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Design And Estimation Of Herbal Anti-Acne Preparations Containing Azadirachta Indica, Ocimum Sanctum & Orange Peel

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Abstract:

Pimples, acne, sunburn mark and pigmentation are issues that affected every individual at least once during life time. Consumer have begun to search for a product that can cure the skin issues and grant them with a good and healthy skin such as anti-acne cream. Nevertheless, most of the anti-acne creams available in the market contain lot of chemical that may have some kind of side effects to the consumers. The present study was conducted to formulate and evaluate the anti-acne cream containing some herbal plant like Azadirachtaindica, Citrus aurantium and Ocimum sanctum extract. Extracts was prepared by Maceration process. And Soxhlet extraction process. Naturally herbal extract in combination can be effectively utilized for the treatment of Acne. Four batches of oil in water (O/W) herbal cream namely F1, F2, F3& F4 were formulated by incorporating different polymers like Stearic acid and Cetostearyl alcohol and Brij 35. All the formulation were evaluated for various parameters like pH, Spreadability, viscosity, drug content, In-vitro drug studies & Acne healing activity etc.

Key words: Anti-bacterial activity, Azadirachtaindica, Citrus aurantium, Ocimum sanctum, Maceration, Diffusion study

Introduction: [1, 2. 3]

Now a day's human being suffer one of the come skin infection is Acne causing pimples and other malfunctions on the face and upper trunk.[1, 2, 3]Acne is a long-lasting, inflammatory skin disorder that causes spots and pimples, especially on the face, shoulder, back, neck, chest, and upper arms. It is most commonly happensin women's during puberty, pregnancy because of hormonal changesand the sebaceous gland activates. acne is caused by a variety of factors and includes immunological hypersensitivity, hormone imbalance, altered follicular keratinization, and bacterial (Propionibacterium-acnes) colonization.[4]

The cosmetic products are the best choice to reduce skin disorders such as hyper pigmentation, acne, pimples and rough skin texture etc. The need of herbal cosmetic day by day is increase, The plant parts used in cosmetic preparation should have varieties of properties like antioxidant, anti-inflammatory, antiseptic, emollient, antiseborrhatic, antikerolytic activity and antibacterial etc. Herbal products claim to have less side effects, commonly seen with products containing synthetic agents. [5,6,7,8]

Azadirachtaindica(neem) have drawn a lot of interest due to their ability to treat acne, it has a long history in traditional medicine and are well-known for their wide range of pharmacological characteristics, which include antioxidant, antibacterial, and anti-inflammatory effects.[4]

Tulsi (Ocimum sanctum) is an anti-inflammatory, anti-oxidant properties and anti-acne activity. The sacred basil, tulsi is renowned for its religious and spiritual sanctity, as well as for its important role in the traditional Ayurvedic and Unani systemic oh holistic health and herbal medicine of the East family Lamiaceae. [9]

Orange peel contain considerable amounts of calcium, copper, magnesium Vitamin A, folate and other Vitamin B and dietary fiber. It has anti-oxidant Activity and anti-acne activity belonging to family Rutaceae. [10]

Method of preparation of extract:

Method of Preparation of Extract Neem (Azadirachtaindica)[11, 12, 13]

1) firstly, dried neem leaves, and crush in mortal pastel and make it dry coarse powder of neem leaves.
2) Accurately weigh 100g of the Neem drug using a digital balance.
3) Place the Neempowder into the maceration jar pour 500ml of Methanol.
4) Stir the mixture thoroughly with a stirring rod to ensure the Neem drug is fully soaked in the Methanol.
5) Seal the maceration jar with a lid or cover to prevent evaporation of the solvent.
6) Store the jar in a cool, dark place at room temperature.
7) Allow the mixture to macerate for a period of 7 days.
8) Stir the mixture daily to enhance the extraction process.
9) After 7 days, filter the mixture using filter paper or muslin cloth and a funnel.
10) Collect the filtrate (Methanol extract of Neem) in a clean, dry container.
11) Then filtrate can be evaporated under reduced pressure using a rotary evaporator to remove excess Methanol and make concentrated extract of Neem.
12) Store the final Neem extract in an amber-colored bottle to protect it from light and prevent Degradation.

