

# Development, Standardization and Evaluation of Antimicrobial Syrup

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## ABSTRACT

Ayurveda states that it takes more than just herbs to accomplish a medicinal impact. Optimizing its composition as a combination of herbs in a certain ratio improves its medicinal impact while reducing toxicity. The purpose of this research was to make a polyherbal syrup using aqueous extracts of *Azadirachta indica*, *Curcuma longa*, *Allium sativum*, *Ocimum tenuiflorum*, and *Punica granatum* in varying proportions in order to provide such an intervention. The agar well diffusion technique was used to screen the formulation for antimicrobial and antifungal activity against various organisms, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, and *Aspergillus niger*, which are representative of gram-positive and gram-negative bacteria, respectively. The syrup underwent review and standardization for multiple assessment criteria. Additionally, this formulation was subjected to an expedited stability test. Research demonstrated that the tested formulation outperformed the gold standard in terms of zone of inhibition. The formulation that has been produced meets all of the requirements, however, additional *in-vitro* and *in-vivo* investigations are needed to confirm its effectiveness.

**Keywords:** Polyherbal, Antimicrobial, Phytoconstituents, Stability studies

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## INTRODUCTION

Antibiotic resistance has been on the rise in recent years, piquing researchers' interest in finding stronger antimicrobials to fight this growing threat. A large variety of complex, pure secondary metabolites and structurally diverse molecules with antibacterial potential can be found in natural products, which continue to be a significant source of novel medications.

There is a dearth of scientific proof supporting the therapeutic effects of polyherbal therapy despite its long history of usage in Ayurvedic, Chinese, and Unani medicine. Polyherbal formulations, as opposed to mono-herbal ones, are more effective in these systems for managing a variety of chronic conditions owing to synergism and reduced adverse effects. In Western medicine, the idea of polyherbal combination has been around for a while and has been quite successful, giving patients new hope. Many studies in the pharmaceutical industry have shown that, as compared to monotherapy, combination therapy with antibiotics and plants is more effective in treating diabetes and cancer.<sup>1</sup> The *in-vitro* antibacterial results of synergy between plant extracts and antibiotics were published in a literature review that covered five years. Combinations of plant extracts and their respective components were more effective than anyone alone. Optimal ratio combinations of herbs with different pharmacological

activities and potencies have shown promise in previous research, suggesting that the combined effects may be stronger than those of the individual herbs used alone.<sup>2</sup> This led to the idea of creating a polyherbal formulation based on plant bioactive compounds to treat a wide range of illnesses. It works synergistically to fight infections caused by microbes and speed up the healing process by killing the microbes.<sup>3</sup>

Steroids, alkaloids, tannins, triterpenes, flavonoids, and anthraquinone *tenuiflorum* glycosides are abundant in the phytochemical composition of tulsi (*Ocimum tenuiflorum*) and *Azadirachta indica* (neem). Both have a long history of traditional medicinal use due to their antibiotic, antimicrobial, antioxidant, skin problem, and wound-healing capabilities, among other uses. *A. indica* has a long history of medicinal use because to its many beneficial effects, including reducing inflammation and fever, warding off malaria, soothing stomach ulcers, lowering blood sugar, and even acting as a neurotransmitter and antibiotic. A number of traditional medicinal uses have been discovered for *O. tenuiflorum*, including its antioxidant, antidiabetic, chemopreventive, antiulcer, anti-carcinogenic, anti-stress, and immune response modulation capabilities. Antifungal, antibiotic, and antibacterial effects are only a few of the traditional uses for *Curcuma longa*'s medicinal qualities. Traditional medicine practitioners have long relied on the antifungal, antimicrobial,

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hypoglycemic, hypolipidemic, and antibacterial effects of *Allium sativum*. You can find medicinal uses for the whole *Punica granatum* L. plant, from the trunk and skin to the seeds, roots, and flowers. When you have diarrhea, you can utilize the blossoms of *P. granatum* L. to help. Bioactive components found in *P. granatum* L. have potential applications in the treatment of a wide range of medical conditions, including but not limited to: oral and cardiovascular illnesses, cancer, diabetes mellitus, skin infections, hemorrhoids, and various bacterial and fungal infections. Peel extract from *P. granatum* L. contains polyphenols that reduce the danger of cancer and cardiovascular disease by inhibiting oxidation, microbial growth, and free radical elimination.<sup>4</sup> Vitamin C is also abundant in the peel extract of *P. granatum* L. The pharmaceutical sector finds it valuable as a raw material due to its antibacterial qualities. The antimicrobial properties of gallic acid, punicallics, and ellagic acid, which were isolated from *P. granatum* L., were found to be effective.<sup>5</sup>

