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Antityrosinase Activities of *Thespesia populnea* Bark and *Phyllanthus emblica* Fruit

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Authors' contributions

This work was carried out in collaboration between all authors. Author HKIP concept and design of the study, obtaining grants, literature search, data collection, analysis and interpretation, manuscript preparation and critical revision of the manuscript. Authors APCP, KDUD, RMUKR and DKLRG collected plant parts and performed the experiments. Author JAVPJ collaborated with the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Currently there is enormous demand for skin-lightening agents, creating a quest for identification of new substances. Tyrosinase has become the main drug target for reducing skin pigmentation. The objective of this study was to detect tyrosinase inhibitory effects of eight medicinal plants that are used to treat skin conditions.
Study Design: *In vitro* assay.
Place and Duration of Study: Department of Biochemistry, Faculty of Medicine, University of Peradeniya, Sri Lanka, from June 2016 to December 2017.
Methodology: Tyrosinase inhibitory effects of methanol extracts of eight plant species that are used to treat various skin conditions were measured using 3,4-Dihydroxy-L-phenylalanine (L-DOPA) as the substrate. Kojic acid was used as the positive control. IC_{50} values of extracts with

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high activity were measured using a range of concentrations. Statistical significance of the differences between the inhibitory effects was calculated using ANOVA.
Results: *Thespesia populnea* bark showed the highest inhibition among the tested, with an IC_{50} of 190 µg/mL. *Phyllanthus emblica* fruit also showed higher inhibitory effect with IC_{50} value of 251 µg/mL. IC_{50} value of kojic acid (3.4 µg/mL) against tyrosinase was significantly lower than that of *P. emblica* and *T. populnea. Even though Santalum album* bark and *Vernonia cinerea* plant showed 36.3 and 32.8% inhibitions respectively at 0.5 mg/mL, these effects did not show a dose-dependent increase. Extracts of *Coscinium fenestratum*, *Persea Americana*, *Vigna radiata* and *Vigna unguiculata* demonstrated only lower inhibitory effects on tyrosinase.
Conclusion: Current study reveals higher antityrosinase activities in methanol extracts of *P. emblica* fruit and *T. populnea* bark.

Keywords: Tyrosinase inhibitors; Thespesia populnea; Phyllanthus emblica.

1. INTRODUCTION

Colour of the human skin is mostly determined by the degree of synthesis of dark macromolecular pigment melanin [1]. Melanin protects the skin from a variety of detrimental agents such as free radicals and UV radiation [2]. However, an increased amount of melanin synthesis occurs as a result of genetic and environmental factors causing pigmentary skin disorders such as melasma, freckles and age spots [3]. Abnormal melanin deposition in the skin has a great impact on the demand for cosmetics and has prompted research and development of agents that could interfere with melanin synthesis [1].

Melanogenesis occurs in melanosomes, a specialized organelle found in epidermal melanocytes through a series of enzyme-catalyzed reactions [4]. Melanogenesis is commenced with the enzyme tyrosinase, which catalyses the rate-limiting step and the remaining reactions will follow spontaneously [4]. Tyrosinase (polyphenol oxidase) is a copper-containing monooxygenase and is located in the membranes of melanosomes. It catalyses two reactions with various phenolic substrates. Two responses are the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and oxidation of L-DOPA to DOPA-quinone [5]. Highly reactive quinones polymerize spontaneously to form high-molecular-weight pigments [5].

Currently, there is a massive demand across Asia for cosmetics/ cosmeceuticals used in skin-lightening, creating a quest for identification of new substances. As the regulatory enzyme of melanin synthesis, tyrosinase has become a widespread drug target used to minimise skin pigmentation in therapeutics and cosmetics [1,6]. Well, known tyrosinase inhibitors such as arbutin and kojic acid have shown only low inhibitory activity against pigmentation in melanocytes in a clinical trial [7]. Hydroquinone used as a whitening compound in some cosmetics is considered cytotoxic and mutagenic to mammalian cells [8]. Therefore, it is necessary to search for new tyrosinase inhibitors from natural resources that do not have side effects [9]. More than 200 medicinal plants that are used to enhance the health and beauty of skin are described in Ayurveda as *varṇya* drugs [10].

