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Review Article

REVIEW ARTICLE: POTENTIAL OF NATURAL PRODUCTS IN INHIBITING PREMATURE SKIN AGING

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ABSTRACT

The objective of this article is to review the phytochemicals that serve as anti-aging materials by developing *in vitro* activity screening test methods. The method used is a literature study from national and international journals, including the original article, research article, etc., with a total of over 40 journals. Secondary metabolite compounds from natural ingredients such as flavonoids, polyphenols, alkaloids, and terpenes have antioxidant activity and have the potential to be anti-aging substances for the skin. The initial stage of searching for natural ingredients that have anti-aging activity can be done by developing *in vitro* activity screening test methods, such as inhibition of collagenase, elastase, hyaluronidase, and tyrosinase enzymes. This enzyme plays an important role as one of the causes of premature aging of the skin. From the literature search, it was found that many plant extracts to active compounds and cosmetic preparations from the extracts can inhibit these enzymes. The group of compounds that are known to have acted as enzyme inhibitors is polyphenolic compounds, especially those with multimer properties, flavonoids, especially those with ortho-OH groups, triterpenes (especially pentacyclic), essential oils, and others. This shows that natural ingredients are one source of potential compounds as raw materials in the formulation of anti-aging cosmetic preparations.

Keywords: Premature skin aging, *In vitro* test, Plant extract, Elastase enzyme, Collagenase enzyme

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INTRODUCTION

The skin is the most visible outermost organ; this makes us pay more attention to the condition of the skin. An aging process is a natural event that occurs in all living things. Irreversible skin aging begins at the age of 20 y, although the signs are not visible for a long time. Aging of the skin is a complex biological process that results from intrinsic aging (from within the body, such as genetics) and changes that develop over time and extrinsic effects caused by environmental factors. Bad environmental conditions and lifestyles can cause premature aging. Extrinsic factors that play a role in premature aging are repetitive facial expressions, poor sleeping positions, smoking, etc. External signs of skin aging include fine wrinkles, thin skin, pigment spots, loose skin, dry skin with or without itching, inability to sweat enough, gray hair, hair loss, etc. Of all these factors, the free radical theory is the theory that is often associated with the cause of premature aging factors. UV radicals are a very potent trigger in the formation of free radicals ROS (*Reactive Oxygen Species*) on the skin. In the skin, free radicals that are produced in excess will damage the collagen in the skin cell membranes, so the skin loses its elasticity and causes wrinkles [1, 2].

Indonesia is a tropical country with year-round sunshine. UV radiation from the sun is absorbed by the skin and becomes one of the causes of premature skin aging so prevention and skincare efforts are needed, such as the use of anti-aging cosmetics. Human desire is to live longer but looks younger so that they can be socially and physically active longer. In the 21st century, with the development of science and technology, plastic surgery techniques and other skin rejuvenation techniques have developed. But there is a tendency for some people to avoid invasive procedures to reduce risks and complications. Humans naturally want to look younger and with as few scars as possible. This has led to increased research on synthetic and natural cosmetic ingredients [3].

MATERIALS AND METHODS

This review focuses on natural ingredients or phytochemicals that serve as anti-aging materials by developing *in vitro* activity screening test methods. This article was used literature studies from Indonesian and international journals, including the original articles, research articles, patents, etc., with a total of over 40 journals.

RESULTS

Skin and premature skin aging

The aging process in humans causes a decrease in various organ functions, including the skin, and also causes several health problems in the elderly. The skin plays an important role as a barrier to the internal and external environment. Other functions of the skin are to maintain homeostasis, regulate body heat, maintain water, electrolyte, and protein balance, sensory perception, and immunological protection. In elderly skin, there is a thinning of the epidermis, a decrease in the supply of blood, fluids, and nutrients to the skin, a slowing of the wound healing process and immune response, disruption of thermoregulation, and a decrease in the number of oil and sweat glands. At the cellular level, there is a decrease in the production of lipids and *natural moisturizing factors* in the stratum corneum. In addition to these changes, there are often comorbid diseases that affect the function of the skin [4].

