International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **15(4),** 26-33, November **(2026)** *Int. Res. J. Biological Sci.*

A Novel Polyherbal Therapeutic Balm for Pain and Inflammation relief: Formulation, Physicochemical characterization and Biological evaluation

Priya M., Neha M. Anand and Anshu Beulah Ram*
Department of PG studies in Biotechnology, Nrupathunga University. Bengaluru-560001, Karnataka, India
anshubeulahram@gmail.com

Available online at: www.isca.in, www.isca.me
Received 28th August 2026, revised 9th September 2026, accepted 4th October 2026

Abstract

Some of the most common ailments that demand safe and effective topical treatments include pain and inflammation. This study aimed to formulate and assess the antimicrobial, anti-oxidative, and anti-inflammatory activity of the polyherbal formulation of Acacia catechu and Vitexnegundo, eucalyptus oil and clove oil. Solvent extraction was employed to prepare extracts, followed by preliminary phytochemical screening and thin-layer chromatography (TLC) that confirmed the presence of bioactive secondary metabolites such as alkaloids, saponins, triterpenoids, flavonoids and glycosides in plant extracts. Beeswax, coconut oil, menthol, camphor, eucalyptus oil, and clove oil were the base ingredients used to formulate the polyherbal balm. The prepared formulation was assessed for the physicochemical properties such as appearance, pH, spreadability and washability, which showed satisfactory properties of the formulation for topical application. Antimicrobial activity was evaluated by broth dilution method against B. subtilis, E. coli, P. aeruginosa, S. aureus and C. albicans. The antibacterial activity of formulation was notable against the bacterium Bacillus subtilis (33 mm), Escherichia coli (24 mm), Pseudomonas aeruginosa (22 mm) but no activity was observed against Staphylococcus aureus. A large degree of antifungal activity was also noted against Candida albicans (35 mm). The antioxidant activity of formulation was observed to have antioxidant activity with 38.88% DPPH radical scavenging activity which showed moderate anti-inflammatory potential and but 19.44% protein denaturation inhibition. In conclusion, the developed polyherbal balm had good physicochemical properties and exhibited promising antimicrobial, antioxidant, and anti-inflammatory activity. Based on these findings, it can be concluded that the formulation can be used as a natural topical herbal formulation for pain, inflammation and microbes infections of the skin. Preclinical and clinical research is suggested to confirm its therapeutic potential and safety.

Keywords: Polyherbal balm, *Acacia catechu*, *Vitex negundo*, Eucalyptus oil, Clove oil, Antimicrobial activity, Antioxidant activity, Anti-inflammatory activity.

Introduction

The following are basic physiological reactions that serve to protect the body from harmful substance, infection and tissue damage—these reactions are pain and inflammation. Acute inflammation is needed to maintain healing and host defense while chronic or uncontrolled inflammation is associated with the development of some pathological conditions such as arthritis, musculoskeletal disorders, cardiovascular diseases, neurodegenerative disorders and autoimmune diseases[1]. Inflammatory pain substantially impacts quality of life and is a huge burden on global health. Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are still the main drugs used, but their long-term use can cause gastrointestinal ulcers, nephrotoxicity, hepatotoxicity and cardiovascular toxicity[2]. These restrictions have led to the pursuit of other therapeutic means that are safer and more effective, such as those that can be obtained from natural sources.

As chronic diseases become an increasing global burden, there has been a surge of interest in Traditional, Complementary, and Integrative Medicine (TCIM), which has become an important component of modern healthcare. The World Health Organization (WHO) estimates that more than one billion people worldwide use traditional medicine for disease prevention, health promotion, and the management of both acute and chronic diseases. The WHO emphasizes that the integration of traditional medicine into national healthcare should be supported by scientific evidence, quality assurance, safety evaluation and appropriate regulatory frameworks. The *WHO Global Report on Traditional, Complementary and Integrative Medicine* (2024) highlights the progress made by Member States in the regulation, research, education, and integration of traditional medicine[3]. Furthermore, the WHO Global Traditional Medicine Strategy 2025–2034 emphasizes the need to invest in research, quality assurance, innovation, and international collaboration to ensure the effective integration of TCIM into universal health coverage[4].

Traditional health care systems have used medicinal plants for the prevention and treatment of many diseases for a long time. Phytochemicals from plants, such as flavonoids, tannins, terpenoids, alkaloids and phenolic compounds, have a variety of biological properties, including antioxidant, anti-inflammatory,

analgesic and antimicrobial effects[5]. Polyherbal formulations are one of the natural therapies, which has received much attention recently as a combination of medicinal plants with complementary phytochemicals may yield effective synergies in therapeutic potential, increase efficacy and decrease toxicity from single herb products[6].

