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# DEVELOPMENT AND CHARACTERIZATION OF POLYHERBAL GEL FOR ANTIBACTERIAL ACTIVITY

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## ABSTRACT
In the Indian system of medicine-Ayurveda, *Albino vereduna*, *Lens culinaris* and *Murraya koenigii* 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 gel containing extracts of *Albino vereduna*, *Lens culinaris* and *Murraya koenigii*. Gel formulations (Formulation PHG1, PHG2, PHG3, PHG4, PHG5 and PHG5) were prepared which comprised of the hydroalcoholic extracts of *Albino vereduna*, *Lens culinaris* and *Murraya koenigii* in a concentration of 1 gm in a base. The prepared formulations were evaluated for appearance, homogeneity, pH, viscosity, spreadability and washability. The formulations were also screened for their antimicrobial activity by disc plate method against Staphylococcus aureus and E. coli. The results of the studies revealed that all formulations under study showed better zone of inhibition as compared with the control. However, formulation PHG5 exhibited maximum activity against the selected strains which may be attributed toits greater amount of herbal extracts as compared to other formulations. 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.

**KEYWORDS:** Antimicrobial activity, Evaluation test, *Murraya koenigii*, Polyherbal gel.

## 1. INTRODUCTION
Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care.[1]

According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy.[2] Plant products and their active constituents played an important role in plant disease control by combating growth and development of pathogens and including resistance in plants. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc.[3]

As these plants and their products are known to posses various secondary metabolites, which showed significant inhibitory effect against the growth of pathogens, therefore, the plant and their products should be utilized to combat the disease causing pathogens. Thus the wide spectra of antimicrobial research are geared towards the discovery and development of noval antibacterial and antifungal agents. The demand for plant based medicines, health products, pharmaceuticals, food supplement and cosmetics etc. are increasing in both developing and developed countries. Since these natural products are non-toxic, have less side effects and easily available at affordable prices they are recognize worldwide.[4]

Acne rosacea a skin disease of adults often affected by women in which blood vessels of the face enlarge indicating a flushed appearance. Rosacea is a common, chronic, incurable, adult acne-like skin condition that is easily controllable and curable medically. Rosacea incline to develop in certain stages and causes to create inflammation of the skin of the face, especially the forehead, cheeks, nose, as well as chin. Symptoms and signs of rosacea are: Redness of the face, tiny red pimples and fine red lines on the facial skin. An enlarged, bulbous red nose. Eye problems, like swollen, red eyelids and conjunctivitis.

*Albina Vereduna* (aka Blankoma, aka White Albino) Some say this one originated in Holland. A (pure) white beet root with green leaves. Roots are tender, mild and very, very sweet. The leaves can be used as greens or cooked gently and added to stews or soups. Shorter shelf -life than other beet varieties. 50-65 days.[5] *Lens culinaris* is the world's third most significant cool-season grain legume. Lentil was first cultivated 8000-10,000 years ago in Southwest Asia. Due to its great nutritional content, particularly protein, the lentil is an important dietary component in many Mediterranean and Asian countries. Lentils have been reported to contain an array of different phyto-chemicals such as phenolics, condensed tannins, carotenoids, toco-pherols, saponins, phytic acid, and phytosterols.[6] Leaves of *Murraya Koenigii(L) Spreng (Rutaceae)* is commonly known as curry leaves or Mittha neem which is widely being used as flavouring agent in curries, and other foodstuffs since ancient times. A small spreading shrub, about 2.5 metres hight; the main stem, dark green to brownish, with numerous dots on it; its bark can be peeled off longitudinally. The former is more popular due to its versatile medicinal properties of leaves, and high acceptance rate it is used from ancient time as a natural flavoring agent in various curries and food items.[7]

Bacterial infection is a chronic inflammatory disorder of the skin which affects approximately 80% adolescent during puberty stage. The increasing frequency of intake of antibiotics to overcome this problem explores several side effects. Therefore it needs to focus on the herbal formulation as a topical first-line treatment. In this study development and characterization of polyherbal gel of *Albino vereduna, Lens culinaris* and *Murraya koenigii*.

## 2. MATERIALS AND METHODS

### 2.1 Collection of plant material

*Albino vereduna, Lens culinaris* seeds were collected from local market in Bhopal and *Murraya koenigii* leaves were collected from medicinal garden of Vindhya Herbal, Bhopal in the month of March, 2022.

