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Research Article

Assessment of Rose Water and Evaluation of Antioxidant and Anti-inflammatory Properties of a Rose Water Based Cream Formulation

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ABSTRACT

Photo-aging is a universal dilemma that occurs in our population due to constant contact with ultraviolet radiation. The utilization of antioxidants is a successful approach to avoid symptoms associated to the photo-induced aging of the skin. In view of this, present study was designed to prepare and evaluate the antioxidant & anti-inflammatory activity of cream comprising the aqueous petals extract of *Rosa damascena* for its radical scavenging and protein denaturation activity. Antioxidant activity assessed using standard ascorbic acid (ferric reducing power assay), and anti-inflammatory activity assessed using standard diclofenac sodium measuring of the %age inhibition of protein denaturation. The rose water contains the major phytoconstituents which are polyphenolic compounds flavonoids, tannins, triterpenoids, saponins which are mainly responsible for the antioxidant and anti-inflammatory properties. Out of three cream formulations (F1, F2, and F3). F1 cream formulation showed the highest antioxidant (81.55%) and anti-inflammatory activity (80.6%) at 1000µg/ml. the result noted to be concentration dependent. The IC_{50} value of F1 formulation cream was 257.39 while for F2 cream formulation 374.41. The present results indicate that the *Rosa damascena* petals extract (Rose water) has a good potential for cosmetic product development.

Keywords: antioxidant, anti-inflammatory, rose water

INTRODUCTION

Ultraviolet (UV) radiation which directly comes from the sun divided into three types UVA (320-400nm), UVB (290-320nm) and UVC (200-290nm)[1]. Skin is the body organ that mainly uncovered and bears an extensive exposure to the ultraviolet radiation. Individual skin persistently and frequently exposed to potentially unsafe compounds and rays for the reason that it serves as a defensive wall between the atmosphere and its in-house organs, thus making it prone to aging[2].Photoaging is a protracted tenure procedure that fallout as of continual contact of the skin to solar radiation. Ultraviolet (UV) radiation has been revealed to cause a variety of lesions in DNA including pyrimidine dimmers of cyclobutane type and other photo products of the nucleic acid base. These photo products are concerned in cellular lethality, alteration, and further biological effects[3]. Thickening, unevenness, coarse wrinkles characterize photoaging, spotted pigmentation and histological changes together with injuring to collagen fibers, unnecessary deposition of abnormal elastic fibers, an increase in glycosaminoglycans[4-6]. Ultraviolet radiations aid the aging procedure and become the reason for the reduction in skin elasticity. For this cause, the formulation investigations for the free radical scavenging and anti-inflammatory activities to know the ability of the formulation to combat or delay the photoaging process[7].

Rosa damascena which furthermore recognized as gul-e-surkh is one of the mainly immediate sweet-smelling and therapeutic plants customarily used for various strength needs. It is an erect shrub up to 2 meters in height.Rose plant is cultivated all over the world for a reason that of its attractiveness and scent. It is the most well-known than any other flower all over the world. It has referred to as a king of flowers. There are over 200 rose species, and more than 180000 cultivars variety of the plant has been known, among them the *Rosa damascena* is one of the most significant generals of *Rosaceae* family. Apart from its uses as a decorative plant in recreational greenhouse and gardens. They predominantly cultivated for use in perfume, medicine, and food industry. The plant has revealed diverse biological and pharmacological actions.It has used in Unani medicine (Tibb-e-Unani)since ancient era [8].

Rose plant used for the management of many complains and described in the ethnobotanical texts, and a range of uses have been reported such as in aching throat, puffy tonsillitis, slimming to women and old people, uterine hemorrhages and urticaria. Locally they are useful to heal aphthae[9]. The most beneficial consequence of *Rosa damascena* in ancient medicine are together with the healing of abdominal chest pain, strengthening the heart, cure of menstrual bleeding and digestive disorders and decrease inflammation particularly of the neck. This plant

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Composition of cream formulation

S.no	Ingredient	Used as	Amount (gram)
01	Cetostearyl alcohol	Emulsifying agent	12.5
02	Cetomacrogol-100	Emulsifying agent	2.5
03	Lanolin	Emollient	20
04	Stearic acid	Emollient	10
05	Glycerin	Humectants	50
06	Liquid paraffin	Barrier	15
07	Methylparaben	Preservative	2
08	Propylparaben	Preservative	0.2
09	Borax	Emulsifier / Preservative Buffering agent	1
10	Active (rose water)	Sun protecting agent	30 g and 50 g
11	Distill water	Vehicle	q.s to make 500 g
12	Total weight		500 gram

Table 1: Phytochemical Analysis of Rose Petal Extract (Rose Water).

