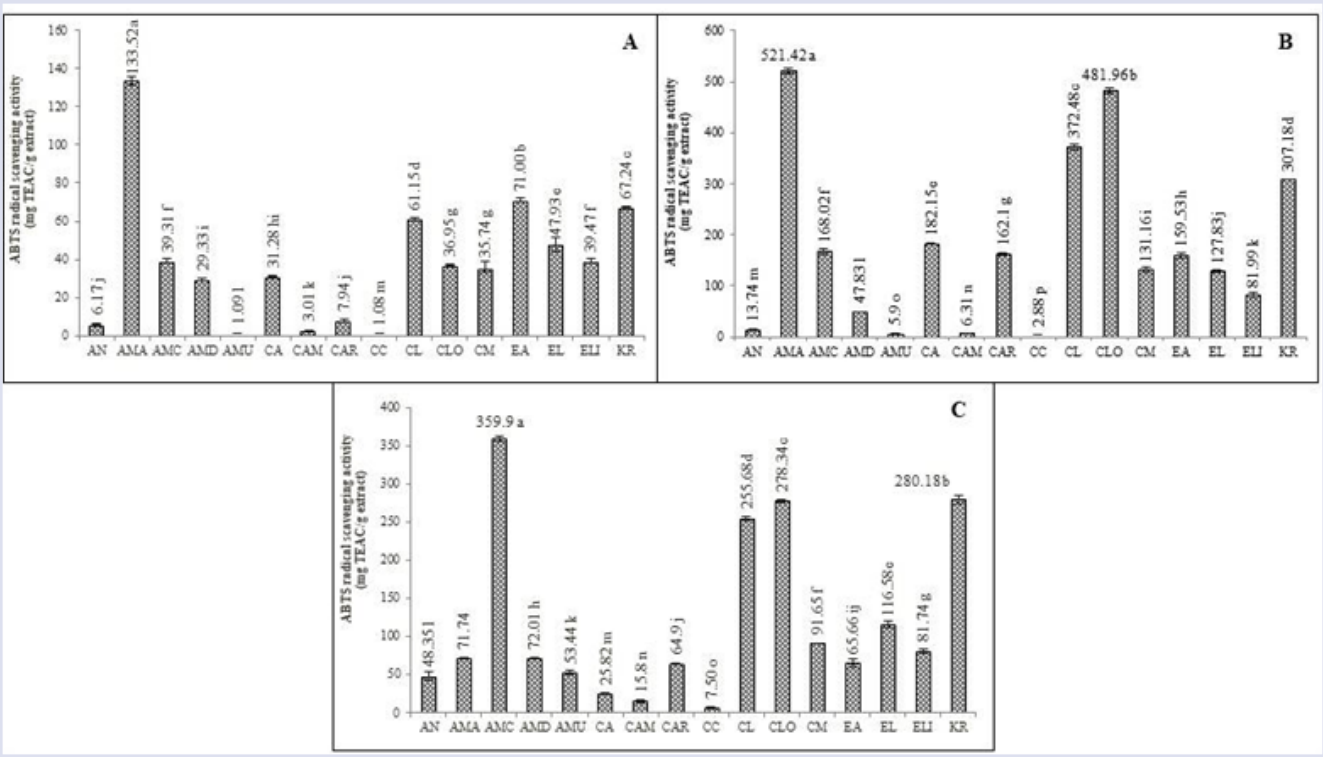

Figure 4: ABTS^{+} radical scavenging activity of selected 16 ginger plants which extracted with n-hexane (A), ethyl acetate (B) and ethanol (C). Mean value with different letters of each peak are significantly different at *P<0.01*; AN =*Alpinia nigra*, AM=*Amomum aculeatum*, AMC=*Amomum coriandriodorum*, AMD=*Amomum dealbatum*, AMU=*Amomum uliginosum*, CA=*Curcuma aeruginosa*, CAM=*Curcuma amada*, CAR=*Curcuma aromatica*, CC=*Curcuma candida*, CL=*Curcuma latifolia*, CLO=*Curcuma longa*, CM=*Curcuma mangga*, EA=*Etlingera araneosa*, EL=*Etlingera elatior*, ELI=*Etlingera linguiformis*, KR=*Kaempferia rotunda*.

