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TO STUDY THE ANTIOXIDANT ACTIVITY OF OPUNTIA FICUS FRUIT EXTRACT IN NIGHT CREAM

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Abstract

Indian climate is categorized by equatorial; being hot, cold, humid throughout the year and Indian folks will expose more ultraviolet rays, ultraviolet rays and other factors will facilitate the aging process and can cause the decrease in skin elasticity, and our skin is at the mercy of the many forces as we age: sun, harsh weather, and bad habits. But we are able to take steps to assist our skin stay supple and fresh-looking. In what way your skin ages will depend upon a range of factors: your routine, diet, heredity, and other own habits. Opuntia ficus-indica Linn could be a perennial plant belonging to the caryophylloid dicot family. Prickly pear fruit is good, oval shape and red colored. Fruit is rich in vitamin C and vitamin E, polyphenols, carotenoids, flavonoid compounds (e.g., kaempferol, quercetin, and isorhamnetin), taurine and pigments. Antioxidants are vital substances which possess the power to shield the body from damages caused by atom induced oxidative stress. this study was undertaken to investigate the presence of various phytochemical constituents and to judge antioxidant activity of Prickly pear fruit in ethanol extracts. Extracts was tested for 1-diphenyl-2-picryl hydroxyl (DPPH) radical scavenging activity and compared with L-antioxidant as standard. The antioxidant activity of those extracts was investigated supported their ability to scavenge (DPPH) stable atom. Phytochemical screening of Prickly pear fruit revealed the presence of carbohydrate, protein, alkaloid, amino acid, flavonoid and vitamin C. Keywords: Prickly pear fruit, Antiaging, Antioxidant, 1-diphenyl-2-picryl hydroxyl (DPPH) radical scavenging activity.

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1.INTRODUCTION

Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules. Oxidation may be a chemical action that transfers electrons from one molecule to an oxidant. Oxidation reactions are known to provide free radicals. These free radicals are highly reactive species which contains one or more unpaired electrons in their outermost shell. Once they're formed, the chain reaction starts. Antioxidant act in response with these radicals and terminates this chain reaction by eliminating free radical intermediates and prevents other oxidation reactions by oxidizing themselves. Though oxidation reactions are crucial always, they'll even be damaging. Plants and animals have a posh system of multiple varieties of antioxidants, like antioxidant and E, furthermore as enzymes, like catalase (CAT), enzyme (SOD), and various peroxidases (1). Oxidative stress plays a key role in causing various human diseases, like cellular necrosis, upset, cancer, upset, Parkinson's dementia, Alzheimer's disease, disease, dystrophy, liver disorder, and even aging (2). Besides, there are some antioxidants within the kind of micronutrients which can't be manufactured by the body itself like tocopherol, β-carotene, and vitamin C, and hence these must be supplemented within the normal diet. (3)

1.1. Natural Antioxidants

Natural antioxidants derive from fruits, vegetables, spices, grains, and herbs. Also, due to toxicological concerns of synthetic antioxidants (4), phenolic compounds in plants were used to minimize or retard lipid oxidation in lipid-based products. Fruits, vegetables

and medicinal herbs are the richest sources of antioxidant compounds such as Vitamin A, C, E, beta-carotene and important minerals. In addition, the call for sustainable source and also the environmentally friendly production is forcing the cosmetics industry to move in that direction. Natural antioxidants either are synthesized in human body through metabolic process or are supplemented from other natural sources, and their activity very much depends upon their physical and chemical properties and mechanism of action. This can be further divided into two categories, i.e., enzymatic antioxidants and nonenzymatic antioxidants.

Enzymatic Antioxidants

Enzymatic antioxidants are uniquely produced in the human body and can be subdivided into primary and secondary antioxidant.

Primary Antioxidants

Primary antioxidants mainly include superoxide \dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)

Secondary Antioxidant

Secondary antioxidant contains glutathione reductase (GR) and glucose-6- phosphate dehydrogenase (G6PDH). G6PDH generates NADPH. GR is essential to recover the reduced glutathione (GSH) by secondary enzyme GR and NADPH.

