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Research Article

Formulation and Evaluation of Herbal Ethosomal Gel for Anti Aging

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Abstract
In the present study, an attempt was made to prepare, characterize and evaluate of topical therapeutic system from *Azadirachta indica* herbal plant. The extraction of *Azadirachta indica* was carried out with ethanol by using soxhlet apparatus. The hydrogel and hydroalcohalic gel formulation of ethanolic acetate extract were designed by using varied concentration of carbopol and sodium CMC polymer. During the trial, the excipients concentrations of carbapol and Sodium CMC were gradually increased and then decreased as several problems like homogeneity, spreadibility and viscosity were encountered. These problems occured in some of the batches (F1, F2, F6 and F7) of polymer based gel containing *Azadirachta indica*. Hence, these batches were discarded and remaining batches (F3, F4 and F5) were characterized for various parameter. The result showed that the developed herbal gel was greenish in color, translucent in appearance and showed good homogeneity with absence of lumps. Formulation F5 had good values of spreadability, viscosity, pH, drug content and during the accelerated stability studies the appearance was clear and no significant variation in spreadability, pH and drug content was observed. Hence hydroalcohalic formulated from hydrogel F5 formulation and its physiochemical study was found to be good.

Keyword *Azadirachta indica*,, topical therapeutic system, hydroalcohalic gel, carbopol.

Introduction
Cosmaceuticals has been used to describe the products that yield benefits traditionally, and active constituents thought to be cosmetic in nature, such as moisturization, as well as product that make marketing claims approaching those of drug products, such as reducing wrinkles, regenerates skin, firms, heal and penetrates into skin. Consumers seek "anti-wrinkle" cosmetic products that treat or delay the visible signs of actual aging and weathered skin, such as wrinkles, lines, sagging, and hyper pigmentation and age spots. The present consumers are highly specific about choice of product; consumers prefer mainly products free of synthetic active constituents, synthetic preservatives and base free of animal derivatives. Unnatural, chemically- synthesized products may be perceived as being environmentally or personally unsafe [1,2].

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In contrast, natural products are perceived as pure, mild, and superior to chemically synthesized products. Natural based products extracted from plants or herbs are believed to contain antioxidant/free-radical scavenging agents that can neutralize the effects of free-radical damage [3,4].

Neem is a fast-growing tree that can reach a height of 15-20 m, rarely to 35-40 m. It is evergreen but under severe drought it may shed most or nearly all of its leaves. The branches are wide spread. The fairly dense crown is roundish or oval and may reach the diameter of 15-20 m in old, free-standing specimens. [5,6]

Neem has been selected as active constituent because it posses antiwrinkle property, since it contains many flavonoids which have high potential of antioxidant activity, thus fight against free radical generations due to ill environmental factors.

Act as preservatives in formulation having antibacterial and antifungal property, it has additional cosmetic value like used for treatment of acne, scars and has additional medicinal value for all type of skin diseases even used for skin cancer. It is easily available through out India, thus proved to be economical also.

Since neem is rich in flavonoids like quercetin and rutin having antioxidant property and limnoids thus for centuries Indians planted this tree in the vicinity of their homes and practiced gentle and daily interaction with this extraordinary plant. Neem leaf powder or crushed leaves incorporated into their face packs provided emollient and anti ageing action. The antiseptic properties of neem leaf extracts helped in controlling pimples,acne,psoriasisNeem oil has numerous remarkably proven medicinal properties also stimulative and antiseptic effect when used for massage of the body. [7,8]

Neem may have an effect in preventing or softening the appearance of wrinkles by providing a natural skin protectant as they contain many antioxidant compounds like polyphenols and flavonoids and moisturizer to the skin. After washing and drying the skin, rub a few drops of neem based cream or oil on areas that are prone to drying and wrinkles. [6-8]

Material and Methods
Collection of plant material
Neem leaves were collected from college campus and whole leaves were collected from same tree. Neem leaves collected were washed by distilled water two times and dried under shade for one month and was authenticated by Dr. S. N. Dwivedi, Prof. & Head, Department of Botany, Janata PG College, APS, University, Rewa, M.P.

Preparation of plant powder

The plant was dried under shade and then powdered coarsely with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use.

