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FORMULATION AND EVALUATION OF ANTI-OXIDANT HERBAL GEL

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Abstract
The present project has been undertaken with the aim to formulate and evaluate of polyherbal gel containing Neem (Azadirachta indica A.Juss, Meliaceae), Turmeric (Curcuma longa L., Zingeberaceae), Aloe (Aloe vera (L.) Burm.f., Asphodelaceae),) and Lemon extract (Citrus limon (L.) Osbeck., Rutaceae) as a cleansing agent, anti-acne and skin nourishing. Natural remedies are more acceptable in the belief that they are suffer with fewer side effects than the synthetic ones. Herbal formulation has growing demand in the world market. The plant has been reported in literature having good antimicrobial, anti-inflammatory, refreshing activity, cleansing agent and anti-oxidant. formulations are prepared by using varied concentration of extract prepared formulation where evaluated for various parameters like color, appearance, consistency, wash ability, pH and Spreadability, Extrudabilty, skin irritation and compared with marketed formulation. It has wide spectrum of antioxidant activity against acne prone skin. The prepare gel is formulated by using carbopol- 934 as gelling agent, herbal extracts are the medicinal agents in formulation. Polyethylene glycol used as a co-solvent, propyl- paraben as a preservative and required quantity of distilled water as a vehicle. On the basis of the results obtained in the present study we conclude that the gel formulation of polyherbal contents showed good activities towards the declared evaluations.
Keywords: Turmeric Extract, Lemon Extract, Neem Juice, Aloe Vera Juice, Carbopol-934, Anti-Acne, Antioxidant Activity.

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INTRODUCTION

The project mainly aims in designing pharmaceutical gel dosage form which is the **"FORMULATION AND EVALUATION OF POLYHERBAL GEL."** it has antioxidant activity, which prevents free radicalsfrom the skin and helps as anti-acne medication.

OBJECTIVES

The objectives of the research work under taken are as

- To perform polyherbal gel characterization.
- To formulate and evaluate the polyherbal gel containing antioxidant activity using gelling agentsand other ingredients.

The anti-oxidant defence is one of the effective skin defence mechanism. Gel formulations have many advantages such as low cost and ease of use,production and scale-up.the criteria behind the formulation is to topical dosage form preparation.

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BACKGROUND AND PURPOSE OF THE PROJECT

Herbal formulation has growing demand in the world market. The plant has been reported in this project having good antimicrobial, antioxidant, refreshing activity, moisturizing activity, cleansing agent. It also helps to add glow to the skin. It also helps to prevent blemish marks and lighten the skin. It also effective for treating pimples. It helping to achieve a flawless complexation and good wash ability, pH, spreadability, skin irritation and compared with marketed formulation.

The given content contains herbal extract which has antioxidant property. Active compound of the plants includes mainly vitamin C which tends to possesses antioxidant property. Composition of the herbalgel prepared from aqueous as well as alcoholic extraction of plant. To keep skin healthy, clear, glossy, a balanced nutrition is required. Apart from the balanced nutrition, hormonal changes especially during puberty in both sexes cause many changes in the body. Among various changes, dryness, roughness and pimples are most common. The pathogenesis of this are bacterial over growth and inflammation. To overcome this problem, the use herbal remedies such as neem, aloe vera, turmeric lemon gel has been formulated.

PLANT PROFILES

Turmeric:

Biological source: it is fresh rhizomes of Curcuma longa.
Family: zingiberaceae
Phytoconstituents: curcumin, curcuminoids.

Neem:
Biological source: it is obtained from fresh leaves of Azadirachta-indica.
Family: Meliaceae
Phytoconstituents: Quercetin, nimbin, nimbidin.

Aloe vera:
Biological source: It is obtained from fresh leaves of plant Aloe berbadensis.
Family: Liliaceae
Phytoconstituents: Berberine , Anthraquinone glycosides.

Lemon:
Biological source: it is obtained from the ripe fruit of Citrus limon.[2,3]
Family: Rutaceae
Phytocontituents: vitamin C, terpens, tannins, citric acid.

