INJ INTERNATIONAL NEUROUROLOGY JOURNAL

Collection And Screening Of Medicinal Plants To Access Its Tyrosinase Inhibition Activity Using HPTLC And GC-MS For Skin Melanogenesis

Arthi Rangasamy*[1], Vinoth kumar[2], Rajesh Raghupathi[3], D. Geetharamani[4]
[1,2]School of Biological sciences, CMS college of Science and Commerce, Coimbatore, India.
[3]CEO, RND Softech Pvt Limited, Harvard Business School.
[4]Dean, Dr.NGP college of Arts and Science, Coimbatore, India

Abstract

Tyrosinase is a key amino acid that catalyzes the synthesis of melanin in plants, microbes, and pigment cells, also known as polyphenol oxidase. The intracellular compound of melanin biosynthesis is helpful for skin lightening agents used in products and for hyperpigmentation disorders. Going to contribute to their role in cancer prevention tissue remodelling. Tyrosinase enzyme has been used as a model organism for assessing plant extract's anti-tyrosinase behaviour. The objective of this work was to examine the inhibitory behaviour of 57 plants using methods of cell death of mushroom tyrosinase. In the very same solvent structures, plant extracts have been prepared in hexane, ethyl acetate, methanol, ethanol, isopropanol, and water-positive kojic acid regulates. Five plants were tested among the screened herbal plants, higher oxidative stress was found in Glycyrrhiza glabra and Mutingia calabura

Key words: Tyrosinase inhibition activity, HPTLC, GC-MS, alkaloids, medicinal plants, Melanin, hyperpigmentation.

Introduction

There is a significant shield that defends our body from problems due to direct contact with the external environment. Melanin is a major skin colouring compound acts by capturing sunlight and eliminating reactive oxygen species, it defends our skin from UV exposure. Approximately 10% of skin cells in the epidermis the outermost layer contains melanin. Melanogenesis is triggered by the protein tyrosinase resulting in the darkening of the skin following exposure to UV radiation (1). In various dermatological illnesses such as melasma, solar lentigines, and ephelides, the hyperpigmentation of skin is observed.

Responding to the needs of customers has become necessary for the cosmetics industry. While product quality and security are important, sensory features can increase the acceptance of customers and marketing expenses. Ingredients were therefore not only created for technical functions but specific sensory objectives. For example, the inclusion of talc allows the formulation to have an absorbent effect and provides a non-sticky contact. Modified starches boost material consistency and density and leave a pleasant after-feel (2). Nylon 12 (polyamide) incorporates a soft touch with good uptake properties, making it easier to apply and spread. Unfortunately, both above claims are unoptimized, and there is generally a lack of a scientific approach to evaluating skin feeling ingredients.

