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Antiaging Activity of the Skin Cream containing Ethyl Acetate Extract of *Eichhornia crassipes* (Mart.) SOLMS

P.Lalitha* and P.Jayanthi

Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore-43,India.

***Corres.author: goldenlalitha@gmail.com**
Mobile Number: +919842292614

Abstract: *Eichhornia crassipes* (Mart.) Solms possess many antioxidants like glutathione, ascorbic acid etc. Two skin creams of the ethyl acetate extract of *E.crassipes* were evaluated for its antiageing efficacy by DNA damage inhibition assay and DPPH radical scavenging assay. The DNA damage inhibition and DPPH radical scavenging ability offered by the skin creams increased with increase in concentration (1 mg to 20 mg) for both the creams. The results suggest the promising use of the plant in cosmeceutical industry as formulations.
Keywords: *Eichhornia crassipes*, hyacinth, ethyl acetate, skin, antiaging, cream, DNA damage, DPPH.

Introduction

The process of skin aging occurs naturally due to changes in elasticity of the skin over time and also due to external factors like exposure to UV radiation (1) in a similar fashion to that of skin whitening. Oxidative changes are one of the primary causes of aging of skin. UV exposure causes physical changes to the skin due to alterations in the connective tissue via the formation of lipid peroxides, cell contents and enzymes, and reactive oxygen species (ROS) (2). Redox-active transition metal ions such as iron (II) or copper (II) cause severe oxidative stress when ROS are overproduced, and thus damage tissues and the cellular DNA, protein, lipid and carbohydrate constituents within (3). Free radicals interact with the intracellular transcription factors, proteins that bind to specific sequences of DNA and thus control the transcription of genetic information from DNA to RNA. They regulate transcription factors such as activator protein 1(AP-1), and nuclear transcription factor kappa B (NF-κB). AP-1 is in charge for the production of metalloproteinases that break existing collagen which leads to wrinkle formation. NF-κB upregulates the transcription of pro-inflammatory mediators including interleukin-1 (IL-1), IL-6 and IL-8 and tumor necrosis factor alpha (TNF-α). These pro-inflammatory mediators helps to further activate the transcription factors AP-1 and NF-κB, resulting in damage. (4).

Antioxidants arrest the formation of free radicals by terminating the chain reaction thereby neutralizing it. Many assays evaluate the antiaging efficacy of the substance and most noteworthy among them are the DNA damage inhibition assay and DPPH radical scavenging assay. Even under the best of circumstances, DNA is constantly subjected to chemical modifications. DNA is one of the principal targets for UV induced damage in a variety of organisms ranging from bacteria to human. Among ultraviolet radiation, UV-B (280 - 315 nm) is the

most deleterious as it blocks the movement of DNA polymerases on DNA template(5). The percentage inhibition of DNA damage can be correlated to the antiaging property of the plant.

The skin is the largest organ in our body and it plays an extremely important role, providing a vast physical barrier against mechanical, chemical, thermal and microbial factors that may affect the physiological status of the body. Over exposure of skin to sun light and pollution causes the production of reactive oxygen species which can react with DNA, proteins and fatty acids, causing an oxidative damage and impairment of antioxidant system. The formation of ROS in skin leads to various skin diseases predominantly wrinkles, aging *etc.*, Cosmetic products are used for treating such conditions thereby improving the skin condition (6).

Antioxidants like glutathione(7), ascorbic acid(8), polyphenols(9) etc., that exhibits antiaging efficiency (10) are present in *E. crassipes*. Various solvent extracts of *E.crassipes* shows varying antioxidant activity (11-19). Acute oral toxicity studies of the ethyl acetate extract, aqueous extract and methanol fractionate of aqueous extract carried out with Swiss Albino mice indicated that the extract is non-toxic upto a dosage of 2000mg/kg body weight of the animal (20).

The present study however is focused on the determination of the antiaging efficacy of the skin creams prepared with the ethyl acetate extract of *E.crassipes* by DNA damage inhibition assay and DPPH scavenging assay.

Experimental

Plant collection

E.crassipes was collected from Singanallur boat house, Coimbatore,Tamil Nadu in the month of March, 2010. The plant sample was identified by Dr.G.V.S.Murthy, Scientist F & Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore- 641 002 with the number BSI/SRC/5/23/2011-12/Tech.