The objective of this study was to detect tyrosinase inhibitory effects of eight medicinal plants that are traditionally used to treat skin conditions.

2. MATERIALS AND METHODS

2.1 Plant Material

Parts from eight plants that are traditionally used to treat various skin conditions were selected (Table 1). Plants were authenticated by the Deputy Director, National Herbarium, Royal Botanical Garden, Peradeniya. Plant materials were cleaned, dried under shade for approximately one week and powdered using a grinder. Dry powder (10 g) of each plant part was extracted with methanol three times (100 ml) using a sonicator [11]. Filtrate was dried by evaporating methanol below 50°C using a rotary evaporator. Extracts were stored at room temperature until further investigation. Extracts were dissolved in phosphate buffer (pH 6.5) before the assay to make 10 mg/mL extracts. DMSO was used for extracts with poor water solubility to yield 100 mg/mL solutions and then diluted to 10 mg/mL with phosphate buffer.

Table 1. Plants and parts used in the study

Plant	Family	Common name	Part
Coscinium fenestratum	*Menispermaceae*	*Vanival*	Stem
Persea americana	*Lauraceae*	*Avacado*	Fruit pulp
Phyllanthus emblica	*Phyllanthaceae*	*Nelli*	Fruit
Santalum album	*Santalaceae*	*Sudu Handun*	Bark
Thespesia populnea	*Malvaceae*	*Gansooriya*	Bark
Vernonia cinerea	*Compositae*	*Monarakudumbiya*	Whole plant
Vigna radiata	*Fabaceae*	Mung bean	Seeds
Vigna unguiculata	*Fabaceae*	Cowpea	Seeds

2.2 Measurement of Tyrosinase Activity

Tyrosinase activity of the test samples was measured with the method of Vaibhav and Lakshaman with modifications [12]. Fifty microliter of 500 U/mL tyrosinase enzyme from mushroom (Sigma) was pre-incubated with phosphate buffer (pH 6.5) and 50 µl of 10 mg/mL plant extracts for 10 min at 25°C. Respective control was prepared in the absence of extracts and the blanks were prepared by adding 50 µl of phosphate buffer instead of tyrosinase. Then 200 µl of 1.78 mM 3,4-Dihydroxy-L-phenylalanine (L-DOPA) (Sigma) was added and mixed. Total volume of the reaction mixture was 1 mL. Reaction mixtures were incubated for 10 min at 25°C and exactly after 10 min incubation, absorbance was read at 475 nm. Kojic acid (Sigma) was used as the positive control. Each measurement was taken in duplicate and on three separate occasions.

Inhibitory effects on tyrosinase activity were calculated using following formula. IC_{50} values of extracts with high activity were measured using a range of extract concentrations.

Percentage inhibitory effect

= [(Control - Control blank) - (Test - Test blank) X 100 / (Control - Control blank)]

2.3 Statistical Analysis

Means and standard deviations were calculated using measurements taken on three separate occasions. Statistical significance of the differences was calculated using ANOVA. P<0.01 was considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

Percentage inhibitions of the eight methanol extracts on tyrosinase and IC_{50} values of two extracts and positive control are shown in Table 2. *T. populnea* showed the highest inhibition among the tested extracts, with an IC_{50} of 190 µg/mL (Table 2 and Fig. 1). *P. emblica* also showed higher inhibitory effect with IC_{50} value of 251 µg/mL. These effects were significantly higher than those of the other six plants (p< 0.002). Even though *S. album* and *V. cinerea* showed a moderate inhibition at 0.5 mg/mL (36.3 and 32.8% respectively), it was unable to calculate their IC_{50} values as the effects were not dose-dependent (Fig. 2). Extracts of *C. fenestratum*, *P. Americana*, *V. radiata* and *V. unguiculata* showed only lower inhibitory effects on tyrosinase (2.2-14.4%) at 0.5 mg/mL (Table 2). IC_{50} value of kojic acid (3.4 µg/mL) against tyrosinase was significantly lower than that of *P. emblica* and *T. populnea* (p<0.0008).