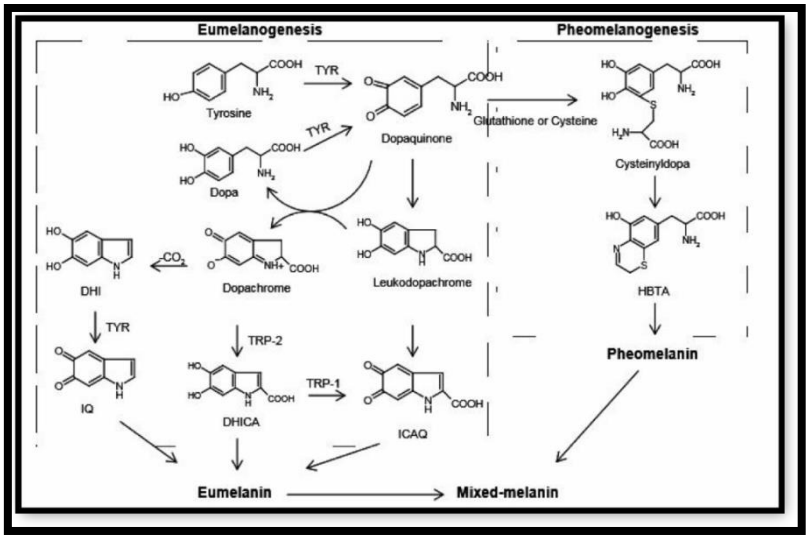

Figure 1 - melanogenesis

Using medicinal herbs as herbal medicines to prevent and remedy several disorders differs from one society to another. The genetic scientific communities have recently caught the attention of traditionally used edible plants (3). Methods of production involve separating medicinally active decimals of plant tissue from dormant/inert components using selective chemicals and removal technology. Solvents spread into the solid tissues of plants and solvent compounds of comparable polarity. Tyrosinase is accountable for mammalian skin pigments, ears, and hair. Tyrosinase is in natural plant tissue chloroplasts in plants, while its substrates are in the lipid membrane. Brushing, peel or smashing plant tissue causes this compartment to lose, causing browning processes induced by tyrosinase to occur. The enzymes are interested in pigmentation and blood clotting. (4). Natural products that contain melanin modulation excitatory activity are of interest, such as skin-whitening or anti-browning preparedness, with their potential cosmetic implementations. In various skin conditions, local hyperpigmentation can be found such as lentigo (a flat brownish pigmented place on the skin), nevus (usually congenital nodular tumor) and ephelis (pimple). Hyperpigmentation can also be caused by autoimmune disorders, such as acne and eczema. Another popular hyperpigmentation is melasma, diffuse hyperpigmentation of the face that occurs during childbirth or hormonal use (5).

In this research, we contrasted tyrosinase excitatory activity using the method of inhibition of mushroom tyrosinase on 57 plants with specific parts obtained from licensed sellers of herbal material. Plant components were derived against well-known good control, kojic acid, with 5 different solvents (Ethylene, ethanol, hexane, isopropanol, and methanol) and deuterium oxide water. Pro-tyrosinase activity qualitative analysis was performed using UV spectrophotometry to calculate dopachrome and intermediate goods in the melanin synthesis path.

Materials And Methods

Plant collection and extraction

The plants were purchased from a from licensed botanist, Coimbatore, India, with their parts. In the study, their fresh and dry parts were used according to the requirement. Water was used to wash the organic materials. They are cut by Mortal pestle and then grounded. In hexane, ethanol, ethyl acetate, methanol, and water, plant extracts were prepared. 25 g of fresh plant materials are collected at 30 ° C for 12 h in a 100 mL solution of hexane, potassium carbonate and ethanol using maceration (cold extraction). Upon filtration, all plant extract is collected. Eventually, for all plant extracts, test sample solutions are prepared (6).

Sigma Chemistry bought L-DOPA and Mushroom Tyrosinase. Mushroom tyrosinase 20 µL (1000 U / mL), phosphate buffer 20 µL (pH 6.8) and study sample 100 µL (20%) combined with 20 µL of plant extracts (known as the enzyme analysis solution). Also, without plant extract, the specimen extract was ready.

Tyrosinase solution preparation

Total of 57 plants were bought from licensed botanist. The selected plants were immersed in 200 mL of phosphate buffer solution (0.2 M, pH 7.2) after decortication and cutting, ground into a substance and filtered with paper. At 4000 g / 5 min, the solution was centrifuged. For the next use, the supernatant has been suctioned and stored at −4 ° C.

Antityrosinase assay

Tyrosinase (the activity of phenoloxidase) that catalyzes the transition of L-tyrosine by hydroxylation into L-DOPA and by combustion into O-dopaquinone. Instead, through a sequence of non-enzymatic reactions, O-dopaquinon is converted rapidly into melanins, calculated in a gas chromatograph at 492 nm. The enzyme-free reaction mixture serves as a blank. The mixture of the reaction with the appropriate solvents (without plant material) provides as a power (7).

High-Performance Thin-layer chromatography (HPTLC)

HPTLC with ethyl acetate: methanol: formic acid: water [20:2.5:0.5:2 (v / v)] as a mobile phase was performed on kitty litter 60 f 254, 20X10 cm HPTLC trays (Merck, Germany-#5642). The standard alternatives (Quercetin, Rutin, Luteolin and Vitexin) (5.0 µL of each 1 mg / mL concentration) were extended as 10 mm bands on the plates, sample implementation with CAMAG-Linomat IV automatic belt spray fitted with 100 µL needle and worked with the following settings: 10 mm band size, 10 sec/µL application speed, 4 mm range, Tray side edge length of 1.5 cm and plate bottom distance of 2 cm (10, 14). Using WIN CATS technology (version 4 X), CAMAG TLC Scanner 3 has been used to measure the band densitometric ally (8).

