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FORMULATION EVALUATION AND VALIDATION OF ANTIACNE HERBAL PREPARATION

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ABSTRACT

Acne is the common skin problem that 85% of the teenagers face today which arise due to over production of oil in sebaceous gland that affect areas including face, back, and trunk. In this study, the poly herbal antiacne gel were prepared by using alcoholic extract of Citrus aurantium peel, Terminalia arjuna bark and Randia dumentorum fruit. gel with purely herbal actives as an effective and safe alternative to harmful antibiotics. The extraction process were carried out in petroleum ether by 60-80^{0}C using Soxhlet apparatus. The existence of flavonoids in the extract of three drugs was confirmed by performing preliminary phytochemical screening it give up lab test and purity of compound was assured by thin layer chromatography, and also spectroscopic analysis such as UV visible spectroscopy and FTIR. The gel were evaluated for various physical properties and skin irritation study. The in-vitro studies were carried out by using Franz diffusion cell and in-vivo skin permeation studies were tested on the rabbite dorsal skin using Franz diffusion cell. From this study, these formulations did not produce any skin irritation and Gel was proved to be stable and considered as an effective herbal formulation for acne treatment.

INTRODUCTION:

Sebum and skin cells and hair follicles bunch together to form a plug and when this plug gets infected by bacteria, the resulting swelling is called acne. Acne vulgaris is the common skin problem that 85 % of the teenagers face today that affecting largest oil glands, including the face, back, and trunk.(Leyden JJ. 1997) Propionibacterium acnes (P. acnes), an anaerobic pathogen, plays an important role in the pathogenesis of acne, which affecting the pilosebaceousfollicles. It arises from the interaction of four pathogenic factors: sebum production, follicular hyper-keratinization (Leyden JJ. 2001) and microbial colonization of the pilosebaceous unit by Propionibacterium acnes, and the release of inflammatory mediators into the follicle and surrounding dermis. (Gollinck et al. 1991)Although not a serious peril to general health, acne is one of the most socially distressing skin conditions, especially for adolescents (Leyden JJ.1995) who must deal with a disfiguring disease that erupts just when sexual maturity makes them most sensitive about their appearance. Additionally, severe acne can lead to permanent scarring of the skin that carries the social distress throughout adulthood. (Degroot H.E.1998)For many years, antibiotics have been used to treat acne vulgaris. However, antibiotic resistance has

been increase to overcome the problem of antibiotic resistance medicinal plants have been studied as alternative treatments for diseases. (plewig G. et al. 1998). The bark of plant *Terminalia Arjuna* belonging to family combretaceae used as astringent, wound healing, cardiac stimulant, heamoptysis, lithontriptic and also useful in bilious infections, diarrhoea and in acne. (Swanson I.K.2003) Citrus family group widely contain metabolites such as flavonoids, ascorbic acid, and cortenpoides. (Nadkamis K.M.2002) Randia dumetorum passes therapeutic properties like antipyretic, anti-inflammatory, anti-allergic,immune-modulatory, analgesic, wound healing. It is also used in treatment of Kushtha(skin diseases), Shoth (inflammation). (Sinchir W.B. 1984) the present studies, topical formulations (Gel) have been developed of above three plants which have been reported for their antimicrobial and antioxidant activity. The developed formulations were examined for Ant acne activity.

MATERIALS AND METHOD

Plant Material:

Terminalia Arjuna bark, *Citrus aurantium* peel and *Randia dumetorum* fruit. The plants were purchased from the local market of Nagpur district. The plant specimens were dried and their herbarium sheets were prepared and as shown in figure 1, 2, 3.

Preparation of extract: The powdered plant materials were defatted with petroleum ether and then subjected to Soxhlet extraction till discoloration to obtain ethanolic extracts of *Terminalia arjuna* bark, *Citrus aurantium* peel and *Randia dumetorum* fruit alternatively. The extracts thus obtained were filtered, concentrated on water bath to a thick paste and dried under vacuum.

Method

Procedure: 1g of carbopol 934 was dispersed in 50 ml of distilled water with continuous stirring. The essential quantity of methyl paraben and propyl paraben were dissolved in 5 ml distilled water by heating on water bath. The solution was cooled, then propylene glycol 400 and polyethylene glycol 200 were added as shown in table 1.

