INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA):IJPS00]
Journal Homepage: https://www.ijpsjournal.com

Research Article

Formulation And Evaluation Of Polyherbal Wound Healing Ointment Using Chromolaena Odorata, Coleus Amboinicus, Strobilanthes Alternata And Solanum Melongena

Sainabi P.[1]*, Meghna[1], Thashreefa P. M.[1], Sheetal Krishna A. T.[1], Vyshnavy Devy D. K.[2]

[1]*Bachelor of Pharmacy, Rajiv Gandhi Institute of Pharmaceutical Sciences and Research, Trikaripur, Kasaragod, India,671310*
[2]*Associate Proffesor, Department of Pharmacognosy, Rajiv Gandhi Institute of Pharmaceutical Sciences and Research, Trikaripur, Kasaragod, India,671310*

ARTICLE INFO

Received: 13 Sep 2024
Accepted: 16 Sep 2024
Published: 29 Sep 2024
Keywords:
Chromolaena odorata, Coleus amboinicus, Strobilanthes alternata, Solanum melongena, DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, antioxidant, anti-inflammatory and antimicrobial activity.
DOI:
10.5281/zenodo.13857577

ABSTRACT

Chromolaena odorata (Asteraceae) is a tropical and subtropical species of flowering shrub, commonly known as siam weed. Coleus amboinicus (Lamiaceae) is a semi-succulent perennial plant, commonly known as Indian borage. Strobilanthes alternata (Acanthaceae) is a prostrate plant with purple-coloured leaves, also known as red-flame ivy. Solanum melongena (Solanaceae) is a plant species grown as brinjal. The aim of the present work is the formulation and evaluation of polyherbal wound healing ointment from the extracts of Chromolaena odorata, Coleus amboinicus, Strobilanthes alternata and Solanum melongena. The leaves were collected from Trikaripur, and subjected to maceration. Later pharmacognostic studies like microscopical evaluation, ash value, and extractive value were carried out. The preliminary phytochemical studies of leaf extract in different solvents like ethanol, ethanol-distilled water and distilled water were carried out. The antioxidant activity of leaf extract was carried out using a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The ointment base was prepared and formulation of ointment was done by incorporating the extracts in the base using levigation method. A total of three formulations are prepared by varying the quantity of ingredients. After completion of formulation, it was evaluated for its physicochemical parameters like colour, odour, pH, spreadability, extrudability, consistency, solubility and washability. By using this result best formulation was selected from the three formulations. The in-vitro studies are carried out to evaluate the antimicrobial activity of polyherbal wound healing ointment. The results shows that polyherbal wound healing

***Corresponding Author:** Sainabi P.
Address: *Bachelor of Pharmacy, Rajiv Gandhi Institute of Pharmaceutical Sciences and Research, Trikaripur, Kasaragod, India,671310*
Email ✉: mail2sainup@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

healing ointment. The results shows that polyherbal wound healing ointment have better antioxidant, anti-inflammatory and antimicrobial activity.

INTRODUCTION

A wound is an interruption to skin integrity caused by physical trauma or disease [1]. Wound healing is a physiologic process that involves four stages such as haemostasis, inflammation, proliferation and remodelling [2]. Healing is a complex process involving co-ordinated interactions between diverse immunological and biological systems. It involves a cascade of carefully and precisely regulated steps and events that correlate with the appearance of various cell types in the wound bed during distinct phases of the healing process [3]. Several factors delay the wound healing process including bacterial infection, lymphatic blockage and diabetic mellitus, generally if the above factor could be altered by any agent, an increased rate could be achieved. Many herbal plants have wound healing property. Plants are more potent healers because they promote the repair mechanisms in the natural way [4]. The present work is to develop polyherbal ointment containing phytopharmaceuticals to be applied in the treatment of wound. Polyherbal formulations are effective for wound healing processs. They can stimulate a variety of physiological functions that accelerates the process of healing [5]. Chromolaena odorata is a traditional medicinal plant that is widely used for its wound healing property. In particular, the several parts of this herb have been used to treat wounds, burns and skin infections. Furthermore, it has also been shown to possess anti-inflammatory, antimicrobial and antioxidant properties. Its phytochemical components are alkaloids, flavonoids, flavanone, essential oils, phenolics, saponins, tannins and terpenoids [6]. Coleus amboinicus is a big succulent aromatic perennial herb belonging to the Lamiaceae botanical family. Its active ingredients have been demonstrated to inhibit AP-1 and TNF and increase wound contraction, resulting in wound healing, increasing deposition of collagen, and shortening epithelialization of the wound [7].

