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DEVELOPMENT OF ANTIOXIDANT CREAM USING EXTRACTS OF TURMERIC LEAVES

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1. Abstract:
Antioxidant is defined as a substance that protects cells from the damage caused by free radicals (unstable molecule made by the process of oxidation during normal metabolism). An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. The antioxidant activity of the turmeric leaves was researched previously by many researchers, but there is no information with formulation of an antioxidant cream. Take this as consideration, the present study is focused to formulate and evaluate the antioxidant cream comprising the aqueous extract of turmeric leaves. The formulation contains 2% of the turmeric leaves extract. The phytochemical analysis of aqueous extract shows presence of alkaloids, saponins, glycosides, flavonoids, tannins and phenolic compounds. The formulation was subjected to standard evaluation process on the following parameters like pH, Homogeneity, viscosity, Irritancy, Spread ability and physical stability of cream. The evaluation of the formulated cream showed good results and can be good potential for cosmetic product development.

Keywords: Antioxidant, Antioxidant cream,Curcuma Longa, Evaluation Parameter, DPPH assay, free radicals, etc.

2.Introduction:

2.1. Turmeric (Leaves):

Curcuma Longa L. (Zingiberaceae), commonly called turmeric, is an herbaceous plant and cultivated in many Asian countries including India and China[1].
Traditionally, turmeric has been used as a medicinal plant with its various biological activities such as strengthening energy, antioxidant, antibacterial, anti-inflammatory, anticancer, and wound healing[2,3].
These functional properties result from curcuminoids, the major components of turmeric, including dimethoxy curcumin, bisdemethoxycurcumin&curcumin[4].
Curcumin, a well-known yellow pigment, is a potential substance that may control oxidative stress-induced cellular damage owing to its radical scavenging activity[3,5].
Similarly, turmeric leaves are an ingredient added to various dishes in South-East Asia, as they are believed to have antioxidant properties[6].
However, turmeric leaves are mostly wasted as byproducts, except for animal feeds after harvesting[7].
According to the research paper[8], turmeric leaves also contain bioactive compounds, such as curcumin, several phenolic compounds, and flavonoids. These compounds have been known to act as an antioxidants owing to its effective radical-scavenging activity[9].
However, there are a limited number of publications detailing the functionality of turmeric leaves. In particular, the effects of turmeric leaves on ROS-induced oxidative stress remain unclear.

Fig. Turmeric Leaves

2.2.Antioxidants:

Antioxidant is defined asa substance that protects cells from the damage caused by free radicals (unstable molecule made by the process of oxidation during normal metabolism).

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules.

Oxidation reactions can form free radicals and these start chain reactions that damage cells.Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions.

Antioxidant is a synthetic or natural substances that prevent or delay deterioration of a product, or are capable of counteracting the damaging effects of oxidation in animal tissues.

Antioxidants a substance that significantly decreases the adverse effects of reactive species such as ROS or RNS on normal physiological function in humans.

Benefits Of Antioxidants:

i. Reducing DNA damage from UV light.
ii. Improving hydration.
iii. Stimulating the production of collagen and elastin.
iv. Reducing the appearance of wrinkles and pigmentation.
v. Supporting healing processes.
vi. Reducing inflammation.
vii. Softening

