International Journal of Applied Pharmaceutics

ISSN - 0975 - 7058 Vol 11, Special Issue 5, 2019

Research Article

ANTI-TYROSINASE ACTIVITY FROM VARIOUS SOLVENTS OF PEANUT SHELL (*ARACHIS HYPOGAEA* L.) EXTRACTS *IN VITRO*

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Received: 15 December 2018, Revised and Accepted: 19 January 2019

ABSTRACT

Objective: This study aimed to determine *in vitro* anti-tyrosinase activity from various solvents of peanut shell extracts and to find out if the activity is better than kojic acid which is a conventional compound used as anti-hyperpigmentation agent.

Methods: Extraction was done by maceration method with various solvents of ethyl acetate, n-hexane, and 70% ethanol. Extracts were made into the series concentration of 25, 50, and 75 µg/ml. Kojic acid with concentration of 50 µg/ml used as positive control and 5% dimethyl sulfoxide used as negative control. Tyrosinase enzyme will react with L-3,4-dihydroxyphenylalanine substrate to produce dopachrome compound. The absorbance of dopachrome read by microplate reader at λ = 492 nm. If the absorbance read by the microplate reader is low, means that the inhibition power of the peanut shell extract against the tyrosinase enzyme is high. Anti-tyrosinase activity seen by the percentage inhibition value. The percentage inhibition value was analyzed with Kruskal–Wallis test followed by Mann–Whitney U-test; all tests were carried out with a confidence level of 95%.

Results: The mean of percentage inhibition value of n-hexane extract ranged from 12.44 ± 1.66% to 39.82 ± 1.33%, 70% of ethanol extract ranged from 39.98 ± 0.85% to 70.19 ± 1.98%, and ethyl acetate extract ranged from 17.85 ± 0.78% to 60.30 ± 0.97%. Kojic acid has mean percentage inhibition value of 78.19 ± 1.97%. IC_{50} of ethanol, ethyl acetate, and n-hexane extracts was, respectively, 40.53 µg/ml, 63.49 µg/ml, and 91.95 µg/ml. Ethanol extract contains flavonoid, tannin, and saponin. Ethyl acetate extract contains flavonoid.

Conclusion: All various solvents of peanut shell extracts have anti-tyrosinase activity but not better than kojic acid. Ethanol extract with concentration of 75 µg/ml has the greatest anti-tyrosinase activity.

Keywords: Peanut shell, Tyrosinase enzyme, Anti-tyrosinase, Anti-hyperpigmentation.

 DOI: http://dx.doi.org/10.22159/ijap.2019.v11s5.T1007

INTRODUCTION

Hyperpigmentation is still a serious problem for Indonesian women. There are many things that trigger hyperpigmentation, including exposure to ultraviolet (UV) light, can make the tyrosinase enzyme overwork to convert L-3,4-dihydroxyphenylalanine (L-DOPA) to dopachrome, which eventually turns into melanin [1].

During this time, hyperpigmentation in Indonesia is overcome using kojic acid or hydroquinone as tyrosinase inhibitors, but kojic acid has side effects including allergies and irritation [2,3], while hydroquinone has side effects including irritation and ochronosis [4]. Kojic acid can be used at a concentration of 1%–4% singly or in combination with other agents. Kojic acid when used alone is less effective than hydroquinone 2% [1].

Natural ingredients that have the potential to be further developed as anti-hyperpigmentation are peanut shells. Peanut shells are products of peanut crop and ones generally disposed of as waste, but it actually contains high antioxidants potential with total polyphenols, flavonoids, contents of amino acids, alkaloids, and terpenes [5,6]. Some previous studies have proven the ability of flavonoids and alkaloids as anti-tyrosinase [7,8]. In this study, we used a variety of solvents to attract various active compounds contained in peanut shells to assess the anti-tyrosinase activity.

