



A BRIEF REVIEW ON POLY HERBAL DRUGS: AS ANTI-MICROBIAL AND ANTI-INFLAMMATORY TOPICAL GEL

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ABSTRACT

This review main aim is to promote an importance of various medicinal herbs used its uses in topical administration as anti-inflammatory and anti-microbial gel and helpful in formulating and evaluating a polyherbal gel. A polyherbal anti-inflammatory and antimicrobial gel involves leveraging the medicinal properties of natural ingredients such as aloe vera, Tulsi, liquorice root, and cardamom. These ingredients are known for their therapeutic effects, including anti-inflammatory and antimicrobial properties. The aim of this study is to develop a herbal gel that provides relief from inflammation and pain, while ensuring safety and minimal side effects compared to synthetic alternatives. The formulation process requires careful selection and optimization of ingredient concentrations to enhance the efficacy and stability of the gel. The evaluation phase includes a series of physicochemical and microbiological tests to determine the cream's stability, anti-inflammatory, and analgesic activities. The polyherbal gel is expected to exhibit significant therapeutic effects, providing a natural and effective solution for managing pain and inflammation. The natural origin of the ingredients ensures that the gel is free from harmful chemicals and preservatives, making it suitable for long-term use. This review aim is to offer an alternative to conventional anti-inflammatory and antimicrobial gel, highlighting the potential of natural herbs in promoting skin health and relieving discomfort. Through rigorous testing and evaluation, the formulated gel is expected to demonstrate strong anti-inflammatory and analgesic properties, contributing to the growing popularity of herbal-based skincare solutions.

KEYWORDS: Anti-microbial, Anti-inflammatory, Polyherbal gel.

INTRODUCTION

Gels are semisolids that range from translucent to opaque and have a high solvent to gelling agent ratio. Gelling agents combine or entangle to create a three-dimensional colloidal network structure when they are distributed in the right solvent.^[1] Typically, polyherbal gel is a blend of herbs that work better together than when taken separately. Herbal formulations are in high demand on the international market. The therapeutic and beauty benefits of plants are widely recognized.^[2] Topical formulations known as polyherbal gels blend extracts from several different plants or herbs to produce a single product that may have antibacterial qualities.^[3] These gels are intended

to treat a range of ailments, particularly those brought on by microbial infections, by utilizing the combined therapeutic qualities of several botanical components.^[4]

Polyherbal gels as antimicrobial agents

They provide a natural substitute for traditional antibacterial therapies, maybe with fewer adverse effects. When many herbs are combined, they may have synergistic effects that increase the antibacterial potency overall. These gels have localized antibacterial activity and can be administered topically.^[5]

Poly herbal gels as anti-inflammatory agents

Compared to preparations made from a single herb, polyherbal gels offer a promising way to create multi-component anti-inflammatory medicines with possibly increased efficacy and fewer adverse effects. To completely understand their modes of action and the best clinical uses, more investigation is required.^[6]

METHODS AND MATERIALS

Preparation of herbal extracts

The solvent of choice for removing active ingredients from herbs is usually ethanol. There are several extraction processes used some are listed below.

Types of extraction

Maceration: Soaking coarse powdered herb material in a solvent (Usually ethanol) for at least 3 days. The solvent is then separated from the solid material by filtration or decantation.^[6]

Infusion: This method is comparable to maceration, but it uses finely powdered herb material and requires less time to extract. Ideal for bioactive substances that

dissolve easily.^[7]

Decoction: Water is used as the solvent in a continuous hot extraction process. Used for plant materials that are heat-stable and water-soluble.^[8]

Percolation: Extracting plant material with successive solvent applications over time using a percolator equipment.^[9]

Soxhlet extraction: Using a Soxhlet device, continuous hot extraction is accomplished. Ideal for plant substances that only partially dissolve in the selected solution.^[10]

CO2 extraction: The solvent is either supercritical or subcritical carbon dioxide can be adjusted to target parts without subjecting them to extreme heat.^[11]

Ethanol extraction: This technique uses ethanol as the solvent and is frequently used in conjunction with other techniques, such as distillation, to create extracts that don't require a solvent.^[11]

Table 1: Herbs used in Anti-inflammatory and Anti-microbial activity.