© International Neurourology Journal

ISSN:2093-4777 | E-ISSN:2093-6931
DOI: 10.5123/inj.2024.2.inj103
Vol. 28 Iss. 2 (2024)

INJ INTERNATIONAL NEUROUROLOGY JOURNAL

Results And Discussion

The reading with or without extract was taken at 475 nm, using a spectrophotometer (Jasco V-530) to test all four forms of cannabinoids. The proportion of inhibition was calculated with the help of a given method. Figure 1 displayed all the percentage inhibition values.

The proportion of tyrosinase inhibition has been determined as follows:

$$\% \text{ tyrosinase inhibition} = \frac{(A - B) - (CD\ D)}{(A - B)} \times 100$$

Where,

A = blank solution absorbance of the protein
B = blank solution absorbance without protein
C = absorption of the enzyme test solution
D = sample solution absorbing without enzyme

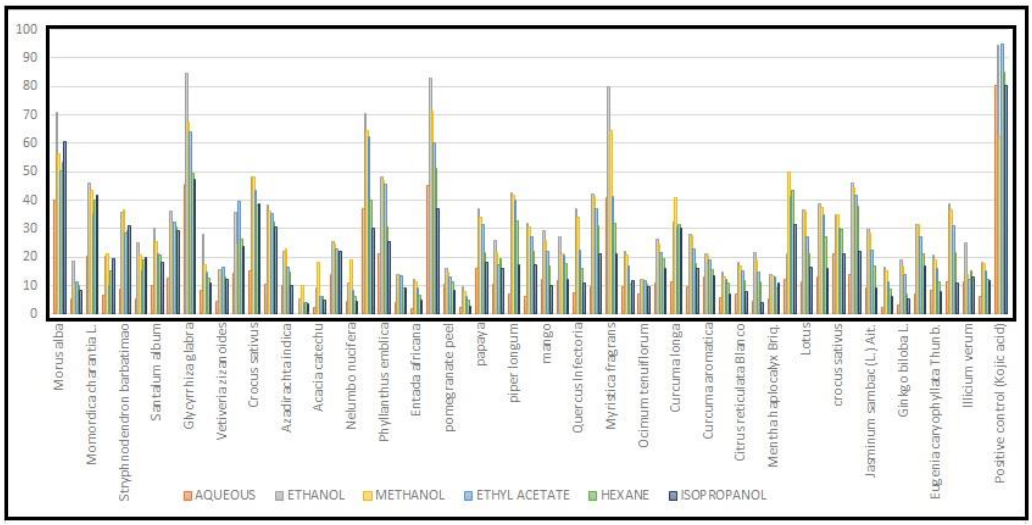

Figure 1 - Inhibition percentages of different organic solvents and aqueous extracts.

With the aid of a spectrophotometer, all six forms of compounds are measured using a formula proportion of repression. Figure 2 here displays the rate of inhibition. Inhibition of tyrosinase by kojic acid (positive control) varied by types of organic solvents, from 85% for kojic acid in hexane to 96% for kojic acid in ethyl acetate. Four of the above 59 plant species had a good tyrosinase secretion with stimulation of 60 to 90% equivalent to that of kojic acid. Five plant components with relatively high levels of tyrosinase in three various organic solvents and deionized water are ethyl acetate, ethanol, and isopropanol. Similarly, plants that exhibited good inhibition activity are *Morus alba, Glycyrrhiza glabra, Zingiber officinale, Mutingia calabura, Myristica fragrans*.