Nonenzymatic Antioxidants

They are a class of the antioxidants which are not found in the body naturally but are required to be supplemented for the proper metabolism. Some of the known nonenzymatic antioxidants are minerals, vitamins, carotenoids, polyphenols, and other antioxidants.

Minerals

Minerals are required in the body cells for the proper functioning of the enzymes. Their absence is known to affect the metabolism of many macromolecules. They include selenium, copper, iron, zinc, and manganese. They act as cofactors for the enzymatic antioxidants.

Vitamins

Vitamins form the class of micronutrients required for the proper functioning of the body's antioxidant enzyme system, such as vitamin A, vitamin C, vitamin E, and vitamin B. They cannot be synthesized in our body and hence need to be supplemented in the diet.

Polyphenols

Polyphenols could be a class of the phytochemicals that possess marked antioxidant activities. Their antioxidant activities rely upon their chemical and physical properties which successively regulates the metabolism looking on their molecular structures. These comprises phenolic acids, flavonoids, gingerol, curcumin, etc.(2). Flavonoid could be a major class of polyphenolic compound and is usually found in vegetables, fruits, grains, seeds, leaves, flower, bark, etc.[5]

4. Active Profile

Active name: Opuntia ficus
Common name: Prickly pear
Family: Cactaceae

Genus: Opuntia ficus (indica)

It is the fully mature fruits extracted by the solvent extraction techniques i.e. Maceration from the fruit powder obtained from the Opuntia ficus (indica) belonging to Cactaceae. [17]

Fig no 1. Prickly pear fruit

Plants belonging to the caryophylloid dicot genus spp. are the foremost abundant of the Cactaceae family, grown throughout solid ground also because the central area of the Mediterranean, Europe, Asia, Africa, and

Australia. Opuntia species display flattened stems called "pencas" or cladodes. The cactus pear fruit also called prickly pear fruit is an oval elongated berry, with a thick pericarp, a juicy pulp with a substantial number of seeds and a semi-hard rind with thorns. The pericarp and also the edible pulp may have different colors like green, greenish white, yellowness, gamboge, red, cherry-red, or purple hues. the typical weight of prickly pears fruits varies from 100 to 160 g looking on the origin site and cultivation. The usable a part of the fruit consists of peel (48%–52%) and pulp (48%–52%). The pulp is further subdivided into seeds and strained pulp (44%–45%), the latter being the idea for fruit and juice products. The fruits with white pulp and green rind are preferred for consumption as food, and their domestic production corresponds to almost 95% of the overall production. Mexico is that the main producer of Opuntia ficus-indica (L.) Mill species, and accounts for over 45% of the worldwide production; however, only 1.5% of this production is exported. The cactus pear fruit could also be considered a functional food; this feature has been attributed to its bioactive compounds like antioxidant and E, polyphenols, carotenoids, flavonoid compounds (e.g. kaempferol, quercetin and isorhamnetin), taurine and pigments. [6]

1.2. *Chemical composition of Opuntia ficus indica*.

Composition	100%	Composition	100%
Alanine	87.2	Vitamin C (total ascorbic acid)	12-18/14
Arginine	30.5	Vitamin E	111-115 micro gram
Asparagine	41.6	Vitamin K1	53 micro gram
Glutamic acid	66.1	Niacin (Vit.B3)	Trace amount
Glutamine	346.2	Riboflavin (Vit.B2)	Trace amount/0.06
Glycine	11.3	Thiamine (Vit.B1)	Trace amount/0.014
Histidine	45.2	Methionine	55.2
Isoleucine	31.2	Phenylalanine	23.3
Leucine	20.6	Serine	174.5
Lysine	17.4	Threonine	13.3
Tryptophane	12.6	Tyrosine	12.3

