Chapter

Plant Natural Products: A Promising Source of Hyaluronidase Enzyme Inhibitors

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Abstract

Hyaluronidase enzyme degrades hyaluronan, the primary component of the extracellular matrix found in connective tissues animals and on the surface of certain pathogenic bacteria. The degradation of hyaluronan is linked to a wide range of physiological and pathological process. Inhibiting the hyaluronidase enzyme is thus significant as an approach to treat a variety of diseases and health conditions such as anti-fertility, anti-tumor, antimicrobial, and anti-venom/toxin agents. HAase inhibitors of different chemical types have been identified include both synthetic compounds and constituents obtained from naturally sources. Plant natural products as HAase inhibitors are unique due to their structural features and diversity. Medicinal plants have historically been used as contraceptives, antidote for snakebites and to promote wound healing. In recent years, small molecules, particularly plant natural products (alkaloids, flavonoids, polyphenol and flavonoids, triterpenes and steroids) possessing potent HAase have been discovered. A number of plant species from various families, which have folk medicinal claims for these ailments (related to hyaluronan disturbances) were scientifically proven for their potential to block HAase enzymes.

Keywords: hyaluronidase inhibitors, natural products, medicinal plant, phytochemicals

1. Introduction

Hyaluronan/hyaluronic acid (HA) is a biologically important polysaccharide molecule found in the animal kingdom, most notably in the extracellular matrix (ECM) of connective tissues and on the surface of certain pathogenic bacteria. Although HA is found in nearly every tissue of vertebrates, it is abundantly present in the extracellular matrix of soft connective tissues. In mammals, it's predominantly found in the connective tissue of skin, testes, umbilical cord and synovial fluid. HA is composed of a linear polymeric chain with a uniform repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine linked through (1,3 and 1,4) glycosidic bond. HA is a megaDalton molecule, synthesized as free polymer by the plasma membrane at its inner face [1–3].

The molecular function of hyaluronan in the body include interaction with HA receptors on the surface of the same cell or ECM molecules of the surrounding cells [4, 5]. When newly secreted, HA interacts with a variety of cell surface receptors

(CSRs) which give rise to important physiological functions such as signal transduction, building of pericellular matrix and the degradation endocytosis of HA via receptor-mediated internalization [6–8].

The metabolism of hyaluronan involves hyaluronidase enzyme, which is a class of glycosidase that predominantly degrades hyaluronan (HA). Karl Meyer coined the word Hyaluronidases (HAases), and over the years of research, the importance of HAases in controlling the physiological and pathological function of HA in animals has been established [9]. In mammals, the HAases hydrolyze the glucosaminidic β-1,4-linkages of hyaluronic acid and produces tetrasaccharide fragments. Three types of HAase enzymes act in concert to degrade HA biochemically; first, intact HA is acted on by endoglycosidase HAases, resulting in oligosaccharides with varying chain lengths that serve as substrates for the other two HAase enzymes (exoglycosidases), namely -glucuronidase and -N-acetyl hexos [10, 11].

The enzyme hyaluronidase and its substrate (Hyaluronan) perform a critical biological function in human body and their imbalance has been linked with various pathological processes and disease states including skin diseases and cancer [1]. The biological role of HA depends on the type of product formed after degradation and the circumstances under which it is synthesized [6, 12]. The involvement of HA has been established in various physiological and pathological processes include embryogenesis [7, 13, 14], immune surveillance, inflammation [15–18], wound healing [19], multi-drug resistance [6], cancer, water homeostasis and viscoelasticity of ECM [6, 8, 11, 20, 21]. Thus, it is critical to maintain HA homeostasis by balancing the action of HAase enzymes involved in anabolic and catabolic activities using various approaches such as hyaluronidase enzyme inhibitors (HAIs). The biological and therapeutic potential of HAase inhibitors (HAIs) is receiving significant attention, and an increasing amount of research is being conducted to develop potent hyaluronidase inhibitors for a variety of health conditions, including contraceptives, anti-tumor, antimicrobial, and anti-venom/toxin agents [22–24]. Hyaluronidase inhibitors of different chemical types are increasingly being reported, which include synthetic and plant derived bioactive compounds, polysaccharides, fatty acids, proteins, glycosaminoglycans and others [23, 25–30].

In this chapter, we have discussed and presented an updated overview of studies on important natural product agents (small molecules, and plants extracts) of various chemical forms derived from medicinal plants, which have been reported as potent hyaluronidase inhibitors. The search engines, such as, Google Scholar and PubMed were used to search the literature using key words such as natural products, medicinal plants, phytochemicals with hyaluronidase inhibitors, and anti-hyaluronidase. Majority of the data covered in this study are research published during the last fifteen years and studies with incomplete data or doubtful peer review system were excluded.

2. Hyaluronidases

Hyaluronidases are a family of endoglycosidase enzymes found in both eukaryotes and prokaryotes and prevalent across the animal kingdom [31]. It was first observed by Duran-Reynals in mammalian testis extract and termed it “spreading factor” as it has the property of breaking down the hyaluronan structure and facilitating tissue permeability and spreading [32]. Karl Meyer later classified hyaluronidases into three groups depending on chemical analysis and end products formed, which included mammalian, leech, and bacterial hyaluronidases.

