Formulation and Evaluation of Antifungal Activity of Polyherbal Gel

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Abstract

Typically, plants have been used to cure various ailments in accordance with their traditional uses. Throughout the world, the usage of drugs derived from plants is growing in popularity. Thus, the current study's objective was to create a polyherbal gel using aqueous extracts of several plants and assess its antifungal effectiveness.The gel formulation consisted of *Argemonemexicana, Terminalia arjuna,* and *Azadirachtaindica*'s aqueous extract. The resulting formulation's antifungal activity was tested using the disc diffusion method on isolated fungi. Additionally, the formulation's appearance, homogeneity, pH, viscosity, and rheological tests, spreadability, and percentage of drug release were all physically evaluated.The created formulation F3 had the highest zone of inhibition among all the prepared formulations, according to stability and physicochemical assessments as well as microbiological testing.Our study led us to the conclusion that these formulations had effective antifungal properties, making them safe for use on human skin.

Keywords: Anti-fungal, *Azadirachtaindica*, Candida, Polyherbal gel, *Terminalia arjuna.*

1. Introduction

Herbal remedies have historically served as the foundation for the treatment and healing of a variety of illnesses in India. [1] Additionally, Indian folk medicine includes a variety of prescriptions for treating various medical conditions, including ulcers, ulcerative colitis, leprosy, scabies, diarrhea, and venereal diseases.[2] According to Babu (2002), more than 80% of the world's population still uses conventional treatments for a variety of skin conditions.[3]

Due to their extensive usage and unclear benefit/risk ratio, herbal therapies administered topically have drawn a lot of interest. [4] Numerous medicinal herbs are used extensively to treat skin conditions and are also known to have antibacterial properties.[5]In comparison to cream and ointment, topical administration of gels at pathological locations offers substantial benefits in a quicker release of a medicine straight to the site of action.

To treat fungi-related illnesses, there has been a significant rise in the demand for herbal remedies, skin care products, and even cosmetics. Since the skin is the area of our bodies that is most exposed to pathogens, it needs to be protected against skin conditions, particularly

fungus. Overuse of antibiotics results in resistant strains of disease-causing bacteria. This complex change depends on several variables in the host, such as hormones, stress, and the organism's receptivity to treatment. The potential benefits of herbal therapy treatments for this condition have been investigated. The herbal extracts were modified and created as poly herbal gel since they couldn't be utilized directly for the therapy.

A. indica, A. mexicana, and *Terminalia arjuna* were medicinal plants used for this experiment. According to studies by Rahman (2004),[6] and More (2016),[7] these three key herbs contain considerable amounts of phytochemicals that exhibit antibacterial and anti-antifungal effects. We developed a polyherbal topical preparation and evaluated its anti-fungal efficacy in response to the rising popularity of natural and herbal medicines, the simplicity of obtaining raw materials, their affordability, and the rarity of reported adverse reactions.

2. Methodology

2.1 Plant Material Collection

Fresh leaves of *Argemonemexicana, Terminalia arjuna,* and *Azadirachtaindica* were collected, washed and dried in shade at room temperature, powdered mechanically and sieved through 40 no. mesh sieve. These fine powders of the leaves were stored in an air-tight container until further use.

2.2 Preparation of Aqueous extract

Aqueous extracts of all the 3 leaves were prepared using the maceration technique. The extract weighed 500 g, and water was added separately. The whole mixture was shaken often every two hours while being stored in dark settings for seven days. The extract was filtered after seven days, and the filtrate was then concentrated by distilling the solvent to a third of its original volume. The extract was then heated to no more than 60°C and concentrated over a water bath. The extract was subsequently dried over a desiccant for an additional night before being utilized in the study.

2.3 Phytochemical analyses of prepared extract

Qualitative tests were conducted to determine the existence of flavonoids, tannins, saponins, alkaloids, phenolics, and carbohydrates. [8-10]

2.4 Preparation of the polyherbal gels

Gel was preparedutilizing extract concentrations of 1%, 2%, and 5%. Carbopol 934 was evenly dissolved in distilled water in a different beaker while being continuously stirred, and it was allowed to soak for 24 hours. Propyl and methyl parabens were dissolved in distilled water in another beaker. The extracts were added to this solution and thoroughly triturated.The aforesaid combination was then thoroughly mixed into the carbopolmixture. Finally, triethanolamine and propylene glycol were added, and the pH was set to 6.8 to 7. Table 1 shows the compositions of gel formulations.

