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PRELIMINARY SCREENING OF TELEGRAPH (CODARIOCALYX MOTORIUS) PLANT EXTRACT FOR SKIN WHITENING PROPERTY AND CYTOTOXICITY ACTIVITY

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ABSTRACT

A perfect skin can be remained as a dream if it does not maintain properly, therefore most of the young women are tempting to skin whitening products that can be composed of harmful chemicals that cause dullness, uneven skin tone or acne breakout instead of making skin healthy and blooming. Natural products are safe for consumption and will work on skin naturally and effectively by balancing skin tone and eliminating harmful effects. This study was carried out to determine skin whitening property and cytotoxicity activity of Codariocalyx motorius. C. motorius is commonly known as "Pranajeewa" in Sinhala or "Telegraph Plant". It is widely consumed as an antidote, cardiac-tonic and for wound healing. The antityrosinase activity and cytotoxicity activity of the methanolic extract of leaves of Telegraph plant have been studied on in-vitro models. The antityrosinase activity was evaluated based on inhibition of mushroom tyrosinase and cytotoxicity activity was evaluated based on brine shrimp lethality bioassay. The IC50 value of antityrosinase activity for methanolic extract of leaves of Telegraph plant was 282.2907299 ±4.521 µg/ml and SASAKI Whitening Body Skin Serum as positive control was 478.800757±3.1567 µg/ml. Value of inhibition of tyrosinase was significantly higher than to positive control. The IC50 value of cytotoxicity activity for methanolic extract of leaves of Telegraph plant was 1516.0538± 2.407µg/ml. This analysis was revealed IC50 value of methanolic extract is nontoxic toxic to brine shrimps. Therefore, it can be concluded that Codariocalyx motorius leaves possess highly active antityrosinase substances which can be consumed for remedy of healthy and brighten skin.

Keywords: Telegraph (Codariocalyx motorius), Antityrosinase activity, Cytotoxicity activity,

Mushroom tyrosinase

INTRODUCTION

Skin is an important component of body image and has immense physiological importance for both women and men. Skin pigmentation can be a source of significant emotional distress in individuals (Stratigos and Katsambas, 2004). Both men and women have been struggling with skin pigmentation

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(hyper/hypo-pigmentation). Therefore most of the young women are tempting to use skin whitening products that can be composed of harmful chemicals that cause dullness, uneven skin tone or acne breakout instead of making skin healthy and blooming and also in long term usage, these chemicals might lead to tumor and other unbearable diseases instead of making skin healthy and glowing. Even someone has a naturally light-skinned, external factors can generate melanin production. Some of external factors are pollution, harmful UV rays from the sun, stress, and unhealthy diet, hectic lifestyles, and many others. Considering these circumstances, the best solution is to consume natural ingredients that are safe to use. And some of benefits of consuming natural ingredients are skin brightening, balancing skin tone, reducing blemishes and marks, photo-protection, and eliminating harmful bacteria.

Tyrosinase (E: C: 1.14.18.1) is an essential enzyme, which contributes towards pigment formation in mammalian's body as well as in plants, microorganisms and fungi (Momtaz et al., 2008). In humans and other mammals, the biosynthesis of melanin takes place in a lineage of cells known as melanocytes, which contain the enzyme tyrosinase (Robb, 1984). Tyrosinase (phenol oxidase) is known to be a key enzyme for melanin biosynthesis. This enzyme is mainly involved in the initial steps of the pathway which consist of the hydroxylation of the p-monophenolic amino acid l-tyrosine (monophenolase activity of tyrosinase) and the oxidation of the product of this reaction, the o-diphenolic amino acid L-DOPA (diphenolase activity), to give rise to o-dopaquinone (Bourin et al.,2002) . This o-quinone is transformed into melanins in a series of non-enzymatic reactions (Prota, 1988, 1992). Melanin plays an important role in protecting human skin from the harmful effects of ultraviolet (UV) radiations by absorbing UV sunlight, removing reactive oxygen species (ROS) and scavenging toxic drugs and chemicals. Melanin also induces the expression and synthesis of a variety of cytokines, primarily of keratinocyte origin, that act in a paracrine fashion to further induce melanogenesis (Yaar et al., 2006). The type and amount of melanin synthesized by the melanocytes and its distribution in the surrounding keratinocytes determine the actual color of the skin (Kim and Uyama, 2005).If there's formation of more melanin in the skin, the darker the skin tone will be arisen. Therefore, tyrosinase inhibition is the most common approach to achieve skin hypopigmentation as this enzyme catalyses' the rate-limiting step of pigmentation (Solano et al., 2006). Arbutin, a naturally occurring beta-d-glucopyranoside of hydroquinone used traditionally for depigmentation, involves inhibition of melanosomal tyrosinase activity. Some other known natural compounds such as kojic acid, kojic acid octanoates, salicylhydroxamic acid, catechins, hydroquinone, and more recently resveratrol and oxyresveratrol were also described for their tyrosinase inhibition properties (Bourin et al., 2002). And some existing natural derivatives have got limitations in term of high toxicity. Therefore, in this study, Codariocalyx motorius (Telegraph Plant) was investigated to determine its potential of decreasing skin pigmentation and cytotoxicity by carrying out shrimp lethality assay. The shrimp lethality assay was proposed by Michael et al., and later developed by Vanhaecke et al., and Sleet and Brendel. It is based on the ability to kill laboratory-cultured Artemia nauplii brine shrimp. The assay is considered a useful tool for preliminary assessment of toxicity, and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria

