



AN IN VITRO STUDY OF ANTI- ELASTASE AND ANTI-HYALURONIDASE ACTIVITIES OF SANDALWOOD EXTRACT

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ABSTRACT Sandalwood has many beneficial effects on health. The anticancer and skin protective effects of sandalwood have been proposed to be due to its anti-inflammatory, antioxidant, antimicrobial and antiprotease effects. The present study aimed to assess the antielastase and antihyaluronidase effects of sandalwood. Aqueous extract of sandalwood (50 mg/ml) was added to the reaction mixtures of elastase and hyaluronidase enzyme assays to test its inhibitory effect. This in vitro study demonstrated that sandalwood extract inhibited elastase activity and hyaluronidase activity by 81% and 72% respectively. Applying aqueous extract of sandalwood after grinding it against a stone with water, is a common practice in India. This religious practice has to be seen from scientific perspective of skin protecting effects of sandalwood.

KEYWORDS :

INTRODUCTION

The Indian sandalwood, *Santalum album L.*, referred to as 'royal tree' in India, belongs to the family santalaceae. The wood of this root hemiparasitic tree is highly aromatic and is known for its medicinal properties since ancient times. Sandal wood has antiinflammatory, anticancer, antioxidant, antimicrobial and antiseptic properties. Various molecular mechanisms have been proposed in beneficial effects of sandalwood.^{1,2}

Proteases in the skin perform essential functions vital for the health. However, defective regulation of the activities of proteases is proposed to contribute to many skin diseases. The inhibitors of proteases now considered to play important role in preventing skin ageing and skin diseases. Phytochemicals present in food sources and medicinal plants have been studied for their role in preventing skin diseases by inhibition of proteases such as elastase.^{3,5} Sandal wood is one plant which is being investigated for its antiprotease actions. Alpha.santalol, an active component of sandalwood oil, has been shown to have apoptotic effect.⁵

Hyaluronic acid, an important glycosaminoglycan in the extracellular matrix, is degraded by hyaluronidase. Regulation of hyaluronidase is necessary for normal tissue organization and extracellular matrix-mediated functions. Inhibitors of hyaluronidase have immense applications as anti-ageing, anti inflammatory and antimicrobial agents.^{4,5}

There is paucity of studies on antielastase and antihyaluronidase effects of sandal wood aqueous extract. The present study has made an attempt to evaluate these effects in vitro.

MATERIALS AND METHODS

The present in vitro study was done at Father Muller Medical College, Mangalore.

Preparation of Sandalwood extract :

For the study purpose, sandal wood which is used for religious purposes, was procured from the local market. The sandal wood was ground finely and strained through muslin cloth. 1 gram of the sample obtained was soaked for 2 hours in distilled water (50 mg/ml). The sample was then centrifuged and the supernatant was collected, and it served as aqueous extract for the further studies.

The chemicals required for the assays were procured from Sigma Aldrich.

Positive Control : For the assay of hyaluronidase and elastase, 50 micromoles/ liter epigallocatechin gallate] (EGCG) was used as the positive control (Sigma-Aldrich).

Elastase activity : The continuous spectrophotometric rate determination at 410 nm was based on hydrolysis of the substrate N-Succinyl –alanine-alanine-alanine-P-Nitroanilide (peptide) by elastase to release –Nitroanilide. In a 3 ml reaction mixture, the final concentrations were : 0.20 mM N- Succinyl –alanine-alanine-alanine-P-Nitroanilide and 0.02-0.05 unit of elastase. One unit of elastase was defined as the enzyme activity required to hydrolyze 1 micromole of the substrate per minute at a pH of 8.0 at 25 degree C.⁸

Hyaluronidase activity : This is a turbidometric method, measuring absorbance at 600 nm. It is based on hydrolysis of hyaluronic acid to give di and monosaccharides and smaller hyaluronic fragments. In a 2 ml reaction mixture, final concentrations were : 0.015% (w/v) hyaluronic acid, 150 mM sodium phosphate, and 25 units of hyaluronidase. One unit of hyaluronidase causes a change of 0.33 absorbance per minute at a pH of 5.35 at 37 degree C.

Determination of percentage inhibition of elastase and hyaluronidase : The effect of sandalwood on the activity of hyaluronidase and elastase was assayed by conducting the reactions with sandal wood extract. The percentage inhibition is the percentage difference in the activity of hyaluronidase/ elastase without and with the presence of sandalwood extract in the reaction.

RESULTS AND DISCUSSION

There was a significant inhibition of the activities of hyaluronidase and elastase by sandal wood extract. The activity of hyaluronidase decreased by 72% and the activity of elastase decreased by 81%.

In the present study we have used only one concentration of sandalwood extract. It exhibited significant inhibition of elastase and hyaluronidase. Sandalwood is known for its anti-inflammatory, antimicrobial, antioxidant and anticancer effects.^{1,2} Applying aqueous extract of sandalwood after grinding it against a stone with water, is a common practice in India. This religious practice has to be seen from scientific perspective of skin protecting effects of sandalwood.

The potential of various herbs to treat different skin diseases, to adorn and improve the skin appearance is well-known. Considering the skin protecting effects of sandalwood, it has been used in oils, soaps, and other cosmetic agents applied on the skin. Sandalwood oil has been extensively studied for its anticancer and skin protecting effects.^{10,11}

There is paucity of literature on antielastase and antihyaluronidase effects of sandal wood aqueous extract. Ours is a pilot study in this direction. Further detailed studies assessing the anticancer effects of aqueous extract of sandalwood are required.

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