Original Article

Lack of *in vitro* antihyaluronidase activity of methanolic leaf extract of *Indigofera tinctoria* L and methanolic stem bark extract of *Stereospermum suaveolens* DC

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Abstract

Objective: To assess the antihyaluronidase activity of methanolic leaf extract of *Indigofera tinctoria* L (*I. tinctoria*) (family: Fabaceae/Leguminosae) and stem bark extract of *Stereospermum suaveolens* DC (*S. suaveolens*) (family: Bignoniaceae) *in vitro* with a view to develop an antiaging skin formulation. **Materials and Methods:** The antihyaluronidase activity of different concentrations (0.19 mg/mL, 0.38 mg/mL, 0.75 mg/mL, 1.5 mg/mL, and 3.0 mg/mL) of methanolic leaf extract of *I. tinctoria*, methanolic stem bark extract of *S. suaveolens*, and reference drug epigallocatechin gallate (EGCG) of different concentrations (12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, and 200 µg/mL) were determined spectrophotometrically using hyaluronic acid (from rooster combs) and bovine testicular hyaluronidase. **Results:** There is no *in vitro* antihyaluronidase activity in the methanolic extracts of *I. tinctoria* leaves and *S. suaveolens* stem bark even at high concentrations. On the contrary, EGCG, the reference agent, showed marked concentration-dependent ($r^2 = 0.92$) antihyluronidase activity [in terms of percentage inhibition: half maximal inhibitory concentration (IC_{50}) 92.64 ± 0.64 µg/mL]. **Conclusion:** It is unlikely that skin antiaging effects of *I. tinctoria* leaves and *S. suaveolens* stem bark, as claimed in traditional and folk medicines in Sri Lanka, are mediated via antihyaluronidase activity.

Key words: Antihyaluronidase activity, *Indigofera tinctoria*, skin aging, skin antiaging, *Stereospermum suaveolens*

INTRODUCTION

Indigofera tinctoria L (*I. tinctoria*) (nilawari in Sinhala, asidni in Tamil, and indigo in English) (family: Fabaceae/Leguminosae) is a branching shrub that grows to a height of 2 m, with slightly angular branches bearing alternate, compound leaves containing 7-11 leaflets. The leaves are dark green when fresh and greyish black when dried. The fruits are cylindrical and many seeded. The plant is found in Africa, the Philippines islands, India, and Sri Lanka.[1] The leaves of this plant (in the form of juice, decoction, or skin application) are used in Ayurvedic, traditional, folk, and homeopathic medicines to treat several ailments, including liver and spleen enlargements, hepatitis, nervous disorders, renal disorders, bronchitis and asthma, and whooping cough.[2,3] Also, the leaves are used to treat rabies and act as a remedy for snakebite and scorpion stings.[1] Interestingly, in Sri Lankan ethnomedicine, it is believed and claimed that the leaves promote hair growth and give complexion to the skin (by impairing the aging process). Thus, to achieve these goals, topical application of extracts, ointments, or oils made from its

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leaves are recommended.[1] Experimentally, the leaves are shown to possess antibacterial, antioxidant, cytotoxic, anti-inflammatory, antidiabetic, nephroprotective, and antinociceptive properties.[2,3] Phytochemically, the leaves are shown to contain polyphenols, flavonoids, tannins, and alkaloids, but no steroids and saponins.[2,4]

Stereospermum suaveolens (Roxb) DC (*S. suaveolens*) (palol in Sinhala, ambu in Tamil, trumpet in English) (family: Bignoniaceae) is a deciduous tree, 10-20 m tall with opposite imparipinnate compound leaves having three or four pairs of leaflets. The stem possesses a thick woody bark. The tree is found in India and Sri Lanka.[5] In traditional and folk medicines, decoction of the stem bark and the roots are used as analgesic, antiseptic, astringent, diuretic, liver stimulant, and anti-inflammatory agents.[5-7] Interestingly, some traditional healers of Sri Lanka recommend the use of extracts of the root and the stem bark of this tree to have a wrinkle-free, young-looking skin. Experimentally, the stem bark is shown to have antiulcerogenic, gastroprotective, wound healing, and antidiabetic properties.[8,9] Further, the bark extracts are shown to contain flavonoids, polyphenols, tannins, saponins, and alkaloids.[8,9]