Preparation of extracts:

The dried powder of plant leave was extracted with Ethanol using Soxhlet apparatus.

About 250 gm of dried powder leave of plant was subjected to soxhlation. It was first defatted with petroleum ether then exhaustively extracted with solvent in a Soxhlet apparatus for 36 hours. The temperature was maintained at 40-50 degree centigrade. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract.[9]

FORMULATION OF SUITABLE TOPICAL THERAPEUTIC SYSTEM

Preparation of Hydrogel and Hydroalcohalic gel Containing Extract :

Hydrogel

Different proportions of Carbopol 934 and Sodium CMC 3:0, 3:1, 2:1, 1:1, 0:3, 1:3 and 1:2 were dispersed in 50 ml of distilled water with continuous stirring. 5 ml of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath and cooled. Propylene glycol 5 % w/v was added and then mixed with first solution. 1.0 gm of *Azadirachta indica* plant extract was dissolved in minimum quantity of alcohal and mixed to the polymer mixture .The volume was made up to 100 ml with distilled water. Finally all the ingredients were then mixed properly with the Carbopol 934 gel with continuous stirring. Triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel of required consistency (Table 1). The same method was followed for preparation of control sample without adding *Azadirachta indica* plant extract. Turbidity and lumping occured in some batches (F1, F2, F6 and F7) of polymer based gel containing *Azadirachta indica* . Hence, these batches were discarded and remaining batches (F3, F4 and F5) were considered for further studies.

Hydroalcohalic gel

1:2 proportions of Carbopol 934 and Sodium CMC were dispersed in 50 ml of distilled water with continuous stirring. 5 ml of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath. The solution was cooled and then propylene glycol 5 % w/v was added and mixed with first solution. 1.0 gm quantity of *Azadirachta indica* plant extract was dissolved in minimum quantity of ethyl acetate and 30 ml of ethanol added then mixed to the above polymer mixture. The volume was made up to 100 ml with distilled water. Finally all the ingredients were then mixed properly with Carbopol 934 gel with continuous stirring. Triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel of required consistency (Table 2.1& 2.2). The same method was followed for preparation of control sample without adding any *Azadirachta indica* plant extract.[10-12]

CHARACTERIZATION AND EVALUATION OF FORMULATION

Evaluation of Gel Formulation[10-12]:

All prepared formulations of gel were characterized for:

Physical Evaluation

Physical parameters such as color and appearance of the herbal gel were observed manually.

Measurement of pH

The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average value was calculated.

Spreadibility

Spreadibility was determined by the apparatus which consists of a wooden block, provided with pulley at one end. By this method spreadibility was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2g) under study was placed on the ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A one kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped of from the edges. The top plate was then subjected to pull of 80 gms weight with the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm was noted. A shorter interval indicates better spreadibility. Spreadibility was calculated using the formula given below:

$$S = M \times L / T$$

Where, S = Spreadibility, M = Weight in the pan (tied to the upper slide), L = Length moved by the glass slide and T = Time (in sec.) taken to separate the slide completely each other.

Consistency:

The measurement of consistency of the prepared gels was done by dropping a cone attached to a holding rod from a fix distance of 10cm in such way that it should fall on the centre of the glass cup filled with the gel. The penetration by the cone was measured from the surface of the gel to the tip of the cone inside the gel. The distance travelled by the cone was noted after 10sec.

Homogeneity

All the developed gels were tested for homogeneity by visual inspection after setting the gels in the container. They were observed for their appearance and presence of any aggregates.

Viscosity

Viscosity of gel was measured by using Brookfield viscometer with spindle No. 7 at 50 rpm at room temperature. The gels were rotated at 0.3, 0.6 and 1.5 rotations per minute. At each speed, the corresponding dial reading was noted. The viscosity of the gel was obtained by multiplication of the dial reading with factor given in the Brookefield Viscometer manual.

Drug content

1 g of the prepared gel was mixed with 100ml of suitable solvent. Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and the drug content was determined measuring the absorbance at 415 nm using UV/Vis spectrophotometer (Shimadzu UV 1700).

