DEVELOPMENT OF INVASOMAL GEL FOR THE TREATMENT OF ACNE VULGARIS

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INTRODUCTION

Acne vulgaris (commonly called acne) is the most prevalent skin complication of different causes with a higher prevalence in adolescents. Acne is a chronic inflammatory disease characterized by skin with scaly red skin (seborrhea), blackheads and whiteheads (comedones), pinheads (papules), large papules (nodules), pimples and scarring. It may be inflammatory and non-inflammatory form. The changes in pilosebaceous unit results in androgen-induced increased sebum production, altered keratinisation and bacterial colonisation of hair follicles on the face, neck, chest and back by *Propionibacterium acnes*.

A.TYPES OF ACNE

1) **Scarring:** refers to a fibrous process in which new collagen is laid down to heal a full-thickness injury. It affects 30% ofthose with moderate or severe acne vulgaris. It is particularly common in nodulocystic acne, acne conglobata and acnefulminans.
2) **Acne rosacea:** Persistent redness in the face in which small blood vessels on nose and cheeks often swell and becomevisible. Swollen, red bumps may sometimes contain pus.
3) **Chloracne:** Eruption of blackheads (open plugged pores), cysts, and pustules (pimples) associated with exposure to certain halogenated aromatic compounds.

B.CAUSES

The main cause of acne is not well known. There are numerous related factors due to:

- Hereditary
- Hormonal activity
- Bacteria
- Inflammation

MATERIAL AND METHODS: MATERIAL

Sr. No.	Excipients	Manufacturer
1.	Phospholipon 90 G	Lipoid, GmH Germany.
2.	Citral	SRL Fine Chemicals Ltd., Mumbai
3.	Sodium Hydroxide	Loba Chemicals, Mumbai
4.	Potassium dihydrogen Orthophosphate	Loba Chemicals, Mumbai
5.	Carbopol 934 P	Loba Chemicals, Mumbai
6.	Ethanol	Loba Chemicals, Mumbai
7.	Potassium Chloride	SRL Fine Chemicals Ltd, Mumbai
8.	2,2-diphenyl-1-picrylhydrazyl(DPPH)	Sigma Aldrich
9.	Methanol	Sisco Research Laboratory Pvt. Ltd., Mumbai
10.	Gallic acid	Sigma Aldrich
11.	Foiln's Coicalteau Reagent	Hi Media
12.	Propylene alcohol	Sisco Research Laboratory Pvt. Ltd., Mumbai
13.	PEG 400	S.D. Fine Chemicals Ltd. Mumbai

14.	Quercetin	Sigma Aldrich
15.	Nutrient Agar	Hi Media
16.	Dialysis membrane	Hi Media
17.	L-ascorbic acid	S.D. Fine Chemicals Ltd. Mumbai

LIST OF EQUIPMENTS :

Sr.No.	Instruments	Name of manufacturer
1.	Electronic weighing balance	Shimadzu, Japan
2.	Digital pH meter	Elico Pvt. Ltd India
3.	Water Purification System	Bio-Age Equipment & Services, Mohali, Punjab
4.	Bath Sonicator	PCI analyticals Pvt. Ltd.,India
5.	Vortex Mixer	Impact, Icon Instruments Company, India
6.	Brookfield Viscometer	Viscolead-one, Fungilab
7.	UV Visible Spectrophotometer	UV-1800 Shimadzu, Japan
8.	Rotary Evaporator	B-100, Buchi
9.	FTIR Spectrophotometer	IR Affinity-1S, Shimadzu, Japan
10.	X-ray diffractometer	Brucker AXS D8 Advance, Germany
11.	Diffrential Scanning Calorimetry	Mettler DSC, Mettler Toledo, Switzerland
12.	Zetasizer (Particle Size Analyzer)	Malvern zeta sizer 2000, Malvern
13.	Probe Sonicator	Sonics Vibra Cell™, USA
14.	Ultracentrifuge	Remi C24 Plus, Electrotechnik Limited,VasaiMumbai
15.	Franz Diffusion assembly	Orchid Scientific, Nashik
16.	HPLC	Agilent technologies 1100
17.	FE-SEM	JSM-7610F FESEM
18.	Homogenizer	IKA Ultra turrex T18, Germany

Experimental studies Pre-formulation Study
Extraction of drug and its identification

Procurement and Collection:
The bathua leaves have been procured and collected from the local vendor. The leaves were washed thoroughly and kept for drying. The dried leaves were coarsely powdered and stored in an air tight container.