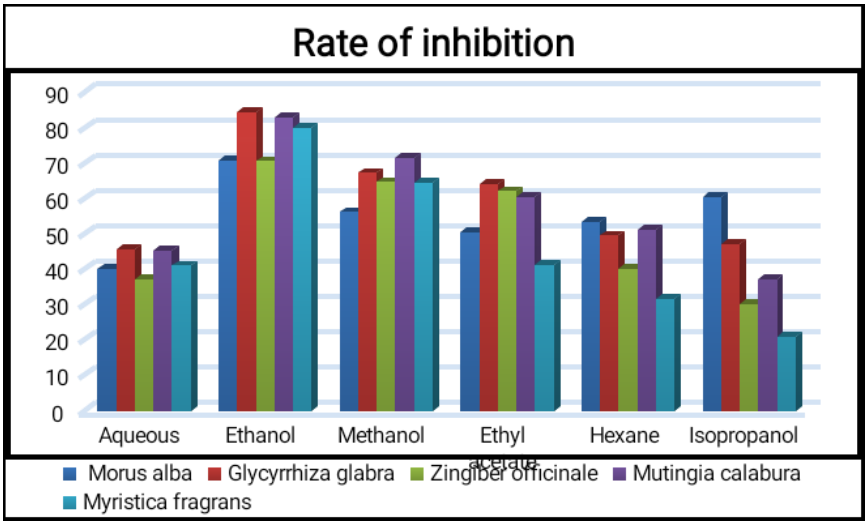

Figure 2 – Plants that exhibited inhibition from different solvents

© International Neurourology Journal
DOI: 10.5123/inj.2024.2.inj103
ISSN:2093-4777 | E-ISSN:2093-6931
Vol. 28 Iss. 2 (2024)

Melanogenesis is a significant biological phenomenon in melanocytes to prevent infection against free radical attacks that can cause skin cell injury. Excessive melanin production from melanocytes, though, occurs in hyperpigmentation diseases, i.e. skin darkening. Figure 2 demonstrated the effects of the capacity for tyrosinase activation of a common solvent derived from two medicinal herbs.

Substances found in plants with strong activation of antityrosinase were reported to be already connected with α– arbutin. An anomer of the found naturally arbutin is α–Arbutin, or 4-hydroxy phenyl α-D glucopyranoside. The drug, without significant adverse reactions, is a powerful suppressor of melanin synthesis in human skin. α-arbutin has just been confirmed to inhibit human tyrosinase much more strongly than arbutin does (9).

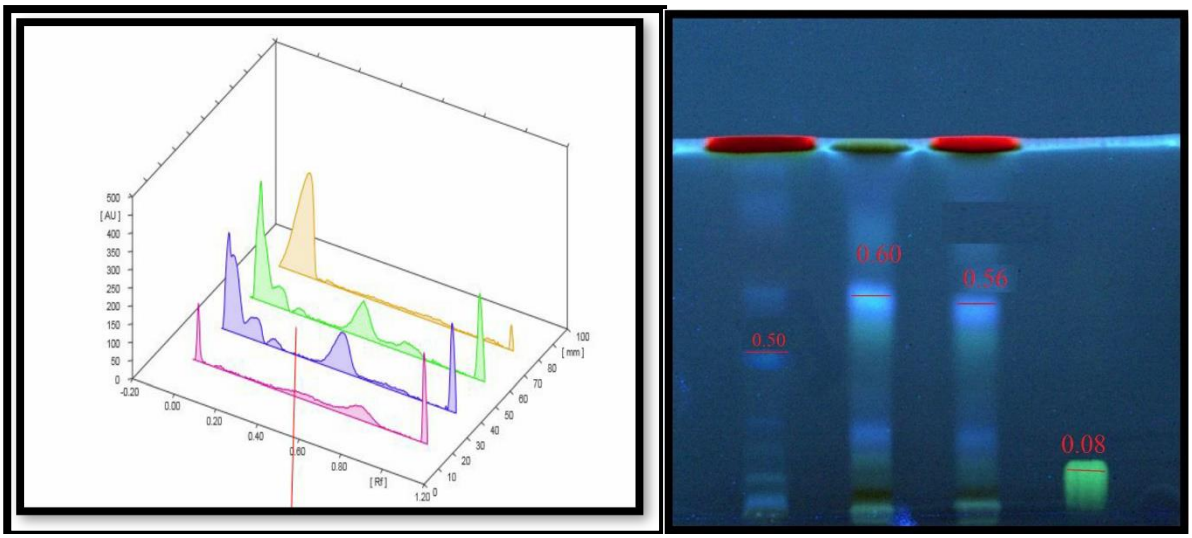

Figure 3a - High-Performance Thin-layer chromatography (HPTLC) for sample 3 (Equal quantity of *mutingia Calabria* and *Glycyrhizza glabra*). 3b - Chromatograms obtained from the separation of plant extracts