Mammalian hyaluronidases are endo-β-Nacetlyhexosaminidases which arbitrary cleave hyaluronan glycosidic ate β-1-4 position, yielding even numbered tetra- and

hexa oligosaccharides as the major end products along with N-acetylglucosamine at the reducing end of the product. These hyaluronidases exhibit both hydrolytic and transglycosidase activity and are found in spermatozoa, mammalian cell lysosomes, and bee, snake, and reptile venoms [33].

The second type of HAases are leech hyaluronidase, which cleave glucoronate linkages of hyaluronan and are inert towards other glycosaminoglycans. These group of HAases are hyaluronate-3-glycanohydrolases are endo-β-D-glucuronidases. Tetra- and hexasaccharides are the main end products with glucuronic acid at the reducing end. This group of enzymes are present in salivary glands of leeches and hook worms [34].

The third type is microbial hyaluronidases, which are distinguished from mammalian and leech HAases by their lack of hydrolysis activity. These HAases catalyze the cleavage of HA at the 1–4 glycosidic bond, resulting in the formation of 4 and 5 member unsaturated oligosaccharides. Enzymes in this class includes HA lyases from *Streptococcus pneumoniae* (S. PHL) and *S. agalactiae* [34, 35].

In humans, six hyaluronidase-like genes known as hyaluronoglucosaminidases (Hyals1–6) have been identified. Of the six Hyal genes, Hyal1 and 2 are the primary hyaluronidases responsible for the catabolism of HA in somatic tissue, while Hyals3 to 6 are inactive and likely do not participate in HA cleavage [36]. Although inactive, hyal3 is widely expressed in chondrocytes, testis, and bone marrow, and its expression increases when fibroblasts differentiate into chondrocytes. Inflammatory cytokines such as IL-1 and TNF- (tumor necrosis factor-alpha) upregulate the Hyal2 and Hyal3 genes, but not the Hyal1 gene [37].

3. Plant derived natural products as hyaluronidase inhibitors

In the regulation of biological processes, inhibition of enzyme activity can be as essential as the activity itself. Many diseases are caused by overactivation of enzymes, which can be regulated with enzyme inhibitors since blocking the enzyme is more efficient in active catabolic reactions than stimulating the synthesis of substrates such as the high molecular weight polymeric hyaluronan contained in the extracellular matrix [38]. This is particularly true when a rapid response or finely regulated temporal and spatial ECM activities are required. Mio and his colleagues have identified the first inhibitor of the hyaluronidase enzyme in human and mouse serum [39].

For centuries, nature has been a source of medicinal products, with numerous useful medicines have been derived from plant sources [40]. Their therapeutic utility in treating a variety of illnesses have been investigated in various conventional medical systems, and their role as a biological modulator has been recognized throughout human history [41]. Natural products' effectiveness as enzyme inhibitors is attributed to their product biosynthetically in living organisms, which enhances their chances of interacting effectively with a variety of biological targets [42]. The inherent steric complexity, more number of rings and chiral centers, as well as the presence of more oxygen and the ability to form more hydrogen bonds, increases drug-likeness property of natural products from synthetic ones [43, 44]. The following section discusses recent research on various plant extracts and phytoconstituents as potential sources of hyaluronidase inhibitors.

3.1 Anti-hyaluronidase phytoconstituents

Various class of natural products derived from different plants species documented as hyaluronidase inhibitors include alkaloids, flavonoids, polyphenols, terpenes and steroids as shown in the **Table 1**. Natural products derived from plants

Class of Natural Products	**Compounds**	**Source of HAase enzyme**	**IC_{50}/%Inhibition**	**Ref.**
Alkaloids	Aristolochic acid	*Naja naja* venom	50 μM	[28]
	Ajmaline		-	
	Reserpine		-	
	Nuciferine	Testicular	>100 μM	[45]
	Nornuciferine		22.5 μM	
	N-methylasimilobine		>100 μM	
	Asimilobine		11.7 μM	
	Pronuciferine		>100 μM	
	Armepavine		>100 μM	
	Norarmepavine		26.4 μM	
	N-methylcoclaurine		>100 μM	
	Coclaurine		11.4 μM	
	Norjuziphine		24.3 μM	
	Aristolocic acid	Naja naja venom	1.43 μM	[46]
	3-[(4-methylpiperazin-1-yl)methyl]-5-phenyl-1H-indole	Testicular	23% (5 μM)	[47]
Flavonoids/ polyphenols	Flavone Tannic acid Quercetin	Naja naja venom	50 μM	[28]
	Tannin	Honey bee, scorpion, snakes and cobra venoms	0.9%	[53]
	Kaempferol		21.0%	
	Silybin		24.8%	
	Myriceetin		31.5%	
	Morin		33.0%	
	Quercetin		33.9%	
	Butein		37.8%	
	Phloretin		41.1%	
	Catechin		42.5%	
	Flavone		46.9%	
	Rutin		40.5%	
	Isoquercitin		42.1%	
	Apigenin		15.0%	
	Apigenin Kaempferol Luteolin Tannic acid	Honey bee, scorpion, snakes and cobra venoms		[54]