Strobilanthes alternata belongs to the Acanthaceae family and is commonly known as red flame ivy, purple waffle plant, and Murikooti. Historically, the leaves of this plant have been used for wound healing. The leaf extract can be applied to fresh cuts to help them cure quickly. Strobilanthes alternata is reported to contain many phytoconstituents such as alkaloids, flavonoids, carbohydrates, steroids, triterpenes, phenols and amino acids. They have antimicrobial, antioxidant, analgesic, and anti-inflammatory activities [8]. Solanum melongena (Solanaceae), commonly known as eggplant is one of the most consumed, economically valuable food source in various parts of the world. Solanum melongena has medicinal features such as analgesic, anti-inflammatory, antipyretic, antioxidant, antifungal, antiasthmatic, antidiabetic, hypocholesteremic, hypolipidemic, hypotensive and antihemorrhoidal effects [9]. By incorporating these four plants leaf extracts we make a polyherbal wound healing ointment with antioxidant and anti-inflammatory activity. For evaluating this activity, we use agar well diffusion method and DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

MATERIALS AND METHODS

Collection of plant material

The fresh leaves of Chromolaena odorata, Strobilanthes alternata, Coleus amboinicus and Solanum melongena were collected from Kasaragod and Kannur district, Kerala (India) in the month of April 2024.

Authentication of plant material

The plant material was collected from Kasaragod and Kannur district, Kerala (India) in the month of April 2024. The plant material was identified and authenticated by Dr.K.M. Sreekumar, Professor (Entomology) Collage of Agriculture Kerala Agricultural University, Padnekkad, Kasaragod (Dt), Kerala.

Extraction of plant materials

Extraction of Chromolaena odorata, Strobilanthes alternata, Coleus amboinicus and Solanum melongena was carried out separately by maceration.

Fresh leaves of chromolaena odorata washed and dried under shade at room temperature at 27°c for 3 weeks. Then leaves powdered by using electrical blender. About 70g of powder macerated in 110ml of 80%(v/v) ethanol were allowed to stand for 24hours.The extract were filtered and concentrated in vacuum [10].

Strobilanthes alternata(10g) dried leaves macerated with ethanol-water mixture (1:3 ratio). The solution was then kept in a dark place for 24 h at a temperature of 4 °C. Then the extract was filtered through Whatman filter paper [11].

A 20 g of dried Coleus amboinicus leaves was powdered finely and boiled in 100 ml of sterile double distilled water for 3 min. The extract is filtered through Whatman filter paper [12].

The fresh leaves of solanum melongena were air dried and powdered in the food processor. About 500 g of powdered sample was boiled in hot water for 30min and allowed to cool. After which it was filtered through Whatman filter paper. This filtrate is used to detect various biologically active constituents present in each plant extract [13].

Preliminary phytochemical screening

The extracts of selected plants were subjected to preliminary phytochemical screening to detect the various phytoconstituents such as alkaloids, carbohydrate, flavonoids, saponins, glycosides, tannins and phenols [14].

Formulation of polyherbal wound healing ointment

Three different formulations were prepared with varying concentration of ointment base named as F1 to F3. Concentration of ointment base ingredients was mentioned in Table 1 and concentration of polyherbal ointment ingredients was mentioned in Table 2.

Procedure

a. Initially ointment base was prepared by weighing accurately grated hard paraffin which was placed in evaporating dish on water bath. After melting of hard paraffin remaining ingredients were added and stirred gently to aid melting and mixing homogeneously followed by cooling of ointment base.
b. Herbal ointment was prepared by mixing accurately weighed Chromolaena odorata, Strobilanthes alternata, Coleus amboinicus and Solanum melongena leaves extract to the ointment base by levigation method to prepare a smooth paste with two or three times its weight of base, gradually incorporating more base until to form homogeneous ointment, finally transferred in a suitable collapsible tube [15].