MATERIALS AND METHODS

Given formulation is developing with different herbal extracts as well as with the help of some syntheticingredients which has necessarily use in the formulation of gel preparation.

Table no. 1- List of herbal ingredients.

Sr. No	Herbal Ingredients	Activity
1.	Neem leaves(Azardirachta indica.) meliaceae.	Antioxidant,anti-fungal

2.	Aloe vera leaves (Aloe berbadensis) liliaceae.	Moisturizing agent
3.	Turmeric rhizomes(Curcumalonga) zingeberaceae.	Antioxidant
4.	Lemon peel(Citrus limon) Rutaceae.	Antioxidant
5.	Rose water	Mask to the odour

COLLECTION AND PREPARATION OF EXTRACTS

Leaves of **Neem**, are collected from the tree **Azadirachta indica** belongings to **Meliaceae** family.it is an flowering plant and normally starts fruiting after 3/4 years.the leaves are upto 30 cm. the leaves are collected and then washed thrice with cold water. And kept for soaking overnight (12 hours) in water. Ratio of water and leaves is for 1 kg. leaves 1 litre.

Leaves of **Aloe vera** collected and washed and then the fresh juice of aloevera collected and stored in container with 0.1 amount of glycerine to maintain its viscosity and moisturizing property as well.[2,3]

Rhizomes of turmeric (Curcuma longa, zingeberaceae) are collected and dried in hot airoven at temperature 50^{o}c. and then powderd. Obtained powder used to further extraction with the help of soxhlet apparatus . methanolic extract was collected and evaporate. Evaporation was done by using electronic water bath. Filtrates were allowed to evaporate in evaporating pan at 600 C temperature until the desired concentration of the extract was obtained.

Lemon peel collected from the fruit of lemon and then juice of the peel were collected and used forpreparation

All obtained fresh extracts are used in the formulation of polyherbal gel.

Ingredients Other Than Active Herbal Extracts

These are the ingredients which has the different properties to formulating gel other than active extracts. Example; thickening agents, gelling agents etc.

Table no.2- List of ingredients other than active herbal extracts.

Ingredient	**Role**
Carbopol-940	Gelling agent
Propyl Paraben	Preservative
EDTA	Chelating agent
Triethanolamine	Nutrilizer
PEG-400	Gelling Base
Cellulose [CMC]	Thickning agent

As per the given introduction related to the formulation. given formulation is developing with different herbal extracts as well as with the help of some synthetic ingredients which has necessarily use in the formulation of gel preparation.Table for formulation(20 gm.)

Table no. 3 List of synthetic ingredients

Ingredient	**Quantity Sufficient**	**Role**
Carbopol-940	0.3 gm	Gelling agent
Propyl Paraben	0.2gm	Preservative

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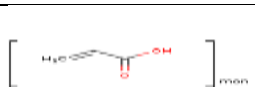
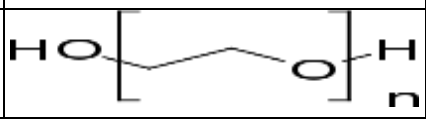
EDTA	0.2 gm	Chelating agent
Triethanolamine	0.010 gm	Nutrilizer
PEG-400	0.5 gm	Gelling Base
Cellulose [CMC]	0.4 gm	Thickning agent

Excipient Profile

Table no. 4 Excipient profile.

Name	Nonproprietary name	Structural formula	Uses
Carbopol	BP-carbomers PhEur-carbomer USP-NF-carbomer		Gelling agent (0.5-2.0)
PEG	BP-Macrogol JP-macrogol 400		Plasticizer,solvent

COMPOSITION OF POLYHERBAL GEL FORMULATION

Here is the table given for the all four formulations with their ingredients as well as quantity ofingredients with active herbal extracts.

