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Formulation and evaluation of antioxidant and antityrosinase activity of *Polygonum amplexicaule* herbal gel

Nauman Aziz[1], Abdul Sami[1], Ammara Jabeen[1], Muhammad Gulfraz[2], Rahmatullah Qureshi[3], Tanveer Ibrahim[1,4], Ammad Ahmad Farooqi[5], SM Saqlan Naqvi[1] and M Sheeraz Ahmad*[1]

[1]Department of Biochemistry, Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan
[2]Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad, Pakistan
[3]Department of Botany, Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan
[4]National Institute of Health, Islamabad, Pakistan
[5]Institute of Biomedical and genetic Engineering (IBGE), Islamabad, Pakistan

Abstract: Medicinal plants are long been used for pharmaceutical and cosmetic industry. Among medicinal plants, *Polygonum amplexicaule* of family polygonaceae has traditional use in medicines and skin care. *P. amplexicaule* belongs to genus Polygonum that contains several important phytochemicals and considered as a rich source of antioxidants. The present study was designed to formulate herbal gel containing *P. amplexicaule* extract and evaluate its different physical properties as well as antioxidants and antityrosinase activities. Chitosan gel base was used as gelling agent and different gel formulations were prepared by different concentrations of extracts and polymers. Physical properties like pH, colour, odour, appearance and homogeneity, spreadability, extrudability and stability were optimized and analysed. A stable gel formulation containing 1% chitosan gel base and 5% plant extract was prepared that showed good appearance and homogeneity, easily spread ability and excellent extrudability. This gel formulation was tested for antioxidant and skin whitening properties by DPPH free radical scavenging assay and tyrosinase inhibition assay respectively and ascorbic acid was used as reference standard. DPPH scavenging activity with an IC_{50} value of 0.446 mg/mL and tyrosinase inhibition activity with an IC_{50} value of 0.805 mg/mL was observed and results indicated that this herbal gel formulation has a good potential for cosmetic use.

Keywords: *Polygonum amplexicaule*, herbal gel, antityrosinase, chitosan.

INTRODUCTION

In the modern world it is the desire of every person on earth that he or she looks beautiful or improve the visual appearance. Some people are naturally beautiful whereas some use various cosmetic products to achieve this goal. In the present world only ladies are not conscious about visual appearance but gents also use cosmetics (Jain *et al*., 2010). Cosmetics and pharmaceuticals when combine, produce cosmaceuticals. Anti-aging creams and moisturizers are the products that usually come under the category of cosmaceuticals.

Biologically active drugs and cosmaceuticals are obtained from nature having less toxicities compared to synthetic compounds (Hussain *et, al.,* 2009a,b). To cure the diseases and to whiten the skin, many plants have been used by man from a long time. Research around the world showed that the source of natural products is mostly obtained from medicinal plants. Any plant which contains the chemical substance in roots, leaves, stem, flowers, fruit and seeds and are used for therapeutic purposes are known as medicinal plant (Khan, 1991, Gulfraz *et, al.,* 2014, Ahmed *et, al.,* 2015).

Plants that are known as knotweed or smartweed are included in the family Polygonaceae which contains flowering plants. *P. amplexicaule* is a flowering plant. Other names of *P. amplexicaule* include *Persicaria amplexicaule* or red bistort. Local name of *P. amplexicaule* is Masloon. Genus of *P. amplexicaule* is Polygonum. On the basis of the genus name, the name of the family based (Jussieu, 1789) and the name Polygonum is given to the plants included in this family because on the stem many swollen nodes are present. Two Greek words generate the name Polygonum; poly which means many and goni which mean joint. About 300 species are present in the genus Polygonum and most species like *P. bistorta* and *P. multiflorum* are medicinal plants. The plants in the genus *Polygonum* produce high content of antioxidants like cardiac glycosides, phenolics, terpenoids and flavonoids are present in high concentration in the plants of the genus Polygonum (Ahmad *et al*., 2013, Batool *et al*., 2015). This plant of genus Polygonum is found in the range of Himalayas. Length of the *P. amplexicaule* plant is 1.2m (4ft) and it is herbaceous perennial plant. In summer rose-red or white colour flower are produced by *P. amplexicaule.* Its leaves are heart shaped, pointed and wide. Evidences are present that many diseases like pain, fractures, haemorrhage, dysentery, inflammation, blood circulation and diuretic can be treated by *P. amplexicaule* (Mudasir *et al*., 2012). The plants in the genus *Polygonum* produce high content

**Corresponding author:* e-mail: dr.sheeraz@uaar.edu.pk

of antioxidants like cardiac glycosides, phenolics, terpenoids and flavonoids (Espin *et al*., 2000; Luo *et al*., 2002).

