Plant Archives Vol. 19, Supplement 2, 2019 pp. 1151-1155 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ANTIAGING AND ANTIWRINKLE POTENTIAL OF *GLYCYRRHIZA GLABRA*

Chanchal Garg, Priyanka Khurana and Munish Garg*
Department of Pharmaceutical Sciences, Maharshi Dayanand University,
Rohtak, Haryana, India-124001
Corresponding Author: Email: mgarg2006@gmail.com;
Ph: +911262-393222, +919812588857

Abstract
Since traditional plants and their extracts are long used for their various therapeutic effects and for the protection of skin against several environmental damages due to their skin protective properties. The present study was designed to evaluate the anti-aging and anti-wrinkle potential of *Glycyrrhiza glabra* through *in vitro* models. Methanolic extract of *Glycyrrhiza glabra* was prepared by maceration and further fractionated to get ethyl acetate, n-butanol and aqueous fractions. The obtained extracts were then subjected to antioxidant, Hyaluronidase, MMP-1/collagenase and elastase inhibition activities to assess the anti aging and anti wrinkle potential of *Glycyrrhiza glabra*. The data obtained showed that the methanolic extracts of *Glycyrrhiza glabra* exhibited a maximum total antioxidant activity of 88.76 at 100µg/ml as compared to ascorbic acid (standard) which was found to be 90.12%. This was further confirmed by calculating the IC_{50} values and was found that the methanolic extract (34.08) showed the maximum antioxidant activity. *Glycyrrhiza glabra* was also evaluated for various enzymatic activities and it was found that methanolic extract exhibited the maximum enzymatic activity for hyaluronidase, elastase and MMP-1/ collagenase at 100µg/ml. The IC_{50} values of the methanolic extract for hyaluronidase, elastase and MMP-1/ collagenase were found to be 13.57, 18.90, 14.40µg/ml. Whereas the % inhibition exhibited by the standard (catechin) was found to be 95.72%. The IC_{50} value of standard catechin was found to be 21.52µg/ml. The present study demonstrated that *Glycyrrhiza glabra* can be beneficial as a potent antiaging agent as illustrated by the *in-vitro* analysis. Among the various test samples, methanolic extract of *Glycyrrhiza glabra* showed the maximum activity as a potent antioxidant and also exhibited the maximum enzymatic inhibitory activities.
***Keywords*:** Skin ageing, *Glycyrrhiza glabra*, enzymatic, hyaluronidase, elastase, collagenase, and antioxidant

Introduction

Aging of skin is a gradual, deleterious, biological action that takes place in response to several environmental factors such as excessive exposure to ultraviolet rays, air pollution etc leading to various structural, functional and biochemical changes (Gilchrest, 1990) involving diminution of cellular response to growth and proliferation and reduced span of life of skin cells (Yaar and Gilchrest, 1990). Skin appears leathery with wrinkles, uneven pigmentation and brown spots (Berneburg *et al*., 2004). Excessive exposure to UV rays results in the generation of reactive oxygen species (ROS) or free radicals which cause oxidative stress leading to changes in dermal and epidermal layers of the skin. The collagen and elastin found in the extracellular matrix (ECM), of skin is degraded leading to skin aging and cellular damage (Bhavan *et al*., 1992). Previous studies demonstrate that decrease in skin elasticity directly corresponds to the enhanced activity of elastase and hyaluronidase enzymes responsible for maintaining the flexibility and strength of skin (Bissett *et al*., 1987; MacKay and Miller, 2003). Also, the enzyme matrixmelloproteinase-1 (MMP-1) is responsible for causing skin aging due to the breakdown of collagen fibers (Losso *et al*., 2004). Thus, skin aging can be controlled by inhibiting the action of these enzymes (Mukherjee *et al*., 2011).

The herbal or natural remedies have been used since ages to moisturize and reduce the skin aging. The constituents present in the natural resources are capable of reducing or retarding the activity of certain dermal enzymes that exhibit a dominant role in skin aging. Thus, they can be utilized effectively in many cosmetic preparations to curb the effects of photoaging (Maquert *et al*., 1999). Moreover, the natural herbs are less toxic and safe which marks their usefulness in cosmetic industry more captivating and desirable (Farooqi *et al.,* 2016).

