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Development and Evaluation of Herbal Gel by Using *Matricaria recutita* for Treatment of Mouth Ulcer



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ABSTRACT

Mouth ulceration is one altogether the foremost customary issues caused due to an expansion of etiological factors. The formulation of herbal gel with exploitation chamomile flower extract. Flavouring medicines are still the mainstay of eightieth of the world's population, notably in growing countries, for primary health care owing to compatibility with body, cultural satisfactoriness. The main aim is to developed and evaluate flavouring gel for mouth ulceration treatment of dried powder of chamomile flower. The dried flower of chamomile includes several flavonoids and apigenin tributary to its medical properties. All the ready gel system was evaluated for various properties like hydrogen ion concentration, appearance, stability study, viciousness, in-vitro unharness. The gel had been ready by the employment of Carbopol 934 as a gelling agent. The formulated gel was proper appearance and uniformity acceptable viscosity, stability, spreadability extrudability properties. The herbal gel formulation was safe and effective for the treatment of mouth lesions.



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INTRODUCTION:

Mouth ulcers are small sores or an abrasion that develops in the mouth or at the base of the gum. A mouth ulcer is also known as a canker sore or aphthous ulcer (**Fig 1**). A break or branch in the mucus membrane, that line within the mouth is also recognized as a mouth ulcer. It generally arises as a yellow colour depression in mouth ulcers. Mouth lesion square measure typically very uncomfortable and create troublesome for some folks to eat, drink, and brush their teeth. There are three major types of mouth ulcers first, a minor lesion that measure tiny oval or spherical ulcers that heal within a one-ton period with no scarring. Second, Major lesions measure larger and deeper than minor ones. Third, Herpetiform in this kind of lesion cluster of dozens of smaller sores regarding the scale of pinheads [1].

Gels square measure generally semi-solid formulation having a liquid part that has been thickened. Gel square measure glorious formulation for many routes of administration. Gels are often clear formulation once all of the particles fully square measure dissolve in an exceeding dispersion medium. Gel formulations are used to deliver the drug topically because of easy application, increase contact time, minimum side effects as compared to other topical preparation and oral administration [2].



Figure no 1: ulcer in oral cavity

Herbal medicine used for the treatment of mouth ulcer hygienic agent square measure historically employed by healer and autochthonic healers for the hindrance and treatment of the lesion. The anti-ulcer properties of the foremost ordinarily utilized flavorer medicines and their known active constituents. Biology compounds with anti-ulcer activity embody flavonoids (quercetin, naringin anthocyanosides, and sophoradin derivative), tannins (from linderæumbellatae), gum mucilage. (Gums and myrrh). Among flavorer drugs liquorice, aloe

vera, and shrub (chilli) are used extensively. The ethnomedical system employs many plant extracts for the treatment of lesions [3].

Chamomile (*Matricaria recutita*) (Fig 2) is one of every big healing spices. Chamomile, a well-known drug from the old-time, is known by an array of names, such as Baboonig, Babuna camornile, Babunj, German chamomile, Hungarian chamomile, Roman chamomile, English chamomile, Camomila, Flo's chamomile, Single chamomile, sweet false chamomile, pinheads, cented mayweed, suggesting its widespread use [4,5]. Chamomile is employed both internally and externally to treat an exhaustive list of conditions. It's used externally for wounds, ulcers, eczema, gout, skin irritation, neuralgia, sciatica, rheumatic pain, haemorrhoids, mastitis, and leg ulcer [6,7]. Additionally, it's used externally to treat dermatitis, cracked nipple, chickenpox, and conjunctivitis, and as a dyestuff and conditioner. Chamomile is additionally extensively consumed as a tea or tonic. It's used internally to treat anxiety, hysteria, nightmares, insomnia, and other sleep problems, convulsion, and even DTs [8,9,]. One of the most roles is as a multipurpose digestive aid to treat gastrointestinal disturbance including flatulence, indigestion, diarrhoea, anorexia, kinetosis, and nausea and vomiting. Chamomile is believed to heal the ulcer and act as a herbal bitter to stimulant the liver [10-11]. In children, it's wont to treat colic, croup, and fevers [12,13]. In women's health, it's used as an emmenagogue and uterine tonic. Chamomile's volatile oil is additionally a treatment for helminth infection, cystitis, cold, and flu [14,15]. It contains active compound classes. Sesquiterpenes, flavonoids, coumarins, and polyacetylenes square measure thought of as the foremost very important constituent of the herb-drug [16]. Eleven bioactive synthetic resin compounds, like chlorogenic acid and caffeic acid (phenylpropanoids), apigenin, apigenin-7-O-glucoside, luteolin, and luteolin-7-O-glucoside (flavones), quercetin and rutin (flavanols), and naringenin (flavanone)are found in herb extract [17].