Naturally produced antioxidants revealed the attraction of researchers all over the world because during oxidative stress these antioxidants play an important role. Among the patient and physicians there is an increased trend to be treated with products containing herbs. Skin disorders are mostly treated with herbal products (Jurga *et al*., 2011). Millions of chemical compounds obtained from aromatic and medicinal plants show many useful properties such as: antimicrobial, antifungal, antityrosinase, antioxidant and many other properties. In the literature millions of such plants that contain different properties are present in cosmetic industry. There is a wide scope to explore the properties of such plants and thus to be used in cosmetics (Chih *et al*., 2011). In the present study, based on the phytochemical components of the *P. amplexicaule* an attempt was made to formulate and evaluate the herbal formulation for skin care.

MATERIALS AND METHODS

Plant collection

The whole plant of *P. amplexicaule* was collected from hills of Murree and Miran Jani Abbottabad. The healthy rhizomes from the plant were washed and cut into pieces. For drying, plant material was kept into shade. After drying, plant material was finely grinded into powder and stored in well closed container. All the experimental work was conducted at Biochemistry Labs of PMAS-Arid Agriculture University Rawalpindi and Chemicals were purchased from Local suppliers. The animal testing was performed with ethical approval from Institutional Ethics Committee (IEC), PMAS-Arid Agriculture University Rawalpindi.

Preparation of plant extract

For herbal gel preparation, the Crude Methanolic Rhizome Extract (CMRE) was prepared. CMRE was prepared by soaking 100 g of the powdered rhizome in analytical grade methanol (300 mL) and placed in shaker for 24 hours with constant shaking using shaker incubator, after shaking, suspension was filtered and then evaporated in a rotary evaporator to obtain a thick paste. Concentrated extract was stored in air tight bottles until analysed.

Preparation of herbal formulation

Herbal formulation was prepared using chitosan as gel base by a method described by (Patil *et al*., 2012). Chitosan is preferred gelling agent because of its unique properties such as antimicrobial character, non-toxic, non-immunogenic and biodegradable. Chitosan may increase the bioavailability of drug which minimizes the dose and administration frequency (Kas, 1997; Hiroshi *et al.,* 2005). Different concentrations of chitosan were tried with different concentrations of plant extracts (as mentioned in Table: 1). 100ml of lactic acid (2% solution) was added and allowed it to swell. Then 20 % glycerine was added in the swollen gel base. In 20 ml of ethanol, plant extract was dissolved and mixed with previous mixture. Then for 5 min it was stirred using mechanical stirrer. After that 0.3ml of glutaraldehyde (0.5%) solution was added and again was stirred for 30 min. Triethanolamine was added drop wise to the formulation for adjusting the required skin pH (pH = 6-7) and the formulation was allow to stand for 48 hours to settle (fig. 1). The same method was followed for the preparation of control sample without adding plant extract (fig. 1).

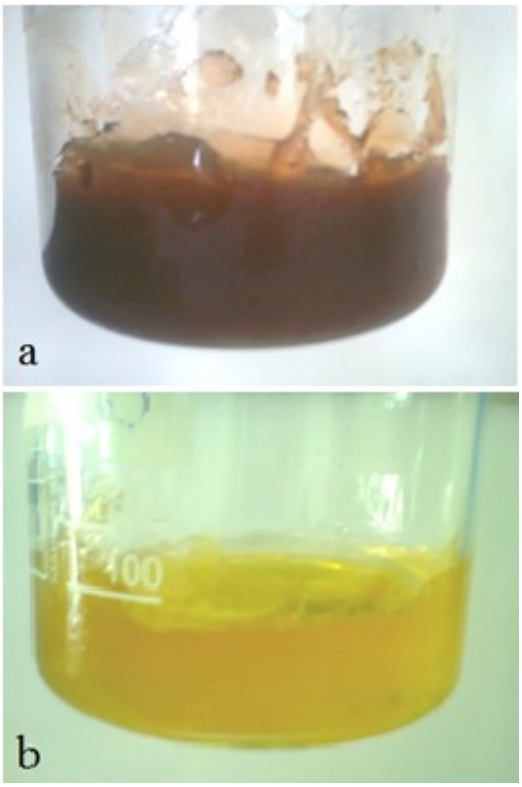


Fig. 1: Herbal gel preparation, (a) Herbal gel formulation that contains 5 % plant extract and 1 % chitosan gel base (b) Control sample that contains no plant extract in it.

Physical evaluation of gel formulation

The gel formulation was subjected to physical evaluation for following parameters.

Determination of pH

The pH was determined by dissolving 2.5gm of formulation in 25ml of distilled water and pH was measured by pH meter (Kuntal *et al*., 2012).

Determination of colour

Colour of the formulation was checked with naked eyes by simply putting the formulation against white background (Kuntal *et al*., 2012).

Determination of gel appearance and homogeneity

The prepared herbal formulation was tested for physical appearance and homogeneity simply by visual observations and by touch and was observed carefully for the presence of any clumps, aggregates etc. Homogeneity of the formulation was ranked as: +++ = excellent, ++ = very good, + = good, - = poor (Saad *et al*., 2013).