MATERIALS AND METHODS

Materials

Peanut plants were obtained from the village of Sumowono, Semarang, Indonesia. Peanut shells were taken from 3-month-old peanut plants. N-hexane solvent (technical grade), dimethyl sulfoxide (DMSO) solvent, pH 6.8 phosphate buffer made from KH2PO4 and NaOH, ethyl acetate solvent, and 70% ethanol solvent were obtained from PT. Brataco, Indonesia. Tyrosinase enzyme, kojic acid, and levodopa or L-DOPA were obtained from Sigma-Aldrich. Dragendorff reagent made from $Bi(NO_3)_3.H_2O$, HNO_3, and KI; Mayer reagent made from $HgCl_2$ and KI; HCl 1%; NaCl 2%; $FeCl_3$ 2%; NaOH; and gelatin 1% were obtained from PT. Brataco, Indonesia.

Peanut shells extraction

Twelve kilograms of peanuts were separated from the outer skin. Furthermore, the peanut seeds were cleaned with a cloth, then dried in an oven at a temperature of 40°C for approximately 15 min until completely dry which is indicated by the peanut shells crushed when squeezed by hand. From that process, we obtained 300 g of peanut shell. The dry peanut shells were pollinated using a blender and measured the water content with a moisture balance tool. Dry water simplicia requirements are <10% [9]. One hundred grams of simplicia was macerated using 750 ml of 70% ethanol for 5 days followed by 250 ml of 70% ethanol for 2 days. The same thing was done also using n-hexane and ethyl acetate solvents. Liquid extract was concentrated using a rotary evaporator to obtain a thick extract. Yield of 70% ethanol, ethyl acetate, and n-hexane extracts was obtained, respectively, 15.47%, 6%, and 6.09%.

The phytochemical screening test

Alkaloid test

Two grams of extract were heated in a large test tube with 1% hydrochloric acid for 30 min in a boiling water bath. The suspension

is filtered into the test tube A and B as much. Test tube A added three drops of Dragendorff reagent and test tube B added three drops of Mayer reagent.

Tannin test

Fifty milligrams of extract is heated with 10 ml of water for 30 min over a water bath. Then filtered, filtrate plus 1 ml of NaCl% solution. If deposits are formed, then filtered. The filtrate is added 2 ml of 1% gelatin.

Saponin test

Add the aquadest to the test tube containing 100 mg extract, cover, and shake vigorously for 30 s. Leave the tube in an upright position for 30 min.

Flavonoid and phenolic compound test

Five milligrams of extract was dissolved in 2 ml of 96% ethanol and then divided into two test tubes. Test tube A added three drops of 2% $FeCl_3$ solution and test tube B added three drops of 0.2 N NaOH solution.

The anti-tyrosinase activity test

This test adopts a method from Masuda *et al*. [10]. First, prepared 8 wells consisting of A group (consists of 3 wells), B group (consists of 1 well), C group (consists of 3 wells), and D group (consists of 1 well). A group consists of 120 µL of pH 6.8 phosphate buffer and 40 µL of tyrosinase enzyme (496 units/mL). B group consists of 160 µL of pH 6.8 phosphate buffer. C group consists of 80 µL of pH 6.8 phosphate buffer, 40 µL of 496 units/mL tyrosinase enzyme, and 40 µL of tested group. D group consists of 120 µL of pH 6.8 phosphate buffer and 40 µL of tested group. Each mixture was incubated at 25°C for 10 min. Furthermore, each mixture was added 40 µL of 2.5 mM L-DOPA then incubated at a temperature of 25°C for 10 min. Furthermore, the mixture is measured using a microplate reader at a wavelength of 492 nm. Anti-tyrosinase activity is seen by the percentage inhibition value, which is calculated using the following formula:

$$\frac{([A-B]-[C-D])}{(A-B)}\times 100\%$$

Where, (*A*): The absorbance of negative blank solution with tyrosinase enzyme; (*B*): The absorbance of negative blank solution without tyrosinase enzyme; (*C*): The absorbance of tested group solution with tyrosinase enzyme; and (*D*): The absorbance of tested group solution without tyrosinase enzyme.

The percentage inhibition data then were tested for normality using the Shapiro–Wilk test and tested for homogeneity using Levene's test, then tested using one-way ANOVA and least significant difference tests at 95% confidence level.

