Industrial Crops & Products 118 (2018) 290–295

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Investigations of anti-aging potential of *Hypericum origanifolium* Willd. for skincare formulations

Rukiye Boran

Medical Laboratory Program, Department of Medical Services and Techniques, Vocational School of Health Service, Aksaray University, TR 68100, Aksaray, Turkey

ARTICLE INFO

Keywords:
Hypericum origanifolium
Anti-aging
Enzyme inhibition
Antioxidant
Antigenotoxicity

ABSTRACT

Skin aging is caused by increased activation of extracellular matrix disruption enzymes, oxidative stress and DNA damage. Inhibition of these mechanisms by natural plants may be an encouraging approach to prevent skin aging. *Hypericum* is medically important plant and a source of several promising compounds. Nevertheless, there are no studies about the anti-aging potential of *Hypericum origanifolium* Willd (Guttiferae). The purpose of this study was to research the anti-collagenase, anti-elastase and anti-hyaluronidase effects, together with the antioxidant and genotoxic/antigenotoxic activities of *H. origanifolium*. Ethanol extract of *H. origanifolium* harvested from Turkey was tested for anti-collagenase, anti-elastase and anti-hyaluronidase activities. The extract inhibited collagenase and elastase activity. The highest ECM-degrade enzyme inhibition was demonstrated against collagenase (79.39%). The phenolic constituent and antioxidant activity were tested by Folin-Ciocalteu method (FCM), 2,2-dipenyl-1-picrylhydrazyl (DPPH) radical-scavenging and β-carotene bleaching assay. The total phenol content revealed to be 93.4 ± 1.6 mg GAE/g dry extract. IC_{50} values observed in DPPH and β-carotene bleaching assays were 270 ± 0.1 and 230 ± 0.2 µg/mL, respectively. Finally, the extract was investigated for its genotoxic/antigenotoxic effects using Ames *Salmonella*/microsome test system. Results indicated no sign of mutagenicity, and depending upon the concentration, the antigenotoxicity was noted with an inhibition of mutagenicity going from 21.54 to 84.90%. The results suggest that *H. origanifolium* can be considered as new natural sources to be potentially used as anti-aging ingredients in skin care formulations.

1. Introduction

Extracellular matrix (ECM) is the prominent component of the dermis, providing a structural framework for skin growth and flexibility (Bravo et al., 2016). The ECM is made up of integrated proteoglycans with matrix metalloproteins. Collagen is most abundant protein, it's responsible for adherence to connective tissues and tensile strength of skin (Vijayakumar et al., 2017). Elastin provides most of the elastic recoil properties of the arteries, lungs and ligaments as well as skin (Scott and Miller, 2003). Glycosaminoglycans are also among the primary dermal skin matrix components. Hyaluronic acid is the predominant glycosaminoglycan in the skin. Hyaluronic acid has a variety of functions in the skin, such as holding moisture, contributing to the mechanical resistance and flexibility of the skin (Kolakul and Sripanidkulchai, 2017). Breakdown and disorganization of collagen, elastin and hyaluronic acid are the main characteristics of skin aging due to the enhanced activation of ECM degrading enzymes such as collagenase, elastase and hyaluronidase.

In young and healthy skin there is a balance between the synthesis and degradation of collagen, elastin and hyaluronic acid. This complex balance diminishes with age. Therefore, today there are many strategies to delay the symptoms of aging; degradative enzymes responsible for the structural changes in the skin are the target of the new strategies. If the function of these enzymes is inhibited, the skin can maintain the structural integrity and thus reduce the formation and appearance of wrinkles (Fibrich and Lall, 2018).

The induction of DNA damage is also another problem that causes aging (Finkel and Holbrook, 2000). Damage to DNA results in collagen and the elastase breakdown in the dermis layer which eventually leads to the formation of wrinkles and photoaging of the skin (Vijayakumar et al., 2017). There is evidence that both DNA repair responses and antioxidant defense mechanisms slow down with age, which can also lead to increased in mutation accumulation (Turker, 2000; Wilson et al., 2008). The inhibition of ECM-degrading enzymes, the presence of antioxidants and anti-genotoxic agents may be a beneficial attempt to prevent aging problems. Traditional herbs can provide interesting and largely undiscovered resources for the improving of new dermatology and cosmeceuticals to combat skin aging (Manosroi et al., 2010).