Phytoconstituents	Presence
Tannins	+
Triterpenoids	++
Saponins	++
Fixed oil	+
Flavanoids	++

(+) indicates the presence of components in a low amount

(++) indicates the presence of components in a moderate amount

Table 2: Percentage of Ferric Reducing Power Capacity of Cream Formulation F1, F2, and F3.

Concentration µg/ml	% of reduction power capacity Equivalent to Ascorbic Acid		
	F1 formulation	F2 formulation	F3 formulation
25	11.41	8.05	7.38
100	41.30	28.69	14.56
500	74.36	65.38	10.89
1000	81.55	72.82	9.12

also used as a mild laxative[10]. Flowers of the plant are good

for eyes. Relieve a pain, toothache, stomatitis, reimbursement the lungs, kidney and liver. It is also used in reheat of the body, chronic fever, inflammation and intestinal affection and to decrease too much perspiration[11]. Rose water forms a satisfying vehicle for the grounding of lotions and collyrium[12].

MATERIAL AND METHODS

Chemicals

All chemicals and solvents used in the study were of analytical grade and they were purchased locally

Collection and Identification

10 kilogram of *Rosa damascena* flowers purchased from the local market care was taken to select healthy flowers and identified by the expert, and its herbarium No. deposited in the Department of Pharmacognosy.The petals were separated from other parts of the plant and washed carefully with water to remove dust and foreign material and dried under shade.

Extraction

Extraction of rose water was carried out by the method reported by Verma et al .,2011[13]. 60 g of dried rose petals were hydrodistilled with 1.5 liters of distilled water for 4 hours and obtained 800 ml of rose water.The collected extract was stored in airtight container in the refrigerator at 4 ^{o}C for further study.

Qualitative Phytochemical Analysis

The extract subjected to preliminary phytochemical screening of saponin, tannins, triterpenoids, flavonoids and fixed oils.

Formulation of Rosewater cream

Rosa damascena petals extract (rose water),50 g and 30 g were used for the preparation of cream formulation F1 and F2 respectively; the F3 formulation was of placebo cream. The procedure for the rose water cream formulation was as follow. The oil part of the cream formulation that is cetostearyl alcohol, cetomacrogol-100, lanolin, stearic acid, glycerin was collectively uniform at 75 ^{O}C ± 2 with continuous stirring using the hot plate. While for the grounding of aqueous part purify water was heated separately in 2000 ml volume beaker at 80 ^{O}C ± 2. Upon constant mixing put in methylparaben, propylparaben and borax and the heat brought to 75 ^{O}C ± 2. The two phases mixed with continuous stirring for about 1-2 minutes finally rose water was added with constant stirring till cream produced. The temperature was additionally reduced to 45 ^{O}C using cold water bathtub.The cream stored in an airtight amber colored bottle at room temperature for further studies[14].

In Vitro Antioxidant Activity of Cream Formulation

In vitro antioxidant activity of cream formulations F1, F2, and F3 performed by the method described as ferric reducing power assay used by Henneberger et al. (2006)[15].

Preparation of Extract Solutions

Accurately weighed 200 mg of extract and dissolved in 50 ml ethanol to obtain solutions of 2000 µg/ml concentration. This solution was serially diluted separately to achieve the lower concentrations25 .100, 500, 1000 µg/ml. The same procedure is adopted for the preparation of standard ascorbic acid dilutions 25,100, 500, 1000µg/ml in distilled water.

Table 3: Anti-Inflammatory Activity of Cream Formulations F1, F2, F3 and Standard Drug Diclofenac Sodium.

Concentration µg/ml	% Inhibition of Protein Denaturation							
	F1 Formulation		F2 Formulation		F3 Formulation		Standard Drug	
	% inhibition	Viscosity (cps)	% inhibition	Viscosity (cps)	% inhibition	Viscosity (cps)	% inhibition	Viscosity (cps)
50	52.2	0.69	41.4	0.63	-0.375	0.23	35.33	0.98
150	63.9	0.73	42	0.69	17.14	0.09	38.53	1.03
300	65.3	0.82	53.01	0.70	32.41	0.34	64.29	1.04
750	70.3	0.91	57.7	0.78	14.51	0.30	78.49	1.08
1000	80.6	0.95	65.2	0.80	43.67	0.42	86.32	1.10
Mean	66.46	0.82	51.86	0.72	21.47	0.27	60.59	1.04
±S.D	±10.31	±0.11	±10.24	± 0.06	±17.00	±0.12	±23.02	±0.04

