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Formulation and evaluation of antimicrobial polyherbal gel by utilizing plant extracts

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Abstract
This study focuses on the formulation and evaluation of an antimicrobial polyherbal gel utilizing extracts from *Acacia nilotica* and *Ocimum sanctum* plants. With the escalating threat of antimicrobial resistance, there is an urgent need for novel therapeutic agents, particularly those derived from natural sources. The objective of this research is to develop a topical gel formulation that harnesses the combined antimicrobial potential of *Acacia nilotica* Bark extract and *Ocimum sanctum* leaf extracts for potential applications in wound healing and dermatological infections. The formulation process involved the extraction of bioactive compounds from *Acacia nilotica* and *Ocimum sanctum* leaves and bark using suitable solvents. These extracts were then incorporated into a gel base following standard procedures. The resulting polyherbal gel was characterized for its physical properties, including appearance, pH, spreadability, and viscosity, to ensure suitability for topical application. The antimicrobial activity of the polyherbal gel was evaluated against a panel of pathogenic microorganisms, including both Gram-positive and Gram-negative bacteria, as well as fungi. The agar well diffusion method and broth microdilution assay were employed to determine the inhibitory effects of the gel against the test microorganisms. The results revealed significant antimicrobial activity, with notable inhibition zones and minimum inhibitory concentrations against the tested pathogens. Furthermore, the cytotoxicity of the polyherbal gel was assessed using *in vitro* cell viability assays to ensure its safety for topical use. The results demonstrated negligible cytotoxic effects on mammalian cells, highlighting the potential biocompatibility of the formulation. In conclusion, the formulation and evaluation of the antimicrobial polyherbal gel utilizing *Acacia nilotica* and *Ocimum sanctum* extracts offer a promising strategy for the development of natural antimicrobial agents. The synergistic action of these plant extracts provides a valuable alternative to synthetic antibiotics, contributing to the ongoing efforts in combating antimicrobial resistance and promoting sustainable healthcare solutions.

Keywords: *Ocimum sanctum*, *Acacia nilotica*, anti-microbial activity, herbal gel, zone of inhibition, phytochemical, evaluation

1. Introduction
The Tulsi (*Ocimum sanctum*) is one of the most valued and holistic medicinal plant which is having medicinal importance and is used for the preparation of traditional medicines from many years in India. Tulsi has been described as 'Queen of Herbs' and 'Mother of Medicine of Nature because of many useful medicinal properties [10]. This plant is widely growing in India and many other countries of South-East Asia. O. sanctum is commonly known as Tulsi in India. It is traditionally important medicinal herb containing many useful compounds [11].
Ocimum sanctum Linn Known as Holy Basil being medically important plant in the family Lemiaceae. Morphologically O. sanctum is an erect about 75 cm tall, much branched with hairy stems and simple opposite green leaves that are strongly scented. Leaves have petioles, and are ovate, up to 5 cm long, usually slightly toothed [1, 2, 3].
Acacia nilotica / Vachellia nilotica (Wild) is a genus of shrubs and trees belonging to the subfamily Mimosoideae [4], of the family Fabaceae or Leguminosae [5]. *A. nilotica* (Wild) has been used traditionally for decades in the treatment of many diseases such as diarrhoea, dysentery, leprosy, cancers, ulcer, burns, boils, wound ulcer and diabetes [6].
Parts of this plant are also used against inflammation, ophthalmia, haemorrhoid, bleeding piles, and leukoderma problems [7]. Due to the increase in bacterial resistance against the common antibiotics, attention has been focused on finding new or alternative substances that will have a broad- spectrum activity and that will also be readily available and affordable to the common rural inhabitants who are mostly victims of microbial infections [8].

Fig 1: Tulsi (*Ocimum tenuiflorum*)

Fig 2: Babul (*Vachellia nilotica*)

Along with other dosage forms, herbal drugs are also formulated in the form of Herbal gels. Antimicrobial activity of any substance is defined as its ability to either kill bacteria or inhibit the growth of bacteria. Antimicrobial activity is significant with respect to the human body in preventing diseases and skin infections [9].

An antiseptic gel is aimed to destroy or inhibit the growth of bacteria. In an earlier study, medicinal plants have been reported to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort, and scarring to the patient [13].

The aim of the present study was to prepare herbal gel formulation using the extracts of *Ocimum sanctum* and *Acacia nilotica* to investigate the antimicrobial activity of the extracts against the common organisms. Furthermore, to evaluate the stability and phytochemical parameters of the prepared formulations so that they can be further standardized and used commercially.