Class of Natural Products	Compounds	Source of HAase enzyme	IC_{50}/%Inhibition	Ref.
	quercetin 3-O-β-D-glucopyranoside	Bovine testes	20.9 mM	[57]
	quercetin 3-O-β-D-xylopyranoside		22.1 mM	
	kaempferol 3-O-β-D-glucopyranoside		26.5 mM	
	isorhamnetin		55.4 mM	
	Rosmarinic acid	Testicular	309 μg/mL	[57]
	Lithospermic acid B		164 μg/mL	
	Diometin-7-O-β-D-glucopyraanoside		644 μg/mL	
	Apigenin-7- O-β-D-glucuronopyranoside		548 μg/mL	
	Tannic acid	Testicular	4.97 units/mL	[58]
	Apigenin		4.02 units/mL	
	Quercentin		4.28 units/mL	
	Tannic acid	Testicular	0.8 units/mL	[59]
	Gallic acid		5 units/mL	
	Ellagic Acid		4.8 units/mL	
	Chicoric acid	*Escherichia coli* F470	171 μM	[68]
Terpenes/ steroids	Glycyrrhizin	*Streptococcus agalactiae*	0.020–1.300 mM	[52]
	Glycyrrhetinic acid	Bovine testes	0.060–0.260 mM	
	3β-urs-12-en-28-oic acid	Testicular	103.18 ±1.70 μM	[62]
	3β,19,23-trihydroxyurs-12-en-28-oic acid		286.95±10.28 μM	
	3β-acetylolean-12-en-28-oic acid triterpenoid		1466.5± 2.37 μM	
	Steroidal Fraction	*Brevibacterium halotolerans DC1*	5.19 mM	[67]
	Testosterone Propionate	*Escherichia coli* F470	124 ± 1.1 μM	[68]
	Glycyrrhizic acid	*Escherichia coli* F470	175 ± 1.2 μM	[68]

Table 1.
Natural product compounds active against hyaluronidase enzyme.

are well-known as HAase inhibitors due to their unique structural features. As indicated in **Table 1**, many classes of natural compounds produced from various plant species have been recorded as hyaluronidase inhibitors. These classes include alkaloids, flavonoids, polyphenols, terpenes, and steroids.

3.1.1 Alkaloids

Alkaloids are naturally occurring secondary metabolites, which consist of a basic nitrogen atom and produced by various species of animals, plants, bacteria and fungi. Morikawa and his team evaluated aporphine and benzylisoquinoline alkaloids which they have earlier isolated from the flower buds of Sacred lotus (*Nelumbo nucifera*) tably, Among the alkaloids discovered as a hyaluronidase inhibitor, nornuciferine (IC_{50} = 22.5 μM), asimilobine (11.7 μM), norarmepavine (26.4 μM), coclaurine (11.4 μM), and norjuziphine (24.3 μM) have shown potent activity, even higher than the standard atilllergic drug (disodium cromoglycate (IC_{50} = 64.8 μM). Nuciferine, N-methylasimilobine, pronuciferine, armepavine, and N-methylcoclaurine are the other alkaloids with moderate anti-HAase action [45]. Girish and co-researchers tested various compounds including well known alkaloids such as aristolocic acid, reserpine, and ajmaline on hyaluronidase enzymes obtained from the *Naja naja* snake venome and observed a dose dependent inhibition of hyaluronidase enzyme activity in manner. It was further observed that aristolochic acid has completely inhibited HAase, while reserpine and ajmaline inhibited it partially in a non-competitive manner. [28, 46]. Olgen and colleagues have tested as a series of aminomethyl indole alkaloids derivatives against the bovine testes hyaluronidase and found 3-[(4-methylpiperazin-1-yl)methyl]-5-phenyl-1H-indole as the most potent inhibitor of HAase enzyme [47].

3.1.2 Flavonoids and polyphenols

Flavonoids are a large group of polyphenolic compounds having benzo-γ-pyrone structure and are ubiquitously present in various parts of the plants. Flavonoids are a wide class of polyphenolic chemicals with a benzo—pyrone structure that are found in virtually every part of plants. Secondary metabolites of phenolic origin, such as flavonoids, are involved in a variety of pharmacological activities [28]. Based on their structure, flavonoids of different types such as flavones, anthocyanidines, flavones, and chalcones have demonstrated antioxidant, anti-inflammatory, antiviral, and antithrombotic properties, antitumor, hepatoprotective and enzyme inhibitory properties [48–50].

Girish and co-researchers have observed in an *in vitro* study that flavonoids of different structure types such as flavone, tannic acid quercetin were able to inhibit the hyaluronidase enzyme activity obtained from *Naja naja* snake venom [28].

