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ORIGINAL ARTICLE

# Effects of Selected Moraceae Plants on Tyrosinase Enzyme and Melanin Content

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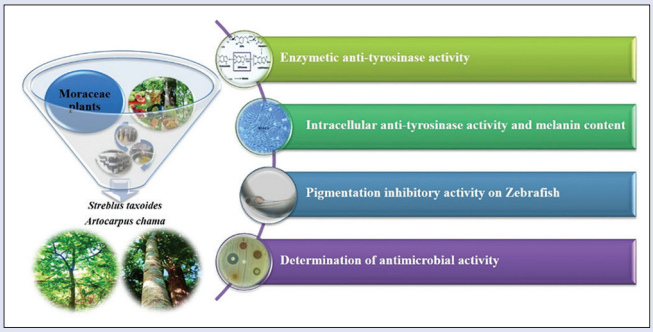
## ABSTRACT

**Background:** Hyperpigmentation is the one cause of skin disorder. The dark-colored skin causes from the increasing of melanin pigment production. It is synthesized by melanogenesis catalyzed by tyrosinase enzyme. Tyrosinase is one of the main causes of melanogenesis; thus, inhibition of the activity of tyrosinase can decrease melanogenesis. Hence, the potential tyrosinase inhibitor could be discovered from natural products. **Objective:** Discovery of tyrosinase inhibitor from natural products by focusing on Moraceae plants. **Materials and Methods:** Forty-eight Moraceae plant extracts were screened for antityrosinase and antibacterial activities; *Streblus taxoides* and *Artocarpus chama* were selected to study in B16F1 melanoma cell; intracellular antityrosinase activity and melanin content. Moreover, pigmentation inhibitory effect on the zebrafish of these samples was studied. **Results:** The extracts of *S. taxoides* and *A. chama* showed the potential activity against tyrosinase enzyme on both intracellular and extracellular enzymatic assays. Moreover, they suppressed pigmentation in zebrafish. Only ethyl acetate extract of these plants could show anti-bacterial activity. **Conclusion:** *S. taxoides* and *A. chama* are potential plants for further study of chemical constituents and biological activities especially the anti-tyrosinase activity of the isolated compound to find out the lead compound for whitening agent from natural product.

**Key words:** *Artocarpus charma*, melanin content, moraceae, *Streblus taxoides*, tyrosinase inhibition

## SUMMARY

- Tyrosinase inhibition and anti-bacterial activity of 48 Moraceae plant extracts were reported
- Tyrosinase inhibition of *Streblus taxoides* woods and *Artocarpus chama* stem were reported for the first
- *A. chama* stem and *S. taxoides* wood extracts exhibited potential activity against tyrosinase enzyme both in enzymatic assay and intracellular assay
- *A. chama* stem and *S. taxoides* wood extracts showed the suppression of pigmentation in zebrafish.




**Abbreviations used:** DMSO: Dimethyl sulfoxide; OD: Optical density; DMEM: Dulbecco's modified eagle medium; SRB: Sulforhodamine B; TCA: Trichloroacetic acid; RIPA buffer: Radioimmunoprecipitation assay buffer; BSA: Bovine serum albumin; PBS: Phosphate-buffered saline; hpf: h postfertilization.

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## INTRODUCTION

The dark-colored skin causes from the increasing of melanin pigment production. This pigment shows brown and black colors.[1] Melanin is distributed in the living organisms of the natural and has many different properties.[2] Melanogenesis initiated from L-tyrosine hydroxylated to L-dihydroxyphenyl-alanine (L-Dopa), then oxidation of L-Dopa to its corresponding *o*-dopaquinone, catalyzed by tyrosinase enzyme. *o*-Dopaquinone can be divided into two different types of reaction to produce eumelanin and pheomelanin.[3,4] Melanin plays an important role in preventing ultraviolet light-induced skin damage, but abnormal melanin (hyperpigmentation) or accumulation of an excessive level of melanin due to the overexpression of tyrosinase leads to skin disorders such as melasma, age spots, and sites of actinic damage.[4] Tyrosinase is the key enzyme in melanogenesis, and then tyrosinase inhibition can decrease melanogenesis, that will be useful for the treatment of hyperpigmentation.

