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PHOTOPROTECTIVE AND ANTIOXIDANT ACTIVITIES ALONG WITH PHYTOCHEMICAL INVESTIGATION OF ROSE WATER

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ABSTRACT: Ultraviolet radiation (UV) from sun has significant detoriating and degenerative effects on human skin and thus responsible for much skin disease. The most authentic and realistic approach to overcome this issue is to use or apply a barrier between sun rays and exposed skin surface using suitable products called sunscreens or UV protectors. Sunscreens usually include both natural and synthetic molecule and have great application in cosmaceuticals. In the present study one such natural product-rose water was evaluated for sun protecting ability of the rose water samples (SPF) using *in-vitro* spectroscopic method. A correlation was also established by evaluating the antioxidant activity in total 12 samples of rose water were evaluated for SPF and antioxidant activity Among all the samples the SPF value of sample #09 showed high sunscreen activity 3.956 followed by sample #08 with SPF value of 3.612. The rose water sample distilled in the lab indicated SPF value as 0.800. All the samples showed antioxidant activity (free radical scavenging ability). The phytochemical investigation indicates the presence of polyphenolic compounds such as triterpenoids, saponin, flavanoids, tannins and fixed oil. The overall SPF and antioxidant activity of rose water may be responsible for the utilization in cosmaceuticals to achieve reasonable skin protecting effect and to prolong the skin aging.

INTRODUCTION: Damask rose botanically known as *Rosa damascena* mill., belongs to the family Rosacea, a very popular family containing many ornamental plants. Rose has more than 200 species and cultivar are also above 18000 [1]. Cultivation of rose is very old about 3000 BC it is reported in China, Northern Africa and Western Asia.

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Rose is a valuable and important main material for fragrance and beautifying purpose. From the rose, rose water, rose oil, concrete and absolute are also extracted [2]. Many naturally occurring materials are good source of antioxidant and used for beautifying purpose for their good sunscreen and antioxidant activities. The outer most part of the body is skin which is in direct contact with sun and ultraviolet radiation which causes serious damage to the skin not only potentiate the oxidation but also accelerate some other diseases such as erythema, photoaging, edema, sunburn and cell formation *etc*. [3] Sun is the main source of ultraviolet radiation and further divided in to UVA (320-400) UVB (280-320) and UVC (200-280).

Majority of light that reaches to the earth that is about 90% is UVA having high penetration power causes immediate tanning of the skin. About 4 - 5% of the ultraviolet radiation is made up of UVB which is 1000 times more potent than UVA to cause skin burn. Whereas most dangerous one is UVC short term exposure, cause serious damage to the skin [4]. Human body has its own force of defense which neutralized the reactive oxidative species and helpful in the repairing of tissues as well as prevention of radical formation. When body are suffering from different diseases and chronic infections these defense forces are become weaker in that conditions, medicinal plants provide strength to the body increase the quality of life in patients [5]. Objectives of present study is to determine the photoprotective and antioxidant activities along with the phytochemical investigation of 12 rose water samples to correlate through application in various forms as available in local market.

FIG. 1: *ROSA DAMASCENA* MILL.

MATERIALS AND METHODS:

Sample Collection and Preparation of Rose Water: Total 12 samples of rose water were used in present study, 6 samples were collected from market, 4 samples were provided by Mohammad Hashim Tajir Surma laboratories and the last sample were prepared in the lab using hydrodistillation method as reported by Verma *et al*., 2011. All samples were filtered through Whatman filter paper and stored at 6 ºC in a refrigerator for further analysis and study. Sample and the voucher specimen number (RD-01-12) is available in Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi herbarium.

Chemicals: Chemicals were utilized in the study is of Analytical grade, methanol (Merck schuchardt OHG Germany), phosphate buffer (sigma Germany) potassium ferricyanide (daejung reagent chemical Korea), trichloroacetic acid (Scharlau Spain), acetic acid and ferric chloride.

