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Evaluation of Hyaluronidase activity of proprietary date seed extract (SesZenNHA™) for skin health applications

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Abstract:

Hyaluronic Acid or Hyaluronan is a glycosaminoglycan that is present in various connective and epithelial tissues. It increases viscosity of the fluid in synovial joints to reduce friction between bones. HA is a major component of the skin. When skin is exposed to UV, hyaluronin is degraded and skin cells reduce production of HA. Hyaluronic acid is degraded by hyaluronidase enzymes. Various biological sources are being explored to source hyaluronidase inhibitors. Polyphenols such as alkaloids, flavonoids and terpenes have demonstrated inhibition against HAases. In this study we have evaluated the HAase inhibition activity of date seed extract (SesZenNHA™) against the well established plant based HA inhibitor which comes from Senna seeds . The polysaccharide content, which is believed to be the phytochemical responsible for HA inhibitory activity of Date seeds, was found to be 18.73% glucose equivalents as against Seena Polysaccharides of 7%. In hyaluronidase inhibition activity,IC20 of date-seed extract was estimated to be 0.5mg/ml whereas IC20 of senna seed extracts was 2 mg/ml. Hence the proprietary ingredient derived from date seed holds 4 times better potential for HA inhibition.

Keywords: Hyaluronan, hyaluronidase inhibitors, bioactive molecules, date seeds, Indian senna

Introduction:

Matrix glycosaminoglycan hyaluronan or hyaluronate (HA) metabolism must be balanced and regulated. Hyaluronan synthase in fibroblasts, endothelial cells, and keratinocytes synthesises HA from D-glucuronic acid and N.-acetyl-D-glucosamine. It is degraded by hyaluronidase enzymes.(EC 3.2.1.35) (Laurent and Fraser 1992; Flannery et al. 1998; Vuillermoz et al. 2005). HA polymers are key components of cartilage, synovial membrane, and synovial fluid.(Fraser et al. 1997). Hyaluronic acid affects extracellular matrix and hydration. Hyaluronic acid may interact with cell migration, proliferation, and gene expression receptors (Bukhari et al. 2018). Hyaluronic acid content is often regulated by hyaluronidase in the skin's dermis

(Papakonstantinou et al. 2012). When hyaluronic acid level reduces, skin hydration and elasticity decrease. Inhibitors of hyaluronidase are strong anti-aging, anti-inflammatory, and anti-microbial agents.

Date seeds *(Phoenix dactylifera)*, contain bioactive compounds and are used in cosmetics and culinary additives (Vayalil 2002). Meer et al. (2017) demonstrated that date fruit extract enhanced skin health significantly (Meer et al. 2017). Date seeds contain 6–8 percent essential oil that can be used to make moisturising soaps/creams, shampoos, and other skincare products (El Hadrami and Al-Khayri 2012; Al Juhaimi et al. 2018). In addition, date seed oil has been utilised in the manufacture of medicines, foods, and even cosmetics (Konkol and Rasmussen 2015; Akbari et al. 2016). Date fruits and seeds have been discovered to contain many phenolic acids and flavonoids, making them an affordable source of natural antioxidants. In addition to sterols, carotenoids, procyanidins, and anthocyanins, date seeds are a rich source of additional phytochemicals, such as carotenoids, procyanidins, and anthocyanins. *P. dactylifera* has been shown to contain significant quantities of anthocyanin and flavonoid, making it a promising source of antioxidant chemicals (Djaoudene et al. 2019). Date seed oil decreases cellular and oxidative rancidity as well as UV-induced skin irritation. Ines et al. (2010) demonstrated that date seed oil concentrations in excess of 30 g/mL were not harmful to human skin (Ines et al. 2010). Lecheb and Benamara produced a cosmetic cream from the aqueous extract of *P. dactylifera* fruit seed oil that demonstrated superior fluidity, viscosity, and rheological behaviour in comparison to other cosmetics (Lecheb and Benamara 2015). Indian senna seeds *(Cassia angustifolia)*, contain a polysaccharide galactomannan which mimics the activity of hyaluronic acid. Senna leaves often used to treat skin infections and inflammations. Phytoconstituents present in the seeds and leaves observed were tannins, flavonoids, steroids and phyloba-tannins (Monkheang et al. 2013; Alsubeie 2022)

Materials and Methods:

Date seed aqueous extract is the proprietary ingredient developed by Zenherb Lab under the trade name of SesZenNHA™

For the experimental purposes, 500mg extracts of date seeds and senna seeds are sonicated for 5 minutes mixed with 100ml water. This solution is filtered and further diluted 1:50. This solution is henceforth referred to as seed extracts.

Estimation of polysaccharide content: (Bhatti et al., 2013):

To estimate the polysaccharide content in the seed extracts, 1ml of 5% phenol is added to the 1ml of extract solution, followed by 5ml of conc. H2SO4. The absorbance was measured after 5 minutes at 488nm against blank. The experiment was carried out in triplicate. The glucose equivalent was calculated using

$$Glucose.eq\ \left(\frac{w}{w}\%\right) = \frac{ppm\ x\ V\ x\ DF\ x\ 100}{wt.of\ sample\ (g) x\ 10\text{^}6}$$

Where,

V = Volume total

DF = Dilution Factor(50)

106= 1000000(parts per million)

Wt of sample in gram

100 = percentage

Estimation of Inhibition of Hyaluronidase enzymes: (Nawy et al., 2001)

To estimate the potential of extracts to inhibit the hyaluronidase enzymes the assay was performed as follows. 50µl of bovine hyaluronidase was mixed with 50µl of the extracts. Hyaluronidase was activated using 12.5mM calcium chloride and mixed with 250µl of sodium hyaluronate (1.2 mg/ml). The mixture was heated to 100°C. After it was subjected to cooling, 1.5ml of p-Dimethylamino benzaldehyde was added and incubated for 20 minutes. The absorbance was measure at 585nm. Oleanolic acid was used as a positive control. The inhibitory activity of the enzymes was calculated.