Preparation of extract:
About 500 grams of plant leaves were grounded and extracted with methanol in a Soxhlet apparatus. Extraction cycle was run at 40-50^{0}C until it gets colourless. Then the methanol was evaporated by rotary evaporator to obtained semisolid extract. The semisolid extract was deep freezed overnight and it was lyophilized to get powder form.

Fig.6.1. a) Soxhlet assembly during drug extraction b) Organic solvent evaporated using rotavapour.

Percent Extract Yield
The yield of dried extracts based on their dry weights was calculated using the following:

$$\frac{W1}{W2} \times 100$$

Where, W1= Weight of extract after solvent evaporationW2= Weight of the dry plant material

HPLC analysis of herbal extract

The individual chromatograms of kaemferol and methanolic extract of CA were recorded. The sample of methanolic extract was injected in the same solvent system to determine the concentration of kaemferol present in the extract.

Quantification of quercetin
The individual chromatograms of quercetin and methanolic extract of CA were recorded. The sample of methanolic extract was injected in the same solvent system to determine the concentration of quercetin present in the extract.

Table no.6.1. HPLC method for kaemferol

Sr.no	Standard	Kaemferol
1.	Column	C18
2.	Mobile phase	Methanol: formic acid (0.1% in water)(75: 25 v/v)
3.	Detection Wavelength	368 nm
4.	Injection volume	20 µl
5.	Flow rate	1.0 ml/min
6.	Temperature	35° C

Table no.6.2. HPLC method of quercetin

Sr.no	Standard	Quercetin
1.	Column	C18
2.	Mobile phase	Methanol: orthophosphoric acid(0.40%) (49:51 v/v)
3.	Detection Wavelength	280 nm
4.	Injection volume	20 µl
5.	Flow rate	1.0 ml/min
6.	Temperature	25° C

RESULTS AND DISCUSSION

7.1. Pre-formulation Study

7.1.1. Authentication of drug

The aerial parts were collected from local vendor in the month of December, authenticated by Botanist. A voucher specimen has been deposited in the Herbarium of Department of Botany, with collection number 10419 was identified as *Chenopodium album* L. (Family-Chenopodiaceae) .

Fig.7.1.1. Authentication of leaves *Chenopodium album* (C A)

Table no.7.1. % Yield of methanolic extract of CA

W1	Weight of plant extract after solvent evaporation	7.75 gm
W2	Weight of the dry plant material	500 gm

The percent yield of methanolic extract of *Chenopodium album* (CAME) was found to be 1.51%.

7.1.2. Solubility Studies

Table no.7.2. Solubility Studies of CA

Solvent	Solubility
Methanol	Soluble
Water	Soluble
Ethanol	Slightly soluble

7.1.3. Phytochemical Screening of Extract

Test		Observation
Carbohydrate	**Molisch's Test**	+++
	Fehling's Test	----
	Benedict's Test	+++
Glycosides	**Libermann Burchard Test**	+++
	Borntragger Test	----
Alkaloids	**Dragendorff's Test**	+++
	Mayer's Test	----
	Hager's Test	+++
	Wagner's Test	----
Flavonoids	**Shinoda Test**	----
	Lead acetate Test	+++
Saponins	**Foam Test**	+++