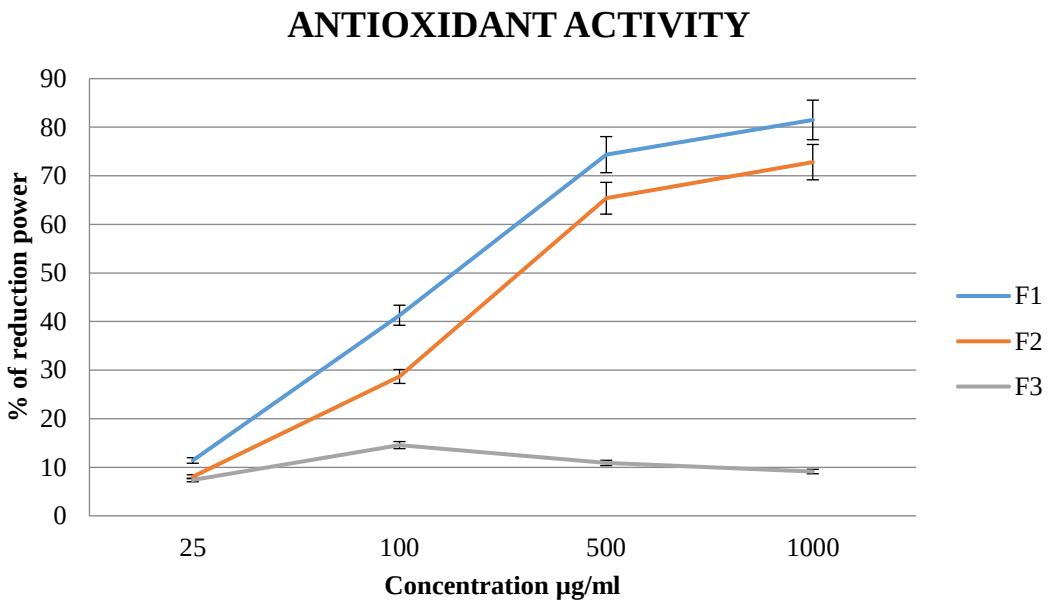

Figure-1: Ferric Reducing Power of F1, F2, F3 Cream Formulation Equivalent to Ascorbic Acid.

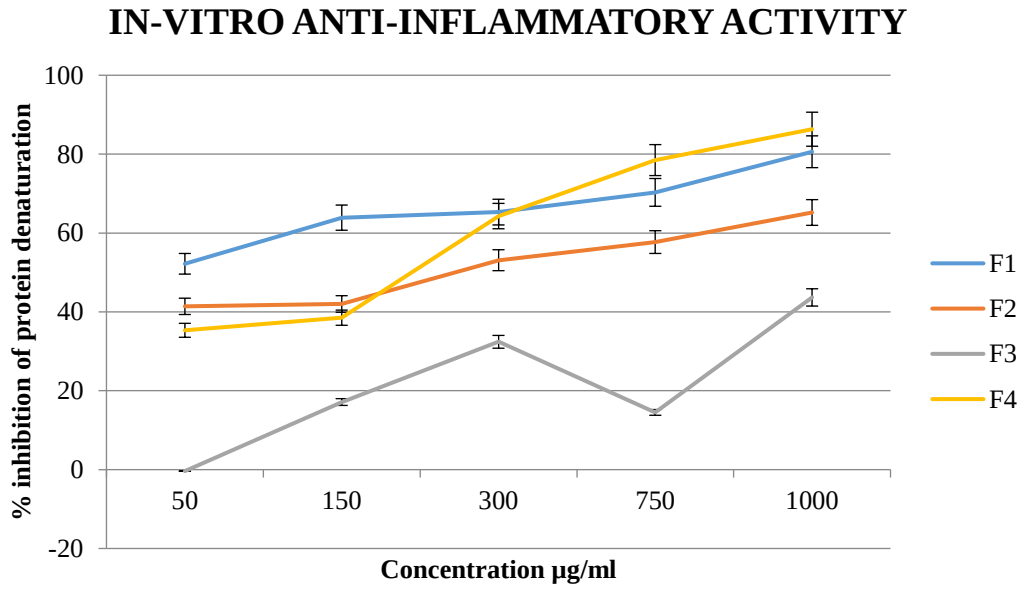

Figure-2: percentage inhibition of diclofenac sodium and formulated creams against denaturation of the protein.