The skin is the largest organ in the human body with the main functions of protection, heat regulation, and the sense of touch. The skin is also the largest organ exposed to ultraviolet radiation and can cause various balance disorders in the skin, such as an increase in the amount of melanin, mild dermatitis, rough texture, wrinkles, premature aging to skin cancer. Nowadays, the awareness to look better with healthy and youthful skin is a necessity and has an impact on a person's quality of life. The aging process in living things will naturally occur over time and the most visible impact of aging is the aging process on the skin because the skin is the most visible external organ. The aging process can also be accelerated by oxidative stress. There are two categories, namely intrinsic and extrinsic oxidative stress. Intrinsic causes are the result of metabolic processes in the body that produce free radicals such as unhealthy foods and unhealthy lifestyles. Extrinsic causes are mainly exposure to solar radiation (*photoaging*), air pollution, and the effects of global warming [5–7].

The skin consists of 3 layers, namely the epidermis, dermis, and subcutaneous. In the dermis, there is an extracellular matrix which is the main component and plays a very important role in the growth and elasticity of the skin. The extracellular matrix contains proteoglycans bound to metalloproteins such as collagen, elastin, and fibronectin produced by fibroblasts. Collagen is the main protein

in the extracellular matrix that is responsible for the strength and elasticity of the skin, connective tissue, hair, and nails. Elastin is an important protein in maintaining skin elasticity to keep it firm and supple. Hyaluronic acid and glycosaminoglycans are important in maintaining skin moisture and thus are linked to skin elasticity [8]. Elastase is a serine protease (endoprotease) and the only enzyme that can break down elastin. When the skin is exposed to UV radiation, the enzymes collagenase and elastase will increase so that they break down collagen and elastin, causing premature aging. Naturally, the amount of collagen will decrease by about 1% per year. Likewise, with elastin, naturally, the amount of elastin will decrease [9].

Efforts to reduce the effects of premature skin aging

There are several ways to reduce the process of premature aging caused by UV rays of the sun, that is avoiding exposure to excessive UV, using clothing that is UV protection, the use of cosmetics containing sunscreen, vitamin A or its derivatives, or cosmetics that contain antioxidants and foods that are antioxidants, maintain a good lifestyle such as exercise, reducing stress, good diet, not smoking and drinking alcoholic beverages, etc. The effects *of photoaging* can also cause dry skin. Exposure to sunlight and high-intensity ultraviolet radiation, especially at 10:00 to 16:00, should be avoided. The skin cells absorb radiation and produce ROS, which can damage DNA and cell membranes. The process of *photoaging* also resulted in the destruction of collagen by the enzyme *matrix metalloproteinase* (MMP) and the accumulation of elastin irregular structure. This interaction produces dry skin, pale, rough, and wrinkled. It is advisable to use a sunscreen that contains *a sun protection factor* (SPF) 30 when exposed to sunlight. Clothing that covers the skin and caps can also reduce exposure to sunlight [4].

Although currently, several synthetic compounds are useful as anti-aging, they generally have side effects such as allergies, irritation, and phototoxicity, so it is necessary to search for active compounds from natural ingredients that have anti-aging properties. Some antioxidants derived from plants and have been studied as anti-aging skin include aloin, ginsenoside, curcumin, epicatechin, asiaticoside, ziyuglycoside, magnolol, gallic acid, hydroxychavicol, hydroxycinnamic acid, etc. [10]. The search for new compounds derived from plants containing phytochemical compounds such as flavonoids, phenolic acids, alkaloids, and terpenes as well as having antioxidant, anti-inflammatory, and anti-aging activities, is currently widely used in the development of cosmetic preparations [11].

The extracellular matrix is the main component in the dermis of the skin, which plays a very important role in the growth and elasticity of the skin. The extracellular matrix contains proteoglycans bound to metalloproteins such as collagen, elastin, and fibronectin produced by fibroblasts. Collagen is the main protein in the extracellular matrix that is responsible for skin attractiveness. Elastin is an important protein in maintaining skin elasticity in the extracellular matrix. Many other vital organs, including arteries and lungs, also contain high amounts of elastin. Physiologically, organisms contain a certain amount of elastin from early development, which may remain physiologically constant because of the very long half-life and very low turnover. The role of hyaluronic acid and glycosaminoglycans is important in maintaining skin moisture and skin elasticity. The enzymes involved in the breakdown of these substances cause the skin aging process [8, 12].