Extraction of the plant material

The root portion was cut off, washed thoroughly to free it from debris and was shade dried for 20 days. The dried plant material was sliced and ground. *E.crassipes* (100g) was extracted successively with ethyl acetate (2000mL) and water (1000mL) twice for 6 h. The extracts were desolvetised to give ethyl acetate extract and aqueous extract.

Preparation of skin cream for antiaging assay

Two skin creams LPR1 and LP3 were prepared for analyzing the antiaging and antioxidant properties of the extract of *E. crassipes*. The weight of the constituents in the skin creams is given in Table 1.

Table 1 Constituents of the skin cream

Sample	Additives/ Extracts	LPR1	LP3
Oil phase	Bees wax	9.71 g	11.49 g
	Emulsifying wax	35.12 g	38.48 g
Aqueous phase	Glycerol	30 mL	30 mL
	Water	97 mL	30 mL
	Ethyl acetate Extract	3.29 g	0.192 g
	Lemon extract	-	1.056 g
	Musk	-	0.057 g

The skin creams were prepared by the addition of aqueous phase to the oil phase with continuous stirring. Paraffin wax and emulsifying wax constitutes the oil phase which was heated upto 70±5 °C. Aqueous phase consisting of glycerol and water was heated upto 80°C and was added to the oil phase drop wise with continuous stirring. The ethyl acetate extract and ethyl acetate extract together with lemon and musk was added

to this mixture respectively yielding LPR1 and LP3. Stirring of the mixture was continued in a magnetic stirrer until homogeneity.

***In vitro* anti-aging property of the skin cream**

Inhibition of DNA damage (21)

The potential of LPR1 and LP3 to prevent DNA damage was carried out by the method of Halliwell and Gutteridge (1981) (22). The skin cream in varying quantity (1.0, 2.0, 5.0, 10.0 and 20.0 mg) was added to the reaction mixture containing 0.5 mL deoxyribose (1 mg/mL in 0.15 M NaCl), 0.5 mL phosphate buffer (0.1 M NaCl) and 0.2 mL ammonium ferrous sulphate (4.8 mM) to make a final volume of 1 mL. The reaction mixture was then incubated at 37 °C for 1 h and the reaction was stopped by the addition of I mL TBA (1%) followed by the addition of 1 mL TCA (2.8%). The tubes containing the reaction mixture were incubated in boiling water for 20 minutes. This was then extracted with butanol and the absorbance was measured at 532 nm in a spectrophotometer.

DPPH radical scavenging activity

The skin creams LPR1 and LP3 were screened for their DPPH radical scavenging activity by the method of Nikhat *et al* , 2009 (23) with 0.25 mM DPPH at different concentrations (2, 10, 20 and 50 mg).

Results and Discussion

Skin aging is a complex and dynamic process which involves genetic, hormonal and environmental factors. In a biochemical level, the aging process is just an inevitable process of oxidation and cellular death. A balanced diet, exercise and basic care such as using antiaging creams will be reflected in the delay of aging (24).

Antiaging creams can be prepared as per the standard procedures and any prepared skin cream in India should adhere to the specifications given in IS 6608:2004. The skin cream was prepared in the present study with the ethyl acetate extract of *E. crassipes* owing to the presence of compounds like long chain fatty acid esters, stigmasterol, glutathione, etc., in this extract. Long chain fatty acid esters are used for the skin cream preparation (25,26), sterols possess antimicrobial (27), anti-inflammatory (28) and other pharmacological properties. Hence, the ethyl acetate extract was chosen among other extracts for the preparation of the skin cream (LPR1). The skin cream made out of ethyl acetate extract, selected quantities of lime and musk (LP3) was prepared to study the synergistic skin whitening action of the composition in the skin cream.