S. no.	Herb	Title	Method of extraction	Conclusion	Reference
1.	Curcuma longa (Turmeric)	Preliminary study on anti-rheumatic activity of curcumin (diferuloyl methane)	Soxhlet extraction	When compared to phenylbutazone, which is employed as a positive control, the results indicate that curcumin may be useful in reducing RA's clinical Instantiations, such as morning stiffness and joint swelling, and in treating RA's inflammation.	[12]
2	Verbena officinalis (Vervain)	Anti-inflammatory and analgesic activity of the topical preparation of Verbena officinalis L	Extracted with 80% aqueous solution of 1,3-buthylene glycol	Studies on carrageenan- induced edema showed that the extract has anti- inflammatory and analgesic effects.	[13]
3	Zingiber officinale	Repeated Oral Administration of a Squeezed Ginger (Zingiber officinale) Extract Augmented the Serum Corticosterone Level and Had Anti- Inflammatory Properties	Soxhlet extraction	Long-term use of the extract has decreased proinflammatory indicators and raised serum corticosterone levels	[14]
4	Rosmarinus officinalis (Rosemary)	A pilot trail evaluation meta050 a proprietary combination of reduced iso-alpha acids, rosemary extract and oleanoic acid in patients with arthritis and fibromyalgia	Hydroalcohol Maceration	In patients suffering from osteoarthritis, extract reduced inflammatory indicators such as hs-CRP.	[15]
5	Borago officinalis	Borage oil reduction of rheumatoid arthritis activity may be mediated by increased cAMP that suppresses tumor necrosis factor-alpha	Moslty Maceration	Borage oil shows anti- inflammatory properties for rheumatoid arthritis (RA)	[16]

6	<i>Boswellia serrata</i>	Modulation of the immune system by <i>Boswellia serrata</i> extracts and boswellic acids	Hydro- distillation	Boswellic acid, the main component of this gum, has the capacity to inhibit C3 convertase and limit the classic complement system pathway. Likewise, it has proven to have anti- inflammatory effects both topically and systemically.	[17]
7	Aloevera	Antimicrobial effects of Aloe Vera on some human pathogens M	Decolorized whole leaf extract method	Demonstrates antioxidant, antibacterial, and anti- inflammatory properties.	[18]
8	<i>Allium cepa</i>	In vitro antimicrobial activity of <i>Allium cepa</i> (dry bulbs) against Gram positive and Gram- negative bacteria and fungi	Red bulbs are crushed and squeeze out the juice, concentrate the extract in alcohol and store in low temperature	Ant scarring, anti- inflammatory activity is observed.	[19]
9	<i>Eucalyptus globulus</i>	Bioactive potential of essential oil extracted from the leaves of <i>Eucalyptus globulus</i> (Myrtaceae)	Hydro- distillation	<i>Eucalyptus</i> gel combinations had a four (4) fold increase in antimicrobial activity against <i>S.epidermidis</i>	[20]
10	<i>Calendula officinalis</i> (Marigold)	Anti-Inflammatory Activity of <i>Calendula officinalis</i> L. Flower Extract	Maceration	Skin wounds and diseases where iNOS contributes to the pathophysiology, such as vitiligo, rosacea, melasma, psoriasis, irritative and allergic contact dermatitis, and cutaneous toxicities from cancer treatment, are treated with calendula oil.	[21]
11	<i>Allium sativum</i> (Garlic)	Antioxidant, anti-inflammatory, and antimicrobial properties of garlic and onions	Aqueous alcohol extraction	Decreases the spotting of the scar and acts as anti- inflammatory activity.	[22]
12	<i>Ocimum sanctum</i> (Holy Basil)	Quantification of Eugenol, Luteolin, Ursolic Acid, and Oleanolic Acid in Black (Krishna Tulasi) and Green (Sri Tulasi) Varieties of <i>Ocimum sanctum</i> Linn. Using High- Performance Thin- Layer Chromatography	Maceration	Has many medicinal values acts as both anti- inflammatory and anti-microbial activity.	[23]
13	<i>Azadirachta indica</i> (Neem)	Antibacterial Activity of <i>Azadirachta indica</i> Leaf Extracts Against Some Pathogenic Standards and Clinical Bacterial Isolates	Maceration	Neem shows promise as an anti-inflammatory medication. It may reduce inflammation in a number of ways, such as by inducing apoptosis and inhibiting NF- κ B.	[24]
14	<i>Tamarindus indica</i>	Antimicrobial, Analgesic and Anti - Inflammatory Activity Reported on <i>Tamarindus indica</i> Linn Root Extract	Ethanol extraction	Tamarind exhibits potent anti-inflammatory properties through a number of routes, including the inhibition of key inflammatory enzymes. Because of its safety profile, it can be employed as a natural anti-inflammatory and anti-microbial agent.	[25]