Instruments: Double beam ultraviolet and visible spectrophotometer (Shimadzoo), centrifuge machine (HERMLE Labortechnik GmbH, Germany), weighing balance (Satorius), pH meter (Systronics) and incubator were utilized in this study.

Phytochemical Analysis:

Qualitative Phytochemical Analysis: Qualitative phytochemical analysis of secondary metabolites was performed as described by the standard procedure to check the presence and absent of the metabolites in samples of rose water, such as test of saponins (foam test), triterpenoids (Libermann burchard test), tannins (lead acetate test, nitric acid test) [6], fixed oil (spot test) [7] and flavonoids (lead acetate test, sulphuric acid test) [8].

FT-IR Analysis: Determination of the functional group in the samples were analyzed through Fourier Transform - Infrared spectro photometer (FT-IR).For liquid samples about 0.1 ml of the solution was spared over sodium chloride plates to form a thin film and observed under FT-IR spectrophotometer the spectrum was recorded between 400 - 4000 cm^{-1} and matched with the library data for functional group conformation [9].

Sunscreen Activity: *In-vitro* determination of SPF (sun protecting factor) was performed as method described by Mansur *et al*., (1986). The absorbance of the extracts was determined from 290 nm to 320 nm at every 5 nm interval using distilled water as blank. The *in-vitro* SPF value was calculated by using the following formula

$$SPF_{spectrophotometric} = CF \times {}^{320}\sum_{290} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where CF (correlation factor) is 10, EE (λ) is erythmogenic effect of radiation with wavelength, (λ), Abs is spectrophotometric absorbance value at wavelength (λ). The value of EE (λ) *I (λ) are constant. The absorbance value is multiplied with EE (λ)*I (λ) and then their summation is taken and multiplied with correction factor to obtain the SPF values [10].

Antioxidant Activity:

Reducing Power Ability (RPA): The reducing power of samples was quantified by the following method. Rose water (5 ml and 10 ml) was mixed with 1 ml of 80% methanol, phosphate buffer (5ml, pH 6.6) and potassium ferricyanide (5 ml, 1.0%) were added, mixtures were incubated at 50 ºC for 20 min. After incubation 5 ml of trichloroacetic acid (10%) was added and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (5 ml) of the solution is mixed with 5 ml distilled water and ferric chloride (1.0 ml, 0.1%), than absorbance of different colored solution was measured through spectrophotometer at 700 nm. The same procedure was followed for ascorbic acid which was used as standard drug at concentration of (50, 100 and 200 µg/ml) [11].

RESULTS AND DISCUSSION:

Phytochemical Screening: The qualitative phytochemical screening of rose water as presented in **Table 1** revealed presence of polyphenolic compounds such as triterpenoids, saponins. flavonoids, tannins and fixed oil in majority of samples.

These phytochemicals are secondary bioactive constituent of plant have both medicinal and nutritional value and are able to provide protection to the plant and also helpful in maintaining human health, working as anti-cancer agent, anti-oxidant, neuro-pharmacological agents and detoxification agent [12].

TABLE 1: PHYTOCHEMICAL SCREENING OF ROSE WATER SAMPLES

Sample●	Detection of tannins	Detection of triterpenoids	Detection of fixed oils	Detection of saponins	Detection of flavanoids
01	+	+	+	+	-
02	+	+	+	+	-
03	+	-	-	+	+
04	+	+		++	++
05	+	+	+	++	+
06	+	++	+	++	++
07	+	+	+	++	+
08	+	++	+	++	+
09	+	++	++	+++	++
10	+	+	+	+	++
11	+	++	++	++	-
12	+	++	+	++	++

●: source & manufacturer's name are available upon request (+) Present, (-) Absent / not present