Today one of the most frequent dermatological concerns, especially in women, is skin aging that results in wrinkles/rhytides.[10,11] There are several antiaging procedures and cosmeceuticals (creams, powders, and lotions) available in the market, which claim to slow or delay the skin-aging process.[12] Unfortunately, most of the antiaging cosmeceuticals, especially the synthetic ones, induce unpleasant side effects such as contact dermatitis, skin irritations, phototoxicity, allergic reactions, and even skin cancer.[10,12] On the contrary, antiaging herbal cosmeceuticals are claimed to be less harmful and more user friendly.[13] Thus, there is a need and demand for the development of novel plant-based antiaging cosmeceuticals.

In view of this need and because in traditional and folk medicines, the leaves of *I. tinctoria* and the stem bark of *S. suaveolens* are claimed to have skin antiaging properties, this study was launched to scientifically investigate the antihyaluronidase activity of the leaves of *I. tinctoria* and the stem bark of *S. suaveolens in vitro*: Impairment of the hyaluronidase activity is considered as one of the main mechanisms of diminishing skin aging.[14]

MATERIALS AND METHODS

Collection of plants materials

Fresh leaves of *I. tinctoria* were plucked from a tree in a home garden at Beliatta (geographical coordinates: 6°1'119" N, 80°45'2700" E) situated in Hambantota District, Southern Province of Sri Lanka in October 2014. Dried pieces of the stem bark of *S. suaveolens* were purchased from the drug outlet of Wickramarachchi Ayurveda Institute in Gampaha District, Sri Lanka in October 2014. The stem bark has been identified by the pharmacognosist/purchasing officer of the drug outlet. The leaves of *I. tinctoria* were identified and authenticated by emeritus professor (Mrs.) A.S. Seneviratne, Department of Plant Sciences, University of Colombo, Sri Lanka. Voucher specimens of the leaves (CS/02/2014) and the stem bark (CS/03/2014) were deposited at the Department of Medical Laboratory Sciences at the Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University, Sri Lanka.

Preparation of methanolic leaf and stem bark extracts

The leaves of *I. tinctoria* were oven-dried at 40°C for 2 days. Both the leaves (moisture content: 13.52%) and the stem bark (moisture content: 11.98%) were crushed into a powder using a grinder. Powdered leaves and stem bark of weight 2.5 g were separately immersed in 25 mL of methanol and left overnight at room temperature (30 ± 2°C). The resulting greenish blue leaf extract and yellowish brown stem bark extract were separately concentrated *in vacuo* and freeze dried. Yields of the leaves and the stem barks were 13.57% and 12.77%, respectively. The freeze-dried extracts were used in the evaluation of *in vitro* antihyaluronidase activity.

Evaluation of *in vitro* antihyaluronidase activity of methanolic leaf and stem bark extracts

Hyaluronidase enzyme inhibitory activity of the methanolic bark and leaf extracts were assessed spectrophotometrically as described by Reissig *et al.* (1995)[15] with some modifications, by measuring the amount of N-acetylglucosamine formed from sodium hyaluronate. A volume of 50 µL of bovine testicular hyaluronidase (Sigma-Aldrich, New York, USA) was dissolved in 0.1 M acetate buffer (pH 3.5) and mixed with 50 µL of different concentrations of the methanolic bark and leaf extracts (bark and leaf assay concentrations: 0.19 mg/mL, 0.38 mg/mL, 0.75 mg/mL, 1.5 mg/mL, and 3.0 mg/mL, $n = 4$) that were incubated in a water bath at 37°C for 20 min. Enzyme was activated by adding 100 µL of 12.5 mM calcium chloride and the mixture was incubated in a water bath at 37°C for 20 min. The reaction was initiated by adding 250 µL of sodium hyaluronate (1.2 mg/mL) (Sigma-Aldrich, New York, USA) dissolved in 0.1 M acetate buffer (pH 3.5) to the calcium activated hyaluronidase and the mixture was incubated in a water bath at 37°C for exactly 40 min. At the end of the incubation period, 100 µL of 0.4 M sodium hydroxide and 100 µL of 0.4 M potassium borate were added and the reaction mixture was incubated in a boiling water bath for exactly 3 min. Finally, the mixtures were cooled to room temperature (30 ± 2°C) and 3 mL of dimethyl benzaldehyde solution (4 g of