Literature reviews have shown that the aforementioned plants have antimicrobial and microbiological effects. This study aimed to prepare a polyherbal syrup by combining water-based extracts of *A. indica*, *C. longa*, *A. sativum*, *O. tenuiflorum*, and *P. granatum*. Then, the formulation was tested for pH, total solids, specific gravity, and viscosity. This formulation was also the subject of an expedited stability test.

## MATERIALS AND METHODS

### Chemicals

The study utilized substances of an analytical grade. Hi-Media Pvt. Limited of Bombay, India, supplied the solid media and broth utilized in the microbial culture.

### Collection and Extraction of Plants Parts

The native regions of Dhule district, Maharashtra, were surveyed in the month when the fresh leaves, peels, buds, and rhizomes were most abundant. We got our *A. sativum* at the neighborhood grocer. The botanical survey in Pune, India, was responsible for the plant's taxonomical identification. To preserve their phytoconstituents, the freshly picked leaves of *A. indica* and *O. tenuiflorum*, as well as the fruit peels of *P. granatum*, roots of *C. longa*, and cloves of *A. sativum*, were dried in a hot air oven set at 40°C. We used a grinding mill to finely ground the plant components after they dried, and then we stored them in an airtight container. The 30 g powder from each specimen was first defatted in a Soxhlet device using petroleum ether at 60 to 80°C and then extracted with water. Extra concentration was achieved using a rotary evaporator, and the resulting extracts were stored in a vacuum dryer until they were needed.

### Phytochemical Screening of Extract

Preliminary phytochemical screening of plant extracts using different chemical test. A variety of phytochemicals, including the tannins, phenols, alkaloids, phytosterols, triterpenoids, anthraquinone glycosides, flavonoids, saponins, cardiac glycosides, carbs, proteins, and more, are tested at this stage.

### Microbial Analysis

Microbial analysis ensures polyherbal formulation safety and quality throughout manufacturing. These medications include plant extracts that promote bacteria growth, endangering consumers. Bacteria, fungi, and molds may damage product stability and safety, posing health risks. Microbiological analysis identifies and quantifies these contaminants, enabling formulators to reduce hazards and meet regulations. By monitoring microbial load during preparation, practitioners may ensure the polyherbal mixture meets microbiological standards. Microbiological analysis keep the product fresh and extends its shelf life, preventing spoilage and ensuring safety. Quality control using microbiological analysis improves polyherbal formulations' efficacy and safety, ensuring customers of a reliable and safe herbal product.

#### Total viable aerobic count

To evaluate each extract, dissolve 10 mg in 100 mL of buffered sodium chloride peptone solution (pH 7). Digital colony counter plate count was assessed, and the aerobic viability of the sample was assessed. Mix 1-mL of pretreatment preparation with 15 mL of liquid casein soybean digest agar in 10 cm petri dishes while the temperature is below 45°C. To get a colony count lower than 300, dilute the pretreatment. Each sample with the same dilution was cultured in two petri dishes at 30 to 40°C for two days. Colonies were counted by the computer. We computed the findings for the plate containing the maximum number of colonies (300 at most) in order to ensure a fair evaluation. Instead of using soybean digest agar for bacterial testing, incubate Sabouraud's dextrose agar with antibiotics at 20 to 25°C for 5 days. This should be done until a more precise count is achieved earlier. Colony counters determined outcomes on plates containing 100 or fewer colonies.

