



Evaluation of Antimicrobial Efficacy of Polyherbal Gel Against Common Bacterial Strains

Ms. C.L.Sindhura, Miss. Kajol Balu Sonawane, Ms. Komal P Bonde,, Ms.Sanika P. Kate, Ms. Shakila S. Shaikh

ABSTRACT

The development of antimicrobial resistance has prompted the search for newer antimicrobials, especially from natural products. The present study investigates the antimicrobial activity of a polyherbal gel prepared from combinations of medicinal plant extracts known to possess antimicrobial activity. The gel was prepared with an appropriate polymeric carrier and was loaded with reported antibacterial drug extracts of herbs. Antimicrobial efficacy of the formulation against representative bacterial pathogens, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, was assessed through well-diffusion and MIC methods. Results showed excellent antibacterial activity of the polyherbal gel, with significant zones of inhibition and MIC values being comparable to control antibiotics. The findings suggest that polyherbal gel formulations could hold potential as efficient alternatives for the management of bacterial infections, providing an antimicrobial resistance-breaking, natural, and efficient solution to the problem of antimicrobial resistance. Additional research is justified to find a balance in formulation parameters and determine clinical efficacy.(1)

KEYWORDS : Herbal medicine, phytotherapy, medicinal plants, ethnopharmacology, antimicrobial activity, anti-inflammatory, phytochemicals, polyherbal formulation, bioavailability enhancement, in vitro and in vivo studies, phytochemical screening, essential oils, Ayurveda, drug safety, and toxicity.

1. INTRODUCTION

Plants contain a broad diversity of secondary metabolites, including tannins, terpenoids, alkaloids, and flavonoids, which in the laboratory have shown antimicrobial activity.(2)

Although 25 to 50% of contemporary medicines are derived from plants, none are being utilized as antimicrobials. Plants were employed by traditional healers centuries earlier to cure or guard against infectious illness; Western medicine is currently attempting to replicate their achievement.(3)

A number of hundreds of plants on earth are utilized in traditional medicine as antimicrobial drugs. Some of them have also been screened in vitro but the effectiveness of such herbal drugs has rarely been clinically tested in controlled clinical trials.1

traditional drugs tend to offer effective antibiotic treatment for bacterial infection but there is also a growing issue of antibiotic resistance and ongoing demand for new options. While natural products are not necessarily safer than synthetic antibiotics, there are some patients who prefer to take herbal medicines.(4)

In the past, plants were employed for the treatment of various diseases. They exhibited potential pharmacological activity. The source of most useful drugs is in the traditional healing practices and with this consideration, it is extremely crucial to study related to screening of the medicinal plants for their claimed biological activity.(5)

Aloe vera (family- Liliaceae) is an herb without a stem. A. vera contains mono- and polysaccharides, tannins, sterols, organic acids, enzymes, saponins, vitamins and minerals, according to reports. A. vera is traditionally

employed in creams and ointments for wound healing, burns, eczema, and psoriasis¹. It has been added to anti-aging and anti-wrinkle creams and moisturizers.⁽⁶⁾

Antibiotics like penicillin are prescription medicines and have saved the lives of humans from otherwise mortal diseases and states since the early 1940s. Meanwhile, humans also seek natural antibiotics to cure them.

The NHS says that 1 in 10 people suffer from side effects that damage the digestive system if they use antibiotics. Approximately 1 in 15 people are allergic to it.

Here we examine the evidence behind seven of the top natural antibiotics. We also examine what to do and when to go to the doctor.⁽⁷⁾

One of the studies identified that tomato seeds inhibits mycelium growth of fungus⁶. The gel can be used in the treatment of sun burns, rashes, burns, wounds, fungal and other dermatological infections. It can also be used in the treatment of superficial mycosis. HPMC and Carbopol 934 was used for making gel. Various concentrations of both were used to achieve optimised formulation.⁽⁸⁾

1.1 Seven best natural antibiotics

1. Garlic

Garlic is a known plant in cultures all around the world because of its curative and prophylactic attributes. Garlic has been discovered to be a good remedy^{Trusted Source} for many types of bacteria, including Salmonella and Escherichia coli (E.coli). Garlic has even been proposed to treat multi-drug resistant tuberculosis.⁽⁹⁾