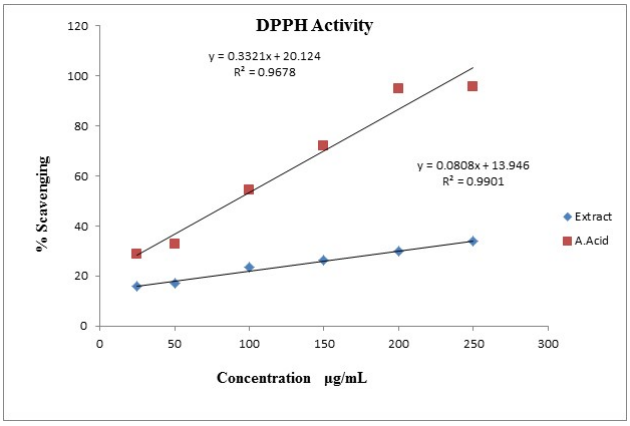


Fig. 2: Radical scavenging effect at different concentrations of Gel formulations

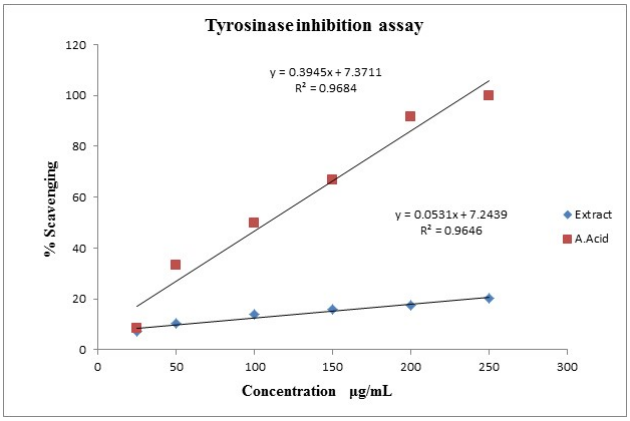


Fig. 3: Tyrosinase inhibition effect at different concentrations of Gel formulations

Determination of odour

For checking the smell of prepared formulation, sufficient amount of formulation was mixed in distilled water and then smell was checked (Saad *et al*., 2013).

Determination of spreadability

The spreadability of the gel formulation was determined by measuring the spreading diameter of formulation. Two glass plates of same dimension were taken. Accurately weighted 1g formulation was placed in between two glass plates in such a way that the formulation was sandwiched between two glass plates. After this two glass plates were compressed to uniform thickness by placing 125g weight on the upper slide for one minute. After one minute, remove the weight and measured the spreadability in milli metres (mm) (Patel *et al*., 2009).

Determination of extrudability

To check the extrudability of formulation, caped collapsible aluminium tubes were taken. The gel formulation was filled in aluminium tubes then tubes were tightly capped and the weight of the tubes was recorded. The tube was placed between two glass slides and 500 g weight was placed over the upper glass slide and then cap was opened. The amount of gel formulation extruded were collected and weighted. The % of formulation extruded was calculated and grades as: (>90% extrudability: excellent, >80% extrudability: good, >70 % extrudability: fair) (Gupta and Gaud, 2005).

Fig. 4: Mice in captivity for skin irritation assay

Stability study

The stability study was carried out at different temperatures. In collapsible tubes, the formulated gel was filled and stored at different temperatures; refrigerator temperature (5±3°C), room temperature (25±2°C) and at 40±5°C for a period of two months and then studied for appearance, pH and spreadability (Mohanata *et al*., 2007).

Preparation of gel extract for antioxidant and antityrosinase assay

Formulation was extracted for different assays to obtain crude methanolic gel extract (CMGE) (Riani *et al*., 2012). Gel formulation was dissolved in methanol and for a period of 24 hours the suspension was kept with constant shaking. After that the extract was filtered and final product was concentrated in rotary vacuum evaporator to obtain methanolic gel extract. The residues were collected and stored for different assays.

Antioxidant activity

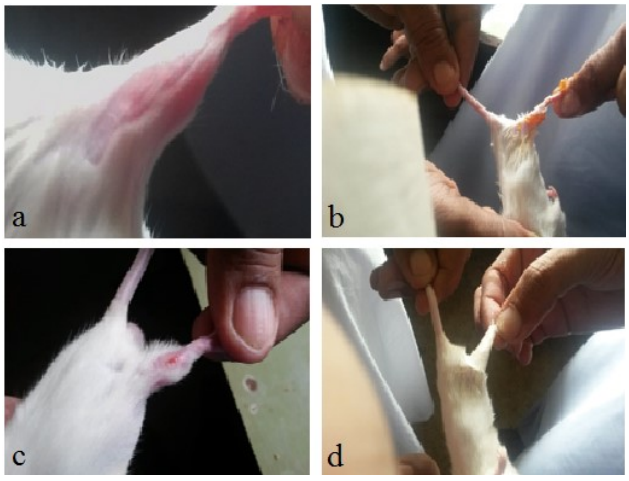
DPPH radical scavenging activity

A stable organic free radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH) is commonly used to detect antioxidant activity of any extract. To carry out DPPH assay the method of (Bidchol *et al.,* 2009) with slight modification was used. In a test tube 2ml of 0.0024% DPPH solution were added followed by the addition of 0.1ml of methanolic gel extract. The reaction mixture was shaked vigorously and then incubated for 30 min at room

temperature in the dark. The absorbance of reaction mixture was measured in spectrophotometer, the wavelength of 517 nm was selected at which DPPH free radicals give maximum absorption. A Solution containing methanol and DPPH solution was prepared as blank. Ascorbic acid was used as a positive control with same concentrations as of extract. The radical scavenging activity was measured as decrease in the absorbance of DPPH by increasing gel extract. % age scavenging effect was calculated using the following equation and graph was plotted to obtain its IC_{50} value (fig. 2).