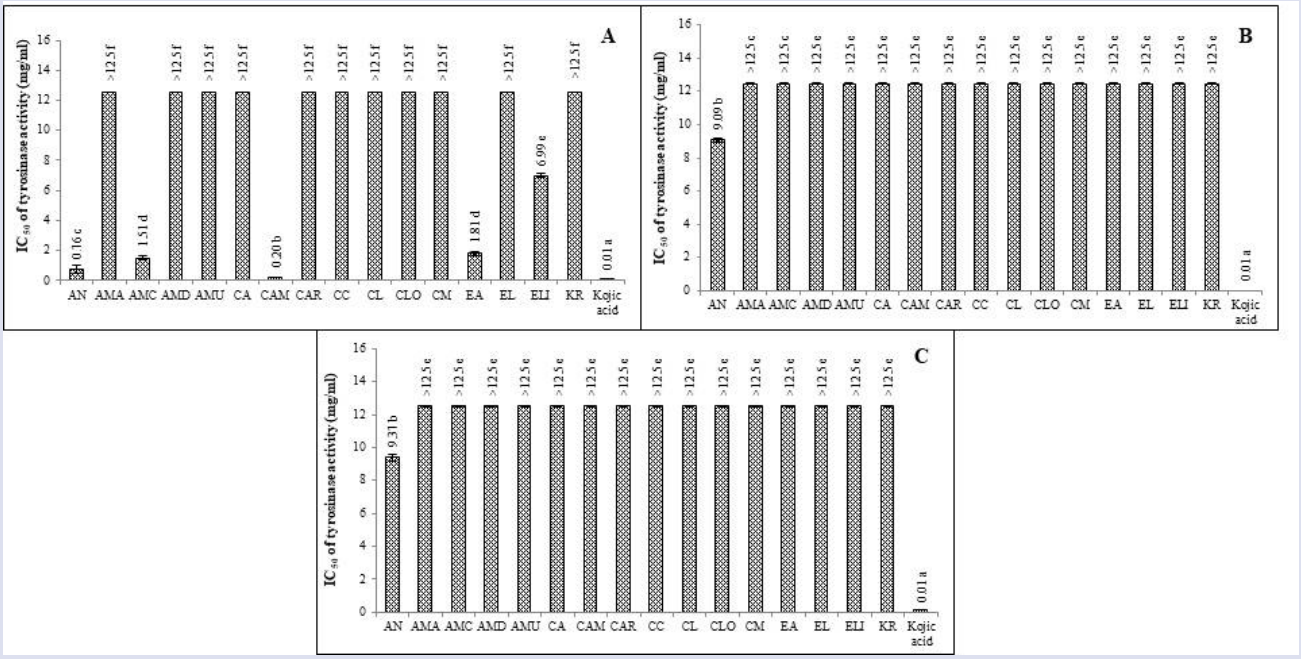

Figure 5: IC_{50} (mg/mL) value of anti-tyrosinase activity of of selected 16 ginger plants which extracted with *n*-hexane (A), ethyl acetate (B) and ethanol (C). Mean value with different letters of each peak are significantly different at *P<0.01*; AN =*Alpinia nigra*, AM=*Amomum aculeatum*, AMC=*Amomum coriandriodorum*, AMD=*Amomum dealbatum*, AMU=*Amomum uliginosum*, CA=*Curcuma aeruginosa*, CAM=*Curcuma amada*, CAR=*Curcuma aromatica*, CC=*Curcuma candida*, CL=*Curcuma latifolia*, CLO=*Curcuma longa*, CM=*Curcuma mangga*, EA=*Etlingera araneosa*, EL=*Etlingera elatior*, ELI=*Etlingera linguiformis*, KR=*Kaempferia rotunda*.

the lowest IC_{50} value at 0.01 mg/mL. The *n*-hexane extracts had IC_{50} value ranged from 0.20 to more than 12.5 mg/mL and *C. amada* had the lowest IC_{50} value at 0.20 mg/mL followed by *A. nigra* (IC_{50}=0.76 mg/mL), *A. coriandriodorum* (IC_{50}=1.51 mg/mL) and *E. araneosa* (IC_{50}=1.81 mg/mL). The ethyl acetate extracts were ranged from 9.09 to more than 12.5 mg/mL. *Alpinia nigra* had the lowest IC_{50} value both in ethyl acetate extracts and ethanolic extracts at 9.09 mg/mL and 9.35 mg/mL respectively. The similar results of IC_{50} value (more than 12.5 mg/mL) among different extracts obtained by three different solvent extractions, because some inhibitors in plant extracts may be active in a high concentration and depend on the each solvent capability.[79] The biological activity of plant extracts depend on active compounds obtained from different solvent extractions. This study demonstrated that the hexane extract had the lowest IC_{50} value, which similar to the previous result, plant hexane extract had the highest in tyrosinase inhibitory activity.[80]

CONCLUSIONS

Our results were indicated that rhizomes of 16 ginger plants (Zingiberaece) could be sources of phenolic compounds and strong antioxidants, which presented in ethyl acetate and ethanolic extracts of *Alpinia*, *Amomum* and *Curcuma*. Their result had antioxidant activity value nearby positive control, expecially Ascorbic acid, which have a strong antioxidants and an efficient antioxidant agent in cosmetic product.[81-82] On the other hand, the *n*-hexane extracts of *Alpinia* (*A. nigra*), *Amomum* (*A. coriandriodorum*), and *Etlingera* (*E. araneosa*) showed anti-tyrosinase properties, while *Curcuma* (*C. amada*) displayed the strongest anti-tyrosinase because its IC_{50} value of tyrosinase nearby kojic acid that use as a positive control. From qualitative and quanlitative value can be used for further research on natural science, food science or pharmaceutical science including with natural product developments.

