Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(3): 500-506
Received: 10-02-2021
Accepted: 16-03-2021

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Isolation, purification and characterization of hyaluronic acid: A concise review

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Abstract
Hyaluronic acid is a naturally occurring linear polysaccharide of the extracellular matrix of connective tissue, synovial fluid, and other tissues. Individuals take hyaluronic acid for different joint issue problems, including osteoarthritis. Hyaluronic acid is utilized in certain eye surgeries like, corneal transplantation, and repair of a disengaged retina and other eye wounds. Hyaluronic acid is additionally utilized as lip filler in plastic surgery. A few people apply hyaluronic acid to the skin for mending wounds, cosmetics, skin ulcers, and as a lotion. Hyaluronic acid has been advanced as a "wellspring of youth." Hyaluronic acid, is extract by different procedures from various sources like pig, rabbit, oxes and human are available. The aim of this review article is to discuss various physiochemical, biochemical, and Pharmaco‐therapeutic uses of HA.

Keywords: Hyaluronic acid, anti-inflammatory, d‐ glucoronic acid, *N*–acetyl‐ d‐ glucosamine

1. Introduction
Hyaluronic acid (HA) is a naturally occurring linear polysaccharide of the extracellular matrix of connective tissue, synovial fluid, and other tissues. It possesses various physiological and structural functions, which include cellular and extracellular interactions, interactions with growth factors and regulation of the osmotic pressure, and tissue lubrication. All these functions help in maintaining the structural and homeostatic integrity of the tissue. Extensive studies on the chemical and physicochemical properties of HA and its physiological role in humans have proved that it is an ideal biomaterial for cosmetic, medical, and pharmaceutical applications

It's no secret that what we eat can affect our skin. We might already try to eat foods high in antioxidants like vitamin A and vitamin E to help improve our complexion. Hyaluronic acid is a compound that occurs naturally in our body, and it is responsible for attracting and retaining moisture. It is thought to improve cell-to-cell interaction and promote collagen synthesis, which can prevent wrinkles and improve our skin's texture [1].

Our body does make its own hyaluronic acid, but as we age, our production of it diminishes. Some people choose to inject hyaluronic acid as dermal filler, which can help add volume to the skin. Others apply it topically, which won't produce as dramatic or lasting results as it can't reach the deepest layers of the skin but can still smooth the appearance of fine lines and wrinkles.

It is naturally synthesized by a class of integral membrane proteins called hyaluronan synthases, and degraded by a family of enzymes called hyaluronidase. As with the joints, doctors also inject hyaluronic acid into the skin to eliminate wrinkles, due to the important part HA plays in collagen health. Additionally, HA is used in skin care creams and lotions since it helps to moisturize the skin and combat the dryness and loss of elasticity that occurs in aging skin that has been depleted of youthful HA stores. Topical hyaluronic acid has been shown to accelerate wound healing - in part by protecting tissue from oxygen free-radical damage in a number of studies. Scientists have noted its beneficial effects both immediately after the injury occur and in long-term wounds as well. HA treatment has been reported to cause a 70 percent reduction in the surface area of wounds [2].

2. History
HA was discovered in 1934 by Meyer *et al.* John Palmer, scientists at Columbia University, New York, who isolated a chemical substance from the vitreous jelly of cow's eyes [3]. They proposed the name HA as it was derived from the Greek word hyalos (glass) and contained two sugar molecules one of which was uronic acid.

3. Chemistry

The precise chemical structure of HA contains repeating units of d‐ glucoronic acid and *N*–acetyl‐ d‐ glucosamine. The primary structure of the polysaccharide comprises of an unbranched linear chain with the monosaccharide's linked together through alternating β1, 3 and β1,4 glycosidic bonds [4]. Hydrophobic faces exist within the secondary structure of HA, formed by the axial hydrogen atoms of about eight carbon‐ hydrogen (CH) groups on the alternating sides of the molecule. Such hydrophobic patches, energetically favor the formation of meshwork‐ like β‐ sheet tertiary structure as a result of molecular aggregation. The tertiary structure is stabilized by the presence of intermolecular hydrogen bonding. The hydrophobic and hydrogen bonding interactions in combination with the countering electrostatic repulsion enable large numbers of molecules to aggregate leading to the formation of molecular networks (matrices) of HA.