In an early study, Rodney and co-researchers evaluated the effect of flavonoids on hyaluronidase and afterwards the effect of 31 flavonoids has been found potent against the activity of bovine testicular hyaluronidase. The inhibitory action of flavonoids on hyaluronidases is dependent on the number of hydroxyl groups and side chain substituents present in the molecules, and flavonoids containing many hydroxyl groups were found to significantly reduce inhibitory activity hyaluronidase enzyme [51]. Plant based flavonoids such as flavones, 2-hydroxy-flavone, apigenin, luteolin, quercetin, and myricetin demonstrated the inhibitory effects on hyaluronidase activity [51].

Herte et al. investigated the effects of several flavonoids on the microbial origin of the hyaluronidase enzyme (Hyaluronate lyases). During their research, they discovered quercetin and myricetin to be the most potent inhibitors, with extra hydroxyl groups at positions 3,3′ (quercetin) and 5′ (myricetin) (myricetin). In addition, glycosylated flavonoids such as rutin, apiin, and silybin have shown a decline in their capacity to inhibit hyaluronate lyase, even when the side groups carried hydroxyl groups themselves [52].

A series of flavonoids were examined by Kuppusamy et al. against hyaluronidase enzyme extracted from the venom of honey bee, scorpion and cobra and found flavonoids such as myricetin, quercetin, luteolin, apigenin, phloretin and kaempferol showing potent anti-HAase effects in *in vitro* assay [53]. In another study the same authors observed a contrast where, sylibin inhibited hyaluronidase activity of bee and scorpion venom supported by the apigenin, kaempferol, luteolin, and tannic acid [54].

Kim and his co-worker isolated flavonols (quercetin 3-O-β-D-glucopyranoside, quercetin 3-O-β-D-xylopyranoside, kaempferol 3-O-β-D-glucopyranoside, and isorhamnetin 3-O-β-D-glucopyranoside) from the *Allium sativum* L. for anti-hyaluronidase properties [55].

Polyhenols are naturally occurring secondary metabolites, largely found in plants and generally involves in the defense of plants against pathogens [56]. Other type of phenolic compounds includes rosmarinic acid, lithospermic acid B, diometin-7-O-β-D-glucopyraanoside, and apigenin-7-O-β-D-glucuronopyranoside reported from the *Meehania fargesii* plant, all of which were effective at suppress HAase activity [57]. Tatemoto and colleagues investigated the effects of tannic acid, apigenin, and quercetin, in *in vitro* fertilization parameters, on hyaluronidase activity, and found that tannic acid was the most active hyaluronidase enzyme activity in a dose-dependent manner at concentrations ranging from 2 to 10 g/ml [58]. The same group of researchers investigated three tannins, tannic acid, gallic acid, and ellagic acid, and found tannic and ellagic acid as potent inhibitors of the hyaluronidase enzyme, effectively preventing polyspermy by suppressing the acrosome reaction induced by sperm-zona interaction during in vitro fertilization of porcine oocytes [59].

3.1.3 Terpenes and steroids

Terpenes are the constituents of pheromones, anti-feedants and flavors, which are composed of isoprene unite (C_5) and their derivatives. Terpenes and terpenoids (oxygenated derivative) are recognized as one of the important class of natural products, are widely distributed in plants and possesses a range of bioactivity, exhibiting a wide bioactivity, such as anticancer, neuroprotection, and anti-inflammation and anti-infective agents [60, 61]. Abdullah and co-authors isolated teriterpenes as HAase blocking agents from *Prismatomeris tetrandra* (Roxb.) K. Schum. The two triterpenoids (3β-urs-12-en-28-oic acid and 3β,19,23-trihydroxyurs-12-en-28-oic acid) were obtained from the chloroform fraction whereas another triterpenoid 3β-acetylolean-12-en-28-oic acid was isolated from the roots of *Prismatomeris tetrandra*. Also, the synthetic analogues of ursolic acid were identified as potential inhibitor of hyaluronidase [62]. The *in-vitro* inhibition of bovine hyaluronidase and hylaluronate lyase was shown by the triterpenes glycyrrhizin and glycyrhetinic acid [52]. However, fatty acid derivative of glycyrrhetinic acid, known as stearyl ester was unable to inhibit the hyaluronidase activity [63]. This difference may be due to the specific structures of the respective enzymes as well as the splitting mechanism of hyaluronic acid as endoenzyme or exoenzyme [64].

Sterols are important structural components in higher organisms. They take part in the regulation of membrane fluidity, permeability and membrane associated metabolic processes [65]. Steroids of different structure types are reported to influence hyaluronidase metabolism [66]. Patil and co-researchers found the steroidal fraction isolated from the leave of *Carissa carandas* as strong inhibitior of hyaluronidase enzyme activity with IC_{50} = 5.19 mM/mL as compared to the standard (quercetin). This steroidal fraction could contain potential hyaluronidase inhibitor and therefore should be considered for further studied as anti-venom agent [67].

In a study, Lengers and team found chicoric acid (IC_{50} = 171 μM) and testosterone propionate (IC_{50} = 124 ± 1.1 μM) as strong inhibitors of Hyal1 expressed over the surface of *Escherichia coli* F470 which was comparable to that of glycyrrhizic acid (IC_{50} = 177 μM) [68].