Table 1 - Compositions of gel formulations

S. No.	Ingredients	F1 (%)	F2 (%)	F3 (%)
1.	Aq. Extract of *T. arjuna*	1	2	5
2.	Aq. Extract of *A. indica*	1	2	5
3.	Aq. Extract of *A. mexicana*	1	2	5
4.	Carbopol	2	2	2

5.	Propylene glycol	2	2	2
6.	Triethanolamine	2	2	2
7.	Propyl paraben	0.1	0.1	0.1
8.	Methyl paraben	0.1	0.1	0.1
9.	Distilled water	q.s	q.s	q.s

2.5 Evaluation of formulated gels

The gel was evaluated using the following criteria.

- **Homogeneity**

After being set in the field, all formulated gels underwent visual homogeneity inspection testing.

- **pH of the gel**

Using a virtual pH meter, the pH of the gelswas determined.

- **Viscosity**

Using a Brookfield digital viscometer, the viscosity of the produced gels was measured. Spindle number 64 was used to test the viscosity at 10 rpm and 25 °C.

- **Extrudability**

Standard collapsible aluminum tubes with caps were filled with the gel compositions, and the ends were crimped shut to seal. The tubes' weights were documented. The tubes were clamped after being positioned between two glass slides. The slides were covered with 0.5 g, and then the cap was taken off. The extruded gel's volume was collected and weighed. Calculated percentages of the extruded gel included excellent (>90%), good (>80%), and fair (>70%).

- **Spreadability**

The two glass plates measuring 5 x 2 cm were used to place the gels. By evenly applying a 100 g weight to the slides, the formulation was made to be sandwiched between the two slides. The extra gel was scraped off as well as the weight that had been added. Only the lower slide became securely secured via the clamp when two slides were kept in place at a 45° angle without even the tiniest movement, allowing the upper slide to slide off easily with the help of a 20-gram weight attached to the upper slide. It was noted how long it took for the upper slide to separate from the lower glass plate. The test was performed three times, and spreadability was computed as follows:

"S = W x L/T"

Where, L =Length of the glass plate, S = Spreadability, T = Time taken (sec), W=Weight tied to the upper plate.

- **In vitro drug release study**

With a 25 mL cell capacity, the Franz diffusion cellular was used for the drug launch testing. One gram of gel is evenly distributed over the surface of the egg membrane in a predetermined area. The receptor chamber was then filled with a freshly produced phosphate buffer (pH 5.8) solution. A magnetic stirrer was used to create movement in the receptor chamber. At regular intervals, 1.0 ml aliquots were removed and flushed with new buffer solution. After appropriate dilutions, samples were examined for drug concentration using a

UV visible spectrophotometer at 280 nm (eugenol) and 342 nm (piperine). Medication secreted over the egg membrane was measured over a period of time.[11]

- **Stability**

Base and formulation stability was investigated under various storage settings and evaluated for physical traits such as appearance, color, and odor (for 30 days). [12]

2.6 Evaluation of the anti-fungal activity

- **Microorganisms**

The *Candida albicans* species and clinically important dermatophytes were used to test the antifungal activity. *Microsporumgypseum*, *Trichophytonrubrum*, and *Epidermophytonfloccosum*were the three dermatophytes utilized for screening.

- **Determination of Antifungal Activityby Disc Diffusion Method**

The Disc Diffusion Method was used to carry out the standard anti-microbial test assay, which involves spreading 100 µl of a solution containing 104 fungus spores per ml on Sabouraud Dextrose Agar (SDA). Whatman filter paper discs of 6 mm in diameter were placed in a Petri dish with a volume of 15–18 µl, the SDA was poured in, and the dish was allowed to cool. With the use of a micropipette, 10 µl of the produced gels (1 mg per ml) were obtained and applied to the SDA-inoculated Petri dish plate.

As a vehicle control, 5% Dimethyl Sulfoxide (DMSO) and 2% Clotrimazole (1 mg per ml) were used. Different gel formulationswere used, and they were poured onto various Petri dish plates. For 72 hours, the infected Petri plates were incubated at 37°C.

The ZOI (Zone of Inhibition), which represents measuring the area where gels inhibit organism growth, was used to screen for antifungal activity. The experiment was repeated with each of the four organisms—*Trichophyton spp., Microsporum spp.,Candida spp.,* and *Epidermophyton spp*.—individually, and the ZOI was assessed with a digital dial caliper. The ZOI was recorded after a 72-hour incubation period, and the experiment was done five more times.