#### Total yeast and mould count

Combine 10 grams of each extract's test mixture with 100 mL of nutrient agar. After adding streptomycin, we incubated the mixture at 40 to 42°C for 4 hours. Incubate 1-mL of material on Sabouraud's dextrose agar medium and 1-mL on corn meal agar for four days at a temperature range of 35 to 37°C.<sup>6</sup>

#### Test for presence of *Escherichia coli*

Add 10 grams of each extract to the test mixture and 100 mL of lactose broth. We incubated the mixture at 35 to 37°C for four hours. To incubate at 43 to 47°C for 24 hours, add 1-mL of sample to 100 mL of MacConkey broth. After preparation, the MacConkey agar media was inoculated with the subculture, and the mixture was incubated at 43 to 47°C for 24 hours. The presence of *E. coli* could be suggested by the growth of red, typically non-mucoid colonies of gram-negative rods.<sup>7</sup>

#### Test for presence of *salmonella typhimurium*

Combine 10 grams of each extract with 100 mL lactose broth for testing. We incubated the mixture at 35 to 37°C for four hours. The next step was adding 10 to 100 mL of tetrathionate bile vivid green broth and incubating it at 42 to 43°C for 18 to 24 hours. Incubate the 1-mL sample at 35 to

37°C for 24 hours on top of the xylose lysine deoxycholate agar medium. Colonies of *Salmonella typhi* that were fully matured and had red coloration with or without a black center were present.<sup>8</sup>

### Preparation of Polyherbal Syrup

These polyherbal syrups are carefully developed by knowing each plant's therapeutic characteristics, compatibility, and synergy. These formulations provide a more thorough therapy and reduce the side effects of high herb dosages. Polyherbal syrups may treat respiratory, gastrointestinal, and immune system problems, highlighting their importance to demonstrate the effectiveness and safety of polyherbal formulations, adding to the data supporting their therapeutic use in contemporary medicine. Polyherbal syrup formulations and development combine traditional herbal knowledge with modern healthcare demands for best patient results, making them an attractive pharmaceutical research option.<sup>9</sup>

#### Preparation of simple syrup (Syrup base)

A sucrose sample with a concentration of 66.7% by weight was measured and combined with filtered water. The mixture was then heated and stirred intermittently until the sucrose completely dissolved. Exactly enough boiling water was added to provide a total volume of 100 mL.

#### Preparation of polyherbal syrup

Their notable activity was the deciding factor in the selection of extracts. Three distinct formulations, designated as formulations F1, F2, and F3, were prepared by completely mixing all of the constituents. Table 1 displays the composition of three developed formulations (F1, F2, and F3) that contain a combination of extracts and excipients.

#### Procedure for polyherbal formulation

A 100 mL volumetric flask with 100 mL distilled water received appropriate solubilizers (Tween 80). The mixture was heated and shaken until dissolved. In order to dissolve the medication, the correct drug extracts were combined and then

heated while occasionally being shaken. Then, while stirring from time to time, the hydrotropic agent (sodium benzoate) and the suspending agent (xanthan gum) were added and heated until dissolved. A 66.7% sucrose solution was added next. After vigorous shaking, the flask was heated until the sucrose dissolved, with occasional shaking. Distilled water was then added to fill the capacity. Allow the mixture to cool before adding the mixed fruit flavoring agent. An airtight jar held the final Polyherbal combination.<sup>10</sup>

### Evaluation Test for Polyherbal Syrup

#### Color

The polyherbal formulation, at a volume of 5 mL, was placed in watch glasses and tested against a white background using a white tube light. The color was visually noticed without the use of any optical instruments.

#### Odour

Each of the finished polyherbal mixtures was scented with two mL. A 2-minute delay was maintained between each round of sniffing to eliminate the impact of the preceding sniff.

#### Taste

A small amount of the finished polyherbal mixture was tested on the tongue to see how it felt on the taste receptors.

#### Determination of pH

A pH meter was used to find the pH of the polyherbal mixture. To provide consistent results, pH meter was calibrated with distilled water and buffer solutions at pH 4 and 9. The physical stability and palatability of the polyherbal formulation were assessed by measuring its pH at different time intervals using a digital pH meter.

#### Determination of density

To determine the density of the polyherbal formulation at room temperature, 10 mL of the mixture was weighed. The density of the polyherbal mixture was ascertained by means of density flasks. By dividing the mass of the polyherbal formulation (in grams) by its volume (in mL), the density of the formulation was calculated.

#### Viscosity

Another metric for evaluating the physical stability and transportability of polyherbal formulations is viscosity. The Ostwald viscometer was used to measure the viscosity of the polyherbal compound.

