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Exploring Medicinal Plants as Natural Inhibitors of Collagenase, Elastase, and Hyaluronidase: A Novel Approach for Cosmeceutical Innovation

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The market value of natural cosmetics continues to rise due to their reduced side effects, resulting in their increasing amalgamation into cosmetic formulations. Plant-derived materials are a rich source of bioactive compounds that can serve as anti-aging and anti-wrinkle agents, as well as therapeutic agents for various dermatological conditions. In this study, 140 medicinal plant materials were collected, taxonomically authenticated, and subjected to ethanolic extraction. The resulting extracts were screened for their inhibitory activity against *Clostridium histolyticum* collagenase (ChC), Porcin pancreatic elastase (PpE), and Bovine testes hyaluronidase (BtH) for identifying potential anti-aging agents suitable for cosmetic applications. The highest total phenolic content (TPC) (31.95 ± 0.045 mg/ml) was observed in *Terminalia arjuna* bark. Overall, 71, 42, and 59 plant extracts exhibited anti-ChC, anti-PpE, and anti-BtH activities, respectively. Among all inhibitory activity exhibiting plants, *Terminalia arjuna* bark exhibited the highest inhibitory activity against ChC, PpE (99.95 ± 1.3% and 99.98 ± 1.5% at 100 μg/ml TPC), and BtH (98.80 ± 2.4% at 150 μg/ml TPC). *Woodfordia fructicosa* flower, *Peltophorum pterocarpum* leaf, and *Bergenia ligulata* root exhibited maximum inhibitory potential against aging enzymes. The inhibitory activities of plant extracts were found to be dependent upon the concentration of TPC. A one-way ANOVA test revealed plant extracts exhibit significant inhibitory potential ($p<0.05$) against aging enzymes. The findings indicate that medicinal plants exhibiting effective inhibitors of collagenase, elastase, and hyaluronidase could be useful for application in cosmetic formulations.

Keywords: Terminalia arjuna, Woodfordia fructicosa, Peltophorum pterocarpum, Bergenia ligulata, collagenase, elastase, hyaluronidase.

Skin, the outermost and essential barrier of the body, performs various biophysical and biochemical functions for maintaining overall human health.[1] Skin aging is an inevitable and irreversible process, broadly divided into extrinsic and intrinsic pathways, both are responsible for unexpected changes in skin structure and elasticity. Extrinsic skin aging is caused by environmental factors, such as pollutants, sunlight exposure (photoaging), and various lifestyle habits (i.e., smoking and diet). whereas, intrinsic skin aging is a natural process and is influenced by genetic

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programming and molecular mechanisms inherent to the body.[2] Among these, the skin's exposure to UV-radiation is responsible for the overproduction of reactive oxygen species (ROS) that induce lipid peroxidation, protein denaturation, and DNA damage.[3] The excess accumulation of ROS accelerates ECM (extracellular matrix) degradation and induces skin aging with consequences such as roughness, wrinkling, elasticity loss, and patchy pigmentation.[3,4]

The dermis, the structural foundation of the skin, is predominantly composed of ECM, which comprises structural proteins (collagen and elastin) along with fibroblasts that regulate their synthesis and remodeling.[5] Collagen is a high molecular weight and significant structural component of the ECM, maintaining tensile strength and elasticity of the skin. Subsequently, elastin, also a structural component in connective tissue as elastic fibers, provides elasticity and recoiling properties to the skin. These are the crucial components of the skin, playing a vital role in maintaining the skin's flexibility, plumpness, and overall integrity of freshness and health.[6,7] However, prolonged skin exposure to solar radiation (photo-aging) enhances ROS accumulation that triggers the activation of collagenase and elastase, which are responsible for unwanted degradation of collagen and elastin.[8,9] Therefore, the expression of collagenase and elastase causes skin aging with visual appearances such as freckles, wrinkles, severe atrophy, sallowness, loss of elasticity, and rough texture.[10]

Hyaluronan, or Hyaluronic acid (HA), is a high molecular weight polymer found in tissue and body fluids. It is predominantly available in the epidermal and dermal layers of the skin. It maintains moisture content, promotes skin rejuvenation, impairs selective permeability of extracellular fluid, and increases viscosity.[11] It has a prominent water-holding capacity; therefore, its highly concentrated surface aids in the smoothness, emollience, and youthful appearance of the skin.[10] Hyaluronidase enzyme decreases the level of HA by cleaving it into small oligosaccharide molecules during the aging process, leading to loss of strength, moisture, and flexibility, decreasing fluid thickness, and facilitating substance movement through connective tissues.[12]

Clostridium histolyticum collagenase (ChC), a bacterial enzyme, can degrade collagen, mainly types I, II, III, and IV, by breaking peptide bonds present in the collagen triple helix.[13] Its amino acid sequence is moderately similar to the amino acid sequence of human collagenase (specifically MMP-1); both are zinc-dependent metalloproteinases, and their zinc-binding catalytic domain performs protease activity for collagen cleavage. This domain has a conserved glutamate and histidine amino acid residue motif, which coordinates with zinc ions for enzymatic catalysis.[14] These proteolytic enzymes recognize the same specific cleaving site at glycine-proline peptide bonds on collagen and share sequence homology in the range of 20-30%, suggesting conserved functional similarities among them.[15]

Porcine pancreatic elastase (PPE) and human neutrophil elastase (HNE) are serine proteases capable of degrading elastin and extracellular matrix proteins. Both enzymes share structural and functional similarities, and their active sites (catalytic triad), which consist of serine, histidine, and aspartate residues, are responsible for proteolytic activity.[16] Despite their structural and functional similarities, they share approximately 43% amino acid sequence identity, with slight differences in their non-catalytic domains that affect their regulation and tissue distribution.[17]

Bovine testis hyaluronidase (BtH) and human hyaluronidase are both enzymes that catalyze the hydrolysis of hyaluronic acid, a glycosaminoglycan found in the ECM. These enzymes share excellent structural and functional similarities and possess a conserved catalytic domain that contains a histidine-aspartate dyad necessary for enzymatic activity.[18,19] These enzymes have similar substrate specificity, cleaving hyaluronic acid and contributing significantly to wound healing, tissue remodeling, and inflammation. Despite their structural and functional similarity, they share amino acid sequence identity with differences in their non-catalytic regions, which are involved in tissue distribution and regulation.[20] These enzymes (ChC, PpE, and BtH) are not human isoforms, but they are widely applied as model enzyme in preliminary enzyme inhibitor studies due to their structural and mechanistic similarity to enzymes originated from human. Collagenase, elastase, and hyaluronidase are considered skin-aging enzymes, and their inhibitors have gained attention as essential components in the cosmetic

industry as well as for controlling skin aging. The use of these inhibitors for the formulation of cosmetic products offers significant strategies for reducing visible signs of aging and improving skin health.[21]

Plant-synthesized polyphenols have received larger awareness in the pharmaceutical and food industries owing to their health benefits, reducing the chronic diseases due to their anti-cancer, anti-inflammatory, anti-oxidant, cardio-protective, and anti-proliferative properties.[22] It has been investigated that some plant-derived polyphenols, like resveratrol, quercetin, and curcumin, exhibit significant anti-aging properties by improving skin barrier function, promoting collagen synthesis, and reducing signs of photoaging.[23,24] Natural products are highly preferred as remedies for their safety profile and management of different types of diseases with the efficiency of skincare properties. Numerous plant-derived therapeutic products exhibit additional beneficial properties, including barrier repair, moisturizing, antioxidant, anti-inflammatory, and photoprotective or skin-whitening effects, as evidenced by traditional and local usage. These properties support the utilization of plant extracts in anti-aging skincare formulations, offering multifunctional benefits for skin health. Therefore, plant-derived products suggest a promising opportunity for exploring their potential as therapeutic agents against skin aging. This study was aimed at screening medicinal plant extracts for their inhibitory activities against PpE, (ChC), and BtH and at determining the inhibitory potential of plants showing inhibitory activities.

MATERIALS AND METHODS

Porcine pancreatic elastase (PpE), *Clostridium histolyticum* collagenase (ChC), Bovine testes hyaluronidase (BtH), Azocasein, Hyaluronic acid, and N-Succinyl-Ala-Ala- Ala-*p*-nitroanilide (AAAPVN) were purchased from Sigma Aldrich. Tris, X-ray film, Hydrochloric acid (HCL), Sodium phosphate, Sodium chloride, Sodium acetate, Calcium chloride, Cetylpyredium chloride, Sodium carbonate, and Ethanol were purchased from RANKEM. Gallic acid and Folin-Ciocalteau reagent were obtained from Hi-MEDIA and SRL, respectively. The different medicinal plant materials (140) belonging to various families were procured from the local market of Chhatrapati Sambhaji Nagar (MS), India and Dr. B. A. M. University campus, Chhatrapati Sambhaji Nagar (MS), India. Each plant specimen was authenticated by a taxonomist from the Department of Botany, GNA College, Barshitakli, Dist., Akola (MS), India (Table 1).

Preparation of extracts

The collected different plant samples were completely dried at 37 ^{0}C in an oven, pulverized into fine powders by a grinder mixer, and stored in a moisture-free compartment at room temperature for further use. All powders were extracted using 70% ethanol, with a few modifications to the previously reported,[25] procedure. Each plant powder was extracted by soaking in solvent (1:10 w/v) and stirring for an hour with a magnetic stirrer. Thereafter, suspensions were filtered through Whatman filter paper (No. 1), and filtrates were preserved at 4 ^{0}C for further testing.

