Current Applied Science and Technology Vol. 21 No. 3 (July-September 2021)

Tyrosinase Inhibiting Extracts from Coastal Plants as Potential Additives in Skin Whitening Formulations

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Received: 6 July 2020, Revised: 23 September 2020, Accepted: 19 January 2021

Abstract

Coastal plants produce more secondary metabolites than normal terrestrial plants and this means that they may possess many bioactive compounds that are worth studying. The study described antioxidant, *in vitro* tyrosinase inhibition activity and cytotoxicity of six coastal plants including *Sonneratia alba, Rhizophora apiculata, Syzygium grande, Rhizophora mucronata, Hibiscus tiliaceus* and *Bruguiera gymnorhiza*. Leaves of these coastal plants were sequentially extracted using dichloromethane (DE), ethyl acetate (EE), acetone (AE) and methanol (ME). Most AE and ME extracts exerted high free radical scavenging activity which ranged from 124 to 454 mg AA/g extract. However, *R. apiculata, R. mucronata* and *H. tiliaceus* showed stronger *in vitro* tyrosinase inhibition, in which *H. tiliaceus* DE exhibited the lowest IC_{50} value, followed by *R. mucronata* DE and *R. apiculata* ME. Tested with B16F1 murine melanoma cells, 9 out of 24 extracts studied for 24 h incubation and 4 out 24 extracts for 72 h incubation showed low cytotoxic. Tyrosinase inhibiting extracts with low cytotoxicity such as *H. tiliaceus* are potential additives in skin-whitening formulations and should be further studied for their melanogenesis inhibition.

Keywords: Malaysian coastal plants; antioxidant; anti-tyrosinase; cytotoxicity; B16F1 murine melanoma cell
DOI 10.14456/cast.2021.48

1. Introduction

Coastal forests are considered a vital natural heritage of Malaysia as much of Malaysia's rural population living along its 4,675 km coastline are dependent on coastal forests for their livelihood. The role of mangroves, as nursery and feeding areas, was well documented by Jusoff [1] and

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Neamsuvan *et al*. [2]. Despite their great diversity and ecological function, coastal forests are under constant threat of being converted into oil palm estates [3].

Coastal forests occur in relatively harsh condition characterised by high salinity and intense UV radiation. In order to survive in difficult environment, coastal plants produce more secondary metabolites than normal terrestrial plants [4, 5]. This makes the coastal plants good candidates for bioprospecting which can provide an additional reason for preserving coastal forests. Such efforts are in line with Goals 9 and 14 of the UN Sustainable Development Goals. Wood from coastal plants, including the trunk and the woody roots of mangroves, are often used for firewood and charcoal production, construction of dwellings as well as production of furniture, boats, and fishing gear. The leaves are often discarded as waste and they can be safely harvested without felling the tree. Thus, the leaves of the coastal plants have been used for the study of their bioactivity.

Six coastal plants including *Sonneratia alba*, *Rhizophora apiculata*, *Syzygium grande*, *Rhizophora mucronata*, *Hibiscus tiliaceus* and *Bruguiera gymnorhiza* were used in this study (Figure 1). *Sonneratia alba*, also known as Mangrove apple, belongs to family Sonneratiacea. It has rounded and leathery leaves and can be found in the Pacific [6]. *Rhizophora apiculata* and *R. mucronata* are the major species of *Rhizophora*. In Malaysia, *R. apiculata* and *R. mucronate*, locally known as Bakau minyak and Bakau kurap, are the famous mangrove plant widely used in charcoal industry [5].

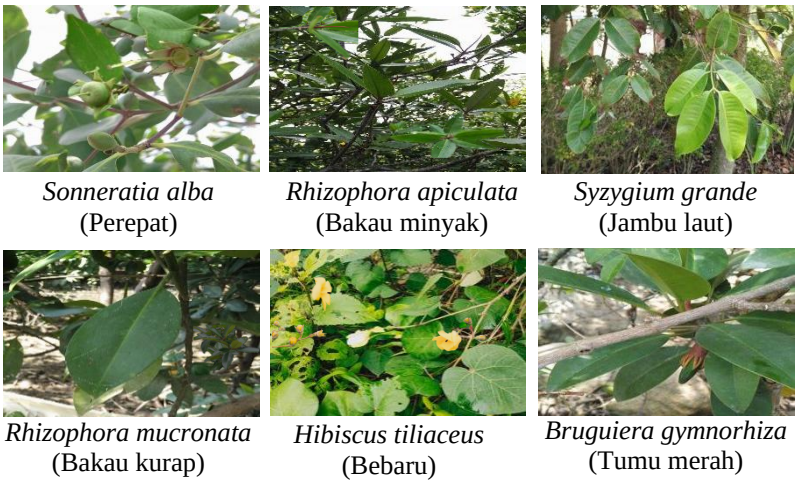


Figure 1. Photos of selected coastal plants taken from the field

Sonneratia grande, also known locally as Sea apple or Jambu laut, is a common coastal plant that is often used for roadside plantings. *Hibiscus tiliaceus*, or Sea hibiscus, is an evergreen plant which normally grows up to 3-10 meters in height [7]. *Bruguiera gymnorhiza* or Tumu merah is a widespread mangrove species in the Pacific [6]. Some parts, like fruits, leaves and shoots of these chosen coastal plants, are edible. Some of these coastal plants were reported to have medicinal and bioactive properties such as antimicrobial, anti-tumour and anti-depressant activities. This may be related to the wealth of bioactive phytochemicals such as flavonoids, triterpenes, phenols and hydrocarbons present in the selected plants [5, 7].