Method of Preparation of Extract Tulsi: [11, 12, 13]

1) firstly dried Tulsi leaves, and crush in mortal pastel and make it dry coarse powder of Tulsi leaves.
2) Accurately weigh 100g of the Tulsi drug using a digital balance.
3) Place the tulsi powder into the maceration jar pour 500ml of Methanol.
4) Stir the mixture thoroughly with a stirring rod to ensure the drug is fully soaked in the Methanol.
5) Seal the maceration jar with a lid or cover to prevent evaporation of the solvent.
6) Store the jar in a cool, dark place at room temperature.
7) Allow the mixture to macerate for a period of 7 days.
8) Stir the mixture daily to enhance the extraction process.
9) After 7 days, filter the mixture using filter paper or muslin cloth and a funnel.
10) Collect the filtrate (Methanol extract of Tulsi in a clean, dry container.
11) Then filtrate can be evaporated under reduced pressure using a rotary evaporator to remove excess methanol and make concentrated extract of Tulsi.
12) Store the final extract in an amber-colored bottle to protect it from light and prevent Degradation.

Method of preparation of Leemon peel Extract: [11, 12, 13]

1) Orange peels were cut into small sized piecesand heated in an oven at a temperature of 100^{o}C for 30 min.
2) The apparatus used in Soxhlet extraction consisted of three main parts: the extraction chamber, a round-bottom flask containing the solvent, and a condenser.
3) The dried orange peel 100g, had been extracted and was placed in a porous thimble (cellulose material).
4) The round-bottom flask containing the methanol solvent was heated, causing it to evaporate.
5) The vapor was then condensed in the condenser and dropped back into the solid sample in the extraction chamber. As the solvent dropped back into the extraction chamber,
6) it gradually dissolved the solid sample of drugs. The dissolved drugs in the solvent gradually accumulated in the round-bottom flask as the process continued.
7) The extraction process continued until the concentration of the drugs in the solvent reached a saturation point.
8) Filter the concentrated drug extract, and store in cool and dark place.

Table No. 01:Compositionof Polyherbal Anti-Acne Cream

Sr. No	Formulation Batches% w/w				
	Ingredients mg	**F1**	**F2**	**F3**	**F4**
1	Neem Extract	0.5	1	1.5	1.5
2	Tulsi Extract	0.5	0.5	0.5	0.5
3	Orange Peel Extract	0.5	0.5	1	0.5
4	Brij 35	3	3	3	2.5
5	Stearic Acid	2	2	2	2
6	Cetyl alcohol	4	4	4	4
7	Linseed oil	1	1	1	1
8	Propylene glycol	3	3	3	3
9	Methyl paraben	0.02	0.0.2	0.02	0.02gm
10	Triethanolamine	Q.S.	Q.S.	Q.S.	Q.S.
11	Dist. Water	Q.S	Q.S	Q.S	Q.S

Preparation of Polyherbal Formulation Containing AzadirchtaIndica:

Formulation Preparation:

1) **Oil Phase:** The oil phase consist of Stearic acid ,Brij 35, cetyl alcohol and linseed oil, were dissolved in the oil phase. The oil phase was placed inside the beaker in the water bath. The temperature of water bath was set to 70 ^{o}C during the heating time.
2) **Aqueous Phase:** The water-soluble component and preservatives such as propylene Glycol, methyl paraben, triethanolamine and distilled water were dissolved in the aqueous phase and heated in the same water bath at temperature 70 ^{o}C.
3) The oil phase was melted up to 60 ^{o}C on a water bath followed by addition of extracts(Neem, Tulsi and Orange peel extract) and mixed well.
4) On other hand aqueous phase also heated up to the same temperature.
5) Oil phase was added gradually drop wise to the aqueous phase with continues stirring until the cooling of emulsifier take place,
6) It was allowed to stand for 15 minutes to cool down at room temperature.

Results and Discussion:

pH determination[14]

The pH was measured in each of the Cream of Polyherbal formulation, using a calibrated digital pH meter. All the formulations are required pH range suitable for absorption and no irritation and compatibility with the skin was observed.

Table No. 02: pH of prepared *Liquid Crystalline Cream Polyhedral Formulations*

Formulation code	*F1*	*F2*	*F3*	*F4*
1	**6.2**	**6.8**	**6.3**	**7.1**
2	**6.4**	**7.3**	**6.6**	**7.4**
3	**6.1**	**6.5**	**7.1**	**6.8**
Mean	**6.2**	**6.8**	**6.6**	**7.1**

The pH of the formulation was found to be Satisfactory in the range of 6 to 7.5.

Type of Smear [15,16]

It was determined by applying the cream on the skin surface of human volunteer. After application of cream, the type of film or smear formed on the skin were checked.

Table No.03: Types of Smear

Sr.no	Cream batches	Types of smear
1	F1	Non-greasy
2	F2	Non greasy
3	F3	Non-greasy
4	F4	Non-greasy

It was found that the cream produced non-greasy film on the skinsurface.