Methods of evaluation

Following evaluation parameters were performed to ensure superiority of prepared ointment;

Physical parameter

The Physical parameter includes its state, colour, odour and texture which were evaluated manually.

pH

pH of prepared herbal ointment was measured by using digital pH meter. The solution of ointment was prepared by using 100ml of distilled water and set aside for 2hrs. pH was determined in triplicate for the solution and average value was calculated.

Spreadability

The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability. Lesser the time taken for separation of two slides results better spreadability.

Spreadability was calculated by following formula

$$S=MxL/T$$

Where, S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides

Extrudability

Extrudability test is the measure of the force required to extrude the material from a collapsible tube when certain amount of force has been applied on it in the form of weight. In the present study the quantity in percentage of ointment extruded from the tube on application of certain load was determined. The extrudability of prepared siam weed, red ivy, Indian borage, eggplant leaves extract containing ointment formulations was calculated by using following formula [16]:

Extrudability = Amount of ointment extruded from the tube x100/Total amount of ointment filled in the tube.

Solubility

Solublity was evaluated in boiling water, alcohol, ether, chloroform.

Washability

Formulation was applied on the skin and then ease extend of washing with water was checked.

Screening for antimicrobial activity

The required amount of agar was prepared and inoculated into it by microorganisms (E. coli). Then agar solution was poured into the petri plates and allowed to stand to solidify for a few minutes. After, solidification a sterilized well digger was used to generate the appropriate size of wells. The ointment samples are then filled in. The plates were incubated in inverted condition at 37°C for 48 hours. After 48 hours, the plates were observed for the presence of inhibition of bacterial growth, and it was indicated in the form of a clear zone of inhibition. The zone of inhibition obtained for the developed herbal ointment was compared with the standard. Ampicillin was used as a standard [17].

Screening for antioxidant activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method for evaluating the antioxidant activity of various substances.

Principle:

DPPH is a stable free radical that absorbs light at 517nm. When an antioxidant is added. It donates an electron to DPPH, neutralizing the free radical and reducing absorbance [18].

Procedure:

1. Prepare the DPPH working solution (0.1-0.2 mM) in methanol.
2. Add 100-200 µL of the DPPH solution to each well in a microplate.
3. Add 10-50 µL of the antioxidant sample (plant extracts) to each well.
4. Mix well and incubate for 30 minutes to 1 hour in the dark.
5. Measure the absorbance at 517 nm using a spectrophotometer.
6. Ascorbic acid was used as reference standard and dissolved in methanol to make the stock solution with the same concentration.
7. Control sample was prepared containing the same volume without any extract and reference (ascorbic acid).
8. Methanol was used as blank.
9. Calculate the percentage inhibition of DPPH using the formula:

% Inhibition = ((Abs control- Abs sample) / Abs control) × 100

- Where, Abs control is the absorbance of DPPH radical + ethanol
- Abs sample is the absorbance of DPPH radical + plant extract.

RESULTS AND DISCUSSION

Extraction of plant materials

The extraction of dried leaves of Chromolaena odorata, Strobilanthes alternata, Coleus amboinicus and Solanum melongena were carried out by maceration process by using suitable solvent. The extracts obtained were collected and concentrated which was weighed and kept in a

desiccator until it was used for further studies. It is shown in Figure 1.

Preliminary phytochemical screening

The plant leaves were macerated with different solvents. Macerated products were subjected to preliminary phytochemical screening to detect the following photochemical constituents in Table 3. The preliminary phytochemical study revealed the presence and absences of certain phytochemicals in the extract of Chromolaena odorata, Strobilanthes alternata, Coleus amboinicus and Solanum melongena.

Formulation of polyherbal wound healing ointment

Polyherbal wound healing ointment were prepared shown in Figure 2 and transferred in collapsible tube shown in Figure 3.

Evaluation of polyherbal ointment

Organoleptic evaluation

The organoleptic parameter such as state, colour, odour, texture was checked and results showed that the developed ointment were semisolid state, greenish in colour, characteristics in odour, smooth in texture which showed in Table 4.