Further required quantity of fractioned extracts of Terminalia Arjuna, Randia dumetorum, citrus aurantium shown in table 2 were mixed to the above mixture and volume was made up to 100 ml by adding remaining distilled water. Finally all ingredients were mixed properly to the carbopol 934 gel with continuous stirring and triethanolamin was added drop wise to formulation for adjustment of required skin pH (6.8-7) and to obtain the gel of required consistency. The same method was followed for preparation of control sample without adding any extract.

Experimental work:

Preliminary phytochemical screening:

The preliminary phytochemical screening of isolated compound of *Terminalia Arjuna, Citrus aurantium*and, *Randia dumetorum* was carried out for the detection of major Phytoconstituents such as glycosides, alkaloids, flavonoids, phenols, steroids and result are disputed in table 3:

Thin layer chromatography:

Chromatographic pattern of active extract of *Terminalia Arjuna, Citrus aurantium, Randia dumetorum*was studied by thin layer chromatography. For applying test samples on TLC plates, the spots were applied near one end of the plate about 1.5-2.0 cm from the edge of the plate and air dried. The spots of the samples were marked on the top of the plate to know their identity. The development of plates started immediately after the spots were dried. The spot wheresequence visualized by UV light (UV chamber), iodine vapours and spraying reagent $Fecl_3$ and H_2SO_4 the RF values of the spots were calculated and result disputed in table: 4

Solubility of isolated compounds of TA, CA and RD

Solubility of isolated compounds of *Terminalia arjuna*bark, *Citrus aurantium*peeland *Randia dumetorum*was determined in distilled water, methanol, ethanol and DMSO respectively.

Solubility studies were performed by taking excess amount of API in different apparatus containing the solvents. The mixture was shaken at regular intervals. The solutions were filtered and analyses spectrophotometrically at 271, 272 and 277.2nm

UV visible spectral analysis:

A fractionated extract solution of *terminalia Arjuna*, *citrus aurantium* and *Randia dumetorum* scanned in the range of 200 to 600 nm. The fractionated extract exhibited the λmax at 274 nm, 266 nm, 269 nm, and showed reproducibility. From the standard cure of TA, CA and RD in phosphate buffer pH 7.4 it was observed that the TA, CA and RD obeys beers-lambert's law in the range 20-100µg/ml in the medium as shown in the fig: 4, 5, 6.

Fourier transforms infrared spectroscopy (FTIR)

A comparative FT-IR spectrum analysis of isolated compounds of *Terminali aarjuna* bark, and *Randia dumetorum*was performed to observe any promising interaction at molecular level in samples using Fourier transform infrared spectrophotometer (Model: FTIR-8300, Shimadzu, Kyoto, Japan). Result are shown in fig: 7, 8.

Formulation of gel

The gel were prepared by using Carbopol 934, Tween 20, Propylene glycol, Methyl paraben, Propyl paraben and combination of three fractionated extract of TA, CA and RD. The prepared gels were characterized for various physicochemical parameters such as physical appearance, pH, viscosity, drug content, Spreadability, In-vitro release and exract permeation study.

Evaluation of gel: These topical formulation were tested for colour, appearance, viscosity, pH, Spreadability, stability, drug content and in vitro diffusion and result are disputed in table 5:

In-Vitro **Drug Release Study:** *In-vitro* diffusion study was carried out in a Modified Franz diffusion cell using cellophane membrane which is heated for 1 hr. in boiling water. The membrane was tied to the donor compartment and mounted on the reservoir compartment of Franz diffusion cell containing 21 ml of pH 7.4 phosphate *buffer*. 1g of gel was placed over the cellophane membrane of donor compartment. Whole set was placed on the magnetic stirrer. The study was carried out at 37± 0.5^0C and 100 rpm. Samples were withdrawn from the sampling port of reservoir compartment at regular intervals and absorbance was measured using Jasco 200 to 600nm UV visible spectrophotometer at 274nm, 266nm and 269nm for TA, CA and RD respectively. Results are given in fig. 9, 10, and 11.