Turkey is an important places for *Hypericum* populations, because there are 89 *Hypericum* species, of which 43 are endemic in the flora of

E-mail address: rukiyeboran@aksaray.edu.tr.

https://doi.org/10.1016/j.indcrop.2018.03.058
Received 8 February 2018; Received in revised form 22 March 2018; Accepted 27 March 2018

Turkey (Davis, 1988). *Hypericum* have been used as sedative, wound healing, anti-inflammatory, antispasmodic and antiseptic, against to ulcer, diabetes, and stomach diseases in Turkish folkloric medicine (Bingol et al., 2011; Özkan and Mat, 2013). Increase in raw material demand due to increased market share for Hyperici herba products has been causing significant research to determine the pharmacological properties of *Hypericum* genus (Cirak et al., 2015). For this reason a number of scientific papers have been published that show the therapeutic utility of Hyperici herba in diseases such as anxiety, depression, cut, burn, inflammation-related disorders, cancer, viral and bacterial infections, and neurodegenerative diseases (Klemow et al., 2011).

Hypericum origanifolium Willd. (Guttiferae) is a species belong to *Hypericum* genus endemic to Turkey, Armenia and Georgia (Yaşar et al., 2013). *H. origanifolium* has been found to contain chlorogenic acid, quercetin, rutin, pseudohypericin, hyperforin, hypericin and hyperoside (Kitanov, 2001; Çırak et al., 2007; Yaşar et al., 2013). The *H. origanifolium* has recently been considered as a new valuable species, and this species have a probable substitute of the well-established EU market position of *Hypericum perforatum* (Saint John's wort) (Bertoli et al., 2015).

Although several phytochemical studies have previously been conducted to identify *H. origanifolium* components (Kitanov, 2001; Çırak et al., 2007; Yaşar et al., 2013; Bertoli et al., 2015), there are only a few studies of pharmacological properties. These studies have demonstrated their antioxidant activity (Öztürk et al., 2009), protective effect against renal ischemia/reperfusion injury (Sentürk et al., 2013), and central nervous system activities (Yaşar et al., 2013). However, there are no studies about the anti-aging potential of *H. origanifolium.* For this reason, the present study was designed to research the anti-aging property of endemic *H. origanifolium* by means of anti-elastase, anti-collagenase, anti-hyaluronidase, and antioxidant and genotoxic/anti-genotoxic activity.

2. Materials and methods

2.1. Materials

Bovine hyaluronidase, *Clostridium histolyticum* collagenase, N-(3-[2-Furyl]-acryloyl)-Leu-Gly-Pro-Ala (FALGPA), porcine pancreatic elastase, sodium hyaluronate, epigallocatechin gallate (EGCG) and N-Succinyl-Ala-Ala-Ala-*p*-nitroanilide, 4-nitro-o-phenylenediamine (4-NPD), D-biotin, L-histidine, D-glucose, butylated hydroxytoluene (BHT), linoleic acid, DPPH, β-carotene and ascorbic acid were bought from Sigma-Aldrich Chemicals Company (St. Louis, USA). *p*-dimethyl amino benzaldehyde, sodium azide (NaN_3), Gallic acid, Folin–Ciocalteau reagent (FCR), other chemicals and solvents were bought from Merck Chemical Co. (Darmstadt, Germany). Culture mediums were purchased from Merck Chemical and Difco (New Jersey, United States).

2.2. Plant material and extraction

The aerial parts of *H. origanifolium* were collected in Adana (37°26′56″N 35°07′14″W) (Turkey), in July 2013 and identified by Dr. Olcay Ceylan and a voucher specimen (ARB-H09) was stored in the Department of Biology, Mugla Sitki Kocman University, Turkey. Samples were dried in the shade for 5 days. Twenty five g *H. origanifolium* were extracted with ethanol in a soxhalet device. After cooled, extract was filtered and concentrated. Then it was kept at a low temperature of −20 °C before finally dissolving in dimethyl sulfoxide (DMSO).