***In-vitro* drug release study of optimized formulation of Hydro gel and Hydroalcohalic gel gel containing Azadirachta indica plant extract :**

Franz diffusion cell (fabricated in our Lab.) with a diameter 3.7 cm was used in in-vitro release studies. A glass tube with both end open, 10 cm height and 3.7 cm outer diameter was used as a permeation cell. A one gram sample was accurately weighed and placed on a semipermeable cellophane membrane to occupy a circle of 3.7 cm diameter. The loaded membrane was stretched over the lower open end of a glass tube of 3.7 cm diameter and made water tight by rubber band. The tube (donor compartment) was immersed in a beaker containing 100 ml of phosphate buffer pH 6.8 (receptor compartment) .The cell was immersed to a depth of 1 cm below the surface of buffer. The system temperature was maintained at 37°±1° and speed was maintained at 30 rpm throughout the experiment by magnetic stirrer (Fig.1). Samples 3 ml were withdrawn at intervals of 15, 30, 45, 60, 90, 120, 180, and 240 min, the volume of each sample was replaced by the same volume of fresh buffer to maintain constant volume. Samples were analyzed without dilution or filtration for Azadirachta indica herbal drug content spectrophotometrically at 415 nm. [11,12]

Accelerated Stability Studies

The optimized formulations were subjected to a stability testing for six months as per ICH norms at a temperature and RH of 40°C ± 2°C/75% RH ± 5% RH respectively. The selected formulations were analyzed for the change in appearance, spreadibility, pH and drug content.[13]

Result and Discussion

The use of medicinal plants as raw materials in the production of new drugs is ever increasing because of their potentials in combating the problem of drug resistance in micro-organisms. Demand for medicinal plants is increasing in both developing and developed countries. Research on medicinal plants is one of the leading areas of research globally.

Extraction

The dried powder of plant was extracted with ethanolic solvents. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract.

Formulation Characterization and Evaluation of Suitable Topical Therapeutic System

Evaluation of Gel Formulation:

During the trial, the excipients concentrations of carbapol and Sodium CMC were gradually increased and then decreased as several problems like homogeneity, spreadibility and viscosity were encountered. These problems occurred in some of the batches (F1, F2, F6 and F7) of polymer based gel containing *Azadirachta indica* . Hence, these batches were discarded and remaining batches (F3, F4 and F5) were considered for further study.

The result showed that the developed herbal gel was greenish in color, translucent in appearance and showed good homogeneity with absence of lumps. Formulation F5 had good values of spreadability, viscosity, pH, drug content and during the accelerated stability studies the appearance was clear and no significant variation in spreadability, pH and drug content was observed. Hence Hydroalcohalic and Ethosomal gel was formulated from hydrogel F5 formulation and its physiochemical study was found to be good.

***In-vitro* drug release study**

Percentage drug release of hydrogel containing EE extract was observed to be- 9.8% (at 30 min.) and 47.63% (at 240 min.) respectively where hydroalcohalic gel containing EE extract was observed to be- 13.62% (at 30 min.) and 60.81% at 240 min.. It was observed that addition of ethanol in formulation increase the release by increasing permeation properties of gel. The Hydroalcohalic gel containing Ethanolic extract showed maximum drug release as compared to others formulation.

Standard calibration curve of Azadirachta indica plant extract for its active constituent

Standard calibraction curve of *Azadirachta indica* extract was determined by plotting absorbace vs concentraction at 415 nm and it follow the beer's law.

Stability Study

The formulated gels were subjected to stability studies. No color fading was observed for all prepared gels. The pH of all formulations remained unchanged and was found to be within the range of 6.2-7.2. The viscosity and spreadability of all gels remained unaltered and found to be within the range. The drug content was found to be in the limit 90% -103% for all gel formulation.

Conclusion

In the present study, an attempt was made to prepare, characterize and evaluate of topical therapeutic system of *Azadirachta indica* herbal plant. Various formulations such as hydrogel and hydroalcohalic gel were designed and optimized.

The result showed that the developed herbal gel was greenish in color, translucent in appearance and showed good homogeneity with absence of lumps. Formulation F5 had good values of spreadability, viscosity, pH, drug content and during the accelerated stability studies the appearance was clear and no significant variation in spreadability, pH and drug content was observed. Hence hydroalcohalic were formulated from hydrogel F5 formulation and its physiochemical study was found to be good. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market.

