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INHIBITORY EFFECTS OF *ALOE VERA* EXTRACTS ON ANTI-TYROSINASE, ANTI-COLLAGENASE AND ANTI-ELASTASE POTENTIAL

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Abstract

In the present work, *in vitro* inhibitory effects of aqueous and methanolic extracts of *Aloe vera* leaves on tyrosinase, elastase and collagenase, which are enzymes responsible for the maintenance of skin texture were studied. The leaf gel and skin aqueous and methanolic extracts were individually examined for tyrosinase and collagenase inhibitory activities. While the skin methanolic extract was studied only for its elastase inhibitory activity. The inhibitory activity of the extracts increased in a dose–dependent manner. *A. vera* gel extracst showed a promising inhibiton effect for tyrosinase and collagenase activity. The ability of *A. vera* extracts to inhibit tyrosinase, collagenase and elastase suggests their potential use as skin care additived in natural remedies and cosmetics.

Introduction

Aloe vera (L.) Burm. f. (Xanthorraceae, previously Aloeaceae and Liliaceae) which is called "the miraculous plant", has been used for centuries worldwide for various medicinal, cosmetic and nutritional purposes. It is a perennial, drought-resistant plant with thick, conical, green lanceolate, juicy, basal, sharp pointed and rough and edged leaves.

When cut the brown exudate or latex flowing from the bottom of a leaf contains anthraquinones, mainly aloe-emodin; a polysaccharide-rich mucilage gel. The pulp portion of a longitudinally sliced leaf chiefly contains acemannan (Choche *et al*. 2014). The leaf pulp of the plant consists of large thin-walled parenchymal cells containing clear aqueous-mucous extract called *Aloe vera* gel (AVG). These "*Aloe vera* gel" leaf pulp components are district from Aloe latex ("Aloe extract") that is obtained from whole leaf (Pressman *et al*. 2019). Although 95% of the gel is water, it contains more than 75 active compounds. Based on dry matter, *Aloe vera* is made of 55% polysaccharides, 17% sugar, 7% protein, 4% lipid, 16% mineral, and 1% phenolic compounds (Ahlawat and Khatkar 2011). The laxative and cathartic effects of the plant are attributed to its anthraquinone derivatives, while the polysaccharides exhibit an immunostimulant effect. Other than these effects, antidiabetic, antitumor, antiinflammatory, antioxidant, and antibacterial effects of *A. vera* are also reported (Akev *et al*. 2015).

There is a need to revolutionize the use of medicinal plants from traditional and empirical techniques to scientific evidence. In this regard, the *A. vera* plant has great potential. Although several beneficial effects of the plant have been extensively documented, the mechanisms as well as the chemical compounds responsible for the effect are being under studied. Nevertheless, some researchers suggest that the synergistic effect of the compounds in the plant's gel is significant for the healing effects of the plant (Raksha *et al.* 2014).

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Many therapeutic drugs act by inhibiting enzymes that have important role in metabolic pathways. Therefore, the mechanism of action of active compounds in plant extracts can be unraveled by examining their inhibitory effects on certain enzymes, thus facilitating the discovery or identification of potential new drugs.

Although the biological activity of aloe extracts and active compounds have been investigated for years, studies on their effects on enzymatic activities or inhibition are scarce. Among these are α-glucosidase (Ghaznavi *et al.* 2019), α-amylase (Tekulu *et al.* 2019), hyaluronidase (Ge brelibanos *et al.* 2014) and cyclooxygenase (Lindsey 2002). Recently, Sacan *et al.* (2017) investigated the inhibition of α-amylase, elastase, and neuraminidase by aloe extracts. Also, the burn and wound healing properties of *A. vera* are well documented (Dutta Gupta 2010). Coupled with its emollient effect, the mucilaginous gel of the leaves is used in a variety of cosmetic formulations. As brown spots due to UV light and aging continue to be a challenge for pharmaceutical and cosmetic industries, the search for formulations capable of blocking melanin synthesis has gained scientific interest.

Tyrosinase (TYR) is an important copper containing oxidase enzyme commonly found in animals, plants and microorganisms. It catalyzes the hydroxylation of phenol derivatives such as tyrosine or tyramine to corresponding catechol derivatives. Tyrosinase-bound protein 1 (TYRP1) and tyrosinase-induced protein 2 (TYRP2) are involved in the biosynthesis of melanin, a pigment responsible for the colour of skin, hair and eyes (Lai *et al.* 2018). Therefore, tyrosinase plays a key role in both mammalian melanogenesis and fruit or fungi enzymatic browning. Hyperpigmentation in human skin as well as enzymatic browning in fruits are undesirable facts. Tyrosinase inhibitors have become increasingly important in the cosmetic industry for the prevention of hyperpigmentation of the skin (Chang 2009). Plant extracts having this effect are preferred over whitening agents such as arbutin, kojic acid, hydroquinone, and azelaic acid due to their lesser side effects. Previous studies have demonstrated the potent inhibitory effect of *A. vera* gel on mushroom tyrosinase (Dutta Gupta and Masakapalli 2013).