Plant profile

Plant profile of Tulsi

Botanical Name: *Ocimum tenuiflorum*
Family: Lamiaceae **Kingdom:** Plantae **Order:** Lamiales
Genus: *Ocimum*

Common name

1. English: Holy Basil or Tulsi 6. Hindi: तुलसी (Tulsi)
2. Bengali: तु लसी (Tulsi) 7. Tamil: துளசி (Tulasi)
3. Telugu: తులసి (Tulasi) 8. Kannada: ತುಳಸಿ (Tulasi)
4. Malayalam: തുളസി (Tulasi) 9. Gujarati: તુલસી (Tulsi)
10. Marathi: तुळशी (Tulshi) 13. Punjabi: ਤੁਲਸੀ (Tulsi)
11. Urdu: تلسی (Tulsi) 14. Nepali: तुलसी (Tulasi)
12. Sanskrit: तुलसी (Tulasi) 15. Sinhala: තුලසි (Tulasi)

Chemical constituent

- **Eugenol:** A phenolic compound with a characteristic spicy aroma. Eugenol possesses antioxidant, anti-inflammatory, and antimicrobial properties.
- **Ursolic acid:** This compound is known for its anti-inflammatory, antimicrobial, and antioxidant effects. It's also believed to have potential anti-cancer properties.
- **Rosmarinic acid:** A polyphenol with antioxidant properties. It scavenges free radicals, protecting cells from oxidative stress. Rosmarinic acid also exhibits anti-inflammatory effects.
- **Apigenin:** A flavonoid known for its antioxidant and anti-inflammatory properties. It's believed to have potential anti-cancer effects as well.
- **Orientin:** A flavonoid with antioxidant properties. It helps in scavenging free radicals and reducing oxidative stress in the body.
- **Vicenin:** Another flavonoid with antioxidant properties. It contributes to the overall antioxidant activity of holy basil.
- **Beta-sitosterol:** A plant sterol that may help in reducing cholesterol levels and possesses anti- inflammatory properties.
- **Luteolin:** A flavonoid with antioxidant and anti-inflammatory properties. It may also have neuroprotective effects.
- **Quercetin:** A flavonoid with antioxidant properties. Quercetin scavenges free radicals and exhibits anti-inflammatory effects.
- **Caffeic acid:** A phenolic acid with antioxidant properties. It helps in neutralizing free radicals and protecting cells from oxidative damage.

Medical use

- **Antimicrobial properties of aqueous extract of *O. sanctum:*** Holy Basil or *Ocimum sanctum* has been investigated to possess various pharmacological properties like anti-toxic, antioxidant, anti- cancer, antimicrobial, antihypertensive, anti-inflammatory, anticoagulant analgesic and anti- thyroid [18, 19]. The Phenolic constituents of *O. sanctum* leaf extract like isothymusin, apigenin, rosmarinic acid, cirsineol and eugenol [20]. The aqueous extracts of *O. sanctum* leaves are more effective against pathogens as compared to methanolic extract [21, 22].
- **Antifungal Properties:** Aqueous and Acetone extract of Tulsi (*O. sanctum*) has been found to be antifungal activity against many fungi such as *Curvularia penniseli, Alternaria tenuis* and *Helminthosporium* spp. [23].

- **Antiviral Properties:** The different types of Holy Basil extract (*O. sanctum*) contain many useful secondary metabolites (such as Eugenol, Urosolic acid, Apigenin, Linalool etc.) which act as antiviral agents against various viruses. The aqueous extract and essential oil of Tulsi (*O. sanctum*) were evaluated for patients suffering from viral encephalitis [24].
- **Antioxidative Properties:** Tulsi extract and essential oil are natural antioxidants. The antioxidant activity of *O. sanctum* extract and essential oil has been found by many researchers [25, 26].

Plant profile of babul
Botanical Name: *Acacia nilotica*
Family: Fabaceae
Kingdom: Plantae
Order: Fabales
Genus: Acacia
Species: nilotica

Fig 2: Babul (*Vachellia nilotica*)

Common Name:
1. English: Nile acacia, Egyptian thorn, Gum arabic tree Tamil: காட்டு
2. த ொட்டி (Kaatu Thotti)
3. Arabic: طنس, طنس ضيبأ, رمس (Sunt, Sant Abiad, Samr)
4. Kannada: ಬಬ್ಬು ಗಿಡ (Babbu Gida)
5. Hindi: बबूल (Babool)
6. Malayalam: ബാഭൂൽ (Baabhool)
7. Bengali: বাবল (Babal)
8. Gujarati: બાવળ (Baval)
9. Telugu: నల్ల తుమ్మ (Nalla Tumma)
10. Marathi: बबूळ (Babul)

Chemical constituent
- **Tannins:** Tannins are polyphenolic compounds known for their astringent properties. They help in wound healing, diarrhea management, and have antioxidant effects.
- **Flavonoids:** Flavonoids are antioxidants that help in scavenging free radicals, reducing inflammation, and supporting cardiovascular health.
- **Alkaloids:** Alkaloids have diverse pharmacological effects, including analgesic, anti-inflammatory, and antimicrobial properties. They may also have effects on the nervous system.
- **Saponins:** Saponins have detergent-like properties and are often used for their expectorant and anti-inflammatory effects. They also have potential immunomodulatory properties.
- **Glycosides:** Glycosides are compounds that are often metabolized into active forms in the body. They may have various pharmacological effects depending on their structure.
- **Phenolic Compounds:** Phenolic compounds include a wide range of antioxidants that help in scavenging free radicals and reducing oxidative stress in the body.
- **Proteins:** Proteins found in *Acacia nilotica* bark may have various biological activities, including antimicrobial and wound healing properties.
- **Terpenoids:** Terpenoids are secondary metabolites with diverse pharmacological properties, including antimicrobial, anti-inflammatory, and antioxidant effects.
- **Gums:** Gums are complex polysaccharides that have adhesive and thickening properties. They may be used in traditional medicine for their demulcent effects.
- **Resins:** Resins are hydrophobic substances produced by plants. They may have antimicrobial and wound healing properties and are often used in traditional medicine for their protective effects.