Glycyrrhiza glabra, belonging to family leguminosae is widely used in several ayurvedic preparations and medicines. The root and underground stem of *Glycyrrhiza glabra* has been utilized for innumerable therapeutic and curative purposes such as bronchitis, sore throat, cough, venereal problems and weakness, skin problems, hoarseness, jaundice and ulcers. It is an antioxidant, antimicrobial, anti-inflammatory and hepatoprotective also (Yasui *et al*., 2011). The curative and healing properties of *Glycyrrhiza glabra* can be mainly attributed to the presence of certain polyphenolic and flavonoidal constituents. Glycyrrhizin, the main constituent of *Glycyrrhiza glabra* demonstrate an excellent property of quenching the free radicals and prevents the lipid peroxidation. Thus, it can be potentially used to reduce the effects of photoaging. Present investigations were carried out to evaluate the antiaging and anti wrinkle potential of *Glycyrrhiza glabra* as a potent inhibitor of various enzymes i.e. elastase, hyaluronidase and MMP-1 or collagenase which play a profound role in skin photoaging.

Materials and Methods

Collection and Authentication of Plant Material

Glycyrrhiza glabra root was collected from local market, Rohtak and was authenticated from the Department of Botany, Maharshi Dayanand University, Rohtak. The voucher specimen (Ph.cog/2017/202) is preserved in the Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak.

Extraction and Fractionation of Plant Material

Powdered *Glycyrrhiza glabra* (250g) was subjected to maceration in a closed conical flask for 7 days using methanol as a solvent to get the methanolic extract. The suspension obtained after maceration was collected and filtered. The supernatant liquid was then evaporated and concentrated. The methanolic extract obtained was subjected to fractionation by suspending it in water and partitioned successively with ethyl acetate and n-butanol to get the respective fractions in a separating funnel. Ethyl acetate fraction was concentrated while n-butanol and aqueous

fractions were lyophilized.

***In vitro* Screening studies**

Methanol, ethyl acetate, n-butanol and aqueous extracts of roots of *Glycyrrhiza glabra* were evaluated for its antioxidant as well as for various enzymatic activities.

Antioxidant Studies

DPPH radical scavenging assay

The ability of scavenging 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical by the extract was estimated by the method described by Nickavar et al (Nickavar *et al*., 2006). Stock solution of sample (1.0 mg/ml) was prepared and further diluted with methanol to get the solutions of concentrations 10, 20, 40, 60, 80 and 100 µg/ml. Ascorbic acid was taken as a standard and diluted in methanol to get the similar concentrations. 2.5 ml of each test solution and standard solution was taken and 1ml of a 0.3mM DPPH in methanol was added to them. The reaction mixtures were then kept at room temperature in dark for 30 min. After this the absorbance was measured at 517 nm by UV spectrophotometer. The increase in the DPPH radical scavenging activity is the measure of the decrease in the absorbance of DPPH solution. The percentage scavenging activity was estimated using the equation as follows:

% Inhibition of DPPH radical = [(Ac - As)/Ac] X 100

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

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

Enzymatic Studies

Hyaluronidase Inhibitory Assay

Take 50µl bovine hyaluronidase prepared by mixing 7900 units/ml in 0.1M acetate buffer of pH 3.5 and mix with 50µl of sample of different concentrations (25µg/ml, 125µg/ml, 375µg/ml and 500µg/ml) prepared by dissolving 5% Dimethyl Sulfoxide (DMSO). All the above prepared samples were incubated for 20 minutes at 37ºC. The control group contained 50µl of DMSO instead of plant extract. Calcium chloride (50µl of 12.5mM) was added to activate hyaluronidase in the reaction mixture and kept for 20 minutes in the incubator at 37ºC. 250µl of sodium hyaluronate (1.2 mg/ml dissolved in 0.1 M acetate buffer, pH 3.5) was then added to Ca^{+2} activated hyaluronidase and incubated for exactly 3 minutes in water bath at 100ºC. The resulting reaction mixture was then cooled to room temperature. After that 1.5ml of p-Dimethyl amino benzaldehyde, prepared by dissolving 4gms of PDMAB in Glacial acetic acid (350 ml) and 10N HCl (50 ml), was added to the reaction mixture and incubated at room temperature for 20mins (Lee *et al*., 1999). The absorbance was measured at 585 nm using UV spectrophotometer. %Inhibition was calculated using the following formula:

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

Where O.D = Optical density.