FT-IR Analysis: The FT-IR absorbption spectrum of rose water samples are given in **Fig. 2 - 13** respectively. For the analysis of functional groups fourier transformer infrared spectroscopy is a safe and time saving method. The FT-IR spectrums revealed that the peaks arise in the range 3600cm^{-1} to 920 cm^{-1}. The tested samples showed intense peaks from 3400 cm^{-1} to 3600 cm^{-1} due to the characteristic stretching vibration of N-H and O-H from alkaloids, polyphenols amino acids, while the absorption peaks from 2800 cm^{-1} to 2900 cm^{-1} were appeared from the C - H symmetric stretching of CH_3 and CH_2 group of lipid region and ester group. While the peak at 2600 cm^{-1} revealed the strong broad O-H stretching of carboxylic acids structure, the absorbance peaks from 1700 cm^{-1} to 1850 cm^{-1} from C = O stretch bending to indicated the presence of conjugated aldehyde and carbonyl group. Peaks from 1450 cm^{-1} to1470 cm^{-1} showed the alkane group. The absorbance peaks from1100 cm^{-1} to 1200 cm^{-1} were appeared in sample from C - O stretching vibration, indicated the presence of alcohol, ether, carboxylic acid and anhydride. Results are shown in **Table 2**.

Sunscreen Activity (SPF): The healing property of different medicinal plants is depending upon the presence of their secondary metabolites such as alkaloids, glycosides, tannins, saponin and flavonoids [13]. In the present decade compound obtained from the natural sources gained the considerable attention as a photoprotective agent. There are wide Varity of chemicals which act as sunscreens and show their absorbance in certain part of the UV spectrum. Extracts of plant had a wide range of natural compounds which cover the full range of UV spectrum wavelength. Due to genotoxicity of ultraviolet radiation cause mutation

in the gene was the first step in the development of skin cancer .Ultraviolet radiation penetrate in to the skin cause DNA damage and free radicals formation [14]. In this research 12 extracts of *Rosa damascena* were evaluated for their SPF by *in-vitro* spectroscopic method as described by Mansur *et al*., 2011. The SPF value ranges from 0.386 of sample # 02 to 3.956 of sample #09. Sun protecting activities of samples was represented in **Table 3** and graphical representation was mentioned in **Fig. 14**.