Estimation of total phenolic content (TPC)

By using the slight modification of the previously reported,[26] procedure the TPC from each plant extract was estimated. At room temperature, twenty microliters of each plant extract (diluted up to 1.5 ml with distilled water) was incubated with Folin-Ciocalteau reagent (0.5 ml) for 3 min. To this test, 1ml of sodium carbonate (10% w/v) was added for neutralization. All tests were incubated at room temperature until color development. Thereafter, the optical density was measured at 650 nm using a spectrophotometer, the TPC was calculated using a gallic acid standard graph, and its amount was expressed as gallic acid equivalent (GAE mg/ml).

Screening of plant extracts for inhibitory activities against ChC and PpE

All plant extracts were screened for inhibitory activities by using a spot test on X-ray film[27]. The principle of this method is based on the digestion of gelatin coated on X-ray film and the appearance of a blue spot against the overall surface of the film. In a classic reaction mixture, 10 µl ChC (200 µg/ml prepared in 50 mM Tris-HCL buffer pH 7.5, 50 mM $CaCl_2$, 100 mM NaCl) was combined with 10 µl buffer, 10 µl of each plant extract and incubated at 37^{0}C for 5 min. For control of enzyme activity, an aliquot was prepared without plant extract. A ten-microliter sample from each mixture was spotted on X-ray film and incubated for 20 min at 37 ^{0}C. After incubation, X-ray films

were washed under tap water and completely dried at room temperature. Inhibition of the ChC was assessed by comparing the control activity of ChC. The location of spots where unhydrolyzed gelatin revealed the plants containing inhibitory activity, and spots that appeared as hydrolyzed gelatin/blue spots indicated the absence of inhibitory activity in plant extracts. Photographs of X-ray films were taken by a megapixel camera. The same procedure was applied for screening of all plant extracts for inhibitory activities against PpE (50 µg/ml prepared in 0.1 mM Tris-HCL buffer pH 8).

Screening of plant extract for inhibitory activities against BtH

Inhibitory activities of plant extracts against BtH were screened using the previously reported[28] procedure with a few modifications. The principle of this technique is based on the measurement of undigested hyaluronic acid by its property to form turbidity with BSA (Bovine Serum Albumin) reagent. The absorbance of turbid hyaluronic acid provides the concentration of undigested hyaluronic acid and hence can be related to the enzyme activity. The solutions of BtH (1 mg/ml) and hyaluronic acid (1.2 mg/ml) were prepared in 0.1M sodium phosphate buffer (pH 5.3, 0.15M NaCl). For the enzymatic reaction, a mixture containing 50 µl BtH and 50 µl plant extract was incubated at 37ºC for 10 min. After incubation, 0.5 ml of hyaluronic acid was added and incubated at 37ºC for 20 min; finally, the hyaluronidase activity was stopped by the addition of BSA reagent. Simultaneously, one aliquot was prepared without plant extract for control. The plant extracts containing inhibitory activities were confirmed by the formation of turbidity after the addition of BSA and vice versa.

Inhibitory assay of plant extracts against ChC

The inhibitory potential of plant extracts against ChC was assessed by azocaseinolytic assay, using the earlier applied[29] method with slight modification. In 2 ml centrifuge tubes, containing 40 µl ChC (0.2 mg/ml) along with 140 µl buffer (50 mM Tris-HCL pH 7.5, 5 mM $CaCl_2$) and 20 µl plant extract (100 µg TPC/ml of each plant extract), were incubated at 37^{0}C for 10 min. Thereafter, the enzymatic reaction of each mixture was started by adding 50 µl azocasein (1% w/v) and incubated at 37^{0}C for 1 hour. The proteolytic activity from each aliquot was terminated by mixing of 60 µl TCA (5 %), and all mixtures were allowed to centrifuge at 6000 rpm for 15 min. The supernatant (150 µl) of each mixture was transferred into a microtiter plate containing an equal volume of 1 N NaOH, and absorbance was measured on a microtiter plate reader at 405 nm. The following equation was applied for the calculation of the percent inhibition.

$$\% \text{ Inhibition} = [A_{control} - A_{sample}] / A_{control} \times 100$$

Where $A_{control}$ is the absorbance of ChC activity on azocasein and A_{sample} is the absorbance of ChC activity on azocasein in the presence of plant extract.

Inhibitory assay of plant extracts against PpE

A synthetic substrate (N-Succinyl-Ala-Ala- Ala-p-nitroanilide) was used for the determination of the inhibitory potential of plant extracts with slight modification to the previously reported[30] method. The solution of PpE (50 µg/ml) and substrate (2 mM) was made in 0.1 mM Tris-HCL buffer pH 8. A reaction mixture containing 50 µl PpE, 900 µl buffer, and 50 µl plant extract (100 µg TPC/ml of each plant extract) was incubated at 25^{0}C for 10min. After incubation, 50 µl substrate was mixed and kept at 25°C for 90 min. Thereafter, the absorbance was measured at 410 nm on a UV-VIS spectrophotometer. Simultaneously, one aliquot was also kept for a control activity without plant extract. The following equation was used for calculating the percent inhibition.

$$\% \text{ Inhibition} = [A_{control} - A_{sample}] / A_{control} \times 100$$

Where $A_{control}$ is the absorbance of PpE activity on substrate, and A_{sample} is the absorbance of PpE activity on substrate in the presence of plant extract.

Inhibitory assay of plant extracts against BtH

The inhibitory potential of plant extracts against BtH was determined using the procedure described in the above screening section. Briefly, 50 µl BtH was mixed with 50 µl plant extract (100 µg TPC/ml of each plant) and incubated at 37^{0}C for 10 min; finally, the reaction was terminated by the addition of 2 ml BSA reagent. After the formation of turbidity, the optical density of all tests was measured at 540 nm. The amount of turbidity

formed from hyaluronic acid is considered as blank (100% enzyme inhibition). The following equation was used for calculating the percent inhibition.

$$\% \text{ Inhibition} = [A_{blank} - A_{sample}] / A_{blank} \times 100$$

Where A_{blank} is the absorbance of turbidity formed by hyaluronic acid, and A_{sample} is the absorbance of BtH activity on hyaluronic acid in the presence of plant extract.

Statistical analysis

Assessment of total phenolic content (TPC) and inhibitory assay was carried out in triplicate readings. Mean TPC values, inhibitory potentials, and their standard deviations were calculated using MS Excel.

RESULTS

Total phenolic content (TPC) in plant extracts

The estimated total phenolic contents (TPCs) for all plant extracts are presented in Table 1. Based on their concentrations (mg/ml), the plant extracts (140) were categorized into five groups: very high (>5 mg/ml TPC; 8 plants), high (2-4 mg/ml TPC; 37 plants), moderate (1-2 mg/ml TPC; 18 plants), low (0.1-1 mg/ml TPC; 65 plants), and very low (<0.1 mg/ml TPC; 12 plants). The highest concentration of TPC (31.95 ± 0.045 mg/ml) was observed in *T. arjuna* bark, while the lowest concentration of TPC (0.02 ± 0.015 gm/ml) was observed in *G. arborea* leaf extract. The other maximum TPC was found in *H. spicatum* rhizome (18.5 ± 0.49 mg/ml), *A. vera* leaf (17.85 ± 0.056 mg/ml), *V. rosea* stem (11.15 ± 0.03 mg/ml), *A. lebbeck* bark (10.4 ± 0.37 mg/ml), *G. glabra* stem (8.86 ± 0.024 mg/ml), *P. somniferum* stem (6.45 ± 0.004 mg/ml), *P. kurroa* stem (5.12 ± 0.009 mg/ml), *S. robusta* leaf (4.96 ± 0.04 mg/ml), and *A. catechu bark (*4.55 ± 0.030 mg/ml). The significant variation was observed in TPC among plant extracts. That indicates the diverse nature of phenolic compounds among the selected plants.

Inhibitory activities in plant extracts

Based on visual observation of X-ray films, out of 140 plant extracts, 71 exhibited anti-ChC activities, while 42 exhibited anti-PpE activities (Fig-1 and -2). The turbidity assay of hyaluronidase activity revealed that the 59 plant extracts exhibited anti-BtH activities. Thirty-six plants exhibited inhibitory activities against all three enzymes (ChC, PpE, and BtH). In most cases, the inhibitory activity of plant extract was found to be dependent upon the concentration of TPC (Table 1). The number of inhibitory activities showing plants would have increased if the concentration of TPC in plant extracts had been higher during screening. Some plants (*C. roseus* flower, *H. indicus stem, C. borivilianum* leaf, *B. montanum* leaf, *S. nuxvomica* leaf, *O. tenuiflorum* seed, *A. sativum* leaf, *C. pungens* stem, *C. hyanae* root, *B. prionitis* stem, *B. cristata* root, and *A. reticulata leaf*) contain adequate concentrations (>0.5 mg/ml) of total phenolics, but they did not exhibit the inhibitory activities against any enzyme. It indicates that different types of phenolic compounds may be present in these plants, or the phenolics of these plants may not have inhibitory properties. It was observed that 14 plants (*W. somenifera* stem, *P. betel* stem, *M. aquatica* leaf, *D. regia* flower, *B. racemosa* bark, *A. salviifolium* leaf, *R. dumetorum* fruit, *P. kurroa* stem, *I. racemosa* root, *C. serratum* stem, *S. indicum* leaf, *C. frutescens* leaf, *C. deodara* leaf, and *G. obtusa* fruit) exhibited only anti-ChC activity, while 2 plants (*T. adscendens* leaf and *C. tora* root) and seven plants (*A. marmelos* fruit, *T. ammi* seed, *C. wightii* fruit, *O. sanctum* flower, *P. nigrum* seed, *C. paniculatus* seed, and *M. pinnata* seed) exhibited only anti-PpE and anti-BtH activities, respectively (Table 1). These findings recommend that these plants may have specific inhibitory activities against enzymes.