Table 2. Percentage tyrosinase inhibitory effects of plant extracts

Plant extract	Mean (%)	SD	IC_{50} (µg/mL)
Coscinium fenestratum	10.79	1.80	NA
Persea americana	14.42	4.23	NA
Phyllanthus emblica	53.35*	1.65	251
Santalum album	36.36	4.23	**
Thespesia populnea	70.54*	6.33	190
Vernonia cinerea	32.82	3.31	**
Vigna radiata	2.26	1.14	NA
Vigna unguiculata	7.06	2.61	NA
Kojic acid			3.4***

*Mean inhibitory effects were obtained at 0.5 mg/mL. *Inhibitory effects of P. emblica and T. populnea were significantly different from others (p<0.002). ** S. album and V. cinerea did not show a dose-dependent increase in inhibitory activity. *** IC_{50} of Kojic acid was significantly lower than that of P. emblica and T. populnea (p<0.0008). NA: IC_{50} values not available*

3.2 Discussion

In the present study, parts from eight plants used as remedies for various skin ailments or as beautifying agents were selected to evaluate for their efficacies against tyrosinase activity.

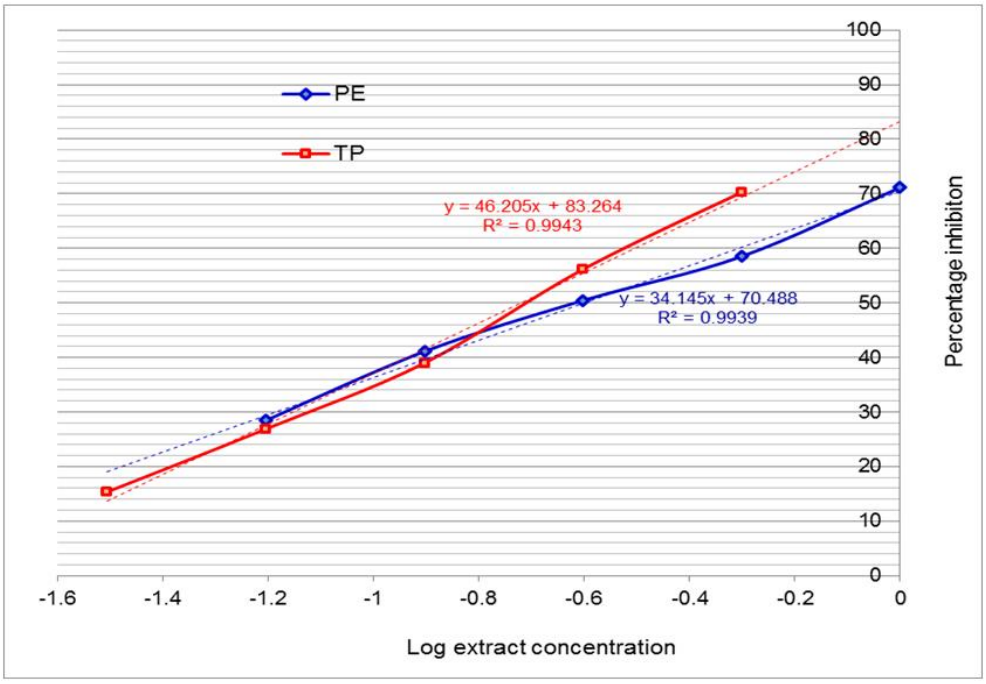

Fig. 1. Tyrosinase inhibitory effects of PE and TP at different concentrations

PE: Phyllanthus emblica, TP: Thespesia populnea. Concentrations of the extracts used were, 0.03 to 0.5 mg/mL for TP and 0.06 to 1 mg/mL for PE