15	Citrus limon	Chemical analysis and bioactivity evaluation of Citrus limon leaves volatile oil from Palestine: investigating phytochemical, anti-inflammatory, antimicrobial, and cytotoxic properties	Solvent extraction	Significant anti-inflammatory activity is shown by citrus limon, both by itself and in combination with ginger, suggesting the possibility of natural anti-inflammatory treatments.	[26]
16	Oenothera biennis (Evening Primrose)	The sterols isolated from Evening Primrose oil modulate the release of proinflammatory mediators.	Oil extraction	Because the sterols in primrose oil, like β -Sitosterol and Campesterol, have modulator effects on nitric oxide (NO), TNF- α , IL-1 β , and thromboxane B2 (TXB2), which lower COX-2 gene expression, primrose oil has a higher anti-inflammatory effect.	[27]
17	Harpagophytum procumbens (Devil's Claw)	Anti-inflammatory activity of Devil's claw in vitro systems and their active constituents.	Solvent extraction	It has been demonstrated that the extract from devil's claw root can reduce NO, PGE2, and inflammatory cytokines (IL-6, IL-1 β , and TNF- α). Additionally, it can prevent the synthesis of eicosanoid and the metabolism of arachidonic acid, which inhibits COX-2 and lowers inflammation.	[28]
18	Strombosia grandifolia	Evaluation of the Antimicrobial Activity of Strombosia grandifolia Hook.f. ex Benth Extract Hand Sanitizer Formulation	Maceration	Based on its ethnomedical application in the management of infectious disorders, this investigation supported the antibacterial activity of <i>S. grandifolia</i> as documented in the literature.	[29]
19	Rosa canina	The anti-inflammatory capacity of Rose-hip is strongly dependent on the seeds - a comparison of animal and human studies	Solvent extraction	People with OA who were treated with this plant reported significant improvements in their CRP, pain, stiffness, and need for rescue drugs. The anti-inflammatory properties of rosehip seeds should be mentioned.	[30]
20	Urtica dioica	Randomized controlled trial of nettle sting for treatment of base-of-thumb pain	Ultrasound-assisted extraction	Through randomized controlled trials, the topical efficacy of nettle leaf has been evaluated in thumb osteoarthritis; notable reductions in pain, stiffness, and the need for anti-inflammatory and analgesic therapy have been noted.	[31]
21	Uncaria tomentosa	Randomized double blind trial of an extract from the pentacyclic alkaloid-chemotype of uncaria tomentosa for the treatment of rheumatoid arthritis.	Hydroalcoholic extraction	A 24-week double-blind, placebo-controlled study was conducted to assess the impact of a highly purified extract of <i>U. tomentosa</i> in RA patients. The extract was given in conjunction with either hydroxychloroquine or sulfasalazine, and the treatment group demonstrated a slight improvement in joint pain, swelling, and tenderness when compared to the placebo group.	[32]
22	Salvia officinalis	Topical anti-inflammatory activity of Salvia officinalis	Pressurized hot water extraction	Chloroform extract of sage leaves has shown atopic anti-inflammatory	[33]