Spreadability [17]

The efficacy of topical therapy depends on the spreading ability of the formulation in an even layer to deliver a standard dose. The optimum consistency of such formulation helps to ensure that a suitable dose is applied or delivered to the target site. The delivery of the correct dose of the drug depends highly on the spreadability of the formulation so spreadability is directly proportional to efficacy. Based on this it was concluded that formulated cream was having more efficacy.

Fig.No. 01:Spreadability test

Table No. 04: Spreadability of cream

Formulation code	F1	F2	F3	F4	Marketed formulation
Spreadabillity (gm.cm/sec) 1	16.4	13.5	12.9	12.4	12.1
2	14.5	15.7	14.6	12.1	12.7
3	15.1	14.3	15.8	11.7	11.5
Means	15.3	14.5	14.4	12.06	**12.1**

The efficacy of topical therapy depends on the spreading ability of the formulation. The spreadability studies showed that all formulation has better spreadability when compared with the marketed cream. All formulation gave good spreadability. Among all F4 possess better value, and this F4 batch have comparable spreadability with marketed cream.

Viscosity:[18]

The measurement of viscosity of prepared creams were carried out with Brookfield Viscometer. The measurement was over speed setting of 100 rpm at 25°C using Brookfield viscometer. The values of each formulation are depicted in table 7.10

Table No.05: Viscosity Test

Formulation code	F1	F2	F3	F4
Readings 1	2900	3100	2400	2300
2	2300	2800	3200	2500
3	2500	2200	2900	2400
Means	2566	2700	2833	2400

The viscosity of the all 4 batches was taken by using spindle no. 62 and viscosity was found mention in the above table, in which F4 trail batch was optimized because it show the thixotropic and pseudoplastic flow

Drug Content: [19, 20]

Each formulation (1gm) was accurately weighed and transferred to 100 ml volumetric flask to which about 70 ml of methanol was added. After shaking, the volume was made up to 100 ml with methanol. The content was filtered through a suitable filter paper. 1ml filtrate was taken and suitable diluted and the drug content (extract) was estimate by using UV Visible spectrophotometer at 250nm for cream. Result shown in Table 7.7.

Table No. 06: Drug content

Formulation code	Drug content
F1	88.3
F2	94.1
F3	92.9
F4	95.7

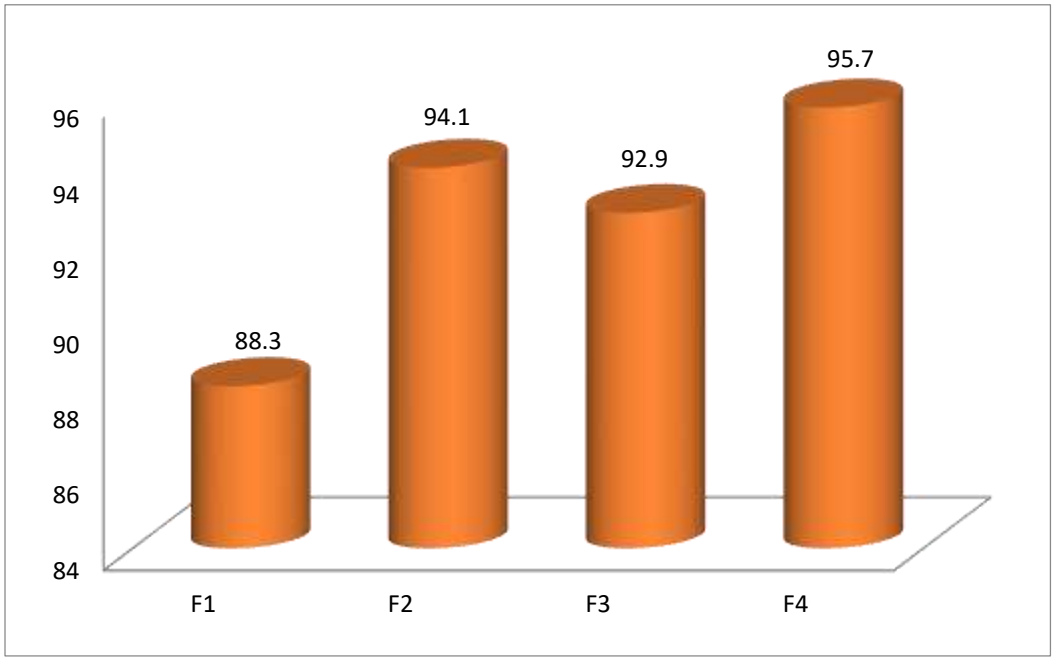

Fig. No. 02: Drug content

The percentage drug content of all prepared topical formulation was found to be in the range of 88-98%. Therefore uniformity of content was maintained in all formulation.