Figure no 2: Flower of Chamomile

More than 120 chemical constituents have been identified in chamomile flowers as secondary metabolites, including 28 terpenoids, 36 flavonoids, and 52 additional compounds with potential pharmacological activity [18,19,20]. Components, such as α -bisabolol and cyclic ethers are antimicrobial, umbelliferon is fungistatic, whereas chamazulene and α -bisabolol are antiseptic [21,22]. The chamomile was found to have the most effective antileishmanial activity [23]. Their flower and flower heads are mainly orange for the production of essential oil. Chamazulene is an artifact formed from matricine, which is naturally present in the flowers during hydrodistillation or steam distillation. The dry flower of chamomile also is in great demand to be utilized in herb tea, baby massage oil, for promoting the gastric flow of secretion and for the treatment of cough and cold [24].

In the present study, an attempt has been made to develop herbal gel by using *Matricaria recutita* for treatment of mouth ulcer and further characterized for various parameters. Stability study and anti fungal activity for the prepared formulation were also studied.

MATERIAL AND METHODS:

Collection of plant material:

The dried flower of *Matricaria recutita* (chamomile) was purchased from Dutipata palace, Bhatenda (E) Rajarhat Chowmatha, Kolkata. The dried flowers were grounded into a coarse powder and stored in an airtight container.

Chemicals:

n-hexane, Carbopol940(Pallav chemical &service Pvt.Ltd), Methyl paraben (Pallav chemical &service Pvt.Ltd), Propyl paraben (Loba chemiev Pvt.Ltd), Propylene glycol (Purenso global), Triethanolamine (Sulab reagent), Distilled water.

Preparation of plant extract:

A pattern of 5gm dried chamomile powder was extracted by 150 ml n-hexane using a Soxhlet apparatus until totally depleted. The whole process took 8 hr. Furthermore, the solvent was evaporated under a vacuum and the obtained extract stored in a glass bottle at 4-5°C [25].

Phytochemical screening

A preliminary phytochemical projection of the flower powder was performed for the appearance of alkaloids, flavonoids, carbohydrate, glycoside, terpenoids, tannins, phenol, saponins using a standard process which is as follow [26]:

Test for alkaloids

Dragendorff's test: To about 3 mL of extract solution, some drops of Dragendorff's reagent were added. (Positive test shows orange-brown coloured precipitate).

Mayer's test: To regarding 3 mL of extract, some drops of Mayer's chemical agent were added. (Positive check shows cream coloured precipitated).

Test for Flavonoids:

Ammonia test: 5 mL of dilute ammonia have been brought to a part of the crude extract observed via way of means of the addition of focused H_2SO_4 . The formation of a yellow colouration withinside the extract shows the presence of flavonoids. The yellow colouration withdraws after a few times.

Alkaline test: The plant extract was treated with 2-3 drops of sodium hydroxide solution. The formation of intense yellow colour, which becomes colourless with the addition of a few drops of sulphuric acid, indicates the presence of flavonoids.

