20 µg/mL >30%, including *Artocarpus altilis* (branch), *A. integer* (branch), *A. chama* (wood), *A. chama* (stem), *Ficus benghalensis* (branch), *F. benghalensis* (wood), *Ficus foveolata* (wood), *Ficus superba* (leaf), *Morus alba* (branch), *M. alba* (leaf), *Streblus ilicifolius* (wood), *S. taxoides* (wood). The wood extracts of *S. taxoides* and *S. ilicifolius* showed the highest tyrosinase inhibition with 58.59% ±1.90% and 69.05% ±5.00%, respectively. However, chemical constituents and biological activities; anti-tyrosinase and antimicrobial activities were already reported for *S. ilicifolius*.[18] Only, the extracts of *S. taxoides* and *A. chama* which showed the potential activity against tyrosinase enzyme were selected for further study and the following successive extraction was used.

The dried materials were extracted with petroleum ether, ethyl acetate, methanol and water, respectively. Percent antityrosinase activities of these crude extracts are shown in Table 2. The ethyl acetate and methanol extract of *S. taxoides* wood and *A. chama* stem showed the most potent effects against the tyrosinase enzyme.

Cell viability

From the results of the enzymatic investigation, several extracts from *S. taxoides* wood and *A. chama* stem showed antityrosinase activity. Thus, the investigations were extended to cellular experiments. First, the extracts were determined for the inhibition of melanogenesis on cultured melanocytes. Then, the effect from the extracts on cell viability was measured. The results indicated that all sample extracts were not considerable cytotoxic in B16-F1 melanoma cells. Cell viability was still >80% at the concentration 100 µg/mL except ethyl acetate extracts of *A. chama* and *S. taxoides,* cell viability was >80% at the concentrations of 5 and 50 µg/mL, respectively.

Intracellular antityrosinase activity and melanin content

The effect of intracellular antityrosinase activity and melanin content on B16F1 melanoma cells were determined. The extracts were prepared at 100 µg/mL except ethyl acetate extract of *A. chama* was prepared at 5 µg/mL and ethyl acetate extract of *S. taxoides* was prepared at 50 µg/mL which followed the concentrations of cell viability results. After 48 h incubation with all sample extracts, the supernatant were measured antityrosinase activity, the results showed that the extracts from *A. chama* and *S. taxoides* exhibited antityrosinase activity, especially ethyl acetate extract from both plants which were prepared at lower concentration [Figure 1]. The ethyl acetate extract of *A. chama* showed 64.41% ±1.27% inhibition at 5 µg/mL, while the ethyl acetate extract of *S. taxoides* showed 54.37% ±1.55% inhibition at 50 µg/mL. Due to the inhibition of tyrosinase enzyme would result to reduce melanin content which results obtained as shown in Figure 1.

Table 2: Enzymatic anti-tyrosinase activity of petroleum ether, ethyl acetate, methanol and water extracts of *Streblus taxoides* wood and *Artocarpus chama* stem at 20 µg/mL

Plant	Sample extract	Percentage anti-tyrosinase activity
A. chama	Petroleum ether	9.35±5.29
	Ethyl acetate	77.53±2.17
	Methanol	70.29±3.24
	Water extract	17.35±1.90
S. taxoides	Petroleum ether	7.36±0.66
	Ethyl acetate	57.15±3.33
	Methanol	75.53±0.48
	Water extract	13.74±5.32
	Water extract of *A. lakoocha*[P]	91.96±0.97
	Kojic acid[P]	84.38±1.54

[P]Positive control. *S. taxoides*: *Streblus taxoides*; *A. lakoocha*: *Artocarpus lakoocha*; *A. chama*: *Artocarpus chama*

Pigmentation inhibitory activity on zebrafish

Zebrafish was used for screening of pigmentation inhibitory effect by measuring the size of black spot on zebrafish. The results showed that at concentration 200 µg/mL [Figure 2a] extracts could inhibit pigmentation while petroleum ether extracts of *A. chama* stem could stimulate pigmentation. However, ethyl acetate extract of *A. chama* stem, ethyl acetate, and methanol extracts of *S. taxoides* wood showed toxicity to zebrafish by the detection of coagulation of the embryo, nondetachment of the tail, lack of somite formation, and lack of heartbeat. After decrease, the concentration to 50 µg/mL, ethyl acetate and methanol extracts of *S. taxoides* wood suppressed the pigmentation on zebrafish [Figure 2b].