In-vitro Drug Release Study [21, 22]

In-vitro release study of the formulated in-situ cream was carried out by using diffusion cell through egg membrane as a biological membrane. Diffusion cell with inner diameter 1.4 cm was used for the study. The formulation 1 ml were placed in donor compartment and freshly prepared 100 ml pH 6.8 phosphate buffer in receptor compartment. Egg membranes were mounted in between donor and receptor compartment. The position of the donor compartment was adjusted so that egg membrane just touches the diffusion medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37 °C ± 0.5 °C. 1 ml of sample is withdrawn from receiver compartment after 5 min, 10 min, 15 min, 30 min, 45 min, 60 min and same volumetric flask with pH 6.8 buffer and analyzed by UV-Visible Spectrophotometer at 276 nm.

Table No. 07: *In-vitro* Drug Release

Time (in minutes)	F1	F2	F3	F4
0	0	0	0	0
5	30.235	33.273	31.186	35.653
10	36.964	47.392	42.736	48.237
15	44.657	56.382	59.295	60
30	61.649	67.732	69.218	72.632
45	83.653	79.554	78.629	84.383
60	91.653	85.352	89.542	90.432

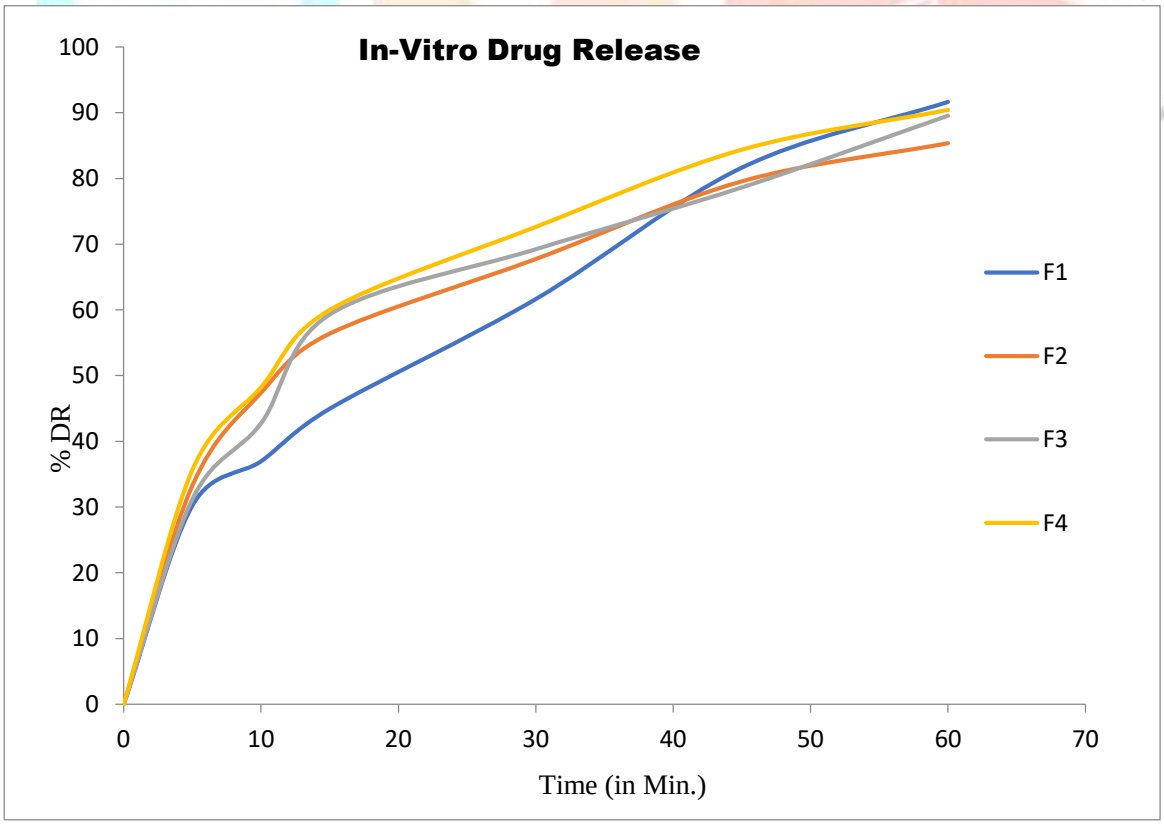

Fig. No. 03: In-Vitro Drug Release

Formulation F1, F2, F3 and F4 has almost 80 %was released in 45 min itself.[40,41]