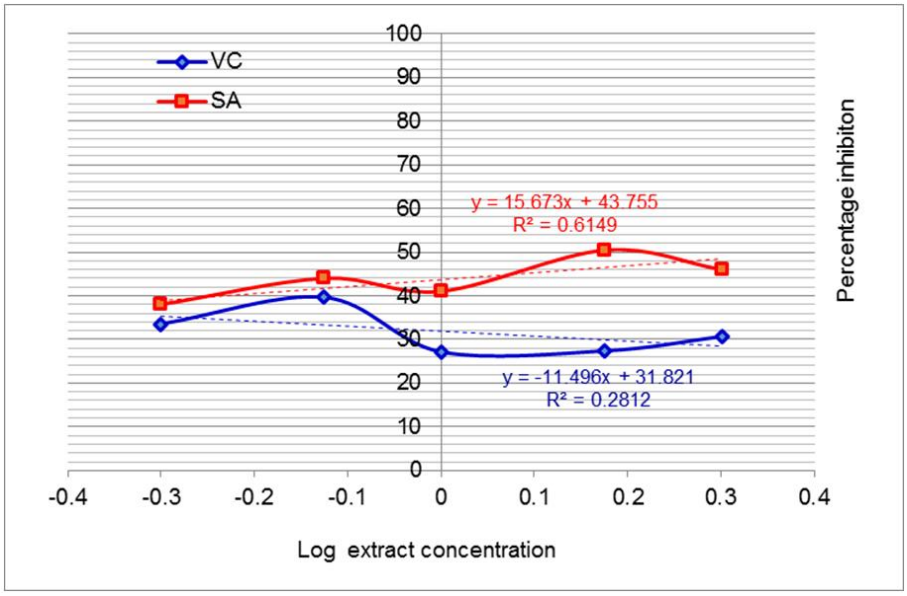

Fig. 2. Tyrosinase inhibitory effects of SA and VC at different concentrations

SA: Santalum album, VC: Vernonia cinerea. Concentrations of the extracts used were 0.5 to 2.0 mg/mL

Phenolic compounds have demonstrated scientific evidence on multiple beneficial effects on skin. They eliminate factors causing skin ageing and damage [13]. There is a wide range of evidence to support the antioxidant, anti-inflammatory and antimicrobial effects, anti-ageing and skin renewal properties and stimulatory effects on synthesis of collagen and

elastin [13]. Phenolics were shown to suppress melanin production in skin cell cultures [13]. Being substrate (tyrosine) analogues, some phenolics such as resveratrol have demonstrated to be effective in inhibiting tyrosinase [13]. Quercetin exhibited the strongest tyrosinase inhibition (IC_{50} 14.29 µM) among eight bioactive compounds isolated from *Hypericum laricifolium* [14]. Licuraside, isoliquiritin, and licochalcone A which are flavonoids isolated from *Glycyrrhiza uralensis* and *Glycyrrhiza inflate* were found to be competitive inhibitors of tyrosinase with IC_{50} values of 0.072, 0.038, and 0.026 mM, respectively [15].

P. emblica is long been used in folk medicine to treat number of metabolic, inflammatory and skin diseases and for beauty care [16]. *P. emblica* fruit which is a rich source of phenolic compounds with high antioxidant activity is used in several *varnya* formulations in Ayurveda [17]. A HPLC fraction rich in antioxidants isolated from *P. emblica* fruits has demonstrated skin lightening effects [18]. In another study, a fraction rich in antioxidants and low molecular weight hydrolysable tannins from *P. emblica* fruits inhibited melanin production in melanocyte cultures and *in vivo* [19]. Inhibition of tyrosinase, inhibition of Fe^{2+}/H_2O_2-induced melanogenesis (due to chelation with Fe^{2+}) and inhibition of superoxide anion-induced melanogenesis (by scavenging superoxide anions) were identified as mechanisms of action responsible for skin lightening effects of *P. emblica* fruits [19]. Ethanol and methanol extracts of *P. emblica* branch extracts demonstrated inhibition of tyrosinase activity with lower IC_{50} values (247 and 194 µg/mL respectively) than that of ethanol extracts of *P. emblica* fruits (4347 µg/mL) [20]. Ethanol extract of *P. emblica* branch also inhibited tyrosinase mRNA expression in murine melanoma cells [20]. Gallic acid and vanillic acid were the major phenolic compounds identified in those extracts of *P. emblica* branch [20]. Present study supports the previous evidence on tyrosinase inhibitory effects of the *P. emblica* fruit. However, IC_{50} value obtained against tyrosinase in the current study with methanol extract was much lower (251 µg/mL) than that of values reported for fruit ethanol extract (4347 µg/mL).