Determination of pH

The pH of the prepared formulation was measured using digital pH meter. The pH of the formulation showed in the Table 5.

Spreadability

The spreadability of polyherbal ointment were studied and results showed in Table 6.

Extrudability

The extrudability of formulation were studied and results showed in Table 7.

Solubility

Soluble in boiling water, alcohol, ether and chloroform.

Washability

Washability of the formulation was good.

Screening for antimicrobial activity

The anti-microbial activity of the developed formulation was done by using agar well diffusion method. The ani-bacterial activity was measured in terms of zone of inhibition. The zone of inhibition obtained for polyherbal wound healing ointment was compared with standard Ampicillin. The developed formulations showed anti-bacterial activity against E. coli produced a better zone of inhibition of about 6m which was zone of inhibition produced by standard Ampicillin (10mm). The result of antibacterial activity of poly herbal ointment formulation showed in Table 8.

Screening for antioxidant activity

The anti-oxidant activity of the plant extract was done by DPPH free radical scavenging assay. The anti-oxidant activity was calculated in terms of % inhibition which showed in Table 9. The aim of study is to formulate the polyherbal ointment using leaf extract of Chromolaena odorata, Strobilanthes alternata, Coleus amboinicus and Solanum melongena for wound healing activity. Physicochemical and phytochemical investigations of the leaves of Chromolaena odorata, Strobilanthes alternata, Coleus amboinicus and Solanum melongena have been reported here in this thesis work. The preliminary phytochemical screening of plant shows that the ethanol, ethanol-water mixture and distilled water extracts shows the presence of major constituents such as tannins, flavonoids, saponins, phenols, terpenoids may contribute to antioxidant, antimicrobial, antihemorrhagic and anti-inflammatory activity which is beneficial in the treatment of wound. Phytoconstituents present in plants were extracted by ethanol, ethanol- water mixture and distilled water using maceration. Polyherbal ointment containing herbal extract were prepared and the results revealed that the prepared polyherbal ointment such as F1, F2 and F3 the F1 have good in appearance, homogeneity, and easily spreadable. Lower the value of spreadability greater the consistency. The in-vitro wound evaluations such as antimicrobial activity was done by using agar well diffusion method and

the antioxidant activity was evaluated using DPPH assay. However, in the present work polyherbal formulations reported to have more significant advantages over synthetic formulations. Hence, we conclude that polyherbal ointment for wound healing is effective, safe and ease of manufacturing and in the economic point of view they are cheap when compared to chemical-based ointment.

ACKNOWLEDGEMENT

I would like to acknowledge our guide Mrs. Vyshnavy Devy D K, Assistant. Professor, Department of Pharmacognosy, Rajiv Gandhi Institute of Pharmaceutical Science and Research for her constant guidance, valuable suggestions and encouragement. We are deeply indebted to Prof. Dr. M. Paridhavi, M Pharm, PhD, FABAP, Principal, Rajiv Ghandhi Institute of Pharmaceutical Science and Research, for his valuable advice and support to make the study successful. We also extend our sincere thanks to all teachers, who helped us for the completion of this dissertation.

