Evaluation of *Pterocarpus santalinus* Linn. f. methanolic extract as a natural melanogenesis inhibitor – *in vitro* study in B16F0 melanoma cell lines

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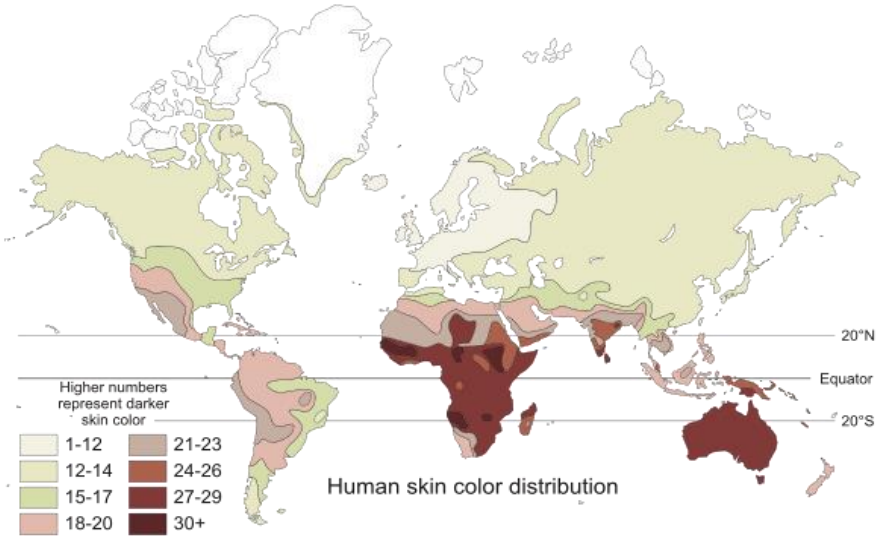
Skin – The first line of defence

- Melanin
- Human skin color
- Darkest brown- pinkish white hues
- Females are evolved lighter than male-
  - ↑ Calcium –pregnancy and lactation

(Muehlenbein, 2010)

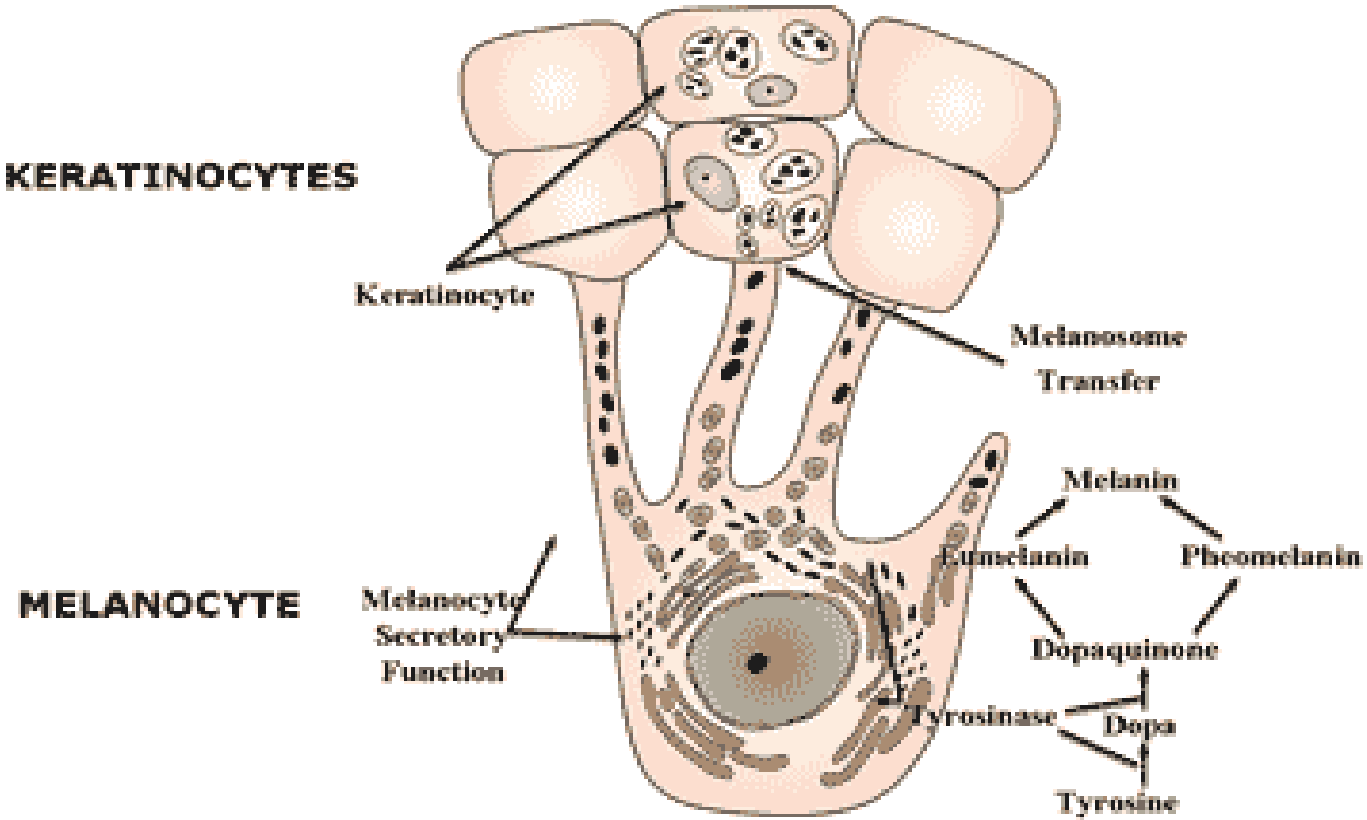
	1	10			19	28	
	2	11			20	29	
	3	12			21	30	
	4	13			22	31	
	5	14			23	32	
	6	15			24	33	
	7	16			25	34	
	8	17			26	35	
	9	18			27	36	