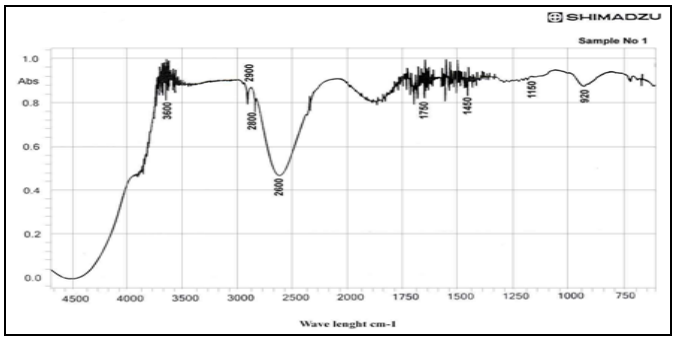

FIG. 2: THE FT-IR SPECTRUM OF ROSE WATER SAMPLE NO. 1

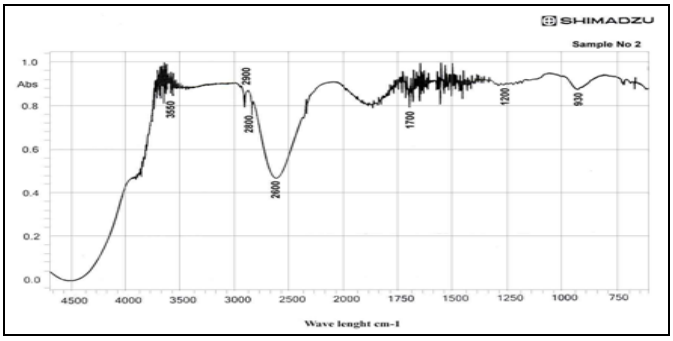

FIG. 3: THE FT-IR SPECTRUM OF ROSE WATER SAMPLE NO. 2

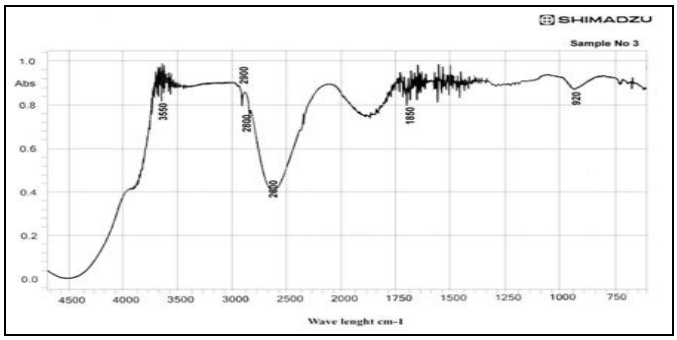

FIG. 4: THE FT-IR SPECTRUM OF ROSE WATER SAMPLE NO. 3

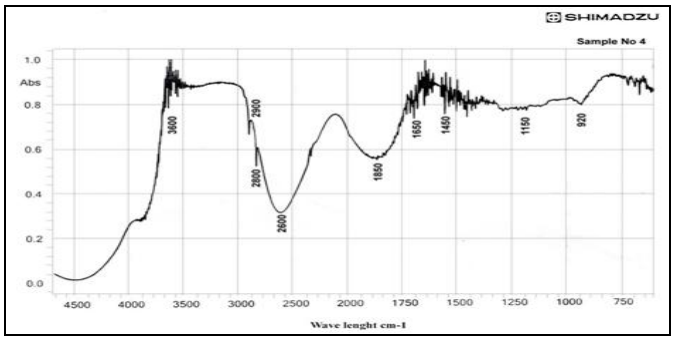

FIG. 5: THE FT-IR SPECTRUM OF ROSE WATER SAMPLE NO. 4

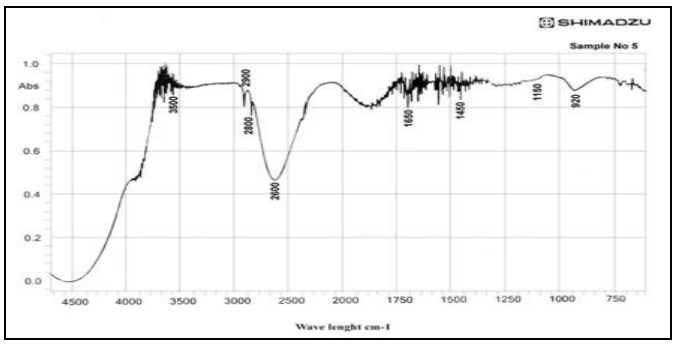

FIG. 6: THE FT-IR SPECTRUM OF ROSE WATER SAMPLE NO. 5

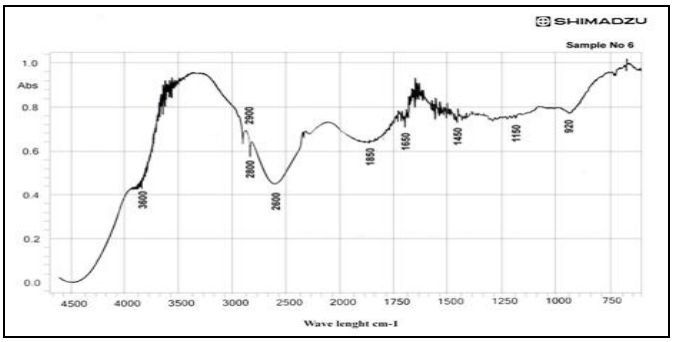

FIG. 7: THE FT-IR SPECTRUM OF ROSE WATER SAMPLE NO. 6

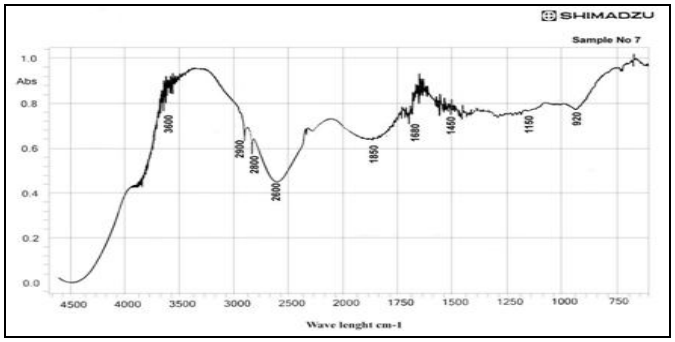

FIG. 8: THE FT-IR SPECTRUM OF ROSE WATER SAMPLE NO. 7

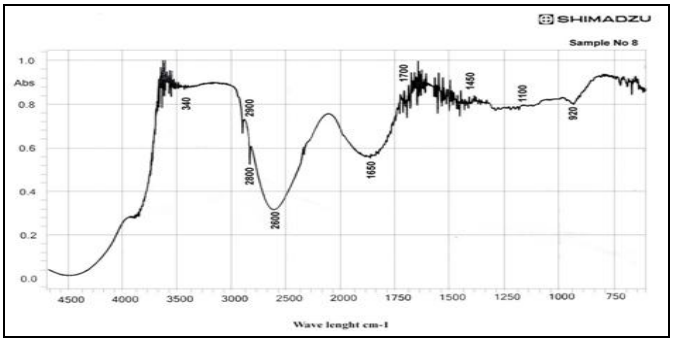

FIG. 9: THE FT-IR SPECTRUM OF ROSE WATER SAMPLE NO. 8

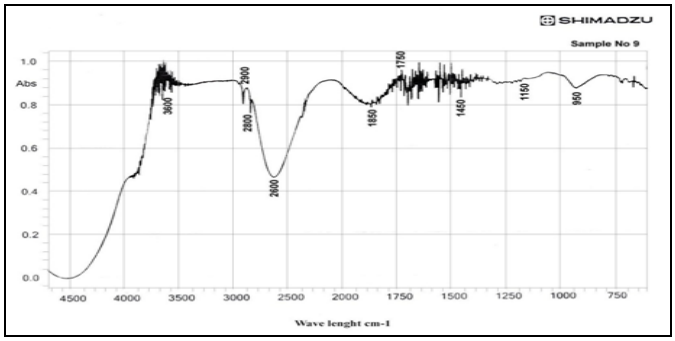

FIG. 10: THE FT-IR SPECTRUM OF ROSE WATER SAMPLE NO. 9

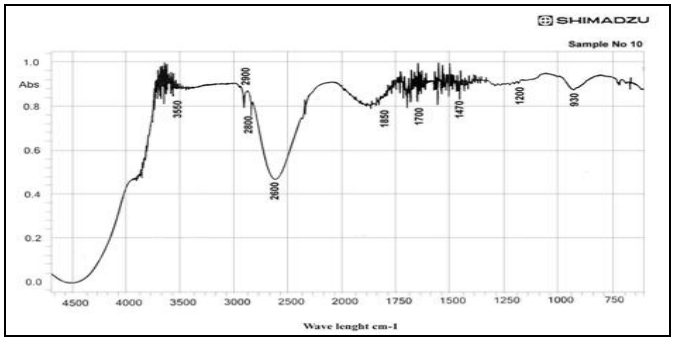

FIG. 11: THE FT-IR SPECTRUM OF ROSE WATER SAMPLE NO. 10

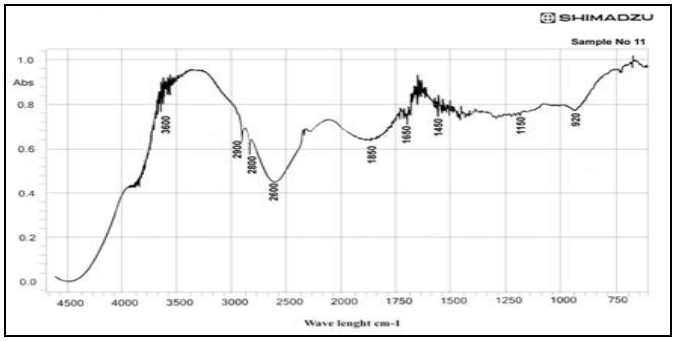