In-Vivo **Skin Permeation Study:** The skin permeation study was carried out with albino rats and weighing about 150-200 gm. By using modified Franz diffusion cell by the same method as described above in the in-vivo drug releases study of gel the skin was carefully checked through a magnifying glass to ensure that the sample was free from any surface irregularity such as tiny holes or crevices in the portion that was used for the permeation studies. The ability of gel to help retain the drug within the skin (i.e. depot-effect) was investigated by determining the amount of drug retained in the skin sample employed in permeation studied. For this, skin from the donor compartment pipette out and dissolved in phosphate buffer. Absorbance was measured by UV spectrophotometer to determine amount of drug retained and remaining to diffuse and result shown in fig.12

In-vitro **release of f2 formulation**

In-vitro Diffusion study of Polyherbal fractionated extract containing gel (F2). The drug release characteristics of the formulation were studied in-vitro conditions by usng artificial semipermiable membrane. The drug release from gel has shown in increasing release of about TA 70.30±2.60, CA 80.70±2.43 and RD 85.48±2.45 at 5hr20min.

Skin irritation study: The skin irritation test was performed on healthy rabbit the animals were kept within limited access facility with environmental condition set to a temp of 25±2ºC, humidity of 60-90% RH.

Figure 1. The specimen voucher no. of *terminalia Arjuna* is 10290

Figure 2. The specimen voucher of *citrus aurantium* 10210

Figure 3.The specimen voucher of *randia dumetorum* is 10243
It was authenticate from the Department of Botany, RTM Nagpur University.

Sr.no.	Contents	quantity
1	Carbopol 934	1.0gm
2	Methyl parabean	0.15
3	Propyl parabean	0.03
4	Propylene glycol	5.0
5	Polyethylene glycol	15.0
6	Distilled water	Up to 100 ml

Table 1.Formulation of gel

S. no.	Plant Extract	Concentration of Extract100gm
1	*Terminalia arjuna*	0.1
2	*Citrus aurantium*	0.1
3	*Randia dumentorum*	0.2

Table 2.plant extract

Test for Phytoconstituent	**Plant Extract**		
	TA	**CA**	**RD**
Phenol	_	_	_
Tannins	_	_	_
Steroids	+	+	+
Glycosides	_	_	_
Flavonoids	+	+	+

Table 3. Phytochemical screening of extract

F. Extract	**Solvent system**	**No. of spots**	**Distance travelled by solute**	**Distance travelled by solvent front**	**RF value**
TA	Ethyl acetate: Acetone 6:4	1	3.9	5.4	0.72
CA	Ethyl acetate: Acetone 6:4	1	4.5	5.5	0.81
RD	Butanol : Acetic acid: Water 4:2:2	1	4.7	6.5	0.72

Table 4.Thin layer chromatography of fractionated extract of TA, CD and RD

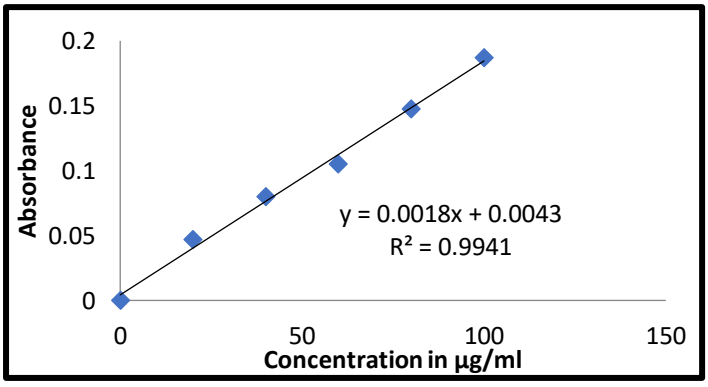


Figure 4. Calibration curve of fractionated extract of TA in phosphate buffer pH 7

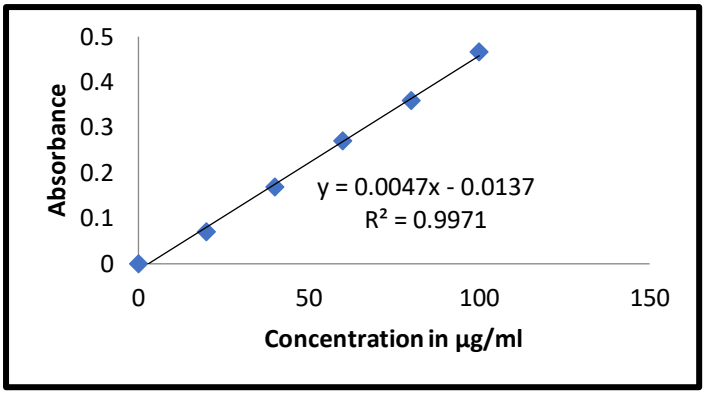


Figure 5. Calibration curve of Fractionated extract of CA in phosphate buffer pH 7.4