Elastase Inhibitory Assay

Elastase inhibition assay was carried out by the procedure described by (Tschesche *et al*., 1991). 0.1ml of a 0.2M Tris-HCl buffer containing 1% albumin, 0.025ml of a substrate solution containing 10mM MAAPVN (N-(methoxysuccinyl)-ala-ala-pro-val 4-nitroanilide), 0.05ml from sample of different concentrations (25, 50, 75 and 100µg/ml) were mixed together. To the above mixture added 0.025ml of elastase (3 units/ml). The reaction mixture was then incubated for 20 minutes at 25ºC. The rate of inhibition was estimated by ELISA reader at 410nm. Catechin served as the positive control.

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

Where, A and B is the absorbance without a test sample and with a test sample respectively.

Matrix metalloproteinase-1/Collagenase Inhibitory Assay

Stock solution of the sample of 10mg/ ml concentration was prepared by dissolving 100mg of dried plant extract in 1ml of Tricine buffer (50mM) and volume made up to 10ml. Subsequent dilutions of concentrations 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml were made from the above stock solution in Tricine buffer (50mM). Standard stock solution of concentration 1mg/ ml was prepared by dissolving 1mg of Catechin in 1ml of methanol and subsequent dilutions were made from the above stock solution. MMP-1 / Collagenase inhibition assay was carried out as per the procedure discussed (Kim *et al*., 2004). According to which, the collagenase enzyme (0.8 units/ ml) from *Clostridium histolyticum* and 2mM of substrate i.e. N-(3-[2-Furyl]acryloyl) -Leu-Gly-Pro-Ala (FALGPA,) were used for carrying out the MMP-1 / Collagenase inhibition assay. The resultant reaction mixture contained 25µl of 50mM Tricine buffer, 25µl of test extract and 25µl of 0.1 units of *Clostridium histolyticum* collagenase enzyme. To this add 50µl of 2mM FALGPA substrate and measure the collagenase inhibition activity immediately at 340 nm using a 96 well micro plate reader. Catechin served as a positive control. The % inhibition was determined by using the formula:

Enzyme inhibition activity (%) = [1 - (B / A)] X 100

Where, A and B described the enzyme activity without test extract and activity in the presence of test extract respectively.

Results and Discussion

Determination of Extractive Value

The extracts were shade dried; stored and extractive value was evaluated. The results are summarized in the table 1.

Table 1 : % Yield Obtained for Different Extracts of *Glycyrrhiza glabra*

Plant Taken for Study	Extract	% Yield (w/w)
Glycyrrhiza glabra	Methanol Extract	24.31
	Ethyl acetate Extract	12.47
	n-butanol Extract	16.91
	Aqueous Extract	32.74

DPPH radical Scavenging assay

The percentage of total antioxidant activity of methanolic, ethyl acetate, n-butanol and aqueous extracts of *Glycyrrhiza glabra* are presented in table 2. The methanolic, ethyl acetate, n-butanol and aqueous extracts of *Glycyrrhiza glabra* exhibited a maximum total antioxidant activity of 88.76, 82.68, 79.82 and 75.12% respectively at 100µg/ml whereas for ascorbic acid (standard) was found to be 90.12% at 100µg/ml. The IC_{50} values of the methanolic, ethyl acetate, n-butanol and aqueous extracts of *Glycyrrhiza glabra* and ascorbic acid were found to be 34.08, 37.59, 41.93, 43.99µg/ml and 29.25µg/ml respectively. The comparative antioxidant potential of standard (ascorbic acid), methanolic, ethyl acetate, n-butanol and aqueous extracts for *Glycyrrhiza glabra* is shown in figure 1.

