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ANTI-AGING AND ANTI-WRINKLE POTENTIAL OF EMBLICA OFFICINALIS

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ABSTRACT

Aim: Present study is aimed to evaluate the anti-aging and anti-wrinkle potential of Emblica officinalis.

Method: The methanolic, ethyl acetate, n-butanol and aqueous extracts of Emblica officinalis were prepared and evaluated for its antioxidant, Hyaluronidase, MMP-1/collagenase and elastase inhibition activity.

Results: The IC50 values of the methanolic, ethyl acetate, n-butanol and aqueous extracts of Emblica officinalis and ascorbic acid were found to be 33.04 µg/ml, 36.86 µg/ml, 38.39 µg/ml, 40.87 µg/ml and 29.25 µg/ml respectively. The IC50 of hyaluronidase inhibition activity values of the methanolic, ethyl acetate, n-butanol and aqueous extracts of Emblica officinalis were 11.50 µg/ml, 51.39 µg/ml, 95.54 µg/ml, 137.63 µg/ml. The IC50 values of the methanolic, ethyl acetate, n-butanol and aqueous extracts were found to be 14.40 µg/ml, 20.90 µg/ml, 37.46 µg/ml, 55.91 µg/ml respectively. The IC50 values of MMP-1/collagenase inhibitory activity of the methanolic, ethyl acetate, n-butanol and aqueous extracts of Emblica officinalis were found to be 25.52 µg/ml, 37.83 µg/ml, 46.75 µg/ml, 58.49 µg/ml and standard catechin was 5.96 µg/ml.

Conclusion: The present study revealed that Emblica officinalis may serve as a potent antiaging agent. Among the test samples, Emblica officinalis methanolic extract showed the most potent antioxidant as well as enzymatic inhibition while aqueous extract showed the least antioxidant as well as enzymatic inhibition.

KEYWORDS : Skin ageing, Emblica officinalis, enzymatic, hyaluronidase, elastase, collagenase, and antioxidant

Introduction

Skin, being the largest organ of the human body forming its outermost covering, is directly affected by the external environment, more precisely the ultraviolet radiations which result in aging known as photoaging. The undue exposure of UV radiations leads to various physical and biochemical changes in the skin due to the formation of reactive oxygen species (ROS).[1] Skin appears leathery, shows brown spots, uneven pigmentation, wrinkle, etc.[2] The extracellular matrix (ECM) is the outermost part of skin comprising of fibroblasts and proteins such as collagen and elastin. Aging of the skin is predominantly correlated to the decreased levels of collagen, elastin and hyaluronic acid due to the increased activity of collagenases/ MMP-1, elastases and hyaluronidases, the key enzymes, which are responsible for the maintenance of flexibility, elasticity and strength of the skin.[3-5] Thus, substances that inhibit the activity of these enzymes are important for the prevention of aging. Although, laser rejuvenation, plastic surgery, and lots of synthetic products, such as sunscreen lotions, creams are available for treating photoaging yet, there is a need for natural, herbal anti-aging products which are economical, safer and effective. Hence, plant extracts are considered safe [6] as they are derived from nature.[7] In addition, the natural herbs have despicable mammalian toxicity and can be handled safely. This makes the use of herbal formulations as skin-care products more attractive and common. The present study is aimed to evaluate the anti-aging and anti-wrinkle potential of *Emblica officinalis*. *Emblica officinalis* comes from family Euphorbiaceae and contains tannins, alkaloids, phenolic compounds and flavonoids.[8] It is traditionally used to treat various diseases and has a potent therapeutic value. Previous studies revealed the use of *Emblica officinalis* as a potent antioxidant and can be used in skin aging. The present work is done to analyze the extract of fruits of *Emblica officinalis* for its effect on matrix-metalloproteinases (MMP-1)/collagenases, elastases and hyaluronidases along with the antioxidant potential.

Materials and methods

Collection and Authentication of Plant Material

The *Emblica officinalis* fruits were collected from local market and authenticated from the Department of Botany, Maharshi Dayanand University, Rohtak and submitted to the Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak with voucher specimen no. Ph.cog/2017/201.