Inhibitory potentials of plant extract against ChC, PpE, and BtH

Out of 140 plants, 36 exhibiting inhibitory activities against all three enzymes were considered to have high inhibitory potential. Therefore, these plants were selected for determining their inhibitory efficacies using a standard solution assay (Table 1). These plants were grouped partwise (bark; fruit, fruit rind, flower, and seed; leaves; stem, root, and rhizome) and sorted based on inhibitory potential as shown in figs. 3 to 6. One-way ANOVA test analysis revealed the plant extracts exhibited significant ($P<0.05$) inhibitory potentials.

Inhibitory potentials of plant barks

Among all barks as well as all plants considered in this study, *T. arjuna* exhibited the highest inhibitory potential against ChC, PpE (99.95 ± 1.3% and 99.98 ± 1.5% at 100 µg/

Table 1. List of various medicinal plants, their estimation of total phenolic content mg/ml GAE, and screening for inhibitory activities against collagenase, elastase, and hyaluronidase. + indicates the presence of inhibitory activities in the plant extracts, and - indicates the absence of inhibitory activities in plant extracts. The serial numbers of plant samples reported in Fig. 1 and 2 are indicated as superscripts.

Plant Name	TPCmg GAE/ml
[1]*Phyllanthusemblica* fruit C+, P+, H+	3.9± 0.03
[5]*Justiciaadhatoda* leaf C- E- H-	0.12± 0.007
[9]*Centellaasiatica* leaf C+ E- H+	0.57± 0.004
[13]*Trachyspermum ammi* seed C- E- H+	0.95± 0.002
[17]*Eclipta prostrata* leaf C- E- H-	0.05± 0.008
[21]*Commiphorawightii* fruit C- E- H+	0.55± 0.043
[25]*Azadirachtaindica* bark C+ E+ H+	1.85± 0.005
[29]*Cyperus rotundus*rhizome C- E- H-	0.26± 0.005
[33]*Psoraleacorylifolia* seed C+ E- H+	0.85± 0.006
[37]*Tribulus adscendens* leaf C- E+H-	0.45± 0.003
[41]*Piper betel*stem C+ E- H-	1.05± 0.002
[45]*Citrulluscolocynthis* fruit C+ E- H+	0.34± 0.03
[49]*Catharanthusroseus* leaf C+ E+ H+	4.05± 0.003
[53]*Celastruspaniculatus* seed C- E- H+	0.37± 0.002
[57]*Piper longum* stem C+ E- H+	2.55± 0.003
[61]*Randia dumetorum* fruit C+ E- H-	1.65± 0.004
[65]*Leptadeniapyrotechnica* stem C+ E+ H+	3.23± 0.007
[69]*Ricinuscommunis* seed C+ E- H+	1.14± 0.003
[73]*Neltuma juliflora* bark C+ E+ H-	0.75± 0.013
[77]*Papaver somniferum* stem C+ E+ H+	6.45± 0.004
[81]*Pluchealanceolata* leaf C+ E- H+	2.12± 0.011
[85]*Aeglemarmelos* fruit C+ E+ H+	1.54± 0.012
[89]*Capsicum frutescens* leaf C+ E- H-	0.81± 0.007
[93]*Limonia acidissium* bark C- E- H-	0.16 ±0.09
[97]*Nerium indicum* flower C- E- H-	0.10± 0.006
[101]*Acorus calamus* leaf C- E- H-	0.05± 0.008
[105]*Tridax procumbens* leaf C-E-H-	0.03± 0.01
[109]*Erythrena superba* bark C- E- H-	0.07± 0.05
[113]*Cassia pulcherima* leaf C+ E+ H+	1.69 ± 0.008
[117]*Albezia lebbeck* bark C+ E+ H+	10.4 ± 0.37
[121]*Marsdenia tenacissima* stem C- E- H-	0.36 ± 0.01
[125]*Barleria cristata* root C- E- H-	0.50 ± 0.009
[129]*Barleria montana* root C- E- H-	0.46 ± 0.008
[133]*Cassia obstusifolia* leaf C- E-H-	0.16 ± 0.04
[137]*Hyptis suaveolens* leaf C+ E+ H-	1.64 ± 0.042
[2]*Catharanthusroseus* flower C-, E-, H-	0.7± 0.008
[6]*Desmodiumgangeticum* stem C- E- H-	0.13± 0.01
[10]*Neriumoleander* leaf C+ E- H+	0.80± 0.03
[14]*Rauvolfiaserpentine* root C- E- H-	0.15± 0.007
[18]*Convolvulus prostrates* stem C- E- H-	0.06± 0.001
[22]*Bergenialigulata* root C+ E+ H+	2.9± 0.003
[26]*Tinosporacordifolia* stem C- E- H-	0.14± 0.003
[30]*Vinca rosea* stem C+ E+ H+	11.15±0.03
[34]*Bacopamonnieri* leaf C- E- H-	0.26± 0.003
[38]*Cissus quadrangularis* stem C- E- H-	0.15± 0.001
[42]*Menthaaquatica* leaf C+ E- H-	1.41± 0.003
[46]*Delonixregia* flower C+ E- H-	0.65± 0.004

[50]*Terminaliabellirica* fruit C+ E+ H+	1.92± 0.001
[54]*Boerhaviadiffusa* stem C- E- H-	0.42± 0.001
[58]*Alstoniascholaris* stem C+ E- H+	2.55± 0.014
[62]*Picrorrhiza kurroa* stem C+ E- H-	5.12± 0.009
[66]*Inularacemosa* root C+ E- H-	2.25± 0.04
[70]*Solanumindicum* leaf C+ E- H-	0.25± 0.003
[74]*Mesua glabra* bark C+ E+ H+	3.13± 0.012
[78]*Cannabis sativa* seed C+ E- H+	0.82± 0.023
[82]*Terminaliaarjuna* bark C+ E+ H+	31.95±0.045
[86]*Stereospermumsuaveolens* bark C+E+H+	3.31± 0.046
[90]*Coriandrumsativum* leaf C- E- H-	0.05± 0.006
[94]*Diospyros melanoxylon* bark C+E+H+	1.94 ±0.04
[98]*Gliricidia sepium* leaf C- E- H-	0.18± 0.01
[102]*Andrographis paniculata* leaf C- E- H-	0.05± 0.062
[106]*Commiphora mukul* rhizome C+ E+ H+	2.89± 0.01
[110]*Shorea robusta* leaf C+ E+ H+	4.96± 0.04
[114]*Madhuca indica* leaf C- E- H-	0.15± 0.07
[118]*Annona reticulate* leaf C- E- H-	0.99 ± 0.01
[122]*Terminalia chebula* fruit C+ E+ H+	1.9 ± 0.04
[126]*Nyctanthesarbor-tristis* leaf C+ E+ H+	3.4± 0.07
[130]*Nyctanthes arbortristis* leaf C- E- H-	0.37± 0.01
[134]*Clematis hyanae* root C- E- H-	0.67± 0.01
[138]*Barleria obtusa* leaf C- E- H-	0.33± 0.05
[3]*Stenocereuskerberi* leaf C-, E-, H-	0.2± 0.005
[7]*Lawsoniainermis* leaf C+ E+ H+	2.8± 0.003
[11]*Woodfordiafructicosa* flower C+ E+ H+	3.65± 0.06
[15]*Tephrosia purpurea* leaf C- E- H-	0.18± 0.041
[19]*Gloriosasuperba* fruit C+ E+ H+	4.35± 0.012
[23]*Vachellianilotica* stem C+ E+ H+	1.35± 0.003
[27]*Ocimum sanctum* flower C- E-H+	0.45± 0.001
[31]*Justicia gendarussa* leaf C- E- H-	0.15± 0.008
[35]*Withaniasomenifera* stem C+ E- H-	0.62± 0.003
[39]*Pongamia pinnata* fruit C- E- H-	0.15± 0.003
[43]*Rubia cordifolia* stem C+ E- H+	1.07± 0.007
[47]*Solanumxanthocarpum* fruit C+ E- H+	1.33± 0.003
[51]*Bauhinia racemosa* bark C+ E- H-	0.65± 0.005
[55]*Strychnosnuxvomica* leaf C- E- H-	1.75± 0.003
[59]*Hemidesmusindicus* stem C- E- H-	1.35± 0.001
[63]*Aloe vera* leaf C+E+H+	17.85± 0.056
[67]*Terminalia elliptica* bark C+ E- H+	3.75± 0.004
[71]*Saussurealappa* root C- E- H-	0.18± 0.06
[75]*Ficusracemosa* bark C+ E+ H+	2.65± 0.010
[79]*Plumbagozeylanica* leaf C+ E- H+	2.15± 0.003
[83]*Clerodendrumphlomidis* stem C+ E+ H+	2.82± 0.006
[87]*Crataeva Nurvala* leaf C+ E+ H+	4.35± 0.006
[91]*Cassia fistula* Pod C+ E+ H-	2.63± 0.12
[95]*Crocus sativus* flower C- E- H-	0.096 ±0.02
[99]*Cedrus deodara* leaf C+ E- H-	2.48± 0.08
[103]*Peltophorum pterocarpum* leaf C+ E+ H+	3.46± 0.20
[107]*Leucasaspera* leaf C- E- H-	0.09± 0.01
[111]*Bauhinia variegata* bark C- E- H-	0.25± 0.009
[115]*Grewia tiliaefolia fruit C-E-H-*	0.28 ± 0.07
[119]*Terminalia argentea* bark C+ E+ H+	3.68 ± 0.14
[123]*Crossandra infundibuliformis* root C- E- H-	0.38 ± 0.01
[127]*Datura metal* fruit C- E- H-	0.26 ± 0.01
[131]*Ficusbenghalensis* leaf C+ E+ H+	2.85 ± 0.03

