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## Research Paper

# Development and Evaluation of Herbal Formulation (Face Serum) By Using Piper Betle Leaf Extract

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## ABSTRACT

In the Indian system of medicine-Ayurveda, piper betle has been mentioned as a remedy for treatment of various infectious diseases and ailments. Based on the folkloric use, the present study was designed to formulate and evaluate polyherbal serum containing extracts of Piper betle. Serum formulations (Formulation 1, 2 and 3) were prepared which comprised of the methanolic extracts of Piper betle in a concentration of 100mg, 200mg and 300mg, respectively in a base. The prepared formulations were evaluated for appearance, pH, homogeneity, viscosity, spreadability, skin irritation test (patch test) and stability. The formulations were also screened for their antimicrobial activity by well diffusion method against *S. aureus*, and *E. coli*. The results of the studies revealed that all formulations under study viz. A, B and C showed better zone of inhibition as compared with the control. However, formulation C exhibited maximum activity against the selected strains which may be attributed to its greater amount of herbal extracts as compared to formulation A and B. The polyherbal gel formulations were observed to possess antimicrobial action. The effective activity may be attributed to the synergistic action of the plants constituents present in the formulation.

## INTRODUCTION

More than 80% of the world's population still greatly depends upon traditional medicines for treatment of various skin diseases.(1) In the recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries, as herbal medicines have been reported

to be safe with minimal side effects especially when compared with synthetic drugs.(2)(3) Herbal treatments applied topically have gained considerable attention due to their widespread use and ill-defined benefit/risk ratio.(4)(5) There are numerous medicinal plants which are widely used in the treatment of skin diseases and also known to possess antimicrobial activity.(6) Topical

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application of serum at pathological sites offer great advantages in a faster release of a drug directly to site of action as compared to cream and ointment.(7)(8) *Piper betle*, is phytochemically rich in steroids, alkaloids, tannins, triterpenes, flavonoid and anthraquinone glycosides. It has been known to be used traditionally for their various therapeutic properties like antibacterial, antimicrobial, antioxidant, skin disorder, and wound healing activity.(9)(10) Also it has been reported to possess various therapeutic properties like anti-inflammatory, antipyretic, antimalarial, antiulcer, antidiabetic, neuropharmacological effect, anthelmintic activity, antimicrobial and antibacterial effect.(11)(12)(13)(14)(15)(16)(17) As per the literature survey the aforesaid plants have been reported for their antibacterial and antimicrobial effect by different researchers in most of the research articles. Based on this information it was decided to develop a herbal serum containing plant extracts which will possess better activity and prove to be effective against microorganisms. Despite of the fact that these plants possess good antimicrobial action, their use and application on the skin surface in the raw form is difficult. Hence, the present investigation was thus undertaken for preparation of polyherbal serum formulation using methanolic extracts of *Piper betle*, so as to facilitate their effective use to exhibit its antimicrobial action. The prepared formulations were thereafter evaluated for their physical appearance, pH, viscosity, spreadability, skin irritation test, antibacterial activity and stability.

## MATERIALS AND METHODS

### 2.1 Collection and authentication of plant material

The medicinal plant *Piper betle* (leaves) were collected. After cleaning, parts were dry under shade at room temperature till complete dryness.

Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration. Authentication of medicinal plant *Piper betle* was performed by a plant taxonomist in order to confirm its identity and purity. Plant part was authenticated and identified by Dr. Saba Naaz, H.O.D, Department of Botany, Saifia Science College, Bhopal, Madhya Pradesh, and voucher no. 121/Saif./Sci./Clg/Bpl was obtained.

### 2.2 Extraction of plant by soxhlet extraction method

Coarsely powered plant parts of *Piper betle* (300 gm) was then extracted by successive extraction using different organic solvents, defatted with petroleum ether and successively extracted with methanol for 36 hrs using soxhlet apparatus. To ensure complete extraction each extract was evaporated to dryness under reduced pressure by rotary evaporator and the resulted dried residue was stored in air-tight container for further use.

