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# STUDY OF PHYTOCHEMICALS AND FORMULATION OF TOPICAL ANTIMICROBIAL GEL OF MEDICINAL PLANTS

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**Abstract**

The two plant species *Mahonia leschenaultia* and *Chromolaena odorata* are used in this study to formulate antimicrobial topical gel. The leaves of *Mahonia leschenaultia* and aerial part of *Chromolaena odorata* were collected and subjected to extraction by hydroalcoholic solvent. The qualitative & quantitative studies on phytochemicals were also performed. The gel was formulated by standard procedures & evaluated for its various parameters along with antimicrobial activity. The result of the study showed that The percentage yields for *Mahonia leschenaultia* and *Chromolaena odorata* hydroalcoholic were found to be 8.52 and 7.63 % w/w respectively. The *Mahonia leschenaultia* extract contains alakaloid, flavonoid, diterpenes, phenol & proteins. While, *Chromolaena odorata* extract found to have presence of alkaloid, diterpenes , phenol, proteins, carbohydrate & saponins. Total alkaloid & phenol content in *Mahonia leschenaultia* extract was found to be 0.263 mg/ 100mg & 0.745mg/100mg respectively. In case of *Chromolaena odorata* total alkaloid & phenol content 0.374 mg/100 mg & 0.695 mg/100mg of dried extract respectively. Antimicrobial activity showed that the formulation HG5 have greater antimicrobial efficacy against E. coli its zone of inhibition of 14±0.57 at 25mg/ml concentration.

**Key words:** *Mahonia leschenaultia* and *Chromolaena odorata*, Phytochemicals Formulation, Antimicrobial Gel.

## Introduction

Medicinal plants contain numerous biologically active compounds which are helpful in improving the life and treatment of disease. Recently considerable attention has been paid to utilize eco-friendly and biofriendly plant based products for the prevention and cure of different human diseases. It is documented thatmost of the world's population has taken in traditional medicine, particularly plant drug for the primary healthcare. Compounds such as carbohydrates, proteins, enzymes, fats, oils, terpenoids, flavonoids, sterols simple phenolic compounds etc. Natural products are the source of synthetic and traditional herbal medicine and are still the primary health care system. The presence of various life sustaining constituents in plants made scientists to investigate these plants for their uses in treating certain infective diseases and management of chronic wounds [1-3].

Hence herbal formulations for topical use, for treating skin diseases became popular. As the development of bacterial resistance to antibiotics and other synthetic antimicrobials, scientists found literally thousands of photochemical from plants which inhibit different types of microorganisms with different mechanisms and which are safe and broad spectrum antimicrobials in the treatment of resistant microbial strains. Topical application of antimicrobial agent at the site of infection offer greater advantages as compared to systemic therapy. Firstly the required concentration for antimicrobial activity of the drug at the target site can be easily achieved after topical dosing. Secondly, topical administration results in much lower or almost undetectable systemic levels of the active constituents. Also, it can avoid an unnecessary exposure of the gut flora to the antimicrobial agents which may lead to drug-resistance or depletion of the natural bacterial flora of the GIT. Therefore, topical application of antimicrobial agents is considered an important alternative to the systemic delivery of drugs for the treatment of skin diseases [4-6].

The two plant species *Mahonia leschenaultia* and aerial part of *Chromolaena odorata* are used in this study to formulate antimicrobial topical gel. The plant *Mahonia leschenaultii* takeda species of Berberidaceae family is a shrub with rough, greyish-brown, corky bark.

Leaves in circles present at the ends of the slender branches. Flowers are yellow, in long erect racemes. It is Indigenous to Nepal. Temperate Himalaya, Himachal Pradesh to Khasia Hills and Nilgiris. The berries are edible, and considered diuretic, demulcent in dysentery. The root, stem-bark and wood contains Umbellatine and Neprotine [7].

The plant *Mahonia leschenaultia* is known in common parlance as Holy-leaved Berry belonging to family Berberidaceae. This plant is of potential value as a medicine besides its used in religious ceremonies. In Toda term it is called Thovari which means Purifier. The stem bark of the plant is made into paste in water and in administered orally as an effective remedy to newly delivered women against fever, cold and also to arrest other complications during post-natal period. The methanol extracts of the root and root bark have significant activity against all strains the highest against *S. epidermiti* when compared with standard drug. The root bark extract showed more significant activity against the organisms than that of the extracts of the root. The highest activity exhibited by root bark against *S. epidermiti*. The antifungal activity is relatively less as compared to antibacterial activity [8].

