Research Article

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COLLAGENASE INHIBITION ACTIVITY OF INDIAN MEDICINAL PLANTS: AN APPROACH TO MODERATE COLLAGEN TURNOVER

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Abstract: Inhibition of collagenase plays a vital role in protecting unbalanced turnover of collagen in human inflamed or UV-irradiated skin. In recent years, plants have been widely investigated and found to have anti-collagenase activity. Therefore, in the present study we have screened eleven Indian medicinal plants *viz., Aegle marmelos, Acalypha indica, Calotropis gigantea, Nerium oleander, Adathoda vasica, Erythrina indica, Acalypha fruiticosa, Vitex negundo, Morinda citrifolia, Nyctanthes arbortristis* and *Acorus calamus* for gelatinase/collagenase inhibition activity. Anti-collagenase activity of plant extract was assessed by plate assay and gelatin-zymogram methods. Primarily, the plant extracts were subjected to investigation for the presence of various phytochemicals such as alkaloids, tannins, saponins, steroids, carbohydrates, glycosides and flavonoids etc. Out of eleven Indian medicinal plants studied, three plants extracts namely *Erythrina indica*, *Nyctanthes arbortris-tis* and *Acorus calamus* exhibited significant collagenase inhibition activity (at 50µg/ml concentration level). The three plants, *Erythrinaindica*, *Nyctanthes arbortris-tis* and *Acorus calamus* find potential cosmetic applications.

Keywords: anti-collagenase activity, plant extract, gelatin-zymogram, *Erythrina indica, Nyctanthes arbortris-tis, Acorus calamus*, Skin barrier.

INTRODUCTION

In general, skin aging is a biological complex process, instigated due to involvement of both intrinsic (such as genetic, hormonal and metabolism changes) and extrinsic (particularly ultra violet A & B radiation from sun) factors. These factors lead to a deterioration of the skin structure, its appearance (like wrinkles, pigmentation and changes in thickness of skin etc.) and as well as function. Indeed, the scientific understanding of the aging process enabled new test procedures to be developed and applied to natural product research. As a result, anti-aging property of plant extracts can now be assessed by inhibition of specific (key) enzymes (biomarkers), especially elastase, hyaluronidase and matrix metallo proteinases (MMP's) which are involved in the biochemical processes/pathway.

Matrix metallo proteinases (MMPs) are zinc dependent endo-peptidases, which are capable of degrading extracellular matrix (ECM) components. In particular, MMPs are responsible for the degradation of type I collagen in the photo-aging process. MMP-2 and MMP-9 are involved in the breakdown of extracellular matrix both at normal physiological condition (such as embryonic development, reproduction and tissue remodeling), as well as at pathological condition (such as arthritis and metastasis).

Inhibition of collagenase activity plays a vital role in protecting the unbalanced turn over or rapid breakdown of collagen in human inflamed or UV-irradiated skin. In recent years, plants have been widely investigated and found to have anti-collagenase and anti-elastase activities. For instance, Polyphenols (catechin and epigallocatechingallate) isolated from green tea (*Camellia sinensis*) are potent inhibitors of collagenase and elastase [1]. Similarly, polyphenols isolated from persimmon (*Diospyros kaki*) leaf extract shown to exhibit anti-collagenase and anti-elastase activity [2] and 5, 6-dehydrokawain from *Alpini azerumbet* [3]. Colhibin (hydrolyzed rice peptides) reported as collagenase inhibitor from rice grains (*Oryza sativa*). Based on the said reports, in the present study, eleven Indian medicinal plants, viz., *Aegle marmelos, Acalypha indica, Calotropis gigantea, Nerium oleander, Adathoda vasica, Erythrina indica, Acalypha fruiticosa, Vitex negundo, Morinda citrifolia, Nyctanthes arbortristis* and *Acorus calamus,* were screened for gelatinase / collagenase inhibitory activity. In addition, these plant extracts were investigated for the presence of various phytochemicals (such as alkaloids, tannins, saponins, steroids, carbohydrates, glycosides, amino acids and flavonoids etc.).

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MATERIALS AND METHODS

Chemicals

Potassium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium chloride and Coomassie brilliant blue (Molecular Biology grade) were all procured from Merck India, Mumbai, Maharashtra State, India. Filter membrane (0.45 µm) from Sartorius India Private Ltd, Mumbai, Maharashtra State, India. Gelatin from porcine skin (Type A) and *Clostridium histolyticum* collagenase / gelatinase (Type IA) was procured from Sigma-aldrich, USA. All other chemicals (Laboratory grade) were procured from S.D. Fine chemicals, Mumbai, Maharashtra State, India

Plant Materials

The eleven Indian medicinal plants, namely, *Aegle marmelos, Acalypha indica, Calotropis gigantea, Nerium oleander, Adathoda vasica, Erythrina indica, Acalypha fruticosa, Vitex negundo, Morinda citrifolia, Nyctanthes arbortristis* and *Acorus calamus* used in the present study were collected from Pasupathikovil, Mathur and Veliathur villages in Thanjavur District, Tamilnadu, India and authenticated by Dr.T.S Subha, Associate Professor, Bharthi Women's College, Chennai (Tamilnadu, India).

