Bioorganic Chemistry 77 (2018) 159–167

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

In-vitro evaluation of antioxidant, anti-elastase, anti-collagenase, anti-hyaluronidase activities of *safranal* and determination of its sun protection factor in skin photoaging

Madan Kumud *, Nanda Sanju

Department of Pharmaceutical Sciences, M. D. University, Rohtak, Haryana 124001, India

ARTICLE INFO

Article history:
Received 22 September 2017
Revised 27 December 2017
Accepted 30 December 2017
Available online 4 January 2018

Keywords:
Safranal
Antiaging
Antioxidant
Anti-elastase
Anti-collagenase
Anti-hyaluronidase
SPF

ABSTRACT

Safranal, a monoterpene aldehyde, is present as one of the main volatile constituents of *Crocus sativus* Linn. (saffron flowers). This volatile constituent not only contributes to the aroma of saffron but has been reported to possess antidiabetic, antiulcer, antiasthamatic, anticonvulsant, antidepressant, cardioprotective, anticancer and UV protective properties. Most of these therapeutic actions are contributed by its potential to quench reactive oxygen species (ROS). Antioxidant properties of phytoconstituents are now being explored for developing photoprotective skin formulations. These bioactives have the potential to protect the epidermal and dermal layers of the skin which mainly comprises of elastin and collagen. When UV rays penetrate the dermal layers, there is an increased production of *elastase, collagenase* and *hyaluronidase* leading to degradation of collagen, elastin and hyaluronic acid respectively. These dermal components are responsible to provide strength, elasticity and moisture to the skin. Due to frequent exposure to sunlight, these conditions tend to augment leading to wrinkle formation and sagging of skin.

Although antioxidant properties of safranal have been established on various cell lines but till date no studies have been reported regarding the dermal enzyme inhibition activities. In the current research work, a comprehensive *in vitro* evaluation of antioxidant, anti-elastase, anti-collagenase, anti-hyaluronidase activities of *safranal* along with determination of sun protection factor (SPF) was carried out. The *in vitro* antioxidant activity was carried out by diphenylpicrylhydrazyl (DPPH) method and its IC_{50} value was found to be 22.7 μg/ml. The enzyme inhibition IC_{50} values of safranal for anti elastase activity were found to be 43.6 μg/ml, 70 μg/ml for antihyaluronidase activity and 9.4 μg/ml for anticollagenase activity. Photoprotective activity of safranal was determined by UV absorbance method and SPF calculated by Mansur equation which was found to be 6.6.

The significant inhibitory activity of *safranal* on matrix metalloproteinases (MMPs) responsible for aging and a higher SPF established that this bioorganic molecule is a strong photoprotective agent. Its established free radical scavenging capability along with above characteristics make it a valuable component to be incorporated into herbal antiaging formulations.

1. Introduction

Cutaneous exposure to UV radiations cause a chain of reactions which is ultimately responsible for progression of photoaging. Repeated exposure to these solar radiations yields faulty repaired dermal matrix, with a cumulative effect on collagenous organisation. These imperfections in the repaired dermal matrix finally becomes visible in the form of wrinkles and sagging of skin [1]. Changes in skin pigmentation are also directly associated with photoaging or premature aging. Besides this, various forms of photo induced skin cancer are reported to be due to acute and chronic sun exposure [2].

The deleterious effects of sun rays in the skin are mainly due to its ultraviolet rays. The spectrum in the range of 320–400 nm is termed UV-A while the rays in the range from 290 to 320 nm are termed as UV-B rays. The UVA rays penetrate the skin and are absorbed by cellular chromophores like urocanic acid, riboflavins, melanin, bilirubin, heme, porphyrin, and pterins [3]. These photosensitizers absorb photons and converts into a 'triplet' excited state. This excited state then reacts with both DNA and the molecular oxygen, resulting in modification of the DNA and production of reactive oxygen species (ROS), respectively [4] (Fig. 1). The ROS species such as hydrogen peroxide, superoxide, singlet oxygen

* Corresponding author.
E-mail address: kumud_25@rediffmail.com (K. Madan).