Enzymatic Studies

Hyaluronidase inhibitory assay

The percentage of total hyaluronidase activity of methanolic, ethyl acetate, n-butanol and aqueous extracts of *Glycyrrhiza glabra* are presented in table 3. The methanolic, ethyl acetate, n-butanol and aqueous extracts of *Glycyrrhiza glabra* at 500µg/ml exhibited a maximum total hyaluronidase activity of 97.17, 92.44, 83.35, 70.32% respectively. The IC_{50} values of the methanolic, ethyl acetate, n-butanol and aqueous extracts of *Glycyrrhiza glabra* were found to be 13.57, 31.17, 164.47, 248.82µg/ml respectively. The comparative hyaluronidase activity of all the extracts of *Glycyrrhiza glabra* is shown in figure 2.

Elastase activity of Glycyrrhiza glabra

The percentage of total elastase activity of methanolic, ethyl acetate, n-butanol and aqueous extracts of *Glycyrrhiza glabra* are presented in table 4. The methanolic, ethyl acetate, n-butanol and aqueous extracts of *Glycyrrhiza glabra* at 100µg/ml exhibited a maximum total elastase activity of 90.84, 80.05, 75.69, 70.28% respectively. The IC_{50} values of the methanolic, ethyl acetate, n-butanol and aqueous extracts of *Glycyrrhiza glabra* were found to be 18.90, 31.06, 44.70, 59.69µg/ml respectively. The comparative elastase activity of all the extracts of *Glycyrrhiza glabra* is shown in figure 3.

Matrix metalloproteinase-1 / Collagenase inhibitory assay

The percentage of total collagenase activity of methanolic, ethyl acetate, n-butanol and aqueous extracts of *Glycyrrhiza glabra* are presented in table 5. The methanolic, ethyl acetate, n-butanol and aqueous extracts of *Glycyrrhiza glabra* at 100µg/ml exhibited a maximum total collagenase activity of 88.71, 86.07, 79.06, 71.56% 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 *Glycyrrhiza glabra* and standard catechin were found to be 21.52, 27.01, 41.76, 59.01 and 5.96 respectively. The comparative collagenase activity of all the extracts of *Glycyrrhiza glabra* is shown in figure 4.

Table 2 : % Inhibition of Methanolic, Ethyl Acetate, n-butanol and Aqueous Extracts of *Glycyrrhiza glabra* (P< 0.0007)

Concentration (µg/ml)	%Inhibition (Mean±SD)				
	Ascorbic acid (Standard)	Methanolic extract	Ethyl acetate extract	n-butanol extract	Aqueous extract
10	42.74±1.03	36.74±0.82	34.82±1.17	31.19±1.04	30.99±1.00
20	45.22±1.02	43.52±0.85	40.27±1.07	38.16±0.96	37.98±1.01
40	53.73±1.03	55.18±1.05	52.31±0.99	49.97±1.02	48.86±0.94
60	61.28±1.11	60.91±1.02	65.43±1.10	64.81±1.11	63.51±0.94
80	79.61±1.23	78.83±1.03	73.56±1.15	71.38±1.05	70.11±0.97
100	90.12±1.07	88.76±1.15	82.68±1.10	79.82±1.04	75.12±0.98
IC_{50} Value	29.25 µg/ml	**34.08µg/ml**	37.59µg/ml	41.93µg/ml	43.99µg/ml