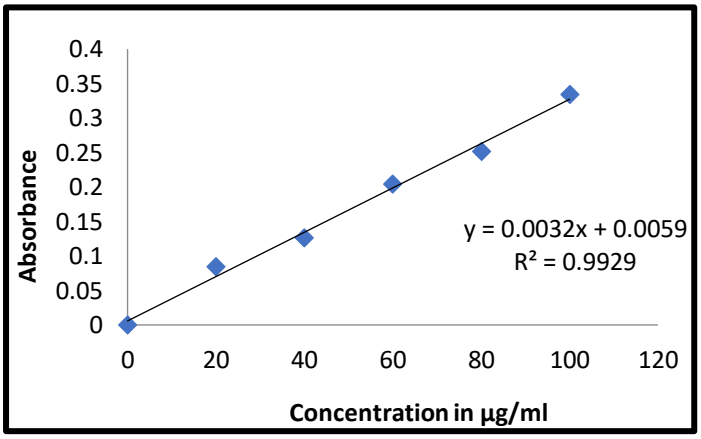


Figure 6. Calibration curve of Fractionated extract of RD in phosphate buffer pH 7.4

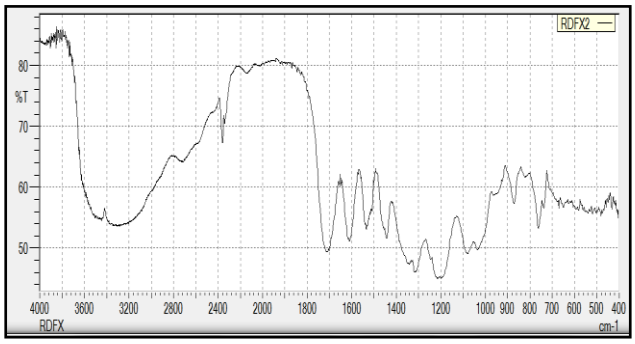


Figure 7. FTIR Spectrum of *randia dumetorum*

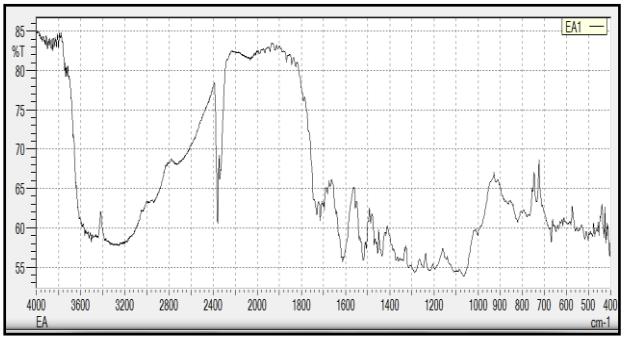


Figure 8. FTIR Spectrum of *Terminalia Arjuna*

Ingredients	Formulation code (%w/v)			
	F1	F2	F3	F4
TA	0.1	0.1	0.1	0.1
CA	0.1	0.1	0.1	0.1
RD	0.2	0.2	0.2	0.2
Carbopol 934	0.5	1	1.5	2
Tween 20	7.5	7.5	7.5	7.5
Propylene glycol	10	10	10	10
Methyl paraben	0.1	0.1	0.1	0.1
Distilled water	Upto 100	Upto 100	Upto 100	Upto 100

Table no. 4 Formulation of gel

Evaluation properties	Gel			
	Formula 1	Formula 2	Formula 3	Formula 4
Colour	Brown	Brown	Brown	Brown
Clarity	Clear	Clear	Clear	Clear
pH	6.6	6.8	6.9	6.8
Homogeneity	No lumps	No lumps	No lumps	No lumps
Skin Irritation	No	No	No	No
Spreadability (g.cm/sec)	9.37	6.81	7.35	5.35
Viscosity (cp)	5904	5842	5954	5986