		L. leaves: the relevance of ursolic acid		effect.	
23	Ribes nigrum	Cytokine and prostaglandin production by monocytes of volunteers and rheumatoid arthritis patients treated with dietary supplements of blackcurrant seed oil	Solid-liquid extraction	Researchers looked into how blackcurrant oil (BCO) affected RA patients over a 6- week period. They found that the experimental group experienced less morning stiffness and that peripheral blood monocytes produced fewer proinflammatory mediators, such as TNF- α and IL-1 β .	[34]
24	Elaeagnus angustifolia	Effects of Elaeagnus angustifolia L. supplementation on serum levels of inflammatory cytokines and matrix metalloproteinases in females with knee osteoarthritis	Polysaccharide extract	In a clinical study involving 90 female patients with osteoarthritis in the knee, the active therapy group showed a significant decrease in proinflammatory mediators TNF- α and matrix metalloprotein-1 (MMP-1), as well as a reduction in the anti-inflammatory cytokine IL-10.	[35]
25	Vaccinium myrtillus	A single supplement of a standardised bilberry (Vaccinium myrtillus L.) extract (36 % wet weight anthocyanins) modifies glycaemic response in individuals with type 2 diabetes controlled by diet and lifestyle	Maceration	After taking one capsule of concentrated bilberry extract (36% w/w anthocyanins) daily, diabetic patients' anti- inflammatory peptides (monocytes chemotactic protein-1) have changed.	[36]
26	Olea europaea	Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil	Maceration	Extra virgin olive oil (EVOO) has been demonstrated to have a positive impact on the regulation of proinflammatory cytokines, TXB2 and LTB4, postprandial plasma lipopolysaccharide, and coronary heart disease risk in both healthy individuals and patients with metabolic syndrome.	[37]
27	Datura stramonium Linn	Phytochemical, preliminary pharmacognostical and antimicrobial evaluation of combined crude aqueous extract	Soxhlet extraction	Datura Stramonium Because of its high and varied flavonoid and phenolic content, linn demonstrated strong anti-inflammatory and antibacterial properties.	[38]
28	Argemone mexicana Linn	Argemone mexicana: chemical and pharmacological aspects	Soxhlet extraction	It can cooperate to fight microbial infection, inflammation, and ROS and speed up the process of skin tissue remodeling by targeting the microorganisms, inflammation, and tissue cell oxidation that lead to wound problems.	[39]
29	Plumbago zeylanica	Indian medicinal plants: an illustrated dictionary	Soxhlet extraction	The plant bioactives of Plumbago zeylanica have been demonstrated to possess antibacterial, antimalarial, and antifungal qualities, and have been traditionally utilized to alleviate inflammation.	[40]
30	Carica papaya	Antimicrobial and anti-inflammatory activity of aqueous extract of Carica papaya	Aqueous extraction	The primary active components that give papaya plant aqueous leaf extract its antibacterial and anti-inflammatory qualities should be	[41]