7.1.4. UV Spectrophotometric analysis of drug

I. λmax determination in water

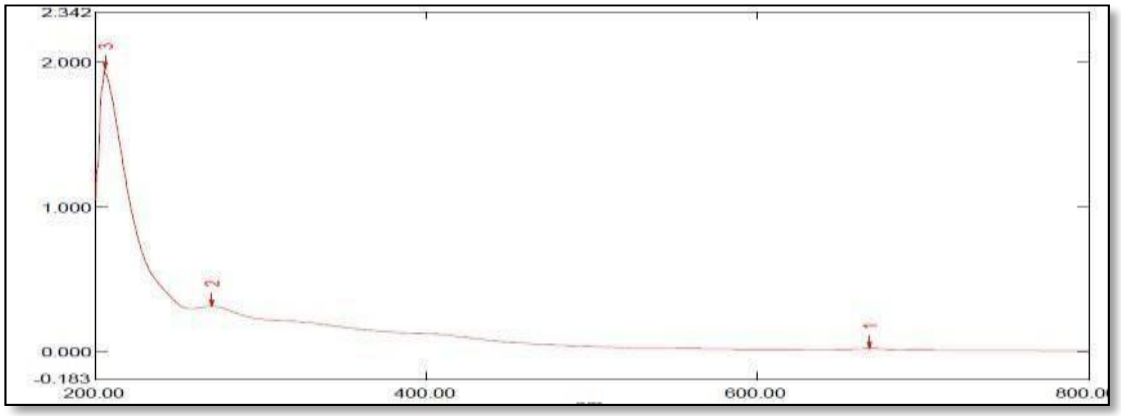


Fig.7.2. UV spectrum of CA in water

Table no.7.3. Standard calibration in distilled water of CA at 204 nm

Sr. no.	Concentration (µg/ml)	Absorbance
1.	0	0
2.	10	0.213

3.	20	0.355
4.	30	0.581
5.	40	0.753
6.	50	0.861
7.	60	1.023

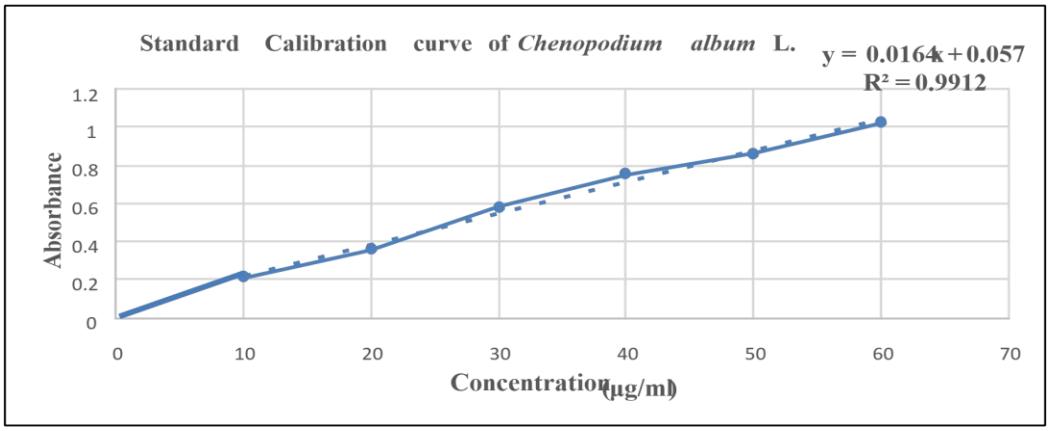


Fig.7.3. Standard calibration curve of CA in distilled water

II. λmax determinationin methanol

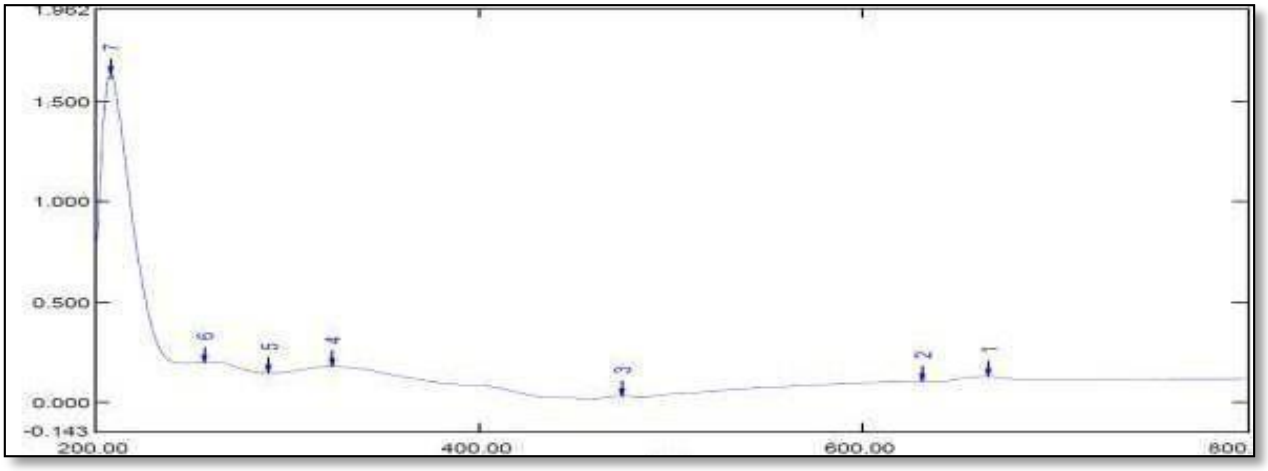


Fig.7.4. UV spectrum of CA in methanol

Table no.7.4. Standard calibration in methanol of CA at 207 nm.