4. Benefits of hyaluronic acid

4.1 Hydrates Your Skin: Hyaluronic acid is common as a way to add moisture to the skin. It can help smooth out skin, reduce dryness and lighten bags under the eyes. Ensuring you have a good concentration of hyaluronic acid can reduce problems like dandruff, overall dryness and skin sagginess [5].

4.2 Decreases the Appearance of Wrinkles: Research suggests that fine lines and wrinkles are most prominent in dry environments. Skin dryness also plays a role in their development. It also suggests that skin creams using hyaluronic acid can decrease wrinkles in two to four weeks. Fillerina is a fairly expensive hyaluronic acid brand and isn't a product we recommend. It can be found on Amazon or on the company's website. [5]

4.3 Wound Healing and Similar Outcomes: Lotions and ointments containing hyaluronic acid are sometimes used to promote wound healing. They are also relevant for issues such as burns, skin ulcers, rashes and the like [6, 7].

4.4 Lowers Joint Pain: Hyaluronic acid is important in all connective tissue, including the joints. It helps to lower wear and tear, playing a buffering role. As a result, hyaluronic acid supplements are often used to decrease joint pain. The compound can even be used for arthritis treatment, normally in injection form. Lower oral doses of the compound can also be effective at reducing some joint pain [8].

4.5 Reduces Dry Eyes and Discomfort: Hyaluronic acid is responsible for most of the fluid in our eyes. This makes it a perfect tool for increasing moisture and relieving dry eyes.

The lubricating effect of hyaluronic acid also helps lower discomfort in your eyes. This is particularly relevant for eye injuries and for anyone having eye surgery.

The benefits are normally seen through hyaluronic acid eye drops, which are easy to use. Such drops can be very relevant if you regularly have dry eyes, use contact lenses or face regular eye strain from computer use.

5. Foods that can promote high hyaluronic acid

There are multiple options, each with their own advantages. Because they promote hyaluronic acid in different ways, the most powerful approach is to include a variety of these foods in your diet [9, 10].

5.1 Bone Broth

Bone broth has become popular for increasing collagen levels and promoting overall health. This includes helping to reduce inflammation and joint pain, along with promoting healthy gut bacteria. Bone broth is also powerful because it offers nutrients that you wouldn't normally get. With so many advantages, bone broth is the best first step for increasing hyaluronic acid levels. It's also very comforting and perfect for the colder months.

1.2 Organ Meats

Meat choices like liver are the most powerful sources of nutrients, including ones that we often miss out on. Yet, we tend to avoid organ meats, partly because of the flavors.

5.3 Leafy Greens

Dark leafy greens like kale, spinach and Swiss chard are another way to increase hyaluronic acid levels. The effect occurs because the greens are high in magnesium.

Magnesium is a key catalyst in the mechanism for producing hyaluronic acid. So, if you don't have enough of the compound, your levels of hyaluronic acid may decrease.

5.4 Almonds and Cashews

Nuts tend to be powerful for health and weight loss offering a dense source of nutrition. Almonds and cashews both offer benefits of their own and they are also significant sources of magnesium. This makes them useful for boosting hyaluronic acid production.

5.5 Soy-Based Foods

Soy tends to be controversial; with concerns about some of the components. Even so, soy may also offer significant health benefits. One of these is increasing hyaluronic acid production.
This helps to increase estrogen levels in the body, which then promotes hyaluronic acid production.

5.6 Root Vegetables

Starchy root vegetables also work for increasing hyaluronic acid levels. This includes sweet potatoes and even just regular potatoes. Root vegetables work well because they contain some hyaluronic acid and they boost production of it as well. In particular, they contain a range of useful nutrients. Many of these help improve hyaluronic acid levels and are a good addition to a healthy diet. Sweet potatoes are a particularly powerful choice. They are higher in magnesium, so their impacts on hyaluronic acid levels should be stronger as well.

5.7 Fresh Fruit

The presence of vitamin C in fruit helps to increase hyaluronic acid levels. Some fruits also contain significant levels of magnesium, which will help as well. Oranges, tomatoes, avocados, cherries, grapes and mangoes are all powerful options. Tomatoes, grapefruit and oranges are also a good source of naringenin. This compound inhibits the enzyme hyaluronidase, which breaks down hyaluronic acid.