***In vitro* anti-aging property of the skin cream**

Free radical medicated DNA damage inhibition assay

Cellular metabolism has been shown to generate the reactive oxygen species such as hydrogen peroxide, hydroxyl radical, and superoxide radical. Trace metals such as copper and iron that are present in biological systems may interact with active oxygen species, ionizing radiation, or microwaves to damage macromolecules. The cleavage of metalloproteins by oxidative damage may lead to increase in the levels of metal ions in biological cells (29). DNA is one of the major targets of free-radical-induced damage. Under physiological conditions, the constant and endogenous rate of production of free radicals may lead to minimal DNA damage, which is needed to induce the defensive systems and DNA repair mechanisms. However, if the production of free radicals increases, they may attack DNA at either the sugar (deoxyribose) or the base level, giving rise to a large number of toxic products. Attack at the sugar level ultimately leads to a strand break with terminal fragmented sugar residues (30-32). Some of the fragmentation products can be detected by adding thiobarbituric acid (TBA) to the reaction mixture, resulting in formation of a pink (TBA) 2-MDA chromogen (22). The Fenton reaction generates hydroxyl radicals ($OH^{\bullet}$), which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component (29, 33). Hydroxyl radicals are the most reactive radicals that are produced via the Fenton's reaction in living systems. These radicals are mainly implicated in the pathology of several diseases such as Parkinson's disease, rheumatoid arthritis, and carcinogenesis (33).

For any skin cream to possess good aging capacity it should possess efficient antioxidant activity. Free radical medicated DNA damage inhibition of the skin cream LPR1 and LP3 assay is given in Table 2. The DNA damage inhibition offered by the skin creams LPR1 and LP3 (Table 2) increases with increase in concentration (1 mg to 20 mg). The DNA damage inhibition offered by the skin creams LPR1 and LP3 reached a maximum at 5 mg and then decreased gradually afterwards. Increase in percentage DNA damage was noted upto 5 mg concentration for LP3. The variation in the pH/concentration of LP3 probably might have reduced the percentage of DNA damage inhibition.

Table 2. Percentage inhibition of DNA damage of LPR1 and LP3

Concentration of cream (mg)	% Inhibition of DNA Damage	
	LPR1	LP3
1	8.07	12.39
2	17.36	14.14
5	27.78	17.26
10	6.78	1.27
20	4.18	Negative

DPPH radical scavenging activity of the skin cream

Liu *et al*, 2010 have demonstrated the DPPH radical scavenging capability of the leaves to be 74.6% and stem to be 62.7%. Surendraraj *et al*, 2011 (34) reported that the ethanolic extract of flower exhibited high DPPH radical scavenging ability. Shanab *et al*, 2007 (14) has reported high DPPH radical scavenging ability of the ethyl acetate extract compared to methanol extract. Enien *et al*, 2011 (15) reported the presence of hydroxyl groups and unsaturated bonds in the structure of the compounds in the extract of waterhyacinth to be responsible for the DPPH radical scavenging ability. Ho *et al*, (16) reported the IC_{50} of the water and methanol extract of *E. crassipes* in DPPH radical scavenging activity to be greater than 2,000 mg.

The percentage inhibition of the DPPH radical scavenging activity of skin creams LPR1 and LP3 is given in Table 3. The DPPH radical scavenging activity of LPR1 and LP3 shows that with increase in concentration (2 to 50 mg), the radical scavenging activity increases (1 to 6%). This indicates the increase in concentration of the compounds responsible for radical scavenging activity with increasing concentration of the skin cream. Both LPR1 and LP3 shows comparable percentage of DPPH radical scavenging activity which indicates that the constituents added (Lemon and Musk) do not alter the nature of the skin cream in terms of its activity but contributed to the pleasant smell and acidic pH of the skin cream.

Table 3 DPPH radical scavenging activity of LPR1 and LP3

Concentration of cream (mg)	% DPPH scavenged	
	LPR1	LP3
2	1.15	1.23
10	2.57	2.71
20	3.45	3.77
50	6.12	5.19

Conclusion

For an antiaging cream to be effective, it should possess many pharmaceutical properties and the most important being the antioxidative property. Even though, several synthetic chemicals that either prevents or controls aging by any one of the many mechanisms are available, recent research focuses on the use of plant derived products as antiageing agents. Prompt screening of the skin creams prepared with plants is much essential. In the present study, the antiageing efficiency of the skin cream prepared with the ethyl acetate extract of *E.crassipes* was evaluated by DNA damage inhibition assay and DPPH radical scavenging assay. Further

validation of the skin cream is warranted to assess the mechanism by which the cream inhibits aging process of the skin.

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