[135]*Gordonia obtusa* fruit C+ E- H-	0.98 ± 0.04
[139]*Cassia tora* root C- E+ H-	1.42 ± 0.01
[4]*Vetiveriazizanioides* root C-, E-, H-	0.15± 0.02
[8]*Madhucalongifolia* leaf C- E- H-	0.15± 0.03
[12]*Aeglemarmelos* fruit C- E- H+	0.85± 0.006
[16]*Tribulusterrestris* thorn C- E- H-	0.05± 0.006
[20]*Alhagicamelorum* leaf C+ E+ H+	2.9± 0.05
[24]*Berberis aristata* stem C- E- H-	0.16± 0.002
[28]*Mesua ferrea* bark C+ E+ H+	4.35± 0.003
[32]*Pipernigrum* seed C- E- H+	0.95± 0.002
[36]*Withaniacoagulans* fruit C- E- H-	0.24± 0.013
[40]*Asparagus racemosus* stem C- E- H-	0.13± 0.003
[44]*Ocimumtenuiflorum* seed C- E- H-	1.23± 0.003
[48]*Vitexnegundo* flower C+ E- H+	0.75± 0.002
[52]*Alangiumsalviifolium* leaf C+ E- H-	2.55± 0.04
[56]*Baliospermummontanum* leaf C- E- H-	0.83± 0.004
[60]*Chlorophytumborivilianum* leaf C- E- H-	2.05± 0.0012
[64]*Glycyrrhizaglabra* stem C+ E- H+	8.86± 0.024
[68]*Clerodendronserratum* stem C+ E- H-	3.11± 0.008
[72]*Millettiapinnata* seed C- E- H+	0.41± 0.004
[76]*Acacia catechu* bark C+E+H+	4.55± 0.030
[80]*Punicagranatum* fruitrind C+E+ H+	3.02± 0.02
[84]*Semecarpusanacardium* seed C+ E+ H+	3.21± 0.008
[88]*Curcuma nurvala longa* rhizome C+ E-H+	0.75± 0.003
[92]*Plumbagoindica* stem C+ E+ H-	3.14± 0.19
[96]*Trichosanthes labata* seed C- E- H-	0.097 ± 0.003
[100]*Gmelina arborea* leaf C- E- H-	0.02± 0.015
[104]*Allium sativum* leaf C- E- H-	0.70± 001
[108]*Hedychium spicatum*rhizome C- E- H-	18.5± 0.49
[112]*Ailanthus excelsa* bark C- E- H-	0.13± 0.05
[116]*Cymbopogoncitrates* leaf C- E- H-	0.38 ± 0.03
[120]*Pithocelibium dulce* bark C+ E- H+	4.23 ± 0.14
[124]*Barleria prionitis* stem C- E- H-	0.51 ± 0.008
[128]*Abieswebbiana* leaf C+ E+ H+	2.84± 0.09

ml TPC), and BtH (98.80 ± 2.4% at 150 µg/ml TPC), followed by *A. catechu, T. argentea,* and *F. racemosa,* which exhibited maximum inhibitory potentials (Fig. 3). *M. ferrea* (65.89 ± 0.15%) and *A. indica* (61.13 ± 0.50%) exhibited moderate inhibitory potential against ChC at 100 µg/ml TPC. *A. lebbeck* (78.22 ± 2.1%) exhibited the maximum inhibitory potential, while *D. melanoxylon* (64.82 ± 2.4%) exhibited the minimum inhibitory potential against PpE at 100 µg/ml TPC. *A. lebbeck* (83.40 ± 0.6%) exhibited the maximum inhibitory potential, while *M. glabra* (70.30 ± 0.2%) exhibited the moderate inhibitory potential against BtH at 150 µg/ml TPC. Among all barks, the lowest inhibitory potential against all enzymes (ChC, PpE, and BtH) was exhibited by *S. Suaveolens*. The remaining plant barks also exhibited remarkable inhibitory activities against all three enzymes (Fig. 3). The inhibitory activities of barks could be due to the presence of polyphenolic compounds.

Inhibitory potentials of fruits, fruit rind, flowers, and seeds

In the case of fruits, fruit rind, flowers, and seeds, the *W. fructicosa* flower exhibited the highest inhibitory potential against ChC (98.37±0.4% at 100 µg/ml TPC), PpE (97.23±0.8% at 100 µg/ml TPC), and BtH (85.53±0.4 at 150 µg/ml TPC), followed by *P. granatum* fruit rind and *T. chebula* fruit, which exhibited the maximum inhibitory potentials against these enzymes. The moderate inhibitory potential against ChC exhibited by *S. anacardium* seed (70.54±1.5%) and *T. bellirica* fruit (63.67±0.50%). *P. emblica* fruit exhibited moderate inhibitory potential (65.61±1.7%)

against PpE. *S. anacardium* seed (21.68±2.7%), *T. bellirica* fruit (42.78±1.3%), and *P. emblica* fruit (40.64±2.1%) exhibited the minimum inhibitory potential against BtH at 150 µg TPC/ml. *G. superba* fruit and *A. marmelos* fruit exhibited the minimum inhibitory potential against all three enzymes (Fig. 4).

Inhibitory potentials of leaves

From inhibitory potentials of leaves, it was found that *P. pterocarpum* exhibits the highest inhibitory potential against ChC (92.64±1.40%). Among all the inhibitory potential of leaves, *A. vera* exhibited the highest inhibitory potential (92.46 ± 2.46%) against PpE at 100 µg/ml TPC. *N. arbor-tristis, S. robusta, A. camelorum,* and *C. roseus* exhibited the maximum inhibitory potential against ChC and PpE. *S. robusta* (75.65 ± 1.5%) and *C. roseus* (80.12 ± 1.5%) exhibited the maximum inhibitory potential against BtH at 150 µg/ml TPC. *C. pulcherima* and *L. inermis* exhibited the minimum inhibitory potential against ChC, PpE, and BtH. *F. benghalensis* exhibited the lowest inhibitory potential against all three enzymes (Fig 5).

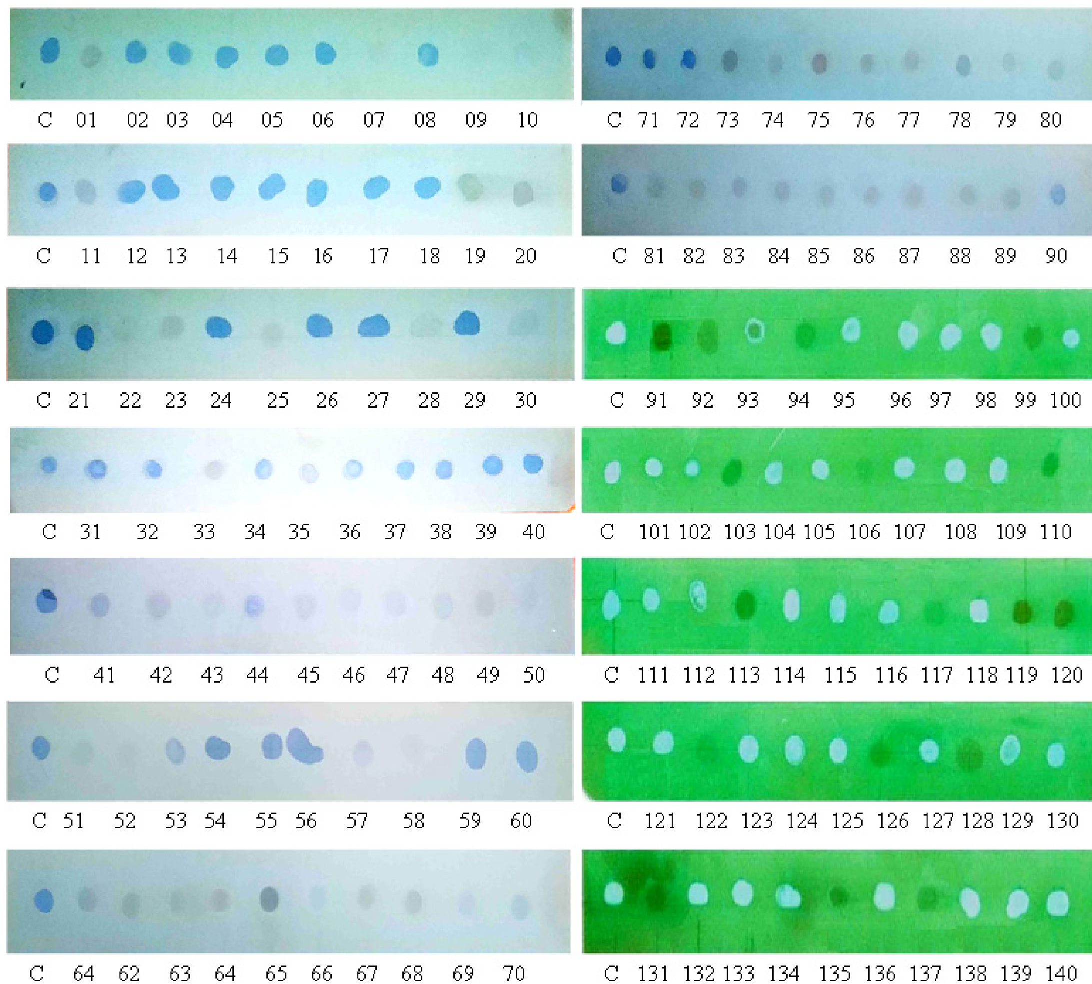

Fig. 1. Screening medicinal plant extracts for inhibitory activities against *Clostridium histolyticum* collagenase (ChC) by dot blot assay on X-ray film. Numbers 1, 2…. up to 140 are serial numbers of plants mentioned in table no. 1 as superscript format. C indicates control activity of enzymes. The inhibitory activity of those plants was tested sequentially as per serial number. Blue spots indicate the gelatinase activity of ChC, and spots without blue color indicate the inhibitory activity of plant extracts