Many problems from current whitening cosmetics have been reported such as dermatitis and skin irritation, melanocyte destruction, postinflammatory pigmentation, and sometimes, we found ochronosis, cytotoxicity, and skin cancer.[5] The discovery of tyrosinase inhibitor from natural sources will be an alternative treatment. It might provide the lead compound of tyrosinase inhibitor and probably develop for whitening agent in cosmeceutical or medicine for hyperpigmentation.



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Pathogenic bacteria are a major cause of human skin disease. Moreover, some micro-organisms can stimulate melanogenesis.[6,7] Antibiotic is the choice for the treatment. However, the use of antibiotics may lead to drug resistance of many bacterial strains. Development of new antimicrobial compounds for resistant organisms is becoming critically important.

The bacterial infection is the one cause of heperpigmentation. Hence, maybe the synergistic effects of antimicrobial and antityrosinase activities from natural products will exhibit the decreasing of melanin pigment production.

Moraceae is the most interesting plant's family for biological especially, tyrosinase inhibition since many isolated compounds from Moraceae plants showed inhibitory effect against tyrosinase enzyme such as resveratrol and artocarpanone which were isolated from *Artocarpus gomezianus* and *Artocarpus integer*, respectively.[8-18]

Hence, this study focused on Moraceae plants. Forty-eight Moraceae plants were selected for antityrosinase and antibacterial screening. Then, *Streblus taxoides* wood and *Artocarpus chama* stem extracts which showed the potential effects were selected for further study on biological activities such as both intracellular and extracellular antityrosinase and antibacterial activities.

# MATERIALS AND METHODS

## Plant materials

Forty-eight Moraceae plant samples were collected from Rajjaprabha Dam, Surat Thani Province; Southern Literature Botanical Garden, Songkhla Province; Botanical Garden, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla Province and Walailuk University, Nakhon Si Thammarat Province [Table 1]. All plant samples were identified by the botanists of each place. All sample specimens were kept in the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. The voucher specimen numbers of two selected Moraceae plants, *A. chama* Buch.-Ham. and *S. taxoides* (Roth) Kurz were SKP 117 01 03 01 and SKP 117 19 20 01, respectively. These plants were selected for further biological studies.

### *Preparation of plant extracts*

Dried powder plant materials were extracted by maceration with absolute ethanol and extracted repeatedly at room temperature for 3 days (X3). The filtrates were pooled and evaporated under reduced pressure at temperature not exceeding 40°C by vacuum rotary evaporator to yield the ethanol extract.

The plants that showed the potential activities of antityrosinase and/or antibacterial activities and have not been reported were selected for further studies through this project.

The dried powder of selected plants was macerated with petroleum ether, ethyl acetate, and methanol at room temperature 3 days (X3, for each solvent separately) and boiled with $H_2O$, respectively, to give petroleum ether, ethyl acetate, methanol, and $H_2O$ extracts.

## Enzymetic antityrosinase activity assay

Antityrosinase activity was determined with the dopachrome method by using L-Dopa as the substrate.[8] Dopachrome is one of the intermediate substrates in melanogenesis. The red color of dopachrome from the oxidation of L-Dopa can be detected by visible light at 492 nm.

140 μL phosphate buffer (pH 6.8), 20 μL sample solution, and 20 μL tyrosinase solution (203.3 unit/mL) were mixed at 25°C for 10 min and then added with 20 μL of 0.85 mM L-Dopa. The visible absorption was measured at 492 nm. The solution was incubated at 25°C for 20 min. After incubation, the amount of dopachrome in the reaction was measured at 492 nm again. Tyrosinase inhibition (T) was calculated as this equation:

Tyrosinase inhibition (%) = (1 – [$OD_{492}$ of sample/$OD_{492}$ of control]) × 100

$OD_{492}$: The difference of sample/control optical density (OD) before and after incubation at 492 nm.