3.2 Anti-hyaluronidase medicinal plant extracts

Plants have remained a major source of medicine for centuries and therapeutic agents derived from natural sources are used traditionally to recover from wound healing, treat snakebites or inflammation as contraceptives. Several studies indicate that plants species from various families, which have folk medicinal claims for these ailments were also scientifically been proven for their potential to block HAase enzymes as shown in **Table 2**.

Plant Name	Plant part/type of extract (active extract)	Biological activity	Source of HAase enzyme	Ref.
Meehania fargesii (Lamiaceae)	Whole plants 80% acetone extract (water soluble fractions)	Anti-HAase	Testicular	[57]
Aesculus hippocastanum (Hippocastanaceae)	Seeds/ aqueous-ethanol	Anti-inflammatory	Testicular	[69]
Hedera helix (Araliaceae)	Leaf/ aqueous-ethanol	Anti-inflammatory	Testicular	[69]
Hygrophila schulli	Leaf/ethanolic extract	Anti-inflammatory	Testicular	[70]
Areca catechu (Arecaceae)	Whole plant/ aqueous methanol	Anti-aging	Testicular	[71]
Dryopteris cassirrhizoma (Dryopteridaceae)	Methanol–water extract of whole plant	Anti-aging/ Anti-inflammatory	Testicular	[71]
Alpinia katsumadai (Zingiberaceae)	Whole plant/ aqueous-ethanol	Anti-aging/ Anti-inflammatory	Testicular	[72]
Cinnamonum cassia (Lauraceae)	Whole plant/ aqeuous-methanol	Anti-aging/ Anti-inflammatory	Testicular	[72]
Curcuma longa (Zingiberaceae)	Methanol–water extract of whole plant	Anti-aging/ Anti-inflammatory	Testicular	[72]
Prunus salicina (Rosaceae)	Root bark/aqueous decoction	Anti-HAase	—	[73]
Anemarrhena asphodeloides (Asphodelaceae)	Rhizom/methanol extract	Anti-inflammatory/ Anti-allergy	Testicular	[74]
Rubus fruticosus/Blackberry (Rosaceae)	Fruits/Methanol	Anti-Inflammatory	—	[75]
Artocarpus altilis (Moraceae)	Bark/ethanol	Anti-aging	Testicular	[76]
Curcuma aromatica (Zingiberaceae)	Rhizomes/ethanol	Anti-aging	Testicular	[76]

Plant Name	Plant part/type of extract (active extract)	Biological activity	Source of HAase enzyme	Ref.
Chamaerhodos altaica (Rosaceae)	Aerial parts/80% acetone extract (aqueous fraction, BuOH)	Anti-HAase	—	[77]
Camellia sinensis (Theaceae)	Leaves, buds/(water brew)	Anti-aging/skin care	Testicular	[78]
Canavalia gladiata White Sword Beans (Fabaceae)	Seeds/80% methanol extracts(fermented and non-fermented)	Anti-inflammatory	—	[79]
Glycine max Soybeans (Fabaceae)	Seeds/80% methanol extracts (fermented and non-fermented)	Anti-inflammatory	—	[79]
Coffee Rubiaceae (Rubiaceae)	Seeds/Coffee silverskin (byproduct of the roasting procedure for coffee beans	Anti-inflammatory/ Anti-allergy	Testicular	[80]
Deutzia coreana (Hydrangeaceae)	Stem/methanolic extract	Anti-inflammatory/ Anti-allergy	Testicular	[81]
Osmanthus insularis (Oleaceae)	Stem/methanol	Anti-inflammatory/ Anti-allergy	Testicular	[81]
Styrax japonica (Styracaceae)	Stem/methanol extract	Anti-inflammatory/ Anti-allergy	Testicular	[81]
Dracocephalum foetidum (Lamiaceae)	Aerial parts/80% aqueous acetone extract (aqueous fraction)	Anti-inflammatory	Testicular	[82]
Keiskea japonica (Lamiaceae)	Aerial part/80% acetone extract	Anti-HAase	Testicular	[87]
Lycopus lucidus (Lamiaceae)	Aerial part 80% acetone extract	Anti-HAase	Testicular	[88]
Lythrum salicaria L (Lythraceae)	Whole Plant/ aqueous extract	Anti-inflammatory	Testicular	[89]
Terminalia chebula (Combretaceae)	Fruit dried/95% ethanol extract	Anti-fertility	Human spermatozoa	[90]
Gaultheria procumbens (eastern teaberry) (Ericaeae)	Leaves/Petroleum ether, chloroform	Anti-inflammatory	Testicular	[91]
Payena dasyphylla (Sapotaceae)	Bark/methanolic extract	Anti-arthritic	Testicular	[92]
Phyllanthus emblica (Phyllanthaceae)	Aqueous extract of fruit	Chondroprotective	Testicular	[93]
Vitis rotundifolia (Vitaceae)	Seed and skin/50% ethanol extract	Anti-HAase	Testicular	[94]
Malaxis acuminata (Orchidaceae)	Leaves, stem/ methanolic extract	Skin-aging	—	[95]
Mimosa pudica (Fabaceae)	Roots/aqueous extract	Anti-ophidian	Snake venom	[96]

