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Evaluation of a dermatological herbal hydrogel integrated with *Ipomea pes-tigridis* for anti acne activity

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ABSTRACT

Plan: *The objective of this research investigation was to formulate and evaluate herbal hydrogel incorporated with the extract of Ipomea pes-tigridis intended for anti acne activity.*

Methodology: *The formulation was evaluated for various parameters like organoleptic characters, pH, skin irritation test by multiple compartment patch, microbial contamination, extrude ability, spread ability, drug content, diffusion studies using pig skin, accelerated stability studies, drug excipient interaction studies by FTIR, in vitro anti acne and in vivo anti inflammatory activity.*

Outcome: *The formulated hydrogel passed all the evaluation parameters. The hydrogel was olive green in colour and had an excellent fragrance. The diffusion studies revealed that the drug release was in controlled release form. The accelerated stability studies revealed that formulation was stable at room temperature whereas its stability reduced with increase in temperature. The FTIR studies showed that there were no drug excipient interactions. The anti acne and anti inflammatory activity showed an activity comparable to that of the standard drugs clindamycin and diclofenac, respectively. Hence it can be concluded that the formulation can be a good substitute for the existing synthetic anti acne agents.*

Keywords: *Carbopol 940, Propionibacterium acnes, Staphylococcus aureus, acne vulgaris.*

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1. INTRODUCTION

Acne vulgaris is a common human skin disease, characterized by areas of skin with seborrhea, comedones, papules, pustules and nodules. It affects mostly skin with the densest population of sebaceous follicles and these areas include the face, upper part of chest and back. Severe acne is inflammatory, but acne can also manifest in non inflammatory forms [1]. Acne develops as a result of blockages in follicles. Hyperkeratinization and formation of a microcomedo is the most primitive change. This may enlarge to form an open or closed comedone. Comedones are formed as the direct result of sebaceous glands' becoming clogged with sebum and dead skin cells. In this environment, the bacterium *Propionibacterium acnes* can cause inflammation, leading to inflammatory lesions in the dermis around the microcomedo or comedone, which results in redness and may result in scarring or hyperpigmentation. *Propionibacterium acnes* and *Staphylococcus epidermidis* are two of the major bacterial strains found in acne lesions and among these only *P. acnes* is implicated in acne inflammation [1-5].

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In the present work we have formulated a herbal hydrogel intended for acne treatment. Hydrogels are hydrophilic polymeric network of three dimensional cross linked structures that absorb substantial amount of water. Cross linking facilitates insolubility in water because of ionic interaction and hydrogen bonding. It also provides required mechanical strength and physical integrity to the hydrogels. Thus, hydrogels can imbibe water nearly 10-20 times its molecular weight and hence become swollen [6]. General benefits include biocompatibility, can be injected, easy to modify, timed release of growth factors and other nutrients to ensure proper tissue growth, entrapment of microbial cells within polyurethane hydrogel beads with the advantage of low toxicity, environmentally sensitive hydrogels have the ability to sense changes of pH, temperature or the concentration of metabolite and release their load as result of such a change, and natural hydrogel materials are being investigated for tissue engineering, which include agarose, methylcellulose, hylaronan, and other naturally derived polymers[6].

Ipomoea pes-tigridis is commonly known as Tiger Foot Morning Glory in English is an annual herbaceous vine, twining, with spreading hispid axial parts. It is widely distributed in Sahel zone from Senegal to Niger and N. Nigeria, tropical Africa, Asia, Australasia, Mascarene Island and Malaysia. Chemical constituents include alkaloids, flavonoids, fatty acids, mucilage, resins, tannins, astringents, cardiac glycosides, saponins, resins and carbohydrates.

The plant is well known for its wide range of medicinal properties which include purgative action, treatment of sores and pimples, haemorrhoids, arthritis, rheumatism, dropsy, swellings, oedema, gout, venereal diseases, boils, carbuncles and dog bites, as diuretic, laxatives, pain killer, antidotes for venomous stings, snake bites, etc[7-9].

The aim of our research investigation is with respect to the traditional claim of *Ipomoea pes-tigridis*, as the folklore of Kerala, India, use the paste of the plant for the treatment of painful, infectious pimples. As leaf paste as such to apply on the surface of the skin is not acceptable by majority of the patients, we decided to formulate and evaluate a hydrogel by loading it with the crude extracts of the plant.