Elastase and collagenase are matrix metalloproteinases (MMPs) and a family of extracellular matrix degrading enzymes which play an important role in the remodeling and repair of tissues. They regulate extracellular matrix destruction and accumulation which is necessary for re-epithelialization of wounds. Moreover, they play a role in the development of many diseases, ranging from cancer to inflammation, as well as in the growth and healing of wounds (Bahadır Acıkara *et al.* 2019). Elastase inhibitors are suggested to influence the treatment of vascular injury due to inflammation. Human neutrophil elastase (HNE) inhibitors like glycosaminoglycans (e.g. heparin) are among profound elastase inhibitors that are effective against lung cancer metastasis (Moroy *et al.* 2012). Aloe gel-rich glycosaminoglycans is supposed to be showcase elastase inhibitory potential.

In the present study, *in vitro* inhibitory effects of the aqueous and methanolic extracts of *Aloe vera* on tyrosinase, collagenase, and elastase enzymes were investigated.

Materials and Methods

Aloe vera leaves (6 big leaves) were weighed, washed and cut from the middle, the gel was separated by scraping with a spoon. The leaf gel was homogenized in phosphate- buffered saline, filtered through cloth, and lyophilized. The leaves without the gel (skin) were cut in small pieces (514 g) and homogenized with phosphate- buffered saline (PBS; pH 7.0, 600 ml) by means of a Waring blender. The extract was kept at 4^{o}C overnight, then filtered through cloth and the filtrate was centrifuged at 45,700 g for 30 mins at 2^{o}C in a refrigerated centrifuge (Cryofuge 20-3 Heraeus-Christ). The green pellet was discarded and the clear yellow supernatant was lyophilized

(Labconco apparatus). Appropriate dilutions were made in certain proportions from the Aloe leaf peel extract (29 g) obtained before use.

Freshly chopped *A. vera* leaves were washed with water to remove dust and impurities, and then drained on filter paper (Whatman 41). The washed leaves were then split in length, and the gel (198.74 g) was removed by scraping with a spoon and homogenized in a Waring mixer. The gel was filtered through cloth and then the filtrate was centrifuged at +4°C, 10,000 rpm, for 30 min. The supernatant was lyophilized (4.63 g**)** and considered to be AVG. The remaining leaves (leaf skins 171 g) were cut into small pieces, homogenized in a Waring blender with 855 ml distilled water, filtered through cloth, and then the filtrate was centrifuged (Thermo) at +4°C, 12.000 rpm, 15 min. The recovered supernatant was lyophilized (11.57 g**)** and considered to be "*A. vera* fresh leaf skin aqueous extract" (Çandöken *et al*. 2017).

Fresh leaf skins (33.67 g) were dried in a ventilated oven at 60°C, for 2 hrs. The dried samples were then ground into powder and stored at room temperature in the dark. The obtained dried leaf skin (4.25 g) and fresh leaf skin (73.4 g) were extracted with methanol for 3 days using a Soxhlet extractor. After extraction, the samples were filtered with filter paper (fresh leaves filtrate 260 ml, dried leaves filtrate 143 ml). The methanol used as solvent was evaporated under pressure using a rotary evaporator within 30 minutes. This resulted in a semi-solid crude extract (3.32 g) of the *A. vera* fresh leaf skin (Çandöken *et al*. 2017).

Tyrosinase inhibitory activity was determined spectrophotometrically (Vanni *et al*. 1990). Kojic acid was used as the standard control compound. Inhibition calculation was made according to the formula given below:

$$\text{Inhibition (\%)} = \left[\frac{(A_c - A_t)}{A_c}\right] \times 100$$

where: A_c = Absorbance of control and A_t = Absorbance of test.

Collagenase inhibitory activity was determined spectrophotometrically (Thring *et al*. 2009). Epigallocatechin gallate was used as a standard compound for control. The percentage inhibition of collagenase was calculated according to the following formula below:

$$\text{Inhibition (\%)} = \left[\frac{(A_c - A_t)}{A_c}\right] \times 100$$

where: A_c = Absorbance of control and A_t = Absorbance of test.