#### Determination of specific gravity

The specific gravity at 25°C was determined using a pycnometer. The loaded weight was deducted from the pycnometer's tare weight. Dividing the mass in air (g) of the amount of polyherbal formulation that fills the pycnometer at the given temperature by the volume (mL) of the pycnometer at the same temperature yielded the mass per milliliter. By dividing the mass of the polyherbal formulation (in grams) by the mass of water (in milliliters), both measured at 25°C, the specific gravity of the finished formulation was calculated.

**Table 1:** Composition of formulation

Ingredients (% w/w)	Formulation codes		
	F1	F2	F3
<i>C. longa</i> extract (mg)	150	200	250
<i>P. granatum</i> extract (mg)	150	200	250
<i>A. indica</i> extract (mg)	150	200	250
<i>Ocimum sanctum</i> extract (mg)	150	200	250
<i>Allium sativum</i> extract (mg)	150	200	250
Sodium benzoate (%)	0.1	0.2	0.3
Sucrose (%)	66.7	66.7	66.7
Tween 80 (%)	0.5	0.5	0.5
Xanthan gum (%)	1	1.5	2
Flavor (Mixed fruit) (%)	0.5	0.5	0.5
Purified water q.s. (mL)	100	100	100

## Antimicrobial Activity of Polyherbal Syrup

### Equipment's

Set of test tubes, autoclave, nichrome wire loop, petri dishes, and a micropipette with many tips. The aseptic cabinet, a 6 mL borer, a surgical cotton roll, and other related items.

### Chemicals

DMSO 70% ethyl alcohol etc.

### Media used

Muller Hinton agar medium (Hi-Media)

### Inoculum size

1 x 10<sup>8</sup> bacteria/mL

### Concentration of standard solution

1-mg/mL, prepared in DMSO.

### Concentration of formulation

1-mL formulation prepared in DMSO.

### Method used

Agar diffusion assay

### Culture used

The bacterial culture of *Pseudomonas aeruginosa* (NCIM 2036), *E. coli* (NCIM 2109), and fungi of *Candida albicans* and *Aspergillus niger* were purchased from NCIM, Pune

### Evaluation of Antimicrobial & Anti-fungal activity<sup>11</sup>

- A sterile petri dish was used to allow approximately 20 mL of bacterial Muller Hinton agar and fungal potato dextrose agar to set.
- Petri dishes were prepared with fungal inoculums (about 0.1 mL) in their respective media, with a set of spore count, cell density, and bacterial inoculums.
- Third, using a sterile borer, we bored 6 mm diameter holes in the agar medium and filled each well with 0.5 mL of plant extracts.
- Bacterial plates were kept at 37°C for 24 hours, while fungal plates were kept at 30°C for 48 hours.
- The zone of inhibition, measured in millimeters, was formed after incubation and used to read the positive antimicrobial activity.
- Each test was conducted three times.

### Stability Study of Polyherbal Formulations

The samples were subjected to accelerated temperature conditions to assess the stability of the developed polyherbal mixture. Separate portions of the finished formula (C) were stored at 4°C, room temperature, and 47° in amber-colored glass bottles. At 24, 48, and 72-hour intervals, the samples were examined for all the physicochemical characteristics, turbidity and homogeneity, to identify any changes.<sup>12</sup>

## RESULTS

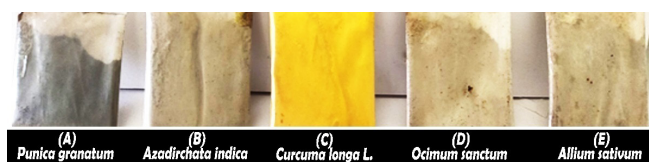
Preliminary identification and authentication were carried out using organoleptic and morphological characteristics on rhizomes of *C. longa* Linn. (Haladi), *A. indica* (neem), *P. granatum* (Pomegranate), *O. tenuiflorum* (Tulasi), and *A.*

**Table 2:** Organoleptic characters drugs extracts

Sample	Color	Odor	Consistency
<i>P. granatum</i>	Cloudy grey	Characteristic	Solid
<i>A. indica</i>	Sorrell brown	Characteristic	Solid
<i>C. longa L.</i>	Orangey yellow	Characteristic	Solid
<i>O. sanctum</i>	Light brown	Characteristic	Solid
<i>A. sativum</i>	sand brown	Characteristic	Solid