Melanocytes, melanosomes and melanin

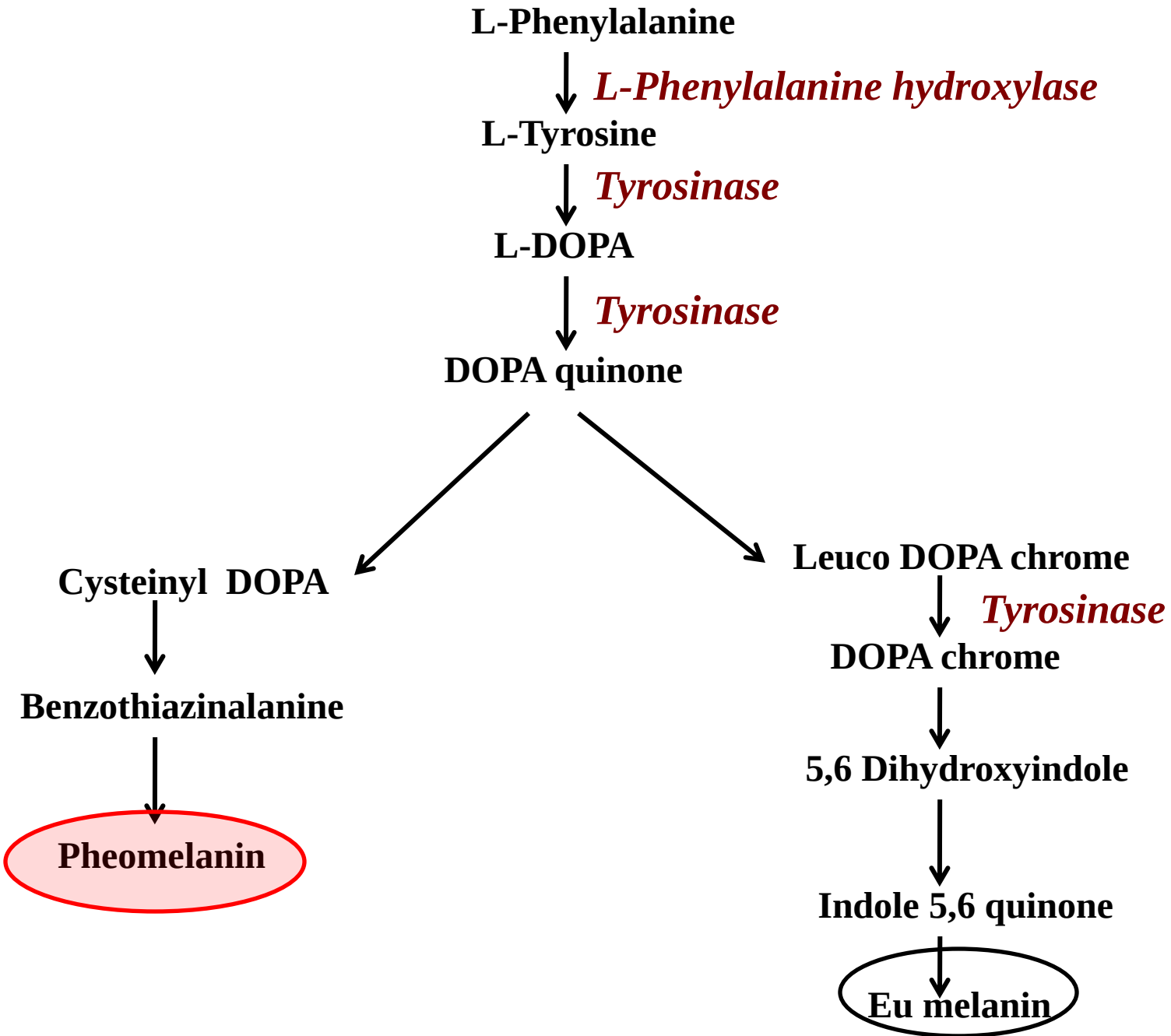
- Melanocytes 1000-2000/sq mm and Melanosome – 500nm
- Melanogenesis – need and role
- Melanin - melas – “black, dark”

Melanin synthesis – tyrosinase (Tishkoff, 2009)



(Chang, 2009)

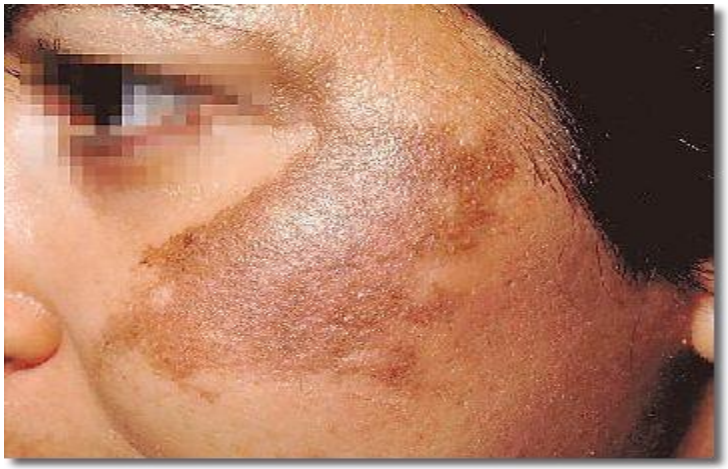
Melanogenesis pathway



Hyperpigmentation – A Major disorder

- Skin pigmentation – melanin in epidermis – defence against solar radiation and absorbing free radicals.
- ↑ melanin – age spots, freckles, chloasma, solar lentigo, melasma, melanoderma incurred by inflammation including eczema, dermatitis and distressing skin problems as well as aesthetic problems.
- causes - hormonal changes, ageing, chronic inflammation, uv light exposure.
- Prevention – reduce exposure to uv and skin lightening agents.

(Uchida et al., 2014; Lin et al., 2007)



Pterocarpus santalinus L.

Common name: raktha chandanam, red sanders wood

Coloring component: santalin

Dye: red

Pharmacological uses:

- The heartwood is rubbed with water, honey, ghee, and oil, applied as collyrium to alleviate defects of vision.
- Treating skin diseases, bone fracture, leprosy, spider poisoning, scorpion sting, hiccough, ulcers, improve complexion, general debility and metal aberrations.