The development of *in vitro* methods to test the inhibitory activities of collagenase, elastase, hyaluronidase, and tyrosinase enzymes is a method that can be used in screening tests for anti-aging activities. *In vitro* assay is a screening test which is a rapid and rational method of reducing the number of *in vivo* trials and risks associated with human subjects; then this can reduce research and development costs [13].

Potentially natural ingredients for anti-aging skin

From many studies of anti-aging activity tests, extracts and isolates of active compounds from natural ingredients show very varied structures. This is an indication that natural ingredients contain potential chemical candidates in improving health and inhibiting premature aging [14]. Plant extracts containing phytochemical compounds such as flavonoids, phenolic acids, tocopherols, alkaloids, and monoterpenes are known to have anti-inflammatory activity and can potentially be used in cosmetic products. The number of pharmaceutical and cosmetic products containing a combination of herbal extracts turned out to have better activity than single extracts [15]. Bravo *et al.* tested the methanol extract of *Rubus compactus* and *R. robustus,* which have strong activity as inhibitors of enzymes that cause premature aging: elastase, and collagenase [8]. Ethanol extracts from ripe and unripe fruit, leaves, and roots of *R. coreanus* have been tested for antioxidant, whitening, and anti-wrinkle properties. The ethanol extract of raw fruit shows a high activity as a whitening and anti-wrinkle agent, so it has the potential to be used as an ingredient in the manufacture of skin care products [16].

The results of a patent search, *R. idaeus* fruit has been patented as an elastase inhibitor by Shiseido Company, Ltd. [17]. *R. fruticosus* leaves have been patented as anti-aging (anti-elastase, anti-collagenase, and anti-hyaluronidase) [18]. Antioxidant and enzyme inhibitor activity tests were carried out on several small and colorful fruit plants that live in the Andean mountains, Colombia. The test results showed that generally, unripe fruit had higher antioxidant and enzymatic inhibitor activity than ripe fruit. The fruit extract fraction of *R. fruticosus* also has hyaluronidase inhibitory activity [19].

Several plant extracts that have been tested for antioxidant, anti-collagenase, and anti-elastase activity *in vitro* and show their potential as anti-aging ingredients, namely tangerine peel/*Citrus reticulata* Blanco [20], cucumber/*Cucumis sativus* [21], essential leaf oil of two varieties *Alpinia zerumbet* (varieties tairin dan shima) [22], white tea leaf (*Camellia sinensis)*, thallus part of algae *Fucus vesicul*osus L, leaves and stems *Galium aparine* L., cabbage rose/*Rosa centifolia* L., green tea leaves *Camellia sinensis*, *Angelica archangelica* L. root, *Illicium verum* Hook, *Punica granatum* L fruit. [6, 23], the pomace of grape/*Vitis vinifera* [24], unripe bokbunja/*Rubus coreanus* [16], *Tagetes erecta* flower [25], *Rubus compactus* and *Ugni myricoides* fruit [8], unripe fruit *R. fraxinifolius* [26]. In several studies of fruit with several levels of ripeness, statistically, it was shown that immature fruit had better antioxidant activity and enzyme inhibitory power [8, 16].

Several cosmetic preparations that have activity as anti-aging of the skin, for example, a mixture of fruit extracts of *Ginkgo biloba, Punica granatum, Ficus carica*, and *Morus alba* have antioxidant, anti-collagenase activity *in vitro* and anti-wrinkle tests *in vivo* [15].

Isolate compounds that have activity as an anti-aging skin

Several bioflavonoid isolates from *Aloe vera* (*Aloe barbadensis*) have strong antioxidant and anti-collagenase activity. The triterpenoid compound boswelic acid isolated from the resin of *Boswellia* spp. has antielastase activity. Polyphenolic and flavonoid compounds isolated from the leaves of *Diospyros kaki* and *Rosmarinus officinalis* have anti-collagenolytic and anti-elastase activity [6]. Polyphenolic catechins and epigallocatechin gallate (EGCG) compounds isolated from green tea (*Camellia sinensis*) have activity as collagenase and elastase inhibitors. Phenolic compounds such as epicatechin, catechin, resveratrol, and procyanidin B2 [24] are also reported to have elastase inhibitory activity.