Table 5 Evaluation of prepared gel

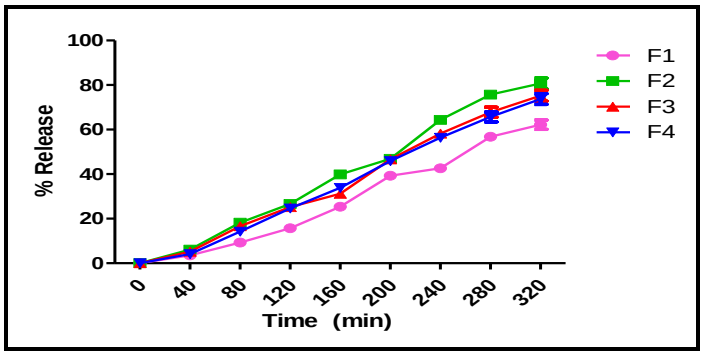


Figure 9. *In-vitro* release of *Terminalia Arjuna*

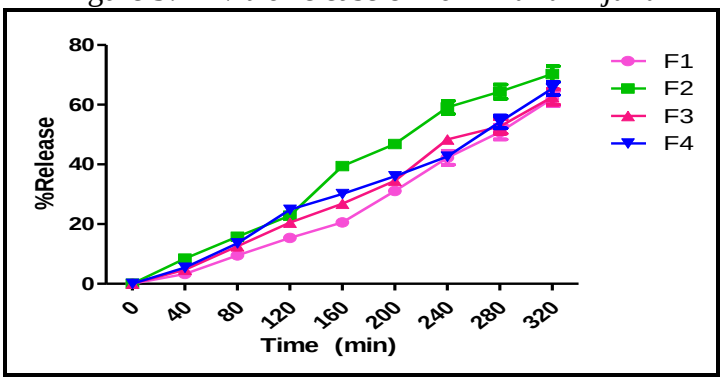


Figure 10. *In vitro* release of *Citrus aurantium*

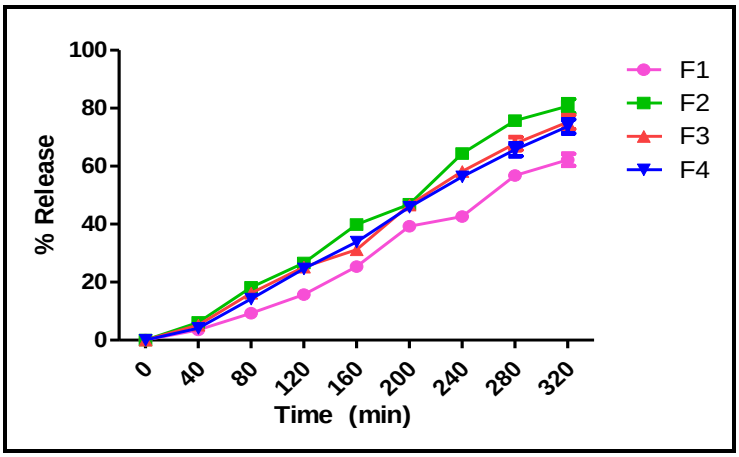


Figure 11. *In vitro* release of *Randia dumetorum*

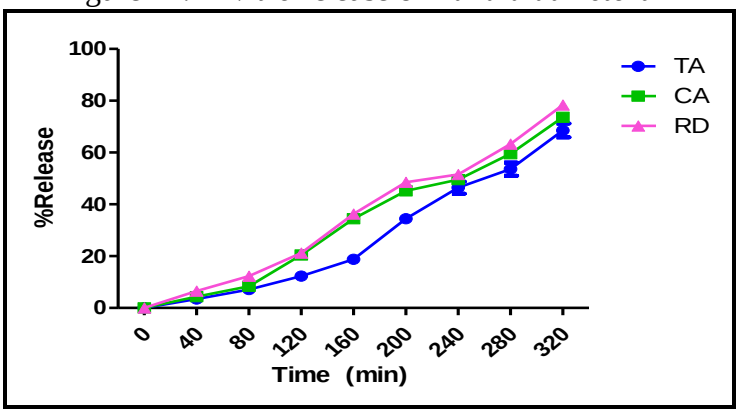


Figure 12: Graph for % Release of Polyherbal Fractionated Extract Containing gel

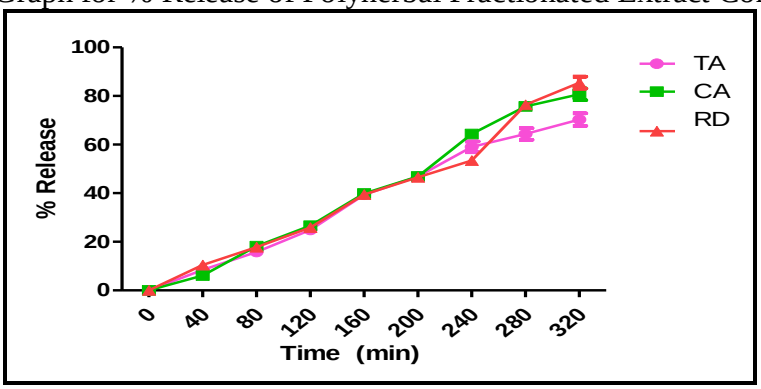
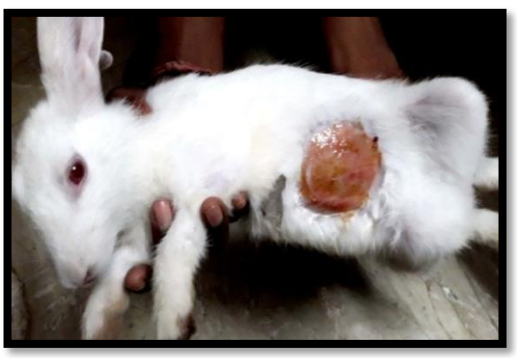


Fig 13 Graph for % *In-vitro* Release of Polyherbal Fractionated Extract Containing gel

Skin Irritation studies



Parameters	STORAGE TEMPERATURE			
	40 ± 2 ºC and 75 ± 5 % RH			
	Initial	10 Days	20 Days	30 Days
Colour	Brown	NC	NC	NC
Odour	Characteristic	NC	NC	NC
Homogeneity	Homogeneous	NC	NC	NC
pH	6.80	6.61	6.77	6.35
Viscosity (cp)	1180	967	1041	932
Spreadability (g.cm/sec)	6.81	7.50	6.81	6.25
Skin irritation	No	No	No	No

Table 6: Stability data of the gel

Source of variation	Sum of squares	Degree of freedom	Mean of squares	F ratio	F crit value
Between Batches	0.04223	3	0.01408	1.265	3.587
Within Batches	0.08900	8	0.01113		
Total	0.1312	11			

ANOVA for Viscosity

Source of variation	Sum of squares	Degree of freedom	Mean of squares	F ratio	F crit value
Between Batches	8571	3	2857	0.4576	3.587
Within Batches	49940	8	6243		
Total	58511	11			

ANOVA FOR VISCOSITY

Source of variation	Sum of squares	Degree of freedom	Mean of squares	F ratio	F crit value
Between Batches	8571	3	2856.8	0.4560	3.587
Within Batches	50118	8	6264.7		
Total	58689	11			

ANOVA for spread ability

Source of variation	Sum of squares	Degree of freedom	Mean of squares	F ratio	F crit value