2. Honey

Since the days of Aristotle, honey has been employed as an ointment that promotes healing in wounds and prevents or extracts infection. Modern healthcare professionals have found it useful in the treatment of chronic wounds, burns, ulcers, bedsores, and skin grafts. For instance, findings of a 2016 study demonstrate how honey dressings promote wound healing. The bactericidal action of honey is typically due to its level of hydrogen peroxide. Manuka honey, however, kills bacteria while having lower hydrogen peroxide levels. In 2011, research indicated that the most well-known kind of honey inhibits about kinds^{Trusted Source} of bacteria. It also implies that honey is effective in curing infected methicillin-resistant Staphylococcus aureus (MRSA) wounds. Aside from antibacterial effectiveness, honey heals wounds by closing them up with a varnish that allows it to promote a moist climate. ⁽¹⁰⁾

3. Ginger

The scientific community also identifies ginger as an antibiotic. Scientific experiments have shown, for instance in 2017^{Trusted Source}, ginger can kill different types of bacteria. Researchers are also studying the efficacy of ginger in treating seasickness and nausea and reducing blood sugar.⁽¹¹⁾

4. Echinacea

Share on Pinterest Since centuries, echinacea has been used to cure infections. For centuries, traditional healers and indigenous American healers have been using echinacea to cure infections and wounds. Now researchers are discovering why. In a study Published in the Journal of Biomedicine and Biotechnology, a report indicates that Echinacea purpurea extract can kill numerous types of bacteria, including Streptococcus pyogenes (S. pyogenes). S. pyogenes causes strep throat, toxic shock syndrome, and the "flesh-eating disease" necrotizing fasciitis. Echinacea also cures inflammation caused by bacterial infection. It can be found in health stores or on the internet. ⁽¹²⁾

1.2 Goldenseal is widely taken in tea or capsule form for respiratory and gastrointestinal illnesses.

It can also cure bacterial diarrhea and urinary tract infection. Additionally, findings of a recent study^{Trusted Source} support the use of goldenseal for the treatment of skin infections. Goldenseal extracts were used in a lab to inhibit MRSA from killing tissue. A patient who is under prescription medication should consult a physician if he/she can take goldenseal since this supplement can affect drugs. Goldenseal also contains much berberine,

a valuable natural antibiotic substance. This alkaloid is not suggested for infants, or pregnant and breastfeeding women. Goldenseal capsules are available from health food stores or online. (13)

Clove

Clove was used in dentistry for centuries. Clove water extract is found to be effective Trusted Source against a variety of other types of bacteria, such as E. coli.(14)

7. Oregano

Oregano is purported to stimulate the immune system and provide antioxidant action. It possibly is an anti-inflammatory agent. As much as scientists have so far not confirmed such assertions, other research has indicated that oregano is one of the enhanced Natural antibiotics, especially when it was produced in oil.(15)

1.3 Risks of natural antibiotics:

Merely because it bears the stamp 'natural,' that is not always the case.

The level and strength of active ingredients differ among supplement brands.

Read it carefully. One should also ask their doctor if they are going to take these supplements. While cooked garlic is okay to eat, studies show that consuming concentrated garlic raises Trusted Source the risk of bleeding. That is an issue for individuals who are undergoing surgery or are on blood thinners. Garlic extracts can also decrease the effectiveness of HIV drugs. Some products are to be avoided, and these include colloidal silver. This is a liquid made up of tiny particles of silver suspended in water. Colloidal silver has been suggested as a cure for many diseases, including the bubonic plague and HIV. (16)However, it is said to be unsafe, and there are no scientifically sound studies Trusted Source to endorse its applications. Colloidal silver supplementation may hinder the action of antibiotics and the medication used for an underactive thyroid gland. Silver also builds up in the body and darkens the skin to bluish-grey. This is known as argyria and is permanent in the majority of individuals.(17)

1.4 Gel :

A gel is a solid or semisolid two- or multiconstituent system, a condensed mass enveloping and infusing a liquid. Gels and jellies consist of small quantities of solid dispersed in fairly large quantities of liquid, but exhibiting more solid-like than liquid-like characteristics. The distinguishing feature of gel and jelly is that there exists some skin structure, thus conferring on them solid-like characteristics.(18)

1.4.1 Types of gel :

1. Organogels
2. Extended-release gels.
3. Hydrophilic gels.
4. Non-aqueous gels
5. Bioadhesive gels
6. Thermosensitive sol-gel reversible hydrogels
7. Complexation gels
8. Hydrogel (19)

1.4.2 Polymers used for the preparation of pharmaceutical gels

The polymer such as natural gum, pectin, Tragacanth, Agar, Alginic acid, semi-synthetic substance, methylcellulose, Hydroxy-methylcellulose, and carbapols is used to shape pharmaceutical gel. Gel are prepared by either the fusion process or a special needed for a specific gallant.(20)