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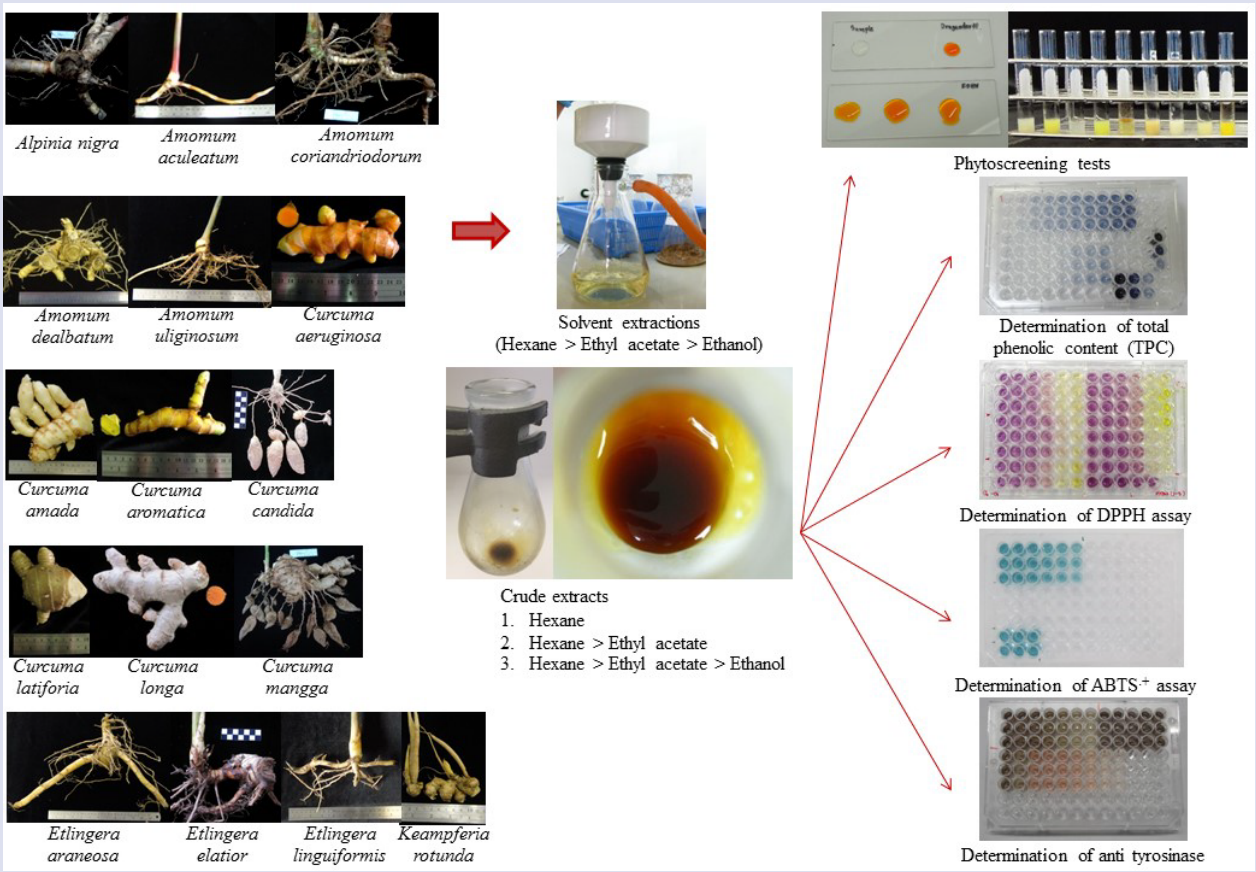
REFERENCES

1. Mamta S, Jyoti S. Phytochemical screening of *Acorus calamus* and *Lantana camara*. Int Res J Pharm. 2012;3(5):324-6. https://irjponline.com/admin/php/uploads/1111_pdf.pdf.
2. Okwu DE. Phytochemicals and vitamin contents of indigenous species of South Eastern Nigeria. J Sustain Agric Environ. 2004;6(1):30-7. https://doi.org/10.0.49.147/ajfn-2-4-5.
3. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem. 2006;99(1):191-203. https://doi.org/10.1016/j.foodchem.2005.07.042.
4. Hamaguchi T, Ono K, Murase A, Yamada M. Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid-beta aggregation pathway. Am J Path. 2009;175(6):2557-65. https://doi.org/10.2353/ajpath.2009.090417; PMid:19893028 PMCid:PMC2789642.