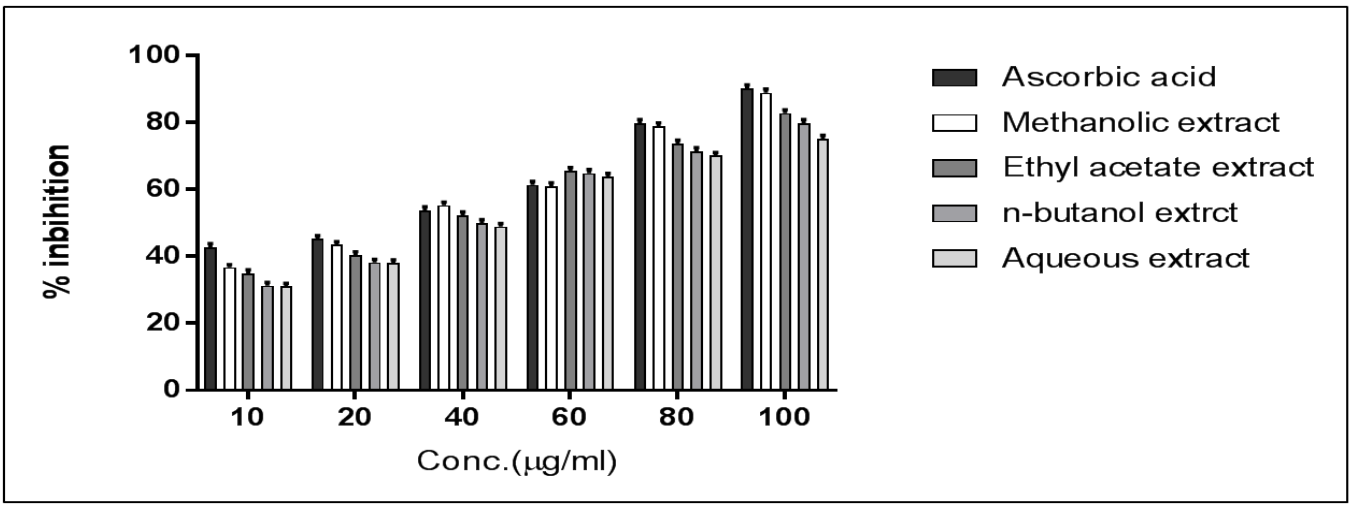

Fig. 1 : Comparative Antioxidant Potential of Different Extracts of *Glycyrrhiza glabra*

Table 3: %Inhibition of Methanolic, Ethyl Acetate, n-butanol and Aqueous Extracts of *Glycyrrhiza glabra* (*P< 0.0003*)

Concentration (µg/ml)	%Inhibition (Mean±SD)			
	Methanolic extract	**Ethyl acetate extract**	**n-butanol extract**	**Aqueous extract**
25	49.37±1.07	52.33±1.25	31.45±1.18	26.95±1.93
125	63.46±1.13	61.33±1.09	50.30±1.10	44.54±0.68
375	82.44±1.04	70.56±1.23	74.31±0.78	61.46±1.20
500	97.17±0.99	92.44±1.05	83.35±1.09	70.32±1.15
IC_{50} Value	**13.57µg/ml**	31.17µg/ml	164.47µg/ml	248.82µg/ml