Tyrosinase is an important enzyme which catalyzes the initial steps of melagenic pathway. It is involved in the formation of ortho-quinone, which then undergoes other reactions and forms melanin, causing skin darkening [8]. To prevent the formation of melanin, tyrosinase inhibition is

the safest way to inhibit melanogenesis. In the literature, antioxidant and anti-tyrosinase properties of several coastal plants, for example, biflavonoids isolated from *Garcinia subelliptica* exhibited strong anti-tyrosinase activity [9, 10]. Possible tyrosinase inhibition ability of the coastal plants may add to the growing body of knowledge on the therapeutic application of coastal forests and promote their conservation.

Solvent extraction is a common technique for sample preparation and involves separation of compounds [11]. The abundant phytochemicals found in these coastal plants can be separated by using different extraction solvents. In this study, we intend to compare the antioxidant and anti-tyrosinase activities of leaves from selected Malaysian coastal plants extracts using different extraction solvent. Leaves are ideal for bioprospecting as they can be safely harvested without felling the tree. The safety of the plant extracts will also be determined based on the cytotoxicity of the extracts on B16F1 murine melanoma cells.

2. Materials and Methods

2.1 Plant Materials

Six coastal plants were chosen for this study: *Sonneratia alba* Sm., *Rhizophora apiculata* Blume., *Syzygium grande* (Wight) Walp., *Rhizophora mucronata* Lam., *Hibiscus tiliaceus* Linn., and *Bruguiera gymnorhiza* (L.) Lam. They were sampled from the vicinity of Pulau Indah and Teluk Gong, Selangor, Malaysia and authenticated by a botanist, Dr. H.T. Chan. All reagents and solvents used in this study were of analytical grade.

2.2 Plant extraction and preparation

The mangrove leaves were dried in an oven at 50°C and ground into powder. For the determination of mode of inhibition of the plants, methanol (300 ml) was used to extract the powdered leaf samples (15 g) followed by continuous swirling for 1 h at room temperature. This was done at a 20:1 (v/w) solvent to sample ratio. For subsequent analysis for determining the tyrosinase inhibition, antioxidant properties and cell viability, a sequential extraction was used to fractionate the phytochemicals according to polarity. The sequential extraction used the following solvents in order: dichloromethane (DE), ethyl acetate (EE), acetone (AE) and methanol (ME), using the same solvent to sample ratio of 20:1 (v/w). All extracts were dried using a rotary evaporator to yield an amorphous solid. The extracts were oven-dried until constant weight and stored at room temperature. A stock solution of 10 mg/ml of each dried extract was then prepared using DMSO and used for the following tests and assays.

2.3 Phenolic contents

2.3.1 Total phenolic contents

Extracts of four types of solvents were analysed for total phenolic content (TPC) and total flavonoid content (TFC). Total phenolic content (TPC) was assayed using Folin-Ciocalteu (FC) assay with modification [12]. Extracts (21 µl) were mixed with 105 µl of FC reagent (10 times dilution) and 84 µl of sodium carbonate (7.5%, w/v). After 30 min incubation in the dark, absorbance was measured at 765 nm and the result was expressed as gallic acid equivalent (GAE) in mg/g of sample. The standard equation for gallic acid was $y = 0.0054x$ ($R^2 = 0.9997$).

2.3.2 Total flavonoid contents

Total flavonoid content (TFC) was evaluated using the aluminium chloride assay [13]. Extracts (21 µl) were mixed with 63 µl of methanol, 42 µl of 1% aluminum chloride, 42 µl of 0.1M potassium acetate and 42 µl of ultra-pure water. The assay solutions were incubated at room temperature for 10 min and absorbance was measured at 415 nm. Total flavonoid content was expressed as quercetin equivalent (QE) in mg/g plant material. The standard equation for quercetin was $y = 0.0031x$ ($R^2 = 0.9996$).

2.4 Antioxidant assay

Antioxidant activity of the extracts was examined depending on the ferrous ion chelatig ability (FIC) and free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

2.4.1 Ferrous ion chelating (FIC) ability

FIC ability was measured using the ferrozine assay as described by Chan *et al.* [14]. $FeSO_4$ (0.2 mM, 35 µl) was mixed with different dilutions of extracts (140 µl), followed by ferrozine (0.5 mM, 35 µL). Absorbance (A) was measured at 562 nm after 10 min. Sample blanks comprised appropriate dilutions of sample with $FeSO_4$ but did not have ferrozine added. Chelating ability of extracts (%) was calculated as $(1 - A_s/A_c) \times 100$ (s = sample, c = control). FIC ability was expressed as chelating efficiency concentration (CEC_{50}) in mg/ml.