Acne Healing Activity

In present studiesitwas determined by applying the cream on the skin surface of human volunteer. After application of cream, showed a better healing activity. The adults' skin treated with the F1, F2, F3& F4 formulation healed in 30 days compared where the healing occurred 26 and 30 days, respectively as shown in Figures

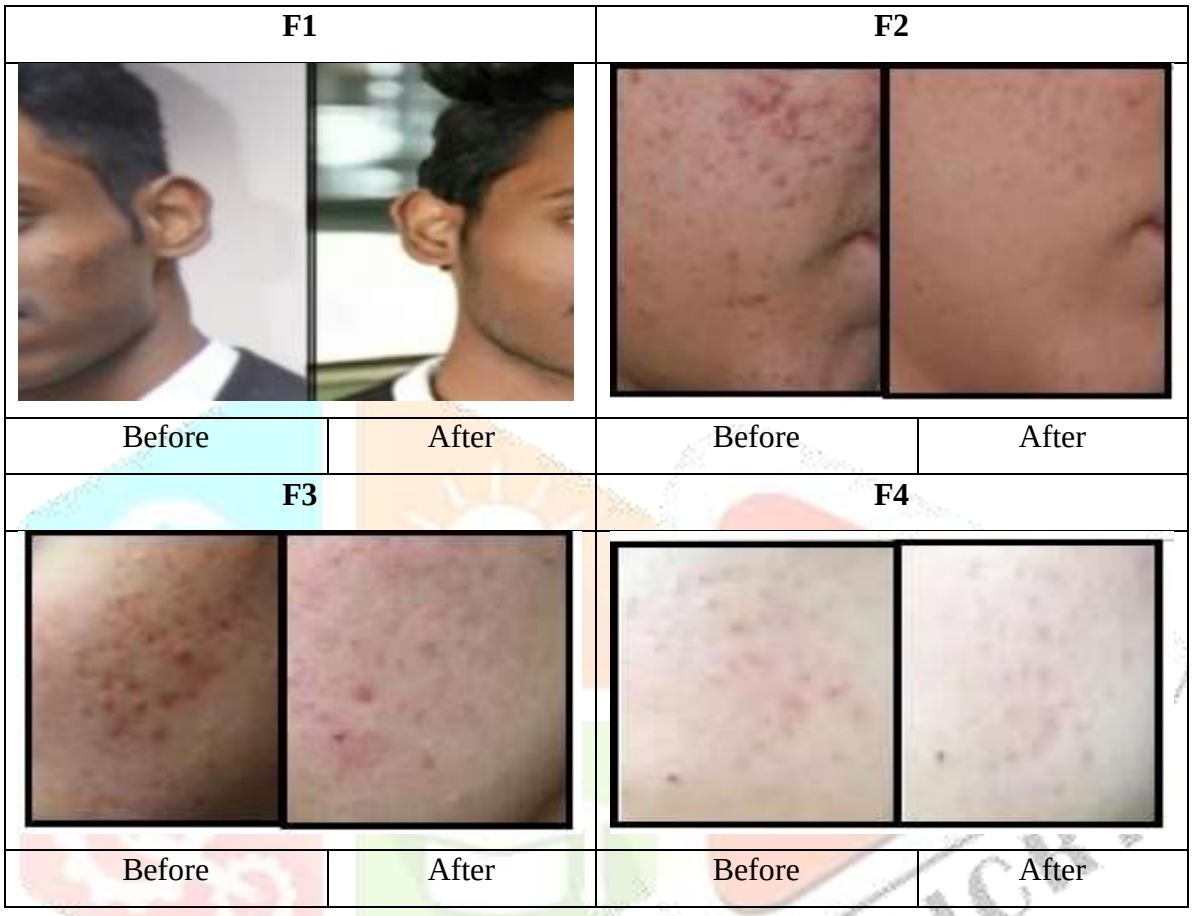

Fig. No. 04: Image of skin surface of human volunteer before the application of cream and after application of Cream

Conclusion:

it is concluded that on combining the extracts of Neem (Azadirachtaindica), Tulsi, Orange Peel in different ratio in formulation of cream to get reduces acne formation and also produce multipurpose effect such as Anti-acne, whitening, antiwrinkle, antiaging etc. we are maintaining its pharmacological properties while improving its tolerance to overcome its side effects and enhancing the efficacy of the existing drugs.

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Table legends

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