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toxins, pesticides, and cytotoxicity testing of dental materials (Carballo, 2002).

LITERATURE REVIEW

Codariocalyx motorius (= D. gyrans (Unnisa et al., 1994; Antkowiak & Engelmann, 1995; Lev-Yadun, 2013; Morse, 2018) = Hedysarum gyrans (Lev-Yadun, 2013; Morse, 2018) = Desmodium motorium (Unnisa et al., 1994; Antkowiak & Engelmann, 1995; Lev-Yadun, 2013; Morse, 2018)), is known as the telegraph plant ("Taxonomy - GRIN-Global Web v 1.10.3.6", 2018; Ileperuma,2015), dancing plant (Ileperuma,2015), or semaphore plant (Ileperuma,2015;"Taxonomy - GRIN-Global Web v 1.10.3.6", 2018). It belongs to the genus Codariocalyx ("Taxonomy - GRIN-Global Web v 1.10.3.6", 2018), family Fabaceae (alt.Leguminosae) (Kim et al., 2014; "Taxonomy - GRIN-Global Web v 1.10.3.6", 2018), subfamily Faboideae ("Taxonomy - GRIN-Global Web v 1.10.3.6", 2018).

C. motorius is widely distributed throughout Asia-Temperate: China (Fujian, Jiangxi, Guangdong, Guizhou, Sichuan, Yunnan, and Guangxi), Eastern Asia: Taiwan, Asia-Tropical :Indian Subcontinent[Bangladesh, Bhutan, India (Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Delhi, Goa, Haryana, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Mizoram, Nagaland, Orissa, Pondicherry, Punjab, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal), Nepal, Pakistan, Sri Lanka],Indo-China (Cambodia, Laos, Myanmar, Thailand, Vietnam),Malesia(Malaysia, Philippines), Australasia :Australia (tropical) ("Taxonomy - GRIN-Global Web v 1.10.3.6", 2018).

This plant is famous for its rapid movement of leaflets and it exists within a period of about three to five minutes. The leaves move up and down rhythmically, as if the plant is dancing or sending out telegraphic messages. Therefore, the necessity of a time lapse camera to see the movements can be avoided, as the rapid movements are observable to the naked eye. The leaf system of Codariocalyx motorius consists of a terminal leaflet and a maximum of two lateral leaflets, all on the same stalk. A set of one larger and two smaller leaflets are connected by a "hinge," which allows the leaflets to lift and rotate themselves. Moreover, the plant also produces small, purple colored flowers (Ileperuma, 2015).

In Siddha medicine, it is used as antidote, cardiac-tonic and wound healing ointment (Trout, 2002; Chidambaram et al., 2013; Kim et al., 2014; Uma et al., 2014). It has also been used in curing snake bite poisons (Chidambaram et al., 2013). The roots are used as emollient, laxative, antidysentric and to treat cough, asthma and fever. The leaves exhibit tonic, diuretic, febrifuge and aphrodisiac properties. In Sikkim, Bengal, Bihar and Orissa, the root extract is used to cure rheumatism (Jain 1991; Devi & Narmathabai, 2011). Its roots possess the highly active antioxidant substance which can be used for the treatment of oxidative stress-related diseases (Chidambaram et al., 2013; Ileperuma, 2015). In Ayurveda, In Jammu, the paste of leaves and fruits are applied on wounds (Bakshi et al. 2001; Devi & Narmathabai, 2011). Tribes of West Bengal, Orissa and Assam use the juice of the flower to treat menstrual disorder, tuberculosis, sexual disability, boils and headache (Barua et al. 2003; Das and Bhadoria 2006; Girach et al. 2007; Devi & Narmathabai, 2011).