5.8 Bananas

Most fresh fruits will help promote hyaluronic acid levels. But, bananas are a particularly good choice. They are one of the few types of fruit that contain some hyaluronic acid directly. They also contain magnesium and vitamin C, both of which help to increase hyaluronic acid production. Despite some claims to the contrary, bananas are also healthy and are a perfect snack for weight loss. It's true that they're fairly high in sugar but this is balanced by the fiber content.

5.9 Peppers

Yellow, orange and red peppers offer another source of vitamin C, helping to increase hyaluronic acid levels.

5.10 Beans

Beans are another powerful option for the nutrition that they contain a good source of magnesium and zinc, with both compounds helping to increase hyaluronic acid levels.

5.11 Origin, body reservoir and metabolism of ha

HA is found in almost all vertebrate organs, but most abundantly in the extracellular matrix of soft connective tissues. In the skin, it has a protective, structure stabilizing and shock‐absorbing role. The estimated total amount of HA in human skin has been reported to be5 g [11]. about a third of the total amount of HA believed to be present within the entire human body. The highest concentrations of HA are found in soft connective tissues (umbilical cord, synovial fluid, skin) and the lowest in blood serum [12].

Most cells of the body are capable of synthesizing HA and synthesis take place in the cell membrane. HA is synthesized in the plasma membrane by a membrane‐bound protein. Synthesized HA is directly secreted into the extracellular space. It is also produced by fibroblasts in the presence of endotoxins.

HA (Hyaluronan) has been identified in all periodontal tissues, being particularly prominent in the non‐mineralized tissues such as gingiva and periodontal ligament and in only low quantities in mineralized tissues such as cementum and alveolar bone. The high molecular weight hyaluronan present in the periodontal tissues is synthesized by hyaluronan synthase (HAS) enzymes (HAS1, HAS2 and HAS3) in various cells from the periodontal tissues, including fibroblasts and keratinocytes in gingiva and periodontal ligament, cement oblasts in cementum and osteoblasts in alveolar bone [13].

The turnover of HA content in the tissues occurs either by lymphatic drainage to the blood stream or by local metabolism. In skin and joints, some 20‐30% of HA turnover occurs by the local metabolism, and the rest is removed by the lymphatic pathways. Upon reaching the blood stream, about 85‐90% is eliminated in the liver. The kidneys extract about 10% but excrete only 1‐2% in the urine. The tissue half‐life of HA ranges from half a day to 2 or 3 days, regardless of its route of elimination [14].

5.12 Properties of ha

HA has unique physiochemical and biological properties, which makes it useful in the treatment of the inflammatory process in medical areas such as orthopedics, dermatology, and ophthalmology.

1. Hygroscopic nature

HA is one of the most hygroscopic molecules known in nature. When HA is incorporated into aqueous solution, hydrogen bonding occurs between adjacent carboxyl and N‐acetyl groups; this feature allows HA to maintain conformational stiffness and to retain water. One gram of HA can bind up to 6 L of water. As a physical background material, it has functions in space filling, lubrication, and shock absorption and protein exclusion [15].

2. Viscoelastic properties

Hyaluronan as a viscoelastic substance assists in periodontal regenerative procedures by maintaining spaces and protecting surfaces. Through recognition of its viscoelastic nature, HA can influence the cell functions that modify the surrounding cellular and the extracellular micro and macro environments. The viscoelastic properties of the material may slow the penetration of viruses, and bacteria, a feature of particular interest in the treatment of periodontal diseases.

3. Bacteriostatic effect

Recent studies on regenerative surgical procedures indicate that reduction of bacterial burden at the wound site may improve the clinical outcome of regenerative therapy. The high concentration of medium and lower molecular weight HA has the greatest bacteriostatic effect,

Particularly on *Aggregatibacter actinomycetemcomitans*, *Prevotella oris* and *Staphylococcus aureus* strains, which are commonly found in oral gingival lesions and periodontal wounds. A clinical application of HA membranes, gels, and sponges during the surgical therapy may reduce the bacterial contamination of surgical wound site, thereby, lessening the risk of postsurgical infection and promoting more predictable regeneration [16].

4. Biocompatibility and non‐antigenicity

The highly biocompatible and non‐immunogenic nature of HA has led to its use in a number of clinical applications, which include: The supplementation of joint fluid in arthritis; as a surgical aid in eye surgery; and to facilitate the healing and regeneration of bone, surgical wounds and periodontal tissue. Modifications to Hyaluronan include esterification and cross‐linking to provide some structure and rigidity to gel for cell‐seeding purposes. These biopolymers are completely biodegradable and support the growth of fibroblasts, chondrocytes and mesenchymal stem.