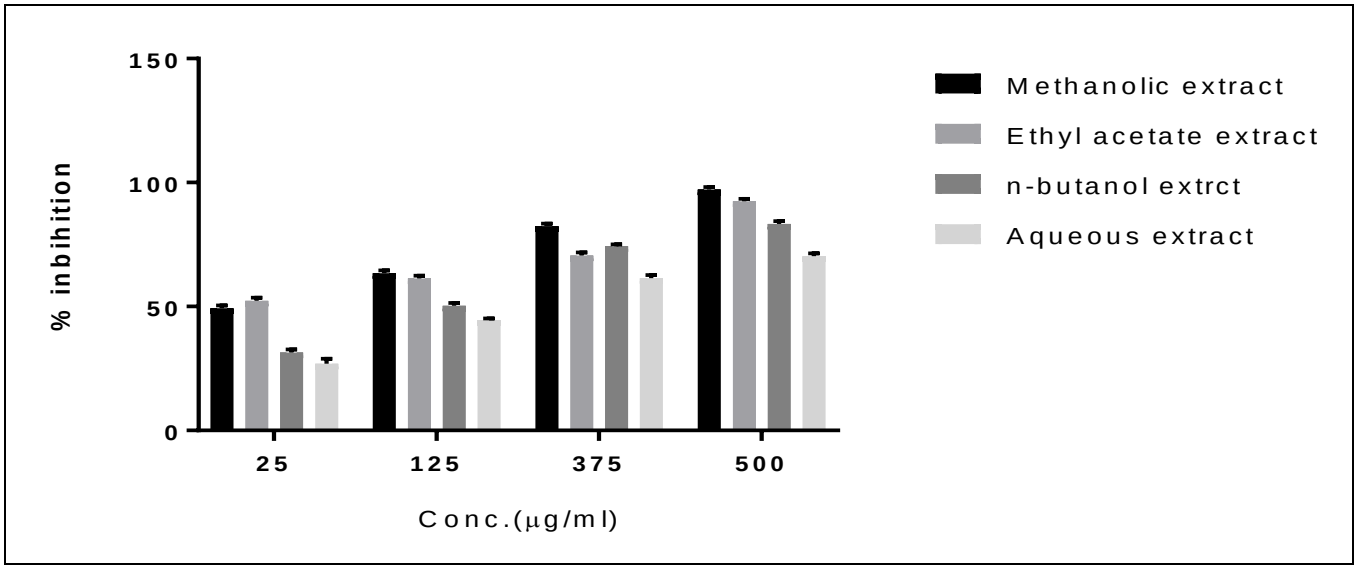

Fig. 2: Comparative Hyaluronidase inhibition of Different Extracts of *Glycyrrhiza glabra*

Table 4 : % Inhibition of Methanolic, Ethyl Acetate, n-butanol and Aqueous Extracts of *Glycyrrhiza glabra* (P< 0.0001)

Concentration (µg/ml)	%Inhibition (Mean±SD)			
	Methanolic extract	**Ethyl acetate extract**	**n-butanol extract**	**Aqueous extract**
25	50.91±0.44	44.72±1.25	37.82±0.41	31.95±1.82
50	68.57±1.13	61.18±0.28	54.91±1.10	48.61±0.68
75	77.29±0.32	69.93±1.23	61.18±0.39	52.82±0.91
100	90.84±0.99	80.05±0.79	75.69±1.09	70.28±1.15
IC_{50} Value	**18.90µg/ml**	31.06µg/ml	44.70µg/ml	59.69µg/ml