2.7 Statistical analysis

All of the data were subjected to a statistical analysis using Statistical Package for the Social Sciences (SPSS) 25, and p-values that were lower than 0.05 were considered to be indicative of statistical significance. The data were all summarized using the mean ±standard deviation (SD).

3. Results

3.1 Phytochemical analysis

When plant extracts were subjected to a preliminary phytochemical study, various phytochemicals were discovered. Coumarins, Phenol, Flavonoids, Alkaloids, Terpenoids, Quinones, Triterpenoids, Carbohydrates, and Saponins were found in the aqueous extract of *T. arjuna*. Coumarins, Tannins, Flavonoids, Alkaloids, Terpenoids, Quinones, Saponins, and Carbohydrates were discovered in the aqueous extract of *A. mexicana*, while Tannins, phenol, Alkaloids, Coumarins, Terpenoids, Quinones, Saponins, and Carbohydrates were discovered in the aqueous extract of *A. indica* (Table 2).

Table 2. Phytochemical screening of plant extracts of *T. arjuna, A. mexicana* and *A. indica*

Secondary metabolites	***T. arjuna***	***A. mexicana***	***A. indica***
	Aqueous Extract	**Aqueous Extract**	**Aqueous Extract**
Coumarins	+ +	+ +	+ +
Tannins	-	+	+ +
Phenol	+ + +	-	+ + +
Alkaloids	+ +	+ + +	+ +
Quinones	+	+	+
Carbohydrates	+ +	+ +	+
Flavonoids	+ + +	+ + +	-
Triterpenoids	+ + +	-	+ + +
Saponins	-	+ +	+ + +
Terpenoids	+ + +	+ +	+ + +

3.2 Organoleptic Properties of polyherbal gels

Table 3 represents the organoleptic properties of all formulated polyherbal gels recorded for a time interval of 1 month. The polyherbal gel formulations F1, F2, and F3 were creamish to off-white in color.All the formulations showed good homogeneity, characteristicodour, and semi-solid appearance. Formulation F2 showed a bad smell and a liquidy appearance at the 4th week indicating the deterioration of the gel.

Table 3. Organoleptic Properties of all formulated herbal gels

Formulation Code	**1 week**	**2 week**	**3 week**	**4 week**
Odour				
F1	Characteristic	Characteristic	Characteristic	Characteristic
F2	Characteristic	Characteristic	Characteristic	Characteristic
F3	Characteristic	Characteristic	Characteristic	Bad smell
Appearance				
F1	Semi-solid	Semi-solid	Semi-solid	Liquidy
F2	Semi-solid	Semi-solid	Semi-solid	Liquidy
F3	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Color				
F1	Creamish	Creamish	Creamish	Off white
F2	Creamish	Creamish	Creamish	Creamish
F3	Off white	Off white	Off white	Off white
Homogeneity				
F1	Good	Good	Good	Good
F2	Good	Good	Good	Good
F3	Good	Good	Good	Good
Extrudability				
F1	Excellent	Excellent	Good	Good
F2	Excellent	Excellent	Excellent	Good
F3	Good Excellent	Good	Good	Good

3.3 Spreadability of herbal gels

The spreadability of formulation F1 was in the range of 8.3-8.5 gm-cm^2, for F2 it was in the range of 9.7-9.8 gm-cm^2, and for F3 it was in the range of 9.2-9.4 gm-cm^{2}respectively. Fewer variations in all three formulations were found with respect to time (figure 1).

Figure 1. Graphical illustration of polyherbal gels spreadbility

Figure1 shows that formulation F2 showed the highest spreadabilityin 3rd week while the least spreadability was seen from formulation F3 in 1st week.

3.4 Viscosity of all formulations

The viscosity of formulation F1 was in the range of 7731-7752 cps, for F2 it was in the range of 7689-7698 cps, and for F3 it was in the range of 7770-7789 cps respectively. Maximum variations in viscosity were seen in F3 with respect to time.

Figure 2. Graphical illustration of polyherbal gels viscosity

Figure 2 shows that formulation F2 showed the highest viscosityin 3rd week while the least viscosity was seen from formulation F3 in 1st week.

3.5 pH of all formulations

The pH of formulation F1 was in the range of 6.82-6.90, for F2 it was in the range of 6.85-6.96, and for F3 it was in the range of 6.75-6.84 respectively. The gels were showing almost neutral pH.