Topical drug delivery systems are defined as those that deliver drugs directly to the site of pain or inflammation and have become an important method for localized pain and inflammatory management to reduce systemic side effects of the drug. Herbal balms are semi-solid preparations that can be used to treat affected tissues and are appropriate for those who suffer from pain, inflammation, muscle pain and minor skin infections[7]. The inclusion of medicinal plants extracts and essential oils into topical formulations provides several therapeutic properties such as antioxidant, anti-inflammatory, antimicrobial and analgesic effects. Although they are widely used in traditional medicine, there is insufficient scientific evidence of their physicochemical properties and biological activity in many herbal balms.

In the present study, a polyherbal therapeutic balm is selected with the components of *Acacia catechu, Vitexnegundo*, clove oil and eucalyptus oil. *Acacia catechu* has been found to be rich in compounds, such as catechins, epicatechins, tannins, quercetin and flavonoids, which have shown strong antioxidant, anti-inflammatory, antimicrobial and wound healing properties due to their antioxidant effect and the ability to inhibit production of inflammatory mediators[8]. *Vitexnegundo* has been utilized in Ayurvedic medication since long for the administration of rheumatism, arthritis, agony and aggravation. It is rich in flavonoids, iridoid glycosides, terpenoids, lignans and essential oils, which have anti-inflammatory, analgesic, antioxidant and antimicrobial properties. Experimental research has shown that *Vitexnegundo* is effective at inhibiting prostaglandin synthesis and inflammatory cytokine production, which helps to decrease pain and inflammation, including swelling and redness[9].

Clove oil contains high amounts of eugenol, β-caryophyllene, and eugenyl acetate, of which eugenol seems to have strong local anesthetic, antimicrobial, antioxidant, anti-inflammatory, and analgesic properties by inhibiting the enzymes of cyclooxygenase, which inhibit inflammatory cytokines[10]. The addition of eucalyptus oil, whose main component is 1,8-cineole, further enhances the formulation with its anti-inflammatory, analgesic, antimicrobial and skin penetration-enhancing properties for enhanced topical drug delivery[11]. The synergistic combination of these medicinal plants and essential oils were formulated to exert complementary therapeutic effects, acting simultaneously on oxidative stress, inflammatory mediators, microbial growth and pain perception. There may be synergistic interactions between their phytoconstituents which enhance the therapeutic usefulness of the formulation than any single herb.

Systematic evaluation of biological activities of herbal formulations is required for scientific validation of these formulations. In vitro assays are useful as a preliminary study on their possible pharmacological potential and are commonly used to test herbal products. Anti-inflammatory activity can be measured by protein denaturation by egg albumin[12] which simulates inflammatory process in biological system. The antimicrobial assays define how much the formulation can reduce the activities of pathogenic microorganisms, and the antioxidant assays define its capacity to neutralize free radicals and reduce the oxidative stress. These are combined to give scientific proof of the biological effectiveness of herbals.

Medicinal plants have individually proved to be effective in terms of antioxidant, anti-inflammatory, analgesic and antimicrobial properties but there are limited studies which investigate the use of selected medicinal plants in a polyherbal topical balm. Most of the published reports have been based on individual use of plant extracts while very little information is available on the synergistic effect in topical formulations. Thus, physicochemical characterization and biological evaluation of the polyherbal therapeutic balm is necessary to prove its therapeutic potential.

In the present study, the physicochemical characterization and in vitro biological evaluation of a polyherbal formulation of *Acacia catechu, Vitexnegundo*, clove oil and eucalyptus oil is discussed and the present study will assist in understanding the activity of polyherbal therapeutics. The results help to understand the synergistic interactions between the selected medicinal plants and their phytoconstituents further to support the use of polyherbal formulations in controlling pain and inflammation. In addition, the study offers experimental data which could be useful for the future formulation of safe and effective and economically feasible herbal topical products for pharmaceutical and complementary medicine.

Hence, the present research was designed to develop and test a polyherbal formulation of therapeutic balm comprising of *Acacia catechu, Vitexnegundo*, clove oil and eucalyptus oil. The developed formulation was evaluated using physicochemical characterization and qualitative phytochemical screening to assess the antioxidant, antimicrobial and in vitro anti-inflammatory activity of the formulation in view of its potential use as a natural/topical formulation to manage pain and inflammation.

Materials and Methods

Plant collection and Authentication: The selected medicinal plants, *Acacia catechu* and *Vitex negundo*, Chikkanayanahalli, Tumkur District Karnataka - 572214 a reliable local sources. Each plant material was confirmed by qualified botanist Dr Suresh Kumar C from Department of Botony, Maharani Cluster University based on morphological characteristics and standard taxonomic features. The authentication was confirmed through

an official authentication certificate issued by the institution. (Authentication Certificate No: MCU/MSCWB/BOT- 37/2026-27).