### 2.2 Drying, size reduction and storage of plant material

The seeds and leaves of plants sample were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. Drying of fresh plant parts were carried out in sun but under the shade. It was pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve No. 20 to maintain uniformity and packed into airtight container and stored in cool and dry place. This material was used for the further study.

### 2.3 Preparation of Extract

100 gram of *Albino vereduna,* 100 gram of *Lens culinaris* and 100 gram of *Murraya koenigii* shade dried plant material were coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in a maceration method. The extraction was continued till the defatting of the material had taken place. Defatted plant materials of *Albino vereduna, Lens culinaris* and *Murraya koenigii* were exhaustively extracted with hydroalcoholic solvent (ethanol: aqueous: 70:30v/v) by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.[8]

### 2.4 Phytochemical Detection

The extracts were subjected to phytochemical screening to test presence of metabolites such as terpenoids, glycosides, alkaloids, phenolic compounds, saponin, flavonoids, protein and carbohydrates.[9,10]

### 2.5. Quantitative estimation of bioactive compounds

#### 2.5.1 Total phenolic content estimation

The total phenolic content of the extract was determined by the modified Folin- Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to standfor 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

#### 2.5.2 Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10- 50µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

### 2.6 Formulation development of polyherbal gel

#### 2.6.1 Method of preparation

Measured quantity of methyl paraben, glycerin, polyethylene glycol and hydroalcoholic extract of *Albino vereduna, Lens culinaris* and *Murraya koenigii* were dissolved in about 35 ml of water in beaker and were stirred at highspeed using mechanical stirrer (or sonicator). Then Carbopol 940 was added slowly to the beaker containing above liquid while stirring.[11] Neutralized the solution by slowly adding triethanolamine solution with constant stirring until the gel is formed. (Table 01).

**Carbopol 940 –** Gelling Polymer.

**Triethanolamine-** Gelling agent, pH Adjusting agent and Neutralizer.

**Distilled Water, Glycerine and Polyethylene Glycol-** Solvents.

**Methyl Paraben –** Preservative.

**Table 01: Formulation of polyherbal gel.**

| Ingredients | PHG1 | PHG 2 | PHG3 | PHG4 | PHG5 | PHG6 |
|---|---|---|---|---|---|---|
| *Albino vereduna* Extract | 1gm | 1gm | 1gm | 1gm | 1gm | 1gm |
| *Lens culinaris* Extract | 1gm | 1gm | 1gm | 1gm | 1gm | 1gm |
| *Murraya koenigii* Extract | 1gm | 1gm | 1gm | 1gm | 1gm | 1gm |
| Carbopol 940 | 0.25mg | 0.5mg | 0.75mg | 1.0 gm | 1.25 gm | 1.5 gm |
| Polyethylene Glycol | 0.2ml | 0.2ml | 0.2ml | 0.2ml | 0.2ml | 0.2ml |
| Methyl Paraben | 0.08mg | 0.08mg | 0.08mg | 0.08mg | 0.08mg | 0.08mg |
| Triethanolamine | 1.0ml | 1.0ml | 1.0ml | 1.0ml | 1.0ml | 1.0ml |
| Distilled Water (q.s) | 100ml | 100ml | 100ml | 100ml | 100ml | 100ml |

**2.7 Evaluation of polyherbal Gel[12]**

**2.7.1 Appearance and consistency**

The physical appearance was visually checked for the texture of polyherbal gel formulations.

**2.7.2 Washability**

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

**2.7.3 Extrudability determination of formulations**

The polyherbal gel formulations were filled into collapsible metal tubes or aluminum collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

**2.7.4 Determination of Spreadability Principle**

An important criterion for anti-bacterial gels is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application to skin. The therapeutic efficacy of a formulation alsodepends on its spreading value.

**2.7.5 Determination of pH**

The pH of the anti-acne gels was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

**2.7.6 Drug content (Phenol content)**

The drug content was determined by taking 1gm of gel in 10 ml volumetric flask diluted with methanol. 3 ml of stock solution was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l)of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

**2.8 In vitro antibacterial activity of polyherbal gel**

**2.8.1 Pathogenic microbes used**

The pathogenic microbes used in the current study are two bacteria obtained from Microbial Culture collection, National Centre forcell science, Pune, Maharashtra, India.

**2.8.2 Media preparation (broth and agar media) Composition of nutrient agar media**

Agar - 1.5 gms
Beef extract - 0.3 gms.
Peptone - 0.5 gms.
Sodium chloride - 0.55 gms.
Distilled water- to make 100 ml.pH – 7

**2.8.3 Method of preparation**

This agar medium was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients are transferred to flask containing required quantity of distilled water and heat to dissolve the medium completely.