Figure 3. Graphical illustration of polyherbal gel formulation's pH value

Figure3 shows that formulation F2 showed the highest pHin 3rd week while the least pH was seen from formulation F3 in 1st week.

3.6 Stability study

Stability tests were performed on the developed gels for one month and all the formulations were found to be stable till 3 weeks. In all prepared formulations, there was no visible colourchanges. All formulations' pH values remained within a limited range, between 6.75 and 6.96. The spreadabilityand viscosity of all gels remained constant and fell within the range.

3.7 In-vitro drug content

Maximum drug release was seen at the 8th hour. Formulation F3 showed the highest drug release as compared with other formulations.

Figure 4. Graphical illustration of polyherbal gel formulation's in vitro drug release

Figure4 shows that formulation F3 showed the highest in-vitro drug release at the 8th hour.

3.8 Antifungal activity

Table 4. Antifungal activity against different fungal isolates

Formulation Code	**Zone of inhibition (mm)**			
	Candida albicans	***Microsporumgypseum***	***Trichophyton rubrum***	***Epidermophytonfloccosum***
Std. drug	19.78 ±0.32	20.56±0.02	19.65±0.11	20.98±0.02
F1	5.89±0.03	6.25±0.14	6.98±0.12	6.48±0.21
F2	6.23±0.56	6.99±0.02	7.59±0.1	6.95±0.21
F3	6.52±0.03	7.65±0.01	10.62±0.01	8.11±0.02

Table 4 represents the zone of inhibition of all formulated polyherbal gels. The antifungal activity of the gels was tested against 4 different fungal isolates; *C. albicans*, *M. gypseum*, *T.*

rubrum, and *E. floccosum*. Ketoconazole was taken as the standard drug. The formulations were mostly active against *T. rubrum* and then *E. floccosum*. The activity of the formulated gels against *C. albicans* was low. Formulation F3 showed more anti-fungal activity as compared to the other formulations.

4. Discussion

Fungal illnesses are a serious threat to global health and one of the leading causes of morbidity and mortality.[13]Infections in humans, especially those that affect the skin and mucosal surfaces, are a severe issue, particularly in tropical and subtropical developing nations.[14] Fungal infections in humans may vary from superficial to very invasive or widespread, and they have sharply risen recently. Mycoses have trailed behind bacterial chemotherapy in terms of treatment, and there are fewer antifungal drugs on the market than there are antibacterial ones. Therefore, it is imperative to look for new antifungal medications.[15]

The most prevalent infectious fungal illness in humans, dermatophytosis, is caused by pathogenic fungi called dermatophytes that may enter keratinized tissues in both people and animals.[16] The majority of clinical isolates (80%) of the pathogenic fungus *Trichophyton rubrum* (*T. rubrum*) are found in humans.[17] There is a need to find novel antifungal medications free from toxicity and side effects due to the rise in drug resistance in human infections and the emergence of undesired effects from certain antimicrobial drugs.

Because of their potential to address the problem of drug resistance in microorganisms, plant extracts or chemicals produced from plants are anticipated to become an important source of novel therapeutic agents. [18]

In the search for novel antifungal medications, the study of plants with putative folkloric antifungal properties should therefore be viewed as a profitable and prudent research strategy. *Terminalia arjuna*, *A. indica*, and *Argemonemexicana* are three medicinal plants receiving significant attention for their antifungal properties.

This study focused on these three plants because of their combined anti-fungal activity. These plants' extracts were combined, and polyherbal gel was created using various extract formulations. The stability, anti-fungal activity, and physicochemical characteristics of the developed gels were evaluated. The anti-fungal activity was assessed using the disc-diffusion technique, and the zone of inhibition was noted.

The formulation F3 showed highest zone of inhibition among all the formulations. Similarly, Kale, P.S., 2022[19] developed polyherbal gel and assessed its antifungal efficacy. The polyherbal gel was made using a mixture of *Tridaxprocumbens* and *Azaridacta indica*, and its effectiveness against *Candida albicans* was examined. *C. albicans* was effectively inhibited by the gels' antifungal properties.

Since the *Azadirachtaindica* tree's whole body is utilized for medicinal and aesthetic purposes, it has also been used to treat several other skin conditions. The chemical constituents of *Azadirachtaindica, Terminalia arjuna*, and *Argemonemexicana* are considered to be antiseptic.