Sr. no.	Concentration (µg/ml)	Absorbance
1.	0	0
2.	10	0.092
3.	20	0.205
4.	30	0.254
5.	40	0.559
6.	50	0.784

7.	60	0.981

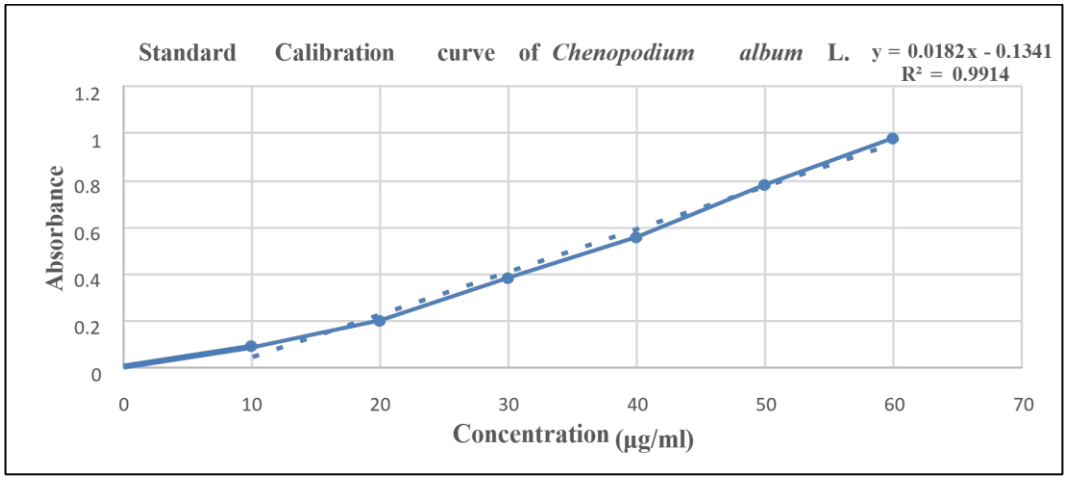


Fig.7.5. Standard calibration curve of CA in methanol

III. λmax determination in phosphate buffer (PBS) (pH 7.4)

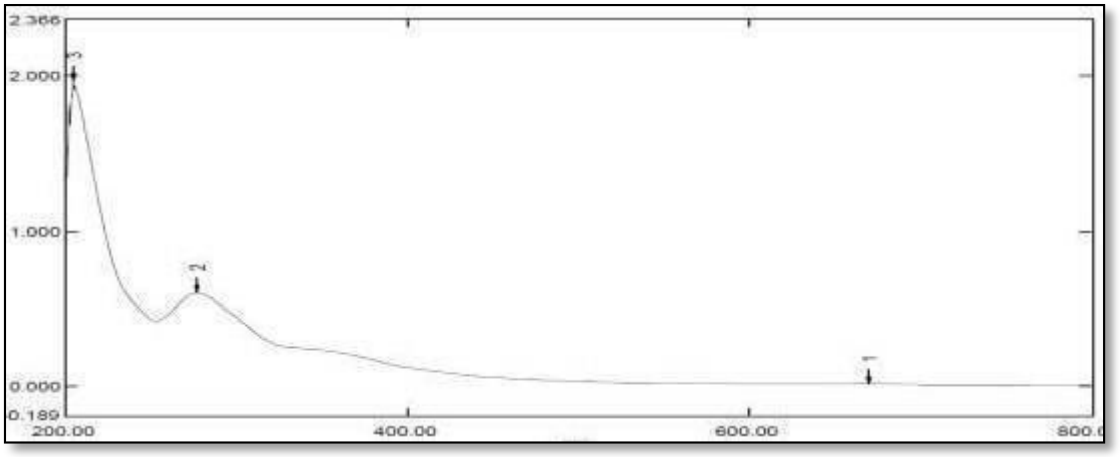


Fig.7.6. UV spectrum of CA in pH 7.4 buffer

Table no.7.5. Standard calibration in PBS of CA at 204 nm

Sr. no.	Concentration (µg/ml)	Absorbance
1.	0	0
2.	10	0.152
3.	20	0.290
4.	30	0.645
5.	40	0.664
6.	50	0.924
7.	60	1.077