Chemicals

1,1-diphenyl-2-picryl-hydrazyl (DPPH), Tris-HCl buffer, Tricine buffer, catechin, Tris-HCl buffer, Collagenase from *Clostridium histolyticum,* Furylacryloyl-Leucine-Glycyl-Propyl-Alanine (FALGPA), N-(methoxysuccinyl)-ala-ala-pro-val 4-nitroanilide (MAAPVN), Albumin, Sodium hyaluronate, Bovine hyaluronidase, Acetate buffer, Dimethyl sulfoxide, Calcium chloride, p-Dimethyl amino benzaldehyde were procured from the Sigma Aldrich Chemicals.

Extraction and fractionation of Plant Material

Powder of *Emblica officinalis* 250 g was extracted by maceration using methanol as a solvent. Maceration was carried out in a closed conical flask for 7 days. The suspension after maceration was collected, filtered, the supernatant evaporated and concentrated. The methanolic extracts of the plant were suspended in water and partitioned successively with ethyl acetate and n-butanol to get the respective secondary extracts using a separating funnel. All the fractions were concentrated and subjected to screening studies.

In-vitro DPPH radical scavenging assay

The ability of the methanol extract and its fractions to scavenge 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical was determined according to the earlier method.[9] Stock solution (1.0 mg/ml) of methanol extract and ethylacetate, n-butanol and water diluted to get the test concentrations of 10, 20, 40, 60, 80 and 100 µg/ml in methanol. Similar concentrations made for ascorbic acid, which acted as a standard. 1ml of a 0.3 mM DPPH in methanol added to the 2.5 ml solution of the test concentrations and standard and allowed to react in dark at room temperature for 30 min. After the reaction time, the absorbances of the test and standard solutions measured at 517 nm spectrophotometrically. Decrease in absorbance by DPPH solution marked the free radical scavenging calculated using the equation:

$$\text{Inhibition (\%)} = [(Ac - As) / Ac] \times 100$$

Where, Ac is the absorbance of the control and As is the absorbance of the extract/standard.

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value is defined as the concentration (µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations.

Hyaluronidase Inhibitory Activity

50 µl bovine hyaluronidase (7900 units/ml dissolved in 0.1M acetate buffer, pH 3.5) mixed with 50 µl of various concentrations of sample (25 µg/ml, 125 µg/ml, 375 µg/ml and 500 µg/ml dissolved in 5% DMSO). Test and control samples incubated at 37° C for 20 minutes. The control group had 50 µl of DMSO instead of plant extract. Hyaluronidases were activated by adding 50 µl of 12.5 mM calcium

chloride in reaction mixture and incubated at 37$_o$C for 20 mins. Then Ca_{+2}activated hyaluronidase subjected to 250 µl of sodium hyaluronate (1.2 mg/ml dissolved in 0.1 M acetate buffer, pH 3.5) and further incubated in water bath at 100$_o$ C, for 3 minutes. Reaction mixture allowed to cool to room temperature. Then 1.5 ml of p-Dimethyl amino benzaldehyde (4 gms) dissolved in 350 ml of glacial acetic acid and 50 ml of 10 N HCl) added to the reaction mixture. The reaction mixture was then incubated at room temperature for 20 mins [10]. The absorbance was measured at 585 nm by using UV spectrophotometer. Inhibitory effect calculated as:

$$\text{Inhibition (\%)} = [(O.D_{\text{of control}} - O.D_{\text{of sample}})/O.D_{\text{of control}}] \text{ X } 100$$

Where O.D = Optical density.

Elastase Inhibitory Activity

Elastase inhibition activity performed as per the earlier method [11] with some modifications. 0.1ml of a 0.2M Tris-HCl buffer comprising albumin (1%), 0.025 ml of a substrate solution 10 mM of N-(methoxysuccinyl)-ala-ala-pro-val 4-nitroanilide, 0.05 ml from sample of different dilutions 25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml for the assay mixed and then 0.025 ml of elastase (3 units/ml) was added. The reaction mixture incubated at 25°C for 20 minutes. The inhibition rate at 410 nm microplate reader.

$$\text{Inhibition rate (\%)} = [1 - (C-D) / (A-B)] \times 100$$

Where, C represents the absorbance with a test sample after incubation. D indicates the absorbance by a test sample before incubation. A indicates the absorbance after incubation without a test sample and B points out the absorbance without a test sample before incubation.