FIG. 12: THE FT-IR SPECTRUM OF ROSE WATER SAMPLE NO. 11

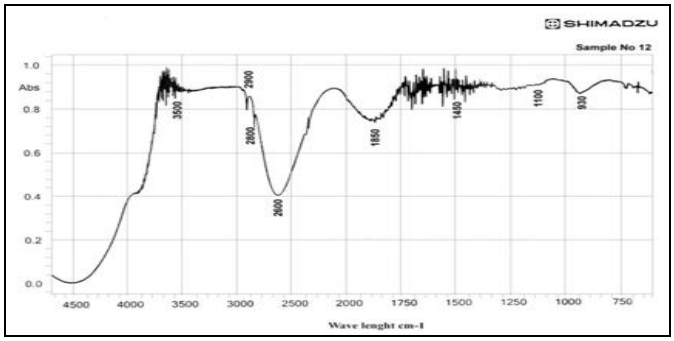

FIG. 13: THE FT-IR SPECTRUM OF ROSE WATER SAMPLE NO. 12

TABLE 2: FT-IR ABSORPTION BAND ASSIGNMENTS

Peak's wave length (cm^{-1})	Possible functional groups	Intensity/ Assignment
3400-3600	O-H and N−H	O-H stretching vibration of hydroxyl groups (mainly lipids and proteins) and N-H stretching vibration mainly carbohydrates proteins
2800-2900	C−H	C-H lipid region, esters groups
2600	O−H	.Strong broad O-H stretching carboxylic acid
1750-1850	C=O	C=O stretching conjugated aldehyde and strong stretching anhydride and carbonyl group
1450-1470	C=C	Weak medium stretching of alkane group, aromatic ring
1100-1200	C-O	C-O stretching of alcohol, ether, ester and carboxylic acid anhydride

The FT-IR spectra of the samples conforms the presence of hydrocarbon, alcoholic, esters, aromatic principle and polyphenolic compounds.

TABLE 3: *IN-VITRO* SUN PROTECTING FACTOR (SPF) OF ROSE WATER SAMPLES

Samples ●	SPF
01	0.637
02	0.386
03	1.373
04	1.854