p-Dimethylaminobenzaldehyde dissolved in 350 mL of 100% acetic acid and 50 mL of 10 M HCl) was added and the reaction mixtures were incubated in a water bath at 37°C for 20 min. Absorbance was measured at 585 nm using 96-well microplate reader (SpectraMax® Plus 384, Molecular Devices Inc, New York, USA. Epigallocatechin gallate (EGCG) (Sigma-Aldrich, New York, USA) was used as the standard. The concentrations of EGCG were 12.5 mg/mL, 25 mg/mL, 50 mg/mL, 100 mg/mL, and 200 mg/mL (n = 4), and the percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = (A_C - A_S)/A_S \times 100$$

where A_C is the absorbance at 585 nm of the control and A_S is the absorbance at 585 nm of the different concentrations of the methanolic bark and leaf extracts.

Statistical analysis

The data are represented as mean ± standard deviation (SD) and half maximal inhibitory concentration (IC_{50}) value was calculated using Microsoft Excel 2007 package. The concentration dependencies were determined using regression analysis with Minitab 14.0 statistical software. Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

This study examined the antihyaluronidase activity of *I. tinctoria* leaves and *S. suaveolens* stem bark using the methanolic extracts and an *in vitro* technique. This *in vitro* technique is simple but validated, reliable, sensitive, and widely used.[15] Thus, the results obtained are valid and reliable. The results obtained are depicted in Tables 1 and 2. As shown, the methanolic extracts of *I. tinctoria* leaves and *S. suaveolens* stem bark did not exhibit any *in vitro* antihyaluronidase activity, even at high concentrations [Table 1]. Conversely, EGCG, the reference agent, showed profound antihyaluronidase activity (range: 7.99-92.98% of inhibition) in a concentration-dependent manner (r^2 = 0.93, $P < 0.05$) with an IC_{50} value of 92.64 ± 0.64 μg/mL.

This is a novel but an unexpected finding with respect to their claimed skin antiaging properties. Since strong associations exist between the degradation of hyaluronic acid and skin aging,[16] impairment of the hyaluronidase activity is considered as one of the main mechanisms of skin antiaging.[14,16] Additionally, bulky polyphenols and flavonoids are reported to act as inhibitors of hyaluronidase[14,16] and these phytoconstituents are reported to be present in both the leaves of *I. tinctoria*[4] and the stem bark of *S. suaveolens*.[8,9] However, the possibility of inducing skin antiaging action by *I. tinctoria* leaves and *S. suaveolens* stem bark through inhibition of collagenase and/or the elastase activity cannot be ruled out; both collagen and elastin fibers play a pivotal role in maintaining the structural and the functional integrity of the dermis and thereby preserve a smooth and youthful appearance of the human skin.[13,14,16,17]

Table 1: *In vitro* antihyaluronidase activity of methanolic leaf extract of *I. tinctoria* and stem bark extract of *S. suaveolens*

Concentration (mg/mL)	% Inhibition	
	Stem bark extract	Leaf extract
0.19	−8.09±1.04	−7.62±0.89
0.38	−11.61±1.27	−7.97±0.74
0.75	−16.99±0.37	−12.03±1.06
1.50	−21.62±1.13	−14.79±0.85
3.00	−21.61±2.36	−18.02±0.59

Data expressed as mean±SD (n=4)

Table 2: *In vitro* antihyaluronidase activity of EGCG

Concentration (μg/mL)	% Inhibition
12.50	7.99±1.31
25.00	9.82±0.73
50.00	42.31±0.81
100.00	62.52±0.47
200.00	92.98±0.54
IC_{50}	92.64±0.64

Data expressed as mean±SD (n=4). EGCG: Epigallocatechin gallate

CONCLUSION

It is unlikely that the skin antiaging effects of *I. tinctoria* leaves and *S. suaveolens* stem bark, as claimed in traditional and folk medicines, are mediated via antihyaluronidase activity.