Determination of antimicrobial activity

From the screening of antimicrobial activity, the ethanol extracts of 48 Moraceae plant samples displayed an inhibition zone against *S. aureus, S. epidermidis, P. acnes,* and MRSA as shown in the Table 3. Then, the extracts from selected Moraceae plants, *S. taxoides* and *A. chama* were determined of MIC and MBC (half-fold dilution; 15.625–2000 µg/ml as the results are shown in Table 4. The results showed that only the ethyl acetate extract of *A. chama* stem and *S. taxoides* wood against these microbes by exhibiting the MIC and MBC lower than 2000 µg/mL. Define what value, for each MIC and MBC, was considered as the extract had antimicrobial activity.

DISCUSSION

A. chama stem and *S. taxoides* wood showed potential activity against tyrosinase enzyme both in enzymatic and intracellular assays. In addition, from *in vivo* study, they also showed the inhibition of melanogenesis by suppressing the pigmentation on zebrafish. Since, Moraceae is the most interesting plant family for biological study, especially, antityrosinase activity because the members of this family have been known to produce stilbenoids and flavonoids of various structural types.[9,28,29] Structure-activity relationships with various flavonoids and stilbenes were demonstrated that 4-substituted resorcinol moiety was essential

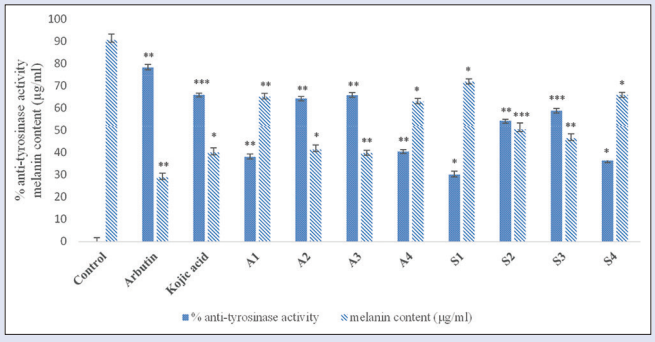

Figure 1: Intracellular anti-tyrosinase activity and melanin content. Arbutin, kojic acid = positive control. A1, A2, A3 and A4 = petroleum ether, ethyl acetate, methanol, water extracts of *Artocarpus chama* stem, respectively. S1, S2, S3 and S4 = petroleum ether, ethyl acetate, methanol and water extracts of *Streblus taxoides* wood, respectively. A1, A3, A4, S1, S3, S4 = 100 µg/mL, A2 = 5 ug/ml, S2 = 50 µg/mL. Data are expressed as mean ± standard deviation from three independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001 indicate a significant difference from control group

Table 3: Anti-bacterial activity of 48 Moraceae plant samples at 2 mg/disc by agar disc diffusion method