Extraction of Plant Materials

Eleven plant materials (leaves of all plants and rhizome of *Acorus calamus*) were shade dried and then pulverized using mixer grinder individually. The powdered plant materials were extracted (soxhlet apparatus) with methanol as a solvent (25 g of each plant material in 100ml solvent respectively).

Phytochemicals Investigation

The presence of phytochemicals like alkaloids, tannins, saponins, steroids, carbohydrates, glycosides and flavonoids were qualitatively determined according to the standard procedures as reported by Dey avijit *et al.*, [4].

Bacterial Collagenase Inhibition Activity Using Simple Plate Method

Bacterial collagenase (or) gelatinase inhibition activity was determined as described by Radhakrishnan *et al.*, [5]. In brief, agar-agar solution (1% w/v) was prepared in gelatinase buffer (50 mMTris-HCl, 10 mM $CaCl_2$, 0.15 M NaCl-pH 7.4) with 0.15% (w/v) of porcine gelatin and allowed to solidify in sterile 60 mm petri plate for 60 minutes at room temperature and upon solidification wells were created. To 50 µl of methanolic extract (1 mg/ml concentration level), 50µl of bacterial (*Clostridium histolyticum* Type-IA) collagenase (0.1 mg/ml of enzyme dissolved in collagenase buffer) was mixed and incubated for 60 minutes. A 50µl of neat collagenase buffer incubated with 50µl of bacterial (*Clostridium histolyticum* Type-IA) collagenase (0.1 mg/ml of enzyme dissolved in collagenase buffer) for 60 minutes acts as a positive control. The resultant reaction solutions were loaded to wells and incubated for overnight at 37°C. The degree of gelatin digestion in agar gel was visualized by Coomassie brilliant blue (CBB) staining. Following destaining, the area of light translucent zone over blue background was determined to estimate gelatinase inhibition activity. Zone of inhibition was expressed in millimeter (mm) and compared with of control.

Gelatin Zymography

The plant extracts shown positive for the above plate method were further confirmed by gelatin zymography, as described by Neely *et al.*, [6]. In brief, samples were prepared by treating the collagenase enzyme (2mg/ml) with 50 µl of the plant extract individually and incubated for half an hour at 37° C. Neat collagenase enzyme along with enzyme buffer was considered as control. After incubation period, 15 µl (approximately 10 µg/ml concentration of protein) were loaded to each well. After electrophoresis, the gel was washed twice with 2.5% Triton X-100 for 60 min in rocker, washed three times with distilled water for 10 min and incubated in an enzyme buffer solution [50 mMTris–HCl, 150mM NaCl, 1 mM $CaCl_2.5H_2O$ (pH 7.4)] under rocking condition for overnight at 37°C. Followed by incubation, drain the enzyme buffer solution, washed with distilled water for 10 min and then stained with (0.25% w/v) Coomassie blue. Following the destaining procedure, the area of light translucent zone over blue background was photographed.

RESULTS AND DISCUSSION

For tissue regeneration and reconstruction, collagenases (MMPs) play an important role. However, it may act as double edged sword, in the sense, if the expression of MMPs is more than the optimum (in the case of diabetic wound patient) or less, the delay in reconstruction and healing has been observed. Thus, Tissue inhibitor of MMPs (TIMP) is produced under *in situ* condition during the healing processes. However, for diabetic patients, the production of TIMP is not regulated properly and thus necessitates the need of external inhibitors. Since, the current research on wound healing focuses the traditional medicines, it is our duty to explore the potency of traditional plants. With regard to anti-collagenase activity of plants, only few studies were available. Hence, an attempt was made in the present study to explore the anti-collegenase activity of Indian traditional medicinal plants. The results of the study revealed interesting observations. The initial experiments on phytochemical analyses of crude methanolic extract of the chosen plants *Aegle marmelos, Acalypha indica, Calotropis gigantea, Nerium oleander, Adathoda vasica, Erythrina*