Four various mobile stages have been verified utilizing silica gel HPTLC plates to separate alkaloids. Sample 1(*mutingia calabura*), sample 2 (*Glycyrhizza glabra*), sample 3 (equal quantity of plant 1 and 2) and specification were the only stage that permitted us to visualize distinctions between the excerpts researched. HPTLC was performed for sample 3 which is *mutingia calabura* and *Glycyrhizza glabra*. The use of these solvent processes guarantees that the flavonoids are well isolated to respectively. Its mobile stage improves on the old method mentioned in Brasseur and Angenot and can substitute the one explained in the last version of the European Pharmacopoeia, Quercetin (Rf values: 0.05, 0.60, 0.65, 0.08) (Figure 3b), combined. Of the compounds obtainable as values, Quercetin was found in many other species of the plant. An amount of flavonoids present in such plants is shown as Sample 3 > Sample 1 > Sample 2 > Quercetin. Many flavonoids ' fluorescence bands are not recognizable at 254 nm wavelength, but at 366 nm they are apparent.

GC-MS analysis

The methanol extract of sample 3 (Sample 1(*mutingia calabura*), sample 2 (*Glycyrhizza glabra*), sample 3 (equal quantity of plant 1 and 2)) was analysed using GC–MS, and a total of 8 compounds were identified by relative matching of the mass values of the GC–MS eluted compounds of the extract and the respective standards given in the GC–MS database. The results demonstrated that the major constituent was myo-Inositol, 4-C-methyl- (14.50%), and that the minor compounds were found to be butane, 1-bromo-2-methyl, 1,3-dioxolane-2-methanol, 2-butanone, 3-meth-oxy-3-methyl 1,3-dioxolane, 2-(2-propenyl), pentane, 1,3-epoxy-4-methyl, 4-methyl-2,4-bis(40trimethylsily-loxyphenyl)pentene-1 and benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester. Among these compounds, 3-O-Methyl-D-glucose was found to be major compound with peak area of 91.89%, whereas phthalic acid, ethyl pentyl ester, 2-butanone, 3-methoxy-3-methyl,2,2-dimethylpropionic acid, cyclopentyl ester, 2-hexen-1- ol, 2-ethyl, 5-hydroxy-2,2-dimethylhexan-3-one, pentanoic acid, 2-methyl and butane, 1-bromo-2-methyl were present in trace amount with lower peak areas less than 1%

© International Neurourology Journal

ISSN:2093-4777 | E-ISSN:2093-6931
DOI: 10.5123/inj.2024.2.inj103
Vol. 28 Iss. 2 (2024)

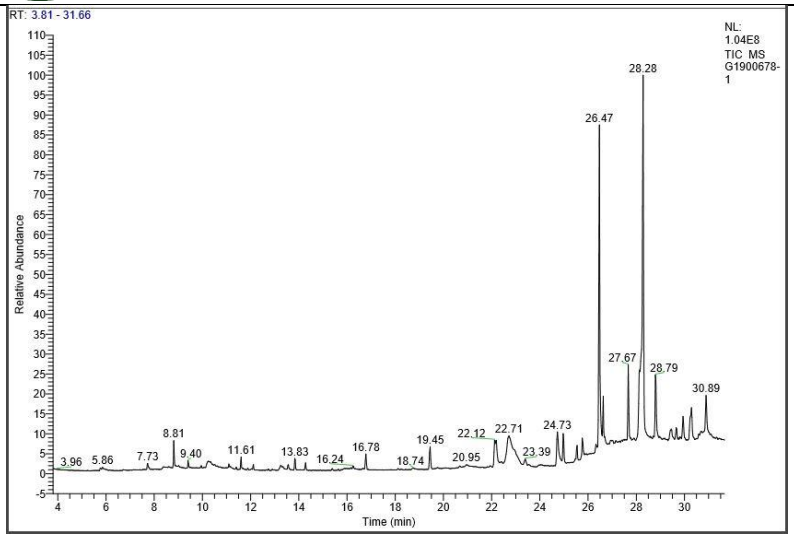

Figure 3 GCMS analysis sample 3 (Sample 1(*mutingia calabura*), sample 2 (*Glycyrhizza glabra*)