2. MATERIALS AND METHODS

2.1. Collection and authentication of plant

The fresh plant of the *I.pes-tigridis* was collected in regions of Bollapally Village of Kattamgur Mandal of Nalgonda district, Andhra Pradesh, South India in the months of November to December 2010. A herbarium of the plant material was prepared and deposited in Department of Botany, Osmania University, Hyderabad, India. The specimen was identified and authentified under the Voucher no: 0396, by Dr.B.Badraiah, H.O.D of Botany department, Osmania University, Hyderabad, India.

2.2. Procurement of Micro organisms

The micro organisms *Propionibacterium acnes* MTCC 1951, *Staphylococcus epidermidis* MTCC 435, *Pseudomonas aerugenosa* MTCC 741, *Bacillus subtilis* MTCC 441, *Escherichia coli* MTCC 443 and *Staphylococcus aureus* MTCC 96 were procured as freeze dried form from Microbial type culture collection, Chandigarh, India.

2.3. Procurement of animals

Wistar Albino Rats (150-250gm) of either sex were procured from National Institute of Nutrition, Hyderabad, A.P, India. The experimental protocol was initially approved from the Institutes animal ethics committee under the reference no. NCOP/IAEC/approval/36/2011 and then experimental studies were undergone according to their rules and regulations. The animals were housed under standard environmental conditions and had free access to standard pellet diet and water *ad libitum*.

2.4. Extraction

The 200gms of shade dried aerial parts of *I.pes-tigridis* were subjected to soxhlet extraction with methanol after defatting with petroleum ether. The extract obtained was then concentrated under vacuum using a rotary vacuum evaporator and subjected to preliminary chemical tests [10,11]. The % yield of the extract was found to be 18.48% w/w.

2.5. Formulation of 2% Herbal Hydrogel

The 1% carbopol 940 was gradually added into pure 96.9mL of water with continuous stirring by using magnetic stirrer until a homogenous mixture appeared. Then the plant extract (2%) was dissolved in little amount of water and mixed with the above homogenous mixture. Finally the mixture was neutralized with triethanolamine. Rose oil was added towards end of preparation to impart high-quality fragrance. The formulation was left undisturbed for 24hrs and was stored at room temperature of 25°C ± 2°C[12-14].

2.6. Evaluation of 2% herbal hydrogel

Organoleptic characters like visual appearance, colour and odour were noted. Presence of foreign particles/grittiness was tested by spreading a small amount of the gel on a glass slide free from greeze and was observed against diffused light to check for presence of foreign particles. pH was determined using a digital pH meter[15-17].Consistency was determined as per standard procedures.

2.6.1. Determination of skin irritancy by patch test (multiple compartment model)

This evaluation was performed on 10 healthy human volunteers including both male and female candidates. The parameter was preceded after getting the written consent of all the volunteers. About 0.5gms of 2% herbal hydrogel was applied to an area of approximately 6cm^2 of skin of hand covered with a gauze patch. The patch was loosely held in contact with the skin by means of a semi-occlusive dressing for the duration of 1 hour and gauze was removed. At the end of the exposure period of 1 hour, residual test substance was removed, without altering the existing response or integrity of the epidermis. Observations were recorded after removal of the patch for 1hr, 3hrs, 6hrs, 12hrs, 24hrs, 48hrs and 72hrs[18].

2.6.2. Microbial contamination test

As the herbal hydrogels are more prone to microbial attack the formulation was subjected for microbial contamination test. Sterilized nutrient agar was prepared and then a small quantity of the formulated 2% herbal hydrogel was spread on it and incubated for 24hrs at 32°C. The growth for any micro organisms was observed.

2.6.3. Extrudability

The formulated 2% herbal hydrogel was filled in standard capped collapsible aluminum tube and sealed by crimping to the end. Weight of the tube was recorded. It was then placed between two glass slides and was clamped. Weight of 500g was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was then calculated (>90% extrudability: excellent,>80% extrudability: good, >70% extrudability: fair) [12-14].

2.6.4. Spreadability

The formulated 2% herbal hydrogel was placed over the glass plate of 20cm × 5cm. Another glass plate of the same dimension was placed on the top of the gel such that the formulation was sandwiched between the two slides by placing a weight of 100g uniformly on the slides. The weight was removed and the excess of gel was scrapped off. Two slides in position were fixed to a stand at a 45° angle without the slightest disturbance so that only the lower slide was held firmly by the clamp, allowing the upper slide to slip off freely with the help of 20g weight tied to the upper slide. The time taken for the upper slide to separate away from the lower glass plate under the direction of the weight was noted as per ICH guidelines 10[24].