1.5 Problem with herbal medicine as an antibiactivity :

The increasing popularity of herbal medicine: problems related to adverse effects and difficulties in safety monitoring. The consumption of herbal medicinal products and dietary supplements has increased dramatically over the last three decades with at least 80% of the world's population depending on them for some part of the first-line treatment.(21)

While these therapies have appeared to be promising with the effectiveness of many herbal drugs having been proven conclusively, they are mostly yet to be tried out and their uses poorly monitored or unmonitored at all. The result of this is an inadequacy of adequate knowledge of their mode of action, possible side effects, contraindications, and interactions with currently available orthodox drugs and functional foods to promote safe and rational application of these medicines. (22) Since safety is a topmost issue with the use of herbal medicines, it becomes imperative, in this regard, that such regulatory agencies put in place proper mechanisms to guarantee public health by seeing to it that all herbal drugs are safe and acceptable in quality.8

1.5.1 Honey(23)

The therapeutic qualities of honey were first described circa 400 B.C. Honey was applied as a cream to heal infections, burns, and wounds. You are not very likely to apply some honey to a skin infection today, but some experiments actually do substantiate that honey used in surgical dressings does help to minimize scarring and heal wounds quicker.(24)

1.5.2 Garlic

Studies have found that garlic is composed of chemicals that are not only antibacterial but also infection-preventative. Garlic kills bacteria in a laboratory test tube, and even kills MRSA and *C. albicans*, two increasingly drug-resistant pathogens. It has also been found to treat effectively bacterial infections by *Salmonella* and *E. coli*, bacteria which are most frequently behind urinary tract infections.(25)

1.5.3 Oregano Essential Oil

Oregano, which belongs to the same family as mint, is of potential medicinal value when it is distilled into a super concentrated essential oil. Essential oil is not oil of oregano, which is less concentrated. Oregano essential oil is useful primarily because of carvacrol, the active phytonutrients.(26)

1.5.3 It can be useful for the following:

- Sinus infections
- Skin fungal infections

1.5.4 Thyme Essential Oil

Thyme has a distinct fragrance that usually differentiates it from home-made organic home cleaning liquids. so often It is found to show up in them due to its bacteriacide property, even drug-resistant ones.(27)

1.6 Myrrh Extract

Myrrh has a warm, aromatic scent. It is used for many purposes, but one of the lesser known purposes is that it can kill pathogenic bacteria, such as:

- E. coli**
- C. albicans**
- P. aeruginosa**
- S. aureus (MRSA)**
- K. pneumoniae (28)**

1.6.1 Echinacea

Echinacea is a naturally occurring plant in North America that was cultivated by aboriginal people for centuries for medicinal purposes. Both topically and internally, Echinacea is utilized. It is marketed as a dietary herbal supplement, and it is typically marketed as an immune system aid product.(29)

1.6.2 Goldenseal

Another widely used herb, goldenseal is usually taken in the form of a capsule or tea for immunity enhancement. It can be useful in bacterial infections leading to diarrhea or respiratory infections and in urinary tract infections.(30)

1.6.2 Turmeric

A dense orange spice shared by most types of spiced food, turmeric contains one powerful active compound: curcumin. Discovered to be an anti-inflammatory with a body-immune-enhancing quality, curcumin is now proven effective against gram-positive and gram-negative bacteria from lab samples as well. Curcumin can also prove effective in the case of bacteria that lead to MRSA as well as E. coli. (31)

Ingredient of antibacterial herbal gel :

1. Ficus Racemosa
2. Azadirachta Indica
3. Aloe Barbadensis Miller(32)

1) Ficus Racemosa :



Fig no 3 ficus racemosa

- Common name : Cluster fig
- Botanical name : Ficus racemosa
- Kingdom : Plantae
- Division : Magnoliophyta
- Class : Dicotyledonae
- Order : Rosales
- Family : Moraceae (33)

2 Azardica Indica (Neem)



Fig no 5 : Azardica Indica (Neem)

- Common name: Neem
- Botanical name: Azadirachta indica
- Kingdom: Plantae
- Division: Tracheophyta
- Genus: Azadirachta
- Species: A. indica
- Family: Meliaceae (34)

3 Aloe Barbadensi Miller



Fig No 6: Aloe Barbadensi Miller

- Common name: Aloe Vera
- Botanical name: Aloe barbadensis miller

- Kingdom: Plantae
- Species: barbadensis miller
- Family: Asphodelaceae (35)

2. Aim :-

The overall aim of this work is the development, formulation and evaluation of the topical herbal gel containing ethanolic extract of Azadirachta Indica, ficus racemosa and aloe barbadensis for antibacterial activity.