Inhibitory potentials of stems, roots and rhizomes

In the case of stems, roots, and rhizomes, the highest inhibitory potential was exhibited by *B. ligulata* root against ChC (94.77 ± 0.2%), PpE (91.98 ± 1.4%), and BtH (90.60 ± 0.72%). *P. somniferum, C. phlomidis,* and *V. rosea* exhibited the maximum inhibitory potential of ChC and PpE. *C. phlomidis V. rosea* and *L. pyrotechnica* exhibited the minimum inhibitory potential against BtH at 150 µg/ml TPC. *L. pyrotechnica* and *V. nilotica* exhibited the minimum inhibitory potential against ChC and PpE. *C. mukul* rhizome exhibited the lowest inhibitory potential against BtH (Fig. 6).

DISCUSSION

Collagenase, elastase, and hyaluronidase are the ECM degrading enzymes, performing various important physiological roles, including tissue remodeling, immune response, and wound ling. In the skin aging process, these enzymes degrade key ECM components excessively, contributing to the visible signs of aging. Collagenase cleaves the collagen fibers, which are essential for maintaining the firmness and structure of skin, leading to wrinkles and sagging.[8,9] Elastase degrades elastin, which is crucial for skin with its elasticity, resulting in loss of skin

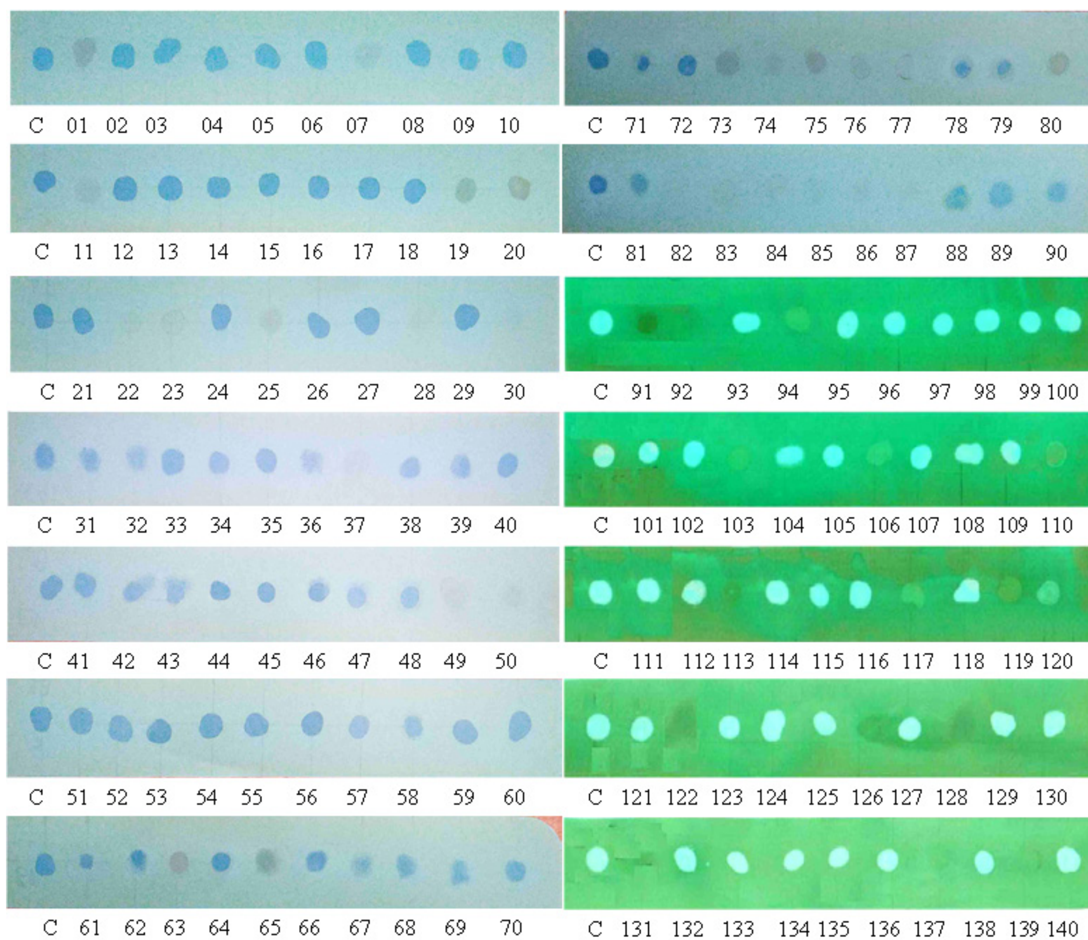

Fig. 2. Screening medicinal plant extracts for inhibitory activities against Porcine pancreatic elastase (PpE) by dot blot assay on X-ray film. Numbers 1, 2…. up to 140 are serial numbers of plants mentioned in table no. 1 as superscript format. C indicates control activity of enzymes. The inhibitory activity of those plants was tested sequentially as per serial number. Blue spots indicate the gelatinase activity of PpE, and spots without blue color indicate the inhibitory activity of plant extracts.

tone and resilience.[10] Hyaluronidase breaks down hyaluronic acid, which is responsible for skin hydration and plumpness, leading to dryness and a decrease in skin volume.[12] The increased activity of these enzymes in aging skin accelerates the loss of structural integrity with the development of fine lines, wrinkles, and reduced skin elasticity. Therefore, the inhibitors of these enzymes are

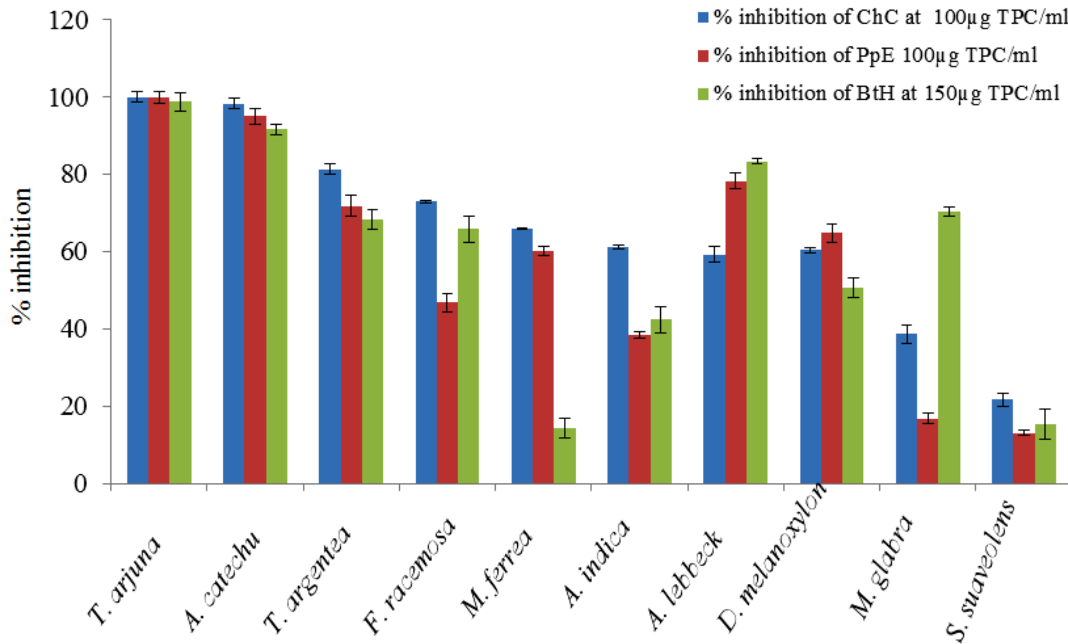

Fig. 3. Inhibitory potential (mean ± S. D.) of plant barks against *Clostridium histolyticum* collagenase (ChC), Porcine pancreatic elastase (PpE), and Bovine testes hyaluronidase (BtH).