$$\text{Scavenging effect}(\%) = \frac{(\text{Ac}) - (\text{As})}{(\text{Ac})} \times 100$$

Whereas:
'As' is the absorbance of test sample
'Ac' is the absorbance of control.
To take mean value, perform the experiment in triplicate and values were averaged.



a) Hairs from the mice skin was cleared for assay
b) Herbal formulation was applied on the cleared mice skin
c) Control sample also applied on mice skin
d) Applied mice skin wrapped with gauze patch for 4 hours.

Fig. 5: Herbal formulation application on mice

Tyrosinase inhibition assay

Cosmetics industries are mostly interested to produce such cosmetics that whiten the skin or in scientific words inhibit the production of melanin. In melanin biosynthetic pathway, tyrosinase enzyme is most important enzyme for the conversion of an amino acid named tyrosine into melanin. The formulated herbal gel was also evaluated for antityrosinase assay to check its suitability in cosmetics. Tyrosinase inhibitory activity was measured at 475 nm by the method described by (Kwak *et al*., 2011). Total reaction mixture was 3 ml. First took 0.3 ml of 192 units tyrosinase solution into each concentrations of 1 ml gel extract. This mixture was incubated at 37°C for 10 minutes. After that 1ml of 1.5mM tyrosine solution was added followed by the addition of buffer to achieve desired volume. This mixture was again incubated for 25 minutes at 37°C. After incubation, the UV absorbance of the reaction mixture was measured at 475 nm. Ascorbic acid, the tyrosinase inhibitor was used as a standard. Control reaction mixture contained buffer, tyrosinase and tyrosine. The percentage of tyrosinase inhibitory activity was calculated by the following equation, and then IC_{50} value was calculated from equation displayed on graph (fig. 3).

$$\text{Scavenging effect}(\%) = \frac{(\text{Ac}) - (\text{As})}{(\text{Ac})} \times 100$$

Whereas:
'Ac' is the absorbance of test sample
'Ac' is the absorbance of control.
Each experiment was performed in triplicate and values were averaged

Primary skin irritation assay

Primary Dermal Irritation index (PDII) of herbal gel was carried according to the method described by Arvind and Arora, 2012. In this study albino mice of either sex weighing 50±2.5 grams were obtained from animal house of National Institute of Health Islamabad. The animals were maintained on standard animal feed with free provision to drinking water. They were kept in standard poly-propylene cages. Photoperiod of mice was maintained at 12 hours cycle of light and dark, room temperature was 24±5ºC and humidity was 45-50%.

Preparation of animals

About one day before the start of experiment dorsal hair at the back of the animals was clipped off (fig. 4). Animals that showed normal skin texture without any damage was used for skin irritation assay and were housed individually in cages.

Application of the herbal formulation

50 mg of the formulation were applied over 1 cm^2 area of intact skin of mice (fig. 5). The herbal formulation was applied for a period of 4 hours and was then removed. Observations were recorded an hour after the removal of the formulation. The animals were observed for four days for any sign of erythema and oedema. Same procedure was repeated for the mice that serve as control and 50 mg of the gel base, i.e. gel formulation that contain no plant extract was applied on the mice skin and in the similar fashion observations were recorded for control animals (fig. 5). Both the animals that serve as control and the test were observed daily for the presence of any redness, irritation and other toxic reactions that includes oedema or erythema. Results of observations were evaluated in between 0-4 where in case of no erythema and escher formation value 0 was awarded and for very slight, well defined, moderate and severe erythema to escher formation values 1, 2, 3 and 4 was awarded respectively. In case of oedema, 0 stood for no oedema and 4 stood for severe oedema (table 7). Classification system based on PDII, PDII = PDII observed after 0.5 + 1 + 2 + 3 days 4

Table 1: Different Formulations of Herbal Gel of *P. amplexicaule*

Gel Formulations	The concentration of different reagents added (%)[123]					
	Plant Extract	Chitosan	Lactic acid	Glycerol	Ethanol	Glutaraldehyde
GF-1	5	1.0	2	20	20	0.3
GF-2	10	1.0	2	20	20	0.3
GF-3	5	2.0	2	20	20	0.3
GF-4	10	2.0	2	20	20	0.3
GF-5	5	2.5	2	20	20	0.3
GF-6	10	2.5	2	20	20	0.3

[1]Each formulation differs only in the concentration of plant extract and gel base. [2]All the formulations were neutralized by triethanolamine to required skin pH. [3] Distilled water was added to make solution 100 %.