indica, Acalypha fruiticosa, Vitex negundo, Morinda citrifolia, Nyctanthes arbortristis and *Acorus calamus* suggested the presence of alkaloids, tannins, saponins, glycosides, carbohydrates, flavonoids and steroids as shown in Table 1. With regard to individual plants, *Acalypha indica* showed the presence of alkaloids, tannins, saponins, glycosides and steroids similar to the reports of Chandra mohan *et al.,* [7] and & Senthil murugan *et al.,* [8]. The presence of phytochemicals observed in *Morinda citrifolia* in the present study correlates well with the findings of Samiraj *et al.,* [9]. And for *Erythrina indica*, the results matches with the study reported by Ramila Devi *et al.,* [10]. Alike the report of Rose *et al.,* [11], in the present study also we observed the presence of phytochemicals such as alkaloids, carbohydrates, flavonoids, steroids and amino acids in *Vitex negundo*. Corresponding to the findings of Balasubramaniam [12], *Nyctanthes arbortris-tis* (methanolic extract) also showed the presence of tannins, saponins, flavonoids, glycosoides & steroids. The presence of alkaloids, tannins, saponins, glycosides, carbohydrates, flavonoids, steroids and amino acids were also observed in the methanolic extract of *Acorus calamus*, similar to the report of Senthilkumar *et al.,* [13]. *Aegle marmelos* and *Nerium Oleander* showed the presence of alkaloids, tannins, steroids, saponins, flavonoids, glycosides, carbohydrates similar to the study report of Uma Devi *et al.,* [14] and Bhuvaneshwari *et al.,* [15]. *Calotropis gigantea* showed the presence of all phytochemicals except carbohydrates [16]. *Adathoda vasica* showed the presence of all phytochemicals except glycosides and these results were on par with the study of Subhasini *et al.,* [17].

Followed by phytochemical analyses, the methanolic extract of the samples were subjected to collagenase/gelatinase inhibition activities and the results were summarized in Table 2. Out of eleven Indian medicinal plants studied, three plants extract namely *Erythrina indica*, *Nyctanthes arbortris-tis* and *Acorus calamus* displayed significant collagenase inhibition activity by simple plate method. Collagenase inhibition activity was further confirmed by gelatin zymogram method and all the three plant extracts shown to inhibit collagenase activity at 50µg/ml concentration level (Fig. 1). The results of the present study well agreed with the study of Benoit [18], who, reported that *Acorus calamus* shown to inhibit collagenase activity. Similarly, *Erythrina indica* & *Nyctanthes arbortris-tis* shown to exhibit collagenase activity at 50µg/ml concentration level respectively and these were on par with the studies of Naomi *et al.,*[19], Masazumi *et al.,*[20], Imadakeisuke [21] and Masazumi *et al.,*[22] respectively.

Table 1: Phytochemical analysis of traditional Indian medicinal plants.

Plants	Alkaloids	Tannins	Saponins	Glycosides	Carbohydrates	Flavonoids	Steroids
Acalypha indica	+	+	+	+	-	-	+
Morinda citrifolia	+	+	-	+	+	+	+
Erythrina indica	+	-	+	+	+	-	+
Vitex negundo	+	+	+	+	+	+	+
Nyctanthesarbortristis	-	+	+	+	-	+	+
Acorus calamus	+	+	+	+	+	+	+
Aegle marmelos	+	+	+	+	+	+	+
Calotropis gigantea	+	+	+	+	-	+	+
Adathoda vasica	+	+	+	-	+	+	+
Nerium oleander	+	+	+	+	+	+	+
Acalypha fruiticosa	+	+	+	-	-	+	-

+ - Positive; - - Negative

Table 2: Gelatinase inhibition assay (Plate method) of the Methanolic extract of the chosen medicinal plants

Plants name	Zone(mm)	Remarks
Aeglemarmelos	12±1	50% inhibition
Acalyphaindica	12±1	50% inhibition.
Calotropisgigantea	12±1	50% inhibition.
Nerium oleander	12±1	50% inhibition.
Adhathodavasaka	15±2	< 25 % inhibition.
Erythrinaindica	10±1	> 50% inhibition.
Acalyphafruiticosa	14±2	< 25 % inhibition.
Vitexnegundo	12±1	50% inhibition.
Morindacitrifolia	15±2	< 25 % inhibition.
Nyctanthesarbortristis	10±0.5	> 50% inhibition.
Acoruscalamus	10±0.5	> 50% inhibition.
Control (Enzyme alone)	24±1	No inhibition.

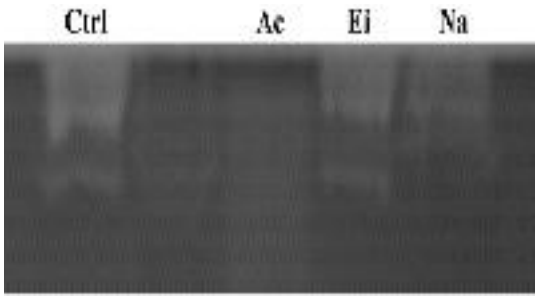

Fig.1: Anti-gelatinase acitivity of plant extract in comparison with control.

Ctrl- Collagenase Enzyme alone (50µl)
Ac- Methanolic extract of *Acorus calamus* (50µl) + Collagenase Enzyme (50µl)
Ei- *Erythrina indica* (50µl) + Collagenase Enzyme (50µl)
Na- *Nyctanthes arbortristis* (50µl) + Collagenase Enzyme (50µl)

CONCLUSIONS

The study concludes that Indian traditional medicinal plants display significant anti-collagenase activity, which, if properly exploited, certainly it will be a material of interest in cosmetic industries.

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