The compound identified as Ziyuglycoside I was isolated from the root extract of *Sanguisorba officinalis*. This compound was tested for free radical scavenging activity, anti-elastase activity, and type I collagen synthesis in normal human fibroblast cells. The results show good results and have potential in anti-aging cosmetic preparations [27]. Cinnamaldehyde compounds from *Cinnamomum verum* have elastase inhibitory activity up to 96.56% [28]. Two triterpenoids derived from ursan from the leaves of *Rubus fraxinifolius* showed inhibitory activity against elastase and tyrosinase enzymes *in vitro* and silico [29].

The flavonoid xanthohumol has also been isolated from the *Humulus lupulus* L. plant which has anti-elastase and anti-collagenase activity [30]. Research from Kim *et al.* 2009 showed that of 5 compounds isolated from *Callistemon lanceolatus* stems, pyracrenic acid had

activity as a strong elastase inhibitor. Lee *et al.* reported that they isolated phenolic compounds in *Areca catechu* L, which have inhibitory activity against porcine pancreatic elastase (PPE), human neutrophil elastase (HNE), and hyaluronidase [31] (fig. 1, table 1).

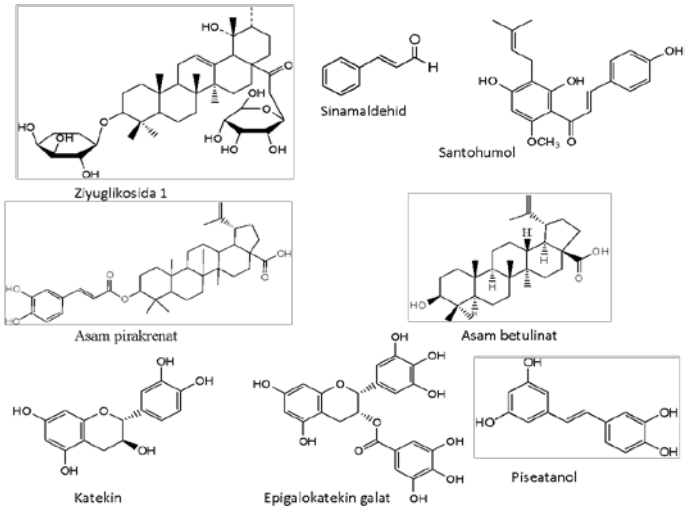
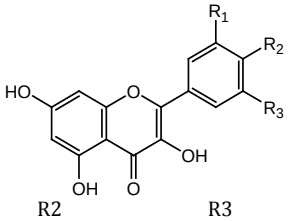


Fig. 1: Various compounds that have anti-aging activity, Several prenyl isoflavones isolated from *Flemingia philippinensis* have been tested and have activity as inhibitors of the neutrophil elastase enzyme with a competitive mechanism [32]

Table 1: Compounds that have anti-aging activity

No	Compounds	Source	Activity	Reference
1	Ziyuglikoside I	*Sanguisorba officinalis* root	Anti-elastase 5.4% in 667 mg/ml Anti MMP-1 6.2% in 100 mg/ml]	[27]
2	Cinnamaldehid	*Cinnamomum verum* Volatile oil	Anti-elastase 96,56%	[28]
3	Flavonoid xanthohumol	*Humulus lupulus* L	Anti-elastase IC_{50} 0.001% Anti MMP-1 IC_{50} 0.25%	[32]
4	Piracrenic acid Betullinic acid Catechin Piseatanol	*Callistemon lanceolatus* stem	Anti-elastase IC_{50} 1.5 µg/ml Anti-elastase IC_{50} 21.6 µg/ml Anti-elastase IC_{50} 20.2 µg/ml Anti-elastase IC_{50} 15.6 µg/ml	[33]
5	Phenolic	*Areca catechu* L	Anti-elastase IC_{50} 26.9 µg/ml Anti-hyaluronidase IC_{50} 210 µg/ml	[31]
6	isoflavone flavanon, chalcone, flavanol	*Flemingia philippinensis* root	Anti-elastase IC_{50}: 1.3–213.1 µM Anti-elastase IC_{50}: 5.3-110.2 µM	[32]
7	Epigallocatechin gallic	*Camellia sinensis*	In 250 µM have activity 35% as elastase inhibitor and 95% as collagenase inhibitor	[6]
8	[+]-gossypol	*Thespesia populnea* bark	IC_{50}= 42,5 µg/ml	[34]
9	2,3-O-ethyleneglycol, 19-hydroxyurs-12-en-23,28-dioic acid 2,3-O-propanediol,19-hydroxyurs-12-en-28-oic acid	*Rubus fraxinifolius* leaf	IC_{50} anti-elastase 122.199 µg/ml and anti tyrosinase 207.79 µg/ml IC_{50} Anti-elastase 98.22 µg/ml and anti-tyrosinase 221.51 µg/ml	[29]