### 2.3 Phytochemical investigation

Experiment was performed to identify presence or absence of different phytoconstituents by detailed qualitative phytochemical analysis. The colour intensity or the precipitate formation was used as medical responses to tests.(18)

### 2.4 Quantitative Phytochemical Estimation

#### 2.4.1 Total phenolic content (TPC)

The total phenolic content of *Piper betle* extract was determined using the Folin-Ciocalteu Assay. The *Piper betle* extract (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured



spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic acid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically.(19)

## 2.4.2 Total flavonoid content (TFC)

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of *Piper betle* extract solution was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated

at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (RE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight.(20)

## 2.5 Formulation of Face Serum

The (o/w) emulsion-based serum was prepared according to the formula given below. The oily component consisting of olive oil, sandalwood oil, tween 20 and coconut oil is mixed together for ten minutes to obtain a uniform solution. At the same time the water phase was prepared by mixing the *Piper betle* extract, glycerine and a small amount of distilled water uniformly. The oil phase is added to the liquid phase drop wise under mechanical vibration at 2500 rpm to obtain o/w based on biphasic emulsion.(21)

**Table1: Composition of face serum formulation**

| S. No | Ingredients     | Formulation 1 | Formulation 2 | Formulation 3 |
|-------|-----------------|---------------|---------------|---------------|
| 1.    | Extract         | 100 mg        | 200 mg        | 300 mg        |
| 2.    | Olive Oil       | 1.8ml         | 1.8ml         | 1.8ml         |
| 3.    | Sandalwood Oil  | 0.02ml        | 0.02ml        | 0.02ml        |
| 4.    | Glycerine       | 5ml           | 5ml           | 5ml           |
| 5.    | Coconut Oil     | 0.4ml         | 0.4ml         | 0.4ml         |
| 6.    | Tween 20        | 0.2 ml        | 0.2 ml        | 0.2 ml        |
| 7.    | Distilled water | Qs to 30 ml   | Qs to 30 ml   | Qs to 30 ml   |

## 2.6 Evaluation parameter of prepared formulation

### 2.6.1 Physical Evaluation

Physical Evaluation (Organoleptic properties) was observed by visual observation. It is the initial evaluation during Preliminary studies which assess the odour and appearance of the substance. The organoleptic properties were checked visually for odour and appearance.

### 2.6.2 Homogeneity

The extracted materials were distributed evenly throughout the formulation. The preparation's homogeneity was confirmed visually by the lack of particulates and physically by touching the result.(22)

### 2.6.3 pH determination

pH of the formulation was determined by using Digital pH meter (EI). The meter was allowed to



stabilize as necessary and properly calibrated, begin by rinsing the probe with deionized or distilled water and blotting the probe dry with lint-free tissue paper. Immerse the sensing tip of the probe in the sample and record the pH reading and Rinse the probe, blot dry and repeat step 2 on a fresh portion of sample. The two readings should agree to within the accuracy limits of the meter. The samples were analyzed in triplicate. If slight deviations in pH were noted, it was adjusted to skin pH using drop wise addition of triethanolamine solution.(23)

#### 2.6.4 Viscosity determination

The viscosity of the herbal serum formulations was determined using Brookfield viscometer at the temperature of 25°C.

#### 2.6.5 Spread ability

Ideal formulations should possess a sufficient spreading coefficient when applied or rubbed on the skin surface. This was evaluated by placing about 1 g of formulation on a glass slide. Another glass slide of the same length was placed above that, and a mass of 50 mg was put on the glass slide so that the formulation gets sandwiched between the two glass slides and spreads at a certain distance. The time taken for the formulation to travel the distance from the place of its position was noted down. Spread ability was determined by the following formula

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

Where, S-Spreadability, g.cm/s M-Weight put on the upper glass L-Length of glass slide T-Time for spreading formulation in sec.(24)

#### 2.6.6 Skin irritation test

The intact skin of Wistar rats of either sex with average weight 150– 200 g was used. The hairs were removed from the rat 2-3 days before the

experiment. The formulation was applied on the properly shaven skin of rat. The animals were treated daily for 2-3 days, and undesirable skin changes, i.e., change in color, change in skin morphology was checked for a period of 24 h and erythema and edema on the treated skin were examined.(25)