Table 2: Physical parameters of prepared formulations (Mean ± STDEV[1])

Gel Formulations	Physical parameters of Gel formulations				pH	Spreading diameter (mm)
	Colour	Odour	Homogeneity[2]	Feel on application		
Control	Yellow	Odourless	+++	Cool and Smooth	6.43	70.09 ± 1.371
GF-1[3]	Brown	OE[5]	+++	Cool and Smooth	6.35	68.72 ± 0.360
GF-2[4]	Brown	OE	+++	Cool and Smooth	6.40	60.98 ± 2.156

[1]Standared deviation, data represents in triplicate value. [2]Homogeneity of the formulation was ranked as: +++ = excellent, ++ = very good, + = good, - = poor. [3]Formulation 1 contain 5 % plant extract. [4]Formulation 2 contain 10 % plant extract. [5]Characteristic odour of extract incorporated.

Table 3: Extrudability of all the formulation at the time of preparation (Mean ± STDEV[1])

Gel Formulations	Extrudability of Gel formulations			Grades[4]
	Weight of formulation in tube (g)	Weight of formulation extruded (g)	Extrudability (%)	
Control	17.10 ± 0.0141	15.60 ± 0.0070	91.22 ± 0.005	++++
GF-1[2]	17.15 ± 0.0212	15.49 ± 0.0565	90.32 ± 0.009	++++
GF-2[3]	17.19 ± 0.0282	14.70 ± 0.0636	85.51 ± 0.010	+++

[1]Standared deviation, data represents in triplicate value. [2]Formulation 1 contain 5 % plant extract. [3]Formulation 2 contains 10 % plant extract. [4]Extrudability were expressed as: Excellent = ++++, Good = +++, Fair = ++.

Table 4: Effect of storage conditions on the stability of GF-1 that contains 5 % plant extract. GF-1 was stored at different temperatures for stability studies (Mean ± STDEV[1]).

Parameters	Monitoring phase	Storage temperature of formulations		
		5 °C	25 °C	40 °C
pH	Initial[2]	6.1 ± 0.070	6.2 ± 0.141	6.1 ± 0.070
	Final[3]	6.1 ± 0.035	6.2 ± 0.035	6.2 ± 0.035
Colour	Initial	Brown	Brown	Brown
	Final	Brown	Brown	Brown
Odour	Initial	OE[4]	OE	OE
	Final	OE	OE	OE
Homogeneity[5]	Initial	+++	+++	+++
	Final	+++	+++	+++
Spreadability (mm)	Initial	68.72 ± 0.360	68.72 ± 0.360	68.72 ± 0.360
	Final	66.28 ± 2.651	68.70 ± 3.302	70.12 ± 1.810
Extrudability[6]	Initial	++++	++++	++++
	Final	+++	++++	++++

[1]Standard deviation, data represents in triplicate value. [2]Monitoring at the time of formulation preparation. [3]Monitoring after two months under different conditions. [4]Characteristic odour of extract incorporated. [5]Extrudability value of formulation was expressed as : Excellent = ++++ , Good = +++ , Fair = ++. [5]Homogeneity of the formulation was ranked as: +++ = excellent, ++ = very good, + = good, - = poor.

Table 5: Radical scavenging effect (%) at different concentrations of gel extract (Mean ± STDEV[1])

S. No.	Extract	Radical scavenging effect at different concentrations of Gel formulations							IC_{50} mg/mL
			25µg/mL	50µg/mL	100µg/mL	150µg/mL	200µg/mL	250µg/mL	
1	CMEG[2]	Abs. 517 nm	0.714± 0.041	0.702± 0.038	0.650± 0.021	0.626± 0.036	0.595± 0.053	0.560± 0.050	0.446
		% inhibition[4]	15.80	17.21	23.34	26.17	29.83	33.96	
2	A.A.[3]	Abs. 517 nm	0.605± 0.040	0.570± 0.038	0.388± 0.021	0.238± 0.036	0.045± 0.053	0.035± 0.050	0.089
		% inhibition	28.65	32.78	54.24	71.93	94.69	95.87	

[1]Standard deviation, data represents in triplicate value. Mean value of absorbance at 517 nm. [2]CMEG = Crude methanolic extract from gel formulation. [3]A.A. = Ascorbic acid. [4]Percent inhibition = (A-B)/A × 100 A = absorbance at 517 nm without test sample. B = absorbance at 517 nm with test sample.