2.4.2 DPPH assay

The free radical scavenging (FRS) activity of samples was determined following Chan *et al*. [12] with modification. Different dilutions of extracts (70 µl) were added to 140 µl of DPPH (5.9 mg/100 ml methanol). Absorbance was measured at 517 nm after 30 min incubation in the dark and IC_{50} was expressed as ascorbic acid antioxidant capacity (AEAC) in mg ascorbic acid (AA)/g, and calculated as $IC_{50}(a)/IC_{50}(s) \times 10^3$ (a = ascorbate, s = sample).

2.5 Determination of mode of inhibition of the coastal plants

Mode of inhibition of methanol extracted samples was assessed using modified dopachrome method with 3,4-dihydroxy-L-phenylalanine (L-DOPA) as substrate [14]. The assay was conducted in a 96-well microtiter plate using a plate reader to measure absorbance (A) at 475 nm with 700 nm as reference. Forty µl of sample (125-500 µg/ml) in 80 µl of phosphate buffer (0.1M, pH 6.8), 40 µl of mushroom tyrosinase (31 units/ml), and 40 µl of L-DOPA (2.5-10 mM) were mixed and absorbance was measured spectrophotometrically at 20 s intervals up to 300 s. Lineweaver-Burk plot was plotted for both enzyme activities without inhibitors and with inhibitors. K_i value and types of inhibition for each inhibitor was identified based on the Lineweaver-Burk plot.

2.6 *In vitro* tyrosinase inhibition assay

Tyrosinase inhibition activity (TIA) of total 24 samples was assessed using the dopachrome method which was mentioned earlier with slight modification [15]. Samples were dissolved in 50% dimethyl sulphoxide (DMSO) and 40 µl of sample was added to 80 µl of 0.1 M phosphate buffer at pH 6.8 with 40 µl of mushroom tyrosinase (31 units/ml). After incubated at 37°C for 1 min, 40 µl of L-DOPA (2.5 mM) was added and absorbance was measured at 475 nm with 700 nm as reference after a 30-min incubation. Each sample was accompanied by a blank that had all the components except

L-DOPA. Results in percentage inhibition (%) were expressed as half-maximal inhibition (IC_{50}) value. Kojic acid was used as a positive control.

2.7 Cell viability assay

The B16F1 murine melanoma cell lines (ATCC® CRL-6323™), obtained from the American Type Culture Collection (USA), were cultured in DMEM supplemented with 5% fetal bovine serum and 1% penicillin (10,000 µg/ml)/ streptomycin (10,000 µg/ml). The cells were placed in CO_2 incubator at 37°C to reach a confluent monolayer.

Cell viability assay of all the samples was performed using 3-(4,5-imethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) as described by Sharif *et al*. [16] with modification. Cells were first seeded in a 96-well plate with 10,000 cells in 0.2 ml growth medium per well and were placed overnight in the incubator. The cells were treated with plant extracts (100 µg/ml) for 24 h and 72 h. Viability of the cells was determined by adding MTT solution (0.5 mg/ml) followed by incubation for 3 h. DMSO was used to solubilise the MTT derivatives and absorbance was measured at 570 nm. A negative control was included to determine cell growth in the absence of any treatment. Cells viability (%) was calculated as (A_{570} of treated cells/A_{570} of control) x 100 and the results were expressed as half-maximal inhibitory concentration (IC_{50}) values.

2.8 Statistical analysis

All experiments were done in triplicate (n = 3) and results were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was analysed using the Tukey Honestly Significant Difference (HSD) test with significant difference at $p < 0.05$. Correlation between different variables were expressed as Pearson's correlation. All the analysis was done using IBM SPSS Statistics 22.

3. Results and Discussions

3.1 Extraction efficiency

All coastal plant leaf sample were extracted with solvents of increasing polarity which were DE, EE, AE and ME (with polarity index 3.1, 4.4, 5.1 and 5.1, respectively) [17]. By using these solvents, compounds of varying polarity were extracted. Methanol extracts were found to obtain higher yield than all other extracts. Methanol extracts of all the coastal plants, except for *S. grande* and *H. tiliaceus*, exhibited more than 15% yield while the other extracts exhibited less than 5% yield. This may be due to the high solubility parameter of methanol in which most of the leftover compounds were soluble and extracted in methanol [17]. Despite having the same polarity index, acetone is less able to extract polar compounds as it only has a single carbonyl oxygen as a hydrogen bond acceptor and no hydrogen bond donor.

3.2 Phenolic contents

Phenolic contents assays include total phenolic contents and total flavonoid contents of the coastal plant extracts as shown in Table 1.