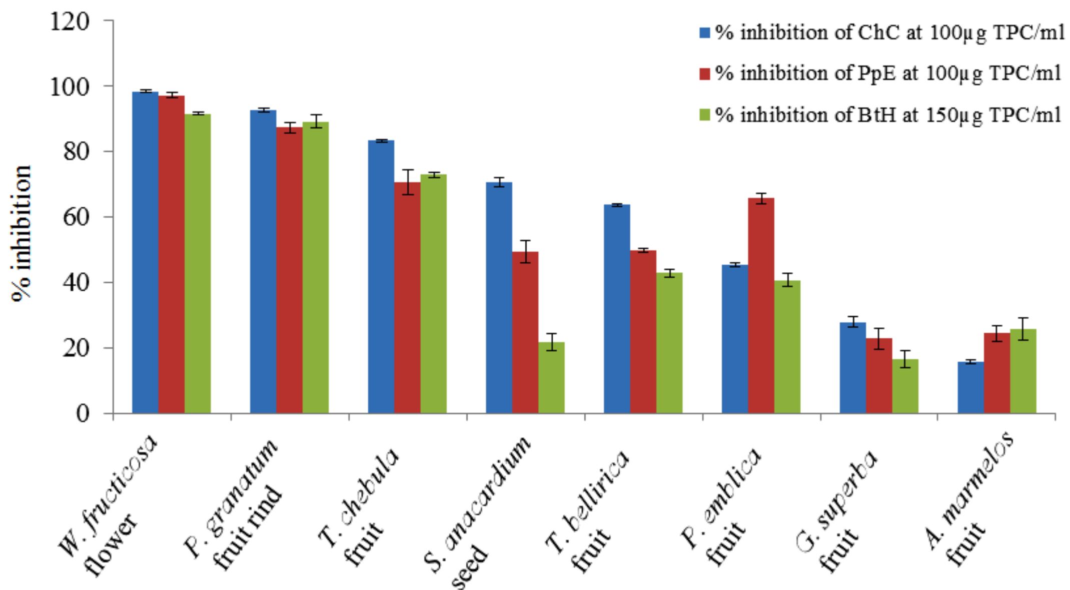

Fig. 4. Inhibitory potential (mean ± S. D.) of plant fruits, fruit rind, flowers, and seeds against *Clostridium histolyticum* collagenase (ChC), Porcine pancreatic elastase (PpE), and Bovine testes hyaluronidase (BtH).

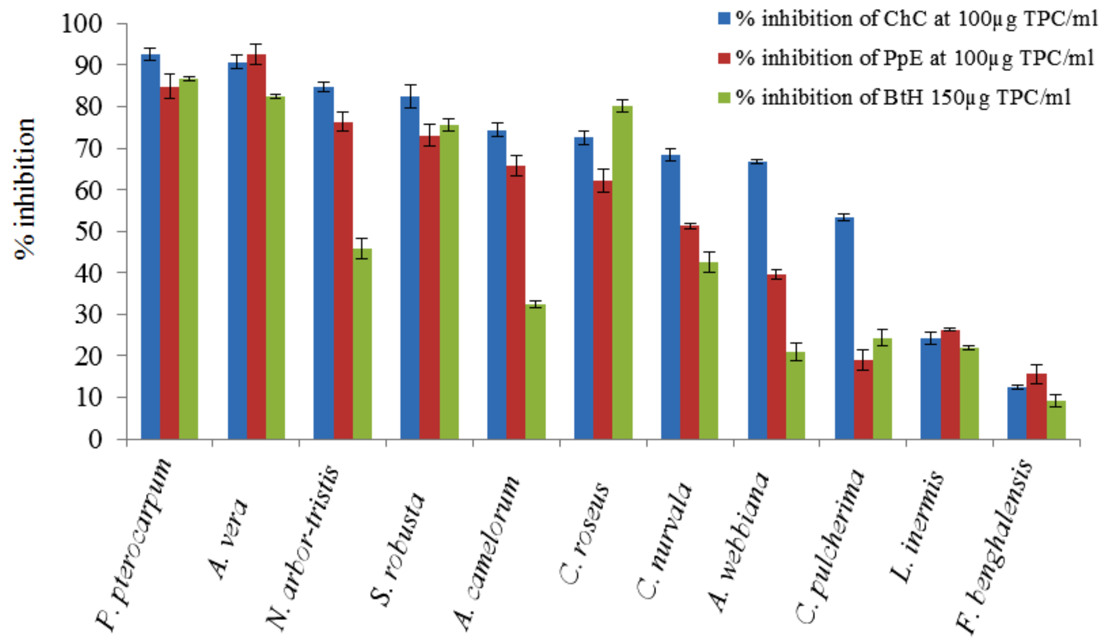

Fig. 5. Inhibitory potential (mean ± S. D.) of plant leaves against *Clostridium histolyticum* collagenase (ChC), Porcine pancreatic elastase (PpE), and Bovine testes hyaluronidase (BtH).

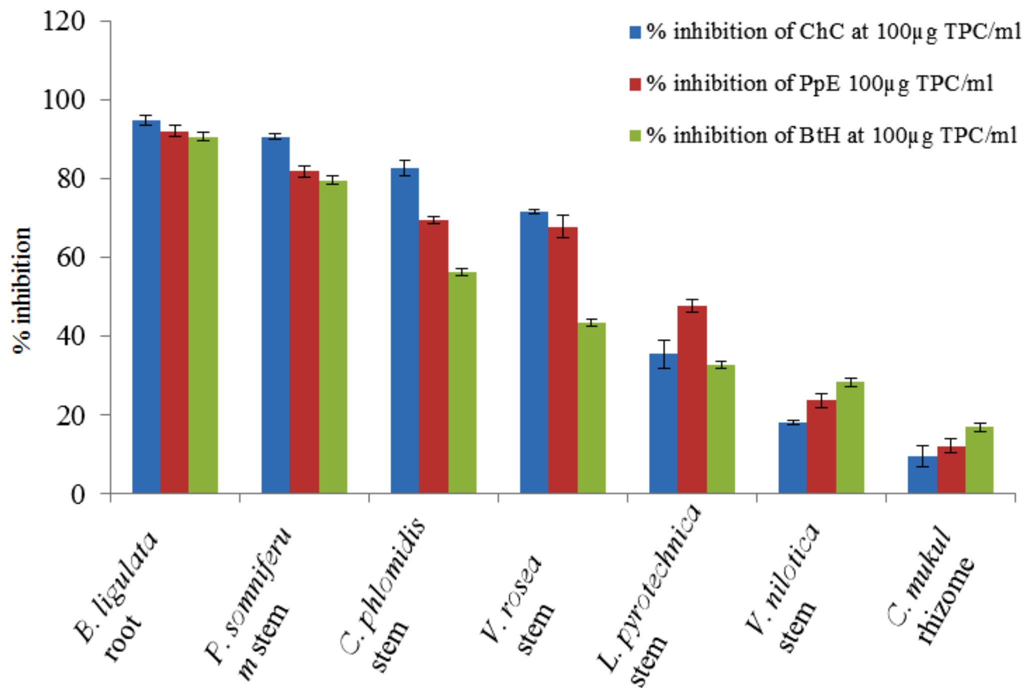

Fig. 6. Inhibitory potential (mean ± S. D.) of plant stems, root and rhizome against *Clostridium histolyticum* collagenase (ChC), Porcine pancreatic elastase (PpE), and Bovine testes hyaluronidase (BtH).

beneficial agents for avoiding and controlling the skin aging related consequences.[21]

Medicinal plants are rich sources of polyphenols, which exhibit antioxidant, anti-inflammatory, anti aging, and photoprotection properties, making them highly important in cosmetics.[31] Plant polyphenols reduce the reactivity of free radicals and guard the skin from environmental damage and oxidative stress. They are applied in cosmetic formulations for their capacity to minimize the external signs of aging by enhancing collagen synthesis and improving skin elasticity.[32] They are involved in skin protection from various UV-radiations; thus, they are important ingredients in sun protection formulations. Because of their antimicrobial and antioxidant properties, they inhibit the formation of acne-prone skin and regulate the sebum production.[33] This study focused on the assessment of anti-aging properties in 140 medicinal plants. In this regard, ethanolic extracts of medicinal plants were screened against skin-aging enzymes (ChC, PpE, and BtH), and the TPC of these plant extracts was estimated. It was observed that plant like; *T. arjuna* bark, *H. spicatum* rhizome, and *A. vera* leaf are rich sources of TPC (Table 1). The obtained TPC from these plant extracts was found to be somewhat similar to those estimated in previous studies. The *T. arjuna* bark is famous for its health beneficial properties in Ayurvedic literature. Saha et al[34] reported that the *T. arjuna* bark aqueous extract contains 44% polyphenols by weight, and the majority of them are polymeric in nature. The previous study reported[34] that *T. arjuna* bark contains flavon-3-ols ((+)-gallocatechin, (+)-catechin, and (")-epigallocatechin), phenolic acids (gallic acid, ellagic, acid and its derivatives), and ellagic acid derivatives (3-*O*-methyl-ellagic acid 4-*O*-â-D-xylopyranoside, and 3-*O*-methyl ellagic acid 3-*O*-rhamnoside). An earlier study showed[35] that *H. spicatum* rhizome is a rich source of total phenolic (12.82 mg/g dry extract) and flavonoid (13.998 mg/g dry extract) contents, and those compounds are responsible for the potential of this plant. The various extracts from the *A. vera* leaf have been reported[36] to contain rich phenolic compounds. Inhibitors from plant resources have been recognized[37] as effective and safe controlling agents for skin aging and related diseases.