Experiment was done in triplicate and spreadability was calculated as follows:

S = M x L/ T, Where, S = Spread ability, L =Length of the glass plate.
W=Weight tied to the upper plate, T = Time taken (sec).

2.6.5. Drug content

To determine the drug content in the formulated 2% herbal hydrogel formulation equivalent to 100 mg was weighed and added in 100 ml of methanol taken in a 100 ml volumetric flask. The volumetric flask was stirred continuously for 24hr on a magnetic stirrer. Dilutions were made suitably and measured for the drug content UV spectrophotometrically by Lambda25 Perkin Elmer UV/Visible Spectrophotometer[15-17].

2.6.6. Diffusion

Phosphate buffer of pH 6.8 was used for *in vitro* release as a receptor medium. Upon receipt the freshly excised skin from albino pigs, was washed gently with 1% (w/w) aqueous detergent, rinsed with deionised water and patted dry with a paper towel before storage in plastic bags at 4°C.Skin was removed from the refrigerator and kept in isotonic solution to hydrate at room temperature one hour before starting the experiment. The dermatomed skin was cut into 10-mm circular pieces with a brass punch and placed epidermis-side-up in Franz diffusion cell[18]. The formulated 2% herbal hydrogel was applied on the skin and then fixed in between donor and receptor compartment of diffusion cell. The receptor compartment contained phosphate buffer (100ml) of pH 6.8. The temperature of diffusion medium was thermostatically controlled at 37° ± 1ºC by surrounding water in jacket and the medium was stirred by magnetic stirrer at 500rpm. The sample at predetermined intervals were withdrawn and replaced by equal volume of fresh fluid. The samples withdrawn were spectrophotometrically estimated at 240nm against their respective blank.

2.6.7. Accelerated stability studies

The formulated 2% herbal hydrogel was stored in different temperatures of 27°C, 50°C and 70°C to accelerate degradation rate for 1 month. Samples were withdrawn periodically every week and observed for drug decomposition by taking the absorbance under UV spectrophotometer. From the concentrations and the temperatures, shelf life of the product was estimated[15].

2.6.8. Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR spectra were obtained on a Perkin Elmer FTIR spectrometer in the transmission mode with the wave number region 2,000–500 cm^{-1}. KBr pellets were prepared by gently mixing 1 mg of test with100 mg KBr[15].

2.7. Anti-acne activity

Screening of anti acne activity was performed by modified method of Hayes and Markovic [28] with slight modifications by standardized filter-paper disc-agar diffusion method. *P. acnes* was incubated in brain heart infusion medium (BHI) with 1% glucose for 72 h under anaerobic conditions. Aliquots of molten BHI with glucose agar were used as agar base. A prepared inoculum was added to the media, mixed and poured over the surface of the agar base and left to solidity.
A concentration of 10mg/ml of the formulated 2% herbal hydrogel was prepared and sterile disc loaded with 100 of this formulation was placed in the inoculated media. Then plates were incubated at 37°C for 72 h under anaerobic conditions. Clindamycin (10mg/ml) was used as the standard and formulation devoid of extract (placebo) was used as the control.

Staphylococcus epidermidis was inoculated in tryptic soy broth (TSB). The procedures were same as mentioned above except the plates were incubated at 37ºC for 24 h under aerobic conditions. All the tests were performed in triplicate and the results were expressed as mean of zone inhibition diameters (mm)[12-14,19].

2.8. Anti inflammatory activity by Carrageenan induced rat Paw edema

Wistar albino rats of either sex weighing 150-250 g were taken and divided into 3groups with 6 animals each. The animals were starved over night and group 1animals were applied topically with 1 ml of placebo (formulation without the crude extract) as control. Group 2 were applied topically with specified quantity of 1g of diclofenac gel as standard. Group 3 was applied topically with specified quantity of 1g of formulated 2% herbal hydrogel. The test and standard drugs were applied to the plantar surface of the left hind paw by gently rubbing 50 times with the index finger to the respective groups 30 minutes prior to carrageenan injection. After 30min 1%w/v of 0.05ml carrageenan was given injected subcutaneously. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediately after injection at 1h, 3h, 6h, 12h and eventually 24h after drug application[20].