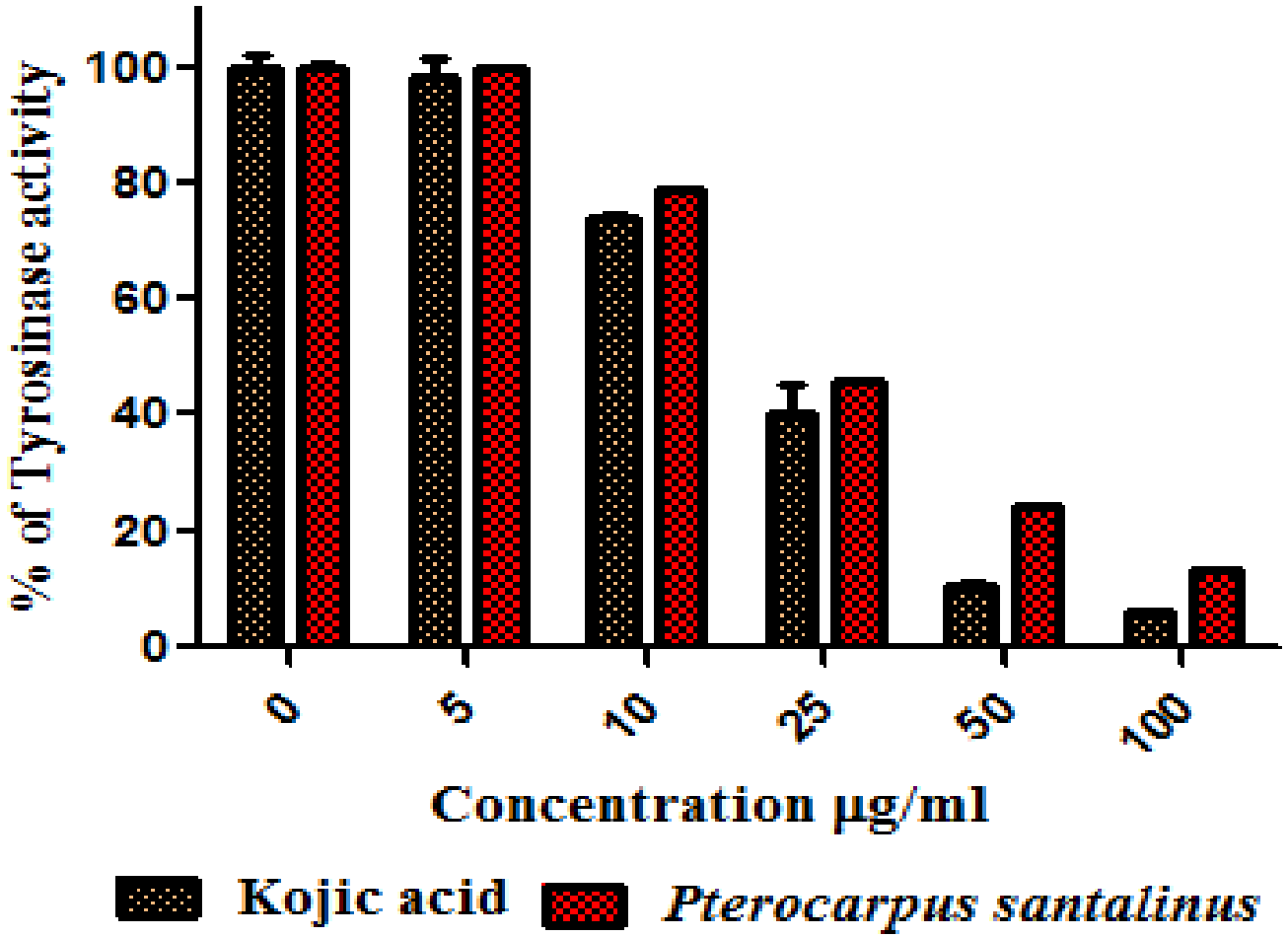
(Siva 2007, Arunakumara et al, 2007)