Table 6: % Tyrosinase inhibition at different concentrations of gel extract (Mean ± STDEV[1])

S. No.	Extract	Tyrosinase inhibition at different concentrations of Gel formulations							IC_{50} mg/mL
			25µg/mL	50µg/mL	100µg/mL	150µg/mL	200µg/mL	250µg/mL	
1	CMEG[2]	Abs. 517 nm	0.707± 0.033	0.685± 0.025	0.658± 0.017	0.642± 0.028	0.631± 0.046	0.609± 0.049	0.805
		% inhibition[4]	7.33	10.22	13.76	15.85	17.3	20.18	
2	A.A.[3]	Abs. 517 nm	0.011± 0.025	0.008± 0.008	0.006± 0.061	0.004± 0.039	0.001± 0.013	0.00± 0.009	0.108
		% inhibition	8.33	33.33	50	66.66	91.66	98.03	

[1]Standered deviation, data represents in triplicate value. Mean value of absorbance at 517 nm. [2]CMEG = Crude methanolic extract from gel formulation. [3]A.A. = Ascorbic acid. [4]Percent inhibition = (A-B)/A × 100 A = absorbance at 517 nm without test sample. B = absorbance at 517 nm with test sample.

Where, PDII = Primary Dermal Irritation Index

According to primary dermal irritation index (table 8) results were classified as; <0.5: non-irritating, 0.5-2.0: slightly irritating, 2.1-5.0: moderately irritating and >5.0: severely irritating.

Table 7: Score assigned to mices on the basis of erythema and oedema formation.

Skin reaction	Score[1]
Erythema formation	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema[2]	3
Escher formation[3]	4
Oedema formation	
No oedema	0
Very slight oedema[4]	1
Slight oedema[5]	2
Moderate oedema[6]	3
Severe oedema[7]	4

[1]These scores are assigned after observing the erythema and oedema in mices. [2]Moderate to severe erythema mean size of erythema is 2 mm. [3]Escher formation mean injuries in depth. [4, 5, 6, 7]Very slight oedema, slight oedema, moderate oedema and severe oedema mean size of oedema rises approximately 1, 2, 3 and 4 mm.

RESULTS

Plant extract preparation

The herbal formulation containing *P. amplexicaule* rhizome extract was prepared and subjected to evaluation of the said physical parameters. The % yield of methanolic extract was found to be 16.38%.

Table 8: Evaluation of Primary Dermal Irritation Index (PDII)

Evaluation	Index[1]
No irritation	0.00
Irritation barely perceptible	0.04-0.99
Slight irritation	1.00-1.99
Mild Irritation	2.00-2.99
Moderate irritation	3.00-5.99
Severe irritation	6.00-8.00

[1]These index value obtained from PDII formula after assigning the erythema and oedema scores to mices.

Herbal gel prepration

Among different combinations of plant extract and gel base, formulation containing 1% chitosan gel base and 5% plant extract was most appropriate for the formulation of gel. Remaining of all other formulations either becomes too hard or too liquid and thus difficult to apply. So herbal formulation containing 1% chitosan gel base and 5% and 10% plant extract was further used for evaluation of different physical properties.

Physical evaluation of formulations

The herbal formulation showed good results for all the physical parameters for which the formulation was analysed (table. 2). The pH of the gel formulation was in the range of 5.9 to 6.5±0.2 which gives cool and smooth feeling on application and produces no irritation. Gel

Table 9: Effect of gel formulation that contain 5 % plant extract on mice skin

S. No	Gel Formulation	Sign of irritation[1]			Score awarded[2]	PDII index[3]
		Redness	Erythema	Oedema		
1	Control	No	No	No	0[4]	No irritation[5]
2	GF-1	No	No	No	0	No irritation

[1]Herbal formulation and control sample was applied on the mice skin for about 4 hours and after the 4 hours formulation was removed and after one hour check for the sign of irritation. [2]Score awarded according to the table 3.2 and 3.3. [3]PDII index was obtained after putting the values of oedema and erythema in PDII formulae. [4]0 mean no erythema and oedema observed in mices. [5]No irritation mean PDII index was 0.00.

formulation was deep brown in colour and have characteristic odour of plant extract incorporated. The appearance of the gel formulation was attractive that form homogenous mixture and produced uniform distribution of extract in herbal gel with absence of any lumps or aggregates. Extrudability was excellent (table 3) while spreading diameter after one minute was in between 60-70 mm. The result of stability studies (table 4) revealed that formulation was stable at normal storage conditions.

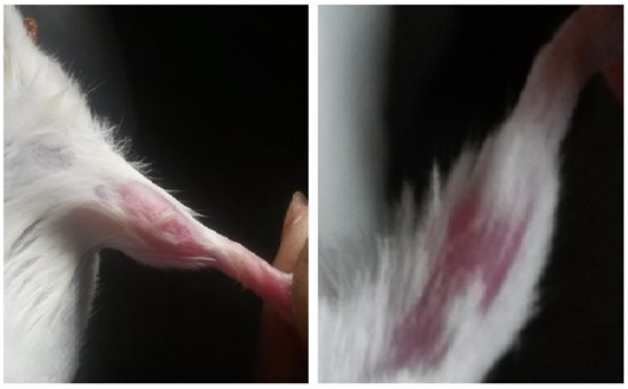


Fig. 6: Herbal formulation did not produce any sign of redness, erythema and oedema on mice skin.

Extraction from herbal formulation

The herbal gel was extracted with methanol to evaluate its antioxidant and antityrosinase activities. The % yield of methanolic extract from the gel was found to be 1.73%. This extract was further used for the antioxidant and antityrosinase activities.