S. album (Sandalwood) oil has been applied topically for centuries in traditional medicine to beautify the skin [21] *S. album* oil is used in number of fairness cosmetics and perfumes [22]. Potent inhibitory effects of sandalwood oil on tyrosinase was revealed with an IC_{50} of 171 µg/mL. In the same study, strong inhibitory effects of α-santalol, the major constituent of the oil on tyrosinase was identified [22]. The present study reveals that the bark of *S. album* possesses some inhibitory effect on tyrosinase even though the effect was not dose-dependent.

T. populnea bark is traditionally used to treat number of skin conditions such as scabies, psoriasis, eczema, ringworm infections [23] and for wound healing [24]. The decoction of the bark is commonly used for the treatment of skin and liver diseases. Bark is known to possess antioxidant, anti-inflammatory activities and hepatoprotective effects [23]. The current study reveals an additional benefit of the *T. populnea* bark extracts with a low IC_{50} value against tyrosinase (190 µg/mL) suggesting its skin lightening effects. Hepatoprotective effects of phenolic acid rich fraction isolated from *T. populnea* bark were demonstrated in rats against CCl_4, acetaminophen and thioacetamide [25]. When methanol extracts of stem barks of five plants, *Ficus benghalensis*, *F. glomerata*, *F. religiosa*, *F. virens* and *T. populnea* were evaluated, highest DPPH radical scavenging action and highest superoxide scavenging potential were revealed in *T. populnea* [26]. Furthermore presence of high amount of tannins and phenolics and good Fe^{3+} reducing power in *T. populnea* were identified [26].

V. cinerea whole plant is used in Ayurveda for curing fever, blisters, boils and as a blood purifier [27]. *V. cinerea* extracts have shown therapeutic effects against chronic skin disorders. This plant has demonstrated wound healing effects, anti-inflammatory effects, immunomodulatory effects, antioxidant effects and antibacterial activity which are beneficial effects with reference to maintenance of skin health [28,29]. The present study reveals some inhibitory effects of *V. cinerea* on tyrosinase. However, the effect was similar to that of *S. album* bark with no dose-dependence on inhibitory effects on tyrosinase.

Seeds of *Vigna* genus are consumed as cheap sources of proteins and are beneficial as low glycaemic index foods [30]. These plants are comprised of number of bioactive compounds such as phytic acid, phenolic compounds and saponins. *V. radiata* is used in Asian countries as a soup to relive heat stress [30]. *V. radiata* paste is used to treat acne, eczema, dermatitis and reduce itchiness [31]. An ethyl acetate fraction obtained from 70% ethanol extract of *V. radiata*

showed highest tyrosinase inhibitory activity when compared with Dichloromethane [31] and n-butanol and residual fractions. Two flavonoids, vitexin and isovitexin, isolated from the ethyl acetate fraction showed inhibitory activities, with IC_{50} values of 6.3 and 5.6 mg/mL, respectively which are considerably higher values [32]. A product from *V. unguiculata* seed extract was patented to be used for various applications including treatment of disorders affecting the skin [33]. In the current study, further investigations were not conducted with *C. fenestratum*, *P. americana*, *V. radiata* and *V. unguiculata* to assess IC_{50} values as the inhibitory effects observed at 0.5 mg/mL against tyrosinase were rather low (2.3 to 14.4%).

