Formulation and stability studies of herbal gel containing Aloe Vera L and Ammi Majus L.

Swapnil V. Shinde¹, Shrinivas K. Mohite²
¹Research Scholar, Shivaji University, Kolhapur, Maharashtra, India
²Professor, Department of Pharmaceutical Chemistry, Rajarambapu College of Pharmacy, Kasegaon, Dist- Sangli, Maharashtra, India.

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*Corresponding author E-mail address: swapnil.shinde@syngeneintl.com

ABSTRACT
The present research has been undertaken with the aim to formulate the gel containing aqueous extract of Aloe Vera L & Ammi Majus L. The gel was prepared by using aqueous extract of Aloe Vera L & Ammi Majus L seeds, Carbopol 934, glycerin, methyl paraben and triethanolmine. The skin pH was maintained by adjusting the pH of formulation at 7.0 (±0.2). Aloe Vera L & Ammi Majus L. Physicochemical parameters of formulation i.e. Colour, homogeneity, Appearance, pH, viscosity, Spreadability and microbiological activity were observed and documented, also stability studies were carried out as per ICH guidelines for 6 months. Experimental data for the gel formulations were found well within specification limit when exposed to accelerated condition 40°C/75% RH.

KEYWORDS
Aloe Vera L, Ammi Majus L, pH, stability, Viscosity, microbiological.
1. INTRODUCTION
Various plant species have been reported to have an effect against skin disease. Herbal medical practitioners can create many different formulas for different types of applications. The use of medicinal plants in the production of drugs is increasing because of their therapeutic safety and effectiveness [1].

*Aloe Vera* is a stemless, perennial, drought resisting, succulent plant and has reportedly been used since ancient times for medicinal purposes. It belongs to the lily (Liliaceae) family, and has stiff grey to bright green lance-shaped leaves containing clear gel in a central mucilaginous pulp. Recent research has shown that the pharmacologically active agent is concentrated in both the gel and the rind of the *Aloe Vera* leaf. The active agents have shown considerable analgesic, antipruritic, wound healing and anti-inflammatory properties [2].

*Ammi majus* L., commonly called as ajwain or ova in India is widely used in the treatment of certain skin diseases like vitiligo since ancient times. It synthesizes furocoumarins such as xanthotoxin and bergaptons, which are used to prepare cream and lotions to cure the skin diseases [3].

2. MATERIALS AND METHODS

2.1. Collection of Plant
Seeds and Leaf were collected from local area, Sangli. The seeds and leaf was identified and authenticated by Dr. S.S. Sathe, an approved Botanist. A specimen voucher no. (RCP/SNG/201, 202) has been deposited in Department of Pharmacognosy, Rajarambapu College of Pharmacy, Kasegaon, Sangli, MS, India.

2.2. Extraction
Aqueous extract of seeds of *Ammi majus* Linn were prepared from shade dried. Aqueous extracts was prepared by 300 gm powder (twice) in 1500 ml of Distilled water for 30 min at 70°C. The extract was filtered through Whatman filter paper (No.1) and then evaporated and concentrated on the water bath at atmospheric pressure to a semisolid condition. This was pouring in flat petridish as thin layer in hot air oven at 25°C for 8 hrs to obtain the extract with 9% yield.

For the aqueous extraction, *Aloe vera* Linn plants were collected from garden, and washed with water. Then the whole leaves were ground using a crusher, then added 500 mL of water and heat the mixer till temperature was gradually increased until reached at boiling point, and was kept constant at an average of 100°C. This process was continued until; finally, approximately 100 mL of the herbal extract was obtained. Centrifugation was used to purify the extracts.

2.3. Animals
Healthy male adult albino mice (25-30 g) obtained from the animal house of Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India was used for the study. Mice were housed in polypropylene cages and fed on standard pellet diet and water ad libitum, and the room maintained under controlled condition (12 h light-dark cycle at 22±2°C). Animals were allowed to acclimatize for 7 days prior to experiments being carried out. Institutional ethics committee
permission was obtained as per CPCSEA guidelines (Registration No: IAEC/ ABCP /15/2015-2016) for carrying out the study in animals.

2.4. Preparation of gel
The Gel was prepared using carbopol-934, glycerin, methyl paraben, Triethanolamine and water in a quantity sufficient to prepare 100 g of gel in case of blank gel. Water required in gel formulation was divided into two parts of ratio (25:75). In one part of 25%, extract (active) was dissolved and to this solution glycerin and methyl paraben was added and dissolved and in other part (75%) of water carbopol-940 was dissolved. Both the solutions were mixed properly in beaker and pH was adjusted to 7.0±0.2°C with tri-ethanolamine.

| Sr. No. | Ingredients                  | Quantity  |
|---------|------------------------------|-----------|
| 1       | Aloe Vera Linn               | 2.5%      |
| 2       | Ammi Majus Linn              | 2.5%      |
| 3       | Carbopol 934                 | 3 gm      |
| 4       | Glycerin                     | 2 ml      |
| 5       | Methyl paraben               | 0.2 ml    |
| 6       | Triethanolmine               | q. s.     |
| 7       | Distilled Water              | q. s.     |

2.5. Evaluation of topical gel formulation [4]

2.5.1. Appearance and Homogeneity
Gel was tested for physical appearance and homogeneity by visual observation.

2.5.2. pH
The pH value of gel formulation was determined by using a pH meter (Sartorious, S0231)

2.5.3. Viscosity
The measurement of viscosity of prepared gel was done with Brookfield viscometer (DV-E viscometer).

2.5.4. Spreadability
The spreadability of gel formulation was determined by sensory evaluation, through feedback given by volunteers.