Kojic acid and water extract of *Artocarpus lakoocha* wood were used as positive controls and Dimethyl sulfoxide (DMSO) was used as a negative control.

## Cell culture

Murine melanoma B16-F1 cells (CLS-400122) were cultured in Dulbecco's Modified Eagle's medium containing 10% heat-inactivated fetal bovine serum at 37°C in a humidified atmosphere with 5% $CO_2$. When cells reach 70%–80% confluence cell viability, cellular tyrosinase activity and melanin content were measured.[19-22]

### *Cell viability assay*

Cell viability was determined by sulforhodamine B (SRB) assay. The cells were seeded in 96-well plate ($5 \times 10^3$ cells/well). After incubation for 24 h, the cells were treated with test samples and 0.5% DMSO for negative control. After 48 h incubation, cells were fixed with 10% trichloroacetic acid and kept at 4°C, 1 h. After that, cells were strained with 0.45% SRB. Then, 10 mM Tris base was added on strained cells and then SRB color was dissolved by shaking. Optical densities were determined at 492 nm. The percent cell viability would be calculated.

### *Intracellular anti-tyrosinase activity and melanin content assays*

The cells were seeded in 12 well plates ($3 \times 10^5$ cells/well) and allowed to adhere at 37°C for 12 h. Cells were treated with test samples while control cells were treated with 0.5% DMSO. After 48 h incubation, cells were lysed with radioimmunoprecipitation assay buffer and centrifuged 14,000 rpm for 20 min (4°C) to separate the supernatant.

### Intracellular antityrosinase activity

The supernatants were collected and the protein content was determined by the Bradford method using bovine serum albumin as standard.[23] The supernatant of lysate cells and 2 mg/mL L-Dopa in phosphate-buffered saline were added to 96-well plate. The mixture was incubated at 25°C for 1 h, OD was determined at 492 nm. Tyrosinase would be calculated.

### Melanin content

Cells pellet were dissolved with 1 M NaOH and incubated at 55°C for 1 h. Melanin concentration was calculated by comparison of the absorbance at 475 nm using a standard curve of synthetic melanin.

## Pigmentation inhibitory activity on zebrafish

Zebrafish (*Danio rerio*) was used to determine the pigmentation inhibitory effect. The method was modified from Le *et al.* 2016.[24] Briefly, embryos at 9 h postfertilization (hpf) were placed individually into 96-well plate filled with 100 μL/well 0.03% sea salt solution and each sample solution. 25 μM 1-phenyl-2-thiourea was used as positive group and 0.03% sea salt solution was used as normal group. The experiment used 20 embryos/group.

At 72 hpf, after embryos hatched, the larvae were put on glass slides and embedded using 2% low melting agarose. The dorsal view of the zebrafish fry was photo-captured, and the size of black spot in the head-dorsal region at 81 hpf was evaluated.

## Antimicrobial activity assay

Micro-organisms: two types of bacteria were involved in this assay; aerobic bacteria were *Staphylococcus aureus* (ATTC 25923), methicillin-resistant