Plant Name	Plant part/type of extract (active extract)	Biological activity	Source of HAase enzyme	Ref.
Oenothera biennis Evening-primrose (Onagraceae)	Aerial Part/50% methanolic extract	Anti-inflammatory	—	[97]
Oenothera paradoxa Evening-primrose (Onagraceae)	Aerial Part/50% methanolic extract	anti-inflammatory	—	[97]
Otostegia fruticosa (Lamiaceae)	Leaf/70% ethanolic extract	Anti-inflammatory	Testicular	[98]
Brown algae (*Eisenia bicyclis* and *E. kurome*)	Crude phlorotaninin extract	Anti-aging	Testicular	[99]
Padina pavonica (Dictyotaceae)	Seaweed/extracts (Pressurized liquid extraction, microwave assisted extraction. Supercritical fluid extraction)	Anti-aging	Testicular	[100]

Table 2.
Medicinal plants with hyaluronidase activity.

In a bioassay directed study, the polar fraction of *Aesculus hippocastanum* L (seeds) and *Hedera helix* L (leaves) were found active against hyaluronidase enzyme and later isolation of triterpene and steroidal saponins and sapogenins also exhibited strong anti-HAase activity when tested against testicular hyaluronidase enzyme [69].

The well-known medicinal plant *Hygrophila schulli*, traditional used as anti-inflammatory and pain treatment in the north Ethiopia and India, possess anti-hyaluronidase activity *in vivo* by the ethanolic leaf extract [70]. In a study by Lee et al. [71], the aqueous methanolic extracts of 150 plant species assayed for their potential as hyaluronidase inhibitors, the extracts of six species found to be most active against HAases were *Areca catechu*, *Alpinia katsumadai*, *Dryopteris cassir-rhizoma*, *Cinnamonum cassia*, and *Curcuma longa*, and the extract of *Areca catechu* showed relatively higher anti-HAase activity. The major constituents identified in *Areca catechu* which include phenolic compounds such as flavonoids and tannins could be responsible for the anti-HAase effect [72].

In another anti-HAase screening study, Tomohara et al. [73] evaluated the decoction extracts of 98 plant species for HAase inhibitory activity in an *in vitro* HAase assay. They observed 17 extracts exhibited moderate to high inhibitory activity (>50% inhibition at 500 μg/mL) and also noted correlation between the total phenol present in the extract and their cumulative effect as anti-HAase activity was varying. From the study, rhizome extract of *Panax japonicus* and root bark extract of *Prunus salicina* were found most potent HAase inhibitors.

Jeong et al. [74] evaluated 100 Korean medicinal plants for their anti-allergic activity. The methanolic rhizome extract of *Anemarrhena asphodeloides* was found active against hyaluronidase enzyme. Marquina and colleagues investigated the anti-inflammatory effect of blackberry fruit extract and fractions. Two of the seven fractions inhibited the hyaluronidase enzyme and shown superior anti-inflammatory effect when compared to aspirin [75].

A study conducted by Liyanaarachchi et al. [76] on fifteen Sri Lankan medicinal plants for their skin aging and anti-wrinkle effect has identified three plant extract with relatively higher anti-HAase activity. The ethanol extract of *Curcuma*

aromatica rhizomes exhibited marked hyaluronidase inhibitory activities (95.0%) inhibition at 500 μg/mL) followed by *Artocarpus altilis* (68.59%) and *Artocarpus nobilis* bark extracts (44.78%) when tested at 500 μg/mL concentration level.

Selenge et al. [77] studied two medicinal plant famous in Mongolian traditional medicine *Chamaerhodos erecta* and *C. altaica* and revealed in the bioassay guided isolation the moderate ability of its constituents as anti-HAase enzyme, suggesting their potential to prevent the extracellular matrix degradation factors.

Similarly, the Sri Lankan origin black tea *Camellia sinensis* L. (Orthodox Orange Pekoe) was evaluated for its potential as cosmeceutical for skin aging by Ratnasooriya et al. [78]. The extract revealed moderate anti-HAase activity (IC_{50} =1.09 ±0.12 mg/mL) compared to standard compound (epigallocatechin gallate) (IC_{50} = 0.09±0.00 mg/mL) in as dose dependent manner.

Han et al. [79] assayed the fermented and non-fermented seed's methanolic seed extract of White Sword Beans (*Canavalia gladiata* DC) and Soybeans (*Glycine max* L. Merrill) and found higher inhibitory activity exhibited by red sword beans (non-fermented/fermented) (1.5–2.6-fold) against HAase enzyme than that of soybeans (non-fermented/fermented). The study suggests that *B. subtilis*-fermented sword beans are potential as potential anti-inflammatory agents for the food industry.