Screening assays of plant extracts revealed that some plants exhibited inhibitory potential; this was based on the phenolic compounds existing in plant extracts. Phenolic compounds are a diverse group of active components in plants synthesized in response to defensive actions and exhibit inhibitory activity against enzymes due to their property to interact with enzymes by different mechanisms, including hydrogen bonding formation with amino acid residue, chelation of metals, hydrophobic interactions, and allosteric inhibition.[38, 39, 40]

Assessment of the inhibitory potential of plant extracts exposed *T. arjuna* bark, *A. vera* leaf, *A. catechu* bark, *W. fructicosa* flower, *P. granatum* fruit rind, *P. pterocarpum* leaf, *B. ligulata* root, and *P. somniferum* stem exhibited the highest inhibitory activities compared to other plants (Figs. 3, 4, 5, and 6). It was observed that *T. arjuna* bark and *A. vera* leaf contained the highest phenolic concentration (>15 mg/ml); therefore, the phenolics of these plants may be involved in inhibitory activity. *T. arjuna*, widely accepted medicinal plant, has been reported to exhibit various biological activities and is a rich source of inhibitory activity showing phenolic compounds, including gallocatechin, catechol, epigallocatechin, gallic acid and ellagic acid.[41, 42] The inhibitory activity of *T. arjuna* was quite comparable with the inhibitory activity reported in a previous study, which investigated[43] that the hydroalcoholic bark extract of *T. arjuna* exhibits significant inhibitory activity against hyaluronidase (greater than 90%) and substantial inhibitory activity against elastase (80.26%) at 150 ìg/ml concentration. *A. vera* is a very popular plant; it is used worldwide for medicinal, nutritional, and cosmetic purposes. Previous studies have shown that *A. vera* exhibits significant inhibition against collagenase, elastase, hyaluronidase, and tyrosinase. Hence, it was suggested[44, 45] that the extract of *A. vera* could be used as natural remedy in cosmetics for controlling the skin aging process. It was reported[46] that *A. vera* exhibits numerous biological properties and has prominent wound and burn healing potentials. Its latex and gel contain biologically important ingredients. Gel isolated from leaves contains polysaccharides, which have health benefit, like anti-inflammatory, anti-diabetic, anti-cancer, and anti-ulcer properties.

Moderate amounts of phenolic concentrations (3 to 6 mg/ml) were observed in *A. catechu* bark, *W. fructicosa* flower, *P. granatum*

fruit rind, *P. pterocarpum* leaf, *B. ligulata* root, and *P. somniferum* stem; however, these plants exhibited high inhibitory potential, indicating the phenolics of these plants possess strong enzyme inhibitory properties. *A. catechu* has been investigated[47] to exhibit various medicinal properties, including antidiarrheal, antihyperlipidemic, antioxidant, antiproliferative, antimicrobial, antinociceptive, antiulcer, antidiabetic, haemolytic, and anti-inflammatory properties due to the presence of bioactive compounds like flavonoids, tannins, and alkaloids. Balaji and Durga[48] reported that red heartwood of *A. catechu* is a source of catechin, a polyphenolic compound, and it exhibits antioxidant and anti-elastase activities. Therefore, the inhibitory activity of *A. catechu* may be due to the existence of phenolic compounds. *W. fructicosa* is a traditional medicinal plant; in ancient periods, it was employed[49] for the treatment of various ailments such as blood infection, dysentery, wounds, fever, inflammation, colds, toothache, leprosy, rheumatic pain, urinary disorders, and menstrual problems. Phytochemical analysis revealed[49] that this plant is a rich source of various bioactive compounds. Therefore, it exhibits various biological activities, including antioxidant, anti-inflammatory, and wound healing. *P. granatum* fruit has been investigated[50] to exhibit various health beneficial properties, including anti-inflammatory and antioxidant activities; thus, this plant fruit is consumed as functional food worldwide. Pomegranate concentrated solution (PCS) has been investigated[50] as a potential functional cosmetic ingredient for skin-whitening and anti-wrinkle effects. It can synthesize hyaluronan in HaCaT cells, decrease procollagenase and elastase activities in HDF-N cells, significantly reduce the UVA-induced MMP-1 activity in HDF-N cells compared to UVA-exposed cells, and suppress melanin production and mushroom tyrosinase activity in Melan-cells.[50] *P. pterocarpum, B. ligulata,* and *P. somniferum* have been reported[51, 52, 53] to have various bioactive metabolites, including phenolic acids, flavonoids, tannins, and terpenoids, which are responsible for exhibiting crucial biological activities. Therefore, the presence of bioactive metabolites, including phenolics, in these plants may be responsible for exhibiting inhibitory activity. The plants including *T. arjuna, A. catechu, W. fructicosa, P. granatum, P. pterocarpum, A. vera, B. ligulata,* and *P. somniferum* showing high inhibitory potential would be recommended as anti-aging candidates in cosmeceuticals for maintaining the skin's elasticity and reducing the visible signs of aging, thereby improving skin health and appearance. This study emphasizes the need for future research on the determination of phenolic inhibitory potential as IC_{50} value, detection of tyrosinase inhibitory activity of these plants as a whitening agent, MTT assay for cell toxicity, and determination of inhibition types as inhibition mechanism. The work on purification and characterization of specific and potent inhibitory compounds from these plants is required for selective inhibitors to reduce side effects and improve therapeutic outcomes, enhancing their potential for clinical application in dermatology and anti-aging treatments.

CONCLUSION

Based on results of this study, it was concluded that medicinal plants, including *T. arjuna* bark, *H. spicatum* rhizome*, A. vera* leaf, and *V. rosea* stem, are rich sources of phenolic compounds. Among the investigated species, the *T. arjuna* bark, *A. catechu* bark, *W. fructicosa* flower, *P. granatum* fruit rind, *P. pterocarpum* leaf, *A. vera* leaf, *B. ligulata* root, and *P. somniferum* stem are prominent sources of ChC, PpE, and BtH inhibitors. These plant extracts, owing to their high inhibitory potential, represent promising candidates for incorporation into cosmeceutical formulations aimed at the prevention and treatment of skin disorders associated with aging, such as premature aging, wrinkling, and intrinsic biological aging. The results of this study provide valuable insights for the cosmetic industry, supporting the development of innovative plant-based cosmeceuticals. Furthermore, the findings highlight the need for future research focused on the isolation, characterization, and mechanistic evaluation of novel bioactive molecules from these plants as potential anti-aging agents.

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Data Availability Statement

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Ethics Statement

This work did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not Applicable.

Author's contribution

Rajesh Dattatray Tak-Research idea and hypothesis, work design, guiding the entire work and manuscript preparation; Ajit Babruvahan Patil-Collection of plant samples and whole laboratory work; Bhimrao Vishwanath Jaiwal-Interpretation of results, manuscript preparation and technical support; Yuvraj Prakash Kale-Cooperation for laboratory work and interpretation of results.

REFERENCES

1. Madison K. C. Barrier function of the skin: "La Raison d'Être" of the epidermis. J. *Invest. Dermatol.,* 2003; 121(2): 231-241.
2. Farage M. A, Miller K. W, Elsner P and Maibach H. I. Intrinsic and extrinsic factors in skin ageing: A review. *Int. J. Cosmet. Sci*., 2008; 30(2): 87-95.
3. Rittié L., and Fisher G. J. UV-light-induced signal cascades and skin aging. *Ageing Res. Rev*., 2002; 1: 705–720.
4. Mukherjee P. K., Maity N., Nema N. K., and Sarkar B. K. Bioactive compounds from natural resources against skin aging. *Phytomedicine.*, 2011; 19: 64–73.
5. Watt F. M., and Fujiwara H. Cell-extracellular matrix interactions in normal and diseased skin. Cold Spring Harb. *Perspect. Biol*., 2011; 3(4): a005124.
6. Pfisterer K, Shaw L. E, Symmank D and Weninger W. The extracellular matrix in skin inflammation and infection. *Front. Cell Dev. Biol*., 2021; 9: 682414.
7. Wang K, Meng X and Guo Z. Elastin structure, synthesis, regulatory mechanism, and relationship with cardiovascular diseases. Front. *Cell Dev. Biol*., 2021; 9: 596702.
8. Chatatikun M and Chiabchalard A. Thai plants with high antioxidant levels, free radical scavenging activity, anti-tyrosinase, and anti-collagenase activity. *BMC Complement. Altern. Med.,* 2017; 17: 487.
9. Popoola O. K, Marnewick J. L, Rautenbach F, Ameer F, Iwuoha E. I and Hussein A. A. Inhibition of oxidative stress and skin aging-related enzymes by prenylated chalcones and other flavonoids from Helichrysum teretifolium. *Molecules*, 2015; 20(4): 7143–7155.
10. Mohiuddin A. K. Skin aging & modern age anti-aging strategies. *Int. J. Clin. Dermatol. Res.,* 2019; 7: 209-240.
11. Leach J. B, Kathryn A. B, Charles W. P. J and Christine E. S. Photocrosslinked hyaluronic acid hydrogels: Natural, biodegradable tissue engineering scaffolds. *Biotechnol. Bioeng.*, 2003; 82(5): 578–589.
12. Žádníková P, Šínová R, Pavlík V, et al. The degradation of hyaluronan in the skin. *Biomolecules*, 2022; 12(2): 251.
13. Shi L, Ermis R, Garcia A, Telgenhoff D and Aust D. Degradation of human collagen isoforms by *Clostridium* collagenase and the effects of degradation products on cell migration. *Int. Wound J.*, 2010; 7(2): 87-95.
14. Jung C. M, Matsushita O, Katayama S, Minami J, Sakurai J and Okabe A. Identification of metal ligands in the *Clostridium histolyticum* ColH collagenase. *J. Bacteriol.*, 1999; 181(9): 2816-2822.
15. Cerveró R. S, Herrero D. G and Hueso F. J. C. Mechanism of action of *Clostridium histolyticum* collagenase for clinical application. *Eur. J. Clin. Pharm. Aten. Farm.*, 2016; 18(4): 263-272.
16. Bode W, Meyer Jr E and Powers J. C. Human leukocyte and porcine pancreatic elastase: X-ray crystal structures, mechanism, substrate specificity, and mechanism-based inhibitors. *Biochemistry*, 1989; 28(5): 1951-1963.
17. Sinha S, Watorek W, Karr S, Giles J, Bode W and Travis J. Primary structure of human neutrophil elastase. *Proc. Natl. Acad. Sci. U.S.A.*, 1987; 84(8): 2228-2232.