It can be stated that that the secondary metabolites azadirachtin, nimbin, mahmoodin,and nimonol found in plant*A. indica* have an impact that is antagonistic to the growth of microorganisms.[20]

In *T. arjuna*, the secondary metabolites methyl gallate, gallic acid, andother flavonoidaland triterpenoidal constituents are the most frequent, and they may be the ones that are responsible for the antifungal activity of the plant while for*A. mexicana*, the secondary metabolites n–Hexadecanoic acid, 9,12-Octadecadienoic acid, Stearic acid, methyl ester, 9-Eicosene, Tetradecanoic acid, Bis(2-ethylhexyl) phthalate are the most frequent, and they may be the ones that are responsible for the antifungal activity of the plant.In in-silico research, these secondary metabolites should be further processed to isolate them for use as a ligand in the process of targeting some of the proteins that serve as targets for the enzymes that they inhibit in the metabolic processes of the fungal organism.

5. Conclusion

A. indica, T. arjuna, and *A. mexicana* has noted a superb anti-fungal action in the polyherbal mixture. Additionally, it has been demonstrated that the combination of multiple ingredients in this formulation exhibits synergism. All of the plant extracts showed antifungal activity, with formulation F3 showingthe greatest zone of inhibition.Despite the fact that a number of researchers have labored to develop traditional plant-based medicinal remedies for a variety of diseases. In light of the preceding discussion, it is evident that the results of the present study are superior to those of conventional research.

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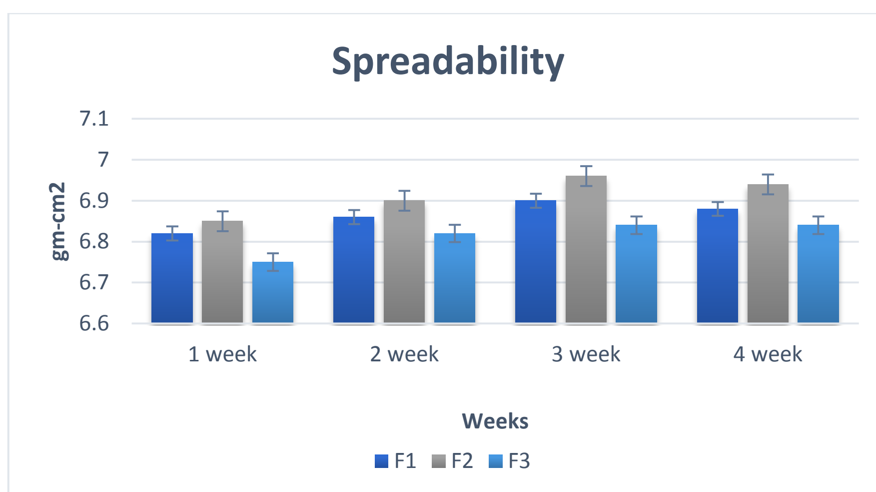