**Table 1:** Anti-tyrosinase activity of 48 Moraceae plant samples at 20 µg/mL

| Number | Plant | Part | Percentage anti-tyrosinase activity |
|---|---|---|---|
| 1 | *A. altilis*[A] | Branch | 48.24±1.10 |
| 2 | *A. altilis*[A] | Leaf | 16.32±1.30 |
| 3 | *A. integer*[A] | Branch | 48.76±2.09 |
| 4 | *A. integer*[A] | Leaf | 23.76±2.48 |
| 5 | *A. rigidus*[B] | Branch | 28.09±5.18 |
| 6 | *A. rigidus*[B] | Leaf | 28.29±6.98 |
| 7 | *A. chama*[B] | Wood | 32.48±5.62 |
| 8 | *A. chama*[B] | Bark | 11.89±1.21 |
| 9 | *A. chama*[B] | Stem | 35.87±3.84 |
| 10 | *A. chama*[B] | Leaf | 14.30±7.00 |
| 11 | *F. benghalensis*[B] | Branch | 32.10±1.66 |
| 12 | *F. benghalensis*[B] | Leaf | −3.80±2.93 |
| 13 | *F. benghalensis*[B] | Wood | 35.44±7.10 |
| 14 | *F. benghalensis*[B] | Bark | −13.97±6.76 |
| 15 | *F. callosa*[B] | Branch | 7.81±2.65 |
| 16 | *F. callosa*[B] | Leaf | 1.22±5.86 |
| 17 | *F. celebensis*[A] | Branch | 14.70±1.66 |
| 18 | *F. celebensis*[A] | Leaf | −0.54±7.14 |
| 19 | *F. chartacea var. torulosa*[B] | Branch | −8.25±2.80 |
| 20 | *F. chartacea var. torulosa*[B] | Leaf | 4.73±3.43 |
| 21 | *F. foveolata*[C] | Wood | 38.49±6.45 |
| 22 | *F. fistolusa*[A] | Branch | −3.18±1.55 |
| 23 | *F. fistolusa*[A] | Leaf | 1.37±2.13 |
| 24 | *F. hispida*[A] | Branch | −28.47±1.80 |
| 25 | *F. hispida*[A] | Leaf | −13.45±2.83 |
| 26 | *F. infectoria*[C] | Wood | 12.05±7.65 |
| 27 | *F. microcarpa*[A] | Branch | −0.89±6.21 |
| 28 | *F. microcarpa*[A] | Leaf | 0.32±6.42 |
| 29 | *Ficus* spp.[C] | Wood | −1.46±5.67 |
| 30 | *Ficus* spp.[C] | Wood | −4.78±1.82 |
| 31 | *Ficus* spp.[C] | Wood | −0.20±4.30 |
| 32 | *Ficus* spp.[C] | Wood | 2.90±3.95 |
| 33 | *Ficus* spp.[A] | Branch | −2.62±1.41 |
| 34 | *Ficus* spp.[A] | Leaf | −2.56±1.74 |
| 35 | *Ficus* spp.[B] | Branch | 15.40±8.95 |
| 36 | *Ficus* spp.[B] | Leaf | 13.64±6.63 |
| 37 | *F. superba*[A] | Branch | 17.40±1.90 |
| 38 | *F. superba*[A] | Leaf | 34.25±2.20 |
| 39 | *F. racemosa*[A] | Branch | 4.65±4.91 |
| 40 | *F. racemosa*[A] | Leaf | 18.49±2.45 |
| 41 | *F. vasculosa*[B] | Wood | −12.90±0.86 |
| 42 | *F. vasculosa*[B] | Bark | −0.35±3.69 |
| 43 | *F. vasculosa*[B] | Leaf | 0.87±7.18 |
| 44 | *M. alba*[A] | Branch | 47.39±4.82 |
| 45 | *M. alba*[A] | Leaf | 49.09±4.71 |
| 46 | *S. ilicifolius*[D] | Leaf | 18.15±2.28 |
| 47 | *S. ilicifolius*[D] | Wood | 69.05±5.00 |
| 48 | *S. taxoides*[D] | Wood | 58.59±1.90 |
| | *A. lakoocha*[P] | Wood | 90.42±0.50 |
| | Kojic acid[P] | - | 81.42±0.34 |

[A]Source: Botanical Garden, Faculty of Pharmaceutical Sciences, Walailuk University, Nakhon Si Thammarat Province, Thailand; [B]Source: Southern Literature Botanical Garden, Songkhla Province, Thailand; [C]Source: Botanical Garden, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla Province, Thailand; [D]Source: Rajjaprapha Dam, Surat Thani Province, Thailand; [P]Positive control. *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*; *A. lakoocha*: *Artocarpus lakoocha*

*S. aureus* (MRSA) (DMST20654), and *Staphylococcus epidermidis* (TISTR 517), whereas anaerobic bacteria was *Propionibacterium acnes* (DMST 14916). Aerobic bacteria were cultured in Mueller-Hinton Agar for 18–24 h at 37°C. Anaerobic bacteria were cultured in Brain Heart Infusion Agar for 72 h at 37°C. The preliminary screening of antibacterial activity was assessed using agar disc diffusion method.[25] The fractions of selected plants were determined for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in 96 well microplate by modified broth microdilution method.[26,27] Oxacillin was used as positive controls except MRSA using vancomycin.