18. Zhang L., Bharadwaj A. G., Casper A., Barkley J., Barycki J. J., and Simpson M. A. Hyaluronidase activity of human Hyal1 requires active site acidic and tyrosine residues. *J. Biol. Chem.*, 2009; 284(14): 9433-9442.
19. Huang H, Hou X, Xu R, et al. Structure and cleavage pattern of a hyaluronate 3-glycanohydrolase in the glycoside hydrolase 79 family. *Carbohydr. Polym.*, 2022; 277:118838.
20. Stern R, and Jedrzejas M. J. Hyaluronidases: Their genomics, structures, and mechanisms of action. *Chem. Rev.*, 2006; 106(3): 818-839.
21. Jiratchayamaethasakul C, Ding Y, Hwang O, et al. In vitro screening of elastase, collagenase, hyaluronidase, and tyrosinase inhibitory and antioxidant activities of 22 halophyte plant extracts for novel cosmeceuticals. *Fish. Aquat. Sci.*, 2020; 23: 1-9.
22. Zagoskina N. V, Zubova M. Y, Nechaeva T. L, et al. Polyphenols in plants: structure, biosynthesis, abiotic stress regulation, and practical applications. *Int. J. Mol. Sci.*, 2023;24(18): 13874.
23. Xie M, Jiang Z, Lin X and Wei X. Application of plant extracts cosmetics in the field of anti-aging. *J. Dermatol. Sci. Cosmet. Technol.*, 2024; 100014.
24. Inchingolo A. D, Inchingolo A. M, Malcangi G, et al. Effects of resveratrol, curcumin and quercetin supplementation on bone metabolism a systematic review. *Nutrients*, 2022;14(17): 3519.
25. Munthe S. W. N, Riskiantos R, Juvi D and Novia J. Antioxidant, total phenolic, and total flavonoid of 70% ethanol extract of avocado seeds (*Persea americana* Mill.). *Pharmacogn. J.*, 2023; 15(4).
26. Ainsworth E. A and Gillespie K. M. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat. Protoc.*, 2007; 2: 875-877.
27. Pichare M. M and Kachole M. S. Detection of electrophoretically separated protease inhibitors using X-ray film. *J. Biochem. Biophys. Methods*, 1994; 28: 215–224.
28. Tolksdorf S and McCready M. H. The turbidimetric assay of hyaluronidase. *J. Lab. Clin. Med.*, 1949; 34: 74–89.
29. Barletta J. P, Angella G, Balch K. C, et al. Inhibition of pseudomonal ulceration in rabbit corneas by a synthetic matrix metalloproteinase inhibitor. *Invest. Ophthalmol. Vis. Sci.*, 1996; 37: 20-28.
30. Kim Y, Uyama H and Kobayashi S. Inhibition effects of (+)-catechinaldehyde polycondensates on proteinases causing proteolytic degradation of extracellular matrix. *Biochem. Biophys. Res. Commun.*, 2004; 320: 256-261.
31. Ur Rehman F, Kalsoom M, Adnan M, et al. Importance of medicinal plants in human and plant pathology: A review. *Int. J. Pharm. Biomed. Res.*, 2021; 8: 1-1.
32. Farhan M. The promising role of polyphenols in skin disorders. *Molecules*, 2024; 29(4): 865.
33. Draghici-Popa A. M, Buliga D. I, Popa I, Tomas S. T, Stan R and Boscornea A. C. Cosmetic products with potential photoprotective effects based on natural compounds extracted from waste of the winemaking industry. *Molecules*, 2024; 29(12): 2775.
34. Saha A, Pawar V. M and Jayaraman S. Characterisation of polyphenols in *Terminalia arjuna* bark extract. *Indian J. Pharm. Sci.*, 2012; 74(4): 339.
35. Kumar M, Pratap V, Singh M. K, Nigam A. K, Kumar P and Kumar J. Evaluation of antioxidant potential of *Hedychium spicatum* rhizome extracts from Bhowali region, Uttarakhand, India. *Int. J. Life Sci. Res.*, 2015; 7(6): 2901-2907.
36. Elferjane M. R, Jovanoviæ A. A, Milutinoviæ V, et al. From *Aloe vera* leaf waste to the extracts with biological potential: Optimization of the extractions, physicochemical characterization, and biological activities. *Plants*, 2023; 12(14): 2744.
37. Khojah H, Ahmed S. R, Alharbi S. Y, et al. Skin anti-aging potential of *Launaea procumbens* extract: Antioxidant and enzyme inhibition activities supported by ADMET and molecular docking studies. *Saudi Pharm. J.*, 2024; 102107.
38. Younis M. M, Ayoub I. M, Mostafa N. M, et al. GC/MS profiling, anti-collagenase, anti- elastase, anti-tyrosinase, and anti-hyaluronidase activities of a *Stenocarpus sinuatus* leaves extract. *Plants*, 2022; 11(7): 918.
39. Lopes G, Andrade P. B and Valentão P. Phytochemical research of traditional uses of medicinal plants in contemporary pharmacology. *J. Ethnopharmacol.*, 2017; 198: 74-87.
40. Shahidi F and Ambigaipalan P. Phenolics and polyphenolics in foods, beverages, and spices: Antioxidant activity and health effects – A review. *J. Funct. Foods*, 2015; 18: 820-897.
41. Maulik S. K and Talwar K. K. Therapeutic potential of *Terminalia arjuna* in cardiovascular disorders. *Am. J. Cardiovasc. Drugs*, 2012; 12: 157–163.
42. Rahman Z, Kohli K, Khar R. K, Lamba H. S, Rathour A and Pahwa R. An overview of *Terminalia arjuna*: Chemistry and pharmacological profile. *Indian Drugs*, 2004; 41(11): 641-649.
43. Satardekar K. V and Deodhar M. A. Anti-

ageing ability of *Terminalia* species with special reference to hyaluronidase, elastase inhibition and collagen synthesis in vitro. *Int. J. Pharmacogn. Phytochem. Res.*, 2010; 2(3): 30-34.

44. Boran R, Ugur A and Sarac N. Investigation of hyaluronidase, collagenase and elastase inhibitory potentials and comparative evaluation of the antimicrobial, antioxidant and homeostatic activities of two natural polysaccharides. *Süleyman Demirel Univ. Fen Bilimleri Enstitüsü Dergisi*, 2018; 22(3): 1182-1189.
45. Sacan O, Akev N and Yanardag R. Inhibitory effects of *Aloe vera* extracts on anti-tyrosinase, anti-collagenase, and anti-elastase potential. *Bangladesh J. Bot.*, 2024; 53(3): 597-603.
46. Jose E, Joseph S and Joy M. *Aloe vera* and its biological activities. *World J. Curr. Med. Pharm. Res.*, 2021; 21-26.
47. Kumari M, Radha, Kumar M, Zhang B, et al. *Acacia catechu* (Lf) Willd.: A review on bioactive compounds and their health-promoting functionalities. *Plants*, 2022; 11(22): 3091.
48. Balaji Surya Durgarao T and Durga Prasad V. K. Y. Isolation and optimization of catechins from *Acacia catechu* and its antioxidant and anti-aging properties in vitro. *Ann. Plant Sci.*, 2017; 6(12).
49. Giri S, Dey G, Sahu R, Paul P, Nandi G and Dua T. K. Traditional uses, phytochemistry and pharmacological activities of *Woodfordia fruticosa* (L) Kurz: A comprehensive review. *Indian J. Pharm. Sci.*, 2023; 85(1).
50. Kang S. J, Choi B. R, Kim S. H, et al. Inhibitory effects of pomegranate concentrated solution on the activities of hyaluronidase, tyrosinase, and metalloproteinase. *J. Cosmet. Sci.*, 2015; 66(3): 145-159.
51. Jash S. K, Singh R. K, Majhi S, Sarkar A and Gorai D. *Peltophorum pterocarpum*: Chemical and pharmacological aspects. *Int. J. Pharm. Sci. Res.*, 2014; 5(1): 26-36.
52. Roychoudhury S, Das D, Das S, et al. Clinical potential of Himalayan herb *Bergenia ligulata*: An evidence-based study. *Molecules*, 2022; 27(20): 7039.
53. Butnariu M, Quispe C, Herrera-Bravo J, et al. A. *Papaver* plants: Current insights on phytochemical and nutritional composition along with biotechnological applications. *Oxid. Med. Cell. Longev.*, 2022; 2022(1): 2041769.