5. Anti‐inflammatory

HA has the anti‐inflammatory effect, which may be due to the action of exogenous Hyaluronic as a scavenger by draining prostaglandins, metalloproteinase and other bio‐active molecules [17].

6. Anti‑ oedematous

The anti‑ oedematous effect of HA may also be related to the osmotic activity. Due to its acceleration in tissue healing properties, it could be used as an adjunct to mechanical therapy [18].

7. Antioxidant

In a somewhat contradictory role, however, hyaluronan may regulate the inflammatory response, acting as an antioxidant by scavenging ROS. Thus, hyaluronan may help to stabilize the granulation tissue matrix [19].

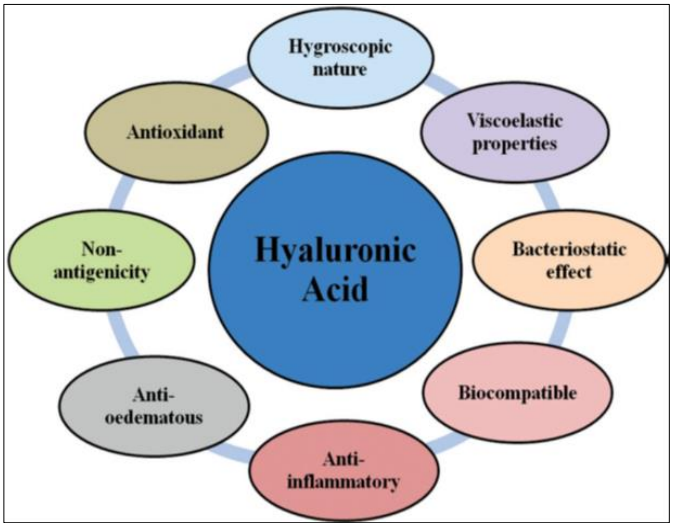

Fig 1: Properties of hyaluronic acid

Table 1: List of terrestrial sources used for ha isolation [20-38]

S. no	Animal	Tissue/body fluid	Literature sources
1.	Rooster	Rooster comb	Kang *et al.* [20]
2.	Human	Umbilical cord	Hadidian *et al.* [21]
		Joint synovial fluid	Balazs *et al.* [22]
		Vitreous body	Nishikawa *et al.* [23]
		Dermis	Postlethwaite *et al.* [24]
		Epidermis	Akiyama *et al.* [25, 27]
		Thoracic lymph	Pethrick *et al.* [26]
		Urine	Toyoda *et al.* [27]
		Serum	Deutsch *et al.* [28]
3.	Rat	Lung	Nettelbladt *et al.* [29]
		Kidney	Hallgren *et al.* [29, 30]
		Brain	Bignami *et al.* [31]
		Liver	Fraser *et al.* [32]
4.	Cow	Bovine nasal cartilage	Cleland *et al.* [46]
5.	Sheep	Synovial fluid	Fraser *et al.* [47]
		Medulla cortex	Dicker *et al.* [33]
		Lung	Postlethwaite *et al.* [24]
6.	Rabbit	Renal papillae	Farber *et al.* [34]
		Kidney	Dicker and Franklin [33]
		Vitreous body	Necas *et al.* [36]
		Renal cortex	Dwyer *et al.* [35]
		Muscle	Necas *et al.* [36]
		Liver	Takagaki *et al.* [37]
7.	Bacteria	Streptococci sp	Seastone *et al.* [38]

6. Extraction methods of hyaluronic acid

1. Extraction by enzyme digestion method

The tissue was defatted with acetone and dried at 60ºC for 24 h. The dried pellet was solubilized in 100 mm sodium acetate buffer, pH 5.5, containing 5 mm EDTA and cysteine. 100 mg of papain was added per gram of tissue, and the solution was incubated for 24 h at 60ºC in a stirrer. After boiling for 10 min, the mixture was centrifuged and three volumes of ethanol saturated with sodium acetate were added to the supernatant and stored at 4ºC for 24 h. The precipitate was recovered by centrifugation and dried at 60ºC [39]. The tissues can also be digested with pepsin [40], pronase, and trypsin [41].