Table 1. Phenolic content and antioxidant activity of coastal plant extracts

Coastal Plants	Extract	Phenolic contents		Antioxidant activity	
		TPC (mg GAE/g)	TFC (mg QE/g)	CEC_{50} (mg/ml)	AEAC (mg AA/g)
Sonneratia alba	DE	22.92 ± 2.22^{dA}	58.31 ± 5.81^{bC}	0.215 ± 0.02^{aD}	9.472 ± 0.13^{dC}
	EE	56.88 ± 2.51^{cA}	75.73 ± 6.65^{aA}	0.179 ± 0.02^{bE}	40.71 ± 0.84^{cB}
	AE	383.0 ± 15.8^{aA}	30.84 ± 2.03^{cC}	0.097 ± 0.01^{cE}	190.9 ± 10.0^{bC}
	ME	186.8 ± 13.4^{bA}	11.26 ± 1.06^{dB}	0.129 ± 0.01^{cE}	357.1 ± 31.1^{aB}
Rhizophora apiculata	DE	8.025 ± 0.72^{dD}	54.62 ± 4.78^{bC}	0.348 ± 0.02^{cB}	17.62 ± 0.59^{cA}
	EE	41.73 ± 2.96^{cB}	75.48 ± 7.30^{aA}	0.979 ± 0.05^{aB}	36.22 ± 1.20^{cC}
	AE	122.0 ± 11.8^{bD}	54.84 ± 5.10^{bA}	0.983 ± 0.08^{aB}	123.6 ± 4.56^{bD}
	ME	184.7 ± 12.3^{aA}	11.82 ± 1.08^{cB}	0.582 ± 0.04^{bC}	327.7 ± 20.3^{aB}
Syzygium grande	DE	15.43 ± 1.31^{dB}	46.48 ± 4.24^{aC}	0.433 ± 0.01^{aA}	9.802 ± 0.43^{cC}
	EE	46.23 ± 2.79^{cB}	49.67 ± 3.65^{aC}	0.048 ± 0.00^{dF}	27.78 ± 2.19^{cD}
	AE	234.4 ± 17.6^{aB}	45.31 ± 4.32^{aB}	0.316 ± 0.01^{bD}	352.6 ± 15.2^{bA}
	ME	172.0 ± 15.5^{bAB}	11.74 ± 1.14^{bB}	0.115 ± 0.01^{cE}	453.6 ± 46.0^{aA}
Rhizophora mucronata	DE	10.21 ± 0.90^{dCD}	12.23 ± 8.94^{cD}	0.297 ± 0.03^{cC}	9.239 ± 0.33^{dC}
	EE	58.98 ± 3.20^{cA}	53.37 ± 5.01^{aBC}	1.232 ± 0.07^{aA}	51.54 ± 3.98^{cA}
	AE	203.4 ± 14.9^{aC}	34.23 ± 2.92^{bC}	0.812 ± 0.05^{bC}	260.1 ± 20.6^{aB}
	ME	156.7 ± 15.6^{bB}	10.89 ± 0.88^{cB}	0.878 ± 0.05^{bA}	175.0 ± 15.4^{bC}
Hibiscus tiliaceus	DE	10.87 ± 1.07^{dC}	85.36 ± 8.12^{aA}	0.096 ± 0.01^{cE}	11.64 ± 0.88^{dB}
	EE	31.12 ± 3.04^{cC}	62.08 ± 5.29^{bB}	0.468 ± 0.03^{bD}	15.28 ± 0.20^{cE}
	AE	55.31 ± 1.88^{bE}	45.12 ± 3.75^{cB}	0.933 ± 0.05^{aB}	34.70 ± 0.84^{bE}
	ME	150.8 ± 8.29^{aBC}	5.740 ± 0.68^{dC}	0.403 ± 0.03^{bD}	153.0 ± 3.15^{aC}
Brugueira gymnorhiza	DE	14.14 ± 1.25^{cB}	70.49 ± 6.84^{aB}	0.199 ± 0.02^{dD}	8.990 ± 0.26^{cC}
	EE	26.62 ± 1.61^{bC}	54.49 ± 5.28^{bBC}	0.693 ± 0.02^{cC}	15.16 ± 0.29^{cE}
	AE	139.2 ± 10.7^{aD}	54.06 ± 5.34^{bA}	1.266 ± 0.06^{aA}	143.7 ± 7.24^{bD}
	ME	130.9 ± 5.60^{aC}	17.25 ± 1.86^{cA}	0.787 ± 0.06^{bB}	155.7 ± 6.23^{aC}

Values represented mean ± SD (n = 3), $p < 0.05$. Within each column of each species, different superscript letters (a-d) and (A-F) indicates significant difference of different plant extracts at $p < 0.05$. Abbreviations: DE = dichloromethane extract, EE = ethyl acetate extract, AE = acetone extract, ME = methanol extract, TPC = total phenolic contents, TFC = total flavonoid contents, CEC_{50} = chelating efficiency concentration, AEAC = ascorbic acid antioxidant capacity

3.2.1 Total phenolic content (TPC)

Natural antioxidants are widely found in medicinal plants in the form of phenolic compounds. According to Table 1, *R. mucronata* AE showed the highest TPC value (364 mg GAE/g extract),

followed by *S. grande* AE (300 mg GAE/g extract) and *H. tiliaceus* ME (261 mg GAE/g extract). Significant difference was observed when different extraction solvents were used.