4. CONCLUSION

The current study reveals significantly high antityrosinase activities in methanol extracts of *P. emblica* fruit and *T. populnea* bark among eight plant extracts investigated. To the best of our knowledge, this is the first evidence reported on the antityrosinase effect of *T. populnea* bark extracts. Further studies are necessary to validate *in vivo* efficacy and safety of these extracts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Matsuda H, Murata K, Itoh K, Masuda M, Naruto S. Melanin hyperpigmentation inhibitors from natural resources. Adv Malig Melanoma – Clin Res Perspect. 2011;171–84.
2. Solano F. Melanins: Skin Pigments and much more—types, structural models, biological functions, and formation routes. New J Sci [Internet]. 2014;2014:1–28. Available:http://www.hindawi.com/journals/njos/2014/498276/
3. Zhang C, Lu Y, Tao L, Tao X, Su X, Wei D. Tyrosinase inhibitory effects and inhibition mechanisms of nobiletin and hesperidin from citrus peel crude extracts. J Enzyme Inhib Med Chem. 2007;22(1): 91–8.
4. Cichorek M, Wachulska M, Stasiewicz A, Tymińska A. Skin melanocytes: Biology and development. Postep Dermatologii i Alergol. 2013;30(1):30–41.
5. Videira IFS, Moura DFL, Magina S. Mechanisms regulating melanogenesis. An Bras Dermatol [Internet]. 2013;88(1):76–83.
[Cited 2017 Nov 14]
Available:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3699939/pdf/abd-88-0076.pdf
6. Wu B. Tyrosinase inhibitors from terrestrial and marine resources. Curr Top Med Chem. 2014;14(12):1425–49.
7. Uchida R, Ishikawa S, Tomoda H. Inhibition of tyrosinase activity and melanine pigmentation by 2-hydroxytyrosol. Acta Pharm Sin B [Internet]. 2014;4(2):141–5.
Available:http://linkinghub.elsevier.com/retrieve/pii/S2211383513001135
8. Mistry N, Kundu RV, Ladizinski B. Widespread use of toxic skin lightening compounds: Medical and psychosocial aspects. Dermatol Clin. 2011;29(1):111–23.
9. Da Silva AP, De Silva NF, Andrade EHA, Gratieri T, Setzer WN, Maia JGS, et al. Tyrosinase inhibitory activity, molecular docking studies and antioxidant potential of chemotypes of *Lippia origanoides* (*Verbenaceae*) essential oils. PLoS One. 2017;12(5):1–17.
10. Matsuura R, Ukeda H, Sawamura M. Tyrosinase inhibitory activity of citrus essential oils. J Agric Food Chem. 2006; 54(6):2309–13.
11. Poongunran J, Perera HKI, Fernando WIT, Jayasinghe L, Sivakanesan R. α-

Glucosidase and α-amylase inhibitory activities of nine Sri Lankan antidiabetic plants. Br J Pharm Res [Internet]. 2015;7(5):365–74.
Available:http://sciencedomain.org/abstract/10063