Elastase inhibitory activity was determined spectrophotometrically as described by James *et al*. (1996). Ursolic acid was used as a standard. Percentage inhibition was calculated according to the formula given below:

$$\text{Inhibition (\%)} = \left[\frac{(A_c - A_t)}{A_c}\right] \times 100$$

where: A_c = Absorbance of control and A_t = Absorbance of test.

Results for enzyme inhibitory activities are given as half maximal inhibitory concentrations (IC_{50} values) calculated by regression analysis prepared from the concentrations of samples.

Results and discussion

Results of *A. vera* skin and gel extracts on tyrosinase inhibition activity are presented in Table 1. A high tyrosinase inhibition activity (57.05%) of leaf gel extract was detected at a concentration of 2 mg/ml. The IC_{50} value was found to be 1.39 ± 0.12 mg/ml. Leaf skin extract showed a good tyrosinase inhibition activity (32.38%) at a concentration of 1 mg/ml. The IC_{50} value was found to

be 2.15 ± 0.05 mg/ml. The tyrosinase inhibition activity of *A. vera* skin methanol extract was found to be 28.32% at a concentration of 1 mg/ml. The IC_{50} value was found to be 2.89 ± 0.43 mg/ml. Tyrosinase inhibitory activity of *A. vera* extracts and that of the standard decreased in the order of Kojic acid > *A. vera* leaf gel > *A. vera* leaf skin extract > *A. vera* skin methanolic extract (Table 1).

The effect of the extracts on collagenase activity is presented in Table 1. The IC_{50} values of the *A. vera* extracts were found to be 12.86 ± 0.15 mg/ml, 45.93 ± 9.66 mg/ml, and 58.89 ± 4.54 mg/ml, for *A. vera* leaf gel, *A. vera* leaf skin methanolic and *A. vera* leaf skin, respectively. *A. vera* leaf gel had the highest inhibitory activity against collagenase (43.64 %) at a concentration of 10 mg/ml, whose IC_{50} value was 12.86 ± 0.15 mg/ml. Also, A. vera gel extracts showed a close inhibitory activity to epigallocatechin gallate when compared with epigallocatechin gallate. Collagenase inhibitor activity of extracts and standard inhibitors decreased in order Epigallocatechin gallate > *Aloe vera* leaf gel > *Aloe vera* leaf skin methanol > *Aloe vera* leaf skin.

The elastase inhibitory activities of *A. vera* extracts presented in Table 1 showed that a lower IC_{50} value is associated with a higher enzyme inhibitory activity. The elastase inhibitory activity of the *A. vera* extracts was dose-dependent. The elastase inhibitory activity of *A. vera* was published previously (Sacan *et al.* 2017). In the present study, methanolic extract exhibited an inhibition value of 29.32% at a concentration of 2000 µg/ml. The elastase inhibitory activity of the methanolic extract of *A. vera* leaf skin was found to be lower than that of ursolic acid (Table 1).

There are many factors that negatively affect the condition of skin during life, causing damage and loss of functionality. Natural aging and environmental factors (referred to as intrinsic and extrinsic aging) cause the depletion of skin elasticity. Ultraviolet (UV) light is one of the key causes of skin damage. For example, many harmful effects such as skin irritation, redness, and burning are caused by UVB light, while UVA is responsible for skin aging and discoloration. More so, other external conditions such as personal care product components, environmental pollution, the electronic device radiation (blue rays) can cause skin damage. Skin allergies, eczema, and psoriasis are considered problems increasingly affecting many people in recent times. Burn injuries due to heat, radioactivity, electricity, friction, or chemicals are other causes of skin damage (Malinowska *et al*. 2019).

Hyperpigmentation disorders result from abnormally high melanin production. This circumstance called melanogenesis results in freckles, melisma, and lentigines. Tyrosinase is the central enzyme in the biosynthesis of the black pigment melanin from tyrosine. Several compounds have been assayed for tyrosinase inhibitors. Among these are hydroquinone, retinoic acid, arbutin, and kojic acid, which are clinically available and used in cosmetic products (Kumari *et al*. 2018). Lyophilized and methanolic extracts from *A. vera* gel were found to exhibit potent mushrooms' tyrosinase inhibitory activity (Dutta Gupta and Masakapalli 2013). In a more recent study, an aloesin derivative in *A. vera* was identified as a tyrosinase inhibitor (Kim *et al*. 2017). Accordingly, the present study shows that gel extract possessed a good tyrosinase inhibitory effect.