Matrix metalloproteinase-1 / Collagenase Inhibitory Activity

The MMP-1/Collagenase inhibition assay was performed according to the method described by Kim et al.[12] Collagenase (0.8 units/ ml) and synthetic substrate, Furylacryloyl-Leucine-Glycyl-Propyl-Alanine (2mM) were used for the assay. Concentrations of 25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml prepared from the stock solution in Tricine buffer (50 mM). The final reaction mixture contained 25 µl of 50mM Tricine buffer, 25 µl of test extract and 25 µl of 0.1 units collagenase enzyme. After adding 50 µl of 2 mM Furylacryloyl-Leucine-Glycyl-Propyl-Alanine substrate, collagenase activity was measured immediately at 340 nm using a 96 well micro plate reader. Catechin was used as a positive control. The % inhibition was calculated as follows:

$$\text{Enzyme inhibition activity (\%)} = [1 - (B / A)] \text{ X } 100$$

A represents enzyme activity without test extract and B represents activity in the presence of test extract.

Statistical analysis

Each concentration was analyzed in triplicates and the results expressed as the mean value ± standard deviation. Linear regression used for determining half maximal inhibitory concentration) (IC_{50}).

Results and Discussion

In-vitro DPPH radical scavenging assay

Antioxidant studies were carried out using UV spectrophotometer. The absorbance was read at 517 nm for all the extracts at different concentrations. The percentage antioxidant activity of methanolic, ethyl acetate, n-butanol and aqueous extracts of *Emblica officinalis* are presented in Figure 1. The methanolic, ethyl acetate, n-butanol and aqueous extracts of *Emblica officinalis* at 100 µg/ml exhibited a maximum total antioxidant activity of 89.78 %, 83.14 %, 81.39 % and 79.98 % respectively. Whereas for ascorbic acid (standard) was found to be 90.12 % at 100 µg/ml. The IC_{50} values of the methanolic, ethyl acetate, n-butanol and aqueous extracts of *Emblica officinalis* and ascorbic acid were found to be 33.04 µg/ml, 36.86 µg/ml, 38.39 µg/ml, 40.87 µg/ml and 29.25 µg/ml respectively as shown in Table 1.

Hyaluronidase inhibitory activity was also carried out using UV spectrophotometer. The absorbance was read at 585 nm for all the extracts at different concentrations. The percentage of total hyaluronidase inhibitory activity of methanolic, ethyl acetate, n-butanol and aqueous extracts of *Emblica officinalis* are presented in figure 2. The methanolic, ethyl acetate, n-butanol and aqueous extracts of *Emblica officinalis* at 500 µg/ml exhibited a maximum total hyaluronidase inhibitory activity of 96.44 %, 90.21 %, 82.34 %, 77.38 %. The IC_{50} values of the methanolic, ethyl acetate, n-butanol and aqueous extracts of *Emblica officinalis* were found to be 11.50 µg/ml, 51.39 µg/ml, 95.54 µg/ml, 137.63 µg/ml as shown in Table 1.

Elastase inhibitory activity was also carried out using ELISA reader. The absorbance was read at 410 nm for all the extracts at different concentrations. The percentage of total elastase inhibitory activity of methanolic, ethyl acetate, n-butanol and aqueous extracts of *Emblica officinalis* are presented in Figure 3. The methanolic, ethyl acetate, n-butanol and aqueous extracts of *Emblica officinalis* at 100 µg/ml exhibited a maximum total elastase inhibitory activity of 92.48 %, 89.48 %, 79.72 %, 73.69 % respectively. The IC_{50} values of the methanolic, ethyl acetate, n-butanol and aqueous extracts of *Emblica officinalis* were found to be 14.40 µg/ml, 20.90 µg/ml, 37.46 µg/ml, 55.91 µg/ml respectively shown in Table 1.

Matrix metalloproteinase-1/Collagenase inhibitory activity was also carried out using ELISA reader. The absorbance was read at 340 nm for all the extracts at different concentrations. The percentage of total Matrix metalloproteinase -1 / Collagenase inhibitory activity of methanolic, ethyl acetate, n-butanol and aqueous extracts of *Emblica officinalis* are presented in Figure 4. The methanolic, ethyl acetate, n-butanol and aqueous extracts of *Emblica officinalis* at 100 µg/ml exhibited a maximum MMP-1/collagenase inhibitory activity of 87.19 %, 80.19 %, 78.38 %, 71.19 % respectively. Whereas the % inhibition exhibited by the standard (catechin) was found to be 95.72 %. The IC_{50} values of the methanolic, ethyl acetate, n-butanol and aqueous extracts of *Emblica officinalis* and standard catechin were found to be 25.52 µg/ml, 37.83 µg/ml, 46.75 µg/ml, 58.49 µg/ml and 5.96 µg/ml respectively. The comparative MMP-1 / collagenase inhibitory activity of all the extracts of *Emblica officinalis.*