RESULTS AND DISCUSSION

The formation of dopachrome products is characterized by the appearance of the brown color produced by the reaction between L-DOPA and the tyrosinase enzyme. The brown color intensity will determine the dopachrome compound that is formed, meaning that the higher the intensity of the brown color, the more dopachrome compounds will be produced. If tyrosinase is inhibited, the formation of dopachrome will also be inhibited. The higher the inhibition of an inhibitor, the lower the intensity of the brown color [11].

After obtaining the percentage inhibition value (Table 1) from the dopachrome absorbance data, then the normal data distribution was analyzed using the Shapiro–Wilk test and the homogeneity was analyzed using Levene's test, produced homogenous data ($p>0.05$) but anomaly distributed data ($p<0.05$) so that it was tested using Kruskal–Wallis test followed by Mann–Whitney U-test; all tests were carried out with a confidence level of 95%. The mean of percentage inhibition value of n-hexane extracts ranged from 12.44 ± 1.66% to 39.82 ± 1.33%, 70% ethanol extracts ranged from 39.98 ± 0.85% to 70.19 ± 1.98%, and ethyl acetate extracts ranged from 17.85 ± 0.78% to 60.30 ± 0.97%. Kojic acid has mean percentage inhibition value of 78.19 ± 1.97%.

All various solvents of peanut shell extract and kojic acid have greater and different meaningful inhibition percentage value than the negative control DMSO (p.0.00). This indicates that peanut shell extracts and kojic acid have antityrosinase activity. Kojic acid has inhibition percentage value that higher than all various solvents of peanut shell extract (p.0.00). The higher the concentration of extract, the higher the percentage inhibition value (p.0.00). Ethanol extract with concentration of 75 µg/ml has the greatest anti-tyrosinase activity (IC_{50}: 40.53 µg/ml) among the whole extracts (ethyl acetate extract has IC_{50} value of 63.49 µg/ml and n-hexane extract has IC_{50} value of 91.95 µg/ml). This is thought to be due to the presence of phenolic compounds in ethanol extract.

The phytochemical screening test resulted that ethanol extract of peanut shell contains flavonoid, tannin, and saponin (Fig. 1); the ethyl acetate extract of peanut shell contains flavonoid (Fig. 2), while the results in n-hexane extract were negative. The addition of gelatin to the extract filtrate produces yellow deposits which indicated that the extract contains tannin compounds. The formation of foam which lasted for 30 min in the extract solution after strong shaking showed the presence of saponin compounds in the extract. The formation of yellow deposits after addition of NaOH to the mixture of extracts and 96% ethanol indicating the presence of flavonoids in the extract. The

Table 1: Percentage inhibition value

Groups	Percentage inhibition value (%) [a]
N-hexane extract 25 µg/ml	12.44±1.66
N-hexane extract 50 µg/ml	28.82±1.33
N-hexane extract 75 µg/ml	39.82±2.32
Ethyl acetate extract 25 µg/ml	17.85±0.78
Ethyl acetate extract 50 µg/ml	37.47±2.03
Ethyl acetate extract 75 µg/ml	60.30±0.97
70% ethanol extract 25 µg/ml	39.98±0.85
70% ethanol extract 50 µg/ml	56.86±1.96
70% ethanol extract 75 µg/ml	70.19±1.98
Kojic acid 50 µg/ml	78.19±1.97
DMSO	−18.5±3.82