Most AE and ME showed high total phenolic content which was more than 50 mg GAE/g extract. This is in accordance with Barchan *et al.* [18] in which polar solvents were more effective in extracting phenolic compounds. This indicates that most phenolic compounds are more polar. Leaves of *H. tiliaceus* and *S. grande* were said to possess higher TPC values than the other plants such as *Hibiscus sabdariffa*, which ranged from 18-30 mg GAE/g extract [19] as well as *Psidium guayaquilense and Psidium rostratum*, which ranged from 6-9 mg GAE/g extract [20]. This suggested that these coastal plants could be a good source of phenolic compounds and antioxidants.

3.2.2 Total flavonoid content (TFC)

For TFC values, the three extracts with the highest TFC values were *H. tiliaceus* DE (85 mg QE/g extract), *S. alba* EE (76 mg QE/g extract) and *R. apiculata* EE (75 mg QE/g extract). For all the coastal samples except *S. grande*, significant difference was obtained for different solvents used. However, no significant difference was observed between different coastal plants using the same extraction solvent.

Flavonoids are regarded as one of the main types of phenolic compounds. However, in contrast with the TPC, most of the ME and AE showed lower TFC values. This indicates that the phenolic compounds of the extracts could be other types of phenolic compounds [21]. The TFC values are comparable to those reported in the leaves of *Eupatorium adenophorum*, which ranged from 19-32 mg QE/g extract and *Cucumis melo*, which was 30 mg QE/g extract [22, 23].

3.3 Antioxidant activity

The antioxidant activity of coastal samples was accessed by FIC assay (CEC_{50}) and DPPH assay (AEAC), and the results are summarised in Table 1. In terms of FIC activity, *S. grande* EE extract showed the lowest CEC_{50}, which was 0.05 mg/ml, followed by *S. alba* AE and *H. tiliaceus* DE, which both exhibited CEC_{50} of 0.10 mg/ml. No fixed pattern was observed for CEC_{50} value of coastal plants extracted by different solvents. For DPPH assay, *S. grande* ME, *S. alba* ME and *S. grande* AE exhibited the top three highest radical scavenging activities with AEAC of 454, 357 and 353 mg AA/g extract, respectively. Most of the AE and ME showed higher antioxidant activity in DPPH assay, which was similar to TPC assay. The antioxidant properties of most of the extracts were comparable to those reported by Silva and Sirasa [24] which ranged from 1-27 mg AA/g.

Phenolic compounds are well known for antioxidant activity due to their capacity to act as reducing agents, hydrogen donors and singlet oxygen quenchers [25]. In correlation analysis, AEAC was positively correlated with TPC with correlation coefficient of r = 0.965 (Figure 2). This indicates that the ability to quench DPPH free radicals increases when phenolic content increases, but levels off to a flat plateau at high phenolic content. While AEAC have weak negative correlation with TFC (r = -0.632) which indicates that flavonoids contributed less or no antioxidant power to the antioxidant capacity of coastal plant extracts. TPC have a weak correlation with TFC (r = -0.534), which might be because flavonoids are just a group of phenolic compounds that consist of other compounds such as phenolic acid, tannins and lignans [26].

However, there was no correlation between AEAC and CEC_{50} (r = -0.087). This might be due to the potential of the phytochemicals to act as free radical quencher but this does not necessarily equal to their ability as metal ion chelator. This could be further examined as no relationship was obtained between CEC_{50} with TPC (r = -0.029) and TFC (r = 0.038). This finding was in accordance with the results reported by Ngo *et al.* [27].

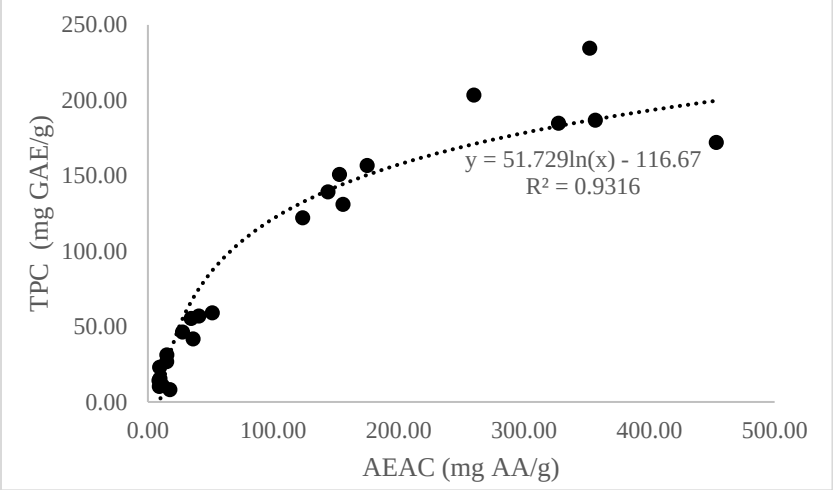


Figure 2. Correlation graph of total phenolic content (TPC) and free radical scavenging (FRS) activity of different coastal plant extracts