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REFERENCES

1. Jayaweera DM. Medicinal Plants (Indigenous and Exotic) used in Ceylon. Vol. 1. 5th ed. Colombo, Sri Lanka: National Science Foundation; 2006. p. 216-17.
2. Motamarri SN, Karthikeyan M, Rajasekar S, Gopal V. *Indigofera tinctoria* Linn-A Phytopharmacological Review. Int J Res Pharm Biomed Sci 2012;3:164-9.
3. Renukadevi KP, Sultana SS. Determination of antibacterial, antioxidant and cytotoxicity effect of *Indigofera tinctoria* on Lung Cancer Cell Line NCI-h69. Int J Pharmacol 2011;7:356-62.
4. Jain S, Nayak S, Joshi P. Phytochemical study and physical evaluation

of *Indigofera tinctoria* leaves. Int J Comprehensive Pharm 2010;4:1-5.
5. Jayaweera DM. Medicinal Plants (Indigenous and Exotic) used in Ceylon. Vol. 2. 5th ed. Colombo, Sri Lanka: National Science Foundation; 2006. p 115-17.
6. Khart V, Chanshetti R, Chavan V, Naik Y, Date N. Evaluation of anti-inflammatory potential of aqueous extract of root bark of *Stereospermum suaveolens* DC. Int J Pharm Pharm Sci 2012;4:494-6.
7. Balasubramanium T, Pramanick K, Chatterjee TN. Diuretic effect of ethanol extract of *Stereospermum suaveolen*. Pharmacologyonline 2009;2:625-35.
8. Muchandi AA, Chandrashekhar VM. Antiulcer and gastroprotective potential of *Stereospermum suaveolens* in Wistar rats. J Pharmacol Pharmacother 2011;2:117-9.
9. Balasubramanian T, Chatterjee TK, Senthilkumar GP, Mani T. Effect of potent ethyl acetate fraction of *Stereospermum suaveolens* extract in streptozotocin-induced diabetic rats. Scientific World Journal 2012;2012:413196.
10. Baumann L. Skin ageing and its treatment. J Pathol 2007;211:241-51.
11. Hori M, Yagi M, Nomoto K, Shimode A, Ogura M, Yonei Y. Inhibition of advanced glycation end product formation by herbal teas and its relation to anti-skin aging. Anti-Aging Med 2012;9:135-48.
12. Antiageing supplements. Available from: http://www.en.wikipedia.org/wiki/Anti-aging_supplements [Last accessed on 2014 Oct 15].
13. Mukherjee PK, Maity N, Nema NK, Sarkar BK. Bioactive compounds from natural resources against skin aging. Phytomedicine 2011;19:64-73.
14. Satardekar KV, Deodhar MA. Anti-ageing ability of *Terminalia* species with special reference to hyaluronidase, elastase inhibition and collagen synthesis *in vitro*. Int J Pharmacol Phytochem Res 2010;2:30-4.
15. Reissig JL, Storminger JL, Leloir LF. A modified colorimetric method for the estimation of N-acetylamino sugars. J Biol Chem 1955;217:959-66.
16. Lee KK, Cho JJ, Park EJ, Choi JD. Anti-elastase and anti-hyaluronidase of phenolic substance from *Areca catechu* as a new anti-ageing agent. Int J Cosmet Sci 2001;23:341-6.
17. Girish KS, Kemparaju K. Inhibition of *Naja naja* venom hyaluronidase by plant-derived bioactive components and polysaccharides. Biochemistry (Mosc) 2005;70:948-52.

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