Preparation of Plant Extract: The leaves of *Vitexnegundo* was collected and washed thoroughly to remove dust and other impurities, Air dried under shade and powdered. Approximately 3g of the dried powdered leaves was subjected to Soxhlet extraction using 70% of 100ml ethanol as the extraction solvent. After 11 cycles the extract was obtained. The obtained extract was concentrated by distillation and the concentrated extract was further dried under reduced pressure at 40 °C using a rotary evaporator[13]. The powdered bark of *Acacia catechu* was extracted using maceration technique in methanol as extracting solvent. 5 g of powdered bark was mixed with 20 mL of methanol and was shaken occasionally and for 48 h. The extracts were filtered thorough Whatman filter paper to obtain a clear filtrate pure extract was allowed for complete solvent evaporation[14].

Preliminary Phytochemical Screening: The preliminary phytochemical screening of the plant extracts was performed using the standard qualitative methods that can indicate presence of major secondary metabolites such as alkaloids, saponins, tannins, flavonoids, cardiac glycosides, triterpenoids, phenolic compounds. Alkaloids were detected by Wagner's reagent, saponins detected by froth test, tannins and phenols detected by ferric chloride test, flavonoids detected by sodium hydroxide test, cardiac glycosides detected by Keller–Killiani test[15] and triterpenoids detected by Salkowski test. The appearance of characteristic colour change and precipitates confirmed the presence of the respective phytochemical constituents[16].

Thin Layer Chromatography: The present plant extracts were separated and identified by using Thin layer chromatography (TLC) using the prescribed method .The stationary phase used was silica gel coated TLC plates. These extracts were spotted on the TLC plates and developed with the suitable solvent systems for the various classes of phytochemicals[17]. The solvents used were chloroform; glacial acetic acid: ethanol: distilled water (5.5:2.7:1.0:0.68 mL) for alkaloids, dichloromethane: methanol: water (6.0:3.9:0.9 mL) for saponins, chloroform: methanol: water (5.9:3.2:0.9 mL) for tannins, ethyl acetate: water: methanol: n-hexane (7.2:0.4:1.3:0.9 mL) for flavonoids, chloroform: methanol: ammonia solution (6.6:2.2:0.9 mL) for cardiac-glycosides and n-hexane: diethylether: glacial acetic acid (7.9:1.9:0.09 mL) for triterpenoids. The TLC plates were then dried in the air and the separated spots were visualized. Retention factor (Rf) values was calculated according to the following equation:

Rf = Distance travelled by the compound / Distance travelled by the solvent front

Development of pain and inflammation promoting polyherbal formulation: Beeswax, coconut oil, menthol and camphor were used as the base ingredients for the preparation of the polyherbal balm. The beeswax was used to make the formulation stiff enough and stable enough in its structure. The extracts of the plants were evenly mixed with coconut oil as a carrier and a natural emollient that enhanced the topical application and hydration of the skin. The use of menthol was added as this is considered as a cooling agent and an immediate analgesic. Camphor was added, because it is well known as being an analgesic, anti-inflammatory and counter-irritant which helps the topical preparations to be more effective. As a whole these excipients helped in the formulation of the polyherbal balm in terms of the stability, spread ability and skin permeation and performance of the balm[18,19].

Active Ingredients Used in the Polyherbal Balm: *Acacia catecú* bark extract, *Vitex negundo* leaf extract, eucalyptus oil and clove oil were the active ingredients used in the preparation of the polyherbal balm. The antioxidant, anti-inflammatory, antimicrobial and wound-healing properties of this plant stemmed primarily from the presence of catechins, flavonoids and tannins, which made *Acacia catechu* a valuable addition to the list. *Vitexnegundo* was chosen due to the large number of flavonoids, terpenoids, and phenolics with potential anti-inflammatory, analgesic, antioxidant, and antimicrobial properties associated with the plant[20]. Eucalyptus oil is present because of its anti-inflammatory, analgesic, antimicrobial and penetration enhancing properties, mainly due to the presence of its major constituent 1,8-cineole (eucalyptol). Clove oil was added as a natural anti-inflammatory and natural analgesic because it contains a high amount of eugenol that also has antioxidant and antimicrobial properties[21,22]. All of these herbal ingredients were used together, and the treatment of pain and inflammation was targeted topically with a synergistic therapeutic effect expected.

Procedure: Polyherbal balm Formulation was formulated using fusion method. The amount of all ingredients was precisely measured with a digital analytical balance. Fresh beeswax was melted in a double boiler at 60-70°C and coconut oil was added and shaken well to ensure a uniform base for the mixture. Then the extracts of Acacia catechu and Vitexnegundo were added to the molten base and stirred until evenly dispersed. Menthol and camphor were then added and mixed well so that they dissolved. Lastly, eucalyptus oil and clove oil was added in formulation with constant stirring to obtain the even spreading of all the ingredients throughout. The molten balm was then poured into sterile containers and allowed to cool down to room temperature until it hardened. The formulation was then prepared and stored in sealed containers in the cool, dry environment until further physicochemical and biological evaluations.

