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In vitro Hyperpigmentant activity on the bark of *Dalbergia sissoo* Roxb.

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ABSTRACT

Vitiligo also known as Leucoderma is caused by the loss of pigment, resulting in irregular pale patches of skin. Vitiligo develops patches of depigmented skin appearing on extremities. The objective of the present study was to evaluate the cytotoxicity and *in-vitro* melanogenic activity on bark of *Dalbergia sissoo* Roxb (Fabaceae). The various successive bark extracts have been individually evaluated for trials of spontaneous melanin content, and cell viability by the MTT assay in murine B16F10 melanoma cells in-vitro. Based on the percentage of cell viability assay, graded concentration of extracts were taken for invitro melanogenic activity. The result indicated that Ethyl acetate extract of bark of *Dalbergia sissoo* was found to be non-toxic and increased melanin activity as compared to Hexane and Ethanol extracts. From the above result, it can be concluded from this study that the bark of *Dalbergia sissoo* stimulates B16F10 melanogenesis at very low concentrations. These findings support the folk medicinal use of Dalbergia sissoo on the treatment of hypopigmentation diseases, such as vitiligo.

Keywords: Leucoderma, Dalbergia sissoo, Ethanol.

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INTRODUCTION

About 0.5 to 1 percent of the world's population, or as many as 65 million people, haveVitiligo[1]. Most develop vitiligo before their fortieth birthday! The disorder affects both sexesand all races equally. However, it is more noticeable in people with dark skin[2]. Leucoderma is the most common chronic depigmentation disorder or hypopigmentation [3].

It includes the loss of functioning melanocytes which causes the appearance of white patches on the skin[4.] Melanin is a pigment produced by melanocytes, which are the cells located in the outer layer of skin called epidermis. Melanin gives color to the skin and also protects it from ultraviolet rays.When melanocytes lose their power of producing melanin, discoloration of the skin starts taking place and appears as white patches. This is called vitiligo

Till now researchers have not identified any causative factor for leucoderma. It can also be familiar. i.e. hereditary factor also has some role in the prevalence of leucoderma. Being emotionally upset may also precipitate or aggravate the complaint in certain circumstances. Some suspect skin , lack of sun exposure , infection , etc. to be the reason for the problem. All these risk factors only, but the real cause is still obscure or unknown.

The main causes of leucoderma are said to be excessive mental worry, chronic or acute gastric disorder, and impaired hepatic function such as jaundice, worms or other parasites in the alimentary canal, typhoid and defective perspiratory mechanism and burn injuries.

Dalbergia sissoo belongs to the family Fabaceae is commonly known as nukku kattai[5]. Decoction of the leaves is helpful in gonorrhea and leprosy[6]. Decoction of the bark used in leprosy. Leaf extracts has been used to treat sore throats, heart problems, dysentery, syphilis and gonorrhea. Sissoo oil is used to treat itching, burning on the skin and scabies. It is a Indian medicinal plant which has a variety of uses in folk medicine Aphrodisiac, Abortifacient , Expectorant ,Anthelmintic ,Antipyretic, Emesis, Ulcers,Dysentery, Stomach troubles and Leucoderma [7,8,9].

MATERIALS AND METHOD

Plant collection and Authentication

The fresh bark of the plant *Dalbergia sissoo* was collected from Komaneri, Tuticorin district, Tamil Nadu, India and it was botanically identified and authenticated by Dr.V.Chelladurai, Research Officer-Botany (Scientist-C), Central Council for Research in Ayurveda and Siddha, Government of India.

Preparation of bark extract

The dried coarsely powdered plant material of bark of *Dalbergia sissoo* Roxb were successively extracted using a soxhlet apparatus with solvents of increasing polarity such as Hexane, Ethyl

acetate, Ethanol at 60-70°C for 18 hours. All the extracts were redistilled and concentrated under rotary vacuum evaporator.

Cell line maintenance and culture

Normal Chang liver cell lines were obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100µg/ml) in a humidified atmosphere of 50µg/ml CO_2 at 37˚C. Cultures were maintained by weekly passage and the culture medium was changed twice a week.

Cytotoxicity screening by tetrazolium (MTT) assay

The Cytotoxicity of samples on B16F10 melanoma cells was determined by the MTT assay (Mosmann et al., 1983). Cells ($1x10^5$ cells/well) were plated in 5ml of medium/well culture plates and incubate it for 24-48hrs at 37°C, in the presence of Kojic acid, Hexane, Ethyl acetate and Ethanol at the concentrations of 1000, 500, 250, 125, 62.5, 31.2, 15.62, 7.81µg/ml. After removal of the sample solution and washing with phosphate buffered saline (pH 7.4), 1ml/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide cells (MTT) and phosphate buffered saline solution was added. After 4hrs incubation, 0.04M HCl/isopropanol were added. Viable cells were determined by the absorbance at 570nm. The absorbance at 570nm was measured with a UV Spectrophotometer using wells without sample containing cells as blanks. The effect of the sample on the proliferation of B16F10 melanoma cells was expressed as the % cell viability, using the following formula:

% cell viability = A570 of treated cells / A570 of control cells × 100%.

Determination of melanin content in melanocytes

Extracts influence on the production of melanin in melanocytes was determined using the modified method of Tsuboi et al method. Cells were added into the wells of a 24 well plate (4 x 10^4 cells per well). After 48 hour, different concentrations of bark extracts and kojic acid (1mM as a reference drug) were added to the cells and incubated at 37°C in 5% CO2, humidified atmosphere for 4 days. Control group was incubated just with DMEM or DMEM plus extract vehicle (0.02% ethanol). Then, the medium was removed and cells were lysed with 500µl of NaOH 1 N in DMSO at 80°C for 1 hour. The relative melanin content was determined by measuring the absorbance at 490 nm in plate reader.