Conclusion

Totally 57 plants were selected with 6 different solvents. Initially first screening was performed for 6 plants with 6 different solvents from this we chose 2 plants(Sample 1(*mutingia calabura*), sample 2 (*Glycyrhizza glabra*))and 1 solvent (Ethanol). Finally, we used plant 1 as sample 1 and plant 2 as sample 2 and the third sample as equal quantity of sample. Sample 1 and sample 2 were further used for HPTLC and sample 3 is taken for GC-MS analysis. *Glycyrrhiza glabra, Mutingia calabura*, was found to be superior. With the different extract of five various solvents in cosmetic application to provide the advantages of skin lightening and anti-aging once dermal security is guaranteed.

References

1. G. Balakrishnan, L. Janakarajan, A. Balakrishnan, and B. S. Lakshmi, "Molecular basis of the anti-inflammatory property exhibited by cyclo-pentano phenanthrenol isolated from *Lippia nodiflora,*" Immunological Investigations, vol. 39, no. 7, pp. 713–739, 2010.
2. A. M. Abbasi, M. A. Khan, M. Ahmad, M. Zafar, S. Jahan, and S. Sultana, "Ethnopharmacological application of medicinal plants to cure skin diseases and in folk cosmetics among the tribal communities of North-West Frontier Province, Pakistan," Journal of Ethnopharmacology, vol. 128, no. 2, pp. 322–335, 2010.
3. A. K. Basu, P. Chakraborti, and P. K. Sanyal, "NodifloretinA new flavone from 6 Lippia nodifloria," Journal of Indian Chemical Society, vol. 46, no. 4, pp. 271–272, 1969. [22] F. A. Tom´as-Barber´an, J. B. Harborne, and R. Self, "Twelve 6-oxygenated flavone sulphates from *Lippia nodiflora* and L. canescens," Phytochemistry,vol.26,no.8,pp.2281–2284,1987.
4. A. G. R. Nair, P. Ramesh, and S. Nagarajan, "New flavone glycosidesfromLippianodifloria," IndianJournalofChemistry, vol. 2, pp. 1316–1317, 1973. [24] S. D. Elakovich and K. L. Stevens, "Volatile constituents of *Lippia nodiflora*," Journal of Natural Products, vol. 48, no. 3, pp. 504–506, 1985.
5. V. Ravikanth, P. Ramesh, P. V. Diwan, and Y. Venkateswarlu, "Halleridone and Hallerone from *Phyla nodiflora* as taxonomic markers," Biochemical Systematics and Ecology, vol. 28, no. 9, pp. 905–906, 2000.
6. B. S. Siddiqui, F. Ahmed, S. K. Ali, S. Perwaiz, and S. Begum, "Steroidal constituents from the aerial parts of *Lippianodi flora* Linn.," Natural Product Research, vol. 23, no. 5, pp. 436–441, 2009.
7. Gyamfi MA, Yonamine M, Aniya Y (2002). Free radical scavenging action of medicinal herbs from Ghana Thonningia sanguine on experimentally induced liver injuries.Gen. Pharmacol. 32: 661-667.
8. Pinnell SR (2003). Cutaneous photodamage, oxidative stress, and topical antioxidant protection. J. Am.Acad. Dermatol.48, 1-19.
9. Noguchi N, Niki E (2000). Phenolic antioxidants: A rationale for design and evaluation of novel antioxidant drug for atherosclerosis. Free Radic. Biol. Med.28, 15381546. 11. Mayo JL (1998). Black cohosh and chasteberry: herbs valued by women for centuries. Clinical Nutrition Insights 6, 1–4.
10. Kubo, I.; Nihei, K.; Shimizu, K. Oxidation products of quercetin by mushroom tyrosinase. Bioorg. Med. Chem. 2004, 12, 5343-5347.