2. Extraction with organic solvents and sodium acetate

The tissue was homogenized with acetone and incubated for 24 h in refrigerator. After 24h, the acetone was squeezed from the tissue material. This step was repeated 10 times in 24-h intervals. This material was extracted 10 times successively with a 5% solution of sodium acetate; each time the viscous fluid was squeezed through several layers of cheesecloth. 1.5 volumes of ethyl alcohol were added to the aqueous extracts. The precipitates formed were pooled, centrifuged, redissolved in 5% sodium acetate solution and recentrifuged. Protein was removed from the supernatant solution by shaking it with chloroform four times and then with a chloroform–amyl alcohol (1:4 parts to 1:2 parts) mixture several times until a gel no longer formed. The final solution was dialyzed; sodium acetate crystals were added to make a 5% solution. Following acidification to pH 4.0, the solution was precipitated with ethyl alcohol and the precipitate was desiccated in vacuum over calcium chloride. The final dried material will be pure white and fibrous in appearance [42].

3. Microbial production

HA can be prepared in high yield from bacterial sources by fermenting the bacteria under anaerobic conditions in an enriched growth medium containing glucose, glycogen, yeast extract, try tone, KH2PO4, K2HPO4, MgSO4. 7H2O, (NH4)2SO4, and polystyrene. HA was precipitated by mixing with three volumes of ethanol and centrifuged at 5000_g for 10 min. The sediment was resuspended with one volume of NaCl (1.5 M) and three volumes of ethanol and precipitated by centrifugation at 5000_g for 10 min. Finally, this last sediment was redissolved in distilled water for HA.[43] The yield, purity, and low cost of the hyaluronic acid produced by the bacterial sources also permit it to be used in ways not previously described or contemplate for hyaluronic acid obtained from mammalian or low yield bacterial sources.

4. Supplementary methods

The separation of the acid mucopolysaccharides into sulfated and no sulfated fractions was achieved with a cetylpyridium complex, [44] and final purification of the fractions was obtained by use of aDEAE-Sephadex anion exchanger A-25. The centrifugal precipitation chromatography [45], electrode position, and ultrafiltration–diafiltration are the other means of separation of HA fragments.

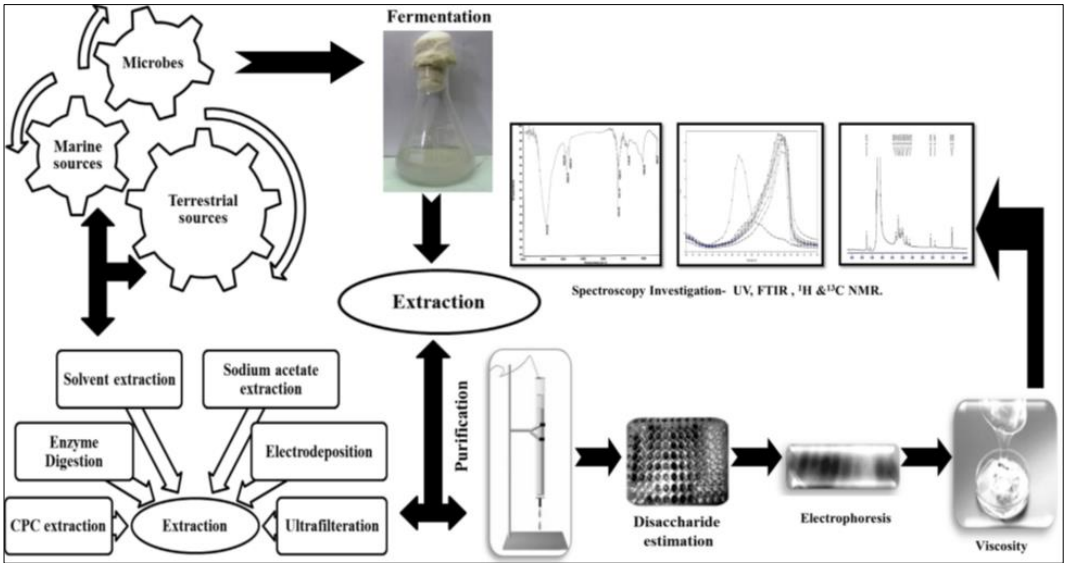

Fig 2: Schematic representation of HA isolation and characterization.

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