05	0.242
06	0.439
07	0.704
08	3.612
09	3.956
10	0.218
11	0.460
12	0.800

●: source & manufacturer's name are available upon request

Antioxidant Activity: The phytochemical screening is helpful in the determination of the bioactive metabolite which play their role in the scavenger for free radicals which not only effect the internal organs of the body by producing different types of diseases but also effect the topical part of body (skin), specially that area which is in direct contact of sunlight. When the oxygen and nitrogen species are imbalance in the body the body is in the state of oxidative stress which is due to the presence of free radical's.

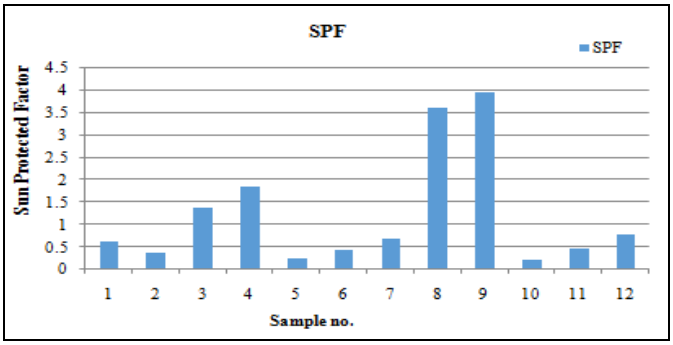

FIG. 14: GRAPHICAL REPRESENTATION OF SPF VALUES OF ALL SAMPLES

TABLE 4: SPF RATING CHART

SPF rating	% of UV radiation blocked from the skin
2	50
4	75
5	80
10	90
15	93
25	96

With the Help of table 4 it was estimated that rose water samples had ability to stop 25-75% of the ultraviolet radiation penetration in to the skin.