2.9. Statistical analysis

Results are expressed as Mean ± SEM. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA) followed by Dunnett's Multiple comparison test (control vs all) using the soft ware Graph Pad Instat.

3. RESULTS AND DISCUSSION

The aerial parts of *I.pes-tigris* after extraction with methanol gave a greenish brown colored powdery extract. The preliminary chemical tests performed revealed the presence of alkaloids, carbohydrate, cardiac glycosides, tannins, phenolic compounds and flavonoids. Topical gel preparations are anticipated for skin application or to certain mucosal surfaces for local action or percutaneous penetration of medicament or for their emollient or defending action. Gels are typically formed from a liquid phase that has been thickened with other components. The continuous liquid phase allows free diffusion of molecules through the polymers scaffold and hence release should be equivalent to that from a simple solution. Of the many available brands, those which are listed as "water-based" or "oil-free" are generally a better choice [13]. Hence with this point in mind we decided to formulate a cosmetic hydrogel by incorporating crude extracts of *I.pes-tigridis* intended for treatment of mild to severe form of acne. The formulated 2% herbal hydrogel was observed to be translucent with a pleasant olive green colour and rose fragrance which is considered to be the two important factors governing the patient compliance. The formulation was observed to be free from grittiness or foreign particles which designate that no abrasions or uneasiness will occur on its application on the dermis. As pH was observed to be 6.8 which were within the range of cosmetic preparation for gels, the formulated hydrogel can be considered to be complementary. The consistency reflects the capacity of the hydrogel, to get ejected in uniform and desired quantity when the tube is squeezed [13].

Skin irritation was performed on human volunteers by taking a written consent from them. We observed that irritation test performed by multiple compartment model did not shown any kind of irritation like erythrema, itchiness, inflammation, redness or burning sensation which showed that neither the plant extract nor the excipients used create any kind of allergic reactions.

The 2% herbal hydrogel was prepared using carbopol 940 as the polymer. These polymers are reported to have a long history of safe and effective use in topical gels, creams, lotions and ointments. They are also supported by extensive toxicology studies. Carbopol polymers have been shown to have extremely low irritancy properties and are non-sensitizing with repeat usage [22, 25]. The observations are represented in Table I and figure1.

The formulated 2% herbal hydrogel was found to be totally free from microbial attack. When applied a weight of 500g, 11.04 out of 12g of hydrogel extruded out which was about 92%. This denotes excellent extrudability of the formulated 2% herbal hydrogel. The extrudability is a useful empirical test to the measure the force required to extrude the material from a tube. Since the packing of gels have gained a considerable importance in delivery of desired quantity of gel from jar or extrusion of gel from collapsible tube, therefore measurement of extrudability becomes an important criterion for gels[14]. The spread ability is an important parameter as it denotes the area that can be covered by the hydrogel. It is also an indication whether the viscosity is appropriate or not. The spread ability value obtained for the formulated 2% herbal hydrogel was very good. When 1g of the formulated 2% herbal hydrogel subjected to spread ability produced 31.05±0.012cm which showed excellent and even spreading nature of the formulation. The drug content in one gram was found to be in excellent percentage which will be sufficient to attain the required anti acne activity. The 1g of formulated hydrogel was found to contain 84.8% of the active principle. The variability of physic chemical properties of drug formulations designed for assuring accurate biocompatibility, stability and bioavailability are some of the important factors to be considered while designing the in vitro drug release tests [26].

For the diffusion studies pig skin model was used. Animal models are generally used for transdermal permeation studies. The characteristics of excised skin from mice, rats, rabbits, and pigs are widely used. However, animal skin is different from human skin in several features. Indeed, the main barrier to drug permeation through skin is the stratum corneum, which has been reported to differ in terms of lipid composition, water content and morphological characteristics (thickness, number of pores, and follicles) on the basis of species. Pig stratum corneum is the most similar to human stratum corneum in terms of lipid composition, but it presents a marked difference in terms of thickness. On the other hand, the thickness of newborn pig stratum corneum is considerably thinner than that of adult pig and more similar to that of human skin, even if the number of hair follicles is higher than that of human or adult pig skin [16,21]. The *in-vitro* diffusion studies on pig skin have performed which showed sustained drug release (Figure 2).