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A juicy extract from leaves and flowers of this plant utilized as a home remedy to treat wounds (Ileperuma, 2015). In China, it has been widely used in Ayurvedic medicine as 'Codariocalyx motorius ohashi' and the main constituent of herbal tea (Sharma et al., 2003; Chidambaram et al., 2013;Uma et al., 2014).And also it has traditionally been used in Chinese Medicine to treat various ailments such as rheumatism, cough, malaria, pyrexia, dysentery, hepatitis, haemoptysis, etc. (Sharma et al.,2003;Ma et al.,2011;Kim et al., 2014;Uma et al., 2014; Ileperuma, 2015) . Extract from D. motorium (known as ''anti-aging tea'') was used in some parts of ancient Thailand to prevent several diseases such as cancer of the stomach and intestines, in curing physical nerve damage and strengthening the immune system. (Devi & Narmathabai, 2011)

C.motorius has been reported to contain antioxidant properties (Alam et al.,2013;Chidambaram et al., 2013;Gopalakrishnan and Rajameena, 2013), anti-inflammatory properties (Lalramnghinglova 2003; Devi & Narmathabai, 2011; Kim et al., 2014) , antihyperglycemic properties (Pullaiah, 2002; Devi & Narmathabai, 2011; Uma et al., 2014), antimicrobial properties (Alam et al.,2013) , antidysenteric property (Ghosal et al., 1972;Uma et al., 2014), secondary metabolites of high medicinal value (Ghosal et al. 1972; Trout 2004; Panda 2004;Devi & Narmathabai, 2011), few alkaloids including

hypaphorine, phenethylamines and 5-Methoxy-N,N-dimethyltryptamine

(Trout,2002;Chidambaram et al.,2013;Kim et al., 2014), 5-methoxy-N,N-dimethyltryptamine (Ileperuma, 2015), flavonoids(Ma etal.,2011;Kim et al., 2014), terpenoids (Ma et al., 2011;Kim et al., 2014), steroids (Ma et al., 2011;Kim et al., 2014), phenols (Ma et al., 2011;Kim et al., 2014), phenylpropanoids (Ma et al., 2011;Kim et al., 2014), glycosides (Ma et al., 2011; Kim et al., 2014), and a number of volatile oils (Ma et al., 2011; Kim et al., 2014), anthraquinones (Gopalakrishnan and Rajameena, 2014), saponins (Gopalakrishnan and Rajameena, 2014), tannins (Gopalakrishnan and Rajameena, 2014), reducing sugars (Gopalakrishnan and Rajameena, 2014), xantho proteins (Gopalakrishnan and Rajameena, 2014), amino acid (Gopalakrishnan and Rajameena, 2014)

C. motorius was categorized under Fabaceae family. Glycyrrhiza glabra (liquorice) (Nerya et al., 2003), Robinia pseudoacacia (black locust) (Chung et al., 1999), Glycine max (soybean) (Lai et al., 2013) are some plant of Fabaceae family. These plants have been reported to possess skin whitening properties. Considering previous evidence of skin whitening properties of plant of Fabaceae family, in this study was carried out to be discovered that it has been possessed skin whitening properties.

MATERIALS AND METHODOLOGY

3.1. Materials

L-DOPA (L-3, 4-dihydroxyphenylalanine), mushroom tyrosinase, DMSO (dimethyl sulphoxide) were obtained from Sigma-Aldrich. SASAKI whitening body skin serum was purchased from beauty parlor (Borella, Sri Lanaka).

3.2. Plant material and preparation of the extract

Fresh leaves of Telegraph plant, Codariocalyx motorius, Pranajeewa (local name) were collected on 6th January 2018 from Nilgala forest is located in Monaralagala district, Uva Province of Sri Lanaka. The collected plant part of C.

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motorius was identified and studied according to their families at the Bandaranayake Memorial Ayurvedic Research Institute, Nawinna, Colombo, Sri Lanka

The leaves were air-dried in shade and the dried material was made to a fine powder using an electric grinder. The resulting residue was dried and extracted further methanol. The methanolic extract (C. motorius) was filtered and concentrated in a chamber for 3-5 days to give a 95% methanolic extract of C. motorius .Inhibition of tyrosinase activity and cytotoxicity activity were observed in the methanolic fraction

3.4. Inhibition of Tyrosinase Activity

Leaves of C.motorius extracts and SASAKI whitening body skin serum were dissolved in DMSO (dimethyl sulphoxide) to a final concentration of 20 mg/ml separately. These stock solutions were then diluted to 600 µg/ml in 50 mM potassium phosphate buffer (pH 6.5). In 96-well plate, 70 µl of each extract dilution was combined with 30 µl of tyrosinase (333 Units/ml in phosphate buffer) in triplicate. After incubation at room temperature for 5 min, 110 µl of substrates (12 mM L-DOPA) were added to each well. Incubation commenced for 30 min at room temperature. SASAKI whitening body skin serum used as reference. Optical densities of the wells were then determined at 492 nm with the BIO-TEK Power Wave XS multi-well plate reader. The concentration of plant extract and SASAKI whitening body skin serum solution at which half the original tyrosinase activity is inhibited (IC50), were determined for. The percentage of inhibition of tyrosinase activity was calculated as follows:

Inhibition %= (A - B)/A × 100

Where A=absorbance at 492 nm without test sample, and B=absorbance at 492 nm with test sample.