5. Kazlowska K, Hsu T, Hou CC, Yang WC, Tsai GJ. Anti-inflammatory properties of phenolic compounds and crude extract from *Porphyra dentata*. J Ethnopharmacol. 2010; 128(1):123-30. https://doi.org/10.1016/j.jep.2009.12.037; PMid:20051261.
6. Oueslati S, Trabelsi N, Boulaaba M, Legault J, Abdelly C, Ksouri R. Evaluation of antioxidant activities of the edible and medicinal *Suaeda* species and related phenolic compounds. Ind Crops Prod. 2012;36(1):513-8. https://doi.org/10.1080/14786419. 2014.947497; PMid:25143148.
7. Rodrigues JA, Vanderlei EdSO, Silva LM, AraÚjo IW, Queiroz IN, Paula GA, et al. Antinociceptive and anti-inflammatory activities of a sulfated polysaccharide isolated from the green seaweed *Caulerpa cupressoides*. Pharmacol Rep. 2012;64(2):282-92. https://doi.org/10.1016/s1734-1140(12)70766-1; PMid:22661177.
8. Oliveira ACd, Valentim IB, Goulart MOF, Silva CA, Bechara EJH, Trevisan MTS. Fontes vegetais naturais de antioxidantes. Quím Nova. 2009;32:689-702. https://doi.org/10.11590 /S0100-40422009000300013.
9. Kim YJ, Uyama H. Tyrosinase inhibitors from natural and synthetic sources: Structure, inhibition mechanism and perspective for the future. Cell Mol Life Sci. 2005;62(15):1707–23. https://doi.org/10.1007/s00018-005-5054-y; PMid:15968468.
10. Rangkadilok N, Sitthimonchai S, Worasuttayangkurn L, Mahidol C, Ruchirawat M, Satayavivad J. Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. Food Chem Toxicol. 2007;45(2):328–36. https://doi.org/10.1016/j.fct.2006.08.022; PMid:17049706.
11. Delin W, Larsen K. Zingiberaceae. In: Wu ZY, Raven PH, editors. Flora of China. MO: Science Press and Missouri Botanical Garden Press; 2000. p. 322-77. http://flora.huh. harvard.edu/china/mss/volume24/ZINGIBERACEAE.published.pdf.
12. Kantayos V, Paisooksantivatana Y. Antioxidant activity and selected chemical components of 10 *Zingiber* spp. in Thailand. J Dev Sustain Agr. 2012;7(1):89-96. https://doi.org /10.11178/jdsa.7.89.
13. Larsen K, Larsen SS. Gingers of Thailand. Chiang Mai: Queen Sirikit Botanic Garden, The Botanical Garden Organization; 2006.
14. Oonmetta-aree J, Suzuki T, Gasaluck P, Eumkeb G. Antimicrobial properties and action of galangal (*Alpinia galanga* Linn.) on *Staphylococcus aureus*. LWT-Food Sci Technol. 2006;39(10):1214-20. https://doi.org/10.1016/j.lwt.2005.06.015.
15. Akinola A, Ahmad S, Maziah M. Total anti-oxidant capacity, flavonoid, phenolic acid and polyphenol content in ten selected species of Zingiberaceae rhizomes. Afr J Tradit Complement Altern Med. 2014;11(3):7-13. https://doi.org/10.4314/ajtcam.v11i3.2; PMid:25371557 PMCid:PMC4202413.
16. Chan EWC, Lim YY, Wong SK. Antioxidant properties of ginger leaves: An overview. Free Rad Antiox. 2011;1(1):6-16. https://doi.org/10.5530/ax.2011.1.3.
17. Nishat FS, Nilima N, Saikia BM. Phytochemical analysis of *Lasia spinosa* and *Alpinia nigra*, potential medicinal plants of Assam. Phytochemistry. 2012;4(3):170–3. https://doi.org/10.5958/j.0975-4261.4.3.021.
18. Anjusha S, Gangaprasad A. Phytochemical and antibacterial analysis of two important *Curcuma* species, *Curcuma aromatica* Salisb. and *Curcuma xanthorrhiza* Roxb. (Zingiberaceae). J Pharmacogn Phytochem. 2014;3(3):50-3. https://www.phytojournal.com /archives/2014/vol3issue3/PartA/27.1.pdf.
19. Oghenejobo M, Opajobi OA, Oghenejobo BUS, Uzoegbu U. Antibacterial evaluation phytochemical screening and ascorbic acid assay of turmeric (*Curcuma longa*). MOJ Bioequivalence Bioavailab. 2017;4(2): 232-9. https://doi.org/10.15406/mojbb.2017.04. 00063.