Number	Plant	Part	*S. aureus*	*S. epidermidis*	*P. acnes*	MRSA
1	*A. altilis*	Branch	-	-	-	-
2	*A. altilis*	Leaf	8.48±0.16	9.63±0.25	15.15±0.71	7.78±0.46
3	*A. integer*	Branch	-	-	-	-
4	*A. integer*	Leaf	11.20±1.35	-	-	7.78±0.16
5	*A. rigidus*	Branch	10.60±0.62	9.73±0.52	8.80±0.22	6.77±0.25
6	*A. rigidus*	Leaf	-	-	-	-
7	*A. chama*	Wood	-	-	-	-
8	*A. chama*	Bark wood	10.83±0.42	9.75±0.52	9.43±0.15	9.93±0.32
9	*A. chama*	Stem	7.23±0.12	-	7.03±0.10	6.98±0.10
10	*A. chama*	Leaf	-	-	9.15±0.43	7.92±0.16
11	*F. benghalensis*	Branch	10.10±0.36	-	-	9.15±0.05
12	*F. benghalensis*	Leaf	-	-	-	-
13	*F. benghalensis*	Wood	-	-	-	-
14	*F. benghalensis*	Bark wood	11.03±0.49	-	-	9.40±0.40
15	*F. callosa*	Branch	10.53±0.60	-	-	8.47±0.31
16	*F. callosa*	Leaf	-	-	-	-
17	*F. celebensis*	Branch	8.57±0.40	-	-	9.57±0.45
18	*F. celebensis*	Leaf	-	-	-	-
19	*F. chartacea* var. *torulosa*	Branch	9.63±0.25	-	-	6.68±0.10
20	*F. chartacea* var. *torulosa*	Leaf	-	-	-	-
21	*F. foveolata*	Wood	-	10.78±0.96	15.57±0.81	7.42±0.26
22	*F. fistolusa*	Branch	-	-	-	-
23	*F. fistolusa*	Leaf	-	-	-	-
24	*F. hispida*	Branch	-	-	-	9.50±0.56
25	*F. hispida*	Leaf	-	-	-	
26	*F. infectoria*	Wood	-	-	-	-
27	*F. microcarpa*	Branch	-	-	-	8.77±0.85
28	*F. microcarpa*	Leaf	-	-	-	-
29	*Ficus* spp.	Wood	-	-	-	-
30	*Ficus* spp.	Wood	8.03±0.38	6.83±0.60	-	-
31	*Ficus* spp.	Wood	-	-	-	-
32	*Ficus* spp.	Wood	8.67±0.15	6.83±0.31	-	7.02±0.26
33	*Ficus* spp.	Branch	-	-	-	7.63±0.84
34	*Ficus* spp.	Leaf	-	-	-	-
35	*Ficus* spp.	Branch	8.07±0.40	-	-	-
36	*Ficus* spp.	Leaf	-	-	-	-
37	*F. superba*	Branch	-	-	-	-
38	*F. superba*	Leaf	-	-	-	-
39	*F. racemose*	Branch	10.87±0.57	7.27±0.40	-	11.58±0.19
40	*F. racemose*	Leaf	-	-	-	-
41	*F. vasculosa*	Wood	-	-	-	-
42	*F. vasculosa*	Bark wood	9.03±0.35	8.35±0.23	-	7.28±0.30
43	*F. vasculosa*	Leaf	-	-	-	-
44	*M. alba*	Branch	-	-	-	-
45	*M. alba*	Leaf	-	-	-	-
46	*S. ilicifolius*	Leaf	-	-		-
47	*S. ilicifolius*	Wood	8.47±0.31	9.25±0.56	-	-
48	*S. taxoides*	Wood	6.85±0.67	6.70±0.53	7.50±0.45	ND
	Oxacillin[P]		20.93±0.25	21.67±0.61	22.68±0.41	ND
	Vancomycin[P]		ND	ND	ND	14.57±0.06

[P]Positive control. ND: Not determined; -: Inactive; *S. aureus*: *Staphylococcus aureus*; *S. epidermidis*: *Staphylococcus epidermidis*; *P. acnes*: *Propionibacterium acnes*; *A. altilis*: *Artocarpus altilis*; *A. integer*: *Artocarpus integer*; *A. rigidus*: *Artocarpus rigidus*; *A. chama*: *Artocarpus chama*; *F. benghalensis*: *Ficus benghalensis*; *F. callosa*: *Ficus callosal*; *F. celebensis*: *Ficus celebensis*; *F. chartacea*: *Ficus chartacea*; *F. foveolata*: *Ficus foveolata*; *F. fistolusa*: *Ficus fistolusa*; *F. hispida*: *Ficus hispida*; *F. infectoria*: *Ficus infectoria*; *F. microcarpa*: *Ficus microcarpa*; *F. superba*: *Ficus superba*; *F. racemose*: *Ficus racemose*; *F. vasculosa*: *Ficus vasculosa*; *M. alba*: *Morus alba*; *S. ilicifolius*: *Streblus ilicifolius*; *S. taxoides*: *Streblus taxoides*; MRSA: Methicillin-resistant *S. aureus*

for showing the strong inhibitory activity against tyrosinase activity.[30,31] The example of Moraceae plant, which showed the strong tyrosinase inhibition was *Artocarpus lakoocha*. Hence, water extract of the wood from this plant was used as positive control in the anti-tyrosinase assay. It exhibited the highest tyrosinase inhibition with >90%. The potent tyrosinase inhibition of *A. lakoocha* extract consorted with the previous reports as suggested that of oxyresveratrol (2,3',4,5'-tetrahydroxystilbene) seems to justify as the active component to show the high tyrosinase inhibition.[30,31]