Taxonomic classification

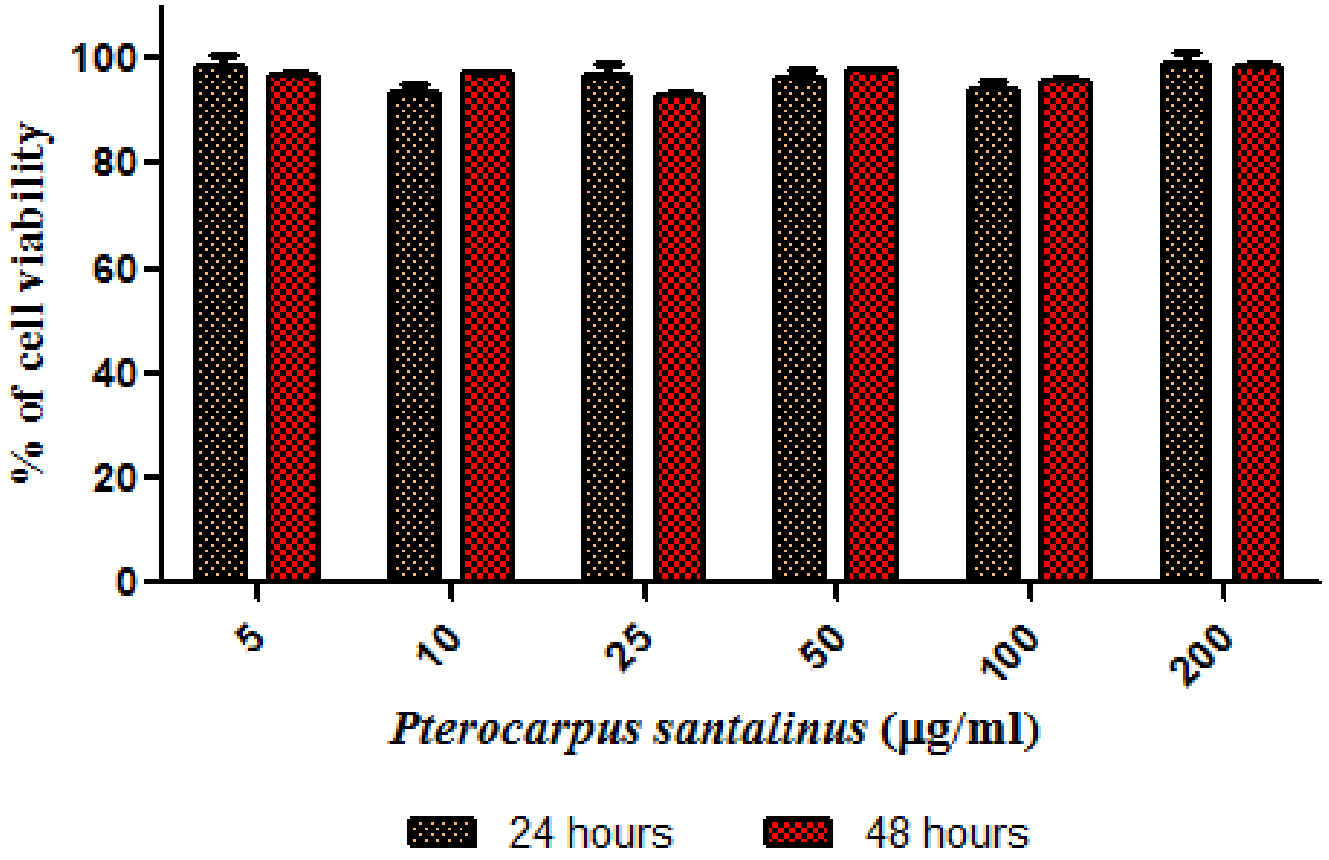
Kingdom:Plantae
(unranked):Angiosperms
(unranked):Eudicots
(unranked):Rosids
Order:Fabales
Family:Fabaceae
Subfamily:Faboideae
Tribe:Dalbergieae
Genus:*Pterocarpus*
Species: *santalinus L.*

Methodology

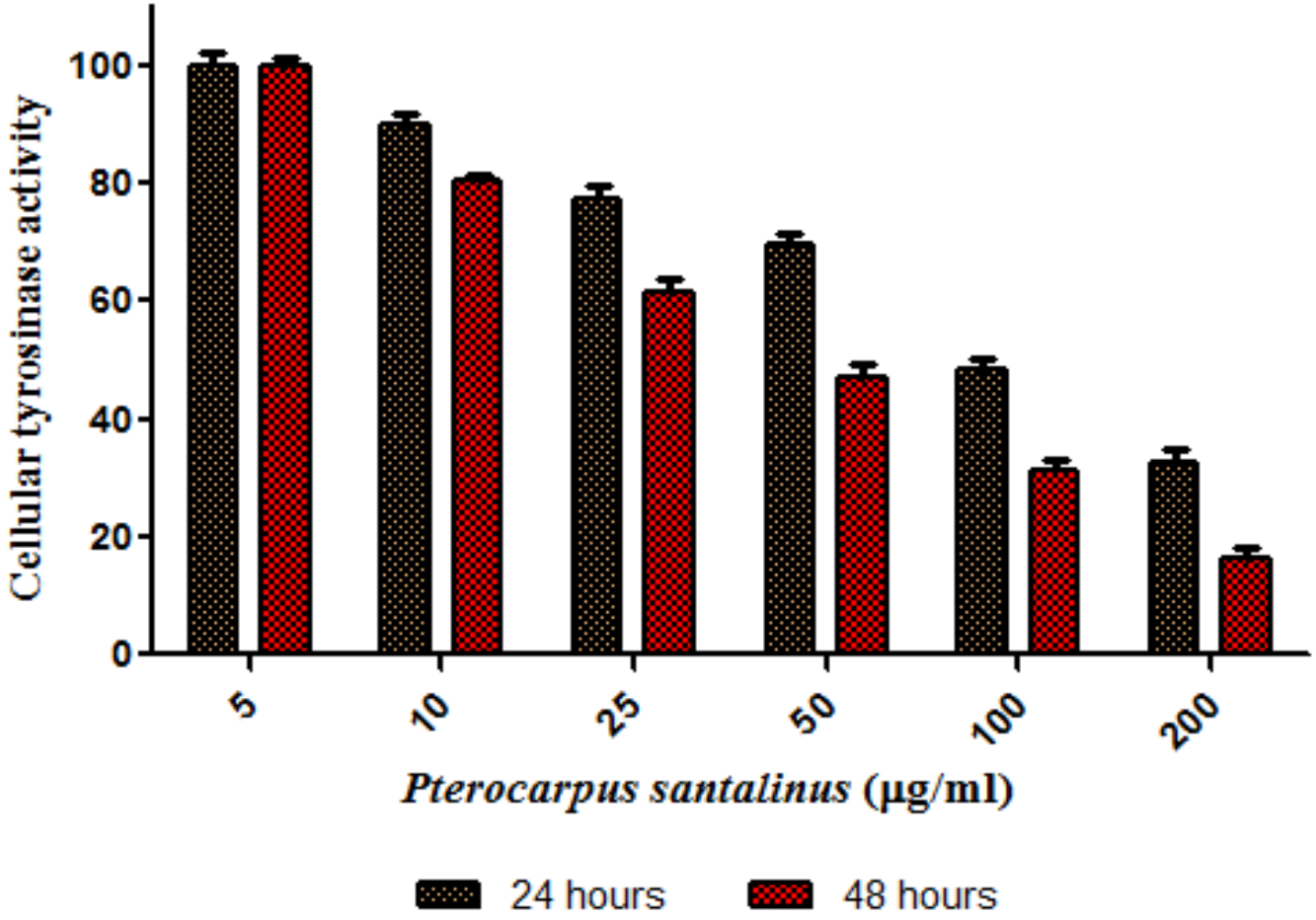
1. Extraction of *Pterocarpus santalinus* methanolic extract
2. Effect of *Pterocarpus santalinus* on cell free tyrosinase activity
3. Cell viability, cellular tyrosinase, melanin content of *Pterocarpus santalinus* extract treated B16F0 melanoma cells
4. Melanogenic gene expression – RT PCR
5. Acute dermal toxicity