Collagen and elastin are two fibrillar proteins responsible for the strength and elasticity of the skin. Collagenases are metalloproteinases degrading proteins of the extracellular matrix (ECM) which include collagen, fibronectin, and laminin. Elastase in turn is a serine protease involved in the breakdown of elastin and other ECM proteins. Skin hydration is mainly provided by glycosaminoglycans (Thring *et al.* 2009). The ECM proteins also play a role in tissue repair and wound healing, thus their maintenance is important for the integrity of the cell. Inhibition of enzymes involved in the breakdown of collagen and elastin is believed to have a positive effect in preventing the undesirable effects of aging or a beneficial effect on wound healing (Boran *et al.* 2018).

Table 1. Tyrosinase, collagenase and elastase inhibitory activities of *Aloe vera* extracts.

Plant extracts and standard	Conc. (mg/ml)	Tyrosinase inhibition (%)*	IC_{50} (mg/ml)*	Concentration (mg/ml)	Collagenase inhibition (%)*	IC_{50} (mg/ml)*	Conc. (µg /ml)	Elastase inhibition (%)*	IC_{50} (µg/ml)*
Aloe vera leaf gel	0.1	23.30 ± 0.41		0.1	19.09 ± 0.91				
	0.5	45.80 ± 0.94	1.39 ± 0.12	1	30.46 ± 1.37	12.86 ± 0.15	-	-	-
	2	57.05 ± 1.88		10	43.64 ± 0.00				
Aloe vera leaf skin	0.1	17.48 ± 0.41		50	42.62 ± 3.28				
	0.25	23.58 ± 0.41	2.15 ± 0.05	100	54.10 ± 1.64	58.89 ± 4.54	-	-	-
	1	32.38 ± 0.47		500	76.50 ± 1.89				
Aloe vera leaf skin methanolic	0.01	16.67 ± 0.41		0.1	25.25 ± 0.87		250	5.71 ± 1.07	
	0.5	22.22 ± 1.24	2.89 ± 0.43	10	39.39 ± 0.00	45.93 ± 9.66	1000	9.87 ± 0.27	3631.27 ± 255.15
	1	28.32 ± 2.04		50	76.50 ± 1.89		2000	29.32 ± 2.19	
Kojic acid	0.001	4.53 ± 0.91							
	0.01	7.90 ± 0.55	0.68 ± 0.07	-	-	-	-	-	-
	0.1	12.18 ± 0.01							
Epigallocatechin gallate	-	-	-	0.01	38.52 ± 3.24	0.46 ± 0.13	-	-	-
				0.1	45.59 ± 5.09				
				1	59.80 ± 0.85				
Ursolic acid							0.1	62.52 ± 0.68	
	-	-	-	-	-	-	1	72.78 ± 1.02	0.079 ± 0.0006
							10	76.52 ± 1.23	

*Mean ± SD

Plants contain a wide variety of compounds including polyphenols such as flavonoids, tocopherols, phenolic acids, and tannins which were found to exhibit collagenase inhibitory action. Twenty- three plant extracts were examined for their anti-collagenase and anti-elastase activities, one dozen of which were found to exhibit high inhibitory activities for both enzymes (Thring *et al.* 2009). Natural polysaccharides like tragacanth and locust beam gum have been tested for their inhibitory activity against hyaluronidase, collagenase, and elastase (Boran *et al.* 2018).

The leaf pulp gel is rich in polysaccharides, with acemannan mainly found to be effective on the skin. The scar healing capacity of the plant is explained by the fact that some of its components increase the crosslinking of tissues and the synthesis of collagen by stimulating the production of cytokines and macrophages (Michayewicz 2018). They also promote the proliferation of fibroblasts, and as well the production of hyaluronic acid and hydroxyproline in these cells (Zhang and Tizard 1996).

In a recent study, *A. vera* gel was demonstrated to inhibit the overexpression of matrix metalloproteinases (MMPs) produced by UV-B irradiation in mice (Saito *et al.* 2016). Chitra *et al.* (1998) suggested that the wound healing efficacy of *A. vera* was by increasing the collagen content of the granulation tissue, as well as its degree of crosslinking. Among the extracts investigated in the present study, gel extract displayed the highest collagenase inhibitory activity, which is higher when compared to the IC_{50} value of epigallocatechin gallate. Among the various components of *A. vera*, glycoproteins, lectins, and acemannan were proven to stimulate wound healing and induce cell proliferation for tissue regeneration (Sánchez-Machado *et al.* 2017). Besides, AVG was found to exert apoptotic activity on a type of skin cancer, B16F10 murine melanoma (Çandöken *et al.* 2017). Since melanin biosynthesis is a result of tyrosinase activity, the tyrosinase inhibitory activity of the extracts could also support the anticancer activity of the extracts on malign melanoma cells. The findings of the present study suggest that *A. vera* leaves are potential candidates as enzyme inhibitors,and that further studies are required in order to to elucidate the reaction mechanisms of isolated compounds.

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