Another plant *Chromolaena odorata* L. belongs to Asteraceae family (sunflower family) is an important and serious perennial herb in the world, while this weed also acts as medicinal plant. Several parts of this plant widely used to treat wound, burns, skin infections as well as to possess anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial, and antioxidant properties. Native to North America and has been introduced to Asia, West Africa and Australia. Siam weed is one of common name of *Chromolaena odorata* L. grown as medical herbs and ornamental plant. The medicinal values of *Chromolaena odorata* L. lie in their phytochemicals component, the dried leaf of *Chromolaena odorata* contained flavonoid aglycones (flavanones, flavonols, flavones) including acacetin, chalcones, eupatilin, luteolin, naringenin, kaempferol, quercetin, quercetagetin, and sinensetin, terpenes and terpenoids, essential oils, and other phenolic compounds, which produce define physiological action in our body [9].

*Chromolaena odorata* has been reported to exhibit antibacterial, antiplasmodic, antiprotozoal, antitrypanosomal, antifungal, antihypertensive, anti-inflammatory, astringent, diuretic, hepatotropic, immunomodulatory and anticancer effects. It is also applied topically as an antidote to the sting of the spine of the common sea catfish. Traditionally, fresh leaves or a decoction of *C. odorata* have been used throughout Vietnam and other tropical countries for the treatment of leech bites, soft tissue wounds, burn wounds, skin infections, rashes, diabetes and periodontitis, and as an insect repellent . A poultice of the leaves is traditionally applied to cuts or wounds to stop bleeding and promote healing. Eupolin, a product made from Chromolaena spp., has already been licensed for use in Vietnam for the treatment of soft tissue burns and wounds. An aqueous decoction of the roots is used as an antipyretic and analgesic remedy, and a leaf extract with salt is used as a gargle for sore throats and cold. By keeping this view this study deals with formulation of topical gel with these two plant with potent antimicrobial effect [10].

## Material and Methods

### Collection of plant material

*Leaves of Mahonia leschenaultia and* aerial part of *Chromolaena odorata* were collected from rural area of Bhopal (M.P), India in the months of February, 2022.

### Methods

### Extraction by maceration process

65.84 gm dried powdered *leaves of Mahonia leschenaultia and* 58.12 gm dried powdered aerial part of *Chromolaena odorata* has been extracted with hydroalcoholic solvent (Ethanol: water; 80:20) using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40°C.

### Phytochemical Screening

The chemical tests were performed for testing different chemical groups present in extract [11].

## Total alkaloids content estimation

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered . This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract [12].

## Total phenolic content estimation

The total phenolic content of extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25mg/ml was prepared in methanol. 1mg of dried extract was extracted with 10 ml methanol, filter, and make up the volume up to 10 ml. One ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract or 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 stand for 15 min. for colour development. The absorbance was measured at 765 nm using a spectrophotometer [13].

## Formulation development of herbal gel

## Method of preparation

In a beaker, measured amounts of methyl paraben, glycerin, polyethylene glycol, hydroalcoholic extract of *Mahonia leschenaultia* and *Chromolaena odorata* were dissolved in roughly 35 ml of water and swirled at high speed using a mechanical stirrer (or sonicator). Then, while stirring, Carbopol 940 was gently added to the beaker containing the aforementioned liquid. The solution was neutralized by progressively adding triethanolamine solution while stirring constantly until the gel was formed [14] .

**Carbopol 940 –** Gelling polymer

**Triethanolamine-** Gelling agent, pH adjusting agent, neutralizer

**Methyl Paraben -** Preservative

**Distilled Water, Glycerin and Polyethylene Glycol-**solvents

**Table 1: Formulation of herbal gel**

| **Ingredients (%)** | **HG1** | **HG2** | **HG3** | **HG4** | **HG5** | **HG6** |
|---|---|---|---|---|---|---|
| *Mahonia leschenaultia* extract | 1gm | 1gm | 1gm | 1gm | 1gm | 1gm |
| *Chromolaena odorata* extract | 1gm | 1gm | 1gm | 1gm | 1gm | 1gm |
| Carbopol 940 | 0.5mg | 0.75mg | 1.0 gm | 1.25 gm | 1.5 gm | 2.0 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 |

**HG= Herbal gel**

**Evaluation of herbal gel**

**Appearance and consistency**

The physical appearance of herbal gel formulations was visually examined for texture, and observations were made.

**Washability**

Formulations were applied to the skin, and then the ease and extent of washing with water were physically evaluated.

**Extrudability determination of formulations**

Herbal gel compositions were placed in collapsible metal tubes or collapsible aluminum tubes. The tubes were pushed to extrude the material, and the formulation's extrudability was tested by Aslani *et al.,* (2013) [15].