### 2.7 Anti-microbial activity of serum formulation by Well diffusion assay

#### a. Preparation of Nutrient Agar Media

28 g of Nutrient Media was dissolved in 1 litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121°C at 15 lbs pressure for 15 minutes. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

#### b. Well Diffusion Assay

The bacterial suspension of *E. coli* was standardized to  $10^8$  CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing  $10^8$  CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate<sup>70</sup>. The agar plate was inoculated by spreading the inoculums with a sterile spreader, over the entire sterile agar surface. Three wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. The wells were then formed for the inoculation of the formulation, formulations (1mg/ml) solution. 100 µl of the sample was loaded. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37° C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the



inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well.(26)

## 2.8 Stability studies

The extract loaded herbal serum formulation was packed and were placed in the stability test chamber and subjected to stability studies at accelerated testing ( $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and  $60 \pm 5\%$  RH) and ( $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and  $70 \pm 5\%$  RH) for 3 months. The formulation was checked for evaluation parameter physical appearance, pH and viscosity studies at the interval of 30, 45, 60, 90 days (3 month) months. The formulation was tested for stability

under accelerated storage condition for 3 months in accordance to International Conference on Harmonization (ICH) guidelines. Formulation was analyzed for the change in evaluation parameter physical appearance, pH and viscosity studies.(27) All Results were compared against final formulation of 0 days as the reference.

## RESULTS AND DISCUSSION

### 3.1. Percentage Yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Piper betle* is shown in Table:2

**Table 2: Percentage Yield of crude extracts of *Piper betle* extract**

| S. No | Plant name         | Solvent   | Theoretical weight | Yield(gm) | % yield |
|-------|--------------------|-----------|--------------------|-----------|---------|
| 1     | <i>Piper betle</i> | Pet ether | 300                | 1.36      | 0.45%   |
| 2     |                    | Methanol  | 284.25             | 6.58      | 2.31%   |

### 3.2 Preliminary Phytochemical study

**Table 3: Phytochemical testing of extract**

| S. No. | Experiment                      | Presence or absence of phytochemical test |                    |
|--------|---------------------------------|-------------------------------------------|--------------------|
|        |                                 | Pet. Ether extract                        | Methanolic extract |
| 1.     | <b>Alkaloids</b>                |                                           |                    |
| 1.1    | Dragendroff's test              | Present                                   | Present            |
| 1.2    | Mayer's reagent test            | Present                                   | Present            |
| 1.3    | Wagner's reagent test           | Present                                   | Present            |
| 1.3    | Hager's reagent test            | Present                                   | Present            |
| 2.     | <b>Glycoside</b>                |                                           |                    |
| 2.1    | Borntrager test                 | Absent                                    | Present            |
| 2.2    | Legal's test                    | Absent                                    | Present            |
| 2.3    | Killer-Killiani test            | Absent                                    | Present            |
| 3.     | <b>Carbohydrates</b>            |                                           |                    |
| 3.1    | Molish's test                   | Absent                                    | Present            |
| 3.2    | Fehling's test                  | Absent                                    | Present            |
| 3.3    | Benedict's test                 | Absent                                    | Present            |
| 3.4    | Barfoed's test                  | Absent                                    | Present            |
| 4.     | <b>Proteins and Amino Acids</b> |                                           |                    |
| 4.1    | Biuret test                     | Present                                   | Absent             |
| 5.     | <b>Flavonoids</b>               |                                           |                    |
| 5.1    | Alkaline reagent test           | Absent                                    | Present            |



|     |                                            |         |         |
|-----|--------------------------------------------|---------|---------|
| 5.2 | Lead Acetate test                          | Absent  | Present |
| 6.  | <b>Tannin and Phenolic Compounds</b>       |         |         |
| 6.1 | Ferric Chloride test                       | Absent  | Present |
| 7.  | <b>Saponin</b>                             |         |         |
| 7.1 | Foam test                                  | Present | Absent  |
| 8.  | <b>Test for Triterpenoids and Steroids</b> |         |         |
| 8.1 | Salkowski's test                           | Present | Absent  |
| 8.2 | Libbermann-Burchard's test                 | Present | Absent  |