3.4 *In vitro* tyrosinase inhibition assay

Table 2 summarises the *in vitro* tyrosinase inhibition activity based on IC_{50} values, and types of inhibition of plant extracts. Tyrosinase inhibition percentage increased in dose-dependent manner as inhibition percentage increased with increased extract concentration. Lower IC_{50} value indicates higher tyrosinase inhibition capacity. It was found that *H. tiliaceus* DE inhibited the tyrosinase enzyme the most with the lowest IC_{50} value (0.13 mg/ml), followed by *R. mucronata* DE (0.15 mg/ml) and *R. apiculata* ME (0.28 mg/ml). Tyrosinase inhibition properties of these three extracts were comparable with α-mangostin reported by Hassan *et al.* [28], but they were much lower than positive control (kojic acid) used in this study. All EE exhibited low inhibition towards tyrosinase, which suggested that ethyl acetate is not a good solvent for extracting tyrosinase inhibiting compounds.

In general, antioxidants were said to be possible tyrosinase inhibitor as they can prevent the initial reaction of tyrosinase by reacting with oxygen. They can also reduce *o*-quinone back to *o*-diphenol [29]. However, there was no correlation between antioxidant and anti-tyrosinase activity (r = -0.271). For example, *H. tiliaceus* DE which is the best tyrosinase inhibitor among all the extracts, exhibited low AEAC (12 mg AA/g extract). This indicates that the tyrosinase inhibiting compounds may not possess antioxidant properties. Melecchi *et al.* [30] reported on a number of compounds in dichloromethane fractionated extract from *H. tiliaceus*. However, bioactivity guide fractionation and structural elucidation would be required to link these compounds to specific bioactivity.

In terms of type of inhibition, inhibitors can be categorized into competitive, non-competitive, uncompetitive as well as mixed inhibitors. This can be determined by the Lineweaver-Burk plot in which the plot of *H. tiliaceus* was shown in Figure 3. Competitive inhibitors commonly bind to enzymes at the substrate binding site, preventing the interaction of the enzymes with substrates while non-competitive inhibitors bind enzymes at the site other than active site to inhibit the enzyme. Uncompetitive inhibition occurs when inhibitors bind to enzyme-substrate complex and enhance the binding of substrates to enzymes, results in decreasing of the rate of reaction. Mixed inhibitors resemble the activities of both competitive and uncompetitive inhibitors [31].

Table 2. *In vitro* tyrosinase inhibition activity of different coastal plant extracts expressed in IC_{50} value (mg/ml) and the type of inhibition identified.

Coastal plant	DE	EE	AE	ME	K_i (mg/ml)	Type of inhibition
Sonneratia alba	0.60 ± 0.05^{bA}	1.78 ± 0.06^{aB}	0.55 ± 0.04^{bC}	0.65 ± 0.06^{bC}	0.45	Un-competitive
Rhizophora apiculata	0.60 ± 0.03^{bA}	1.21 ± 0.11^{aD}	0.64 ± 0.06^{bB}	0.28 ± 0.02^{cE}	0.14	Non-competitive
Syzygium grande	0.44 ± 0.04^{bcB}	1.94 ± 0.05^{aA}	0.38 ± 0.02^{cD}	0.48 ± 0.03^{bD}	0.38	Un-competitive
Rhizophora mucronata	0.15 ± 0.01^{cC}	1.37 ± 0.14^{aC}	0.66 ± 0.04^{bB}	0.79 ± 0.04^{bB}	0.33	Non-competitive
Hibiscus tiliaceus	0.13 ± 0.01^{cC}	0.74 ± 0.07^{aE}	0.54 ± 0.04^{bC}	0.46 ± 0.05^{bD}	0.60/0.20	Mixed
Brugueira gymnorhiza	0.62 ± 0.02^{cA}	1.48 ± 0.05^{aC}	1.30 ± 0.08^{bA}	1.30 ± 0.08^{bA}	0.62	Un-competitive
Control (kojic acid)	0.01 ± 0.00					

Values represent mean ± SD (n = 3), $p < 0.05$. Within each column of each species, different superscript letters (a-c) and (A-E) indicates significant difference of different solvents and types of plant at $p < 0.05$. Abbreviations: DE = dichloromethane extract, EE = ethyl acetate extract, AE = acetone extract, ME = methanol extract

Rhizophora apiculata and *R. mucronata* are non-competitive inhibitors and *H. tiliaceus* is a mixed-type inhibitor. Non-competitive inhibitor may be good for the future application as it works regardless of substrate concentration whereas mixed-type inhibitor indicates that there may be more than one type of inhibitors (active compounds) in the extracts [32]. Among all the coastal plants, *R. apiculata* was found to exhibit lowest K_i value (0.14 mg/ml) which indicated the strongest binding towards the enzyme. This may be because *R. apiculata* non-competitively inhibited the enzyme, binding to a site other than the active site [33].