Physicochemical Evaluation: Physicochemical evaluations of the prepared Polyherbal balm formulation revealed their appearance, color, odor, texture, consistency, homogeneity, pH,

spreadability and washability. These characteristics are important for ensuring the formulation overall performance.

The visual characteristics of the formulation for appearance, color, odor, texture, consistency and uniformity were assessed. The physical characteristics of the balm such as uniformity, smooth texture, absence of lumps, phase separation and foreign particles were inspected to ensure they are acceptable.

Determination of pH of the formulation: The formulation pH was determined with a calibrated digital pH meter. About 1g of the balm was added to 10mL of distilled water and stirred to make a uniform mixture. The dispersion was allowed to stand for 10–15 min, filtered on muslin cloth and the pH of the filtered solution was measured. The three readings for all measurements were taken and the average pH was noted[23].

Spreadability: The glass slide method was used to determine spreadability. A standard amount of balm was placed between two glass slides, and a standard weight pressed on it to make a uniform film. The top slide was then slided and the time taken for the slides to separate completely was noted with the help of the applied weight. The spreadability was determined according to the following equation:

$S = (M \times L) / T$

The equation has S as the spreadability, M as the weight attached to the upper glass slide, L as the length of the glass slide and T as the time needed for separation of the glass slides.

Washability: A small amount of the balm was applied onto the skin and washed off easily by using running water, so that it was also washable. The formulation was evaluated for the ease and effective washing without leaving any residue on the washable articles[24].

Antimicrobial Activity: Polyherbal balm formulation was tested for antimicrobial activity by agar well diffusion method against Gram-positive bacteria (Staphylococcus aureus and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungal organism (*Candida albicans*).

The bacterial strains were grown on Nutrient Broth medium (NBM) and Luria–Bertani (LB) medium and *Candida albicans* was grown on Sabouraud Dextrose Agar (SDA). Media were prepared as per manufacturer's instructions, sterilized by autoclaving at 121°C and 15 psi for 20-25 min and poured in sterile Petri dishes. The growth of bacterial strains was done at 37°C for 24h whereas the growth of fungal strain was done at room temperature for 72hrs to obtain the fresh microbial cultures[25].

The anti-microbial activity was tested by the agar well diffusion method. Uniform microbial culture was used in agar plates through the use of sterile cotton swabs. A sterile cork borer was then used to aseptically punch holes into the agar, creating wells. The standard used was 2.5µL Nitrofurantoin for the bacteria and 2.5µL Clotrimazole for the fungus. Bacterial plates were grown at 37°C for 24 h and fungal plates were grown at room temperature for 72h. The antimicrobial activity was determined through measuring the diameter of the zone of inhibition (mm) that surrounded each well[26].

In Vitro Antioxidant Activity: DPPH Radical Scavenging Assay: The antioxidant activity of the polyherbal balm was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity method. Ascorbic acid has been chosen as the standard antioxidant. A formulation stock solution was prepared at the concentrations of 200 to 1000 µg/mL. For 2, 4, 6, 8 and 10 mL aliquots of each concentrations, 2 mL of freshly prepared DPPH solution was added to the test tubes respectively. Samples were placed in the dark at room temperature for 25 min. The absorbance was measured at 517nm and distilled water and the corresponding sample used as a blank were used in the UV–Visible spectrophotometer. The control was made by adding 2 mL of DPPH solution to 2 mL of methanol. The DPPH radical scavenging activity (RSA%) was determined by using below equation[27].

DPPH Radical Scavenging Activity (%) = $[(A_a - A_1) / A_a] \times 100$

Where: A_a is the absorbance of the control sample and A_1 is the absorbance of the test sample.

In Vitro Anti-inflammatory Activity: Determination of the egg albumin assay: The egg albumin denaturation assay was used to assess the anti-inflammatory activity of the polyherbal balm formulation in vitro. Aspirin (0.1%) was used as the standard drug. The reaction mixtures were made up of 2.8 mL of phosphate-buffered saline (PBS) (pH 6.4) and 0.2mL of fresh hen egg albumin in addition to 2mL of the test formulation (at concentrations of 100, 200, 300, 400 and 500µg/mL). The reaction mixtures were incubated at 37 ± 2°C for 15 min and then heated at 70°C for 10 min. The absorbance was measured after the samples cooled to room temperature at 660 nm in UV–Visible spectrophotometer using PBS as blank. The percent inhibition of protein denaturation was determined using the equation below[28].

Percentage Inhibition (%) = $[(A_a - A_1) / A_1] \times 100$

Where: A_a is the absorbance of the control and A_1 is the absorbance of the test sample.