Furusawa et al. [80] investigated the silverskin coffee beans (a by-product during roasting) for its anti-inflammatory and anti-allergic effects. The results indicated a potent inhibitory effect against hyaluronidase (IC_{50}=0.27 ±0.04 mg/mL) as compared to the standard disodium cromoglycate (IC_{50} = 0.31±0.05 mg/mL). The strong effect is argued possibly due to the presence of acidic polysaccharides present in the extract, which is mainly composed of uronic acid present in Silverskin coffee beans extract.

A major screening study on 500 Korean Medicinal plants as HAase inhibitors identified the stem extract of three species possessing relatively higher anti-HAase activity include plant specied *Styrax japonica* (57.28%), *Deutzia coreana* (53.50%), and *Osmanthus insularis* (53.19%). The study further explores that the HAase inhibition could be due to presence of multifunctional compounds and may be effective in preventing allergic reactions and inflammation [81].

Dracocephalum foetidum Bunge, is a medicinal plant traditionally used by Mongolian nomads for various infections and suppurative disease and fever. Its chemical and physiological role was investigated by Selenge et al. [82] and found the aqueous acetone extract of the aerial part possess potent anti-hyaluronidase activity Acetone extract (IC_{50} = 0.27 ± 0.01 mg/mL) compared to the standard (disodium cromoglicate, IC_{50} = 0.33 ± 0.02 mg/mL) and further phytochemical analysis of its aqueous fraction resulted into compounds of various class as highly potent HAase inhibitors.

Załuskia et al. [83] found strongest inhibitory effects in the freshly dried fruits of *Eleutherococcus senticosus* (IC_{50} = 0.58 ± 0.01 mg/mL) and *E. henryi* (IC_{50} = 0.61 ± 0.05 mg/mL), compared to positive control (Methyl indole-3-carboxylate, (IC_{50} = 07.11 mM). *Eleutherococcus senticosus* Maxim. called as Siberian ginseng, and has been used from ancient times in Northeastern Asia and Eastern Russia as a tonic and anti-fatigue agent. In northeast China, the ethanolic roots extract is a popular health supplement for weakness, rheumatism, impotence and hemorrhoids [84, 85]. *E. senticosus* products are imported in Europe and it is one of the ten popular herbal dietary supplements in North America [86].

Murata and co-researchers investigated three the 80% acetone extracts of three medicinal plants, *Keiskea japonica, Lycopus lucidusand* and *Meehania fargesii* for their inhibitory effect against hyaluronidase. From bioassay directed study of these plants, the phenylpropanoids and flavone glucuronide from aerial part of *Keiskea japonica* [87], phenylpropanoids from aerial parts *Lycopus lucidas* [88] and

spermidine alkaloids flavone glycosides from dried whole plant extract of *Meehania fargesii* [57] showed strong hyaluronidase inhibitory activity.

Piwowarski and group examined tannin-rich aqeous extract of twelve plant for their ability to inhibit hyaluronidase materials based on their use in traditional Polish medicine for external treatment of skin and mucosal diseases. Among the plants, *Lythrum salicaria* L. extract has shown strongest inhibition of hyaluronidase (IC_{50} = 8.1 ± 0.8 μg/mL) compared to the heparin (IC_{50} = 62.1 ± 7.5 μg/mL) which was used as standard control [89].

Terminalia chebula, an Indian medicinal plant was assessed for its role as male antifertility agent using hyaluronidase inhibition enzyme assay. The 95% ethanol dried fruit extract of the plant showed in vitro HAase inhibitory activity of the human spermatozoa (~93% inhibition,) (IC_{50} = 0.8579 mg/ml) and rat caudal epididymal spermatozoa (~86% inhibition) (IC_{50} = 1.6221 mg/ml) at 30 mg/mL compared to the standard tannic acid (IC_{50} =299.6 μM). In the *in vivo* study on rates showed statistically significant ($P < 0.001$) inhibition of hyaluronidase activity of HAase enzyme extracted from testis (50 mg/kg dose, −47% decrease) and caused a further decrease (−72% decrease) at 100 mg/kg dose. The anti-HAase activity of the extract against caput and cauda epididymal spermatozoa extracted enzyme exhibited significantly better ($P < 0.001$) activity at 50 mg/kg dose (−41% each) and 100 mg/kg dose (−65% and −77%, respectively) when given orally for 60 days [90].

Michel and colleagues investigated the anti-inflammatory properties of Eastern Theaberry (*Gaultheria procumbens*) [91], found the chloroform (IC_{50} = 282.15 ± 10.38 μg/mL) and pet-ether (IC_{50} = 401.82 ± 16.12 μg/mL) fractions of the plant leaf extract as potent hyaluronidase inhibitors compared to the standard drug heparin (IC_{50} = 366.24 ± 14.72 μg/mL) which was higher than the activity they observed in nine most active constituents present in the sample.