Table no. 5 For composition of 20 gm. of Gel formulation

Sr no.	Name of ingredients	Quantity taken				Role
		F1	F2	F3	F4	
1	Neem extract (gm)	1	1.5	1	1.5	Antibacterial
2	Turmeric extract(gm)	1	1.5	1	1.5	Antioxidant
3	Aloe vera extract(gm)	1	1.5	1	1.5	Moisturizing Agent
4	Lemon extract(gm)	1	1.5	1	1.5	Antioxidant
5	Carbopol -940(gm)	1	1.5	1	1.5	Gelling agent
6	Propyl paraben(gm)	1	1	1	1	Preservative
7	EDTA(gm)	0.2	o.2	0.2	0.2	Chelating agent
8	Triethanolamine (gm)	0.10	0.20	0.10	0.20	Neutralizer
9	Carboxy methyl cellulose(CMC) (gm)	1	1.5	1	1.5	Thickening agent
10	Polyethylene glycol(PEG)-400 (gm)	2.50	2.50	2.50	2.50	Gelling base
11	Distlled water(Q.s)	100(q.s)	100(q.s)	100(q.s)	100(q.s)	Diluent

12	Rose water(Q.s)	0.7	0.7	0.7	0.7	Flavourant

METHOD OF PREPARATION OF GEL CONTAINING EXTRACT

1 g of carbapol 940 was dispersed in 50 ml of distilled water kept the beaker a side to swell the carbapol 940 to form gel. Take 5ml of distilled water and required quantity of propyl paraben were dissolved by heating on water bath solution was cooled and propylene glycol 400 and CMC added. Further required quantity of extract was mixed to the above mixture and add this solution into the carbapol 940 gel with continuous stirring and add triethanolamine was added dropwise to the formulation for adjustment of required skin pH and to obtain the gel at required consistency.[9]

Figure No.1 carbopol gelling base

Figure No.2 consistency of carbopol

EVALUATION PARAMETERS OF GEL FORMULATION

Visual inspection:

The gel were examined for their physical properties by visual inspection of colour, clarity, homogenesity, odour etc.

Appearance:

All the formulation of antioxidant gel was pale yellow/yellow in colour.

Consistency:

The consistency was checked by applying on the skin.

Greasiness:

The greasiness was assistes by the application on the skin and the slide.

Determination of pH:

The pH of gel was determined using digital pH meter by dipping the glass electrode completely into the gel and by using pH paper.

Determination of viscosity:

Viscosities of the formulated gels was determined using viscometer.(speed 60 rpm. At 25^0 c.was

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used for gels, corresponding dial reading on the meter was noted.

Determination of spreadability:
Spreadability was measured on the basis of "slip" and "drag" charactristics of the gels.a ground glass slide was fixed on wooden block.an weight is provided by pulley ground slide was fixed on this block.2gm. of gel placed on the slide make sandwich.weight of 100 gm. placed on the top of the two slides for 5minutes. excess of gel (about 2 gm.)was scrapped out from edges.top plate subjected to pull of 20 gm. weight with help of stirring attached hook. Time in seconds required by the top slide cover a distance 7.5was noted.[10,11]
Spreadibility was determined using following formula.
S=M.L/T
Where S is the spreadability in grams.cm/sec.,M is the mass in grams, T is time in seconds.


Fig No.4- homogenesity and clarity of the gel

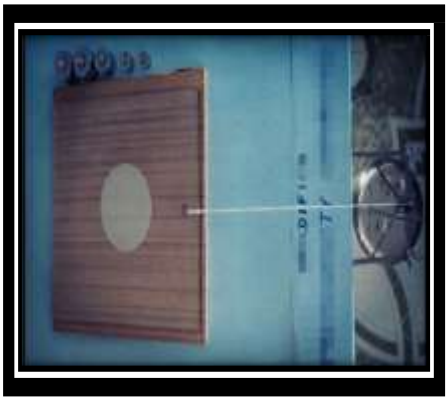
Fig No. 3. Spreadability testing of gel.