## RESULTS

### Enzymatic antityrosinase activity

Among 48 Moraceae plant samples [Table 1] investigated for antityrosinase activity, 12 samples showed antityrosinase activity at

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

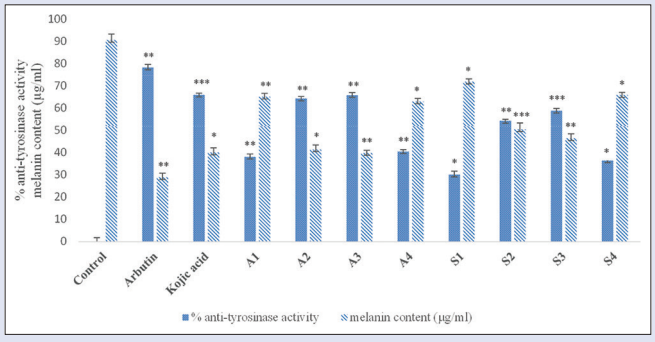


**Figure 1:** Intracellular anti-tyrosinase activity and melanin content. Arbutin, kojic acid = positive control. A1, A2, A3 and A4 = petroleum ether, ethyl acetate, methanol, water extracts of *Artocarpus chama* stem, respectively. S1, S2, S3 and S4 = petroleum ether, ethyl acetate, methanol and water 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*

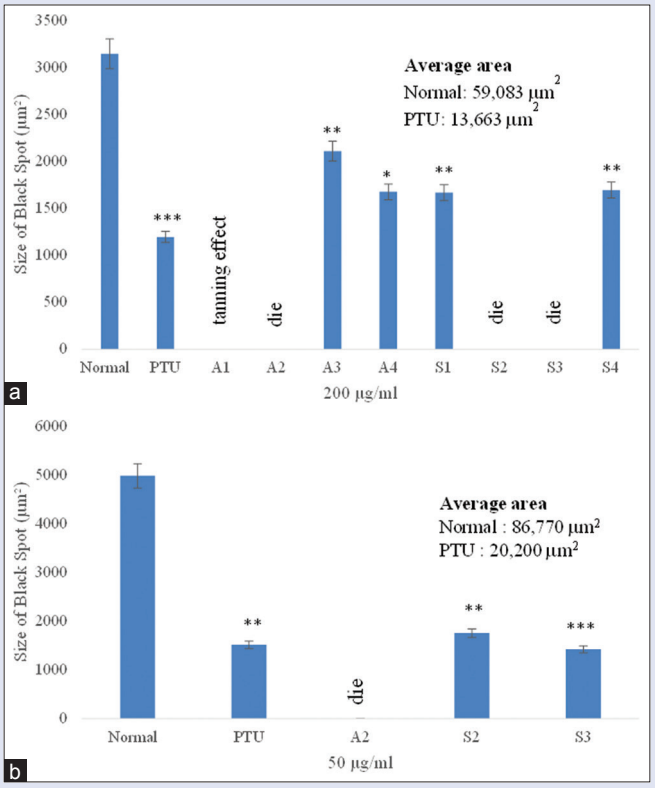


**Figure 2:** Pigmentation inhibitory on Zebrafish. PTU (phenylthiourea) = positive control. A1, A2, A3 and A4 = petroleum ether, ethyl acetate, methanol, water extracts of *Artocarpus chama* stem, respectively. S1, S2, S3 and S4 = petroleum ether, ethyl acetate, methanol and water extracts of *Streblus 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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