**2.8.4 Sterilization culture media**

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch$^2$ (121$^o$C) for 15 minutes.

**2.8.5 Preparation of plates**

After sterilization, the nutrient agar in flask was immediately poured (20 ml/ plate) into sterile Petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37$^o$C overnight to check the sterility of plates. The plates were dried at 50$^o$C for 30 minutes before use.

**2.8.6. Revival of the bacterial cultures**

The Bacterial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques, the lyophilized cultures are inoculated in sterile nutrient broth than incubated for 24 hours at 37$^o$C. After incubation the growth is observed in the form of turbidity. These broth cultures were further inoculated on to the agar plates with loop full of bacteria and further incubated for next 24 hours at 37$^o$C to obtain

the pure culture and stored as stocks that are to be used in further research work.

Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the 100 mg/ml concentration of phyto extract used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37$^{o}$C for 24hours. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture.

The well diffusion method was used to determine the antibacterial activity of the extracts prepared from the powdered seeds of *Albino vereduna*, *Lens culinaris* and leaves of *Murraya koenigii* using standard procedure. There were 3 concentrations used which are 25, 50 and 100 mg/ml for antibiogram studies. The plates were incubated at 37$^{o}$C for 24 hr. and then examined for clear zones of inhibition around the wells with particular concentration of drug.

## 3. RESULTS AND DISCUSSION

Bacterial infections disorder of the skin affects approximately 80% adolescents during their puberty stage. Medicinal plants are used all over the world to treat various diseases due to its variety of phytochemical constituents. Ideally, Topical application of gels at pathological sites offers great advantages in a faster release of a drug directly to site of action as compared to cream and ointment. Ideally, topical therapy is the first line treatment for many skin diseases. Among the various topical formulations, gels have been considered as a potential vehicle due to its nonsticky nature, stable and greater aesthetic value.

Table No. 02 showed the percentage yield of *Albino vereduna*, *Lens culinaris* and *Murraya koenigii* were found 8.25%, 7.43% and 6.44% respectively. From the Table No. 03, it could see that, flavonoids, saponins, alkaloids, glycosides, carbohydrate and phenol were present in hydroalcoholic extract of *Albino vereduna*. The phytochemical screening of *Albino vereduna* revealed negative results for diterpenes.

The phytochemical tests revealed various bioactive secondary metabolites which might be responsible for their medicinal attributes. The hydroalcoholic extract of *Lens culinaris* had revealed the presence of alkaloids, flavonoids, glycosides and phenol.

Phytochemical screening of leaves extract of *Murraya koenigii* showed the present of glycosides, flavonoids, diterpenes, phenol carbohydrate and saponins.

In the above formulations of gels, it has been noted that all of them has clear colour, no clogging, good homogeneity and smooth texture. In the above formulations of gels, they have good washability as well as extrudability. In all above formulations of gel the spreadibility of PHG5 is good. The above formulation of topical gels has different pH value for different formulation. In the above formulations the viscosity of different sample of gel were determined and found that there is increase in viscosity. The formulation PHG5 has good viscosity. In the above formulation of different gels the percentage of drug content was found that PHG5 has maximum percentage of drug content (Table No. 05).

The present investigation in this research work antimicrobial activity of polyherbal gel of Albino vereduna, Lens culinaris and Murraya koenigii were evaluated against pathogens used under present study. The polyherbal gel obtained from plants used to suitably dilute upto the concentrations of 100, 50 and 25 mg/ml and applied on to the test organism using well diffusion method (Table No. 06-07).

Acne is the most common disorder treated by dermatologists. As many as 80-90% of all adolescents have some type of acne and 30% of them require medical treatment.It is an inflammatory disease of the pilosebaceous unit characterized by the formation of open and closed comedones, papules, pustules, nodules, and cysts.

The present study was conducted to investigate the *in vitro* antibaterial activity of three selected medicinal plant extracts. Activity of *Albino vereduna*, *Lens culinaris* and *Murraya koenigii* was further evaluated so that they can be developed into formulations and used commercially for treatment of bacterial infections.