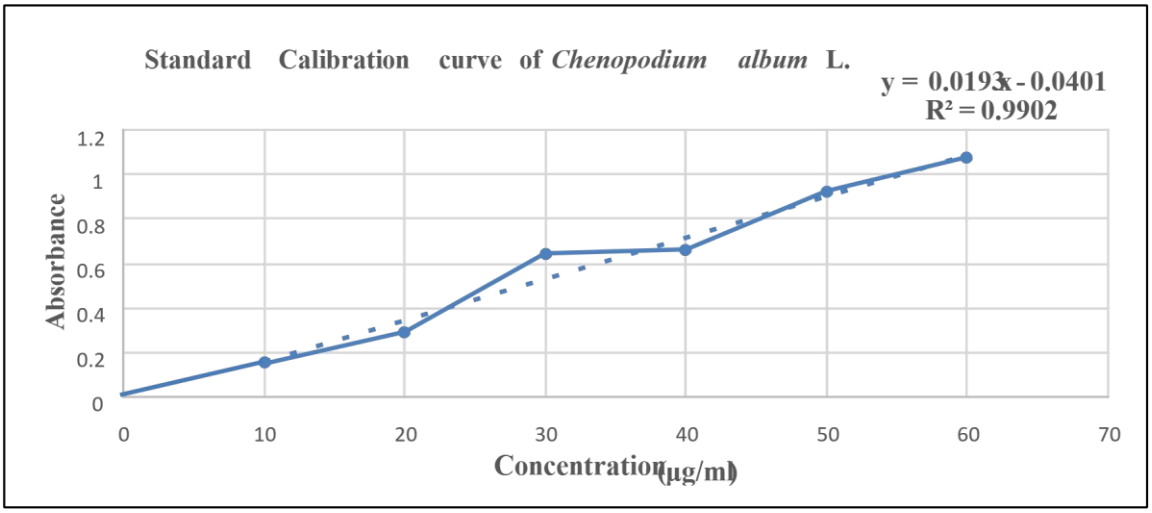


Fig.7.8. Standard calibration curve of CA in PBS (7.4)

Table no 7.6. λ maxof drug in different solvent

Sr. No.	Solvent	λ max (nm)
1.	Methanol	207
2.	Distilled water	204
3.	Phosphate buffer pH 7.4	204

Depending on the above absorbance of drug at 204 nm, 207 nm and 204 nm; the calibration curve in distilled water, methanol and PBS respectively. The plotted graph shows that it follows Beer-Lambert's law with regression value (R^2) of 0.9912, 0.9914and 0.9902.

7.1.5. HPLC of Extract

HPLC analysis revealed the presence of quercetin and kaemferol in CAME. The chromatograms of standard kaemferol and quercetin are depicted in Fig.7.8 and 7.10. The chromatograms of CAME solution depicted in Fig.7.9 and 7.11 shows the peaks due to presence of kaemferol and quercetin, respectively. The amount of quercetin and kaemferol was found to be 0.0031% and 0.193% w/w, respectively.