Ferric reducing power assay

2.5 ml of the F1 cream formulation (ranges 25- 1000 µg/ml) were individually assorted using 2.5 ml of 0.2 M phosphate buffer pH 6.6 and 2.5 ml of (1%,w/v) potassium ferricyanide. The combination incubated at 50 ^{O}C for 20 minutes.At the ending of incubation, 2.5 ml of (10 %, W/V) trichloroacetic acid (TCA) added to the mixture then centrifuged at 3000 rpm for 10 minutes. Afterward, 2.5 ml of supernatant mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1 %, W/V), the absorbance was recorded at 700 nm using UV-VIS spectrophotometer. Ascorbic acid used as a reference standard. The percentage reduction calculated by using the following formula[16].

Percentage of reduction power (%) =1- [1- As/Ac] x 100

Where: Ac absorbance of the standard at the different concentration tested

As: absorbance of the sample

The similar procedure was adopted to calculate the reduction power of F2 and F3 formulations.

In vitro anti-inflammatory activity of cream formulations

Inhibition of albumin denaturation method as described by Gautam et al., 2013[17]. Moreover, previously reported by Mizushima and Kobayashi 1968[18]. was adopted to confirm the anti-inflammatory activity of cream formulations

Preparation of stock solution

250 mg of extract was weighed and transferred into 100 ml volumetric flask to make 2500µg/ml stock solution further dilution (50,150,300,750, 1000 µg/ml) prepared from that solution.

Antiinflammatory activity method

2ml of egg albumin from the fresh hen egg, 28ml phosphate buffer pH 6.4 and 20ml (50ug/ml,150ug/ml,300ug/ml,750ug/ml and 1000ug/ml) dilutions of F1 ,F2 ,F3 and Standard) .Incubated the

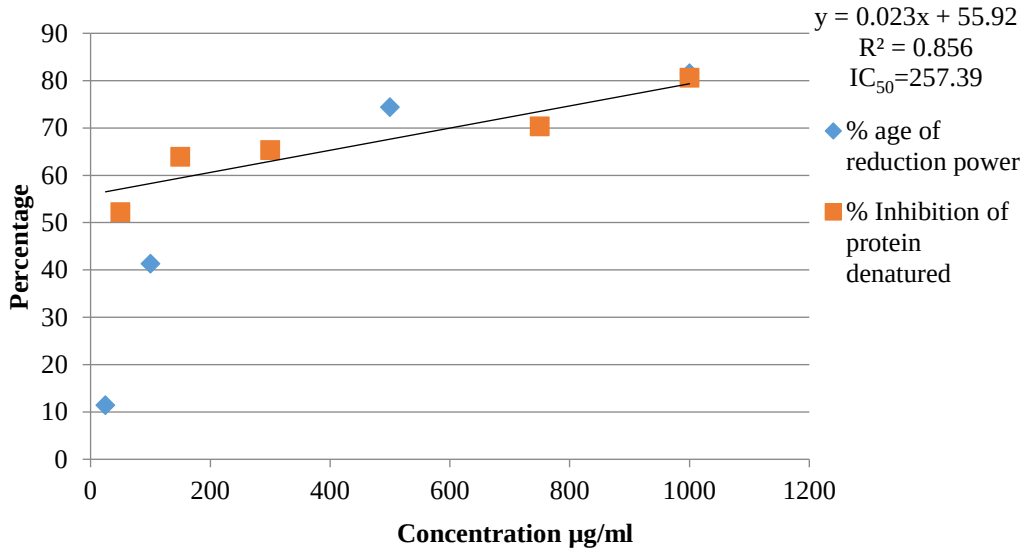

Figure-3: Relationship between Ferric Reducing Power Antioxidant Activity and Percentage Inhibition of Protein Denaturation of Cream Formulation F1.