3.5. Cytotoxicity Assay

a) Brine shrimp lethality assay Hatching brine shrimp

1. 30 ml of sea water were measured using measuring cylinder and poured into a petri dish which was half covered by aluminum foil
2. About 1g of brine shrimp eggs was added at the top water level of the petri dish
3. A light (60-100 Watt bulb) was switched on and placed a few inches away from the petri dish
4. After 12-24 hours, the nauplii were hatched
5. The eggs and nauplii were observed
6. The nauplii were collected after the next 24 hours
7. Hatched nauplii were separated from the empty egg. It was done by switching off the lamp. The empty eggs floated while the brine shrimp concentrated in the water column.
8. 10 nauplii were transferred to a test tube using a Pasteur pipette

 Toxicity testing

9. The nauplii were exposed to different concentrations of the plant extract
10. The number of survivors were counted and calculated the percentage of death after 24 hours

For each tube, count the number of dead and number of live nauplii, and determine the % death,

Percentage of Death (%): (Total naupii – Alive naupii) x100%/Total naupii

DATA ANALYSIS AND DISCUSSION

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4.1. Antityrosinase IC50 Value

Table 1: IC_{50} value of tyrosinase Inhibition of Codariocalyx motorius methanol extract and SASAKI Whitening Body Skin Serum

	IC_{50} Value
Codariocalyx motorius methanol extract	282.2907299 ±4.521 µg/ml
SASAKI Whitening Body Skin Serum (Reference)	478.800757±3.1567 µg/ml

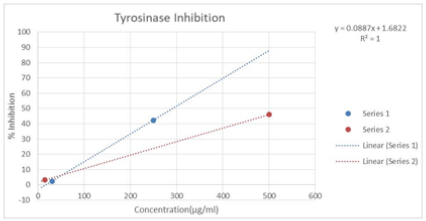

Figure 1: % Tyrosinase Inhibition of Codariocalyx motorius methanol extract

In here, C.motorius methanol extract was screened to determine its inhibitory activities of tyrosinase which key enzyme in melanin synthesis with reference which is a commercially available SASAKI Whitening Body Skin Serum. Its composition is alpha arbutin, glutathione, vitamin C, E, B3. IC50 value of C.motorius methanol extract and SASAKI Whitening Body Skin Serum were given as follows respectively, 282.2907299 ±4.521 µg/ml and 478.800757±3.1567 µg/ml. This study revealed that C.motorius methanol extract have higher anti tyrosinase activity when compared to SASAKI Whitening Body Skin Serum. Methanolic extract from the leaves of C.motorius was verified a significant capability of inhibiting mushroom tyrosinase, are described for the first time for this biological property. For further studies human melanocytes must be done in order to clarify antityrosinase activity.

4.2. Cytotoxicity Assay

The yield of extracts from plant used ranged between 3.906-3000µg/ml. Brine shrimps with LC50 value is 1516.0538± 2.407µg/ml.

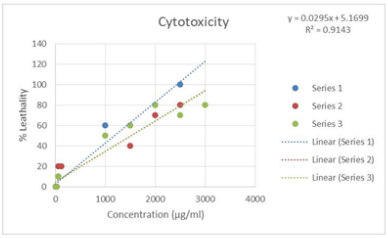

Figure 2: % Cytotoxicity of Codariocalyx motorius methanol extract

Due to the ethical issues in toxicological tests, substituting animals with alternative models is very important (Hamidi et al., 2014). The effectiveness of the C. motorius bioassay for predicting the toxicity of plant extracts was evaluated by comparing the LC50 results for the brine shrimps. According to Meyer's toxicity index, extracts with LC50 < 1000 µg/ml are considered as toxic, while extracts with LC50 > 1000 µg/ml are considered as non-toxic (Meyer et al., 1982). Clarkson's toxicity criterion for the toxicity assessment of plant extracts classifies extracts in the following order: extracts with LC50 above 1000 µg/ml are non-toxic, LC50 of 500- 1000 µg/ml are low toxic, extracts with LC50 of 100 - 500 µg/ml are medium toxic, while extracts with LC50 of 0 -100 µg/ml are highly toxic (Clarkson et al., 2004). The LC50 value obtained for C. motorius methanol extract using the Brine Shrimp Lethality Assay is 1516.0538± 2.407µg/ml that indicating according Meyer's toxicity index, while it is nontoxic.

CONCLUSION

The leaf extracts of C. motorius revealed cytotoxic activity against the brine shrimp and considered as containing nontoxic components and also possess highly active antityrosinase substances which can be consumed for remedy of healthy and brighten skin. This

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preliminary screen can be leaded of manufacturing skin whitening cosmetics and therapeutic drugs by developing out of this research.

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