20. Arora RB, Kapoor V, Basu N, Jain AP. Anti–inflammatory studies on *Curcuma longa* (turmeric). Indian J Med Res. 1971;59(8):1289–95. PMid:5132235.
21. Lee YY, Lee S, Jin JL, Choi HSY. Platelet anti–aggregatory effect of coumarins from the roots of *Angelica genuflexa* and *A. gigas*. Arch Pharmacal Res. 2003;26(9):723–6. https://doi.org/10.1007/bf02976681; PMid:14560920.
22. Khunlad P, Tundulawessa Y, Supasiri T, Chutrtong W. Tyrosinase Inhibitory Activity of Curcuminoids from Powder of Turmeric (*Curcuma longa* Linn.). SWU Sci J. 2008;24(1): 125-39. http://www.thaiscience.info/Journals/Article/SSCJ/10894883.pdf.
23. Shahid-Ud-Daula AFM, Kamariah AS, Lim LBL, Ahmad N. Phytochemical screening, antioxidant, and antimicrobial activities of leaves, stems, and rhizomes of *Etlingera coccinea* (blume) S. Sakai & Nagam. Int J Pharmacogn Phytochem Res. 2015;7(5):873-83. http://impactfactor.org/PDF/IJPPR/7/IJPPR,Vol7,Issue5,Article1.pdf.
24. Habsah M, Lajis NH, Sukari MA, Yap YH, Kikuzaki H, Nakatani N. Antitumour-promoting and cytotoxic constituents of *Etlingera elatior*. Malays J Med Sci. 2005;12(1):6-12. PMid: 22605941 PMCid:PMC3349407.
25. Chan EWC, Lim YY, Wong LF, Lianto FS, Wong SK, Lim KK, et al. Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. Food Chem. 2008;109:447-83. https://doi.org/10.1016/j.foodchem.2008.02.016.
26. Lotulung PDN, Minarti, Kardono LBS, Kawanishi K. Antioxidant compound from the rhizomes of Kaempferia rotunda L. Pak J Biol Sci. 2008;11(20):2447-50. https://doi.org/ 10.3923/pjbs.2008.2447.2450; PMid:19137858.
27. Kumar A, Kumar S, Navneet. Antimicrobial activity and phytochemical analysis of Kaempferia rotunda L. rhizomes. Der Pharma Lett. 2015;7(9):389-95. https://www.scholarsresearchlibrary.com/articles/antimicrobial-activity-and-phyto chemical-analysis-of-kaempferia-rotunda-l-rhizomes.pdf.
28. Lotulung PD, Minarti, Kardono LBS, Kawanishi K. Antioxidant compound from the rhizomes of *Kaempferia rotunda* L. Pak J Biol Sci. 2008;11(20):2447-50. https://doi.org/10.3923/pjbs.2008.2447.2450; PMid:19137858.
29. Ibrahim H. *Kaempferia* L. In: de Padua LS, Bunyapraphatsara N, Lemmens RHMJ. editors. Plant Resources of South East Asia No. 12(1): Medicinal and Poisonous Plant 1. Leiden: Backhuys Publishers; 2003. p. 331-5. https://doi.org/10.1021/np 990726c.
30. Numan EM, Jyad JS, Alazawi AHI, Al-Jumaily EF, Jwad AN, Zehrawo HM, et al. Comparison of different extraction methods of (*Zingiber officinale*) on chemical composition, antioxidant activity. Int J Pharm Pharm Sci. 2016;5(3):215-23. https://www.researchgate.net/publication/323074302_Comparison_of_Different_Extraction_Methods_of_Zingiber_officinale_on_Chemical_Composition_Antioxidant_Activity.

31. Pranav KAVR, Deepak SK, Beura S, Sreenivas RGR, Uday BGG, Potbhare MS. Comparative evaluation of effect of extracting solvents on therapeutic activities of *Curcuma aromatica* rhizomes. Iran J Pharm Sci. 2013;9(4):83-97. http://www.ijps.ir/article_ 10261_4bb983e843386a0596f138bb1b9fe962.pdf.
32. Natta L, Orapin K, Krittika N, Pantip B. Essential oil from five Zingiberaceae for anti food-borne bacteria. Int Food Res J. 2008;15(3):337-46. http://www.ifrj.upm.edu.my/ 15%20(3)%202008/12.%20Natta%20L.pdf.
33. Roy B, Swargiary A, Giri BR. *Alpinia Nigra* (Family Zingiberaceae): an anthelmintic medicinal plant of North-East India. Adv Life Sci. 2012;2(3):39–51. https://doi.org/ 10.5923 /j.als.20120203.01.
34. Srivastava AK, Jain PC. Instrumental approach to chemical analysis. 4th ed. Ram Nagat. New Delhi. 2008.
35. Joy B, Rajan A, Abraham E. Antimicrobial activity and chemical composition of essential oil from *Hedychium coronarium*. Phytother Res. 2007;21(5):439-43. https://doi.org/10.1002/ptr.2091; PMid:17245683.