Composition	Pulp	Skin	Composition
Water content	94.40±2.61	90.33±0.21	18.05±2.53
Protein	1.45±0.08	1.45±0.08	4.48±0.01
Lipids	0.7±0.08	1.06±0.08	3.66±0.21
Saccharose	0.19	2.25	0
Glucose	29	14	0
Fructose	24	2.29	0
Fiber	0.02-3.15/3.6	-	-

Table No 1. Chemical composition of Opuntia ficus-indica fruit samples

Benefits of product

1) Especially effective are night creams that may help restore your skin's radiance overnight. These creams can even hone in on skin issues like fatigue, fine lines and dark spots.
2) Night creams formulated with antioxidants can help take your skin care routine to the following level. Antioxidant night cream to assist boost your skin's moisture levels.
3) Repairs Damage and Regenerates Skin Cells. Vitamins C and E are known to be the building blocks of the assembly of collagen, a substance which forms the bottom of skin cells. a daily dosage of both may help regenerate skin cells leading to a minimized appearance of wrinkles and repair any damaged section of your skin.

2.MATERIAL METHODS

2.1. Plant materials and extraction

2.1.1. Collection and authentication of fruit

The fruits of cactus Prickly pear were collected from Warvandi village hills, Teal- Deola, Dist- Nashik, and

Maharashtra, India by random selection method. The fruit was authenticated in Department of Botany, Karmaveer Ramraoji Aher Arts, Science and Commerce College, Deola. Nashik, Maharashtra.

2.2. Preparation of extracts

Fruits of cactus Prickly pear were washed and turn over very small pieces and so dried under the shade at temperature for 10 days and later dried in an oven at 450C for complete removal of moisture to get constant weight then subjected to size reduction.200g of air dried powered fruit material was successively extracted in cold Maceration extraction technique. [8,9,31,36] for the duration of this process, the full or coarsely powdered crud drug is placed during a stoppered container by the solvent and permitted to face at temperature for a period of a minimum of 4 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the mark (the damp solid material) is pressed, and therefore the combined liquids are clarified by filtration after standing. 'Opuntia ficus' Fruit powder are extracted with ethanol. the majority of extraction of fruit containing material still performed by cold Maceration extraction technique and therefore the extract treated with Ultra Sonication technique for 10 minutes [10].

Fig no.2. Prickly pear fruit

Fig no.3. Prickly pear fruit powder

Fig .no.4 Ethanolic extract

Fig .no.5 Extract filtration

2.3. Preliminary Phytochemical Screening

All extracts were subjected to preliminary phytochemical screening for evaluation of phytochemical constituents such as carbohydrate, protein, amino acid, alkaloids, tannins, fats and oil, flavonoids, Vitamin C using standard procedure of analysis [18, 19, 20].

2.4. Determination of antioxidant activity of cactus Prickly pear fruit extract by DPPH method [21]

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compound. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H* [11].The DPPH is reacted with methanol or absolute ethanol to yield purple color. The presence of antioxidants in the sample scavenge the formed DPPH radical and decrease in color is observed

which is Spectrophotometrycally measured at 517nm [12, 13]. In one cuvette 3ml of methanol was taken and kept as a Standard for all the extracts. In other cuvette 3ml of DPPH was taken. Absorbance for the blank samples at 517 nm was determined [14]. Cuvette of methanol was not disturbed. At this time in another cuvette 3ml of DPPH was retained aside for 5 min.To this cuvette ascorbic acid was added in microliter in several concentrations. Absorbance at 517nm was read for each concentration. Scavenging activity was expressed as the % inhibition. Now ascorbic acid was replaced by extracts and followed same procedure. The percentage of inhibition can be calculated using the formula.

$$\% \text{ Antioxidant activity} = \frac{\text{Absorbance of the control - Absorbance of the test}}{\text{Absorbance of the control}} \times 100$$

2.5. Selection of base

Cosmetic antioxidants night cream has higher concentrated product based on a water and oil. When using concentrated we get not only quickly cosmetics effect but also physiological satisfaction after the treatment because result will be seen practically immediately. Antioxidants protect skin by limiting the production of free radicals, which can damage skin cells. Our night cream boosts collagen in your skin. The cream also helps in better blood circulation. The crinkles and further fine lines on your face get reduced.