Lead acetate test: The extract was treated with some drop of lead acetate solution yellow precipitated indicate the presence of flavonoids. Orange to crimson colour shows the presence of flavonoids.

Test for carbohydrate

Molisch test: To 2mL of the extract, two ml of Molisch reagent was added and mixed. 2 mL concentrated sulphuric acid was added to this solution formation of the red-violet ring at the junction of the solⁿ and its disappearances on the addition of excess alkali solⁿ indicate the presence of carbohydrate.

Fehling's test: Take 2mL of extract, more 2mL of Fehling's resolution A and Fehling's resolution B to that. Keep it in boiling water for regarding 10 minutes. If there's the formation of red precipitate then the presence of saccharide is confirmed.

Test for glycoside

For Anthraquinone: 5-10 mL of dilute HCl was added in 1 gm of the drug, then boiling it for 10 min in a water bath and filtered with benzene then add an equal amount of ammonia solution to filtrate and shake. The pink-red colour indicates the presence of glycoside.

Test for Saponins

Foam test:

a small amount of the extract turned into shaken with 2ml of water. The persistence of froth produced for ten mins suggests the presence of saponin.

Test for tannins

Ferric chloride test: Treat the extract with 3-4 drops of ferric chloride solution. Formation of bluish-black colour. Indicate the presence of phenol.

Test for terpenoid

Salkowski test: To 2 ml of extract, 2 ml of chloroform, 2 ml of concentrated sulphuric acid were added and shake, red colour at lower layer indicated the presence of steroids.

Preparation of gel formulation:

1 gm of Carbopol 940 was dispersed in 50 ml of distilled water with continuous stirring. The 5ml of distilled water was taken and the required quantity of methylparaben and propylparaben were dissolved by heating on a water bath. Cool the solution, and then add propylene glycol. A further required quantity of extract was added to the above mixture and this solution was mixed properly to the Carbopol 940 gel with continuous stirring. Finally, volume made up to 100 ml by adding remaining distilled water and triethanolamine was added dropwise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel of required consistency. Four gel formulations were prepared using *Matricaria recutita* flower extract [27].

Evaluation test for herbal gel:

The results of different parameters for the evaluation of herbal gel are provided as follows:

The percentage yield of gel:

Weight the empty container in which the gel formulation was stored then again then the container with gel formulation. To obtain the practical yield subtract the weight of the empty container from the container with gel formulation. Then the percentage yield was calculated by the formula given below:

$$\text{Per centage yield} = (\text{practical yield/theoretical yield}) \times 100$$

Physical analysis of the preparation of herbal gel:

The prepared formulation was evaluated for physical parameters such as colour, odour, appearances.

Measurement of pH:

The pH of the natural gel method was determined with the aid of using the use of a virtual pH meter. 1gm of gel dispersed in 10ml of water and kept apart for two hours. Then the pH of the formulations was done using a pH meter and the values are reported [28].

Extrudability:

A closed collapsible tube containing approximately 20g of gel pressed firmly on the crimped and a clamp turned into implemented to save you any rollback. The cap turned eliminated and the gel turned into extruded. The quantity of the extrude gel turned amassed and weighed. The percentage was calculated [29].

Viscosity measurement:

The measurement of viscosity of the formulated gel was determined by Brookfield viscometer with spindle no. 1 at 25°C. The gel was rotated at speeds of 0.3, 0.6, 1.5 rpm. And at each speed, the reading was noted [30].

Spreadability:

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel that is placed in between the slides under the direction of a certain load. If the time taken for the separation of two slides is less then better the spreadability [31]. Spreadability is calculated by using the formula:

$$S = M \times L/T$$

Where M = weight tried to upper slide

L= length of glass slide

T= time taken to separate the slide

Gel strength:

Gel strength was determined by the time in the second required by the weight to penetrate the gel. A 3.5 gm weight was placed on the surface of the 5 gm formulation gel. Gel strength was determined by reporting the time in seconds required to be the weight to penetrate 0.5 cm in the gel [32].