The graph for the drug release shows linear path for 12 hours. It is reported that the polymer carbopol used in the formulation is an excellent vehicle for drug delivery. Due to their extremely high molecular weight, they cannot penetrate the skin or affect the activity of the drug. The % drug diffused was found to be in a uniform manner and by 12th h 72% drug release was observed. The shelf life of the formulated 2% herbal hydrogel was found to be 26.25 months if stored at 27 °C, 21.57if stored at 50 °C and 19. 34 months if stored at 70°C.

The above table revealed that the stability of the formulation reduced when exposed to higher temperatures. The stability testing at higher temperature was performed to identify its stability if the formulation is accidentally exposed to fire or any other situations where the temperatures conditions will be elevated (Table2, Figure 3).

Purpose of accelerated stability testing is to provide evidence how quality varies with time under influence as temperature, humidity and light. Purpose of accelerated stability testing is to establish re-test period for drug substances, to establish shelf life for drug products and to recommend storage conditions [23,24]. During the stability studies the appearance was clear and no significant variation in pH was observed. Considering the accelerated stability studies and physiochemical parameters, the formulated gel is stable and if stored at higher temperature or accidental exposure to higher temperature the formulation was observed to be stable with only difference in a reduction of two months.

The FTIR studies revealed that there were no extract-excipient interactions. Compatibility of the drug with excipients was determined by FT-IR spectral analysis, this study was carried out to detect any changes on chemical constitution of the drug after combining it with the excipients. The peak values (wave number) and the possibility of functional group are shown in spectra which compare with extract, physical mixture and placebo (Figure: 4A-D). The wave numbers 3340 cm^{-1}, 3367 cm^{-1}, 3307 cm^{-1} and 3238 cm^{-1} are present in the formulation, similar peak numbers were also observed in extract (3379 cm^{-1}, 3340 cm^{-1}) and physical mixture (3342 cm^{-1}, 3306 cm^{-1}) which showed that there is no drug exipient interactions. These peak values represent the O-H or N-H, hydrogen bond stretching. These peaks were found to be absent in placebo, which confirms that these peak values are that of the extract. The peak values 2042 $cm^{-1}$and 2019 cm^{-1} were seen in formulation, placebo and physical mixture confirming that these are the peaks of the excipients and there were no interactions. These values represent alkyne bond.

Infrared spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present.

The formulated 2% herbal hydrogel showed exhibited a potent anti bacterial activity against *S. epidermidis* and *P. acnes* by producing a zone of inhibition of 22.2± 0.05mm and 17±0.04 mm. The standard clindamycin was found to possess excellent activity with inhibition zone of 28.5± 0.004mm and 21.2± 0.003mm. It was observed that the placebo (control) did not possess any anti acne activity, which strengthens the result obtained for the herbal hydrogel that the activity was merely from the herbal extract. The credits for this potency can be attributed to the alkaloids and flavonoids which are well known for the anti microbial activity. Generally acne is mostly an inflammatory reaction of our body to skin lesions caused by sebum outbreaks to cells lining the hair follicles, and by similar lesions inflicted by chemicals produced by the development of acne bacteria within blocked pores. Acne treatment must repair skin lesions and address inflammation, blocked pores and acne bacteria, and always try to avoid loss of tissues as in ice pick or pitted acne scars or abnormal fibrotic or keloid scars[2-4]. The *in vivo* anti inflammatory was screened and a potent activity in drug dependent manner was observed for the formulated 2% herbal hydrogel. By third hour of treatment potent reduction in the inflammation was observed and the paw volume was found to be1.435±0.01mL and 1.41±0.006mL for the test and the standard, respectively. The anti inflammatory activity was found to be present till 24h and by the end of this time the test had shown better activity than the standard and the paw volume was measured to be 0.986±0.01 mL and 1.02±0.005mL for test and standard, respectively(Figure:5).

The formulation also showed a potent anti-inflammatory activity that can be comparable with that of the standard and the main active chemical entity responsible may be the flavonoids as they are established anti inflammatory agents. As the excipients were devoid of any pharmacological activity we can confirm that the obtained results are due the active principles in the herbal formulation. This shows that the polymer carbopol 940 used has no effect on the biological activity of the drug. Drug release across the skin is an effective and targeted therapy for the infected area, used in local dermatological treatment [27]. The formulated hydrogel has produced a potent action due to enhanced diffusion, drug content and suitable pH. The carbopol used also played an important role as these are used to permanently suspend the active ingredients in transdermal reservoirs as well as in topical gels and creams.