https://doi.org/10.1016/j.bioorg.2017.12.030
0045-2068/

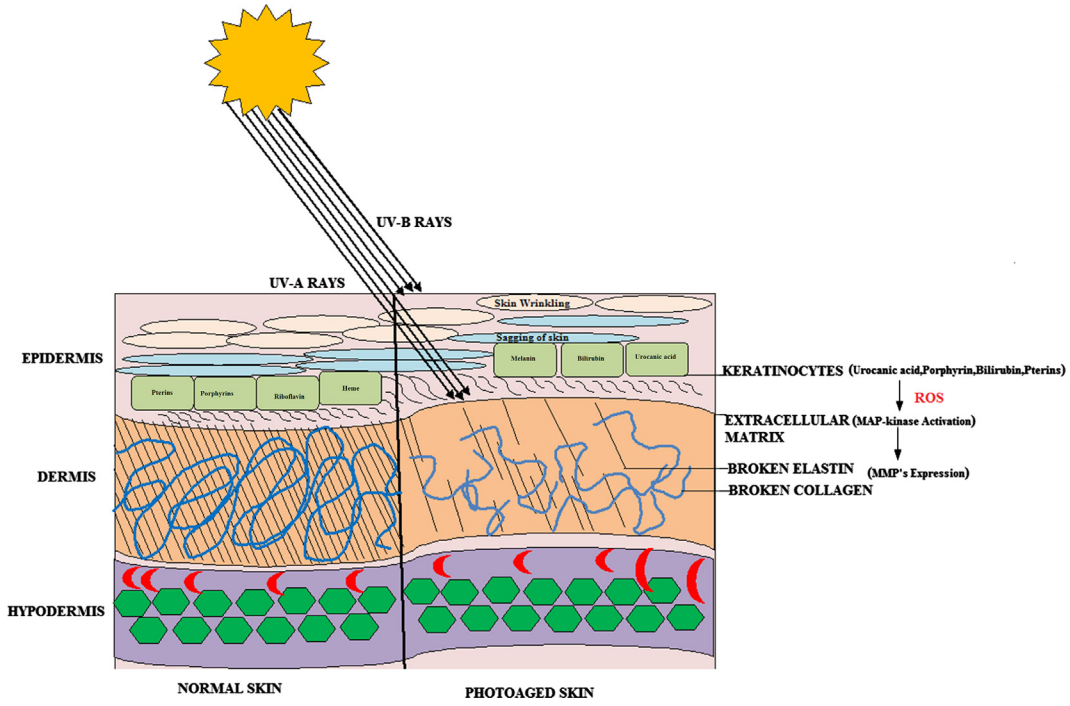

Fig. 1. Photoaging and its mechanism.

or hydroxyl radicals act as secondary messengers, resulting into the activation of MAP-kinasep38, ERK (extracellular signal-regulated kinase) and JNK (c-Jun amino-terminal kinase). This leads to the expression of the transcription factor activator protein1 (AP-1) culminating in the expression of matrix metalloproteinases (MMPs). These endopeptidases are secreted by keratinocytes and dermal fibroblasts in response to multiple stimuli such as cytokines, oxidative stress and UV radiation.

As a result of this, the production of collagenase, gelatinase and stromelysin-1 is stimulated leading to the deterioration of collagen, elastin and other components of the dermal extracellular matrix [5]. AP-1 also inhibits transforming growth factor-β (TGF-β) which is a major regulator for the production of procollagen type I in human skin [6]. The AP-1 mediated MMP expression also leads to cellular damage [7]. Alteration in the structure of elastic fibres, subsequently reduces elastic properties of the skin and results in wrinkle formation [8].

Although chronological aging cannot be treated, photoaging can be treated by compounds or products with antioxidant properties. Antioxidants, which are free radical scavengers too, are being explored to prevent photoaging because of their ability to inhibit the expression and activity of MMPs [6]. Presently, research based on antiaging compounds especially from natural sources, is greatly expanding. Phytochemicals such as triterpenes, polyphenols and sterols are being constantly investigated for their potential to protect the skin from harmful UV radiations [9]. These herbal constituents also have the potential to inhibit the enzymes responsible for degradation of elastin and collagen in the skin. The important role of elastase inhibitors in UV-induced wrinkle formation has been established by various studies.

In one of the studies by Genji Imokawa, extract of *Zingiber officinale* (L.) was identified as a safe and potent elastase inhibitor, specifically for fibroblast elastases. In the clinical study conducted using human facial skin, it was found that the aqueous extract of *Zingiber officinale* (L.) Rose prevented wrinkle formation in the area around the corners of the eyes [8]. In another study, *Phyllanthus emblica* L. (amla), *Silybum marianum* (silymarin) and *Manilkara zapota* (sapota) have been explored for their antioxidant potential. The phenolic compounds and flavonoids present in amla, sapota and silymarin have proven to possess potential antiaging properties and showed anti elastase and anticollagenase (MMP1 and MMP2) activities [10]. Similarly, phenolic and flavanoid content present in Thai plants was responsible for their antioxidant and antienzymatic activity as determined by Moragot Chatatikun and Anchalee Chiabchalard [11]. However, the activity was dependent on the solvent used for extraction. The ethanol extract showed highest activity followed by dichloromethane and petroleum ether extracts. Cocoa pod extract has also been investigated for its potential as a cosmetic ingredient due to its anti-wrinkle, skin whitening and sunscreen effects. This has been attributed to the presence of carboxylic acid, phenolic acid, fatty acid, flavonoids, stilbenoids and terpenoids, as determined by the LC-MS studies [12]. In another study, extracts of pomace from Riesling grapes were analyzed for their inhibitory properties on collagenase and elastase. This dose dependent enzyme-inhibitory activity with IC_{50} values of 14.7 μg/ml (elastase) and 20.3 μg/ml (collagenase) was attributed to presence of polyphenols in the extract [13]. Similarly, pomegranate concentrated solution (PCS) exhibited significant antihyaluronidase activity when tested on human keratinocytes, HaCaT cells [14].

Nema et al. studied the matrix metalloproteinase, hyaluronidase and elastase inhibitory potential of standardized extract of *Centella asiatica*; a valuable shrub described in Ayurveda [15]. The enzymatic activities were evaluated using ursolic acid and oleanolic acid as standards. Antielastase and antihyaluronidase activities with IC_{50} of 14.54 ± 0.39 μg/ml and 19.27 ± 0.37 μg/ml respectively, proved it be a potential antiaging agent in skin care.

Human skin has a network of protective antioxidants. These include endogenous enzymatic antioxidants such as GSH peroxidase, superoxide dismutase (SOD), catalase and nonenzymatic low-molecular- weight antioxidants such as vitamin E, vitamin C, Glutathione (GSH), uric acid, and ubiquinol [16]. Acute exposure to UV induces oxidation of biomolecules present in human skin alongwith deterioration of endogenous antioxidants. Although