Kanashiro *et al.* researched anti-elastase on several flavonoids and obtained the results of activity of quercetin>myrisetin>kaempferol = galangin. These studies have revealed that the number and position of free hydroxy groups are important structural features for the compound's modulatory activities on oxygen-dependent neutrophil functions as oxidative burst. This proves the importance of the catechol group on ring B of flavonoids in anti-elastase activity. The structure of the flavonoid test can be seen in fig. 2 [35].



Flavonoid	R1	R2	R3	antielastase
Galangin	H	H	H	+
Kaempferol	H	OH	H	+
Quercetin	OH	OH	H	+++
Miricetin	OH	OH	OH	++

Fig. 2: The structure of some flavonoids that have been tested for antielastase [35]

The results of the research of Küpeli Akkol *et al.* 2015 showed that the root methanol extract of Rubus sanctus Schreber contained a new compound quercetin-3-O-β-galactoside and was named hyperoside. Cinnamaldehyde compounds from Cinnamomum verum have elastase inhibitory activity up to 96.56% [28]. Xanthohumol flavonoid compounds have also been isolated from the Humulus lupulus L. plant, which has anti-elastase and anti-collagenase activities. [30]. Research from Kim *et al.* 2009 showed that of the 5 compounds isolated from Callistemon lanceolatus stems, pycrarenic acid had activity as a strong elastase inhibitor [33].

Isolation of the compound was guided by the elastase enzyme inhibitory activity test from the herb R. sanctus, namely, the methanol extract of the herb was fractionated using column chromatography with silica gel followed by preparative TLC and structural elucidation resulted in a quercetin-3-O-β-galactoside and named hyperoside [36].

Phytochemical compounds from plants that have potential as anti-aging skin

Plant extracts rich in phytochemical compounds such as flavonoids, phenolic acids, tocopherols, alkaloids, monoterpenes, antioxidant, and active anti-inflammatory compounds are also commonly used in the development of topical cosmetic preparations [37]. Several studies have shown that anti-skin aging and antioxidant activity in plants is also related to the plant defense system, consisting of terpenes and polyphenols or phenolic compounds [8, 39] as shown in fig. 3. *In vitro* studies have shown that phenolic compounds can reduce oxidation levels and inhibit the work of collagenase, elastase, hyaluronidase, and tyrosinase enzymes. The strength of the activity of the polyphenol group depends on the position and the phenol group. Otherwise, the contrary research on 20 plants containing high tannin levels showed no correlation with anti-elastase and anti-hyaluronidase activity [38].

Terpenes are known to be metabolites in the face of oxidative stress in plants through the mechanism of reducing free radicals [10]. The *in silico* test also showed that the aromatic group, which has an ortho-dihydrate group, is one of the important structures for elastase inhibition [39]. The greater the degree of polymerization of polyphenols, indicating an increase in the activity of polyphenols to inhibit the elastase enzyme, this inhibition is reversible and competitive. Docking simulations and molecular dynamics show that the polyphenol tetramer structure has more contact points with amino acids on the active site of the enzyme. The hydrogen-bonding interactions and hydrophobic effects formed between the polyphenol groups and the side chains of the enzyme residues stabilize and support this binding mode. This research is relevant to the study of the antinutritional effect caused by dietary tannins on digestive enzyme activity, reducing food digestibility and nutrient absorption [40].

In vitro and *in vivo* testing of several pentacyclic triterpenes as inhibitors of human neutrophil elastase [HNE] showed reversible and competitive activity. Further, a molecular docking test was carried out and it was concluded that the carboxyl group at position 28, and the double bond at the appropriate location in the pentacyclic ring system of the triterpene contributed to the binding of HNE [41].