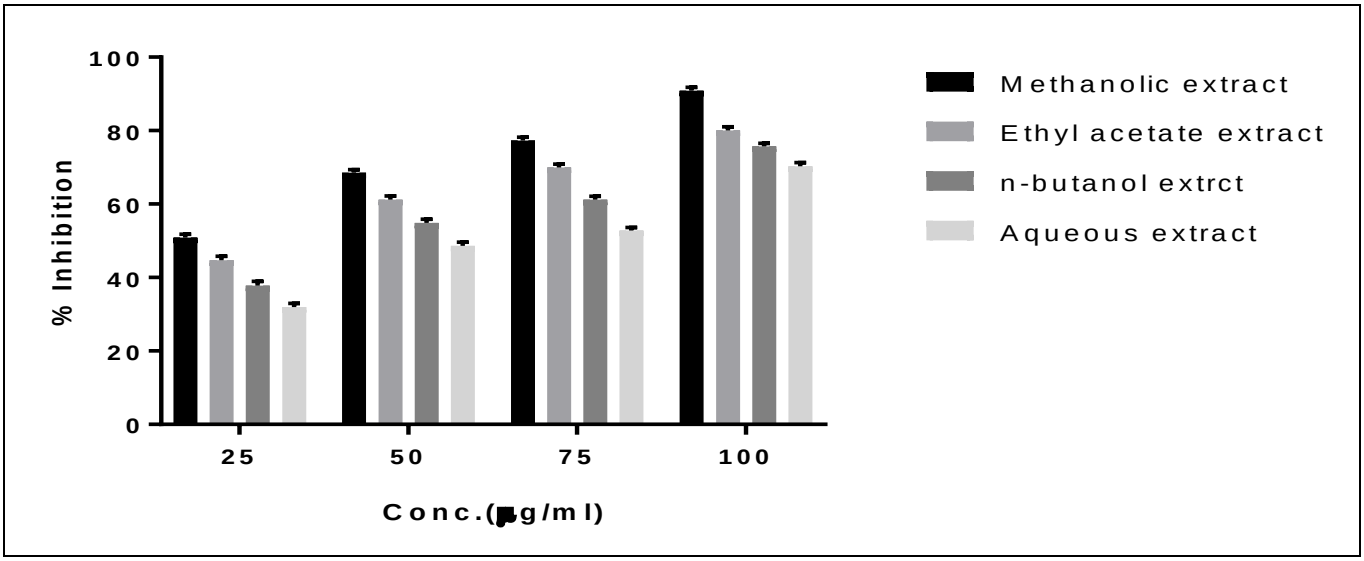

Fig. 3 : Comparative Elastase inhibition of Different Extracts of *Glycyrrhiza glabra*

Table 5 : % Inhibition of Methanolic, Ethyl Acetate, n-butanol and Aqueous Extracts of *Glycyrrhiza glabra* (*P< 0.0003*)

Concentration (µg/ml)	%Inhibition (Mean±SD)				
	Catechin (Standard)	Methanolic extract	Ethyl acetate extract	n-butanol extract	Aqueous extract
25	58.40±1.03	50.58±0.51	47.18±1.13	39.91±1.16	36.02±1.02
50	72.91±1.02	67.38±0.85	65.88±1.07	58.73±0.97	46.74±1.16
75	86.37±1.03	75.81±1.05	73.62±0.99	65.52±1.04	53.91±0.94
100	95.72±1.11	88.71±1.09	86.07±1.05	79.06±1.13	71.56±0.98
IC_{50} Value	5.96µg/ml	**21.52µg/ml**	27.01µg/ml	41.76µg/ml	59.01µg/ml

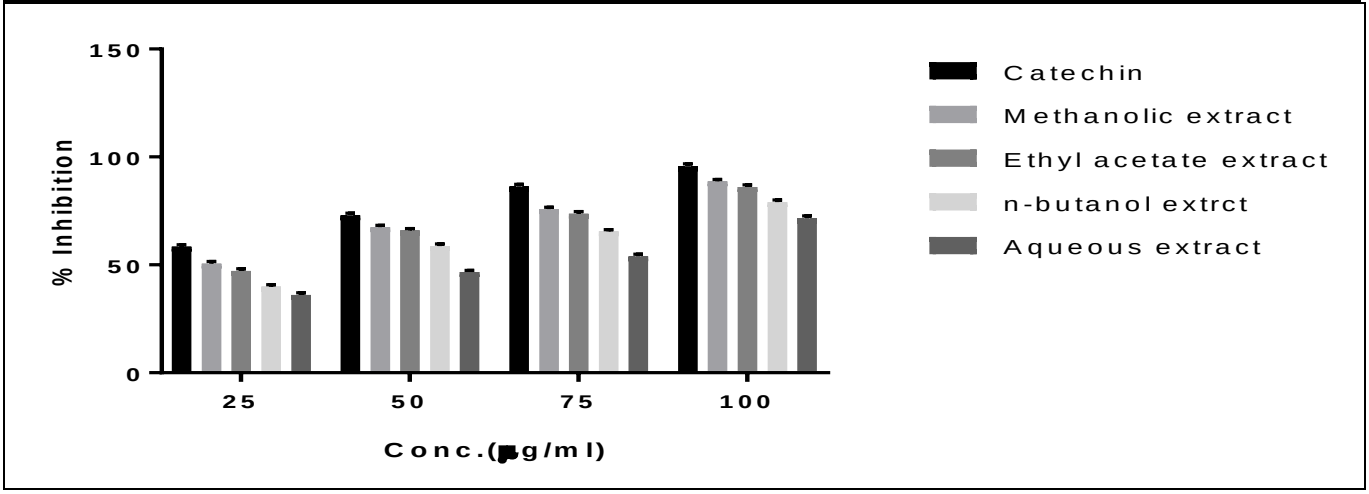

Fig. 4 : Comparative MMP-1/Collagenase inhibition of Different Extracts of *Glycyrrhiza glabra*