**Determination of Spreadability**

Two standard-sized glass slides (6 ×2) one of the slides was covered with the herbal gel formulation that was to be tested for spreadability. The second slide was positioned above the first in such a manner that the formulation was sandwiched between them for a total distance of 6 cm down the slide. The herbal gel mixture between the two slides was traced uniformly to produce a thin layer by placing 100 grams of weight on the upper slide.

The excess of the herbal gel formulation clinging to the slides was scraped off and the weight was removed. The bottom slide was attached to the apparatus's board, and one end of the top slide was linked to a string to which a 20-gram force could be imparted using a simple pulley. The time it took for the upper slide to travel 6 cm and separate from the lower slide under the weight's direction was recorded. The experiment was performed six times, with the average of the results determined for each gel formulation.

**Determination of pH**

A digital pH meter was used to determine the pH of the herbal gels. One gram of gel was dissolved in 25 ml of distilled water, and the electrode was dipped in the gel mixture until a steady reading was obtained. It was also reported that she was always reading. Each formulation's pH readings were repeated two times [16].

**Drug content**

1 gram of gel was placed in a 10 ml volumetric flask and diluted with methanol to assess the drug concentration. 1 ml Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) sodium carbonate were combined with 2 ml stock solution. The mixture was vortexed for 15 seconds before being let to sit for 15 minutes to develop color. A spectrophotometer was used to measure the absorbance at 765 nm.

## Anti -microbial activity

The antimicrobial activity of both the plant extracts & formulated gel was checked against E.coli by Well diffusion method [17].

## Results and Discussion

*The percentage yields for Mahonia leschenaultia* and *Chromolaena odorata* hydroalcoholic were found to be 8.52 and 7.63 % w/w respectively. *The Mahonia leschenaultia* extract contains alakaloid, flavonoid, diterpenes, phenol & proteins. While, *Chromolaena odorata* extract found to have presence of alkaloid, diterpenes , phenol, proteins, carbohydrate & saponins. Total alkaloid & phenol content in *Mahonia leschenaultia* extract was found to be 0.263 mg/ 100mg & 0.745mg/100mg respectively. In case of *Chromolaena odorata* total alkaloid & phenol content 0.374 mg/100 mg & 0.695 mg/100mg of dried extract respectively. The freshly produced formulations were light green. Clogging was determined to be absent in all formulations, and all formulations HG1, HG2, HG3, HG4, HG5, and HG6 had acceptable homogeneity and texture. The ease and extent of washing with water were physically assessed after the formulations were applied to the skin. Because of their non-greasy characteristics, all of the formulations were easily washable and left no residues on the skin when washed with water. All of the gel formulations were determined to have average extrudability and washability. Spreadability of the formulations HG1, HG2, HG3, HG4, HG5 and HG6 were studied and found to in the range of 19±7, 17±2, 24±4, 18±3, 15±2 and 20±2respectively. The Formulation HG5 showed the good Spreadability 6.93±8 among all formulation. The pH of the produced mixture was found to be comparable to the skin pH of 6.8 in the range of 6.8±0.2 to 7.5±0.3. Because the pH of all formulations was determined to be close to that of the skin, all formulations were classified as non-irritant. The viscosity of different gel samples was measured in the aforesaid formulas, and it was discovered that the viscosity increased. The viscosity of the HG5 formulation is excellent. The viscosity of formulations HG1, HG2, HG3, HG4, HG5 and HG6 were found to be 1583±8, 2641±9, 1854±6, 1978±5, 2796±7 and 1163±3. The phenolic content of prepared formulations was found to be 0.385±0.001, 0.652±0.002, 0.421±0.003, 0.245±0.005, 0.895±0.001 and

0.541±0.002 percentage for formulations HG1, HG2, HG3, HG4, HG5 and HG6 respectively. Antimicrobial activity showed that the formulation HG5 have greater antimicrobial efficacy against *E. coli*.

**Table 2: Physical characteristics of hydroalcoholic extract**

| Extract | Consistency | Colour | Odour | % Yield (w/w) |
|---|---|---|---|---|
| *Mahonia leschenaultia* | Solid | Dark green | Pungent | 8.52 |
| *Chromolaena odorata* | Solid | Dark green | Pungent | 7.63 |