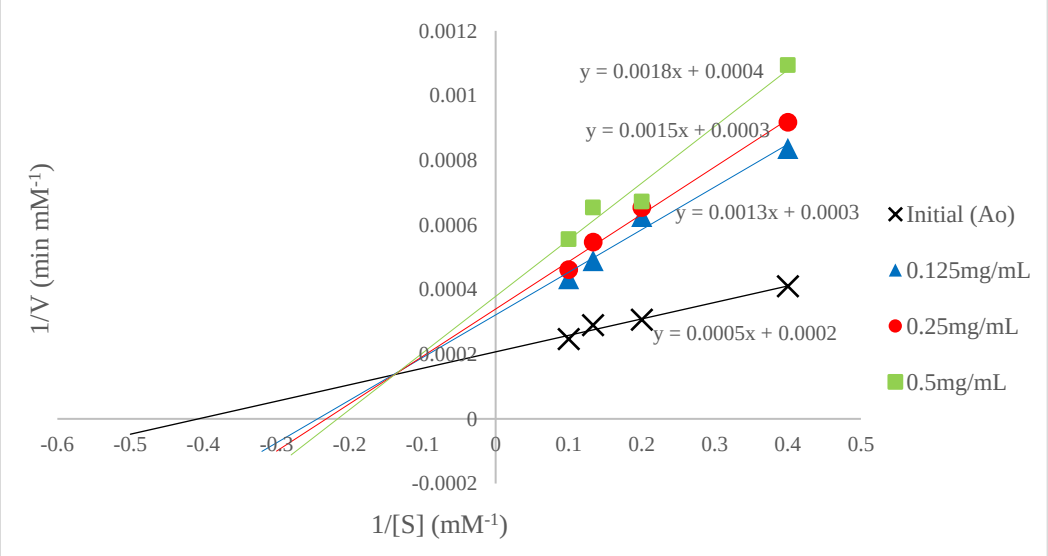


Figure 3. Tyrosinase inhibitory effect of *Hibiscus tiliaceus*

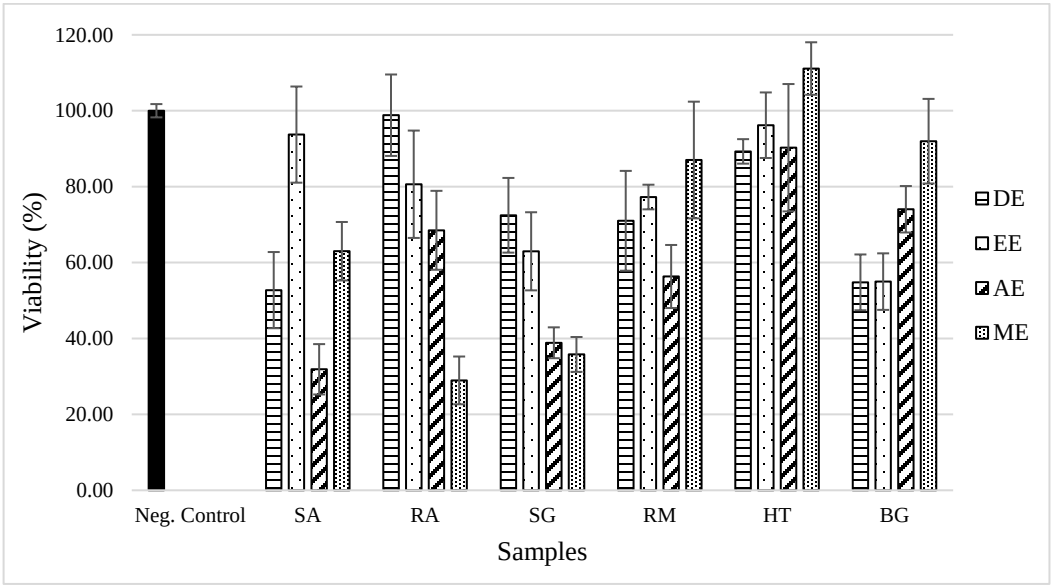
3.5 Cell viability assay

No studies of cytotoxicity on B16F1 murine melanoma cells have been done on the selected coastal plants. It is necessarily to determine the cytotoxicity of the extracts because they may lead to inflammation. The cytotoxicity of all the extracts were screened against B16F1 murine melanoma cells for 24 and 72 h, and the results obtained were reported in Figure 4. In the study of cytotoxicity of the extracts, higher cell viability was preferred. From the results of 24 h, it was noticed that 9 out of 24 extracts had more than 80% cell viability which is considered as low cytotoxic. The most promising extracts for 24 h were *H. tiliaceus* ME (111%), *R. apiculata* DE (99%) and *H. tiliaceus* EE (96%). For MTT assay for 72 h, only 4 out of 24 extracts exhibited more than 80% cell viability.