				identified by assessing its immunopharmacological significance in vivo.	
31	<i>Cassia sieberiana</i>	Analgesic and anti-inflammatory activity of aqueous root extract of <i>Cassia sieberiana</i> DC (Caesalpiniaceae)	Cold maceration	Traditional drug to treat pain And Inflammation	[42]
32	<i>Cussonia paniculata</i>	Anti-inflammatory and analgesic activities of the aqueous extract of <i>Cussonia paniculata</i> stem Bark.	Soxhlet extraction	Extensively used against pain, inflammation, infections	[43]
33	<i>Diospyros variegata</i>	Analgesic and anti-inflammatory activities of methanol extract from <i>Desmodium triflorum</i> DC in Mice.	Methanol extraction	Use in relieving complications and inflammation	[44]
34	<i>Glycine tomentella</i>	Analgesic and anti-inflammatory activities of aqueous extract from <i>Glycine tomentella</i> root in mice.	Aqueous extraction	Treating degenerative diseases and joint aches	[45]
35	<i>Hypericum canariense</i>	Analgesic and topical anti-inflammatory activity of <i>Hypericum canariense</i> L. and <i>Hypericum glandulosum</i> Ait.	Maceration	Used in fibromyalgia, arthritis, muscular pain and fatigue, seditious and painful conditions	[46]
36	<i>Hypericum glandulosum</i>	Analgesic and topical anti-inflammatory activity of <i>Hypericum canariense</i> L. and <i>Hypericum glandulosum</i> Ait.	Maceration	Used in arthritis, muscular pain and atory and painful conditions	[46]
37	<i>Margaritaria discoidea</i>	Anti-inflammatory, analgesic and wound healing activities of the leaves of <i>Memecylon edule</i> Roxb	Cold maceration	Dinghies are used to relief toothache, post-partum pains, relieve stomach aches	[47]
38	<i>Pergularia daemia</i>	Analgesic and anti-inflammatory activity of a crude root extract of <i>Pergularia daemia</i>	Aqueous extract	Used as antihelmintic, laxative, antipyretic and expectorant, and is also used to treat infantile diarrhoea and malarial intermittent complications, inflammation.	[48]
39	<i>Pfaffia glomerata</i>	Analgesic and anti-inflammatory activity of a crude root extract of <i>Pfaffia glomerata</i> (Spreng) Pedersen.	Pressurized liquid extraction	Used in fever and reduce Inflammation.	[48]
40	<i>Rheedia longifolia</i>	Analgesic and antiinflammatory activity of the aqueous extract of <i>Rheedia longifolia</i> Planch & Triana	Aqueous extract	Different plant part from rheedia species used to treat inflammation, pain and infections	[49]
41	<i>Saraca indica</i>	Verma A, Jana GK, Chakraborty R, Sen S, Sachan S, Mishra A: Analgesic activity of various leaf extracts of <i>Saraca indica</i> Linn. <i>Der Pharmacia Lettre</i> 2010; 2: 352- 357.	Soxhlet extraction	To treat painful conditions, improves digestion and assimilation, alleviates excessive thirst, to kills infectious agents and in blood complaint, inflammation.	[50]
42	<i>Spathodea campanulata</i>	Experimental evaluation of the analgesic and antiinflammatory properties	Solid liquid extraction	Part of Plant is used as astringent and to relief for painful flammatory conditions	[51]

		of a traditional remedy.			
43	Verbena tenuisecta	Some pharmacochemical investigations on Verbena Tenuisecta.	Maceration	Folk drug against diarrhea, gastrointestinal diseases, fever, pain, inflammation	[52]
44	Zizyphus lotus	Anti-inflammatory and analgesic activities of flavonoid and saponin fractions from Zizyphus lotus (L.) Lam.		Used in inflammation, stress, tooth pain	[53]
45	Cassia fistula	Antibacterial and antifungal activity of Cassia fistula L.: An ethnomedicinal plant	Ultrasonic- assisted extraction	Methanol and ethanol extracts of Cassia fistula on microbes had high antibacterial and anti-fungal potential	[54]
46	Terminalia catappa (Almond Tree)	Antimicrobial herbal soap formulation	Supercritical fluid extraction	The leaves of Terminalia catappa (Almond Tree) have antioxidant, sudorific and antibacterial, and used as remedy for leprosy	[55]
47	Garcinia indica	Garcinia indica, IUCN red list threat. Species.	Soxhlet extraction	Garcinia indica rinds generally Appertained to as Kokam are rich source of organic acids primarily hydroxyl-citric acid and other garcinol factors.	[56]
48	Salvadora persica (sticks)	Antibacterial, anticandidal and antioxidant activities of Salvadora persica and Juglans regia extracts.	Aqueous extraction	The reported pharmacological activities of S. persica are anti-inflammatory, analgesic, antipyretic, antirheumatic anti-microbial, anti-plaque, diuretic effects	[57]
49	Xeromphis spinosa	Anti-inflammatory activity of f Xeromphis spinosa.	Fractionation	Used in pain, inflammation, complications and as aphrodisiac, antiemetic, carminative	[58]
50	Smilax china	Anti-inflammatory and antinociceptive activities of Smilax china L. aqueous extract.	Aqueous extraction	It is bitter, acrid, anodyne, anti-inflammatory, digestive and used in dyspepsia, flatulence, colic, skin Disorder, and fever.	[59]