Citalingam and Co-researchers have screened different extracts prepared from the bark and leaves of *Payena dasyphylla* medicinal plants for their potential as anti-hyaluronidase inhibitors. It was found to exert higher activity (IC_{50} = 100 μg/mL) against bovine testicular hyaluronidase The *Payena dasyphylla* extract also showed strong inhibition of HAase expression (IC_{50} = 100 ng/mL) in the cultured human chondrocyte cells in response to IL-1β. Similarly, the ethyl acetate fraction of the plant has strongly exhibited inhibited the HYAL1 and HYAL2 mRNA gene expressions (IC_{50} = 100 μg/mL) [92].

Phyllanthus emblica is a rejuvenating plant famous in Ayurvedic medicine has been evaluated by Sumantran et al. for its ant-arthritic property [93]. The aqueous decoction extract fruit (powder) was found to inhibit the activity of HAase enzyme effectively at IC_{50} = 0.15 mg/ml.

The muscadine grape (*Vitis rotundifolia*) is scientifically known for its anti-inflammatory properties. Bralley and co-authors have tested the ethanol extract of fruit skin and seed for their inhibitory potential against hyaluronidase enzyme. In their study they observed the bronz IC_{50} = 0.3 mg/mL for) and purple IC_{50} = 0.6, mg/mL) mascadine seed extracts as highly potent compared to the fruit skin extracts of the two mascadine types (IC_{50} = 1.0, 1.0 mg/mL respectively) [94].

Malaxis acuminata, an important medicinal plant known in Ayurvedic medicine was evaluated for its effect on skin aging and related enzyme activity. The researchers found the in vitro- isolated leaf extract (Methanolic) as strong inhibitor of hyaluronidase activity (IC_{50} = 60.36 ± 1.6 μg/mL) compared to the standard compound oleanolic acid-known for skin protective effect (IC_{50} = 32.45 ± 1.7 μg/mL) [95].

Girish et al. demonstrated that the aqueous root extract of *Mimosa pudica* reduced the hyaluronidase activity of three Indian snake venoms; *Naja naja* (2.16 x 10^{-3} ± 0.04), *Vipera russelii* (1.25 x10^{-3} ± 0.045) and *Echis carinatus* (1.1 x10^{-3} ± 0.072) units/min/mg protein [96].

Plants bearing high amount of tannin are known to block Hyaluronidase enzyme. A study conducted by Granica et al. discovered the extracts made from aerial part of two plants Oenothera paradoxa Hudziok and *O. biennis* L, which are rich in macrocyclic ellagitannin showed strong inhibition (97.3 ± 3.0% and 97.9 ± 1.7% respectively) of the HAase enzyme activity at 50 μg/mL compared to the stander heparine (62.1 ± 7.5 μg/mL) [97].

In a recent study on *Otostegia fruticose*, a medicinal plant traditionally used in Ethiopia to treat different ailments including inflammatory disorders. In the study, Bahta and co-researchers [98] found the ethanolic leaf extract and its fractions a dose depended anti-HAase activity. The crude ethanolic extract and chloroform fraction exhibited highest hyaluronidase inhibition (79.20% and 85.75% respectively), compared to standard drug indomethacin (95.52%) at the concentration of 100 μg/mL.

Brown algae are a nutrient-dense and potential source of bioactive secondary metabolites. In a study conducted by Shibata and co-workers [99], the crude phlorotaninin extract of two brown algae (*Eisenia bicyclis* and *E. kurome*) exhibited stronger anti-HAase activity (IC_{50} = 0.03 and 0.035 mg/mL respectively) compared to the two standard compounds Epigallocatechin gallate (IC_{50} = 190 mM/mL and Sodium cromoglycate (IC_{50} = 270 mM/mL). In the same study they observed its constituents possessing strong inhibitory activity.

In another study on algae, Fayad and co-researcher have used capillary electrophoresis-based enzymatic assay method to assess the anti-skin aging property of a macroalga (*Padina pavonica*). In their study, the water extract was found strongly inhibiting the hyaluronidase activity (IC_{50} = 0.04 ± 0.01 mg/mL) compared to the literature reported value of phlorotannin fractions of *Einsenia bicyclis* (IC_{50} = 0.03 mg/mL) [100].

4. Conclusion

The modulation of hyaluronidase enzyme and its substrate HA throughout the body is critical to maintain hyaluronan homeostasis as HA degradation is associated with pathogenesis of various health conditions. The literature survey carried out in this study found an increasing number of studies reported on HAase inhibitors derived from various biological sources and majority of the discoveries were from medicinal plants which have ethnobotanical claims for ailments associated with hyaluronan. Various class of natural products identified include alkaloids, flavonoids and terpenes have shown potent inhibitory activity against HAases in the *in vitro* studies. Similarly, a number of medicinal plant extracts and their fractions were found active against hyaluronidases and could serve as potential reservoirs for HAase inhibitors. These preliminary findings need further research to identify the active constituent(s) present in the extracts and establish their mechanism of action, safety profile and appropriate dosage of the active agents in animal and human studies. Hence, HAase inhibitors could be effective in controlling diseases involving uncontrolled degradation of HA and may serve as chondroprotective, anti-aging, antitumor, antimicrobial contraceptive agents, and anti-venom alternative.

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Conflict of interest

The authors declare that they have no conflict of interest.

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