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IN-VITRO HYALURONIDASE INHIBITION PROPERTIES OF *ALOE CAMPERI, ALOE PERCRASSA* AND *SENNA SINGUEANA*

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ABSTRACT: In folk medicine, *Aloe camperi*, *Aloe percrassa* (Aloeceae), and *Senna singueana* L. (Fabaceae) are used in the management of infectious and inflammation-related disorders. The objective of this work was to evaluate the *in-vitro* hyaluronidase inhibition activities of extracts from these three medicinal plants. Various concentrations (10, 50, and 100 µg/ml) of leaf latex from the two *Aloe sp* and hydro alcoholic extract of *S. singueana* leaves were screened using hyaluronidase inhibition assay. The results showed that all the three extracts displayed concentration-dependent inhibitory activities with IC_{50} values of 771.78, 664.47 and 630.83 µg/ml for *A. camperi*, *A. Percrassa* and *S. singueana,* respectively. Indomethacin, used as a standard drug in this study, showed IC_{50} value of 27.95µg/ml. Hyaluronidase hydrolyzes hyaluronic acid in the extracellular matrix during tissue remodeling. Since, oligomers from hyaluronic acid degradation are associated with induction of inflammation and hyaluronidase activity up-regulation occurs in chronic inflammatory conditions, hyaluronidase inhibitors are suggested to have a beneficial role in prevention and treatment of inflammation-related disorders. Thus, the hyaluronidase enzyme inhibition activity of extracts from *A. camperi*, *A. percrassa*, and *S. singueana* could partially contribute to their traditional use against infectious and inflammatory related disorders.

INTRODUCTION: In folk medicine, *Aloe camperi* and *Aloe percrassa* (Aloeceae) are used in the treatment of malaria, wound, eye inflammation, skin, and gastrointestinal problems. Similarly, most traditional claims of *Senna singueana* L. (Fabaceae) focus on managing gastrointestinal disorders, infections, pain, and inflammation.

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In the area of herbal medicine, several types of research are being done to explore newer and safer alternatives to combat several diseases including inflammatory disorders [1]. As an alternative, plants have been extensively studied to obtain an alternative treatment; [2] and the rich wealth of plant kingdom can represent a novel source of newer compounds with significant anti-inflammatory activities [3].

Hyaluronidase inhibitory assay is one of the different models used to study anti-inflammatory activities. The principle is that hyaluronidase is an enzyme that degrades hyaluronic acid and chondroitin sulfate which are components of the

extracellular matrix of connective tissue. By degrading the components of connective tissue, hyaluronidase promotes the spread of inflammatory mediators throughout these tissues, thereby contributing to the pathogenesis of inflammatory diseases [4]. Thus, hyaluronidase inhibitors are suggested to have a beneficial role in prevention and treatment of inflammation-related disorders [5]. Accordingly, the present work was intended to evaluate the *in-vitro* hyaluronidase inhibition activities of *A. camperi*, *A. percrassa*, and *S. singueana* as a measure of anti-inflammatory properties since these plants are traditionally used, among others, to manage related inflammatory disorders.

METHODOLOGY:

Collection of the Plant Materials: Plant materials were collected from Tigray, the northern part of Ethiopia. The whole fresh plant of *A. percrassa* and *A. camperi* were collected from a locality called Edagahamus; while leaves of *S. singueana* were collected from around Adwa. Sample specimen of each plant was collected and authenticated at the National Herbarium, Department of Biology, Addis Ababa University, Ethiopia.

Preparation of Extracts: Leaf latex was collected from the Aloe species by arranging the leaves concentrically around a plate, and the collected latex was left in open air for 1-3 days to allow evaporation of water, which yielded a dark brown powder. The hydro-alcoholic extract was prepared from dried, and powdered *S. singueana* leaves using 70% ethanol by maceration, which was then filtered, concentrated under reduced pressure using Rota Vapor and dried in a vacuum oven at a temperature of 35 ºC. The dried extracts were then transferred into vials and stored for further use.

Hyaluronidase Inhibition Activity Evaluation: Prepared extracts were sent to BioGenics Research and Training Center in Biotechnology (India) for anti-inflammatory testing by the method of hyaluronidase inhibition assay. The assay medium consisting of 5U hyaluronidase (from Sigma – Aldrich, Bangalore) in 100 µl of 20 mM sodium phosphate buffer (pH 7.0) with 77 mM sodium chloride, 0.01% BSA was pre-incubated with different concentrations (10, 50, and 100 µg/ml) of the test extracts and standard drug (Indomethacin) for 15 min at 37 °C. The assay was commenced by adding 100 µl hyaluronic acid (from Sigma - Aldrich, Bangalore; 0.03% in 300 mM sodium phosphate, pH 5.35) to the incubation mixture and incubated for a further 45 min at 37 °C. The undigested hyaluronic acid was precipitated with 1ml acid albumin solution made up of 0.1% bovine serum albumin in 24 mM sodium acetate and 79 mM acetic acid, (pH 3.75). After standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm. The absorbance in the absence of enzyme was used as the reference value for maximum inhibition. The inhibitory activity of each test sample was calculated as the percentage ratio of the absorbance in the presence of test compound vs. absorbance in the absence of enzyme. The enzyme activity was checked by control experiment run simultaneously, in which the enzyme was pre-incubated with 5µl DMSO instead, and followed by the assay procedures described above. Samples were tested in a range of 10 µg - 100 µg in the reaction mixture. Indomethacin (Indo) was used as reference standard.