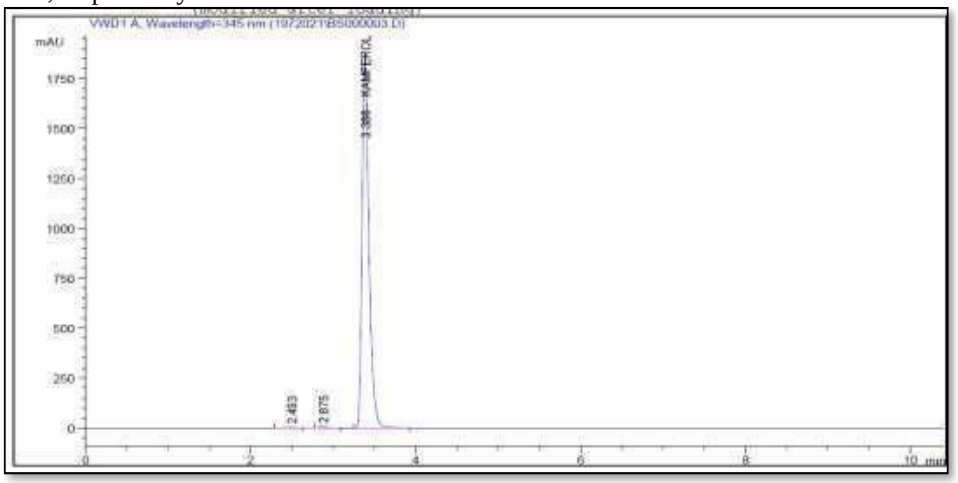


Fig.7.8. Chromatogram of kamferol

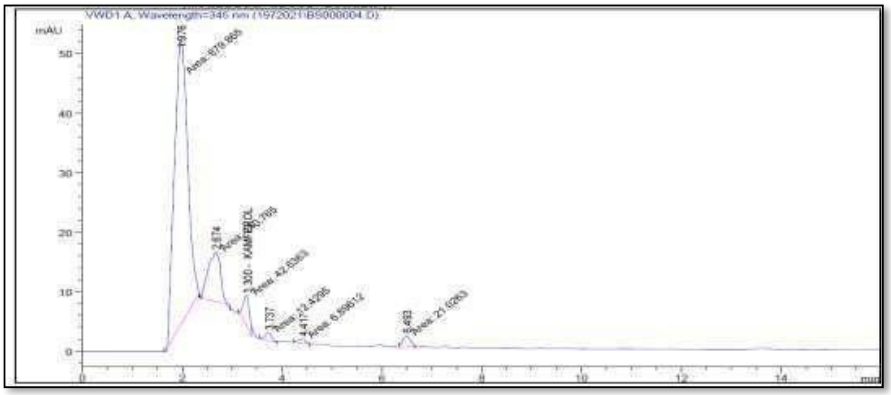


Fig.7.9. Qualitative estimation of kamferol in the CAME

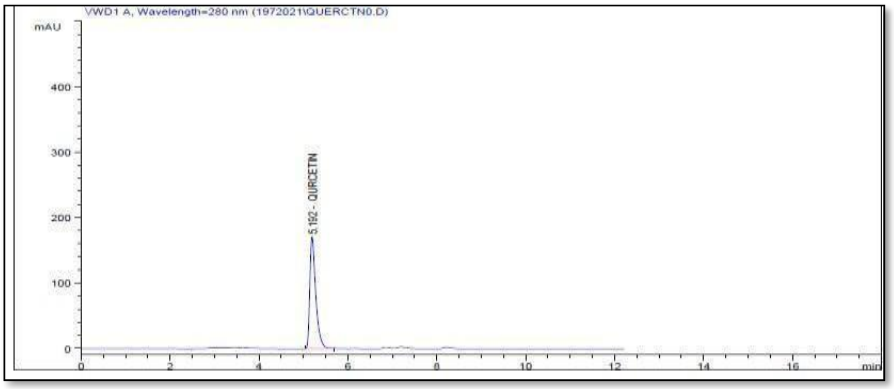


Fig.7.10. Chromatogram of quercetin

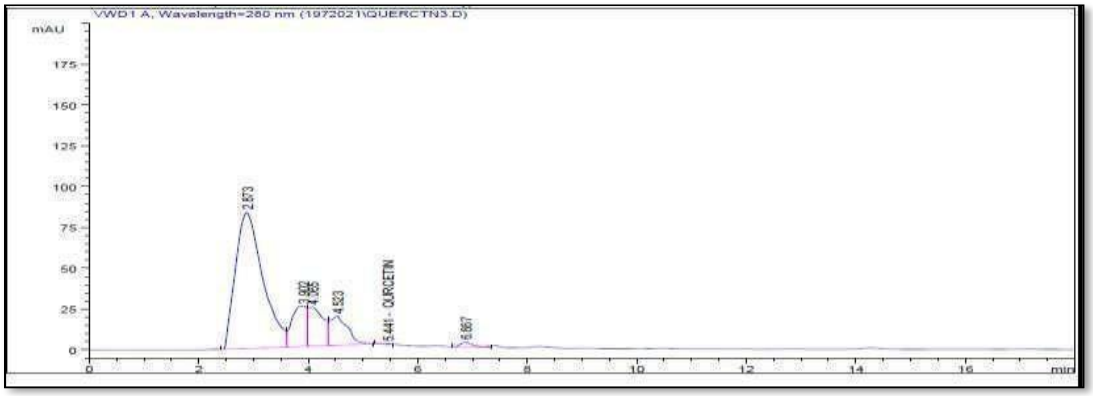


Fig.7.11. Qualitative estimation of quercetin in the CAME

7.1.6. Determination of Total Phenolic Content

Table no.7.7. Calibration curve of gallic acid

Sr. No.	Concentration (µg/ml)	Absorbance
1.	20	0.158
2.	40	0.279
3.	60	0.427
4.	80	0.569
5.	100	0.789