Base preparation

The base gel typically contains the following key components.^[60]

Gelling agent: Carbopol 940 is commonly used as the primary gelling agent.

Solvents: Water, Ethanol, Propylene glycol-400

Preservatives: Methyl paraben, Propylparaben, Sodium benzoate.

PH adjuster: Triethanolamine

Formulation of base gel

Ingredient	Percentage (w/w)
Gelling Agent (Carbomer 934P)	0.5% to 1%
Solvent (Propylene Glycol)	98.5%
pH Adjuster (Tromethamine)	0.5%
Preservative (Parabens)	0.1%
Antioxidant (Butylated Hydroxyanisole)	0.1%
Antioxidant (Butylated Hydroxytoluene)	0.1%

Types of gelling agents

Carbopol 940: This is one of the most commonly used

Antioxidant: EDTA disodium

The required gel strength, texture, stability, and compatibility with the herbal extracts being employed are some of the parameters that frequently influence the choice of gelling agent. To get the appropriate qualities in the finished gel formulation, some gelling agents could require further ingredients, such as cross-linking agents or pH adjusters.

gelling agents for polyherbal gel formulations. It forms a clear gel upon neutralization with

triethanolamine.^[61]

Sodium carboxymethylcellulose (Na-CMC): This is another popular gelling agent used in gel formulations.^[62]

Xanthan gum: A high molecular weight polysaccharide that forms a clear gel when combined with other gums or salts.^[63]

Guar gum: Another polysaccharide that can be used alone or in combination with other gums to form gels.^[64]

Pectin: Derived from citrus fruits, it can be used as a gelling agent in certain gel formulations.^[65]

Agar: Obtained from red algae, it forms a firm gel upon cooling.^[66]

Procedure

Weigh out the gelling agent and mix it with a small portion of warm water to create a uniform dispersion. Measure out the remaining ingredients according to the percentages listed. Combine the gelling agent dispersion with the rest of the ingredients in a suitable container, stir thoroughly. Once thickened, mix the gel thoroughly before packaging.

Evaluation test

- **Physical appearance:** Colour and Transparency
Some formulations, including those containing Asiatic acid-loaded transfersomes made of soybean lecithin with SDC (SDCAATG), may show a minor translucency. Topical gel formulations are usually opaque and white in appearance, or the colour of the extract.^[67]

Texture and Consistency

The semi-solid consistency of topical gels allows them to form a three-dimensional matrix; their higher water content tends to make them less greasy than creams and ointments; and they can be made to feel shiny, light, and silky by adding ingredients like dimethicone- based emulsifiers.^[68]

Appearance stability

Environmental elements including temperature and humidity can have an impact on the look of topical gels; they may experience syneresis during storage, leading to unpredictable shrinking or drying out.^[69]

Viscosity

Viscosity of the pain relief balm was determined using brook field viscometer at 25°C with a spindle speed of the viscometer rotated at 12rpm.^[70]

Spreadability

Spreadability was performed through parallel plate method. Two sets of glass slides of standard

dimensions were taken and the pain relief balm formulation was placed on one of the slides. The other slides are placed on the top of the formulation such that balm was sandwiched between two slides. 100gm of weight was taken on upper slide so that the balm spread between two slides and form two thin layers. The weight was removed and excess of prepared balm adhering two slides was scraped off. A 20gm weight was tie to the upper slide carefully because of the weight the balm spread throughout the slide. The time taken for the upper slide to travel the distance of 7.5cm of the slide was noted. The experiment was repeated 3 times and average was noted.^[70]

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

Where, S= spread ability

M= mass of the weight taken L= length of the slide

T= time taken for the formulation to spread throughout the slide.