36. Taveira FN, Oliveira1 AB, Souza FJD, Braga FC. Epimers of labdane diterpenes from the rhizomes of *Hedychium coronarium* J. Koenig. Rev Bras Farmacogn. 2005;15(1):55-9. https://doi.org/10.1590/S0102-695X2005000100012.
37. Chimnoi N, Pisutjaroenpong S, Ngiwsara L, Dechtrirut D, Chokchaichamnankit D, Khunnawutmanotham N, Mahidol C, Techasakul S. Labdane diterpenes from the rhizomes of *Hedychium coronarium*. Nat Prod Res. 2008;22(14):1249-56. https://doi.org/10.1016/ S0031-9422(00)90417-5.
38. Elgerwi AA, Benzekri Z, El-Magdoub A, El-Mahmoudy A. Qualitative identification of the active principles in Citrullus colocynthis and evaluation of its teratogenic effects in albino rats. Int J Basic Clin Pharmacol. 2013;2(4);438-45. https://doi.org/10.5455/2319-2003.ijbcp20130818.
39. Raaman N. Phytochemical Techniques. 1st ed. Pitam Pura. New Delhi. 2006
40. Sharma AK, Gangwar M, Tilak R, Nath G, Sinha ASK, Tripathi YB, Kumar D. Comparative in vitro antimicrobial and phytochemical evaluation of methanolic extract of root, stem, and leaf of *Jatropha curcas* Linn. Pharmacogn J. 2012;4(30):34-40. https://doi.org/10.5530/pj.2012.30.7.
41. Hossain MA, AL-Raqmi KA, AL-Mijizy ZH, Weli AM, AL-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. Asian Pac J Trop Biomed. 2013;3(9):705-10. https://doi.org/10.1016/S2221-1691(13)60142-2; PMid:23998010 PMCid:PMC37572 78.
42. Ao C, Deba F, Tako M, Tawata S. Biological activity and composition of extract from aerial root of *Ficus microcarpa* L. fil. Int J Food Sci Tech. 2009;44(2):349-58. https://doi.org/10. 1111/j.1365-2621.2008.01732.x.
43. Holtz RW. In Vitro methods to screen materials for anti-aging effects. In: Dayan N, editor. Skin aging handbook an integrated approach to biochemistry and product development personal care & cosmetic technology. New York: William Andrew Inc.; 2009. p. 329-62. https://doi.org/10.1016/B978-0-8155-1584-5.50017-X
44. Cerretani L, Bendini A. Chapter 67 – Rapid assays to evaluate the antioxidant capacity of phenols in virgin olive oil. In: Preedy V R, Watson R R, editors. London: Academic Press; 2010. p. 625-35. https://doi.org/10.1016/B978-0-12-374420-3.00067-X
45. Yen GC, Hsieh CL. Antioxidant effects of dopamine and related compounds. Biosci Biotech Bioch 1997;61(10):1646-9. https://doi.org/ 10.1271/bbb.61.1646; PMid:103362 74.
46. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Bio Med 1999; 26(9-10):1231–7. https://doi.org/10.1016/s0891-5849(98)00315-3; PMid:10381194.
47. Nattapong S, Omboon L. A new source of whitening agent from a Thai Mulberry plant and its betulinic acid quantitation. Nat Prod Res. 2008; 22(9):727–34. https://doi.org/ 10.1080/14786410601130794; PMid:18569714.
48. Lin YS, Chen SH, Huang WJ, Chen CH, Chien MY, Lin SY, Hou WC. Effects of nicotinic acid derivatives on tyrosinase inhibitory and antioxidant activities. Food Chem. 2012; 132(4):2074–80. https://doi.org/10.1016/j.foodchem.2011.12.052.
49. Chen J, Suna H, Tao X, Wanga S, Sun A. Inhibitory mechanism and kinetics study of apple polyphenols on the activity of tyrosinase. Int J Food Prop. 2014;17(8):1694–701. https://doi.org/10.1080/10942912.2012.675606.
50. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 2009;14(6):2167-80. https://doi.org/ 10.3390/molecules14062167; PMid:19553890 PMCid:PMC6254218.
51. Delorenzi JC, Attias M, Gattass CR, Andrade M, Rezende C, Pinto AC, Henriques AT, Bou-Habib DC, Saraiva EM. Antileishmanial activity of indole alkaloid from *Peschiera australis*. Antimicrob Agents Chemother 2001; 45(5):1349–1354. https://doi.org/ 10.1128/AAC.45.5.1349-1354.2001; PMid:11302794 PMCid:PMC90472.