**Figure 1:** Photograph of extracts



**Figure 2:** Photographs of spray-dried extracts

*sativum* (Garlic) gathered from the local market of Dhule. Aqueous extract of *C. longa* Linn, *P. granatum*, *A. sativum*, *O. tenuiflorum* and *A. indica* were prepared by continuous hot extraction method liquid extract are given in Figure 1. The yield and organoleptic properties of the spray-dried extracts were assessed. Color, arrangement, and consistency were used to assess the organoleptic qualities of the extracts. Table 2 details organoleptic properties, and Figure 2 displays images of spray-dried extracts. *C. longa* L.'s extract was orange-yellow, *A. sativum*'s was cloudy-grey, and *A. indica*'s, *O. tenuiflorum*'s, and *P. granatum*'s were reddish orange, according to the results. All of the extracts had a distinct aroma and a non-sticky, powdered consistency. A battery of phytochemical assays, including those for alkaloids, glycosides, saponins, steroids, triterpenoids, carbohydrates, and amino acids, were administered to all *P. granatum*, *A. indica*, *C. longa* L, *O. tenuiflorum*, and *A. sativum* extracts. You may find the results in Table 3. Glycosides, flavonoids, and amino acids were detected in *C. longa* L. extracts, but alkaloids, saponin glycosides, tannins, steroids, and triterpenoids were not. The presence of alkaloids, saponin, carbohydrates, amino acids, and glycosides was demonstrated in the *P. granatum* extract. There were no tannins, steroids, or triterpenoids. No saponin glycosides, carbohydrates, steroids, or triterpenoids were detected in the water-based *C. longa* L. extract. The aqueous extracts of several plants, including *O. tenuiflorum*, *A. sativum*, *P. granatum*, and *A. indica*, included alkaloids and tannins. All of the extracts included glycosides and alkaloids

**Table 3:** Phytochemical screening of extracts

Test	<i>P. granatum</i>	<i>A. indica</i>	<i>C. longa L.</i>	<i>O. sanctum</i>	<i>A. sativum</i>
Mayer's	+	+	-	+	+
Wagner's	+	+	-	+	+
Dragendorff's	+	+	-	+	+
Glycoside General test	-	+	+	+	+
Foam test	-	+	+	+	+
Shinoda test	-	+	+	-	+
FeCl <sub>3</sub> test	-	-	-	+	-
Vanillin HCL	-	-	-	+	-
Froth test	+	+	-	-	+
Liebermann Burchard test	-	+	-	+	+
Molisch's	+	+	-	-	+
Fehling's	+	+	-	-	+
Biuret	+	+	+	+	+
Ninhydrin	+	-	+	+	+

+ = Positive; - = Negative

**Table 4:** Microbial load of extracts

Sample	Total aerobic microbial count	Total yeast and mold count	<i>E. coli</i>	<i>S. aureus</i>
Standard Limit	10 <sup>5</sup> /gm	10 <sup>5</sup> /gm	10 <sup>1</sup> /gm	Absent
<i>P. granatum</i>	10 cfu/g	10 cfu/g	AB	AB
<i>A. indica</i>	NG	NG	AB	AB
<i>C. longa L.</i>	NG	NG	AB	AB
<i>O. sanctum</i>	10 cfu/g	NG	AB	AB
<i>A. sativum</i>	10 cfu/g	NG	AB	AB

NG = No growth, AB = Absent, Cfu = colony forming units

**Table 5:** Evaluation of polyherbal syrup

Parameters	F1	F2	F3
Color	Reddish brown	Reddish brown	Reddish brown
Odor	Aromatic	Aromatic	Aromatic
Taste	Intensely bitter	Intensely bitter	Lightly bitter
pH	5.60 ± 0.05	6.01 ± 0.04	5.95 ± 0.01
Density	1.09	1.14	1.19
Viscosity	0.011 ± 0.03 p	0.15 ± 0.04 p	0.20 ± 0.02 p
specific gravity	1.05 ± 0.01	1.14 ± 0.02	1.22 ± 0.01

P: Poise

except for *C. longa L.*, which is carbohydrate deficient. While *P. granatum* extracts were devoid of flavonoids, those from *A. indica*, *A. sativum*, and *C. longa L.* were found. Saponin and triterpenoids were found in the extracts of *A. sativum*, *A. indica*, and *P. granatum*; however, they were not found in the *C. longa L.* extracts.