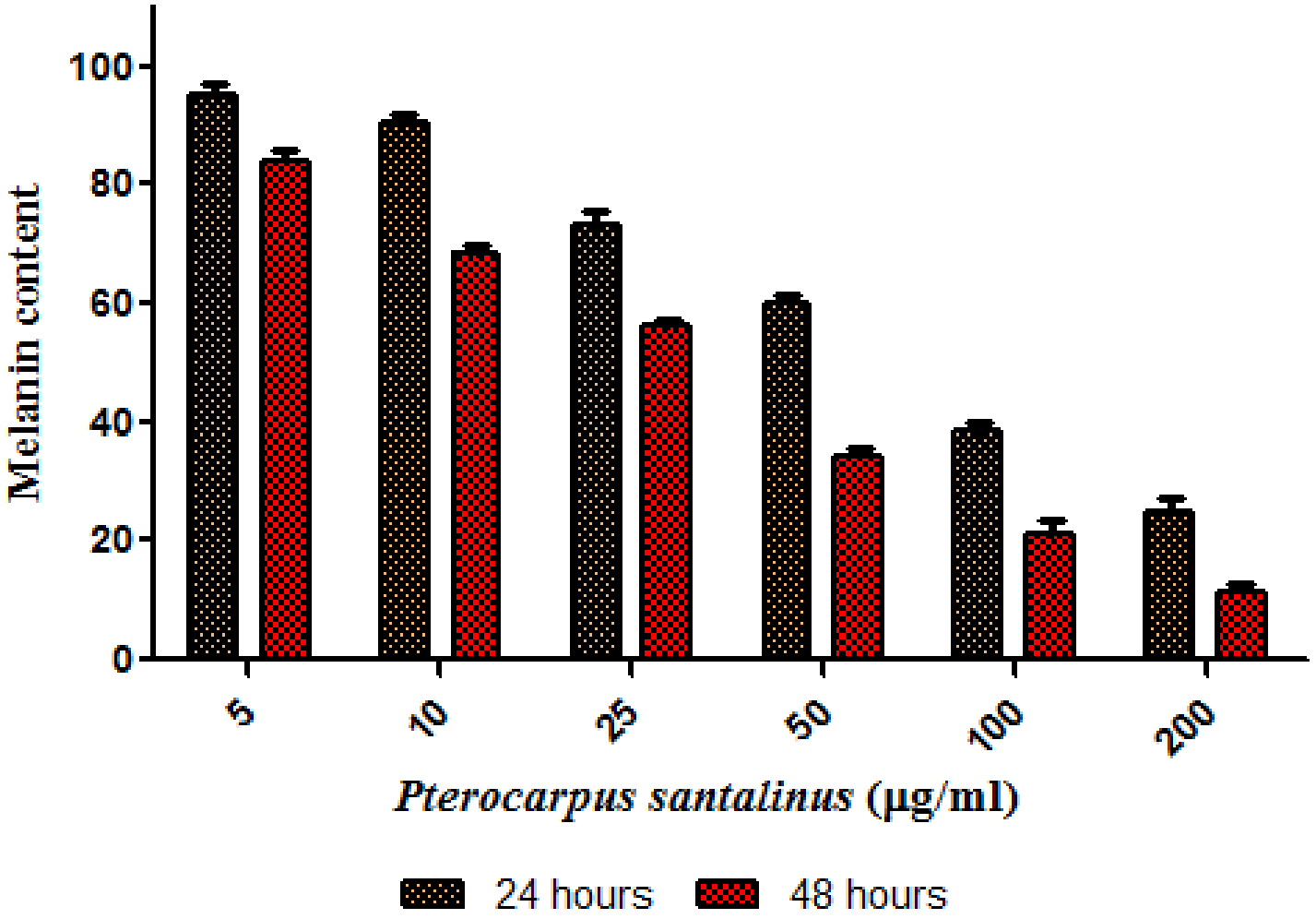
Inhibitory effect of *Pterocarpus santalinus* extract on tyrosinase activity. Data are presented as mean ± SD (n = 3). IC50 value of *Pterocarpus santalinus* 25.63 ± 0.569 µg/ml



The cell viability of B16F0 after treatment with different concentration of *Pterocarpus santalinus e*xtract (5, 10, 25, 50, 100, 200 µg/ml) for 24 and 48 hours



Effect of *Pterocarpus santalinus* extract on cellular tyrosinase activity in B16F0 cells for 24 and 48 hours.