Between Batches	4.049	3	1.350	1.737	3.587
Within Batches	6.215	8	0.7768		
Total	10.263	11			

The area of back of rabbit was shaved prior to the experiment. Gel was applied to shaved area of approximately 6cm^2 of skin. Treated site of rabbit were covered by gauze and the back of rabbit was wrapped with a non-occlusive bandage, the animal then turn to thei rcage. After 24 hr. the bandage and the test animal were removed and 1 hr. later site were examined for skin irritation. The result are given in fig: 13, 14.

Stability study:

The stability study was performed as per ICH guidelines. The formulated gels were filled in the collapsible tubes and stored at fixed temperature and humidity condition, viz. 40oC ± 2oC/ 75% ± 5% RH for a period of three months and studied for appearance, pH, viscosity and Spreadability. Results are depicted in table: 6

Validation of the optimized model

Formulation of batches of anti-acne Gel was prepared in order to validate the One-way ANOVA using the optimized value of the variables and performed all above evaluation for the validation of hypothesis value

RESULT AND DISCUSSION:

Preliminary phytochemical screening

The following phytoconstituents were found to present in the ethanolic extracts of *Terminalia Arjuna, Citrus aurantium, Randia dumetorum* respectively.

Thin Layer Chromatography Study: Spots were detected using UV light (UV Chamber) and spraying reagent $Fecl_3$ and (50%)H_2SO_4

From the results of thin layer chromatography (TLC) Mobile phase with Ethyl acetate: Acetone (6:4) and Butanol: Acetic acid: Water (4:2:2) showed the maximum resolution andreproductive results and it was observed that the spots matched with the results of phytochemical screening.