**Table 02: Results of percentage yield of extract of *Albino vereduna, Lens culinaris* and *Murraya koenigii.***

| Hydroalcoholic extracts | Percentage yield (w/w) |
|---|---|
| *Albino vereduna* | 8.25% |
| *Lens culinaris* | 7.43% |
| *Murraya koenigii* | 6.44% |

**Table 03: Result of Phytochemical screening of extract of *Albino vereduna, Lens culinaris* and *Murraya koenigii.***

| S. No. | Constituents | Hydroalcoholic Extract of AV | Hydroalcoholic Extract of LC | Hydroalcoholic Extract of MK |
|---|---|---|---|---|
| 1. | Alkaloids<br>A) Wagner's Test:<br>B) Hager's Test: | <br>-Ve<br>+Ve | <br>-Ve<br>+Ve | <br>+Ve<br>+Ve |
| 2. | Glycosides<br>A) Legal's Test: | <br>-Ve | <br>-Ve | <br>+Ve |
| 3. | Flavonoids<br>A) Lead acetate Test:<br>B) Alkaline Reagent Test: | <br>-Ve<br>+Ve | <br>-Ve<br>+Ve | <br>+Ve<br>+Ve |
| 4. | Saponins<br>A) Froth Test: | <br>+Ve | <br>+Ve | <br>+Ve |
| 5. | Phenolics<br>A) Ferric Chloride Test: | <br>+Ve | <br>+Ve | <br>+Ve |
| 6. | Proteins<br>A) Xanthoproteic Test: | <br>+Ve | <br>+Ve | <br>+Ve |
| 7. | Carbohydrate<br>A) Fehling's Test: | <br>+Ve | <br>+Ve | <br>+Ve |
| 8. | Diterpenes<br>A) Copper acetate Test: | <br>-Ve | <br>-Ve | <br>-Ve |

**Table 04: Estimation of total phenolic and flavonoids content of *Albino vereduna, Lens culinaris* and *Murraya koenigii.***

| S. No. | Hydroalcoholic extract | Total phenol content (mg/100mg of dried extract) | Total flavonoids content (mg/ 100 mg of dried extract) |
|---|---|---|---|
| 1. | *Albino vereduna* | 0.568 | 0.414 |
| 2. | *Lens culinaris* | 0.741 | 0.548 |
| 3. | *Murraya koenigii* | 0.596 | 0.452 |

**Table 05: Results of Evaluation of gel formulation.**

| Formulation | Spreadability (gcm/sec) | pH | Viscosity (cps) | Phenol content (mg/100mg) |
|---|---|---|---|---|
| PHG1 | 21.20±0.52 | 6.62± 0.11 | 2356±32 | 0.563 |
| PHG2 | 20.18±0.14 | 6.75±0.15 | 3165±31 | 0.745 |
| PHG3 | 16.67±0.47 | 7.02±0.11 | 4025±24 | 0.713 |
| PHG4 | 14.52±0.35 | 7.00±0.14 | 3021±32 | 0.654 |
| **PHG5** | 15.36±0.47 | 7.10±0.12 | 2895±18 | **0.869** |
| PHG6 | 12.74±0.63 | 7.25±0.13 | 3125±20 | 0.745 |

*Mean±S.D., Average of three determinations

**Table 06: Antimicrobial activity of standard drug against selected microbes**

| S. No. | Standard | Microbes | Zone of inhibition (mm) | | |
|---|---|---|---|---|---|
| | | | 25mg/ml | 50 mg/ml | 100mg/ml |
| 1. | Gentamycin | ***Staphylococcus aureus*** | 12±0.86 | 13±0.94 | 15±0.47 |
| | | ***Escherchia coli*** | 11±0.57 | 15±0.5 | 17±0.47 |

**Table 07: Antimicrobial activity of polyherbal gel formulation (PHG5) against selected microbes**

| S. No. | Test | Microbes | Zone of inhibition (mm) | | |
|---|---|---|---|---|---|
| | | | 25mg/ml | 50 mg/ml | 100mg/ml |
| 1. | Polyherbal gel (PHG5) | ***Staphylococcus aureus*** | 06±0.12 | 07±0.94 | 08±0.86 |
| | | ***Escherchia coli*** | 07±0.47 | 09±0.52 | 12±0.57 |

## 4. CONCLUSION

Herbal plants such as *Albino vereduna, Lens culinaris* and *Murraya koenigii* are selected and incorporated into a 1.25%w/w carbopol gel base as a non-oily (aqueous) topical polyherbal formulation. The antibacterial study results of the developed polyherbal topical gel showed the antibacterial activity against *Staphylococcus aureus* and *Escherchia coli*. The study results conclude that the formulated polyherbal gel with hydroalcoholic extract of *Albino vereduna, Lens culinaris* and *Murraya koenigii* can be used for the management of bacterial infections.

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