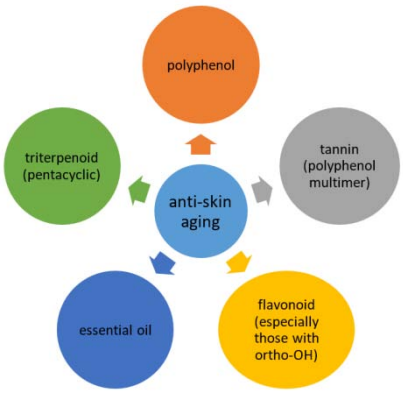


Fig. 3: Phytochemical compounds that have anti-aging activity (anti-elastase, anti-hyaluronidase, and anti-collagenase)

CONCLUSION

In an effort to find natural ingredients both in the form of extracts and isolated compounds with anti-aging properties, *in vitro* screening tests can be carried out, namely inhibition tests on enzymes that affect premature skin aging such as elastase, collagenase, tyrosinase, and antioxidants. Phytochemical compounds that have activity inhibiting the work of these enzymes include polyphenolic compounds, flavonoids, triterpenoids, and other terpenes. Plants containing this compound have the potential to be used as raw materials in the formulation of anti-aging cosmetics.

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AUTHORS CONTRIBUTIONS

YD: conception and design of the work, FF: drafting the article, YN, HR, and NSSA: participated in intellectual discussions and critical revision of the article. All authors approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest in this article.

REFERENCES

1. Kalyana Sundaram I, Sarangi DD, Sundararajan V, George S, Sheik Mohideen S. Polyherbal formulation with anti-elastase and antioxidant properties for skin antiaging. BMC. Altern Complement Med. 2018;18:1-13.
2. Tundis R, Loizzo MR, Bonesi M, Menichini F. Potential role of natural compounds against skin aging. Curr Med Chem. 2015;22(12):1515-38. doi: 10.2174/0929867322666150227151809, PMID 25723509.
3. Binic I, Lazarevic V, Ljubenovic M, Mojsa J, Sokolovic D. Skin aging: natural weapons and strategies. Evid Based Complement Alternat Med. 2013:827248:1–10.
4. Bianti M. Kulit Kering pada Usia Lanjut. Cermin Dunia Kedokteran. 2016;43:737-40.
5. Scharffetter Kochanek K, Brenneisen P, Wenk J, Herrmann G, Ma W, Kuhr L. Photoaging of the skin from phenotype to mechanisms. Exp Gerontol. 2000;35(3):307-16. doi: 10.1016/s0531-5565(00)00098-x, PMID 10832052.
6. Thring TSA, Hili P, Naughton DP. Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. BMC Complement Altern Med. 2009;9:27. doi: 10.1186/1472-6882-9-27, PMID 19653897.
7. Manosroi A, Jantrawut P, Akihisa T, Manosroi W, Manosroi J. *In vitro* and *in vivo* skin anti-aging evaluation of gel containing niosomes loaded with a semi-purified fraction containing gallic acid from Terminalia chebula galls. Pharm Biol. 2011;49(11):1190-203. doi: 10.3109/13880209.2011.576347, PMID 22014267.
8. Bravo K, Alzate F, Osorio E. Fruits of selected wild and cultivated Andean plants as sources of potential compounds with antioxidant and anti-aging activity. Ind Crops Prod. 2016;85:341-52. doi: 10.1016/j.indcrop.2015.12.074.
9. Imokawa G, Ishida K. Biological mechanisms underlying the ultraviolet radiation-induced formation of skin wrinkling and sagging I: Reduced skin elasticity, highly associated with enhanced dermal elastase activity, triggers wrinkling and sagging. Int J Mol Sci. 2015;16(4):7753-75. doi: 10.3390/ijms16047753, PMID 25856675.
10. Mukherjee PK, Maity N, Nema NK, Sarkar BK. Bioactive compounds from natural resources against skin aging. Phytomedicine. 2011;19(1):64-73. doi: 10.1016/j.phymed.2011.10.003, PMID 22115797.
11. Kapoor VK, Dureja J, Chadha R. Herbals in the control of ageing. Drug Discov Today. 2009;14(19-20):992-8. doi: 10.1016/j.drudis.2009.06.014, PMID 19596462.