Stability of herbal gel

Stability studies are performed to observe the environmental condition. Stability for prepared formulations was studied at different storage conditions (accelerated stability condition) for 3 months as per ICH guidelines. The samples were withdrawn for evaluation at 1-, 2- and 3-months intervals and assessed for their physical characteristic, pH, viscosity, Spreadability, extrudability, gelling strength [33].

Antifungal activity

The antifungal activity of all optimized formulation and the blank formulation was carried out by cup-plate method in comparison with marked antifungal formulation (Daktarin oral gel). The antifungal test was performed by using *Candida albicans*. Prepared nutrient brought and poured into sterile Petri plates and kept aside for drying and cooling. After that *Candida albicans* culture was spread by a micron wire loop. A sterile cork borer 6mm diameter was used to drill holes 4 mm deep. Then place 0.5 gm of gel from each formulation into these holes. Plates were then incubated at 27°C for 48hr. Then the zone of inhibition for the antifungal activity (diameter in mm) was measured [34,35].

RESULT AND DISCUSSION:

Plant material extraction:

The extracts were obtained by extracting the dried flower of *Matricaria recutita* using n-hexane. The extracted materials were further tested for their chemical constituents present in the extract.

Phytochemical screening:

Phytochemical screening of the extracts was performed and the results were shown in **table 1**. After the Phytochemical screening of the extract of *Matricaria recutita*, it was confirmed the presence of alkaloids, flavonoids, carbohydrates, glycosides, saponin, and terpenoids.

Table no 1: Preliminary phytochemical analysis

Plant constituent			Test	Observation
Alkaloids			Dragendorff's test	+
			Mayer's test	-
Flavonoids			Ammonia test	+
			Alkaline test	+
			Lead acetate test	+
Carbohydrate			Molisch test	+
			Fehling test	+
Glycoside			Anthraquinone	+
Saponins			Foam test	+
Tannins			Ferric chloride test	-
Terpenoids			Salkowski test	+

Preparation of gel formulations:

The gel was prepared using Carbopol 940 and other excipients. The formulation ratio was shown in **table 2** and the prepared formulations were used for further studies.

Table no 2: Composition of a herbal gel formulation with Carbopol 934.

Ingredients	F1 (1%)	F2 (2 %)	F3 (4 %)	F4 (Gel base)
Extract (<i>Matricaria recutita</i>) (gm)	1	2	4	-
Carbopol 940 (gm)	1	1	1	1
Methylparaben (gm)	0.5	0.5	0.5	0.5
Propylene paraben (gm)	0.2	0.2	0.2	0.2
Propylene glycol (gm)	2	2	2	2
Triethanolamine (ml)	1	1	1	1
Distilled water (ml)	Qs	Qs	Qs	Qs

The percentage yield of gel:

The percentage yield of all the prepared formulations were represented in **table 3**.

Table no 3: Percentage yield of gel formulation

Formulation	Percentage yield (%)
F1	95.985
F2	97.110
F3	94.421
F4 (base)	96.992

Physical analysis of the prepared herbal gel

The freshly prepared base was colourless and the other formulations were yellow to brown. Regarding the base formulation and other formulations, the consistency was good and the odour of all the formulations except the base formulation was characteristic.

Table no 4: Evaluation parameter of prepared herbal gel

Formulation	pH	Viscosity	Spreadability	Extrudability	Gelling strength
F1	6.15	4700	25±0.42	84.76±0.007	35±0.15
F2	6.30	4800	30±0.53	96.12±0.007	41±0.15
F3	6.31	4800	30±0.53	90.54±0.006	39±0.24
F4	6.45	4600	25±0.49	83.68±0.004	28±0.75

Determination of pH:

The pH of the gel formulations was recorded in the range of 6 to 6.5 which is normal pH range of the oral cavity. The result for pH for all formulations is shown in **table 4**.