The results of the phytochemical studies revealed that the lead acetate test gave a pink or red coloration of the solution that indicated the presence of flavonoids. There is dark blue or greenish gray coloration of the solution indicated the presence of tannins in the drug. Yellow or reddish-brown precipitation indicated the presence of alkaloids. The solution pink to red indicates the presence of glycosides. Layer of foam formation indicates the absence of saponins. On phytochemical screening of methanolic extract of

*Piper betle* extract showed presence of alkaloid, flavonoid, saponins and glycoside.

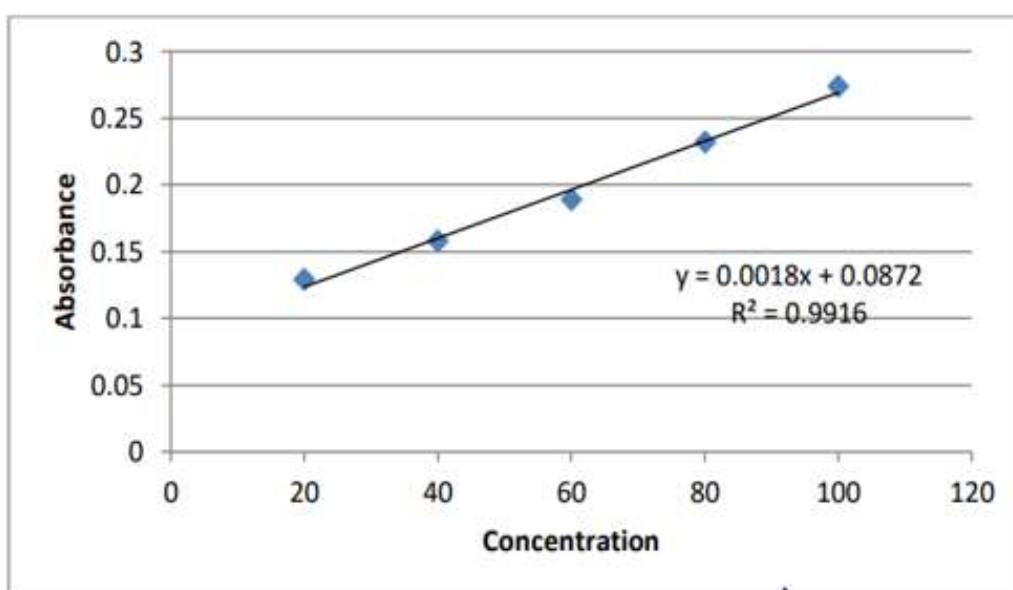
### 3.3 Quantitative Analysis

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

#### 3.3.1 Total Phenolic content (TPC) estimation

**Table 4: Standard table for Gallic acid**

| S. No. | Concentration (µg/ml) | Absorbance |
|--------|-----------------------|------------|
| 1.     | 20                    | 0.129      |
| 2.     | 40                    | 0.158      |
| 3.     | 60                    | 0.189      |
| 4.     | 80                    | 0.232      |
| 5.     | 100                   | 0.274      |



**Figure 1: Graph represent standard curve of Gallic acid**

**Table 5: Total Phenolic Content**

| S. No | Absorbance | TPC in mg/gm equivalent of Gallic Acid |
|-------|------------|----------------------------------------|
| 1     | 0.132      | 54mg/gm                                |
| 2     | 0.157      |                                        |
| 3     | 0.179      |                                        |