12. Le Page A, Khalil A, Vermette P, Frost EH, Larbi A, Witkowski JM. The role of elastin-derived peptides in human physiology and diseases. Matrix Biol. 2019;84:81-96. doi: 10.1016/j.matbio.2019.07.004, PMID 31295577.
13. Roy A, Sahu RK, Matlam M, Deshmukh VK, Dwivedi J, Jha AK. *In vitro* techniques to assess the proficiency of skin care cosmetic formulations. Pharmacogn Rev. 2013;7(14):97-106. doi: 10.4103/0973-7847.120507, PMID 24347917.
14. Argyropoulou A, Aligiannis N, Trougakos IP, Skaltsounis AL. Natural compounds with anti-aging activity. Nat Prod Rep. 2013;30(11):1412-37. doi: 10.1039/c3np70031c, PMID 24056714.
15. Ghimeray AK, Jung US, Lee HY, Kim YH, Ryu EK, Chang MS. *In vitro* antioxidant, collagenase inhibition, and *in vivo* anti-wrinkle effects of the combined formulation containing Punica granatum, Ginkgo biloba, Ficus carica, and Morus alba fruits extract. Clin Cosmet Investig Dermatol. 2015;8:389-96. doi: 10.2147/CCID.S80906, PMID 26203268.
16. Park CM, Joung MS, Yang DO, Choi JW. The study on the effect of Rubus coreanus Miquel extracts as a Comsetic ingredient. J Soc Sci Korea. 2007;33:41-5.
17. Iino M. Elastase inhibitor contains raspberry. Patent No. Tokyo: Shiseido Company, Ltd. (JP); US8491946 B2; 2013. p. US8.491.946 B2. p. US8491946 B2.
18. Herrmann M, Joppe H, Franke H, Vielhaber G, Blackberry. (Rubus fruticosus) Extract as anti ageing; 2008.
19. Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J. Bioactive compounds and antioxidant activity in different types of berries. Int J Mol Sci. 2015;16(10):24673-706. doi: 10.3390/ijms161024673, PMID 26501271.
20. Apraj VD, Pandita NS. Evaluation of skin anti-aging potential of citrus reticulata blanco peel. Pharmacognosy Res. 2016;8(3):160-8. doi: 10.4103/0974-8490.182913, PMID 27365982.
21. Nema NK, Maity N, Sarkar B, Mukherjee PK. Cucumis sativus fruit-potential antioxidant, anti-hyaluronidase, and anti-elastase agent. Arch Dermatol Res. 2011;303(4):247-52. doi: 10.1007/s00403-010-1103-y, PMID 21153830.
22. Tu PTB, Tawata S. Anti-oxidant, anti-aging, and anti-melanogenic properties of the essential oils from two varieties of Alpinia zerumbet. Molecules. 2015;20(9):16723-40. doi: 10.3390/molecules200916723, PMID 26389869.
23. Lianza M, Mandrone M, Chiocchio I, Tomasi P, Marincich L, Poli F. Screening of ninety herbal products of commercial interest as potential ingredients for phytocosmetics. J Enzyme Inhib Med Chem. 2020;35(1):1287-91. doi: 10.1080/14756366.2020.1774571, PMID 32515615.
24. Wittenauer J, Mackle S, Submann D, Schweiggert Weisz U, Carle R. Inhibitory effects of polyphenols from grape pomace extract on collagenase and elastase activity. Fitoterapia. 2015;101:179-87. doi: 10.1016/j.fitote.2015.01.005, PMID 25598188.
25. Maity N, Nema NK, Abedy MdK, Sarkar BK, Mukherjee PK. Exploring tagetes erecta linn flower for the elastase, hyaluronidase and MMP-1 inhibitory activity. J Ethnopharmacol. 2011;137(3):1300-5. doi: 10.1016/j.jep.2011.07.064, PMID 21843618.
26. Desmiaty Y, Elya B. Unripe fruit of rubus fraxinifolius as a potential source of antioxidant and antielastase AGENT. Int J App Pharm. 2021;13 Special Issue 2:78-81. doi: 10.22159/ijap.2021.v13s2.15.
27. Kim YHS, Chung CB, Kim JG, Ko KI, Park SH, Kim JH, Ko K Il. Anti-wrinkle activity of ziyuglycoside I isolated from a Sanguisorba officinalis root extract and its application as a cosmeceutical ingredient. Biosci Biotechnol Biochem. 2008;72(2):303-11. doi: 10.1271/bbb.70268, PMID 18256460.