Extrudability:

The extrudability reflects the capacity of gel, to get rejected in uniform and desire quantity when the tube is a squeeze. From the result, all formulation shows good and excellent; this depends on the polymer concentration.

Viscosity measurement:

The viscosity of all the prepared formulations was measured by Brookfield viscometer. The viscosity of various samples was calculated and tabulated in **table 4**.

Spreadability:

The herbal formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by the distance by the slides. The time taken for the upper slide to travel the distance and separated from the lower slide under the influence of weight was noted. The study of Spreadability was that with increasing the viscosity of formulation Spreadability will be wise –versa. The result was calculated and shown in **table 4**.

Gel strength:

The gel strength was determined by the time and seconds required by the weight to penetrate the gel. A 3.5 gm. weight was placed on the surface of 5 gm formulated gel. Gel strength was determined by reporting the time in seconds required by the weight of penetrating 0.5 cm in the gel.

Stability study:

The stability of the prepared formulation was assessed using accelerated stability conditions. pH, viscosity, Spreadability, extrudability, and gelling strength were studied at an interval of 1, 2, and 3 months. The results for all the parameters were shown in graphs from **Fig 3-7**.

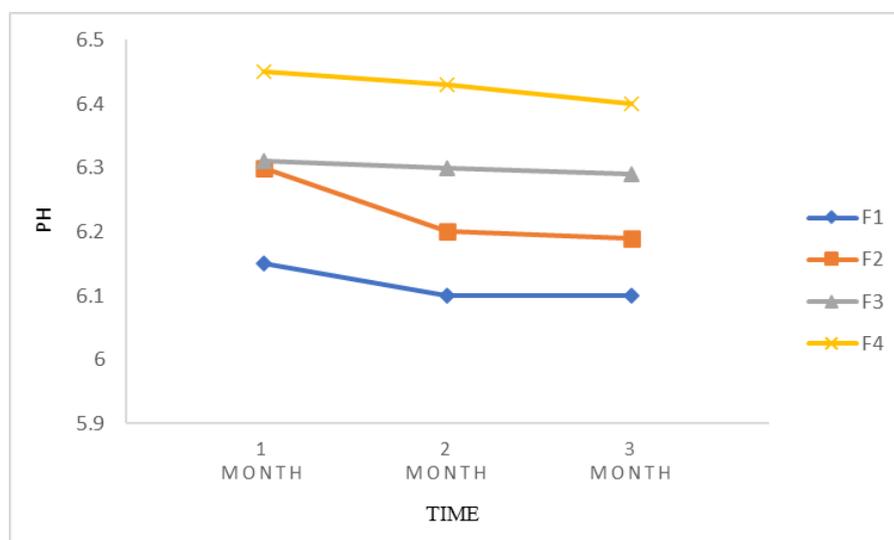


Figure no 3: pH parameter during stability study for all the prepared formulation subjected to accelerated storage condition