12. Vaibhav S, Lakshaman K. Tyrosinase enzyme inhibitory activity of selected Indian Herbs. Int J Res Pharm Biomed Sci [Internet]. 2012;3(3):977–82.
Available:www.ijrpbsonline.com
13. Działo M, Mierziak J, Korzun U, Preisner M, Szopa J, Kulma A. The potential of plant phenolics in prevention and therapy of skin disorders. Int J Mol Sci. 2016; 17(2):1–41.
14. Quispe G, Hwang NY, Wang SH, Lim Z, Sung S. Screening of Peruvian medicinal plants for tyrosinase inhibitory properties: Identification of tyrosinase inhibitors in *Hypericum laricifolium* juss. Molecules. 2017;22(3).
15. Fu B, Li H, Wang X, Lee FSC, Cui S. Isolation and identification of flavonoids in licorice and a study of their inhibitory effects on tyrosinase. J Agric Food Chem. 2005;53(19):7408–14.
16. Adil MD, Kaiser P, Satti NK, Zargar AM, Vishwakarma RA, Tasduq SA. Effect of *Emblica officinalis* (fruit) against UVB-induced photo-aging in human skin fibroblasts. J Ethnopharmacol. 2010; 132(1):109–14.
17. Sharma K, Joshi N, Goyal C. Critical review of Ayurvedic *varṇya* herbs and their tyrosinase inhibition effect. Anc Sci Life [Internet]. 2015;35(1):18.
Available:http://www.ancientscienceoflife.org/text.asp?2015/35/1/18/165627
18. Jain R, Pandey R, Mahant RN, Rathore DS. A review on medicinal importance of *Emblica officinalis*. IJPSR. 2015;6(1):72–84.
19. Chaudhuri RK, Lascu Z, Puccetti G. Inhibitory effects of *Phyllanthus emblica* tannins on melanin synthesis. Cosmet Toilet. 2007;122(2):73–80.
20. Sripanidkulchai B, Junlatat J. Bioactivities of alcohol based extracts of *Phyllanthus emblica* branches: Antioxidation, antimelanogenesis and anti-inflammation. J Nat Med. 2016;68(3):615–22.
21. Moy RL, Levenson C. Sandalwood album oil as a botanical therapeutic in dermatology. J Clin Aesthet Dermatol. 2017;10(10):34–9.
22. Misra BB, Dey S. TLC-bioautographic evaluation of *in vitro* anti-tyrosinase and anti-cholinesterase potentials of sandalwood oil. Nat Prod Commun. 2013;8(2):253–6.
23. Parthasarathy R, Ilavarasan R, Karrunakaran CM. Antidiabetic activity of *Thespesia populnea* bark and leaf extract against streptozotocin induced diabetic rats. Int J PharmTech Res [Internet]. 2009;1(4):1069–72.
[Cited 2017 Dec 2]
Available:http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.550.4939&rep=rep1&type=pdf
24. Fathima NMN, Varnakulendran N. Evaluation of the wound healing activity of *Thespesia populnea* stems bark on Wistar albino rats. Int J Adv Ayurveda Naturop. 2017;2(1):6–13.
25. Yuvaraj P, Subramoniam A, Louis T, Madhavachandran V, Antoney M, Narasu ML. Hepatoprotective properties of phenolic acids from *Thespesia populnea* Soland ex. Correa. Ann Phytomedicine. 2012;1(2):74–87.
26. Anandjiwala S, Bagul MS, Parabia M, Rajani M. Evaluation of free radical scavenging activity of an ayurvedic formulation, *Panchvalkala*. Indian J Pharm Sci. 2008;70(1):31–5.
27. Dogra NK, Kumar S. A review on ethno-medicinal uses and pharmacology of *Vernonia cinerea* Less. Nat Prod Res. 2015;29(12):1102–17.
28. Prabha LJ. Therapeutic uses of *Vernonia cinerea* -A short review. Int J Pharm Clin Res [Internet]. 2015;7(4):323–5.
[Cited 2017 Oct 21]
Available:www.ijpcr.com
29. Saraphanchotiwitthaya A, Sripalakit P. Anti-inflammatory activity of a *Vernonia cinerea* methanolic extract *in vitro*. ScienceAsia [Internet]. 2015;41:392–9. [Cited 2017 Oct 21]
Available:http://www.scienceasia.org/2015.41.n6/scias41_392.pdf
30. Kumari M, Rana A. Bioactive components of Vigna species: Current Prospective. 2017;6:1–13.
31. Tang D, Dong Y, Ren H, Li L, He C. A review of phytochemistry, metabolite changes, and medicinal uses of the common food mung bean and its sprouts

(*Vigna radiata*). Chem Cent J. 2014;8(1): 1–9.

32. Yao Y, Cheng X, Wang L, Wang S, RG. Mushroom tyrosinase inhibitors from mung bean (*Vigna radiatae* L.) extracts. Int J Food Sci Nutr. 2012;63(3):358–61.

33. Msika P, Saunois A, Leclere-Bienfait S, Baudoin C, Inventors; laboratoires expanscience, assignee. *Vigna unguiculata* seed extract and compositions containing same. United States Patent US 8,840,939; 2014.

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