DPPH *assay*

The result of DPPH radical scavenging assay has been presented in table 5. Results had shown that the scavenging effect of methanolic extract from gel containing *P. amplexicaule* rhizome extract on DPPH radicals increased with increasing concentrations of extract. The result was compared with standard curve (fig. 2) of ascorbic acid generated in the range of 25-250 µg/mL. IC_{50} value of ascorbic acid was 0.089mg/mL and for gel extract it was determined to be 0.446mg/mL. The IC_{50} value was calculated graphically with reference to standard curve $y = 0.0808x + 13.946$, $R_2 = 0.9901$ for gel extract and $y = 0.3321x + 20.124$, $R_2 = 0.9678$ for ascorbic acid (fig. 2).

Tyrosinase inhibition assay

The result of tyrosinase inhibition assay has been presented in table 6. This indicates that the gel extract show significant tyrosinase inhibition with increase in concentration of gel extract. The results of tyrosinase inhibition were compared with ascorbic acid which was used as standard tyrosinase inhibitor. The ascorbic acid was used in the same concentrations (25-250µg/mL) as that of gel extract. IC_{50} value of ascorbic acid is 0.108 mg/mL which was calculated graphically with reference to standard curve $y = 0.3945x + 7.3711$, $R_2 = 0.9684$. IC_{50} value of gel extract was found to be 0.805 mg/mL which was also calculated graphically with reference to standard curve $y = 0.0531x + 7.2439$, $R_2 = 0.9646$ (fig. 3).

Primary skin irritation assay

The scores for skin irritation in terms of erythema and oedema had recorded for skin of mice at 0.5, 1, 2 and 3 days according to Organization of Economic Corporation and Development (OECD) scoring system. Results revealed the herbal formulation had not caused any erythema or oedema on the intact mice skin when observed for 3 days (fig. 6). The Primary dermal irritation index (PDII) of the formulation was 0.00, (table 9) hence according to OECD guidelines the formulation can classified as a non-irritant to the mice skin.

STATISTICAL ANALYSIS

The parametric data were expressed as mean ± Standard deviation. The MS Excel 2010 was used for statistical analysis.

DISCUSSION

In general the gel formulation that contains 1% chitosan gel base and 5% plant extract showed best results in terms of physical properties and antioxidant and antityrosinase activities. This formulation is well suited for all physical parameters like pH, spreadibility, extrudability, colour, stability, and odour. The pH of the gel was in the range of 5.9 to 6.5±0.2 which gives cool and smooth feeling on application and produces no irritation. Gel formulation was deep brown in colour and has characteristic odour of plant extract incorporated. The appearance of the gel formulation was attractive that forms homogenous mixture. Extrudability was excellent while spreading

diameter was in between 60-70mm. The result of stability studies revealed that formulation was stable at normal storage conditions.

Antioxidant and skin whitening property of the formulation was also analysed. The formulation containing 5% crude methanolic extract of *P. Amplexicaule* showed good DPPH scavenging and tyrosinase inhibition activity with IC_{50} value 0.446 mg/mL and 0.805mg/mL respectively. Therefore, gel formulation having 1% chitosan gel base and 5% plant extract can be further analysed *in vivo* for other pharmacological and toxicological studies for potential use in cosmetic industry.

CONCLUSION

The data presented in this study demonstrated that *P. amplexicaule* extract in the gel formulation having 1% chitosan gel base and 5% plant extract possesses significant antioxidant and antotyrosinase activities.

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REFERENCES

Ahmad MS, Naqvi SMS, Mushtaq S, Ramzan F, Sami A, Batool S, Ihsan-ul-Haq and Bushra Mirza B (2013). Phytochemical and biological evaluation *of Polygonum amplexicaule* rhizome extract. *Canadian Journal of Pure and Applied Sciences,* **7**(3): 2303-2314.

Ahmed A, Asad MJ, Ahmad MS, Qureshi R, Shah SI, Gul H and Gulfraz M (2015). Antidiabetic and hypolipidemic potential of *Rhazya stricta* Decne extract and its fractions. *International Current Pharmaceutical Journal,* **4**(2): 353-361

Batool S, Gulfraz M, Akram A, Naqvi SMS, Ihsan-ul-Haq Mirza B and Ahmad MS (2015). Evaluation of antioxidant potential and HPLC based identification of phenolics in *Polygonum amplexicaule* extract and its fractions. *Pak. J. Pharm. Sci.,,* **28**(2): 415-419.

Bidchol AM, A Wilfred, P Abhijna and R Harish (2009). Free radical scavenging activity of aqueous and ethanolic extract of *Brassica oleracea*. *Food. Biopro. Technol.,* DOI: doi:10.1007/s11947-009-0196-9.

Chihchien L, Y Chaohsun, W Peyshiuan, K Changchin and C Yishyan (2011). Antimicrobial, antioxidant and antityrosinase activities of aqueous aromatic extract from forty eight selected herbs. *J. Med. Plan. Res.,* **5**(26): 6203-6209.