### Microbial Analysis

*P. granatum*, *A. indica*, *C. longa L.*, *O. tenuiflorum*, and *A. sativum* extracts were tested for microbes, including total viable aerobic count, total yeast and mold, *E. coli*, and *Salmonella typhimurium* counts. You may find the results in Table 4. According to the results, *C. longa L.* extracts did not promote the growth of aerobic bacteria, mold, or yeast. The total number of aerobic microbes detected in the *P. granatum*, *O. tenuiflorum*, and *A. sativum* extracts was 10 cfu/g. While other extracts did not show any growth, extracts of *P. granatum* showed a total yeast and mold count of 10 cfu/g. All five extracts were negative for *E. coli* and *S. typhi*, indicating that the microbiological contamination level was below the recommended range.

### Evaluation of Polyherbal Syrup

Physical criteria such as color, odor, taste, pH, density, viscosity, and specific gravity were used to analyze all batches of the polyherbal mixture. The results showed that trial batch 3 had the best overall flavor and taste. Therefore it's the one that went on to further testing. The following results is given in Table 5 and the prepared polyherbal formulation show in Figure 3.

### Antimicrobial Activity of Polyherbal Syrup

Batch 3 of polyherbal formulation containing extract of *C. longa Linn.* (Haldi), *P. granatum* (Pomegranate), *A. indica*


**Figure 3:** Prepared polyherbal formulation (Batch C)

**Table 6:** Antimicrobial & antifungal activity of batch 3 polyherbal formulation

Microorganisms	Diameter of zone of inhibition (mm)	
	Batch 3 Polyherbal formulation (20 µg/mL)	Standard (Streptomycin) (30 µg/mL)
<i>Gram negative bacteria</i>		
<i>P. aeruginosa</i>	8.3	10.12
<i>E. coli</i>	9.1	9.5
<i>Gram positive bacteria</i>		
<i>Staphylococcus aureus</i>	8.8	12.20
<i>B. subtilis</i>	8.9	11.16
<i>Fungi</i>		
<i>C. albicans</i>	4.8	7.5
<i>A. niger</i>	6.1	9.7

(neem), *O. tenuiflorum* (Tulsi) and *A. sativum* (Garlic) used the agar-well diffusion method to test for antibacterial and antifungal activities. The findings are presented in Figure 4 and Table 6.

Batch 3's polyherbal formulation was tested for its antibacterial efficacy using the disc method against four different bacterial strains: *E. coli*, *P. aeruginosa*, *Staphylococcus*, and *Bacillus subtilis*. Batch 3's synthetic polyherbal formulation had 8.3, 9.1, 8.8, and 8.9 inhibition zones, respectively. The antibacterial activity of the sample was impressive (Figure 5). When testing against fungus, the results indicate that the sample is particularly efficient against *A. niger* and *C. albicans* (Figure 6). The sample's antibacterial property was superior to that of other salts because of the high surface area that allowed for better contact with microbes. In order to shield the DNA from the silver ions, bacteria aggregate around a low-molecular-weight area that forms in the middle of the cell when samples enter the cell.

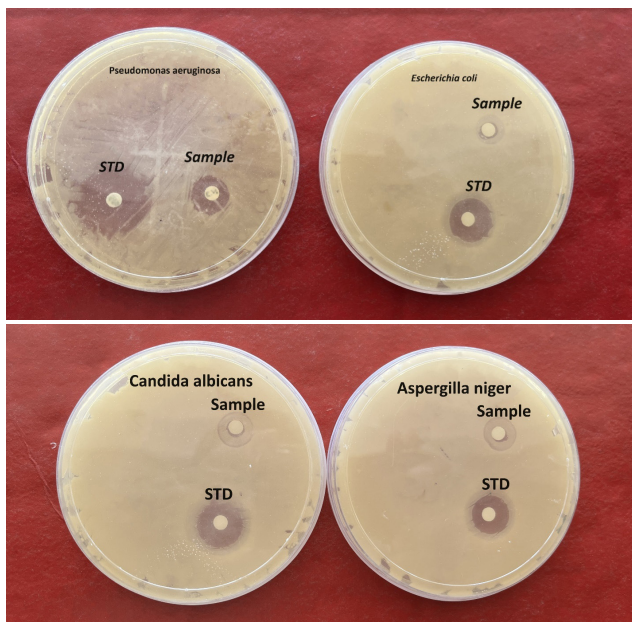


Figure 4: Antimicrobial activity of polyherbal formulation (Batch C)