4. CONCLUSION

Hence the present study states that the 2% herbal hydrogel loaded with the crude methanol extract of aerial parts of *I.pes-tigridis* can be used in the effective treatment of acne. As no skin irritations were observed this formulation can be very well substituted for the synthetic cosmetics used for acne therapy.

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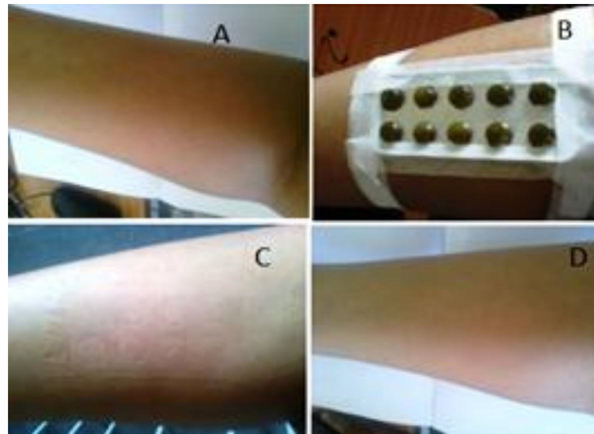

Figure 1: Multiple compartment Patch test for skin irritation

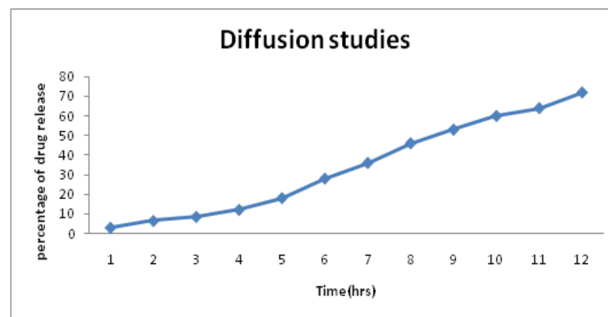

Figure 2: Diffusion studies of 2% herbal hydrogel

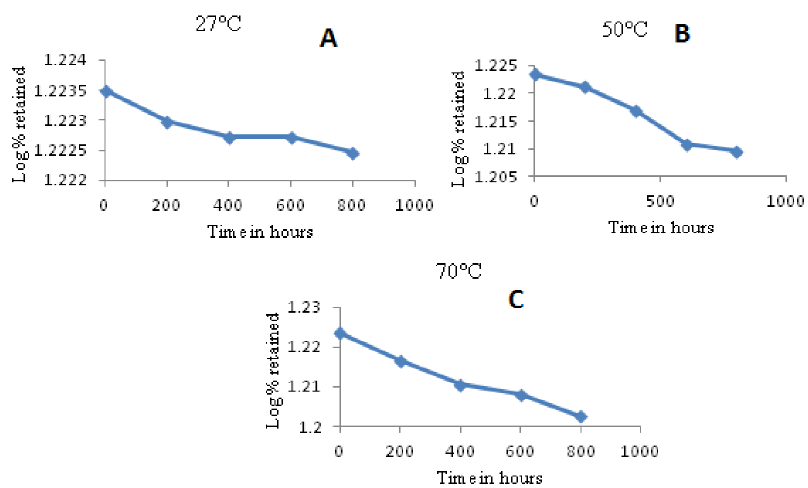

Figure 3: Accelerated stability studies performed on various temperature ranges.