Espin JC, RC Soler and HJ Wichers *et al* (2000). Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuffs. *J. Nat. Prod.,* **48**(5): 1588-1592.

Gulfraz M, Ahamd Dawood, Ahmad MS, Qureshi R, Mahmood RT, Jabeen N and Abbasi KS (2014). Effect of leaf extracts of *Taraxacum officinale* on CCl4 induced Hepatotoxicity in rats, *in vivo* study. *Pak. J. Pharm. Sci.,* **27**(4): 825-829.

Gupta GD and RS Gaud (2005). Release rate of tenoxicam from acrypol gels. *J. Ind. Phar.,* **5**: 69-76.

Hiroshi H, W Keta, RV Ratana and T Seiichi (2005). Preservation of chitosan aqueous gel under neutral conditions. *J. Metals Materials and Minerals,* **15**: 19-21.

Hussain M, Ahmad MS, Siddique A, Hanif M, Ali S, Mirza B (2009a). Dimethyltin(IV) Derivatives of Biologically Potent Substituted Phenylacrylic Acids; Synthesis, Chemical Characterization and Inhibitory Effects on Agrobacterium tumefaciens. *Chemical Biology & Drug Design*, **74**(2), 183-189

Hussain M, Hanif M, Ali S, Shahzadi S, Ahmad MS, Mirza B and Evans HS (2009b). Synthesis and coordination chemistry of Organotin(IV) complexes of 2,3-methylenedioxyphenylpropenoic acid. *Journal of Coordination Chemistry*, **62**(13), 2229-2238.

Jain A, D Subodh, G Alka, K Pushpendra and T Vivek (2010). Potential of herbs as cosmeceuticals. *J. Res. in Ayu and Pha.,* **1**(1): 71-77.

Jurga B, M Ruta, D Julija, P Rimantas, G Roumalda, B Ruta, M Daiva, L Robertas, C Genuvatie, V Saule, M Jan and C Zuzana (2011). Topical application of *Calendula officinales (L):* Formulation and evaluation of hydrophilic cream with antioxidant activity. *J. Med. Plan. Res.,* **5**(6): 868-877.

Jussieu AL (1789). Genera Plantarum, secundum ordines naturals disposita, juxta methodum in horto region parisiensi exararum, p.82.

Kas HS (1997). Chitosan: Properties, preparations and application to micro-particulate systems. *J. Microencapul.,* **14**: 689-711.

Khan AA (1991). Marketing of herbal drugs and its problems. Proc. Nat. Work. NIH. Islamabad, Pakistan, pp.69-72.

Kuntal D, D Raman, UM Manjunath, RE Ugandar and BR Lalitha (2012). Evaluation for safety assessment of formulated vanishing cream containing aqueous *Stevia* extract for topical application. *Ind. J. Nov. Dru. Del.,* **4**(1): 43-51.

Kwak SY, S Lee, HR Choi, KC Park and YS Lee (2011). Dual effects of caffeoyl-amino acidyl-hydroxamic acid as an antioxidant and depigmenting agent. *Bioorg. Med. Chem. Lett.,* **21**: 5155-5158.

Luo XD, MJ Basile and EJ Kennelley (2002). Polyphenolic antioxidants compounds from the fruit of *Chrysophyllum cainto* L. *J. Agr. Food Chem.,* **50**(6): 1379-1382.

Mohanata GP, M Jamal and S Umadevi (2007). Formulation and evaluation of poly herbal wound healing Cream. *J. Ind. Drug,* **44**(4): 281.

Mudasir AT, MM Radwan, S Akbar, IA Khan (2012). 5, 6-Dihydropyranobenzopyrone: A previously undetermined antioxidant isolated from *Polygonum amplexicaule, Chin. J. of Nat. Med.,* **10**(1): 28-31.

Patel RP and R Kamani (2009). Formulation Optimization and Evaluation of Mometazone Furoate Cream. *J. Phar. Res.,* **2**(10): 1565-1569.

Patil SK, VR Salunkhe, DS Ghumte, SK Mohite and CS Magdum (2012). Comparative studies on anti-inflammatory activity of hydrogels containing herbal extracts. *Int. J. Pharma. Chem. Bio. Sci.,* **2**(4): 612-616.

Riani H, E Berna and A Juheini (2012). Formulation and evaluation of antioxidants and tyrosinase inhibitory effect from gel containing the 70% ethanolic *Pleurotus ostreatus* extract. *Int. J. Med. Arom. Plan.,* 2(1): 135-140.

Saad HA, SN Ahmed and EB Mohammad (2013). Formulation and evaluation of herbal cream from *Ziziphus spina* leaves extract. *Int. Res. J. Pharm.,* **4**(6): 44-48.

Sharma A and Arora S (2012). Formulation and *In vitro* Evaluation of Ufasomes for Dermal Administration of Methotrexate, ISRN Pharmaceutics, Volume 2012, Article ID 873653, 8 pages. doi:10.5402/2012/873653