Stability of polyherbal gel:
Stability of formulations (F1, F2, F3, F4.) were studied at different storage conditions and assessed fortheir physical characteristics like odour,appearance and colour.

Determination of extrudability:
It was determined by using a tube filled with the gel, having a tip of 5 mm opening and by measuring theamount of gel that extruded through the tip when a pressure was applied on the tube was noted down.[4,5]

Solubility:
Gel has been tested by dissolving in ethanol, methanol, chloroform and water.

Clarity:
The prepared gel formulation was evaluated in glass container and observed under the glass.

Washability:
Formulation was applied on hand and was observed under running water.

Irritancy test (patch test):
Skin irritation test was performed for the selected gels on human volunteers to find out any irritation problems which could make it unsuitable for topical use. Skin irritation test was

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performed, for each gelon three volunteers. Approximately 1gm of gel was topically applied to the hand near the wrist over 2 square inch area and observed for any lesions, irritation, allergy or edema etc.

DPPH assay for evaluating the antioxidant activity of gel:

An inter-laboratory evaluation study was conducted in order to evaluate the antioxidant capacity if gel using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. These showed that the proposed DPPH assay couldbe used as a standard method to evaluate the antioxidant capacity of the gel.
In this study, the DPPH assay was conducted according to the following procedure.[9,10]
The methods for preparing each reagent were detailed in the analytical procedures.

1. Preparing DPPH Solution

DPPH (7.8 mg) was weighed on a chemical balance with a minimum weighing limit . thereafter ,it was dissolved in 99.5% ethanol to obtain a constant volumeby filling 100 mL of measuring flask or measuring cylinder with a stopper.(0.2 mM DPPH).the absorbance of a DPPH solution is empirically known to decrease with time until approximately 1 h after preparation. Therefore, it waskept in the dark for 2 h until the absorbance stabilized. After 2 hours , 1 mL of the DPPH solution was added into a test tube or sampling tube and then 200 µL of ethanol and 800µL of 0.1 M sodiumphosphate buffer(pH 7.4) were added. After mixing, the absorbance at 517 nm was measured. A mixed solution containing 1.2 mL of ethanol and 800 µL of sodium phosphate buffer was used as blank. When the absorbance was in range of 1.00±0.05, the prepared DPPH solution was used directly for the measurements. The DPPH solution was storedin the dark at room temperature during the assay , and used up on the day of preparation.[5,6]

Preparing sodium phosphate buffer (pH 7.4)

Add 40 mL of 0.2 mol/litre sodium hydroxide to 50 mL of 0.02 mol/litre sodium hydrogen phosphate and dilute to 100 ml.

DPPH assay procedure

After 200 µL of an analytical sample solution and 800 µL of 0.1 M sodium phosphate buffer (pH 7.6) were added into a test tube or sampling tube , 1 mL of DPPH solution was added. Immediately, the solution was mixed with a test tube mixer for 10 seconds thereafter , ot was left at room temperature in the dark. Exactly 30 minutes after the addition of the DPPH solution, the absorbance of the solution at 517 nmwas measured. A mixed solution of 1.2 mL of ethanol and 800 µL of sodium phosphate buffer was used as the blank. The absorbance at the addition of the analytical sample was expressed as **As** , the absorbanceat the addition of ethanol instead of the sample as **Ac** , and the inhibition ratio (%) was obtained from the following equation :

Inhibition ratio (%) = {(Ac – As) / Ac} × 100.

In the analytical procedure distributes, the measurements at six points of concentration , including control , were required. The measurements of the DPPH radical scavenging activity for the analyticalsample solution at each concentration was repeated three times.

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Figure no. 5-final formulations of polyherbal gel.(Each container containing 20 gm of gel)

RESULT AND DISCUSSION

Results are given below in the table

As we performed all evaluation parameters tests , the results are also found for every single tests of the gel formulation. Evaluation of polyherbal gel has been performed with parameters given for theevaluation pupose of the gel. It is evaluated by all factors like physiochemical as well as physiological factors.