52. Pereira CG, Leal PF, Sato DN, Meireles MAA. Antioxidant and antimycobacterial activities of *Tabernaemontana catharinensis* extracts obtained by Supercritical CO_2^+ cosolvent. J Med Food 2012; 8(4):533–538. https://doi.org/10.1089/jmf.2005.8.533; PMid:16379568.
53. Saldana MDA, Gamarra FMC, Siloto RMP. Emerging technologies used for the extraction of phytochemicals for fruits, vegetables, and other natural sources. In: Rosa LA, Alvarez-Parrilla E, Gonzále GA, editors. Fruit and vegetable phytochemicals: chemistry, nutritional value, and stability. Chicago: Blackwell Publisher; 2010. p. 235-70. https://doi.org/10.1002/9780813809397.ch9.
54. Sarker SD, Nahar L. Chemistry of pharmacy students; general, organic and natural product chemistry. 1st ed. Chichester. West sussex. 2007. https://doi.org/10.1002/978111 8687529.
55. Robles-Sardian AE, Bolanos-Villar AV, Aguilar GAG, Rosa LA. Flavonoids and their relation to human health. In: Rosa LA, Alvarez-Parrilla E, Gonzále GA, editors. Fruit and vegetable phytochemicals: chemistry, nutritional value, and stability. Chicago: Blackwell Publisher; 2010. p. 155-76. https://doi.org/10.1002/9781119158042.ch7.
56. De RVV, Morán VFE, Mercadante AZ, Borsarelli CD. Singlet oxygen quenching by anthocyanin's flavylium cations. Free Radic Res. 2008;42(10):885–91. https://doi.org/ 10.1080/10715760802506349; PMid:18985487.
57. Williams RJ, Spencer JP, Rice-Evans C. Flavonoids: antioxidants or signalling molecules?. Free Radic Biol Med. 2014;36(7):838–49. https://doi.org/ 10.1016/j.freerad biomed.2004.01.001; PMid:15019969.
58. Khoo H E, Azlan A, Tang S T, Lim S M. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res. 2017; 61(1): 1-21. https://doi.org/10.1080/16546628.2017.1361779; PMid:28970777 PMCid:PMC5613902.
59. Hwang J M, Kuo H C, Lin C T, Kao E S. Inhibitory effect of liposome-encapsulated anthocyanin on melanogenesis in human melanocytes. Pharm Biol. 2013;51(8):941-7. https://doi.org/10.3109/13880209.2013.771376; PMid:23570521.
60. Jhan JK, Chung YC, Chen GH, Chang CH, Lu YC Hsu CK. Anthocyanin contents in the seed coat of black soya bean and their anti-human tyrosinase activity and antioxidant activity. Int J Cosmet Sci. 2016;38(3):319-24. https://doi.org/10.1111/ics.12300; PMid:26663436.
61. Wang Y, Zhao L, Zhang R, Yang X, Sun Y, Shi L. et al. Optimization of ultrasond-assisted extraction by response surface methodology, antioxidant capacity, and tyrosinase inhibitory activity of anthocyanins from red rice bran. Food Sci Nutr. 2019;8(2):921-32. https://doi.org/10.1002/fsn3.1371; PMid:32148801 PMCid:PMC7020273.
62. Taïeb A, Cario-André M, Briganti S, Picardo M. inhibiors and enhancers of melanogenesis. In: Boroanský J, Riley P. Melanins and melanosomes: biosynthesis, biogenesis, physiological and pathological functions. Weinheim:Wiley-Blackwell. 2011. p.117-65. https://doi.org/10.1002/9783527636150.ch5.
63. Akinjogunla OJ, Yah CS, Eghafona NO and Ogbemudia FO. Antibacterial activity of leave extracts of *Nymphaea lotus* (Nymphaeaceae) on Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Staphylococcus aureus* (VRSA) isolated from clinical samples. Ann Bio Res. 2010;1(2):174–84. https://www.scholarsresearchlibrary.com/articles/antibacterial-activity-of-leave-extracts-of-nymphaea-lotus-nymphaeaceae-onmethicillin-resistant-staphylococcus-aureus-mr.pdf.
64. Lorent, JH, Quetin-Leclercq J, Mingeot-Leclercq MP. The amphiphilic nature of saponins and their effects on artificial and biological membranes and potential consequences for red blood and cancer cells. Org Biomol chem. 2014;12(44):8803–22. https://doi.org/10.1039/c4ob01652a; PMid:25295776.
65. Deshpande SS, Cheryan M, Salunkhe DK. Tannin analysis of food product. Crit Rev Food Sci and Nutr. 1986;24(4):401-49. https://doi.org/10.1080/10408398609527441; PMid:3536314.
66. Grassmann J, Hippeli S, Elstner EF. Plant's defence and its benefits for animal and medicine: role of phenolics and terpenoids in avoiding oxygen stress. Plant Physiol Bioch. 2002;40(6-8):471-8. https://doi.org/10.1016/S0981-9428(02)01395-5.