2.6. Preparation of base

1) All the apparatus should be wash and clean properly.
2) The oil phase ingredients Mineral oil, petroleum jelly, white beeswax, paraffin wax and lanolin. Take in a one beaker and the water phase Borax, Glycerin, water. Ingredients in another beaker. Bothe phase are heated to separate beakers to temperature of about 75°C.
3) Temperature i.e. 75°C. Preservative is solidified in water before heating the mixture.
4) The heating material are stir continuously.
5) Slowly oil phase mixture is added to water phase mixture along with continuous stirring.
6) Perfume and extract is added after the preparation has attained a temperature of about 35°C.
7) Incorporation of Active in Cream Base, The final formulations of cream with cactus Prickly pear fruit extract was prepared by adding varying concentration of cactus Prickly pear fruit extract as 1%, 2% and 3% in formulation 1, 2 and 3 respectively. The formulation table is given in Table 3.
8) The prepare product fill in suitable cream container.

Fig .no.6 Formulated antioxidant night creams

Formulation Table-1

Sr. No.	Ingredients	Quantity 100%		
		A	B	C
1	Mineral oil (lubricant)	34%	33%	31%
2	Petroleum jelly (lubricant)	7%	9%	11%
3	White beeswax (emollient)	12%	12%	12%
4	Paraffin wax (base and lubricant)	2%	5%	4%
5	Lanolin (emollient)	5%	4%	4%
6	Borax (buffer)	3%	4%	4%
7	TEA (buffer)	2%	2%	2%
8	Water (vehicle)	30%	27%	29%
9	Glycerin (humectant)	5%	4%	3%
10	Perfume (Odour)	Q.S.	Q.S.	Q.S.
11	Sodium benzoate (Preservatives)	0.1%	0.1%	0.1%

Table No.2. Formulation of Night Cream.[22,23.]
Formulation Table-2

Sr. No.	Ingredients	Quantity 100%
1	Mineral oil (lubricant)	31%
2	Petroleum jelly (lubricant)	11%
3	White beeswax (emollient)	12%
4	Paraffin wax (base and lubricant)	4%
5	Lanolin (emollient)	4%
6	Borax (buffer)	4%
7	TEA (buffer)	2%
8	Water (vehicle)	29%
9	Glycerin (Humectant)	3%
10	Perfume (Odour)	Quantity sufficient
11	Sodium benzoate (Preservatives)	0.1%
12	*Cactus prickly pear* fruit extract	3%

Table No.3. Formulation of Night Cream with active

Analysis of Cream Base -The analysis of product was carried out by following methods. [32]

A. Physical Appearance
   Colour
   Odour
   Consistency
B. Determination of pH
C. Determination of Thermal Stability
D. Determination of Total Fatty Matter
E. Skin irritation test
F. Spreadability
G. Phytochemical screening of the methanol extracts [18, 19, 20].
H. Determination of antioxidant activity night cream through a cactus Prickly pear fruit extract by DPPH method. [21]

A) Physical Appearance

1) Colour and Appearance: the color and appearance of the formulation was observed visually.
2) Odour: The Odour of the formulation is pleasant / characteristics.
3) Consistency: it's found to be semi-solid with visually observation.

B) Determination of pH

The pH of the developed cream base was measured on the same digital pH meter at temperature by taking adequate amount in a very 50 ml beaker.

C) Determination of Thermal Stability

Thermal stability (200C, 300C and 400C) of the prepared and formulation was determine in line with (BSI) Indian standard guideline.