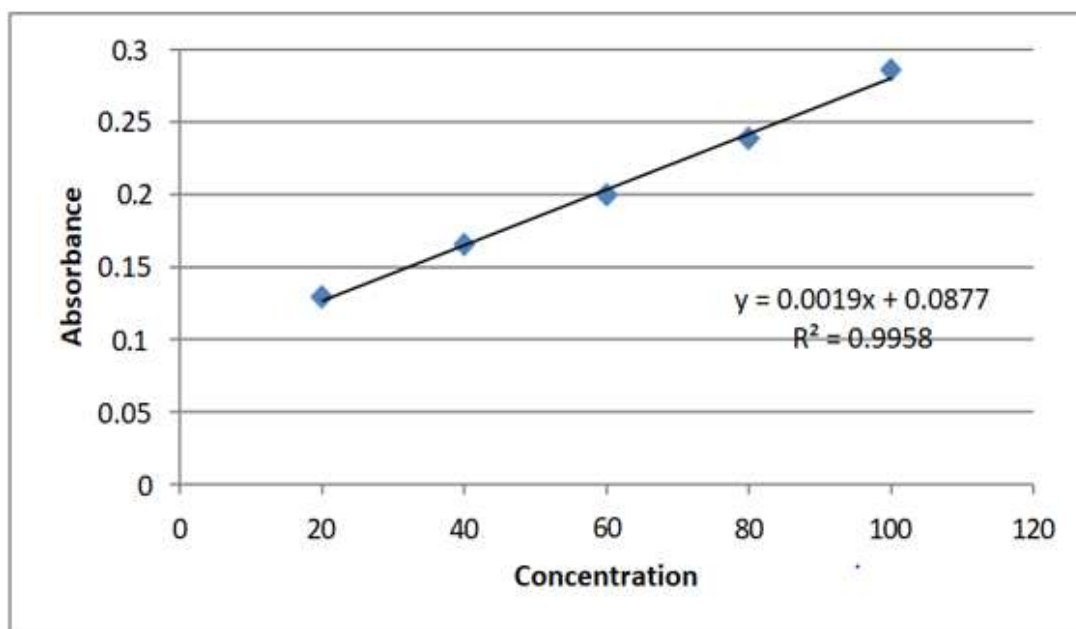
**Table 6: Total Phenolic Content of extract *Piper betle***

| Extracts | Total Phenolic content (mg/gm equivalent of Gallic acid) |
|----------|----------------------------------------------------------|
| Methanol | 54                                                       |

### 3.3.2 Total Flavonoids content (TFC) estimation

**Table 7: Standard table for Rutin**

| S. No. | Concentration (µg/ml) | Absorbance |
|--------|-----------------------|------------|
| 1.     | 20                    | 0.129      |
| 2.     | 40                    | 0.165      |
| 3.     | 60                    | 0.199      |
| 4.     | 80                    | 0.238      |
| 5.     | 100                   | 0.285      |

**Figure 2: Graph represent standard curve of Rutin****Table 8: Total Flavonoid Content**

| S. No | Absorbance | TFC in (mg/gm) equivalent of Rutin |
|-------|------------|------------------------------------|
| 1     | 0.110      | 42.33mg/gm                         |
| 2     | 0.140      |                                    |
| 3     | 0.162      |                                    |

**Table 9: Total Flavonoid Content of extract *Piper betle***

| Extracts | Total Flavonoid content (mg/gm equivalent of Rutin) |
|----------|-----------------------------------------------------|
| Methanol | 42.33                                               |

### 3.4 Formulation of herbal face serum

#### 3.4.1 Evaluation parameter of herbal face serum formulation

**Table 10: Organoleptic properties of herbal serum formulation**

| S. No | Formulations | Physical appearance     | Odour                 | Homogeneity           |
|-------|--------------|-------------------------|-----------------------|-----------------------|
| 1.    | F1           | Brownish to Yellow      | Characteristics odour | Absence of aggregates |
| 2.    | F2           | Brownish to Yellow      | Characteristics odour | Absence of aggregates |
| 3.    | F3           | Brownish to dark Yellow | Characteristics odour | Absence of aggregates |

An evaluation of the herbal face serum, including physical appearance, odour and homogeneity, was conducted. Herbal face serum was discovered to have a yellowish colour to it when tested. Herbal face serum exhibited the same odour, and