From anti-tyrosinase study, *R. apiculata* ME, *R. mucronata* DE and *H. tiliaceus* DE were more potent inhibitors, however, only *H. tiliaceus* DE had more than 80% cell viability for 24 h which is good to be further studied. Interestingly, *R. apiculata* ME showed an increased cell viability (83%) for 72 h. Weak positive correlation was obtained for cell viability of 24 h and 72 h ($r = 0.573$), which inferred that not all samples had decresed cell viability from 24 h to 72 h. This was explained by Prakash *et al.* [34], whose work indicated that the variation in cytotoxicity was with respect to the mechanism of the plant extract.

Some extracts such as *R. apiculata* ME and *S. alba* AE exhibited cytotoxic potential towards melanoma cells with IC_{50} value lower than 100 µg/ml. This is comparable with *Kalanchoe laciniata* (322-639 µg/ml) and *Bryophyllum pinnatum* (552 µg/ml) which were addressed by Sharif *et al.* [16]. Therefore, it is recommended to extend the study on these extracts for their anti-carcinogenic effect.

a)



b)

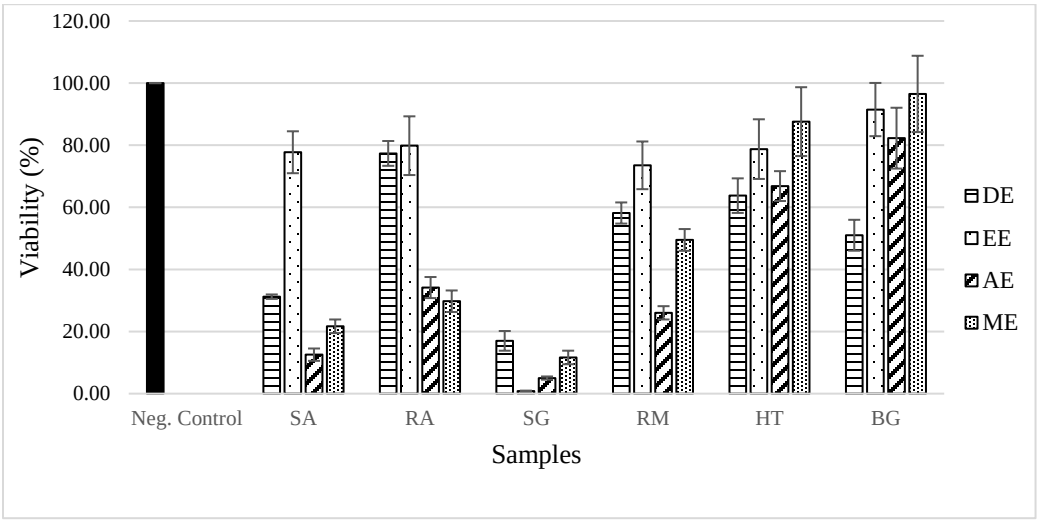


Figure 4. Cell viability in percentage against samples in 100 µg/ml for: a) 24 h and b) 72 h Abbreviations: SA = *S. alba*, RA = *R. apiculata*, SG = *S. grande*, RM = *R. mucronata*, HT = *H. tiliaceus*, BG = *B. gymnorhiza*, DE = dichloromethane extract, EE = ethyl acetate extract, AE = acetone extract, ME = methanol extract. Values represent mean ± SD (n = 3), $p < 0.05$

4. Conclusions

In summary, different bioactive constituents from the six selected coastal plants were extracted with different solvents according to their polarity. It can be concluded that AEAC of the extracts was positively correlated with TPC. Most of the AEs and MEs showed high TPC and DPPH radical scavenging activity, but did not show high TFC and FIC abilities. *Hibiscus tiliaceus* DE, *R. mucronata* DE and *R. apiculata* ME were found to possess high anti-tyrosinase properties. Out of these three extracts, *H. tiliaceus* DE exhibited the lowest cytotoxicity on B16F1 murine melanoma cells, and is thus considered the safest to use. Tyrosinase-inhibiting extracts with low cytotoxicity are potential additives in skin-whitening formulations and should be further studied for their melanogenesis inhibition, and subsequent pre-clinical skin irritation and absorption studies.

5. Acknowledgements

The authors would like to thank Dr H.T. Chan from International Society for Mangrove Ecosystems, Faculty of Agriculture, University of the Ryukyus, Japan who helped for the determination of coastal plants. The author would also like to express their gratitude to the Centre of Excellence for Research, Value Innovation and Entrepreneurship (CERVIE) for the financial support of this study under Pioneer Scientist Incentive Fund (PSIF) with the code of Proj-In-FAS-052, UCSI University, Malaysia.

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