Table no. 6- physicochemical characteristics of gel

Sr.No	Parameters	Observation
1	Colour	Yellow
2	Odour	Aromatic
3	Acid value	2.60
4	Ester value	37.20
5	Solubilty in ethanol	Freely soluble
6	Density	1.02g/ml
7	Refractive index	1.48

Table no. 7: some basic parameters like; pH, appearance, spreadability, extrudability, homogenesity as wellmeasured as per IP.

Formulation	Appearance	pH	Spreadability	Extrudability %	Homogenisity	Drug content
F1	Pale yellow	5.5	18.20	92.13	Good	78.8
F2	Yellow	4.9	18.16	93.12	Good	68.34
F3	Yellow	5.0	17.47	94.14	Very good	79.81
F4	Yellow	4.4	17.45	90.23	Very good	69.78

Table No.8: inhibition ratio (%) of antioxidant activity determination by DPPH assay

Sr. no.	Concentration(ml)	Absorbance ofsample (As)	Absorbance ofcontrol (Ac)	Inhibition ratio (%)
1	0.2 ml	1.2	1.67	29.37 %
2	0.4 ml	1.19	1.72	30.81 %
3	0.6 ml	0.92	1.66	44.57 %
4	0.8 ml	1.0	1.67	40.11 %
5	1.0 ml	1.0	1.72	41.86 %

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Table No. 9: determination of antioxidant activity of gel.

Sr. no.	Antioxidant activity
10 – 20 %	Poor
20 – 40 %	Fair
40 – 60 %	Good
60 – 80 %	Very good
80 % <	Excellent

Antioxidant activity found- as per the results inhibition ratio of the sample of gel formulation havinggood (40- 60 %) antioxidant activity.[7]

Result of Activity Determination:

As we have performed DPPH assay procedure for the determination of antioxidant activity of the gel.By performing this procedure absorbance was recorded for the sample as well as control group.[7,12]

Table No. 10: stability testing of formulation of polyherbal gel.

Sr. No	Duration parameters	Storage conditions					
		7 days		15 days		30 days	
		8^{o} c	40^{o} c	8^{o}c	40^{o}c	8^{o}c	40^{o}c
1	Appearance	semisolid	semisolid	semisolid	Semisolid	Semisolid	Semisolid
2	Colour	yellow	Yellow	yellow	Yellow	Yellow	Yellow
3	Odour	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic

As per the results are shown in Table no.10 , formulation showed no significant changes in appearance ,odour and colour after 30 days. The gel has good stability.

DISCUSSION

The procured lemon oil was characterized for the following parameters-
Acid value-2.601
Density-1.02gm/ml Refractive index-1.48

The formulation was developed by using lemon oil and turmeric extract as well of same concentration and carbopol at different concentrations. all the formulations were pale yellow to yellow in colour and had some characteristic odour of lemon oil.

The pH of all formulations ranged from 4 to 5.5 which is slightly alkaline and which is totally suitable for all type of skins as per topical formulations.

The spreadability of gel was found 15.50-17.20 gm-cm/sec, confirming that this gel may spread smoothly and uniformly.

The formulations were glossy and translusent, the homogeneity and tube extrudability of all formulations was good. As we checked it out by putting the container downward angle. As gel should not flow easily it must be semisolid in nature. We found our formulation has good homogenesity and consistency as well. The drug content of formulations was ranged from67.4-

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70.20%. as we checked it by addition of ethanol and single point standardization with the help of one standard sample which is marketed formulation.

The gel formulations of antioxidant agents showed good physicochemical properties as well as good drug content . has good stability profile as per different storage conditions.

These antioxidant gel formulations further selected to the antioxidant activity determination as per DPPH assay method for antioxidant activity determination. By the help of DPPH assay method we found the good results with the help of UV spectrophotometer.