Results and Discussion

Yield of plant samples extracted: After the complete evaporation of the solvent, the extraction yield of *Acacia catechu* and *Vitexnegundo* were calculated. The percentage yield was calculated from the weight of dried plant material and

weight of dried extract. *Vitexnegundo* demonstrated the highest extraction yield (24.3%) while *Acacia catechu* had an extraction yield of 8.8%. *Vitexnegundo* yield shows enhanced extraction when the extraction was carried out under the selected extraction condition, which is due to the higher level of extraction of soluble phytoconstituents.

Preliminary Phytochemical Screening: The preliminary phytochemical screening was carried out to determine the major classes of secondary metabolites present in the *Acacia catechu, Vitexnegundo*, Eucalyptus oil and Clove oil extracts and essential oils which were employed in the formulation. The qualitative analysis singled out a number of active phytochemicals namely, the alkaloids, saponins, cardiac glycosides, triterpenoids, and flavonoids but with different distribution in the tested samples. The result showed that *Acacia catechu* was positive for alkaloids, saponins, triterpenoids while *Vitexnegundo* was positive for saponins, cardiac glycosides, and triterpenoids. In this study, it was found that the Clove oil had saponins, flavonoids and cardiac glycosides, while the Eucalyptus oil had saponins and cardiac glycosides. The developed polyherbal balm formulation has therapeutic potential owing to the antiviral, antioxidant, anti-inflammatory activities of these phytochemicals.

Thin Layer Chromatography (TLC): For the selected plant extracts and essential oils for the formulation, the phytochemical fingerprints were carried out using the technique of thin layer chromatography (TLC). Chromatograms were developed with suitable developing system and the retention factor was calculated by using standard retaining formula.

The analysis using TLC technique revealed the characteristic phytochemical constituents of ingredients which were selected. *Acacia catechu* showed alkaloids (Rf = 0.85), saponins (Rf = 0.71), and triterpenoids (Rf = 0.72). In the present study, *Vitexnegundo* leaf extract yielded saponins (Rf = 0.53), cardiac glycosides (Rf = 0.75) and triterpenoids (Rf = 0.08). In Eucalyptus oil, saponins (Rf = 0.65) and cardiac glycosides (Rf = 0.70) were detected while the cardiac glycosides (Rf = 0.53) and flavonoids (Rf = 0.65) were found in clove oil. The list of the selected ingredients was confirmed by TLC profile with the presence of bioactive phytochemicals supporting the suitability for preparation of polyherbal balm.

Polyherbal Balm Formulation: A polyherbal balm was successfully prepared by using ethanolic extracts of *Acacia catechu* and *Vitexnegundo* apart from the eucalyptus and clove oil. The bases used in the formulation were beeswax and coconut oil, and the ingredients added were menthol and camphor to provide a cooling effect and analgesic properties. The formulation was prepared by fusion method and had a smooth, homogeneous and semi-solid herbal balm with a pleasant herbal odor. The prepared formulation had good physical appearance and was found appropriate for subsequent physicochemical, antimicrobial, antioxidant, anti-inflammatory and user acceptability assessment.



Figure-1: Polyherbal Balm Formulation.

Evaluation Parameters: Organoleptic Evaluation: The prepared polyherbal balm was assessed for its organoleptic properties such as appearance, colour, odour, texture, consistency and homogeneity. The formulation was smooth, semi-smooth and had light green colour with pleasant aromatic odour. The balm was smooth without a gritty texture, homogenous and without any visible lumps and no phase separation was observed. These observations show that the plant extracts and essential oils are incorporated in the balm successfully and it is suitable for topical application.

pH Evaluation: To check the suitability of the prepared polyherbal balm for topical application, the pH was measured by using a calibrated digital pH meter. The pH of the formulation was 6.43, a range that is within the normal pH of human skin (4.5-6.5). This finding shows that the formulation may be used topically without causing any irritation.

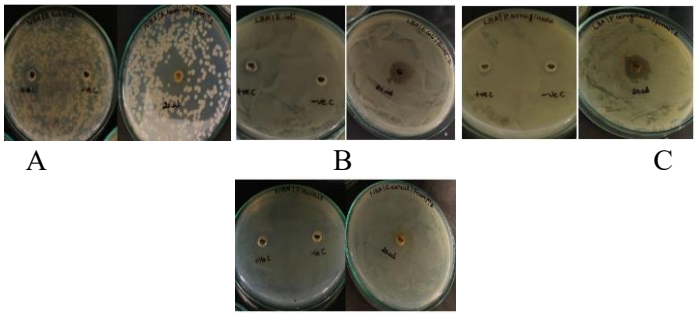
Spreadability: Spreadability was evaluated to determine the ease of application of the balm on the skin surface. The spreadability value of the formulation was found to be 25 g·cm/s. This demonstrates the ease of spreading and uniform distribution of the balm on the skin and allows for easy application and increased patient acceptability.