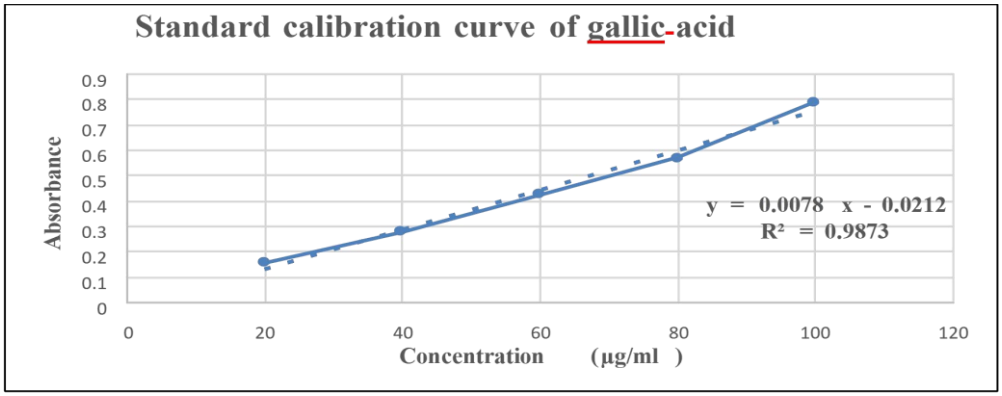


Fig.7.12 Standard calibration curve of gallic acid at 760nm

Quantitative estimation of total phenols was done on the basis of a standard curve of gallic acid and linearity of the calibration curve was achieved between 20 to 100 µg/ml concentration for gallic acid. The regression coefficient (R^2) was found to be 0.9873. The results showed that, the phenolic contents in the CA was found to be 11.23 mg GAE/g.

7.1.7. Total Flavonoid Content

Table no.7.8. Standard Calibration curve of quercetin

Sr. no.	Concentration (µg/ml)	Absorbance
1.	20	0.170
2.	40	0.286
3.	60	0.392
4.	80	0.474
5.	100	0.587

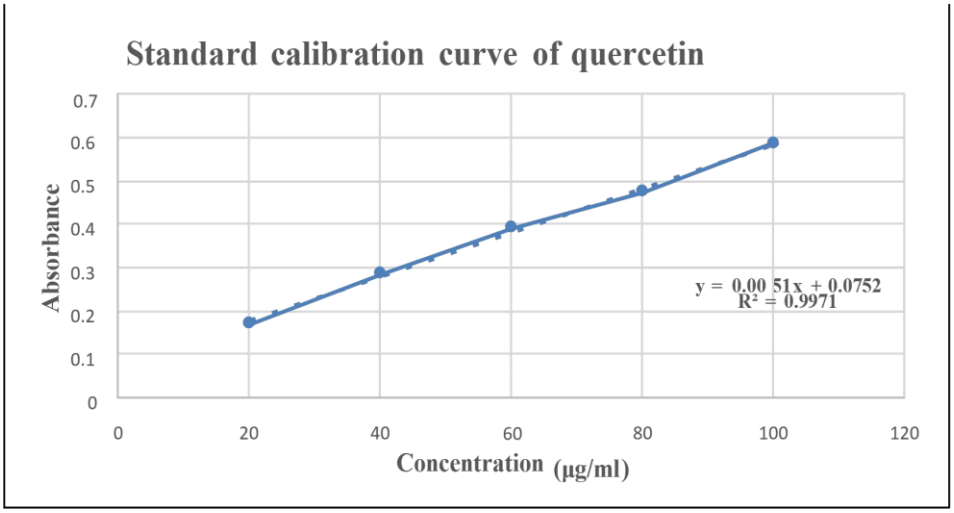
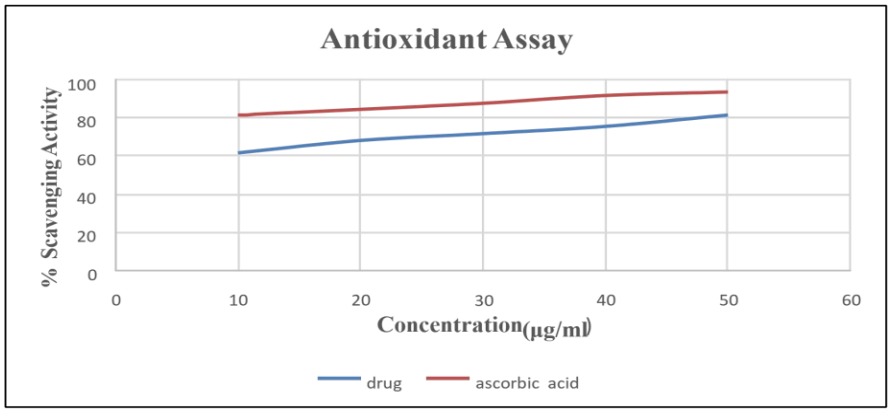