[a]Mean±SE, n=3. DMSO: Dimethyl sulfoxide, SE: Standard error

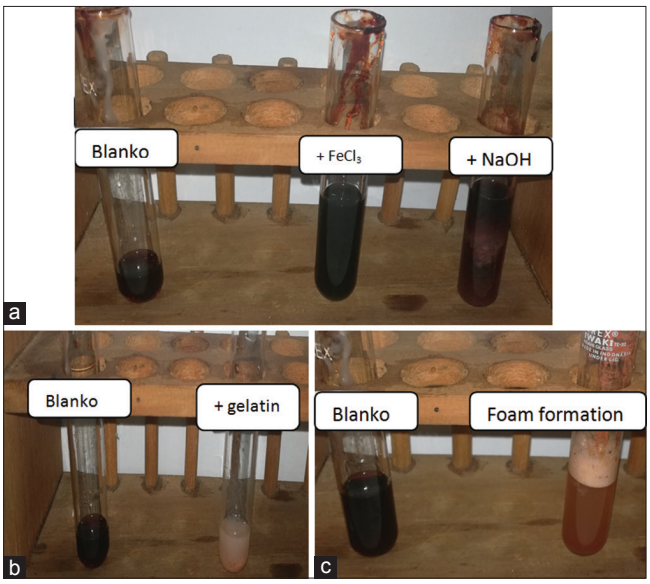

Fig. 1: The phytochemical screening of the ethanol extract of peanut shell, (a) flavonoid and phenolic compound test, (b) tannin test, (c) saponin test

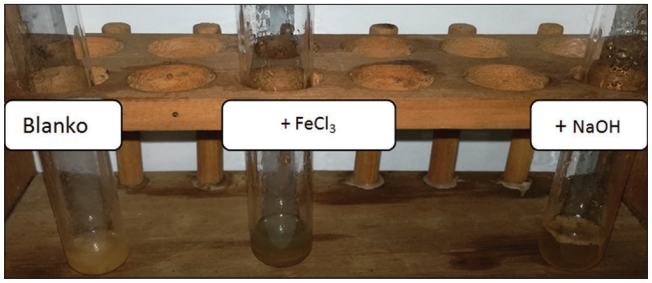

Fig. 2: The flavonoid and phenolic compound test of ethyl acetate extract of peanut shell

formation of blue-green color after adding $FeCl_3$ to the mixture of extract and 96% ethanol showed the presence of phenolic compounds in the extract.

Flavonoid, tannin, and saponin are phenolic compounds. Several studies have proven the existence of phenolic compound in peanut shells. Dean *et al*. identified 425 ± 33 mg FA/100 g phenolic compounds in acetone extract of peanut shell [12]. Adhikari *et al*. identified 142 mg of flavonoid compound on methanol extract [5]. Win *et al*. identified that phenolic composition of peanut shell is p-hydroxybenzoic acid, resveratrol, and luteolin [6].

Phenolic compounds have been proven through several studies regarding their activity in inhibiting tyrosinase. Alam *et al*. have carried out research on the anti-tyrosinase effect of *Pleurotus ferulae*, which has an effect of 19.81%–60.10%. The extract contains 11 phenolic compounds and gallic acid is the largest content of 40 µg/g [13]. Corradi *et al*. investigated that gallic acid was found to be the key compound related to the tyrosinase activity detected in *Schinus terebinthifolius* extracts [14]. The inhibition of tyrosinase effect might be due to the hydroxyl groups of the phenolic compounds that could form a hydrogen bond at the active site of the enzyme, leading to a lower enzymatic activity [15].

This study will be continued to examine the *ex vivo* anti-hyperpigmentation activity of peanut shell ethanol extract on guinea pigs, which were previously irradiated with 311 nm UV-B light to make black spots on guinea pig skin. If proven to have anti-hyperpigmentation effect, it will be continued to be formulated and retested to obtain an extract formula which is optimal as an anti-hyperpigmentation.

CONCLUSION

All various solvents of peanut shell extracts have anti-tyrosinase activity ($p<0.05$) but not better than kojic acid. Ethanol extract of peanut shell with concentration of 75 µg/ml has the greatest anti-tyrosinase activity and the smallest IC_{50} among the whole extracts. This effect is thought to be due to the presence of phenolic compounds in ethanol extract of peanut shell. Thus, the study clearly indicated a promising tyrosinase inhibition potential of peanut shell, the by-product of peanut crop. This research really needs to be continued through a series of tests to test the ability of phenolic compounds in peanut shell extract to overcome hyperpigmentation.

CONFLICTS OF INTEREST

All authors have none the conflicts of interest.

ACKNOWLEDGMENT

We would like to thank the overseas seminar assistance program, Directorate General of Strengthening Research and Development, the Ministry of Technology Research and Higher Education of the Republic of Indonesia, for the support to the authors in presenting our research on ICPPS 2019.

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