S. taxoides wood and *A. chama* stem which demonstrated a capability to inhibit tyrosinase activity and they were described for the first. These plants could represent a potential source of new antityrosinase inhibitor. Further biological investigations on human melanocytes must be done to confirm these activities. Then, the toxicity on B16-F1 melanoma cells was evaluated with these samples. The results showed that they were non-toxic. However, only ethyl acetate extract of both plants showed the activity against bacteria. The isolation and the structural elucidation of the active constituents of these two selected plants will be useful

Table 4: Minimum inhibitory concentration and minimum bactericidal concentration of selected plant extracts (15.625-2000 µg/mL)

Plant	Sample extracts	*S. aureus* (µg/mL)		*S. epidermidis* (µg/mL)		*P. acnes* (µg/mL)		MRSA (µg/mL)	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
A. chama	Petroleum ether	>2000	>2000	>2000	>2000	500	2000	>2000	>2000
	EtOAc	15.625	125	15.625	62.5	31.25	31.25	15.625	31.25
	MeOH	2000	>2000	>2000	>2000	>2000	>2000	1000	>2000
	Water	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
S. taxoides	Petroleum ether	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	EtOAc	125	1000	1000	>2000	15.625	15.625	31.25	500
	MeOH	>2000	>2000	500	>2000	>2000	>2000	500	>2000
	Water	2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
Oxacillin[P]		0.25	0.5	0.5	0.5	0.1	0.2		
Vancomycin[P]								0.5	1.0

[P]Positive control. MIC: Minimum inhibitory concentration; *S. aureus: Staphylococcus aureus;* MBC: Minimum bactericidal concentration; *S. epidermidis: Staphylococcus epidermidis*; *P. acnes: Propionibacterium acnes*; *A. chama: Artocarpus chama*; *S. taxoides: Streblus taxoides*; MRSA: Methicillin-resistant *S. aureus*

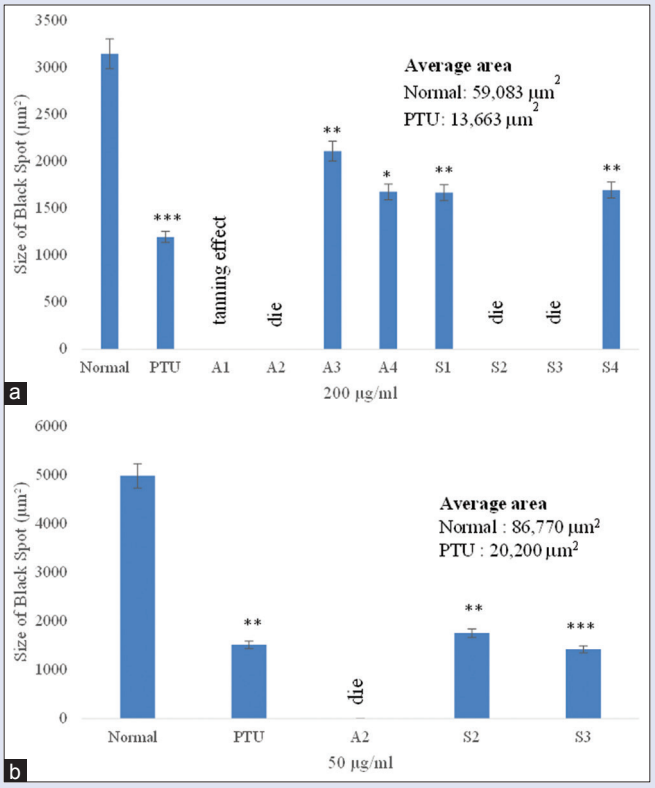

Figure 2: Pigmentation inhibitory on Zebrafish. PTU (phenylthiourea) = positive control. A1, A2, A3 and A4 = petroleum ether, ethyl acetate, methanol, water extracts of *Artocarpus chama* stem, respectively. S1, S2, S3 and S4 = petroleum ether, ethyl acetate, methanol and water extracts of *Streblus taxoides* wood, respectively. (a) At concentration 200 µg/mL, (b) At concentration 50 µg/mL. Data are expressed as mean ± standard deviation from three independent experiments. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 indicate a significant difference from control group

to provide the lead compound in the development of skin-whitening agents.

CONCLUSION

S. taxoides and *A. chama* showed the potency of tyrosinase inhibition and reduction ability of melanin content without cytotoxicity. Then, they will be the are interested plants for further study of chemical constituents and biological activities, especially the antityrosinase activity of the isolated compound to find out the lead compound for whitening agent from natural product.

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Conflicts of interest

There are no conflicts of interest.

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