3. Objective:

1)The aim of the present study is to develop and assess the topical herbal gel of Azadirachta Indica, ficus racemosa and aloe barbadensis leaf extracts for antibacterial activity.(36)

2)To develop transdermal topical gel of Azadirachta Indica, ficus racemosa and aloe barbadensis leaf extracts with carbopol934 that can be effectively used for transdermal topical delivery.(37)

3) To formulate a topical herbal gel with antibacterial, anti-inflammatory as well as soothing effect.(38)

4) The objective of formulating a gel formula is to compare the difference in efficacy of gel formula with other topical preparations.(39)

5) To investigate the action of all 3 best herbal treatments of ayurveda in a single formula(40)

4. MATERIALS AND METHODOLOGY

Experimental work:-

Collection and authentication:-

Fresh leaves of Azadirachta Indica were taken from Smt.SSPCOP botanical garden, Chopda. Leaves were washed and dried in the sun for 4 one week and powdered using home grinder.

Recently collected Ficus racemosa leaves were received from Chopda garden and were washed for removing the dust and fertilizer. Leaves were air-dried for 4 days ; subsequently, the drying was finished by hot air oven storage. Then, Ficus racemosa leaves were ground using grinder.(41)

Fresh young green leaves of Aloe barbadensis were cutted and placed vertically for 3 hours to get de-yellowing from the leaves. Then leaves were Washed and Sun dried. Then some dry leaves were kept in hot air oven to remove excess water again. Dry leaves then were ground by using grinder.(42)

4.1 Preparation of Gel base:-

<u>Ingredients</u>	<u>Quantity in gm/ml</u>
Extract of Azadirachta Indica	1.5gm
Extract of Ficus racemosa	1.5gm
Extract of Aloe <u>barbadensis</u>	1.5gm
Carbopol934	2gm
Polyethylene_glycol	4ml
Propyl paraben	0.5ml
Methyl_paraben	0.05ml
Triethanolamine	15ml
Distilled_water	QS

Table No .1 : Formulation for 50 gm of 1% herbal gel preparation:-

<u>Ingredients</u>	<u>Quantity in gm/ ml</u>
Extract of Azadirachta Indica	1.5gm
Extract of Ficus racemosa	1.5gm
Extract of Aloe vera	1.5gm
Carbopol934	1.5gm
Polyethylene glycol	5ml
Propyl paraben	0.5ml
Methyl paraben	0.05ml

Table No.2 :- Formulation for 50 gm of 2% herbal gel:

Ingredients	Quantity in gm /ml
Extract of Azadirachta Indica	1.5gm
Extract of Ficus racemosa	1.5gm
Extract of Aloe vera	1.5gm
Carbopol934	3gm
Polyethylene glycol	5gm
Propyl paraben	0.5gm
Methyl paraben	0.05gm
Triethanolamine	15ml
Distilled water	q.s.

4.2 Physical Appearance :- The appearance of the formulation was checked visually.

- **Color :** The color of the formulations was checked out against white & black backgrounds.
- **Consistency :** The consistency was checked by applying the gel on to the skin.
- **Greasiness :** The greasiness was assessed by the application on to the skin
- **Odour :** The odour of the gels was checked by mixing a little amount of gel in water and by taking smell. (43)

Formulation code	Colour	Homogeneity	Consistency	Phase Separation
HG1	Green	Excellent	Excellent	None
HG2	Green	Excellent	Excellent	None
HG3	Green	Excellent	Excellent	None
HG4	Green	Excellent	Excellent	None

4.3 Determination of pH:-

pH Values of 1% aqueous solutions of the optimized gel was measured at 25°C using a pH meter (Systronic digital pH meter).(44)

<u>Formulation code</u>	<u>pH</u>
HG1	6.8
HG2	6.7
HG3	6.7
HG4	6.8

4.4 Rheological investigation:

Viscosities of the batches thus prepared were measured using a Cone and Plate Viscometer fitted with spindle 7. Equipment was attached to a thermostatted circulating water bath at 25°C. A definite volume of gel was placed in a beaker that was covered with a thermostatic jacket cover. Gel was stirred at 100 revolutions per minute using spindle (45)

4.5 Spreading coefficient:

Spreading coefficient was also measured on apparatus proposed by Mutimer. It is wooden block, and this wooden block is attached with pulley in one end. Spreading coefficient was measured in relation to 'Slip' and 'Drag' behavior of Gels. Wooden block is mounted with a ground glass slide. 2 gm of investigational gel is taken on the ground slide. Gel preparation was then put between this slide and second glass slide of the same size as that of the fixed ground slide. The second glass slide is fitted with the Hook. 500 mg weight was put on the top of the two Slides for 5 min to expel air and to provide a uniform film of the gel between the two slides. Weight in terms of mass was put into the pan fixed on the pulley by the assistance of Hook. The time (in sec) that was required by the top slide to travel a Distance of 5 cm was recorded. (46)