Appearance as the I.P. requirements for these characteristics and the results were listed in Table no.10

#### 3.4.2 Measurement of pH

**Table 11: pH determination**

| S. No | Formulation   | Results |
|-------|---------------|---------|
| 1.    | Formulation 1 | 5.1     |
| 2.    | Formulation 2 | 5.2     |
| 3.    | Formulation 3 | 5.5     |

The pH of all prepared formulation ranged from 5.1- 5.5. The pH of the prepared herbal serum formulation was considered to be acceptable to

avoid the risk of irritation upon application to the skin. The results were shown in Table .11

#### 3.4.3 Spreadability

**Table 12: Spread ability determination**

| S. No | Formulation   | Results (gm.cm/sec) |
|-------|---------------|---------------------|
| 1.    | Formulation 1 | Easily Spreadable   |
| 2.    | Formulation 2 | Easily Spreadable   |
| 3.    | Formulation 3 | Easily Spreadable   |

Spreadability denotes the extent of area to which the formulation readily spreads on application to skin or the affected part. Spreadability of different serum formulation was studied. The formulations

produced well and easily spreadability and the results were shown in Table no. 12

#### 3.4.4 Viscosity



**Table 3: Viscosity determination**

| S. No | Formulation   | Results (cps) |
|-------|---------------|---------------|
| 1.    | Formulation 1 | 253±0.13      |
| 2.    | Formulation 2 | 229±0.53      |
| 3.    | Formulation 3 | 230±0.74      |

Viscosity is an important property of preparation which describes a liquids resistance to flow and is related to the internal friction within the fluid. This rheological property helps in determining consistency and also the diffusion rate of extract from formulation. The measurement of viscosity

of the prepared herbal serum formulation was done with Brookfield viscometer. The results were shown in Table no.12

### 3.4.5 Acute skin irritation study

**Table 13: skin irritation test**

| S. No | Formulation   | Results               |
|-------|---------------|-----------------------|
| 1.    | Formulation 1 | Not irritant observed |
| 2.    | Formulation 2 | Not irritant observed |
| 3.    | Formulation 3 | Not irritant observed |

Results of skin irritation test indicate that prepared herbal formulation was not produce irritation, redness, or oedema on application and free from dermatological reaction.

### 3.5 Results of antimicrobial activity of all formulations

#### 3.5.1 Antimicrobial activity of herbal serum formulation

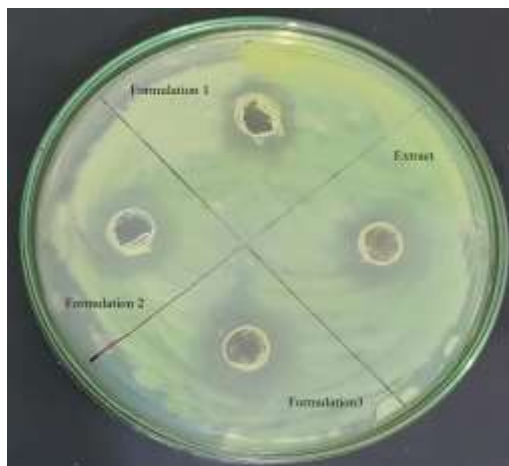
**Table 14: Antimicrobial activity of all formulation (F1, F2 and F3 formulation)**

| S. No | Sample name   | Zone of Inhibition (mm) |
|-------|---------------|-------------------------|
| 1     | Extract       | 5 mm                    |
| 1     | Formulation 1 | 8 mm                    |
| 2     | Formulation 2 | 16 mm                   |
| 3     | Formulation 3 | 19 mm                   |

**Figure 3: Antimicrobial activity against E. Coli**

**Table 15: Antimicrobial activity of all formulation (F1, F2 and F3 formulation)**

| S. No | Sample name   | Zone of Inhibition (mm) |
|-------|---------------|-------------------------|
| 1     | Extract       | 3 mm                    |
| 1     | Formulation 1 | 6 mm                    |
| 2     | Formulation 2 | 10 mm                   |
| 3     | Formulation 3 | 13 mm                   |

**Figure 4: Antimicrobial activity against *S. aureus***

Results obtained from the antimicrobial study showed that all formulations (F1, F2 and F3) have an inhibitory effect on the *E. coli* and *S. aureus*. The *in vitro* antimicrobial activities were calculated in term of the zone of inhibition

diameter (mm) the result recorded in (Table 14 and 15). F3 showed the higher zone of inhibition against *E. coli* and *S. aureus*.