Washability: Washability was evaluated to check the requirement for using water for the removal of balm from the skin. The formulation had moderate washability and can be easily removed after washing with soap and water. The same is desirable of products for topical use since it facilitates skin cleaning after application without leaving excess residue.

Bioactive Assessment against Microbes: The prepared polyherbal balm formulation was evaluated for antimicrobial activity against two gram positive bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*), two gram negative bacteria strains (*Escherichia coli, Pseudomonas aeruginosa*) and one fungal strain (*Candida albicans*) by agar well diffusion method. 2.5µL Nitrofurantoin was used as the positive control for antibacterial activity, while 2.5µL Clotrimazole served as the positive control for antifungal activity. The antimicrobial activity was determined by measuring the zone of inhibiton surrounding each well incubation.

Table-1: Antimicrobial activity of the prepared polyherbal balm formulation.

Treatment	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Positive Control (mm)	18	14	12	18
Formulation (mm)	33	24	22	0



A B C D

Figure-2: Antibacterial activity against A) *Bacillus subtilis* B) *Escherichia coli* C) *Pseudomonas aeruginosa* D) *Staphylococcus aureus.*

In the present study, the prepared poly herbal balm showed different kinds of anti-bacterial activity against the test microorganisms. The largest diameter of inhibition was seen against *Bacillus subtilis* (33 mm) followed by *Escherichia coli* (24 mm) and *Pseudomonas aeruginosa* (22 mm) respectively. None of the zone of inhibition was visible against *Staphylococcus aureus* due to effectiveness of the formulation to work only under experimental conditions. The obtained results indicate that the developed poly herbal balm has wide spectrum anti bacterial activity and the most highly effective one is *B. subtilis*.

Table-2: Antifungal activity pf the prepared polyherbal formulation.

Formulation	*Candida albicans*
Positive Control (mm)	39
Formulation (mm)	35



Figure-3: Antifungal activity against *Candida albicans.*

*Candida albicans*was used to test the antifungal effect of the prepared polyherbal balm. The formulation however yielded a zone of inhibition of 35 mm which was comparable to the positive control which yielded 39 mm which indicates a high antifungal activity against *Candida albicans*. The outcome revealed that the fabricated formulation has an excellent antifungal activity.

In vitro antioxidant activity (DPPH Radical Scavenging Assay): The free radical scavenging activity of prepared polyherbal balm was determined by DPPH free radical scavenging assay. Two methods were used to assess the antioxidant potential by measuring the absorbance at 517 nm.

The formulation of polyherbal balm had 38.88% scavenging activity while the balm base gave only 4.76% scavenging activity. The results obtained showed that the herbal extracts and essential oils had a significant effect on the antioxidant capacity of the formulation when compared to pure base, indicating the presence of bioactive phytochemicals that neutralize free radicals with antioxidant activity.

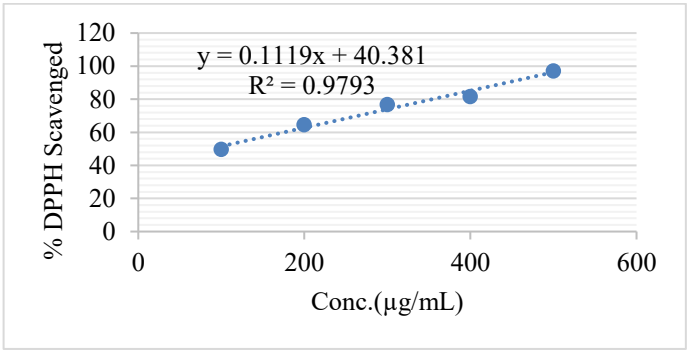


Figure-4: Graph for DPPH antioxidant assay.

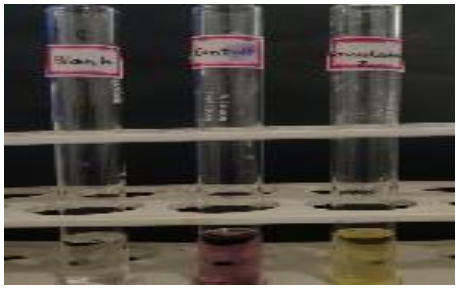


Figure-5: DPPH scavenging activity for formulation.

In Vitro Anti-inflammatory Activity (Egg Albumin Denaturation Assay): The anti-inflammatory activity of the prepared polyherbal balm was determined by using Egg albumin denaturation assay method. The anti-inflammatory activity was assayed by determining the absorbance at 660 nm after heat induced denaturation of proteins.

This polyherbal balm formulation showed the maximum inhibition of protein denaturation of 19.44% while the balm base showed 7.2% inhibition. From these findings, it can be

concluded that the herbal extracts increased the anti-inflammatory activity of formulation than that of the base. The observed anti-protein denaturation activity indicates significant anti-inflammatory activity of the developed polyherbal balm, which could be useful for topical treatment of pain and inflammation.