Formulation code	Spreading (gm. cm / sec)
HG1	18.56±0.75
HG2	17.76±0.57
HG3	15.78±0.70
HG4	414.98±0.86

TableNo. 5 : Spreading Coefficient value of Gel Vs Marketed Gel

4.6. Extrudability Study:

It is a fact-finding empirical test to determine the amount of force necessary to Extrude the material from a tube. To determine the Extrudability the gel product was filled in regular plastic capped collapsible tube. Initial

weight of the tube was taken. The tube was positioned between two glass slides and clamped. 1 g weight was put over the glass slide and then the cap was opened.(47)

The volume of the extruded gel was measured and recorded. The percentage of extruded gel was compared with the commercial gel.

<u>Formulation code</u>	<u>% Extrudability</u>
HG1	<u>88%</u>
HG2	<u>85%</u>
HG3	<u>88%</u>
HG4	<u>86%</u>
Marketed gel	<u>84%</u>

Table No. 6:- % Extrudability of gel vs marketed gel :-

4.7 Antimicrobial Susceptibility test of gel:-

Herbal gel were tested for antimicrobial activity against E. Coli using the disk diffusion method with slight modification (Bansal et al). The E.coli were preserved at department of Microbiology. Muller Hinton agar (MHA) plates were prepared by pouring 15ml of molten media into sterile Petri Plates. A drop of 10 μ l vehicle formulation was placed on Surface of medium and incubated at 37 $^{\circ}$ C for 24 hrs. at the Incubation, inhibition zone were measured around drop with Transparent ruler in mm. this study was performed in Triplicate.(48)

4.8 Stability Studies:-

Stability studies were performed to all the four formulations In four different timings that is on day-0, 15th day and on 30thDay. On these time intervals all the evaluation studies and Skin irritation studies were performed to check the stability of topical herbal gel formulations. (49)

5. CONCLUSION

The goal of this research is to develop and formulate the Antibacterial Herbal Gel that would possess the combined effects of the best traditional herbs. As a majority of healers use with this Azadirachta Indica, Ficus racemosa, and aloe vera to cure many of fungal as well as bacterial diseases, we developed a formula based on the use of the Azadirachta Indica, Ficus racemosa, and the extract of the aloe vera. No change was noted in its pH and other physical characteristics. Irritation studies of skin were noted with all the four formulae. Together with the aforementioned the gel formulation is also have good antimicrobial activity.(50)

The antimicrobial activity of the Azadirachta Indica, aloe vera and Ficus racemosa herbal gel formulations exhibits dose dependent zone of inhibitions in exponential manner i.e HG3 formulations exhibits 1.53 \pm 0.4 cm zone of inhibition it is very much higher than the other three formulations and the standard penicillin.

in comparison to the control drug our formulation gels show enhanced antimicrobial activity. Through this study findings we are concluding that all these four formulations are best in their stability and anti-microbial activity so we can use this formulation for treating microbial infections.(52)

Antifungal activity of the herbal gel product was also intended to obtain by employing the isolated fungal strains as an early measure as possible.(50,51)

6. RESULT AND DISCUSSION

Preformulation studies are conducted on repetitive processes. Solubility of Azadirachta Indica, Ficus racemosa and Aleo vera was determination in some polar and non-polar solvents and results are given as per described in India's pharmacopoeia. Methanol solubility of Azadirachta Indica, ficus racemosa and Aleo vera was 1mg/1000 ml.

Gel can be a good, viable, safe and cost-effective delivery system for herbal on the basis of their biodegradability, biocompatibility, and compatibility for topical application and low immunogenicity.

With a rise in the concentration of gelling agent from 1.0 gm to 2.5 gm the rise in viscosity from 1900 to 2490 cp was observed.

The rise in viscosity with the rise in the concentration of gelling agent can be ascribed due to more amount of gelling agent being present that increased the viscosity of the formulation. The Drug content was found to increase from 91.85 ± 1.35 to 97.30 ± 1.6 on increasing the concentration of Carbopol (gelling agent) from 0.5 gm to 2.0 gm which can be attributed to the increase in the viscosity of the solution which hindered the drug crystals from flowing out of the droplets.

But while further increasing the concentration of gelling agent the drug content was found to decline due to very viscous solution being formed, which was hard to process. The highest concentration of drug was present in formulation HG 3 and the viscosities were also good so this concentration of gelling agent was chosen. Varying amount of oil containing Gel was prepared.

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