2.3. Enzyme inhibition

For the enzyme inhibition activities, *H. origanifolium* ethanol extract (100 μg/mL and 1000 μg/mL) were dissolved in 5% DMSO and for the negative control group, 5% DMSO was used.

2.3.1. Anti-collagenase activity

The principles of this assay are based on the enzyme-substrate interaction of collagenase with synthetic FALGPA and proteolytic degradation leads to a reduction in the absorption of 335 nm in the presence of collagenase inhibitors. Twenty five μl collagenase (0.8 U/mL) derived from *Clostridium histolyticum* was allowed to react with 25 μL test samples in a 96-well microtitre plate containing 25 μL tricine buffer, pH 7.5, (with 10 mM $CaCl_2$ and 400 mM NaCl), in the dark at 37 °C for 20 min. For the positive control group, 25 μl of EGCG was used. After the pre-incubation, an amount of 50 μL of the FALGPA (1.6 mM) working solution was added to each well. Absorbance was determined at 335 nm quickly and at 2-min intervals for 20 min (Barrantes and Guinea, 2003).

2.3.2. Anti-elastase activity

Fifty μl of 200 mM Tris–HCl buffer (pH 8.0) and 25 μL elastase (1 μg) was allowed to react with 50 μl test samples in a 96-well microtitre plate in the dark at 25 °C for 20 min. Positive control was performed with 50 μl EGCG. After the pre-incubation, an amount of 125 μl N-Succinyl-Ala-Ala-Ala-*p*-nitroanilide solution (0.8 mM) was put into wells, and the microplate was further incubated in the dark at 25 °C for 20 min. The fluorescence intensity of the solution in each assay well was determined using a fluorescence microplate reader at the excitation wavelength of 410 nm for 20 min (Lee et al., 1999).

2.3.3. Anti- hyaluronidase activity

Briefly, 50 μl of bovine hyaluronidase (7900 U/mL) and 100 μL test samples pre-incubated at 37 °C for 20 min. Positive control was performed with 100 μL tannic acid. After 20 min incubation with 100 μl $CaCl_2$, 250 μL (1.2 mg/mL) sodium hyaluronate was put into wells and re-incubated for 40 min at 37 °C. 100 μL sodium borate (0.2 M) and 50 μL NaOH (0.2 M) were then added into the samples and incubated in boiled water for 3 min. After cooled, *p*-dimethylaminobenzaldehyde solution was added. After 20 min incubation at 37 °C, the fluorescence intensity was determined at 585 nm (Lee et al., 1999).

The following formula was used to calculate the percentage of enzyme inhibitions:

$$\text{Inhibition (\%)} = [(C - B) - (T - D)]/(C - B) \times 100$$

Here, C: absorbance without the *H. origanifolium*, B: absorbance without the *H. origanifolium* and enzyme, T: absorbance with the *H. origanifolium*, and D: absorbance with the *H. origanifolium* without enzyme.

2.4. Total phenol content

The total phenol content of the *H. origanifolium* was calculated with Folin–Ciocalteu technique (Singleton et al., 1999). One hundred μL of 0.2 N FCR were put into 200 μL of a suspension of extract having 1 mg/mL concentration, the mixture is then vortexed for 60 s and after 3 min, 2 mL of 5% aqueous Na_2CO_3 solution is put into wells to neutralize the suspension. The absorbance was evaluated after 2 h at 760 nm. The standard curve is obtained by applying the same technique for the standard solution of gallic acid as on the sample mixture. The total phenol content was given as milligram Gallic acid equivalent per gram of the dry extract (mg GAE/g extract).

2.5. Antioxidant activity

2.5.1. Radical scavenging assay

For radical scavenging activity assay 1 mL DPPH solution and 1 mL diverse dilutions of test samples (100 μg/mL–1000 μg/mL) were mixed, and then left for 30 min in the dark. The absorbance was later evaluated at 517 nm (Ebrahimabadi et al., 2010). BHT and ascorbic acid were used as references. The DPPH radical-scavenging ability was calculated