67. Gutierrez-Lomeli M, Toro-Sanchez DCL, Rodriguez-Sahagun A, Castellanos-Hernandez OA. Natural products extracts: terpenes and phenolics. In: Orphan IE. editor. Biotechnological Production of Plant Secondary Metabolites. Dubai: Bentham Science Publishers; 2012. p. 21-35. https://doi.org/10.2174/978160805114411201010021.
68. Berger A, Jones PJ, Abumweis SS. Plant sterols: factory affecting their efficacy and safety as functional food ingredients. Lipids Health Dis. 2004;3:1-19. https://doi.org/10.1186/1476-511X-3-5; PMid:15070410 PMCid:PMC419367.
69. Springob K, Kutchan TM. Introduction of the different classes of natural products. In: Osbourn A, Lanzotti V. Plant-derived Natural Products: Synthesis, Function, and Application. New York: Springer; 2009. p. 1-50. https://doi.org/10.1007/978-0-387-85498-4_1.
70. Ananta S, Bishnupada R. Phytochemical screening and antioxidant property of shoot extracts of *Alpinia nigra* (Gaertn.) Burtt (Family – Zingiberaceae). Medicinal Plants – Int J Phytomed Rel Ind. 2015;7(1):48-54. https://doi.org/10.5958/0975-6892.2015.00007.6.
71. Lawand R, Gandhi SV. Comparison of *Curcuma caesia* Roxb. with other commonly used *Curcuma* species by HPTLC. J Pharmacogn Phytochem. 2013;2(4):126-31. http://www. phytojournal.com/vol2Issue4/Issue_nov_2013/6.1.pdf.

72. Donipati P, Sreeramulu SH. Preliminary phytochemical screening of *Curcuma amada*. Int J Pharm Technol. 2015;7(2):9154-60. https://www.ijptonline.com/wp-content/up loads/2015/10/9154-9160.pdf.
73. Hossan AMd, Ibrahim MMd, Ahsan Q, Aktar FMd, Kuddus R, Chowdhury MMU, Rashid MA. Pharmacological and phytochemical screeing of ethanol extract of *Etlingera linguiformis* (Roxb.) R.M. Sm. growing in Bangladesh. Bangladesh Pharm J. 2013; 16(1):33-7. https://doi.org/10.3329/bpj.v16i1.14488.
74. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun OT. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutat Res-Fund Mol M. 2015;579(1-2):200–13. https://doi.org/10.1016/j.mrfmmm.2005.03.023.
75. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. J Taibah Univ Sci. 2015;9(4):449-54. https://doi.org/10.1016/j.jtusci.2014.11.001.
76. Cai YZ, Sun M, Xing J, Luo Q, Corke H. Structure-radical scavenging activity relationship of phenolic compounds from traditional Chinese medicinal plants. Life Sci. 2006; 78(25):2872-88. https://doi.org/10.1016/j.lfs.2005.11.004; PMid:16325868.
77. Ebanks JP, Wickett RR, Boissy RE. Mechanisms regulating skin pigmentation: the rise and fall of complexion coloration. Int J Mol Sci. 2009;10(9):4066-87. https://doi.org/10.3390/ijms10094066; PMid:19865532 PMCid:PMC2769151.
78. Yamaguchi Y, Brenner M, Hearing VJ. The regulation of skin pigmentation. J Biol Chem. 2007;282(38):27557-61. https://doi.org/10.1074/jbc.R700026200; PMid:17635904.
79. Koodkaew I, Sukonkhajorn P. Anti-tyrosinase and antioxidant activities of Impatiens balsamina L. Songklanakarin J Sci Technol. 2019;41(3):686-92. https://rdo.psu.ac.th/ sjstweb/journal/41-3/27.pdf.
80. Hashemi F, Zarei M A. Tyrosinase inhibitory activity within hexane extract of ten screened plants from Kurdistan province of Iran. Int J Adv Biol Res. 2014;2(11):2795-5. http://www.ijabbr.com/article_10886_4ef14b64d0e4ea3033b78acda20bb64f.pdf.
81. Bendich A, Machlin LJ. Scandurra O, Burton GW, Wayner DDM. The antioxidant role of vitamin C. Adv Free Radical Biol Med. 1986;2(2):419-44. https://doi.org/10.1016/S8755-9668(86)80021-7.
82. Pehlivan FE. Vitamin C: an antioxidant agent. In: Hamza AH. editor. Vitamin C. Rijeka:InTech; 2017. p. 25-35. https://doi.org/10.5772/intechopen.69660.

GRAPHICAL ABSTRACT



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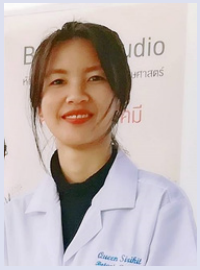

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