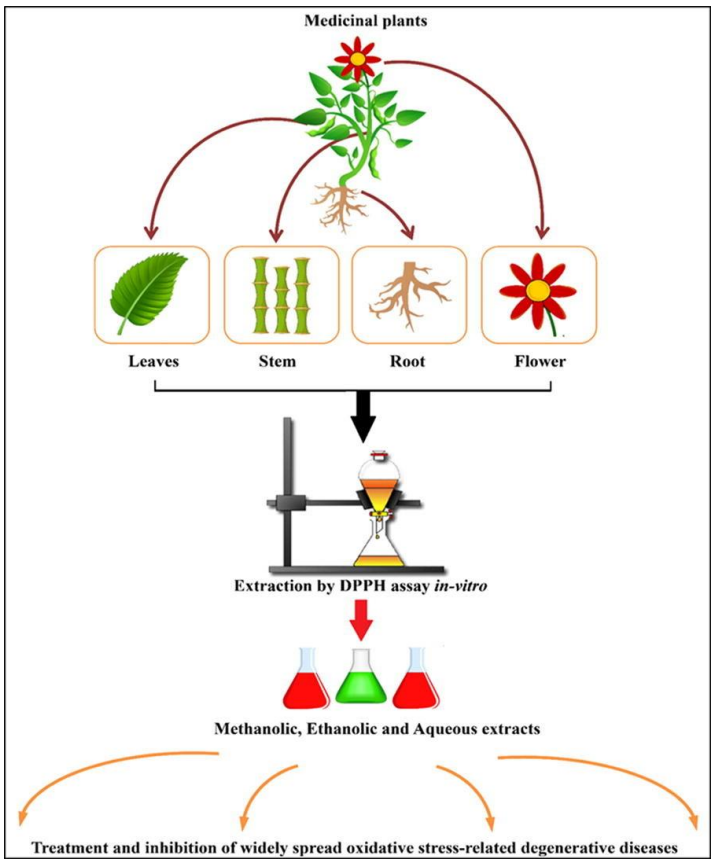


Fig. collection of extract from different parts of plant

3.Aim & Objectives

Aim:

Development of antioxidant cream using extracts of turmeric leaves.

Objectives:

To collect turmeric leaves.
To prepare turmeric leaves crude extract from leaf powder and distilled water as solvent.
To perform phytochemical analysis of crude extract.
To perform evaluation parameter of formulated cream.

4.Materials and Methods

4.1.Plant Materials:

Turmeric leaves were provided from farm. Fresh turmeric leaves were harvested between September and November and washed several times to eliminate soil and impurity. then, the leaves were hot-air dried at 50°C for 24h using a convection oven for storage and further extraction. After that, the dried leaves were ground as powder and stored at room temperature[10].

4.2.Turmeric Leaf Extraction:

The turmeric seeds were sown in April and fresh turmeric leaves were harvested in November. They were washed three times to eliminate impurities, and hot-air dried at 50°C for 24 h. Then, the leaf powder was extracted with distilled water at 85°C for 150 min with 1:25 extraction ratio as an optimal extraction condition. After filtration of the turmeric leaf extract (TLE) with 0.22µm PVDF filter, the leaf extract was diluted with distilled water at 10 mg/mL and stored at -20 °C until use[10].

4.3.Phytochemical Analysis:

The stock solution was prepared from the crude extract using the water as solvent. The obtained stock solution were subjected to preliminary phytochemical screening including[11],