Antioxidants provide protection to the living being from the hazardous effect of reactive oxygen species. There are many medicine which are now a day having radical scavenging mechanism for the treatment of many diseases [15, 16]. Antioxidants are important in the prevention of UV radiation. The major indicator of antioxidant activity is the reducing capability of Fe^{3+}/ ferricyanide complex to ferrous form. The free radicals are inhibiting due to the presence of reductones which perform antioxidant activity by donate their electron to free radicals [17]. The antioxidant activity of Rosa water samples are shown in **Table 5 - 6**. The higher ferric reducing power value the greater the antioxidant activity.

TABLE 5: PERCENTAGE OF FERRIC REDUCING POWER CAPACITY OF ROSE WATER SAMPLES (05 ml) WITH RESPECT TO 50, 100, 200 µg/ml OF ASCORBIC ACID AS STANDARD

Sample●	mean of absorbance	% age of reduction power capacity		
		Equivalent to 50 µg of ascorbic acid	Equivalent to 100 µg of ascorbic acid	Equivalent to 200 µg of ascorbic acid
01	0.023	191.66	6.927	2.33
02	0.034	283.33	10.24	3.45
03	0.055	458.33	16.56	5.59
04	0.064	533.33	19.27	6.51
05	0.023	191.66	6.92	2.33
06	0.005	41.66	1.50	0.50
07	0.008	66.66	2.40	0.81
08	0.022	183.33	6.62	2.23
09	0.03	250	9.03	3.05
10	0.004	33.33	1.20	0.40
11	0.006	50	1.80	0.61
12	0.101	841.66	30.42	10.27

●: source & manufacturer's name are available upon request

TABLE 6: PERCENTAGE OF FERRIC REDUCING POWER CAPACITY OF ROSE WATER SAMPLES (10 ml) WITH RESPECT TO 50, 100, 200 µg/ml OF ASCORBIC ACID AS STANDARD

Sample●	mean of absorbance	% age of reduction power capacity		
		Equivalent to 50 µg of ascorbic acid	Equivalent to 100 µg of ascorbic acid	Equivalent to 200 µg of ascorbic acid
01	0.081	52.59	17.60	13.68
02	0.043	27.92	9.34	7.26
03	0.072	46.75	15.65	12.16
04	0.071	46.10	15.43	11.99
05	0.042	27.27	9.13	7.09
06	0.008	5.19	1.73	1.35
07	0.008	5.19	1.73	1.35
08	0.18	116.88	39.13	30.40
09	0.037	24.02	8.04	6.25
10	0.02	12.98	4.34	3.37
11	0.006	3.89	1.30	1.01
12	0.37	240.25	80.43	62.5

●: Source & manufacturer's name are available upon request

CONCLUSION: Natural antioxidants are very effective for reducing the oxidative stress. It was conformed that consumption of antioxidant substance provide prevention for the formation of free radicals. Free radicals effect the skin as it is in direct contact with the solar light which is the major cause of skin damage and responsible for different types of skin related problems such as sunburn, phototoxicity and photosensitivity [18]. On the basis of present study on rose water shows that due to the presence of polyphenolic compound saponins and flavavoids rose water provide protection from the ultraviolet radiation and work as sun protecting agent by inhibiting the formation of free radicals which is the main cause of skin related problems.

Now a days people avoid to use synthetic antioxidants because of their higher toxicity level so the researchers was focused on the discovery of natural compounds having antioxidant effects, natural products are safe, low in cost and effective [19]. In the present study *in-vitro* SPF and antioxidant method was adopted, as *in-vitro* methods are safe cost effective and not required any ethical approval. *In-vitro* spectrophotometric is the approved method for the analysis of SPF and antioxidant activity of the compound.

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CONFLICT OF INTEREST: There is no conflict of interest of all authors in this study.

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