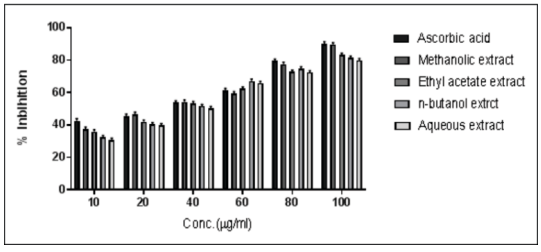

Figure 1. Comparative antioxidant potential of different extracts of Emblica officinalis. All the tests were performed in triplicate and the graph plotted with the average of three observations ± standard deviation. Solution of ascorbic acid served as positive control.

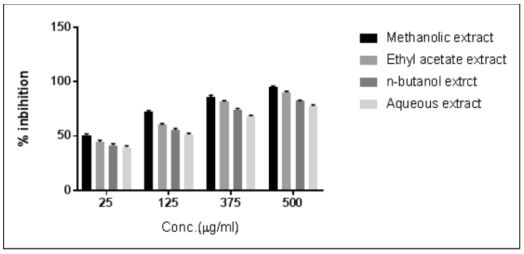

Figure 2. Comparative hyaluronidases inhibition potential of different extracts of Emblica officinalis. All the tests were performed in triplicate and the graph plotted with the average of three observations ± standard deviation.

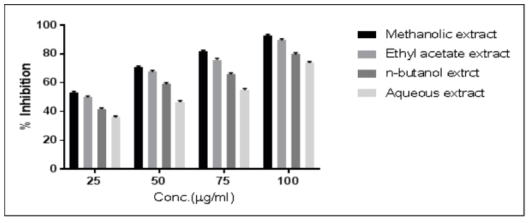

Figure 3. Comparative elastase inhibiton activity of different extracts of Emblica officinalis. All the tests were performed in triplicate and the graph plotted with the average of three observations ± standard deviation.

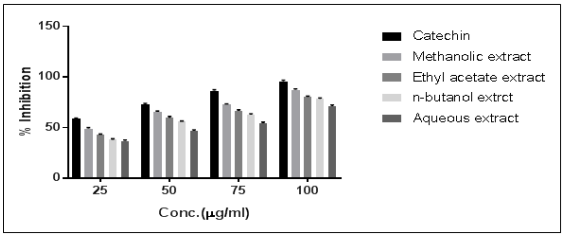

Figure 4. Comparative collagenase inhibition activity of different extracts of Emblica officinalis. All the tests were performed in triplicate and the graph plotted with the average of three observations ± standard deviation.

Table 1. Median inhibition concentration (IC50) of extract of Emblica officinalis extract in different assays.

Assay	Half maximal inhibitory concentration (IC50)			
	Methanol extract	Ethyl-acetate	n-butanol	Aqueous extract
Antioxidant assay	33.04 µg/ml	36.86 µg/ml	38.39 µg/ml	40.87 µg/ml
Hyaluronidase inhibition assay	11.50 µg/ml	51.39 µg/ml	95.54 µg/ml	137.63 µg/ml
Elastase-inhibition assay	14.40 µg/ml	20.90 µg/ml	37.46 µg/ml	55.91 µg/ml
Collagenase-inhibition assay	25.52 µg/ml	37.83 µg/ml	46.75 µg/ml	58.49 µg/ml

The antioxidant and enzyme inhibition activity of different extracts expressed as IC_{50}, calculated by linear regression analysis of mean of three observations.

Conclusion

Emblica officinalis methanolic extracts showed significant antioxidant, anti-collagenase, anti-elastase and anti-hyaluronidase activities. The present study revealed that *Emblica officinalis* may serve as a potent antiaging agent. Among the test samples, *Emblica officinalis* methanolic extract showed the most potent antioxidant as well as enzymatic inhibition while aqueous extract showed the least antioxidant as well as enzymatic inhibition. These results suggested that *Emblica officinalis* could be used as an effective ingredient in cosmetics since it provides protection against various aging enzymes and can be beneficial for skin aging particularly photoaging.

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