Test For Alkaloids:
Mayer's reagent: Add 2-3 ml of filtrate, few drops of Mayer's reagent along sides of the test tube. Formation of white or creamy precipitate indicates the presence of alkaloids.
Wagner's reagent: Add 1-2 ml of filtrate, few drops of Wagner's reagent in a test tube. Formation of reddish-brown precipitate indicates the presence of alkaloids.
Hager's reagent: To 1-2 ml of filtrate, add few drops of Hager's reagent in a test tube. Formation of yellow color precipitate indicates the presence of alkaloids.
Test For Carbohydrates:
Molisch test: The filtrate was treated with 2 – 3 drops of 1% alcoholic alpha naphthol and add 2 ml of concentrated Sulphuric acid along sides of the test tube. Formation of red or purple color.
Fehling's test: To the filtrate add 1 ml of Fehling's A and B and heat in a boiling water bath for 5 – 10 min. Appearance of reddish orange precipitate shows the presence of carbohydrate.
Test For Proteins:
Biuret test: Add equal volume of 5% solution of sodium hydroxide and 1% copper sulphate. Appearance of pink or purple color indicates the presence of proteins and free amino acids.
Test For Glycosides:
Keller-Killiani test: To 2 ml of extract, add specific quantity of glacial acetic acid, one drop 5% ferric chloride and concentrated sulphuric acid. Appearance of reddish-brown color at the junction of the two liquid layers indicates the presence of cardiac glycoside.
Borntrager's test: To 3 ml of extract, add few drops of dilute sulphuric acid, boil and filter. Cool the filtrate andthen add equal value of chloroform. Separate the organic layer and add ammonia. Ammonia layer turns pink or red.
Test For Fixed Oils:
Spot test: Press a small quantity of the extract between two filter papers. Appearance of oil stain on paper indicates the presence of fixed oils.
Test For Saponin:
Foam test: The extract diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.
Test For Tannins and Phenolic Compounds:
Ferric chloride test: Dissolve small quantity of the extract in distilled water. To this solution add 2 ml of 5% ferric chloride solution. Formation of blue, green or violet color indicates presence of phenolic compounds.
Lead acetate test: Dissolve small quantity of the extract in distilled water. To this solution add few drops of lead acetate solution. Formation of white precipitate indicates presence of phenolic compounds.
Test For Flavonoids:
Shinoda's test: To the extract, 5 ml (95%) of ethanol will be added. The mixture then treated with few fragments of magnesium turning, followed by drop wise addition ofconcentrated hydrochloric acid. Formation of pink color indicates the presence of flavonoids.
Flavonoid test: Test solution was taken in test tube and added few drops of dilute NaOH solution, an intense yellow color was appeared in test tube. It became colorless when an addition of few drops of dil. Acid that indicate the presence of flavonoids.
Test For Steroids:
Liberman- Burchard test: The extract is treated with chloroform. To this solution add few drops of acetic anhydride, boil and cool. Add concentrated sulphuric acid through the sides of the test tube. A brown ring is formed at the junction of two layers, if upper layer turned green, indicates the presence of steroids and formation of deep red color indicates the presence of triterpenoids.

4.4. DPPH Assay radical scavenging Activity measurement:
Procedure:

1. 2,2-diphenyl-1-picrylhydrazyl (DPPH).
2. stock solution of DPPH in buffered methanol solution(violet-purple colour).
3. buffered methanol prepared using 40 ml of 0.1 M acetate buffer (pH 5.5) with 60 ml methanol.
4. the test tubes wrapped in aluminium foil and kept at 30°C for 30 min in dark.
5. Absorbance is measured using spectrophotometer at 517 nm.
6. calculation performs using formula

% RSA = [(absorbance of control – absorbance of sample)/ absorbance of control] × 100

5.Preparation of Formulation

5.1.Composition of Antioxidant Cream:

Ingredients	**Amount (%w/w)**	**Uses**
Extract of turmeric leaves	2	Antioxidant
Methyl paraben	0.02	Preservative
Tween 80	2%	Surfactant
Polyethylene glycol	4%	Humectant
Glycerin	2%	Thickening agent
Mineral oil	3%	Lubricant
Stearic acid	6%	Emulsifier
Cetyl alcohol	3%	Emollient
Distilled water q.s.	100%	Solvent

5.2.Method of Preparation:

Turmeric leaves extract was used to prepare the antioxidant cream. Oil in water (O/W) emulsion-based cream (semisolid formulation) was formulated. The composition of the cream was shown in Table.The aqueous phase and oily phase components were heated separately up to 70°C and mixed uniformly using homogenizer by addition of methyl paraben, extract and perfume. Care was taken for even mixing, the remaining Distilled water is added with continuous stirring until the mixture cools and formed as cream. Base cream is prepared in the same method as formulation without extract.

5.3.Formulation:

Fig. antioxidant cream

6.Evaluation of Antioxidant Cream:

The standard procedure was followed to evaluate all the parameters. The following parameters were used to evaluate the antioxidant cream[10].