Anti-Inflammatory test

1. Egg albumin denaturing assay

Preparation of reagents: Prepare a 1% solution of egg albumin. Prepare phosphate buffered saline (PBS) at pH 7.4. Distilled water or dimethyl sulfoxide (DMSO)

Preparation of test samples: Prepare plant extracts using maceration technique with distilled water as solvent. Make dilution series.

Egg albumin denaturation assay protocol: Mix 0.2 mL of 1-2% egg albumin solution, 2 mL of sample extract or standard (diclofenac sodium), and 2.8 mL PBS in a total volume of 5 mL. For control, mix 2 mL of triple-distilled water, 0.2 mL of 1-2% egg albumin solution, and 2.8 mL PBS. Incubate reaction mixtures at 37 ± 2°C for 30 min. Heat samples at 70 ± 2°C for 15 min. Cool and measure absorbance at 280 nm using a UV/Vis spectrophotometer.

Data analysis: Calculate percentage inhibition using formula:

$$\%inhibition = \frac{\text{absorbance of sample}}{\text{absorbance of control}} - 1 \times 100$$

Perform multiple replicates. Use diclofenac sodium as positive control. Compare results across different concentrations. Correlate with other anti-inflammatory assays like membrane stabilization assay.^[71]

2. Membrane stabilization

Materials needed: Human red blood cells (RBCs), Tris-hydrochloride (Tris-HCl) buffer (50 mM at pH 7.4), Phosphate buffered saline (PBS), Test samples (e.g. extracts or formulations), Positive control (e.g. standard anti-inflammatory drug), Distilled water as negative control.

Preparation of RBC suspension: Collect fresh

human blood in a sterile tube containing anticoagulants, Centrifuge at 1000 g for 10 minutes to separate RBCs, Remove supernatant and wash RBCs 3 times with PBS, Resuspend RBCs in Tris-HCl buffer to get a 10% v/v suspension.

Procedure: Add 1 mL of RBC suspension to each centrifuge tube, Add different concentrations of test sample (e.g. 10-50 µg/mL), gently mix and incubate at 37°C for 30 minutes, Centrifuge at 1000 g for 10 minutes, Measure absorbance of supernatant at 540 nm.

Data analysis: Calculate percentage inhibition using formula

$$\%inhibition = \frac{\text{absorbance of sample}}{\text{absorbance of control}} - 1 \times 100$$

Compare results across different concentrations, Compare to positive control and negative control, Use multiple concentrations of test sample, Include positive and negative controls, Perform multiple replicates for each sample, Correlate with other anti-inflammatory assays like egg albumin denaturation.^[72]

Anti-Microbial activity

Select appropriate microorganisms: Choose relevant bacterial, fungal, or viral strains depending on the intended application, Common test organisms include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida* species, etc.

Prepare test Samples and Agar media: Dissolve the antimicrobial compound in an appropriate solvent, Prepare dilution series, Nutrient agar media is prepared.

Set up assay plates: Inoculate agar plates with test organisms.

Incubate: Follow specific incubation conditions for each organism and method, for 24-48hrs at a temperature of 37°C. Measure zone diameters.^[73]

CONCLUSION

In present paper, we summarized various formulated antimicrobial and anti-inflammatory herbs as gel formulations with various herbs and. As per the reports of maceration and Soxhlet apparatus, Carbopol 934 gel with maceration technique reported better antimicrobial activity. So, various formulations with herbal medicaments can be formulated as topical gels for better safety, efficacy and patient compliance in the future. Various research works on herbal formulations with less side effects and contraindications would be formulated, for topical administration gel is the good choice to deliver the drug through skin providing excellent results. With this review paper a excellent knowledge about the uses

of herbal drugs can be achieved and better formulations will be provided to mankind.

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