REFERENCES

1. Nicky Cullum, Hannah Buckley, Jo Dumville, Jill Hall, Karen Lamb, Mary Madden, et al. Wounds research for patient benefit: a 5-year programme of research. National Institute for Health Research. 4, 2016, 1.
2. Frances Strodtbeck DNS, RNC, NNP. Physiology of wound healing. Newborn and Infant Nursing Reviews. 1, 2001, 43-52.
3. T Velnar,T Bailey, V Smrkolj. The wound healing process: an overview of the cellular and molecular mechanisms. The Journal of International Medical Research. 37, 2009, 1531.
4. Durga Nand thakur, Ankita Chourasiya. Evaluation of antimicrobial and wound healing activity of aqueous extract of medicinal plant on wister albino rats. International Journal of Phytopharmacy Research. 14, 2023, 45.
5. Satish Dubey, Ashwini Kumar Dixit. Preclinical evidence of polyherbal formulations on wound healing: A systematic review on research trends and perspectives. Journal of Ayurveda and Integrative Medicine. 14, 2023, 1.
6. Anushika Sirinthipaporn, Wannee Jiraungkoorskul. Wound healing property review of siam weed, Chromolaena odorata. Pharmacognosy Reviews. 11, 2017, 35-36.
7. K Monica, S Rajeshkumar, Ramasubramanian, Abilasha, Ramani, Pratibha, et al. Anti-inflammatory and antimicrobial effects of herbal formulation using karpooravalli, mint and cinnamon on wound pathogens. Journal of Advanced Pharmaceutical Technology and Research.13, 2022, 369-371.
8. Rex Devasahayam Arokia Balaya, Akhina Palollathil, Sumaithangi Thattai Arun Kumar, Jaikanth Chandrasekaran, Shubham Sukerndeo Upadhyay, Sakshi Sanjay Parate, et al. Role of Hemigraphis alternata in wound healing: metabolomic profiling and molecular insights into mechanisms. Scientific Reports. 14, 2024, 1-2.
9. Ceylon Donmez, Funda N Yalcin, Ozge Boyacioglu, Petek Korkusuz, Esra Kupeli Akkol, Emirhan Nemutlu, et al. From nutrition to medicine: Assessing hemorrhoid healing activity of Solanum melongena L. via in vivo experimental models and its major chemicals. Journal of Ethnopharmacology. 261,2020, 1-2.
10. Stanley Anyanwu, Imeobong J. Inyanga, Enosakhare A. Asemota, Okechi O. Obioma, Dorathy C. Okpokam, Victoria O. Agu, et al. Effect of ethanolic extract of Chromolaena odorata on the kidneys and intestines of

healthy albino rats. Integrative Medicine Research. 6, 2017, 293.

11. Jijo Koshy, Dhanaraj Sangeetha. Hemigraphis alternata leaf extract incorporated agar/pectin-based bio-engineered wound dressing materials for effective skincancer wound care therapy. Polymers. 15, 2023, 2.
12. Roshan Patel, Naveen K Mahobia, Ravindra Gendle, Basant Kaushik, Sudarshan K Singh. Diuretic activity of leaves of plectranthus amboinicus (Lour) spreng in male albino rats. Pharmacognosy research. 2, 2010, 86.
13. Laura Aracely Contreras- Angulo, Aldo Moreno-Ulloa, Rommel A. Carballo-castaneda, Josefina Leon-Felix, Jose Geovanni Romero-Quintana, Maribel Aguilar-Medina, et al. Metabolomic analysis of phytochemical compounds from agricultural residues of eggplant (Solanum melongena L.). Molecules. 27, 2022, 13.
14. Junaid R Shaikh, MK Patil. Qualitative tests for preliminary phytochemical screening: An overview. International journal of chemical studies. 8, 2020, 604-607.
15. Swati Siddheshwar Londhe, Mangesh Gautam Bhosale, Amol. Arun Joshi, Geeta Sapkale, Mohini Khandagale, Amitkumar Jadav. Formulation and evaluation of polyherbal ointment. International journal of advances in pharmacy and biotechnology. 6, 2020, 13.
16. Himal Paudel Chhetri, Nisha Shrestha Yogol, Jyoti Sherchan, Anupa K. C., S. Mansoor, Panna Thapa. Formulation and evaluation of antimicrobial herbal ointment. Kathmandu university journal of science, engineering and technology. 6, 2010, 102-107.
17. Rajasree PH, Viswanad V. Formulation and evaluation of antiseptic activity of polyherbal ointment. International journal of pharmacy and life sciences. 3, 2012, 2021-2031.
18. Siddartha Baliyan, Riya Mukherjee, Anjali Priyadarshini, Arpana Vibhuti, Archana Gupta, Ramendra pati Pandey, et al. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. 27, 2022, 1-5.

HOW TO CITE: Sainabi P. , Meghna, Thashreefa P. M., Sheetal Krishna A. T., Vyshnavy Devy D. K. , Formulation And Evaluation Of Polyherbal Wound Healing Ointment Using Chromolaena Odorata, Coleus Amboinicus, Strobilanthes Alternata And Solanum Melongena, Int. J. of Pharm. Sci., 2024, Vol 2, Issue 9, 1518-1524. https://doi.org/10.5281/zenodo.13857577