Plants are considered to be a vital source of potentially useful constituents for the development of new therapeutic agents, as most of them are safe with less or no side effects. Topical application of gels atpathological sites offer great advantages in a faster release of a drug delivery to site of action as compared to cream and ointments. Nowadays gels have been widely used as a vehicle for topical delivery of drugs. Extracts of plants and herbs with specific medicinal properties can be incorporated in this dosage forms as active ingredients in order to additional benefits. However, their application and use in raw form on to the skinsurface is difficult therefore the extracts of these plants were developed in the gel formulation.

Cosmetically, the chemical constituents of Azadirachta indicia, Curcuma longa, Aloe – berbadensis, Citrus sativum are considered to be natural antioxidant, antiseptic.

Herbal cosmetic producs are assumed to be safe for longer period of time. However, quality control for efficacy and safety of herbal cosmetic products is of paramount importance; and quality control tests must therefore be carried out for this preparations. Stability studies and patch test are well known methods which willprove its efficacy and efficiency of the cosmetic herbal formulations. Short term stability studies as per ICH guidelines, revealed that the pH of all the formulations indicated variability at different storage conditions. Viscosity studies, spreadability , extrudability showed minimal variations in the results which proved that all the prepared formulations are stable for 8 and 40^{o} c. applicability of the herbal formulation was proved to be satisfactory from the results of the viscosity and spreadability. In our study it was observed that the prepared formulations readily spread on application to the skin or affected part and homogenesity confirmed no lumps, respectively. Also the physicochemical parameters applied in the testing of stability of cosmetics formulations made apparent consequences that all formulation are relatively better due to better concentration of active constituents. Literature surveys releaved that individually all extracts has potentially been known for their antioxidantactivity. This study clearly indicated that formulations which possessed plant extracts were more more potent than the others. The results of patch test exhibited no irritation, redness on skin after application of all four formulations. Also, the results of washability test proved non-greasy properties of all prepared formulations.the possible explanation for this is is the presence of active constituents of plant which exhibit antioxidant activity. Potency of all the formulation found to be greater.

CONCLUSION AND FUTURE SCOPE

Conclusion-

Results of the studies revealed that the prepared polyherbal gel formulation which comprised of ethanolic extract of azadirachta indica (neem), curcuma longa (turmeric), Aloe berbadensis (aloe vera), Citrussativum (lemon) in different concentrations respectively produced no skin irritation after performing patch test .Also the physical analysis and satability studies of the prepared polyherbal gel proved potency and efficacy. Thus, this formulation can be used safely on human skin. The effective activity exhibited by the polyherbal formulation may be attributed synergistic action of the plants constituents present in the formulation. At concentration (0.6 Ml.) showing

good antioxidant activity.

The given content contains herbal extract which has antioxidant property. Active compound of the plants includes mainly vitamin C which tends to possesses antioxidant property. Composition of the herbal gel prepared from aqueous as well as alcoholic extraction of plant. To keep skin healthy, clear, glossy, a balanced nutritionis required. Apart from the balanced nutrition, hormonal changes especially during puberty in both sexes cause many changes in the body. Among various changes, dryness, roughness and pimples are most common. The pathogenesis of this are bacterial over growth and inflammation. To overcome this problem, the use herbal remedies such as neem, Aloe Vera, turmeric lemon gel has been formulated.

Future scope-

The prepared polyherbal gel formulation has good antioxidant activity. so gel can be useful for treatment of acne prone skin, pigmented skin.

This polyherbal gel has natural constituents which has beneficial effect on skin care andtreatment.

The herbal plants which is used in this formulation can be available very easily in environment.

Extraction process has less or no difficulties. It will be the less time taking procedure.

For those peoples who prefers herbal topical formulations over than chemical ones this formulation will be the good and effective one.

This polyherbal gel formulation can be use not only for treat the pigmentation or acne but also for healthy skin benefits and having lusture to the skin.

Formulation of this polyherbal gel has good stability profile.

This formulation can be available in less price also as it has good benefit for skin.

There is no formulation available in market yet which is containing this four herbal constituents as an active phytopharmaceutical ingredient. Hence this will be the greater and effective antioxidant polyherbal gel.

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