### 3.6 Stability studies

**Table 16: Stability Study of F3 formulation**

| S. No | Time (Days) | 25°C±2 °C and 60 ± 5% RH |     |           | 40°C±2 °C and 70 ±5% RH |     |           |
|-------|-------------|--------------------------|-----|-----------|-------------------------|-----|-----------|
|       |             | Physical appearance      | pH  | Viscosity | Physical appearance     | pH  | Viscosity |
| 1.    | 0           | Brownish to Yellow       | 5.5 | 219       | Brownish to Yellow      | 5.5 | 219       |
| 2.    | 30          | Brownish to Yellow       | 5.5 | 218       | Brownish to Yellow      | 5.4 | 220       |
| 3.    | 45          | Brownish to Yellow       | 5.4 | 219       | Brownish to Yellow      | 5.4 | 220       |
| 3.    | 60          | Brownish to Yellow       | 5.5 | 220       | Brownish to Yellow      | 5.3 | 221       |
| 4.    | 90          | Brownish to Yellow       | 5.3 | 220       | Brownish to Yellow      | 5.4 | 220       |

Formulation were found to be stable, both physically and chemically, for a period of 3 months at accelerated stability conditions (25°C±2 °C and 60 ± 5% RH) and (40°C±2 °C and 70 ±5% RH). Physicochemical parameters, including, viscosity and pH studies were not altered significantly. Results of assay and other evaluation

criteria at periodic time points of stability studies are summarized in Table 16.

### CONCLUSION

The results of the phytochemical studies revealed that the lead acetate test gave a pink or red coloration of the solution that indicated the presence of flavonoids. There is dark blue or

greenish gray coloration of the solution indicated the presence of tannins in the drug. Yellow or reddish-brown precipitation indicated the presence of alkaloids. The solution pink to red indicates the presence of glycosides. Layer of foam formation indicates the absence of saponins. On phytochemical screening of methanolic extract of *Piper betle* extract showed presence of alkaloid, flavonoid, saponins and glycoside. Herbal face serum was discovered to have a yellowish colour to it when tested. The pH of all prepared formulation ranged from 5.1- 5.5. The pH of the prepared herbal serum formulation was considered to be acceptable to avoid the risk of irritation upon application to the skin. Spreadability of different serum formulation was studied. The formulations produced well and easily spreadability. The measurement of viscosity of the prepared herbal serum formulation was done with Brookfield viscometer. Results of skin irritation test indicate that prepared herbal formulation was not produce irritation, redness, or oedema on application and free from dermatological reaction. The antimicrobial study showed that all formulations (F1, F2 and F3) have an inhibitory effect on the *E. coli* and *S. aureus*. F3 showed the higher zone of inhibition against *E. coli* and *S. Aureus*. Formulation were found to be stable, both physically and chemically, for a period of 3 months at accelerated stability conditions ( $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and  $60 \pm 5\% \text{ RH}$ ) and ( $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and  $70 \pm 5\% \text{ RH}$ ). Physicochemical parameters, including, viscosity and pH studies were not altered significantly. From above discussion it is concluded that Natural plant Extract had antimicrobial property. From the above experimental work, the Natural plant Extract showing good activity against bacteria. Finally it was concluded that extract of Natural plant shows antibacterial activity against selected microorganism with increase in concentration the activity is increase therefore it can be incorporated

in cosmetics products. All the parameters showed that they are within the limits and since all the ingredients added have many advantages. From the research of study it was concluded that poly herb containing F3 formulation shows better results than other formulation containing single herb. Thus F3 formulation removes skin pigmentation and improves face complexion.

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