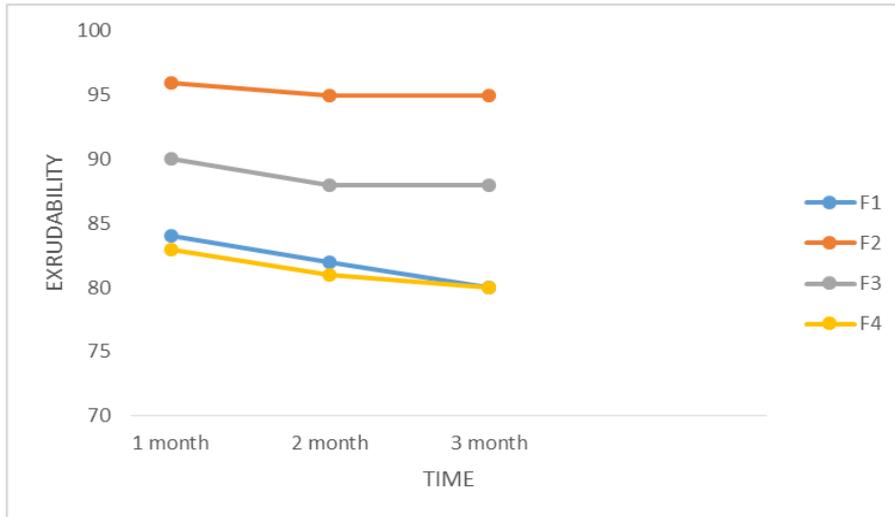


Figure no 4: Extrudability parameter during stability study for all the prepared formulations subjected to accelerated storage condition.

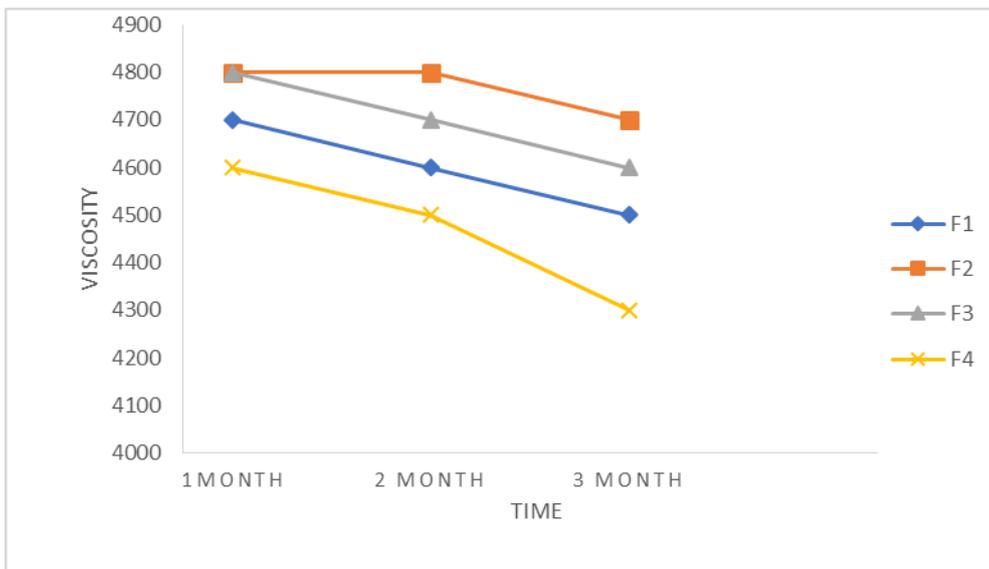


Figure no 5: Viscosity parameter during stability study for all the prepared formulations subjected to accelerated storage condition.