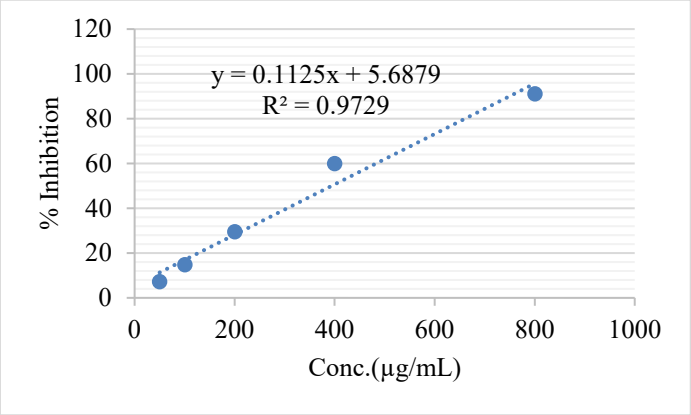


Figure-6: Graph for Protein Denaturation Assay.

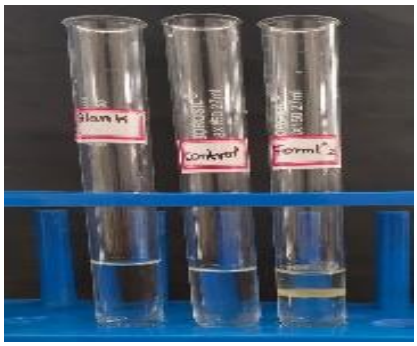


Figure-7: Anti-inflammatory protein denaturation assay.

Conclusion

The present study was successfully developed and the polyherbal balm formulation of *Acacia catechu, Vitexnegundo*, eucalyptus oil and clove oilwas tested for pain and inflammation. The selected herbal ingredients revealed the presence of important phytochemical compounds such as alkaloids, saponins, flavonoids, glycosides and triterpenoids, through preliminary phytochemical screening and thin-layer chromatography, demonstrating the therapeutic potential of the herbal ingredients. The formulated balm exhibited good physicochemical characteristics namely smooth semisolid appearance, pleasant aroma, uniform appearance, acceptable pH (6.43), good spreadability and moderate washability which is suitable for topical application. The activity of the formulation against antimicrobial microorganisms, namely *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*, and the fungus, *Candida albicans* was found to be promising, whereas, no antimicrobial activity was observed against the bacteria, *Staphylococcus aureus*. The best antibacterial activity was against the bacterium *Bacillus subtilis* (33 mm) and the formulation also exhibited good antifungal activity against *Candida albicans* (35 mm). The DPPH radical scavenging assay showed moderate antioxidant potential up to 38.88%, suggesting the potential of the formulation to scavenge free radicals. The formulation showed 19.44% inhibition in the Egg Albumin Protein denaturation assay which confirms the anti-inflammatory effect of the formulation. In general, the results indicated that developed polyherbal balm has good antimicrobial, antioxidant and anti-inflammatory activity and acceptable physicochemical properties making it suitable for further use as a topical herbal product in pain and inflammation management. Further studies, including stability evaluation, toxicity assessment, clinical trials and molecular characterization are recommended to validate the safety and efficacy and validate its therapeutic application.

Acknowledgement

The authors are also grateful to Mr. Aravind Ganessin, Managing Director, Dextrose Technologies Private Limited, for providing the laboratory facilities, technical support, and necessary resources to successfully carry out the experimental work. Special thanks are extended to Dr. Suresh Kumar, Professor, Department of Botany, Maharani Cluster University, Bengaluru, for authenticating the plant material used in this study.

References

1. Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature*, 454(7203), 428–435.

2. Vane, J. R. and Botting, R. M. (1998). Anti-inflammatory drugs and their mechanism of action. *Inflammation Research*, 47(Suppl. 2), S78–S87.

3. World Health Organization. (2024). *WHO global report on traditional, complementary and integrative medicine 2024*. World Health Organization.

4. World Health Organization. (2025). *WHO global traditional medicine strategy 2025–2034*. World Health Organization.

5. Newman, D. J. and Cragg, G. M. (2020). Natural products as sources of new drugs over nearly four decades (1981–2019). *Journal of Natural Products*, 83(3), 770–803.

6. Parasuraman, S., Thing, G. S. and Dhanaraj, S. A. (2014). Polyherbal formulation: Concept of Ayurvedic medicine. *Journal of Traditional and Complementary Medicine*, 4(2), 73–80.

7. Benson, H. A. E. (2005). Transdermal drug delivery: Penetration enhancement techniques. *Current Drug Delivery*, 2(1), 23–33.