Results and Conclusion:

Estimation of polysaccharide content: The polysaccharide content was evaluated for date-seed extract and found to be 18.73% (%w/w).

Hyaluronidase assay: the extracts were evaluated for the ability to inhibit hyaluronidase enzymes. The date seed extract was able to inhibit hyaluronidase enzymes better than senna seed extracts. IC20 of date-seed extract was estimated to be 0.5mg/ml, whereas IC20 of senna seed extracts was 2 mg/ml. Date seed extracts showed 50% hyaluronidase inhibitory activity at 1.25 mg/ml. Oleanolic acid had a mean anti-hyaluronidase activity at 92.53%.

Discussion:

Hyaluronic acid (HA) is an important polymer present in skin, synovial fluid of joints and the eyes. It is a humectant that seals moisture into the skin and reduces friction in the joints. Hyaluronidase enzymes break down hyaluronic acid in the tissues and inhibitors of this enzyme help maintain the hyaluronic acid levels. Date seed and senna seed have been explored for their benefits to the skin, as anti-inflammatory and antioxidant agents. Plants have been a promising source of hyaluronidase inhibitors. Various alkaloids and polyphenols such as tannins, quercetin and ellagic acid amongst a few have shown their potency as hyaluronidase inhibitors. High tannin content in plants is known to inhibit the enzyme hyaluronidase. The extracts from the aerial parts of two plants, *Oenothera paradoxa* Hudziok and *O. biennis* L, which are rich in macrocyclic ellagitannin, were found to strongly inhibit the HAase enzyme activity at 50 g/mL compared to the standard heparine (62.1 7.5 g/mL) in a study by Granica et al (Granica et al. 2013). In this study we have utilised extracts of date seeds and senna seeds to evaluate the hyaluronidase inhibition activity. IC20 of date-seed extract was estimated to be 0.5mg/ml whereas IC20 of senna seed extracts was 2 mg/ml. The. Hence the

proprietary ingredient derived from date seed holds 4 times better potential for HA inhibition.This can also be correlated with the 2x phyto-polysaccharides present in the proprietary date seed extract.

Further studies should be conducted to identify the bioactives responsible for HAase inhibition. We do not know how the different classes of inhibitors respond to different HA sources. If there is a varied response, we would be able to control HA levels in different organs and tissues.

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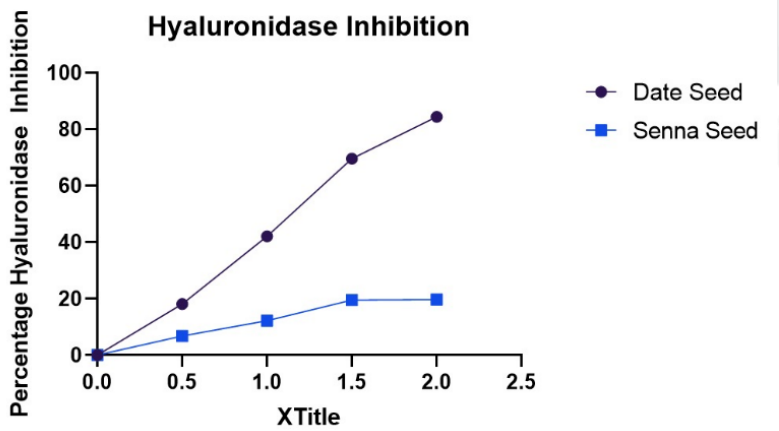
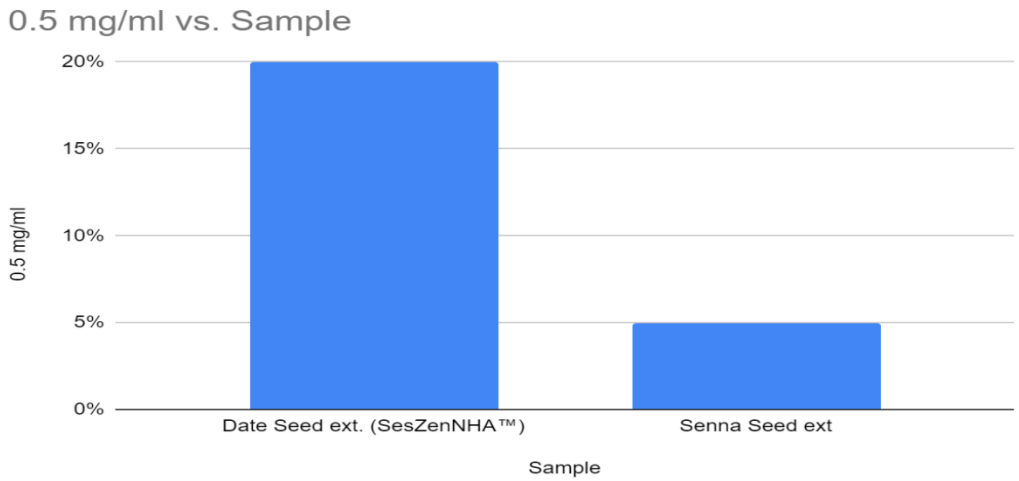
List of figure:



List of Tables:

% HA inhibition at 0.5mg/ml	
Sample	**0.5 mg/ml**
Date Seed extract (SesZenNHA™)	**20%**
Senna Seed extract	**5%**



% HA inhibition at 0.5mg/ml