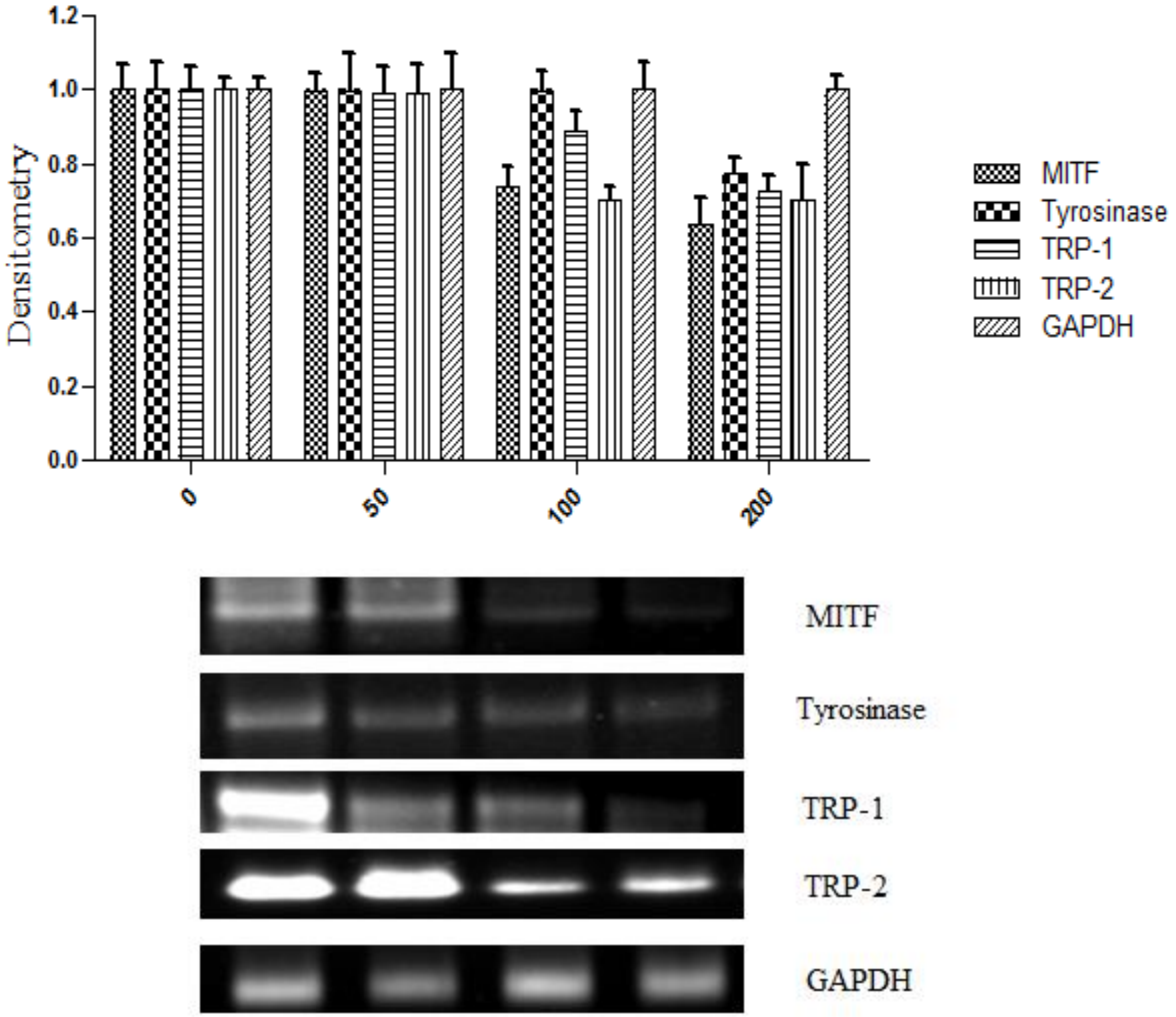
Effect of *Pterocarpus santalinus* extract on melanin synthesis in B16F0 cells at 24 and 48 hours.

RT-PCR

Melanogenic Genes	Forward Primer	Reverse Primer
MITF	5’-AGTACAGGAGCTGGAGATG-3’	5’-GTGAGATCCAGAGTTGTC-3’
TYROSINASE	5’-GGCCAGCTTTCAGGCAGAGGT-3’	5’-TGGTGCTTCATGGGCAAA-3’
TRP1	5’-GGCCTCTGAGGTTCTTTAAT-3’	5’-AATGACAAATTGAGGGTGAG-3’
TRP2	5’-ATGAGAAACTGCCAACCTTA-3’	5’-AGGAGTGAGGCCAAGTTATGA-3’
GAPDH	5’-GTGAAGGTCGGTGTGAACG-3’	5’-CTCGCTCCTGGAAGATGGTG-3’

PCR conditions

40 cycles at 94^{o}C for 30s, 55^{o} C for 30s and 72^{o}C for 30s



Pterocarpus santalinus extract decreases MITF and tyrosinase gene expression in B16 melanoma cells. B16 melanoma cells were treated with the higher concentrations of *Pterocarpus santalinus* extract (50, 100 and 200 µg/ml) for 48 h.

Acute Dermal Toxicity Test OECD guideline number 402

Animal: Wistar rats (*Rattus norvegicus*)
Weight: approximately 200 grams
Gender: Female (6 animal per group) (Monique et al, 2010)

Ethical clearance issue : VIT/IEAC/9/July 26/No.20

Groups	Dose (mg/kg body weight)
Group I (control)	Treatment with vehicle (70% ethanol)
Group II (PS extract)	500
Group III (PS extract)	1000
Group IV (PS extract)	2000

Acute Dermal toxicity test: (OECD guideline number 402)

Week 1 : Acclimitization to housing conditions
Week 2: Day 0 - 10% of hair will be shaved and caged individually (24 hours).
Day 1- extract applied with guaze patch and covered (24hours)
Day 2- rinsed (1,24,48 and 72hours) observed for skin irritation test
Week 3: till day 14- observations and body weight measurement.
Week 4: day 15- sacrifice.

Analysis :

- Morphological analysis : observation of erythema and oedema and its scoring according to Draize scoring system.