Figure 1. Graphical illustration of polyherbal gels spreadbility

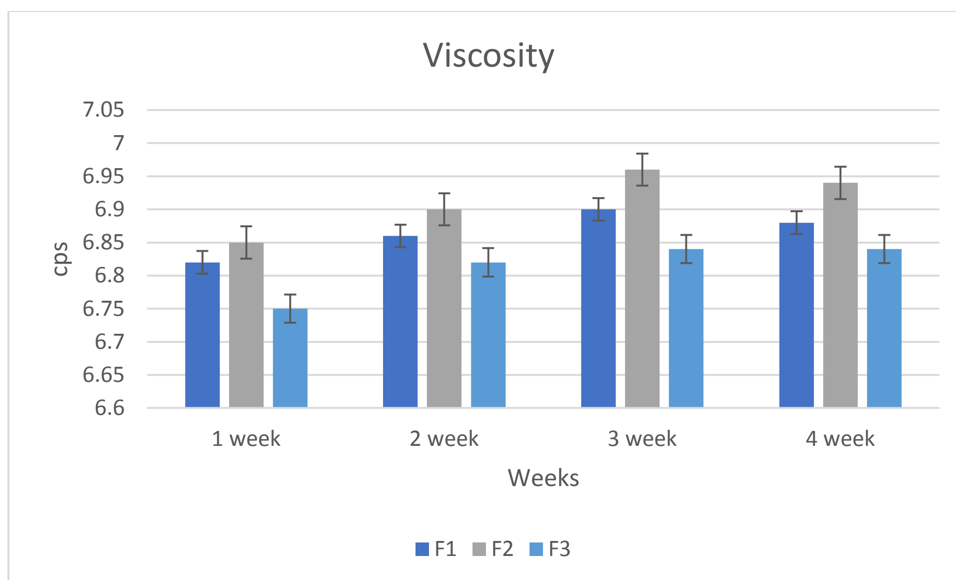

Figure 2. Graphical illustration of polyherbal gels viscosity

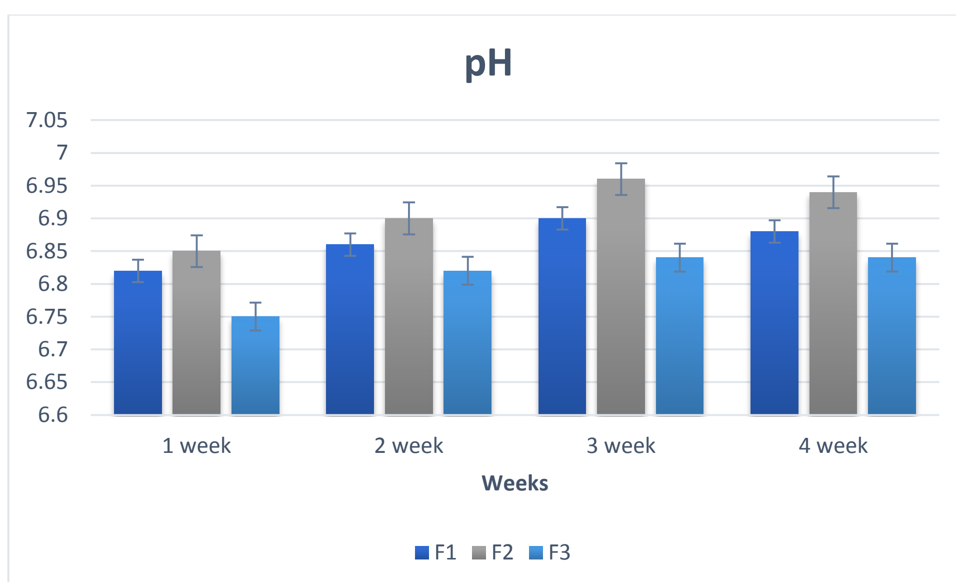

Figure 3. Graphical illustration of polyherbal gel formulation's pH value

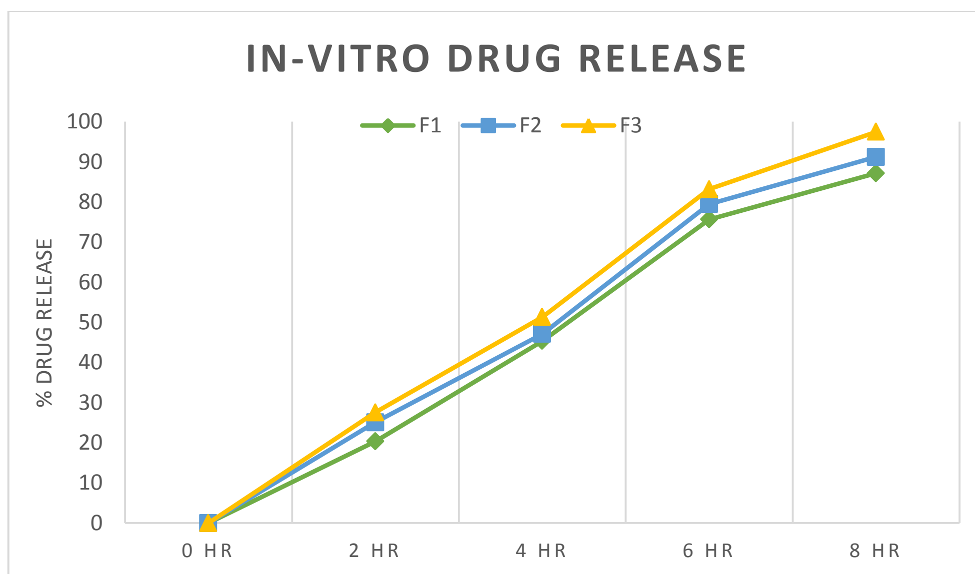

Figure 4. Graphical illustration of polyherbal gel formulation's in vitro drug release