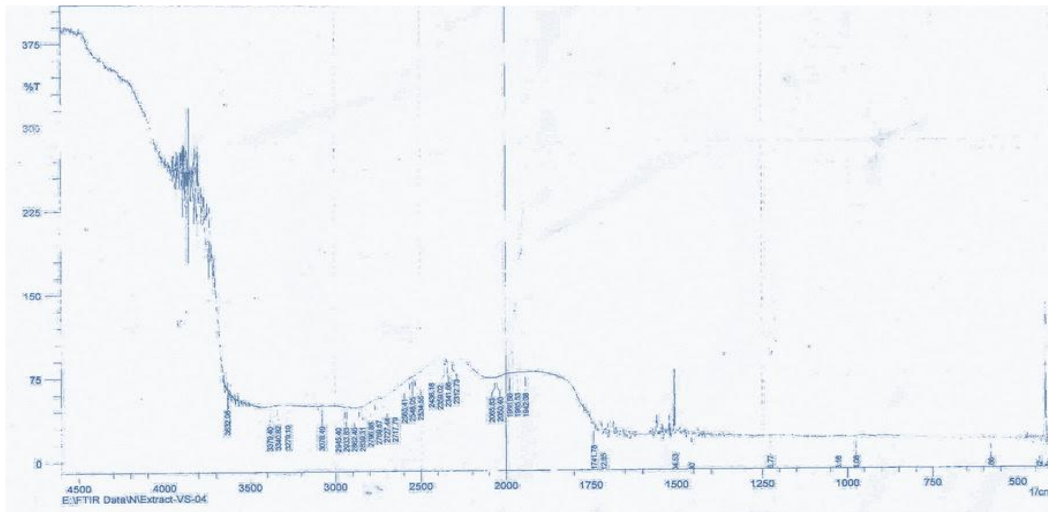

Figure 4A: FTIR studies. A-plant extract

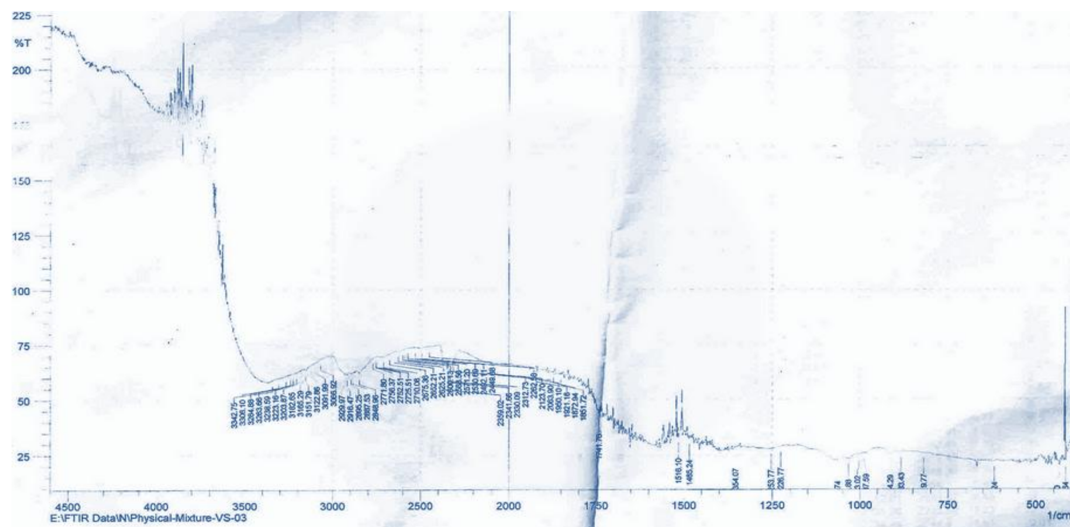

Figure 4B: FTIR studies. Physical mixture

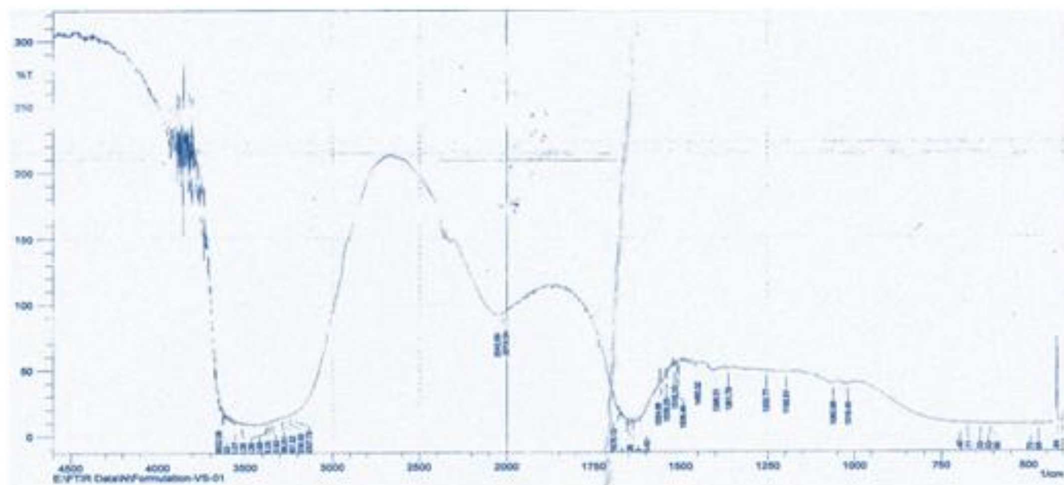

Figure 4C: FTIR studies. 2%hydrogel

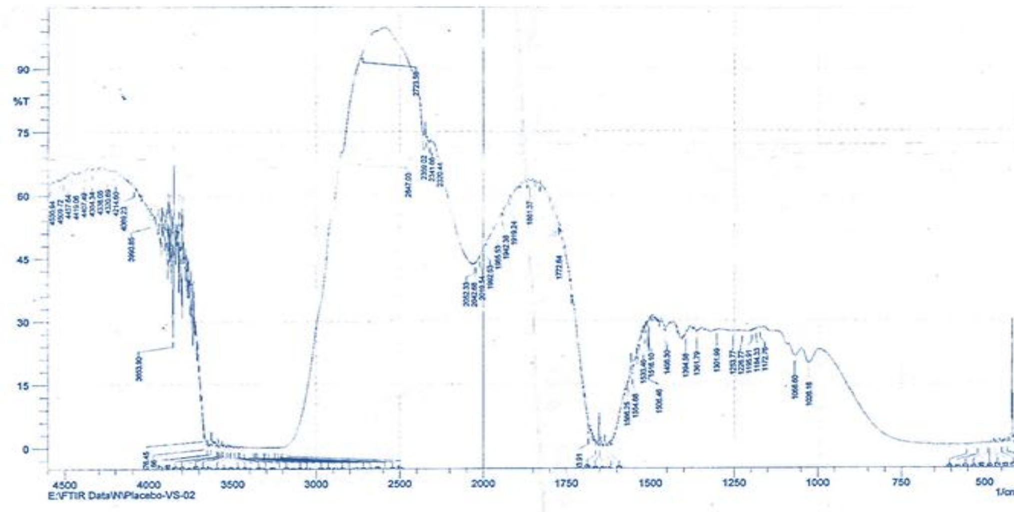

Figure 4D: FTIR studies of excipients

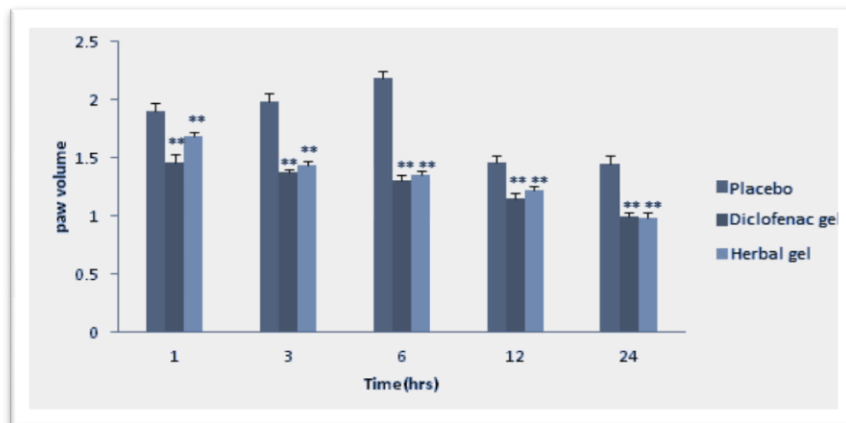

Figure 5: Anti-inflammatory activity for 2% herbal hydrogel

Table 1: Skin irritation by multiple patch test

S.No	*Treatment*	*1hr*	*3hr*	*6hr*	*12hr*	*24hr*	*48hr*	*72hr*
1.	2% herbal hydrogel	A	A	A	A	A	A	A

In the above table, A: no reaction, B: slight patchy erythema, C: slight but confluent or moderate but patchy erythema, D: moderate erythema, E: severe erythema with or without edema.

Table 2: Accelerated stability studies

Weeks	70°C	50°C	27°C
0	16.73	16.73	16.73
1	16.47	16.64	16.71
2	16.24	16.48	16.70
3	16.15	16.25	16.70
4	15.94	16.20	16.69

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