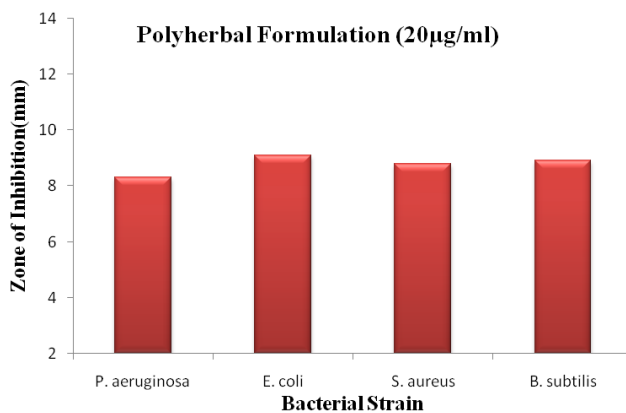


Figure 5: Antimicrobial activity of polyherbal formulation against various bacterial strains at 20 µg/mL

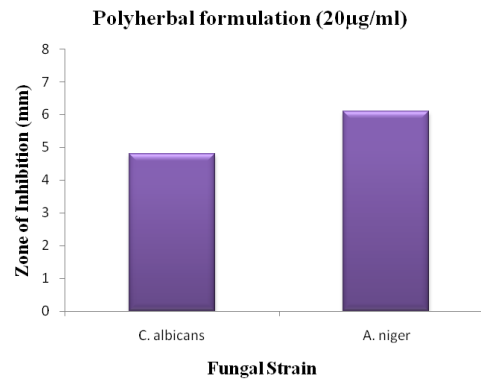


Figure 6: Antifungal activity of polyherbal formulation against different fungal strains at 20 µg/mL

Table 7: Accelerated stability study of F3

Parameters	After 1 month	After 2 month	After 3 month
Color	Reddish brown	Reddish brown	Reddish brown
Odor	Aromatic	Aromatic	Aromatic
Taste	Lightly bitter	Lightly bitter	Lightly bitter
pH	5.95 ± 0.01	5.91 ± 0.02	5.91 ± 0.01
density	1.19	1.19	1.18
Viscosity	0.20 ± 0.02 p	0.19 ± 0.01 p	0.19 ± 0.01 p
specific gravity	1.22 ± 0.01	1.24 ± 0.02	1.24 ± 0.02

### Stability Study of the Polyherbal Formulation

Formulation F3 was found to be stable according to the accelerated stability investigation results, which indicated no significant difference in the physicochemical characteristics, *in-vitro* diffusion study, and *in-vitro* permeability study. The findings are presented in Table 7.

### CONCLUSION

According to folklore, The five-herb polyherbal syrup has hyperlipidemic properties. All of the extracts had a microbial load below the recommended level, meaning they are safe to ingest for both humans and animals. It has been known for some time that chemicals included in polyherbal formulations have antibacterial effects. The antimicrobial activity of the extracted compounds was tested using the well-established agar well diffusion method. The results of the standard *in-vitro* model used to assess the potential antimicrobial and antifungal capabilities of the optimized batch 3 formulation indicate that they are highly effective antibacterial agents. They inhibited the growth of every single bacterial strain that was tested, and they were also tested for their antifungal efficacy against two different types of fungi: *A. niger* and *C. albicans*. They demonstrated size-dependent, strong efficacy against the two fungus species. As a result of fungus's inferior defense mechanisms, they were also discovered to be much more poisonous to fungal strains than bacterial ones. Under these conditions, the formulation remains stable according to the accelerated stability study conducted for three months at 40°C

$\pm 2^{\circ}\text{C}/75\% \text{RH} \pm 5\%$ . The formulation that has been produced meets all of the requirements, however, additional *in-vitro* and *in-vivo* investigations are needed to confirm its effectiveness.

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