- Body weight measurement

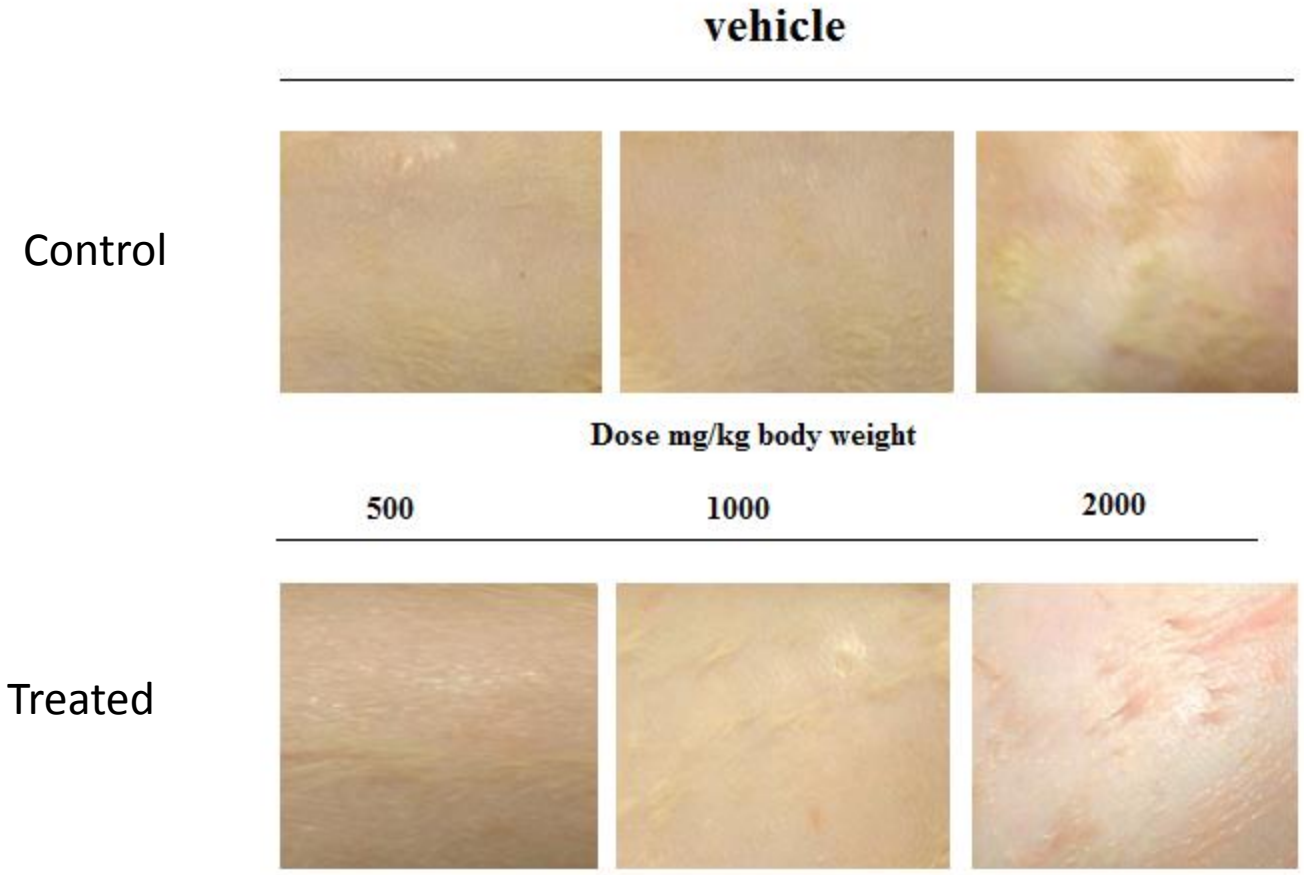
- Histopathological analysis of treated skin (Isbrucker 2006).

Group	Dose mg/kg body weight	Erythema					Oedema				
		1 hour	24 hour	48 hour	72 hour	14th day	1 hour	24 hour	48 hour	72 hour	14th day
1	0 (Control)	0	0	0	0	0	0	0	0	0	0
2	PS 500mg	0	0	0	0	0	0	0	0	0	0
3	PS 1000mg	0	0	0	0	0	0	0	0	0	0
4	PS 2000mg	0	0	0	0	0	0	0	0	0	0

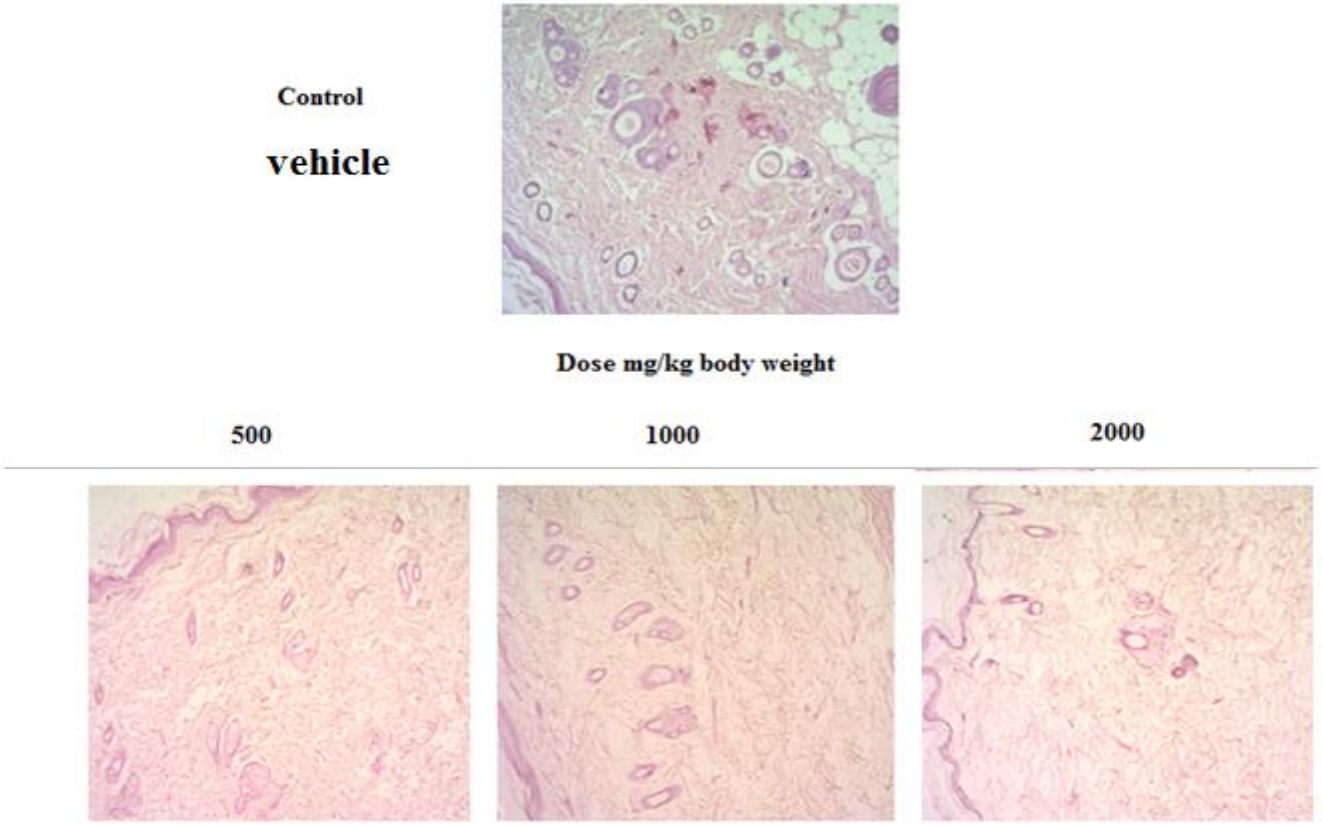
Skin reaction scored for control and treated groups at different time intervals according to draize skin irritation scoring, 0: no erythema or no oedema; 1: barely perceptible erythema or oedema; 2: well defined erythema or slight oedema; 3: moderate to severe erythema or moderate oedema; 4: severe erythema or oedema.