UV-Visible Spectrophotometric Analysis: the maximum absorption value of pure drugs, *Terminalia Arjuna,* was observed at 274 nm, citrus aurantium was observed at 266nm and *Randia dumetorum* was observed at 269nm wavelength in phosphate buffer pH 7.4 therefore 274 nm 266 nm and 269 nm was recorded as λmax of the pure drug TA, CA, RD. the observed λmax value of drugs was found to be complicated with the speciphication0 of indian pharmacopoeia.

Fourier Transform Infrared Radiation (FTIR)

All the prominent and primary peaks were observed in FTIR spectrum of *Terminalia Arjuna, Citrus aurantium* and *Randia dumetorum.* The FTIR spectrum of fractionated extract TA (Fig 7) was carried out. The FTIR spectrum of drug shows prominent peak at 3400cm^{-1} for OH stretching of phenol, 1620cm^{-1} for C=C stretching of variable, 2350cm^{-1}for O=C=O stretching of flavonoids carbonyl group, 1440cm^{-1}for C-H Bending of variable All the prominent and primary peaks were observed in FTIR spectrum of drug which confirms the pure drug. The FTIR spectrum of fractionated extract RD (Fig 8) was carried out. The FTIR spectrum of drug shows prominent peak at 3400cm^{-1} for OH stretching of phenol, 1620cm^{-1} for C=C stretching of variable, 2350cm^{-1}for O=C=O stretching of flavonoids carbonyl group, 1440cm^{-1}for C-H Bending of variable All the prominent and primary peaks

were observed in FTIR spectrum of drug which confirms the pure drug.

IN-VITRO DRUG RELEASE STUDY:

Determination of in vitro release of the Polyhedral Fractionated Extract Containing Gel of TA, CA, RD was separately evaluated and at the end of 5 hr 20 min it was found that the percent release of F2 is greater in all the three extract i.e. TA 70.30±2.60, CA 80.70±2.43, RD 85.48±2.45 respectively. From the above observation no irritancy of skin was found, no oedema and no erythema was observed up to 72hr.

Validation

Validation of Gel: The parameters studied were

➢pH

➢ Viscosity

➢ Spreadability

In order to reveal reproducibility of process optimized batch of selected formulations were prepared. The data obtained was subjected to ANOVA.

pH

ANOVA for pH

Hypothesis (H_0)

F ratio is 1.265 since the calculated F ratio was less than table F value (1.265<3.587), accept hypothesis that there is no significant difference between the pH values of the different batches at 5% level of significance.

Viscosity

(At 100 rpm)

Hypothesis (H_0)

F ratio is 0.4576 since the calculated F ratio was less than table F value (3.587<0.4576), accept hypothesis that there is no significant difference between the viscosity values of the different batches at 5% level of significance.

(At 50 rpm)

Hypothesis (H_0)

F ratio is 0.4560 since the calculated F ratio was less than table F value (0.4560<3.587), accept hypothesis that there is no significant difference between the viscosity values of the different batches at 5% level of significance.

Spreadability

Hypothesis (H_0)

F ratio is 1.737 since the calculated F ratio was less than table F value (1.737<3.587), accept hypothesis that there is no significant difference between the Spreadability values of the different batches at 5% level of significance.

Stability studies

Stability may be defined as the capability of a particular formulation, in a specific container to remain within its physical, chemical, microbiological and toxicological specifications. Stability tests were carried out for 30 days. The conditions were 40 ± 2ºc and 75 ± 5 % RH. The formulation was analyzed for the change in physical properties like colour, odour, homogeneity, pH, viscosity, Spreadability and skin irritation. The results of stability study of the Polyherbal fractionated extract containing gel showed that at the temperature 45ºC and 75% RH, the appearance of the gel did not change. The colour, odour, homogeneity, pH, viscosity, Spreadability and skin irritation did not show significant change at 45ºC. Hence it can be said that the gel has good stability

CONCLUSION

In the present study, the attempt was made to formulate, evaluate and validate a gel using the fractionated alcoholic extracts of TA, CA and RD for their synergistic activity in anti-acne treatment. The flavonoids present in the fractionated extracts of TA, CA and RD can be responsible for the activity against acne. From the results, it is clear that the developed gel is synergistically effective in the treatment of acne.

So in future, attempt should be made to isolate the active constituents from the extracts and incorporate them in a better developed formulation and its development can lead to a new potent formulation in the treatment of acne.

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