D) Determination of Total Fatty Matter

Weigh accurately about 2 g of the fabric into a conical flask, add 25 ml of dilute acid, fit a condenser into the flask, and boil the contents until the answer is perfectly clear. Pour the contents of the flask into a 300-ml separating funnel and permit it to cool down to 280C. Rinse the conical flask with 50 ml of petroleum ether in portions of 10 ml. Pour the Petroleum ether rinsing into the separating funnel, shake the separation funnel well and leave until the layers separate. filtrate the aqueous phase and shake it out with 50 ml portions of Petroleum ether twice. Combine all the Petroleum ether extracts and wash them with water until freed from acid (when tested with methyl 1 orange indicator solution). Filter the Petroleum ether extracts through a paper containing sodium sulfate into a conical flask which has been previously dried at a temperature of 90 + 2oC and so weighed. Wash the sulfate on the filter with Petroleum ether and mix the washing with the filtrate. Distil off the Petroleum ether and dry the fabric remaining within the flask at a temperature of 90 + 2oC to constant mass.

Calculation

Total fatty substance, percent by mass = 100(M1/M2)
Where,
M1 = mass in g of the residue,
M2 = mass in g of the material taken for the test

E) Skin irritation test

Test for irritation was performed on human volunteers with their consent. Five volunteers were selected and 1.0 g of formulated cream was applied on an area of 2 square inch to the back of hand. The volunteers were observed for lesions or irritation.

f) Spreadability

The spreadability of test samples was determined using the following technique: 0.5 g test formulation was placed within a circle of 1 cm diameter pre-marked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. Spreadability refers to the area covered by a fixed amount of cream sample after the uniform spread of sample on the glass slide. The increase in the diameter due to spreading of the test formulation.

G) Phytochemical screening of the methanol extracts

Qualitative phytochemical tests were carried out to identify some bioactive components of the extracts. The main bioactive groups (alkaloids, tannins, saponins, flavonoids, fats & oil and other compounds) were identified in each extract using different standard methods.

Test for Protein [24]

a. **Biuret test** 3mg of crude extract was mixed in 3ml of water. To this 4% sodium hydroxide (1ml) and 5 drops of 1% solution of copper sulphate was added. Appearance of violet or pink colour indicates the presence of proteins. **Test for Amino acid [24]**

a. **Ninhydrin** test 0.1ml ninhydrin in n-butanol solution was added to 2mg of extract. Appearance violet purple colour indicates the presence of amino acids.

Test for Tannins [25] 5mg of crude extract was mixed with 5ml of water and filtrate was used for following tests a. Ferric chloride reagent Ferric chloride reagent was added to 2ml of the above filtrate. Appearance of deep blue-black colour indicates presence of tannin.

Test for flavonoid [15, 26]

a. **Shinoda test** 5ml of 95% ethanol, 2 drops of conc. HCl and 0.5g of magnesium was added to 2mg of dry extract. Appearance of pink color indicates the presence of flavonoid.

Fats and oil test [27]

a. **Spot test** A drop of each extract was placed between folds of filter paper and pressed it. Permanent stains of oils on filter paper indicate presences of fats and oil.

Test for Carbohydrate [28]

a. **Fehling test** 5ml of Fehling's solution which was prepared by mixing equal volume of Fehling's A solution (copper sulphate solution) and Fehling's B solutions (alkaline tartarate solution) was taken in test tube and heated to gentle boiling .To this 2mg of crude extract in 5ml of water was added and boiling was continued gently for a minute . A red precipitate of cuprous oxide if formed shows the presence of reducing sugar.

Test for Vitamin-C 1ml of 2% w/v solution was diluted with 5ml of water and 1drop of freshly prepared 5%w/v solution of sodium nitroprusside and 2ml of dilute sodium hydroxide solution was added. To this 0.6ml of hydrochloric acid was added drop wise and stirred. Yellow color turning to blue indicates presence of Vitamin C. [29, 30]

H) Determination of antioxidant activity

night cream with of cactus Prickly pear fruit extract by DPPH method.