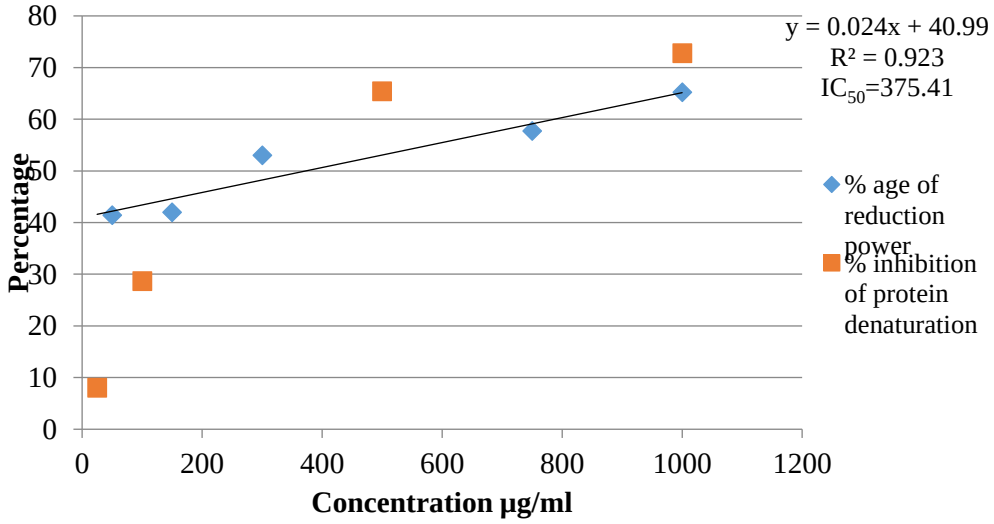

Figure 4: Relationship between Ferric Reducing Power Antioxidant Activity and Percentage Inhibition of Protein Denaturation of Cream Formulation F2.

mixture at 37ºc for about 30min and then heated at 70ºC for about 15 min, cooled the mixture and observed their absorbance at 517, 660 and 700 nm using the vehicle as blank. The percentage inhibition of protein denaturation was calculated from the control using the following formula [19].

$$\% \text{ inhibition} = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100$$

Whereas, Abs: absorbance

RESULTS AND DISCUSSION

Qualitative Phytochemical Studies

The qualitative phytochemical analysis of rose petals extract (rose water) showed the presence of flavonoids, tannins, saponin, fixed oil, and triterpenoids.Represented in Table-1

Ferric Reducing Power Ability (FRPA)

Antioxidant activity of cream formulation F1, F2, and F3(placebo) calculated by using ferric reducing power assay, and the result shown in Table-2.The highest reducing power ability seen at 1000µg/ml F1 (81.55%) and F2(72.82%) formulations.It is seen that by increasing the amount of rose water and concentration of the solution reducing the power of the cream also increases as shown in Figure-1

% inhibition of protein denaturation

In-vitro anti-inflammatory activity of cream formulations. F1, F2, and F3 calculated by using *in-vitro* protein denaturation method.The result is shown in Table-3.It noticed that anti-inflammatory activity was concentration dependent as we increase the concentration the inhibitory effect also increased the highest value recorded at 1000µg/ml F1 (80.6%), F2 (65.2%) and F3 (43.67%).Moreover, when compared with standard drug diclofenac sodium, diclofenac sodium had higher anti-inflammatory activity as compared to F1, F2, and F3 with (86.32%) at 1000µg/ml.The extracts were found to be less active compared to the standard diclofenac sodium, as shown in Figure-2. Thus the result can conclude that the present study proposed that the rose water cream formulation F1 and F2 possess the high potential of antioxidant and anti-inflammatory properties. The observed antioxidant and anti-inflammatory effects can be attributed majorly to the presence of polyphenolic compounds in the rose water. A comparison graph of % of reducing power and % inhibition of protein denaturation is presented in the Figure-3 for F1 and Figure-4 for F2. The 50% inhibitory concentration (IC_{50}) of cream formulation F1 was 257.39 and for F2 375.41.

CONCLUSION

The *Rosa damascena* petal extract commonly known as rose water widely used for its medicinal value in the traditional system of medicine. The rose water contained four major polyphenolic compounds which are flavonoids, tannins, saponin and triterpenoids which are responsible for the antioxidant and anti-inflammatory properties4. The antioxidant creams are extensively used nowadays as they appear to be an attractive way to defend the skin in opposition to oxidative stress caused by a variety of extrinsic sources. A numeral of therapeutic plants used in emergent countries for the management of some disease condition together with pain and inflammatory condition. The validation of folkloric claims of these medicinal plants will present a scientific foundation for the conservation of tropical medicinal resources. The use of rose water as an anti-inflammatory agent also reported (Maleev et al.,1972). As rose water contains a polyphenolic compound which is helpful in reducing inflammation. To sustain the efficiency of antioxidant and anti-inflammatory activity of cream formulation against free radicals and inflammation, it is vital to stabilizing the final formulation on its properties. As a part of synergistic effects, the current practice moves towards in the formulation of different combinations of active ingredient instead of single products. The present study revealed that by increasing the concentration of rose water its antioxidant and anti-inflammatory activities were also increased. Moreover, our study presented that formulation of F1 (50g) is more efficient as compared to F2 (30g). The research work suggests that to ensure the quality and purity of the cream, it must have the consistency and uniformity in the ingredients of the herbal antioxidant cream. The trend of using herbal skin cream is becoming in demand since it is proven that topical application of anti-oxidant cream will be effective against UV radiation and protect the skin from the major consequence of UV damage. In conclusion, the topical application of the formulated cream rose water will help in reducing oxidative damage and give the antioxidant effect to our skin due to its high antioxidant values.

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