Physical Properties:

The cream was observed for color, odor and appearance.
Determination of pH: The pH meter was calibrated using standard buffer solution. About 0.5g of the cream was weighed and dissolved in 50ml of distilled water and its pH was measured.
Determination of Emulsion Type (Dye test): The emulsion type was determined by using dye test. The scarlet red dye is mixed with the cream. Placed a drop of cream on a microscopic slide covers it with a cover slip and examined it under a microscope. If the disperse globules appears colorless the ground is red, the cream is oil in water type. The reverse condition occurs in water in oil type cream. i.e., the disperse globules appear red in the colorless ground.

Homogeneity:

The formulations were tested for the homogeneity by visual appearance and by touch.
After Feel Effect: Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.
Loss on Drying: 1 g of cream was taken in china dish and kept in an oven at 105°C for 2 hours.
Rheological Studies: The formulated cream was found to be nonnewtonian. Take a fixed quantity 10 g of cream in a 10 ml beaker. Keep it impact for 1 hr. The beaker was inclined to one side see whether consistency has changed or not. The beaker was again tilted and checked for pourability of the cream.

Ph Measurement:

The pH of the formulations was measured by a digital pH meter at room temperature (30 ± 2oC), (n=3).

Viscosity And Flow Measurement:

The viscosity and rheological properties of the formulations were assessed using a bob cup Brookfield rheometer (model LVDV-III Ultra, Brookfield Engineering Laboratories Inc., MA, USA) and small adapter. The spindle SC4-31 was used. The measurements were performed at room temperature (30 ± 2°C), (n=3).

Stability Study:

Stability study over a period of three months was conducted. The physical appearance, pH value, drug content, were determined periodically after the 1st, 2nd&3rd month after cream preparations. The stability of the formulatedherbal cream was tested under different temperature which are 2°C, 25°C and 37°C[17].

Homogeneity & Appearance:

After the cream has been set in the container, the formulation was tested for homogeneity by visual appearance and by physical touch. The appearance was determined by examining the pearlescence, theroughness, and the color[18].

Spread ability:

Spread ability is measured by time in seconds utilized by two glass slides to slip off from cream, lesser time taken for separation of two slides, denotes the better the spreadability. Measuring the spreadability was done by adding 3g of the herbal cream between two slides and pressed it to get a thin layer which is uniform and then a 1000g weight was placed for 5 minutes. Using a pan, 10g of weight was added to it. The upper plate was attached to a string which is also attached to a hook so that the plate can be pulled. The time taken for the upper plate to go over the lower one to cover 10 cm of distance was recorded. After that, the spreadability was calculated using the following formula[19].

$S = M \times L/T$

Where M = weight tied to upper slide, L = length of glass slides,
T= time taken to separate the slides

Irritancy Test:

An area (1 cm2) on the dorsal left-hand surface was marked. The cream was applied to the specified area and the time was noted. Irritancy, erythema, edema were checked for regular intervals up to 24 h and reported[20].

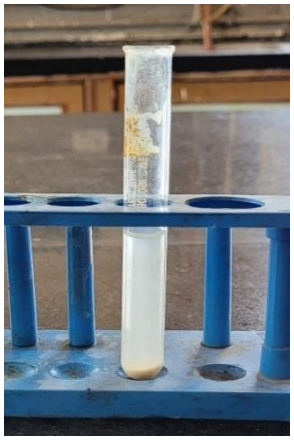
7. Observation and result:

Phytoconstituents	**Leaves Extract**
Alkaloids	Present
proteins	Absent
saponins	Present
carbohydrates	Absent
glycosides	Present
Fixed oils	Absent
Tannins & phenolic compounds	Present
flavonoids	Present
steroids	Absent

7.1.Phytochemical Analysis of The Extract:

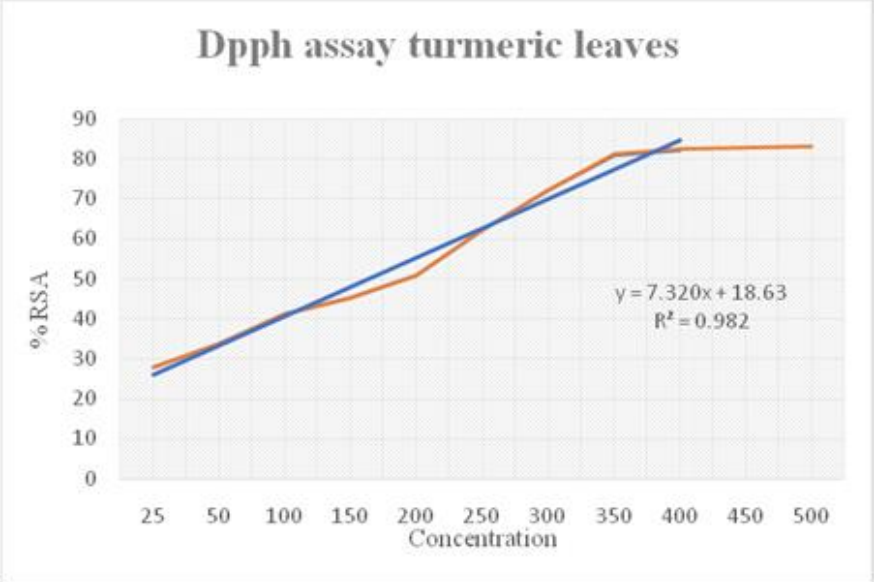
calculation of % radical scavenging and IC50 from DPPH assay					
Absorbance measurement data					
Sr. No.	Concentration (µg/ml)	Control	Sample	%RSA	IC50
1	25	0.52	0.375	27.884	0.86
2	50	0.52	0.345	33.653	4.28
3	100	0.52	0.305	41.346	11.11
4	150	0.52	0.285	45.192	17.94
5	200	0.52	0.255	50.961	24.77
6	250	0.52	0.196	62.307	31.60
7	300	0.52	0.144	72.307	38.43
8	350	0.52	0.098	81.153	45.26
9	400	0.52	0.092	82.307	52.09
10	450	0.52	0.09	82.692	58.92
11	500	0.52	0.088	83.076	65.75

Fig. phytochemical analysis of extract

			
Flavonoids test :- Yellow color became colorless	Lead acetate test:- White ppt	Foam test :- Formation of foam layer	Wagner's test :- Reddish brown ppt

7.2.Calculation of % radical scavenging and IC50 from DPPH assay:

Fig. DPPH radical scavenging activity of turmeric leaves



7.3.Evaluation of the Formulated Cream:

Parameter	Observation

PH	6.4
Homogeneity	Homogenous
Appearance	White color semisolid cream
Odor	Mildly aromatic
Spreadability	Good(15g.cm/s)
After feel	Emollient and slipperiness
Removal	Easily removed with water
Stability	Stable throughout the study
Irritancy	No Irritation
Viscosity	10568 cp at 12 RPM

8.Conclusion:

The study accomplishes that due to the impressiveantioxidant activity, the topical application of theformulated cream from turmeric leaves extract willhelp in overcoming oxidative damage and can beconsidered as another source in cosmeticindustries.Thefree radical scavenging activity was studied byusing stable 2,2 -Diphenyl-1-picrylhydrazyl (DPPH). The turmeric leaves extract showed impressivehigh radicalscavenging activity, the IC50 value shows that leaves of turmeric show good antioxidant properties with 24.77µg/ml. Throughout the study focused on masking the color and aromatic odor of the cream to make it more attractive and tempting the consumer.Moreover, the formulation of the cream can also be focused to increase the potency of the antioxidant activity, which will create more importance in the future cosmetic product development.

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