Result

Preliminary phytochemical screening All the extracts were screened for presence of carbohydrate, protein, amino acid, alkaloid, tannin, fat and oil, flavonoid and Vitamin C. Preliminary phytochemical screening showed the presence of carbohydrate, protein, alkaloid and

flavonoid in ethanol extracts and Vitamin C in extract which is recorded in table no.4

Sr. no	Phytochemical	Test	Result
1	Carbohydrate	Fehling solution test	+ pass
2	Protein	Biuret test	+ pass
3	Amino acid	Ninhydrin test	+ pass
4	Tannin	Ferric chloride reagent	+ pass
5	Flavonoid	Shinoda test	+ pass
6	Vitamin C	Vitamin C test	+ pass
7	Fat and oil	Spot test	+ pass

Table No 4. Phytochemical screening of the ethanolic extracts.

DPPH free radical scavenging activity it was observed that ethanol extract of cactus prickly pear fruit showed DPPH free radical scavenging activity. Different concentrations of L-ascorbic acid were used as standard antioxidant. Readings showed in table no 5.

Conc. of Extract (µL/ml)	Ethanolic extract %Inhibition	Ascorbic acid
5	31.19±0.080	21.03±0.067
10	41.17±0.048	29.95±0.094
15	51.40±0.082	40.10±0.063
20	66.49±0.053	62.80±0.061
IC50	13.22±0.012	16.54±0.018
IC50 (Std.)Ascorbic acid - 60 µg/ml		

Table.No.5 .DPPH free radical scavenging activity readings

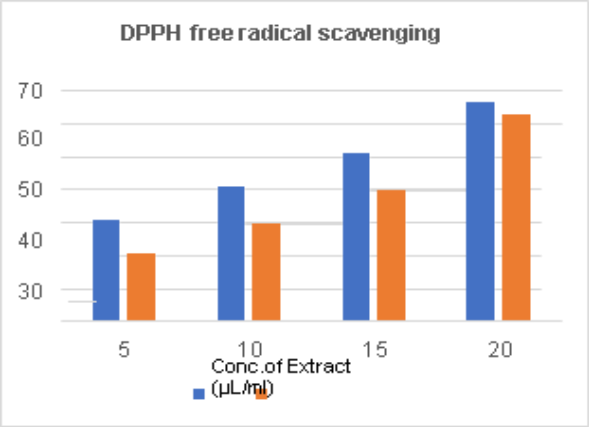

Fig.No.7.DPPH free radical scavenging activity graph.

Evaluation parameters

Sr. No	Parameters	Results		
		1 Week	2 Week	3 Week
1.	Colour	White	White	White
2.	Odour	Pleasant	Pleasant	Pleasant
3.	Consistency	Semi- solid	Semi- solid	Semi-Solid
4.	pH	5.5	5.6	5.6

Table.No.6 .Evaluation parameters

Evaluation of cream according to BIS

Sr.No.	Parameters	Results
1.	Colour	White
2.	Odour	Pleasant
3.	Consistency	Semi-Solid
4.	pH	5.6
5.	Spreadability	6.72 g.cm/sec
6.	Skin irritation test	Non-irritant
7.	Thermal stability	To pass the test(20,30,40^{0}C)
8.	Total fatty matter	4.7

Table.No.7 Evaluation of cream according to BIS

3.DISCUSSION

The phytochemical study of ethanolic extract of Opuntia ficus showed the presence of carbohydrates, proteins, amino acids, tannins, flavonoid and vitamins. The present study demonstrated that ethanolic extract of Opuntia ficus has antioxidant activity as assayed by reduction of DPPH. The antioxidant activity of ethanolic extract in night cream is contributed due to presence of vitamins, tannins and flavonoid.

4.CONCLUSION

The demand for natural antioxidants is growing day by day. Present study showed Opuntia ficus contains tannins, vitamin and flavonoid which are responsible for antioxidant property. Further the result of the present study demonstrated that Opuntia ficus is one of the opuntia species that merits more investigation and research.

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