RESULTS AND DISCUSSION: In the present study the *in-vitro* anti-inflammatory properties of extracts from three medicinal plants: *A. camperi*, *A. percrassa*, and *S. singueana* were evaluated using hyaluronidase enzyme inhibition assay; and the results are summarized in **Table 1**. As can be seen in **Table 1**, all the extracts and the standard drug (Indomethacin) exhibited concentration-dependent hyaluronidase inhibition activities in the concentration range (10-100 µg/ml); yet, the effect of crude plant extracts was found to be less potent as compared with that of standard drug, indomethacin.

TABLE 1: PERCENTAGE INHIBITION OF HYALURONIDASE ENZYME BY TEST EXTRACTS AND INDOMETHACIN

Conc.	% Hyaluronidase Inhibition			
	Aloe camperi	***Aloe percrassa***	***Senna singueana***	**Indomethacin**
10 µg	0	-0.77	1.86	29.72
50 µg	1.86	1.39	4.18	79.26
100 µg	5.88	6.19	8.82	94.74

The concentration required to produce 50% inhibition (IC_{50} value) of each test extract as well as the standard drug was calculated from the dose-response curve by plotting percentage inhibition against treatment concentration by linear regression analysis. The calculated IC_{50} values are summarized in **Table 2**. As can be seen from the results **Table 2**, the hyaluronidase inhibition IC_{50} values of tested extracts range from ≈0.63 to 0.771 mg/ml.

In the literature, ethanol extracts from different parts of *Vitis rotundifolia* were reported to exhibit *in vitro* hyaluronidase activities with IC_{50} values ranging from 0.3 to 1.0 mg/ml.[6] Similarly, phenolic extract of *carum carvi* (caraway*)* exhibited significant hyaluronidase inhibitory activity with an IC_{50} value of 336 µg/ml (0.336 mg/ml) [7]. Therefore, although the screened extracts exhibited relatively lower potency when compared to standard drugs like indomethacin, which showed an IC_{50} value of 27.95 µg/ml (≈0.028 mg/ml), and catechin, a natural hyaluronidase inhibitor with reported IC_{50} of 20 µg/ml;[8] the activities exhibited by the tested extracts can be considered as significant inhibitory activities when compared to the activities of other extracts reported in the literature.

TABLE 2: IC_{50} VALUES OF TEST SAMPLES AND INDOMETHACIN AGAINST HYALURONIDASE ENZYME

Test Samples	IC_{50} values (µg/ml)
Aloe camperi	771.78
Aloe percrassa	664.47
Senna singueana	630.83
Indomethacin	27.95

Hyaluronan (also called hyaluronic acid or hyaluronate or HA) is a biopolysacharide, which has important biological functions in bacteria and higher animals including humans. Hyaluronan synthases naturally synthesize it and degraded by a family of enzymes called hyaluronidases [9, 10]. Hyaluronidases are ubiquitously found in the animal kingdom, bacteria or pathogenic fungi. They are found as components of all types of animal venoms, which may contribute to local tissue damage and act as a spreading factor facilitating the diffusion of toxic venom components. Likewise, microbial hyaluronidases are important virulence factors involved in pathogeneses and the disease progression caused by the pathogen. By degradation of hyaluronan-rich tissues of the host, the bacterial hyaluronidases facilitate the invasion of the pathogen. Additionally, the hyaluronan oligomers created by the enzymes are potent inflammatory agents and promote a microbial-friendly environment [9, 11, 12].

Hyaluronidase hydrolyzes HA in the extracellular matrix during tissue remodeling; and up-regulation of hyaluronidase activity occurs in chronic inflammatory conditions [6]. Thus, since (i) oligomers from hyaluronic acid degradation induce inflammation 5 and (ii) hyaluronidase activity up-regulation occurs in chronic inflammatory conditions, [6] hyaluronidase inhibitors are suggested to have a beneficial role in the prevention and treatment of inflammatory disorders [5, 6]. Accordingly, the hyaluronidase enzyme inhibition activity displayed by the three medicinal plants: *A. camperi*, *A. percrassa*, and *S. singueana* could partially contribute to their traditional use against infectious and inflammatory related disorders.

CONCLUSION: Leaf latex of *A. camperi*, *A. percrassa*, and hydroalcoholic extract of *S. singueana* leaves showed concentration dependent hyaluronidase enzyme inhibition activities; and this could partially contribute to their traditional use against infectious and inflammatory related disorders.

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