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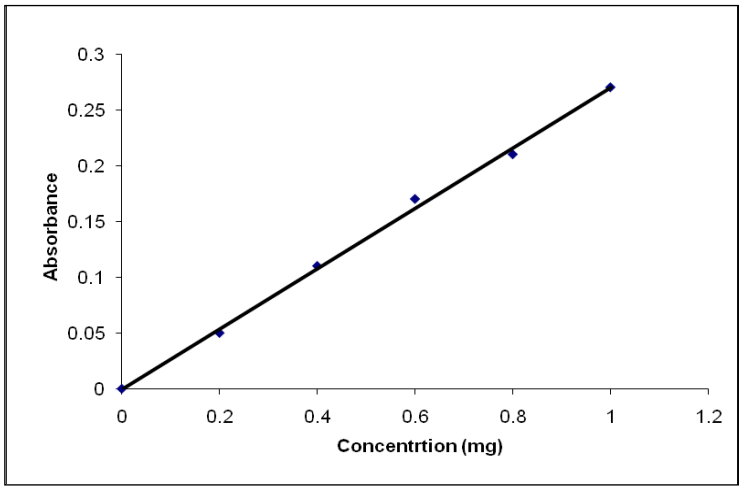
Table No 1: Accelerated Stability study of formulated gel

Batch	Color	Appearance	Spreadibility (gm.cm/sec)	Consistency (60 Sec)	Viscosity (cps)	Ph	Drug content (%)
F3	Greenish black	Homogeneous	14.60	5	22230	6.84	99.77
F4	Dark Greenish	Homogeneous	18.50	6	24010	6.93	98.20
F5	Greenish	Homogeneous	20.15	6	18170	6.90	99.82
FA	Greenish	Homogeneous	23.00	9	16042	6.95	98.95

Table No 2: Physical evaluation of all formulations

Batch	Color	Appearance	Spreadibility (gm.cm/sec)	Consistency (60 mm)	Viscosity (cps)	Ph	Drug content (%)
F3	Greenish	Homogeneous	19.75	5	22410	7.00	99.81
F4	Greenish	Homogeneous	21.65	7	19380	7.00	99.75
F5	Greenish	Homogeneous	21.38	6	24180	7.00	99.95
FA	Greenish	Homogeneous	23.96	8	17053	7.00	99.80
FE	Greenish	Homogeneous	24.21	8	16915	7.00	99.94

Fig No 1: Standard calibration curve at 415 nm.



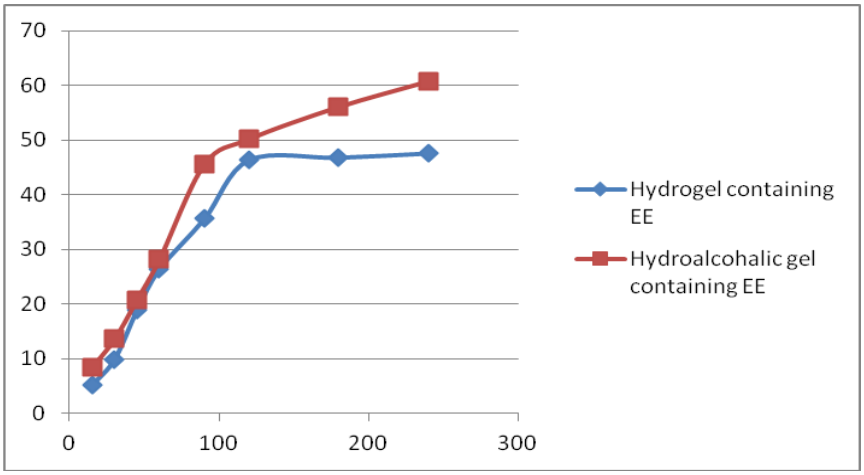


Fig 2: Release profile of Hydrogel and Hydroalcohalic gel containing extract

Table No-3 Release profile of Hydrogel and Hydroalcohalic gel containing extract

Time Interval (Min)	% Drug release of formulations	
	F_7	FA
15	05.12	08.42
30	09.89	13.62
45	18.91	20.81
60	26.41	28.23
90	35.62	45.58
120	46.43	50.24
180	46.85	56.12
240	47.63	60.81