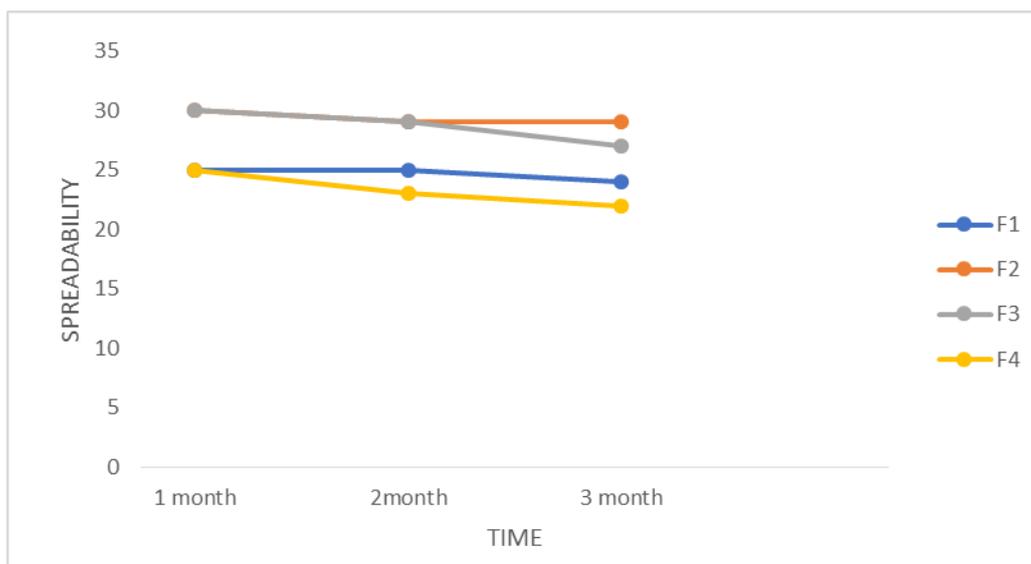


Figure no 6: Spreadability parameter during stability study for all the prepared formulations subjected to accelerated storage condition.

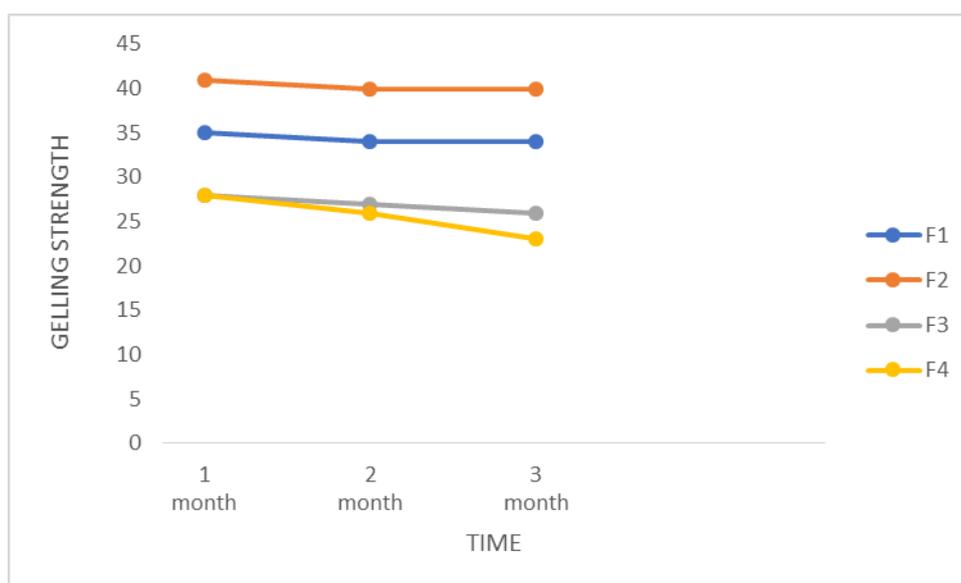


Figure no 7: Gelling strength parameter during stability study for all the prepared formulations subjected to accelerated storage condition.

Antifungal activity:

Among all the prepared formulations containing *Matricaria recutita* formulation, two (F2) was selected for the antifungal activity because it shows good characteristics properties than the other formulations. Antifungal activity was carried out using the cup-plate method. Formulation two was compared with the standard drug (Daktarin oral gel) and base

formulation. The prepared formulation showed antifungal activity against microorganisms (*Candida albicans*) responsible for mouth ulcers. The result was shown in **table 5**.

Table no 5: Antifungal activity

Formulation	Zone of inhibition
Standard drug (Daktarin)	22
F2	18
Base	13

CONCLUSION:

Natural remedies are more acceptable in the belief that they are safer with lesser side effects than synthetic medicines. Nowadays herbal formulations are in demand in the market. In this study herbal gel was prepared with different concentrations of *Matricaria recutita*. The study shows that the developed formulations show suitable for drug delivery and therapeutically active. Stability study shows that the formulations are stable and formulation used for antifungal activity shows good activity. So, it is safe, stable and good for the treatment of mouth ulcers.

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