Conclusion

Glycyrrhiza glabra methanolic extracts showed significant antioxidant, anti-collagenase, anti-elastase and anti-hyaluronidase activities. The present study revealed new alternative for potent antiaging agents. Among the test samples, *Glycyrrhiza glabra* methanolic extract contained high phenolic content and showed the most potent antioxidant as well as enzymatic inhibition while aqueous extract showed the poor antioxidant as well as enzymatic inhibition. These results suggested that *Glycyrrhiza glabra* could be used as an ingredient in cosmetics since they provide protection against various enzymes and can be beneficial for skin aging particularly photoaging.

References

Berneburg, M.; Plettenberg, H.; Medev-Konig, K.; Pfahlberg, A.; Gers-Barlag, H.; Gefeller, O. and Krutmann, J. (2004). Induction of photoaging-associated mitochondrial common deletion in vivo in normal human skin. J Invest Dermatol, 122(5): 1277-1283.

Bhavan, B.V.; Leung, A.Y. and Foster, S. (1992). Selected medicinal plants of India.1992, Bombay, India: Tata Press.

Bissett, D.L.; Hannon, D.P. and Orr, T.V. (1987). An animal model of solar-aged skin: Histological, physical and visible changes in UV-irradiated hairless mouse skin. Photochem Photobiol, 46: 367-78.

Farooqi, A.H.; Sharma, S. and Kumar, S. (2002). Herbal Skin Care Formulation and a Process for the Preparation there of U.S. Patent 6368639; 2002. Available from: http://www.patent.ipexl.com/US/6368639.html. [Last accessed on 14 Oct 2016].

Gilchrest, BA. (1990). Skin aging and photoaging. Dermatol Nurs., 2(2): 79-82.

Kim, D.S.; Park, S.H.; Kwon, S.B.; Li, K.; Youn, S.W. and Park, K.C. (2004). Epigallocatechin-3-gallate and hinokitiol reduce melanin synthesis via decreased MITF production. Arch Pharm Res., 4(27): 334-9.

Lee, K.K.; Kim, J.H.; Cho, J.J. and Choi, J.D. (1999). Inhibitory Effects of 150 Plant Extracts on Elastase Activity, and Their Anti-inflammatory Effects. Int J Cosmet Sci., 21(2):71-82.

Losso, J.N.; Munene, C.N.; Bansode, R.R. and Bawadi, H.A. (2004). Inhibition of matrix mettaloproteinase-1 activity by the soyabean Bowman-Brik inhibitor. Biotechnol Lett., 26: 901-5.

MacKay, D. and Miller, A.L. (2003). Nutritional support for wound healing. Altern Med rev, 8: 359-77.

Maquuart, F.X.; Chastang, F. and Simeon, A. (1999). Triterpenes from Centella asiatica stimulates extracellular matrix accumulation in rat experimental wounds. Eur J Dermatol, 9: 289-96.

Mukherjee, P.K.; Maity, N.; Neema, N.K. and Sarkar, B. (2011). Bioactive compounds from natural resources against skin aging. Phytomedicine, 19: 64-73.

Nickavar, B.; Kamalinejad, M.; Haj-Yahya, M. and Shafaghi, B. (2006). Comparison of the free radical scavenging activity of six iranianachillea. species. Pharm Biol, 44(3): 208–212.

Tschesche, H.; Bakowski, B.; Schettler, A.; Knäuper, V. and Reinke, H. (1991). Leukodiapedesis, release of PMN leukocyte proteinases and activation of PMNL procollagenase. Acta Biomedica Biochimica. 50: 755-761.

Yaar, M. and Gilchrest, B.A. (1990). Cellular and molecular mechanisms of cutaneous aging J Dermatol Surg Oncol, 16(10): 915-922.

Yasui, S.; Fujiwara, K.; Tawada, A.; Fukuda, Y.; Nakano, M. and Yokosuka, O. (2011). Efficacy of intravenous glycyrrhizin in the early stage of acute onset autoimmune hepatitis. Digestive Diseases and Sciences, 56 (12): 3638–47.