Group	Dose mg/kg Body weight	Day 0	Day 7	Day 14
1	0 (Control)	208.6±1.59	225.3±1.52	243.6±3.78
2	PS 500mg	215.3±2.10	236.5±1.98	256.8±3.65
3	PS1000mg	213.3±1.09	233.9±2.87	254.7±2.76
4	PS 2000mg	225.0±1.31	244.3±1.65	272.0±2.24

Body weight of rats treated with *Pterocarpus santalinus* extract on skin. Data are expressed as mean ± S.D, No statistical difference between control and treated group (P>0.005).

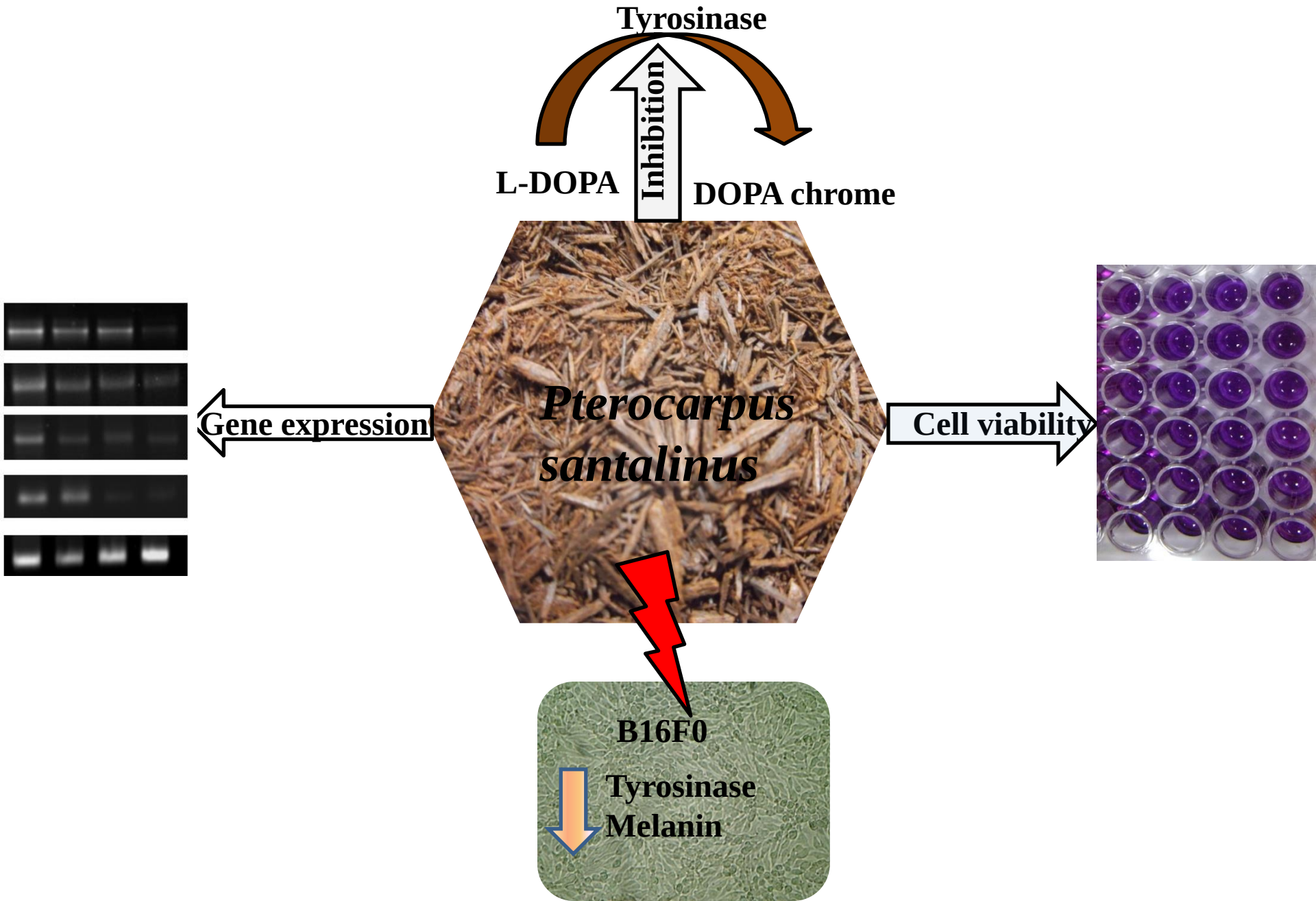


Representative Images of skin irritation test observed post treatment of *Pterocarpus santalinus* extract



Histopathological evaluation of skin tissues treated with *Pterocarpus santalinus* (Magnification 10X)

An overview of the research work



Research Outcomes:

- Significant Anti tyrosinase activity was exhibited by *Pterocarpus santalinus* extract dose dependently

- Decreased cellular tyrosinase activity and melanin content without cytotoxicity to the treated cells time and dose dependently

- Down regulation of Melanogenic genes

- *Pterocarpus santalinus* – dermal application – safe

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