Fig.7.13. Standard Calibration curve of quercetin at 510 nm

Quantitative estimation of total flavonoid was done on the basis of the standard calibration curve of quercetin and the linearity of the curve is achieved between 20 to 100 µg/ml concentration for quercetin. The regression coefficient (R^2) was found to be 0.9971. The result showed that the flavonoid content in the CA was found to be 28.106 mg QE/g.

7.1.8..Antioxidant Assay

Scavenging activity of CAME is shown in fig 7.14. The activity was dose dependent and the maximum scavenging activity 80.580 was observed at 50 µg/ml concentration. The total antioxidant capacity in the leaf extract was determined by the formation of phosphomolybdenum complex, which was able to reduce the stable DPPH radical to the yellow colored diphenylpicrylhydrazine. The IC50 value was found to be 14.661±0.45 in the CAME.



CONCLUSION

The aim of the study was to formulate and evaluate *Chenopodium album* loaded invasomal gel as a topical drug delivery system for the treatment of acne vulgaris. *Chenopodium album* is herbal drug which contains flavonoids that results in the anti- acne activity.

Preformulation study of the drug was carried out by authenticating by botany department. The leaves were collected, washed thoroughly and dried at room temperature then it was extracted by methanol solvent using Soxhlet apparatus and organic solventwas evaporated using rotavapour. The semi solid extract was then lyophilized into solid state. The phytochemical screening was carried to observe the presence of flavonoids, phenols, alkaloids etc. The preformulation studies such as FTIR, DSC and XRD werecarried out. HPLC, TPC and TFC were carried to quantify the amount of flavonoids and free phenols present in the extract.

The minimum inhibitory concentration (MIC) of drug was evaluated against *S.epidermidis* and *S. aureus* by measuring zone of inhibition. CA showed minimum inhibitory activity at 400 µl and CA loaded invasomes showed minimum inhibitory activity against the bacteria. It was also concluded that when CA is compared with CA loaded invasomes then it was found that it starts showing activity at reduced dose.

The drug and PC were prepared in the ratio of 1:1,1:2 and 1:3 by solvent evaporation method. The 1:1 shows better results than 1:2 and 1:3 by comparing percent entrapment efficiency. The concentration of ethanol and terpenes varied with the optimized batch of CA-PC complex (D1) for CA loaded invasome preparation.

The preparation of CA loaded invasomes were done by mechanical dispersion method followed by probe sonication method. The characterization of CA loaded invasomes were done by %EE, particle size, PDI, zeta potential and visual characterization by FE-SEM. Formulation was optimized using 3^2 full factorial design it was observed that concentration of ethanol: terpene ratio (10:1) was found to be optimum in particle size 245.8nm, %EE 70.84%, PDI 0.331 and zeta potential (-) 7.57 mV.

The optimized batch (B3) of CA loaded invasomes were then formulated into topical gel. The formulated gel was found to be yellow in colour with characteristic lemon grass odour with pH 7.308 suited for topical formulation. The *in-vitro* drug release of CA loaded invasomal gel (71.235%) was compared with herbal topical gel (58.856%) and it was found that CA loaded invasomal gel show better sustained drug released than conventional.

The *ex-vivo* permeation studies were performed and it was found that CA loaded invasomal topical gel has increased permeationof 0.9564 with enhancement ratio when compared with plain herbal drug. Animal irritation studies were performed and no irritation of CA loaded invasomal topical gel was observed upto 7 days. Stability studies were performed after 1 month that revealed there is no significant changes in pH, viscosity and drug content. Hence, it was found to be stable at all the temperatures.

From the above research, it can be concluded that *Chenopodium album* loaded invasomal gel is compatible for the treatment of acne. Hence, due to the formulation of invasomes, it has shown increase in penetration of drug and the site of action is achieved.

Future research can be done to study the MIC on *Propionibacterium*

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