**Table 3: Result of phytochemical screening of hydroalcoholic extract of *Mahonia leschenaultia and Chromolaena odorata***

| S. No. | Constituents | *Mahonia leschenaultia* extract | *Chromolaena odorata* extract |
|---|---|---|---|
| 1. | **Alkaloids** Hager's Test: | +ve | +ve |
| 2. | **Glycosides** Legal's Test: | -ve | -ve |
| 3. | **Flavonoids** Lead acetate Test: | +ve | -ve |
| 4. | **Diterpenes** Copper acetate Test: | +ve | +ve |
| 5. | **Phenol** Ferric Chloride Test: | +ve | +ve |
| 6. | **Proteins** Xanthoproteic Test: | +ve | +ve |
| 7. | **Carbohydrate** Fehling's Test: | -ve | +ve |
| 8. | **Saponins** Froth Test: | +ve | +ve |

**Table 4: Estimation of total alkaloid and phenol content of *Mahonia leschenaultia***

| S. No. | Total alkaloid content (mg/ 100 mg of dried extract) | Total phenol content (mg/ 100 mg of dried extract) |
|---|---|---|
| 1. | 0.263 | 0.745 |

**Table 5: Estimation of total alkaloid and phenol content of *Chromolaena odorata***

| S. No. | Total alkaloid content (mg/ 100 mg of dried extract) | Total phenol content (mg/ 100 mg of dried extract) |
|---|---|---|
| 1. | 0.374 | 0.695 |

**Table 6: Results of physical appearance of gel**

| Formulation | Colour | Clogging | Homogeneity | Texture |
|---|---|---|---|---|
| **HG1** | Green | Absent | Good | Smooth |
| **HG2** | Green | Absent | Good | Smooth |
| **HG3** | Green | Absent | Good | Smooth |
| **HG4** | Green | Absent | Good | Smooth |
| **HG5** | Green | Absent | Good | Smooth |
| **HG6** | Green | Absent | Good | Smooth |

**Table 7: Results of washability and extrudability of gel**

| Formulation | Washability | Extrudability |
|---|---|---|
| **HG1** | Good | Average |
| **HG2** | Good | Average |
| **HG3** | Good | Average |
| **HG4** | Good | Average |
| **HG5** | Good | Average |
| **HG6** | Good | Average |

**Table 8: Results of spreadability**

| Formulation | Spreadability (gcm/sec) |
|---|---|
| **HG1** | 19±7 |
| **HG2** | 17±2 |
| **HG3** | 24±4 |
| **HG4** | 18±3 |
| **HG5** | 15±2 |
| **HG6** | 20±2 |

**Table 9: Determination of pH**

| Formulation code | pH |
|---|---|
| **HG1** | 6.8±0.2 |
| **HG2** | 6.9±0.6 |
| **HG3** | 7.2±0.5 |
| **HG4** | 7.5±0.3 |
| **HG5** | 7.1±0.4 |
| **HG6** | 7.3±0.5 |

**Table 10: Results of Viscosity**

| Formulation | Viscosity (cps) |
|---|---|
| **HG1** | 1583±8 |
| **HG2** | 2641±9 |
| **HG3** | 1854±6 |
| **HG4** | 1978±5 |
| **HG5** | 2796±7 |
| **HG6** | 1163±3 |

**Table 11: Results of phenol content using Folin-Ciocalteu method**

| Formulation | Phenol content (mg/ 100 mg) |
|---|---|
| **HG1** | 0.385±0.001 |
| **HG2** | 0.652±0.002 |
| **HG3** | 0.421±0.003 |
| **HG4** | 0.245±0.005 |
| **HG5** | 0.895±0.001 |
| **HG6** | 0.541±0.002 |

**Table 12: Antimicrobial activity of extract and herbal gel formulation (HG5)**

| S. No. | Extract /Formulation | Zone of inhibition (mm) | | |
|---|---|---|---|---|
| | | **100mg/ml** | **50 mg/ml** | **25mg/ml** |
| 1. | *Mahonia leschenaultia* extract | 10±0.47 | 9±0.74 | 7±0.86 |
| 2. | *Chromolaena odorata* extract | 12±0.86 | 10±0.5 | 8±0.57 |
| 3. | Herbal gel | 18±0.47 | 16±0.74 | 14±0.57 |

## Conclusion

From the present study, it was concluded that the hydroalcoholic extract of *Mahonia leschenaultia* and *Chromolaena odorata* was enriched with phytochemicals. The formulations showed acceptable physical properties, and hence, were compatible with the skin. The in-vitro antimicrobial activity showed that the formulated topical gel of *Mahonia leschenaultia* and *Chromolaena odorata* showed good antimicrobial activity as compared to only extracts. In addition, the formulations passed the short-term stability, indicating the physical and chemical stability of the product. Hence, the formulated topical gel of the

hydroalcoholic extract of *Mahonia leschenaultia* and *Chromolaena odorata* were safe and efficient carriers, with potent antimicrobial activity.

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