8. Ismail, S. and Asad, M. (2009). Immunomodulatory activity of *Acacia catechu*. *Indian Journal of Physiology and Pharmacology,* 53*(*1), 25–33.

9. Dharmasiri, M. G., Jayakody, J. R. A. C., Galhena, G., Liyanage, S. S. P. and Ratnasooriya, W. D. (2003). Anti-inflammatory and analgesic activities of mature fresh leaves of Vitexnegundo. *Journal of Ethnopharmacology*, 87(2–3), 199–206.

10. Cortés-Rojas, D. F., de Souza, C. R. F. and Oliveira, W. P. (2014). Clove (Syzygiumaromaticum): A precious spice. *Asian Pacific Journal of Tropical Biomedicine*, 4(2), 90–96.

11. Juergens, U. R., Stöber, M., Vetter, H., & Richter, K. (2003). Anti-inflammatory activity of 1,8-cineole in bronchial asthma. *Respiratory Medicine*, 97(3), 250–256.

12. Mizushima, Y. & Kobayashi, M. (1968). Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *Journal of Pharmacy and Pharmacology*, 20(3), 169–173.

13. Lakshmi, T., Rajeshkumar, S., Gurunathan, D., Geetha, R., Roy, A. and Trishala, A. (2019). *Physicochemical profile of Acacia catechu bark extract—An in vitro study*. *Indian Journal of Public Health Research & Development*, 10(10), 33–37.

14. Tiwari, A. and Tiwari, A. (2024). Flavonoids in Acacia catechu bark extracts and bibliographic analysis in Guna, Madhya Pradesh, India. *International Journal of Pharmacy and Pharmaceutical Sciences*, 16(6), 43–48.

15. Jency, S., Jeya Sundara Sharmila, D. and Patrick Gomez, M. (2013). Phytochemical screening, functional group and elemental analysis of *Crossandra infundibuliformis (L.) Nees* flower extract. *Indian Journal of Natural Sciences,* 4(21), 1442–1447.

16. Shaikh, J. R. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 8(2), 603–608.

17. Bele, A. A. and Khale, A. (2011). An overview on thin layer chromatography. *International Journal of Pharmaceutical Sciences and Research,* 2(2), 256–267.

18. Chavan, S., Salwade, S., Jakhad, V. and Chavan, J. (2025). Formulation and evaluation of pain reliever balm with diclofenac. *World Journal of Pharmaceutical Research*, 14(11), 1144–1162.

19. Patel, M. G., Patel, P. M. and Agnihotri, V. (2025). Formulation and evaluation of poly-herbal pain relief balm. *International Journal of Creative Research Thoughts*, 13(5).

20. Panday, G. and Kumar, R. (2013). A review on phytochemical and pharmacological properties of Vitexnegundo Linn. *Journal of Pharmacognosy and Phytochemistry*, 2(3), 89–95

21. Juergens, U. R., Dethlefsen, U., Steinkamp, G., Gillissen, A., Repges, R. & Vetter, H. (2003). Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: a double-blind placebo-controlled trial. *Respiratory medicine*, 97(3), 250-256.

22. Yedke, A. and Pawar, B. (2025). Formulation and evaluation of herbal balm using clove oil for pain relief and anti-inflammatory. *International Journal of Scientific Research and Technology*, 2(12), 405–411

23. Bindod, H. V., Dafe, V. N., Hatwar, P. R., &Bakal, R. L. (2025). Formulation and evaluation of herbal analgesic balm. *Journal of Drug Delivery and Therapeutics*, 15(8), 27–33.

24. Markad, S. R., Magar, K. S. & Salunke, P. S. (2024). Formulation and evaluation of herbal pain relief balm. *International Journal of Creative Research Thoughts*, 12(5), b793–b809.

25. Dev, S. K., Choudhury, P. K., Srivastava, R., & Sharma, M. (2019). Antimicrobial, anti-inflammatory and wound healing activity of polyherbal formulation. *Biomedicine & Pharmacotherapy*, 111, 555–567.

26. Pradhan, S., Dash, S., Parida, S., Sahoo, B., &Rath, B. (2023). Antioxidant and antimicrobial activities and GC/MS-based phytochemical analysis of Parmotrematinctorum. *Journal of Genetic Engineering and Biotechnology*, 21, Article 59.

27. Kumar, A. and Kumar, D. (2015). Development of antioxidant rich fruit supplemented probiotic yogurts using free and microencapsulated Lactobacillus rhamnosus culture. *Journal of Food Science and Technology*, 53(1), 667–675.

28. Joshi, P., Yadaw, G. S., Joshi, S., Semwal, R. B. and Semwal, D. K. (2020). Antioxidant and anti-inflammatory activities of selected medicinal herbs and their polyherbal formulation. *South African Journal of Botany,* 130, 440–447.