28. Khan MSA, Ahmad I. *In vitro* antifungal, anti-elastase and anti-keratinase activity of essential oils of cinnamomum-syzygium- and cymbopogon-species against aspergillus fumigatus and trichophyton rubrum. Phytomedicine. 2011;19(1):48-55. doi: 10.1016/j.phymed.2011.07.005, PMID 21893402.
29. Desmiaty Y, Hanafi M, Saputri FC, Elya B, Rifai EA, Syahdi RR. Two triterpenoids from Rubus fraxinifolius leaves and their tyrosinase and elastase inhibitory activities. Sci Rep 2021;11:1–9.
30. Philips N, Samuel M, Arena R, Chen YJ, Conte J, Natarajan P. Direct inhibition of elastase and matrix metalloproteinases and stimulation of biosynthesis of fibrillar collagens, elastin, and fibrillins by xanthohumol. J Cosmet Sci. 2010;61:125-32.
31. Lee KK, Cho JJ, Park EJ, Choi JD. Anti-elastase and anti-hyaluronidase of phenolic substance from Areca catechu as a new anti-aging agent. Int J Cosmet Sci. 2001;23(6):341-6. doi: 10.1046/j.0412-5463.2001.00102.x, PMID 18498484.
32. Kim JY, Wang Y, Uddin Z, Song YH, Li ZP, Jenis J. Competitive neutrophil elastase inhibitory isoflavones from the roots of Flemingia philippinensis. Bioorg Chem. 2018;78:249-57. doi: 10.1016/j.bioorg.2018.03.024, PMID 29614436.
33. Kim JH, Byun JC, Kumar A, Bandi R, Hyun C, Ho N. Compounds with elastase inhibition and free radical scavenging activities from callistemon lanceolatus. J Med Plants Res. 2009;3:914-20.
34. Annamalai T, Venkateswara Rao G, Mukhopadhyay T. Isolation of an elastase inhibitor, (+)-gossypol from the bark of Thespesia populnea plant. Pharm Lett. 2013;5:312-5.
35. Kanashiro A, Souza JG, Kabeya LM, Azzolini AECS, Lucisano Valim YM. Elastase release by stimulated neutrophils inhibited by flavonoids: the importance of the catechol group. Z Naturforsch C J Biosci. 2007;62(5-6):357-61. doi: 10.1515/znc-2007-5-607, PMID 17708440.
36. Kupeli Akkol EK, Suntar I, Ilhan M, Aras E. *In vitro* enzyme inhibitory effects of rubus sanctus schreber and its active metabolite as a function of wound healing activity. J Herb Med. 2015;5(4):207-10. doi: 10.1016/j.hermed.2015.09.002.
37. Jadoon S, Karim S, Bin Asad MH, Akram MR, Khan AK, Malik A. Anti-aging potential of phytoextract loaded-pharmaceutical creams for human skin cell longetivity. Oxid Med Cell Longev. 2015;2015:709628. doi: 10.1155/2015/709628. PMID 26448818.
38. Piwowarski JP, Kiss AK, Kozłowska Wojciechowska M. Anti-hyaluronidase and anti-elastase activity screening of tannin-rich plant materials used in traditional Polish medicine for external treatment of diseases with an inflammatory background. J Ethnopharmacol. 2011;137(1):937-41. doi: 10.1016/j.jep.2011.05.039, PMID 21669271.
39. Siedle B, Murillo R, Hucke O, Labahn A, Merfort I. Structure activity relationship studies of cinnamic acid derivatives as inhibitors of human neutrophil elastase revealed by ligand docking calculations. Pharmazie. 2003;58(5):337-9. PMID 12779051.
40. Bras NF, Gonçalves R, Mateus N, Fernandes PA, Ramos MJ, De Freitas V. Inhibition of pancreatic elastase by polyphenolic compounds. J Agric Food Chem. 2010;58(19):10668-76. doi: 10.1021/jf1017934, PMID 20839876.
41. Feng L, Liu X, Zhu W, Guo F, Wu Y, Wang R. Inhibition of human neutrophil elastase by pentacyclic triterpenes. PLOS One. 2013;8(12):e82794. doi: 10.1371/journal.pone.0082794, PMID 24376583.