RESULTS AND DISCUSSIONS

The Cytotoxicity effect of plant extracts and standard was assessed by cell viability. The cell viability was found to be increased with decreasing in concentration of test compounds. Results

are tabulated (Table 1) and graphically represented.Among these test compounds, the ethyl acetate showed a high percentage of cell viability when compared to Hexane and Ethanol.

Melanogenic activity in cultured murine B16F10 melanoama cells is directly related to the quantity of produced melanin in which is estimated through the amount of melanin retained in the cells. All extracts increased melanin formation. Since melanin content was found to be enhanced in response to several concentrations of Hexane, Ethyl acetate, Ethanolic extracts of bark of Dalbergia sissoo(0.01 to 3µg/ml) are tabulated (Table 2). Among these test extracts, the ethyl acetate showed a high amount of melanin content production when compared to Hexane and Ethanol (Figure. 1).

Table 1: Determination of Cytotoxicity effect by using B16F10 melanoma cell line

S.NO	Conc. (µg/ml)	Cytotoxicity study of standard(Kojic acid),and Plant extracts (Hexane, Ethyl acetate, Ethanol)							
		Standard		Hexane		Ethyl acetate		Ethanol	
		Abs (O.D)	% Cell Viability	Abs (O.D)	% Cell Viability	Abs (O.D)	% Cell Viability	Abs (O.D)	%Cell Viability
1	1000	0.10	18.52	0.12	22.22	0.09	16.66	0.10	20.37
2	500	0.13	25.92	0.17	31.48	0.21	38.88	0.17	33.33
3	250	0.21	40.74	0.22	40.74	0.25	46.29	0.23	44.44
4	125	0.28	53.70	0.26	48.14	0.28	51.85	0.27	50
5	62.5	0.33	62.96	0.31	57.40	0.35	64.81	0.29	55.55
6	31.2	0.38	72.22	0.33	61.11	0.39	74.07	0.38	72.22
7	15.6	0.43	81.48	0.38	70.37	0.43	81.48	0.42	79.62
8	7.8	0.49	92.59	0.43	81.48	0.48	90.74	0.45	85.18
9	Cell control	0.54	100	0.54	100	0.54	100	0.54	100

Table. 2.Effects of bark extracts on melanin content production in B16F10 melanoma cell line

S.No	Concentration (µg/ml)	Kojic acid	Hexane	Ethyl acetate	Ethanol
1	1000	18.52	22.22	16.66	20.37
2	500	25.92	31.48	38.88	33.33
3	250	40,74	40.74	46.29	44.44
4	125	53.70	48.14	51.85	50.00
5	62.5	62.96	57.40	64.81	55.55
6	31.2	72.22	61.11	74.07	72.22
7	15.6	81.48	70.37	81.48	79.62
8	7.8	92.59	81.48	90.74	85.18
9	Cell control	100	100	100	100

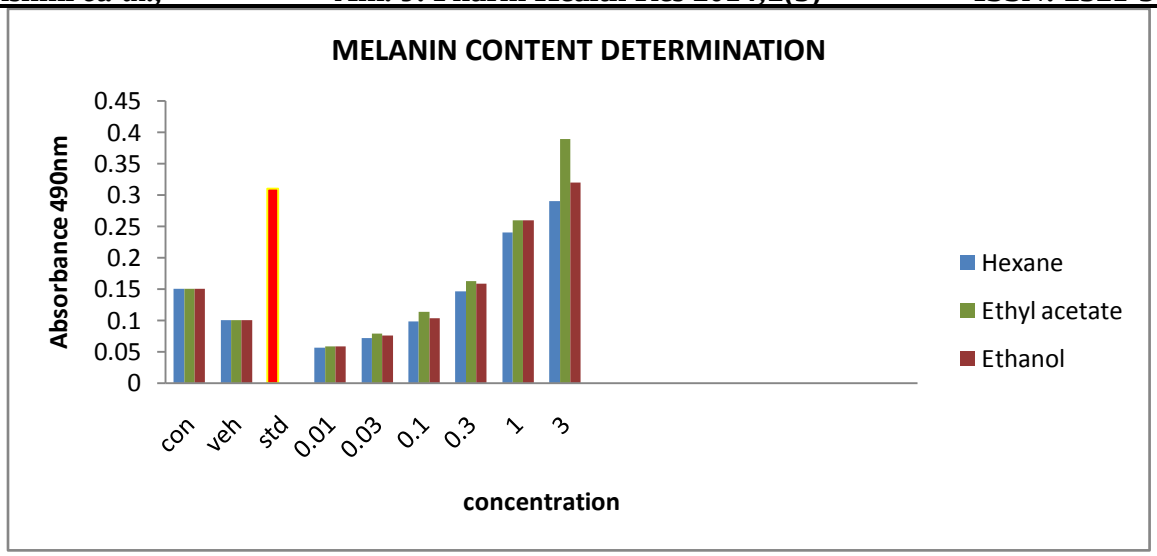

Figure.1.Graphical representation of bark extracts of *Dalbergia sissoo* on melanin content in B16F10 melanoma cell line

Con –control, veh- vehicle, std- standard.

CONCLUSION

The ethyl acetate extract of the bark of *Dalbergia sissoo* may be responsible for protection of the melanoma cells in high amount. Hence ethyl acetate extract has been selected for *in vivo* antileucoderma activity

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