2.5.5. Microbiological Activity
Activity performed for specified Microorganisms Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa.

2.5.6. Stability Studies
Gel formulation is packed and subjected for stability study (Newtronic Inc.) [5]

2.6. Storage conditions and time-points.
2.7. Skin irritation studies

The Wistar rats of either sex weighing 150-200 gm were used for this test. The intact skin was used. The hairs were removed from the rat 3 days before the experiment. The gels containing extracts were used on test animal. Gel base was applied on the back of animal taken as control. The animals were treated daily up to seven days and finally skin was examined visually for erythema and edema [6, 7].

3. RESULTS AND DISCUSSION

The developed herbal gel was greenish in colour, translucent in appearance and showed good homogeneity with absence of lumps. The formulated gel was much clear and transparent. The values of Spreadability indicate that the gel is easily spreadable. Spreadability of gel 18.30 gm.cm/sec and 23.00 gm.cm/sec respectively. pH also maintained throughout the study which was found 7.0. The viscosity of developed gels was measured using Brookfield viscometer with spindle. Microbial tests of formulated gel of Aloe vera L gel and Ammi majus L gel were found complies as per the acceptance criteria. The control and experimental rats showed no signs of inflammation, erythema, and edema.

Table 1. Evaluation of different parameters on formulated gel.

| Parameters     | Results for Aloe vera L gel | Results for Ammi majus L gel |
|----------------|-----------------------------|------------------------------|
| Colour         | Greenish                    | Yellowish                    |
| Homogeneity    | Good                        | Good                         |

Note:
1. Test results should comply with finished product shelf life In house specifications.
2. * In case of significant change observed at sample stored at 40°C ± 2°C & 75 % ± 5 % RH (accelerated condition) storage condition; perform the analysis of sample stored at 25°C ± 2°C / 60 ± 5% RH(Long-term) &30°C ± 2°C / 75 ± 5% RH(Intermediate) storage condition.
3. A= Appearance, pH, Viscosity, Spreadability, homogeneity and microbiological activity
Table 2. Stability profile of *Aloe vera* L gel formulation.

| Evaluation          | Initial       | 1 month  | 3 month  | 6 month  |
|---------------------|--------------|----------|----------|----------|
| Colour              | Greenish     | Greenish | Greenish | Greenish |
| Homogeneity         | Good         | Good     | Good     | Good     |
| Appearance          | Complies     | Complies | Complies | Complies |
| pH                  | 7.0          | 6.9      | 6.8      | 6.9      |
| Viscosity (cps)     | 4360         | 4290     | 4160     | 4040     |
| Spreadability       | Good         | Good     | Good     | Good     |
| Microbiological     | Pass         | Pass     | Pass     | Pass     |
| activity            |              |          |          |          |

Table 3. Stability profile of *Ammi majus* L gel formulation

| Evaluation          | Initial       | 1 month  | 3 month  | 6 month  |
|---------------------|--------------|----------|----------|----------|
| Colour              | Yellowish    | Yellowish | Yellowish | Yellowish |
| Homogeneity         | Good         | Good     | Good     | Good     |
| Appearance          | Complies     | Complies | Complies | Complies |
| pH                  | 7.0          | 6.9      | 6.8      | 6.8      |
| Viscosity (cps)     | 4132         | 4200     | 4050     | 4005     |
| Spreadability       | Good         | Good     | Good     | Good     |
| Microbiological     | Pass         | Pass     | Pass     | Pass     |
| activity            |              |          |          |          |

Table 4. Skin Irritation Study Results

| Treatment           | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
|---------------------|-------|-------|-------|-------|-------|-------|-------|
| Control             | A     | A     | A     | A     | A     | A     | A     |
| *Ammi majus* L gel  | A     | A     | A     | A     | A     | A     | A     |
| *Aloe Vera* L gel   | A     | A     | A     | A     | A     | A     | A     |

*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.*
4. CONCLUSION
It is inferred from results that the Aloe Vera L leaves and Ammi majus L seeds gel formulation are good in appearance, homogeneity and easily spreadable. When stored at Accelerated stability condition (40°C ± 2°C / 75 ± 5% RH) indicated that temperature and humidity has no impact on colour, appearance, homogeneity, pH, viscosity and spreadability. Microbiological activity in the gel was found well within the acceptance limit, upon storage for 6 months at accelerated condition. The topical gel formulated was non irritant upon application on the skin.

5. REFERENCES
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