Medicinal use
- **Anti-hypertensive and anti-spasmodic activities:** A decrease in arterial blood pressure is reported by use of methanolic extract of *A. nilotica* pods and provides evidence of anti-hypertensive activities independent of muscarinic receptor stimulation. In the *in vitro* studies, *A. nilotica* has inhibitory effect on force and rate of spontaneous contractions in guinea-pig paired atria and rabbit jejunum. *A. nilotica* also inhibits K+ induced contractions in rabbit jejunum advocating the antispasmodic action of *A. nilotica* which is mediated through calcium channel blockade and this may also be responsible for the blood pressure lowering effect of *A. nilotica*, observed in the *in vivo* studies.
- **Anti-diabetic activities:** Studies have confirmed anti-diabetic activities. However, pods and tender leaves are considered very beneficial in folk medicine to treat diabetes mellitus [27].
- **Antibacterial and antifungal activities:** The assays of the stem bark extracts confirm the antimicrobial activity against *Streptococcus viridans*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Shigella sonnei* using the agar diffusion method. *A. nilotica* could be a potential source of antimicrobial agents [28]. *nilotica* demonstrates highest activity against three bacterial (*E. coli, S. aureus* and *Salmonella typhi*) and two fungal strain (*Candida albicans* and *Aspergillus niger*) [29].
- **Antiplasmodial activities:** The ethyl acetate extract holds the highest activity on *Plasmodium falciparum*. Phytochemical analysis indicated that the most active phase contained terpenoids and tannins and was devoid of alkaloids and saponins [30]. Crude methanolic root extracts of *A. nilotica* reveals significant activity against chloroquine sensitive strain of *Plasmodium berghei* in mice [31].
- **Antioxidant activity:** Water extract/fractions of *A. nilotica* (L.) in lipid peroxidation assay possess the peroxyl radical scavenging capacity and results prove the anti-oxidant activity of plant. The bark powder of the plant extracts with different solvents found the scavenging activity using maceration extraction [32].

Materials and Methods

Collection of plant materials and other chemicals

The study employed an *in vitro* experimental design. Tulsi leaves were obtained from courtyards and Babul bark powder were obtained from vendors in the local market. White wax, white petroleum and methyl paraben were utilized from college laboratory. Authenticity of plant by Vijaysinha Yadah Arts and Science College, Department of Botany, Peth Vadgaon.

Drying

Leaves were separated from the stem, washed in clear water and dried until they were adequately dry to be ground (dried for 7 days). Dried leaves were powdered separately in an electric grinder until a homogenous powder was obtained [12].

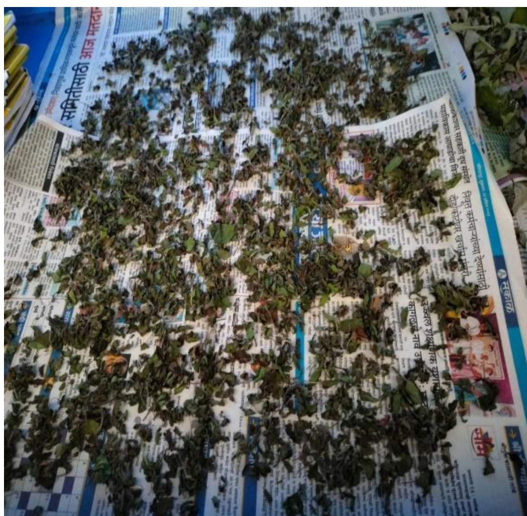

Fig 3: Drying process

Preparation of Ethanolic Extract of Polyherbs

Ethanolic extract was prepared from the powder obtained using "Maceration extraction method" [16]. One hundred grams of finely powdered *Ocimum sanctum* (Linn.) was then used for extract preparation. 40 gm of Tulsi powder dissolved in 150 ml of ethanol. Covered that mixture with the help of aluminum foil. Allow to stand for 72 hours for maceration process. It was then subjected to filtration with Whatman filter paper to obtain a clear filtrate.

Fig 4: Extraction process (Macration method)

Formulation of Placebo Gel (Control formulation)

For the preparation of gel formulation, firstly take carbopol 940 which was then dispersed in distilled water along with methyl paraben, propyl paraben and glycerine kept for overnight. Take the leaves extract of *Ocimum sanctum* and bark extract of *Acacia nilotica* in propylene glycol which was then added in polymer dispersion. Remaining quantity of water was then added and neutralized to pH 7 with triethanolamine by constant stirring for 10 minutes [14]. The control batch formulation is shown in Table 1.

Development of Herbal gel formulations

For the preparation of gel formulation, firstly take carbopol 940 which was then dispersed in distilled water then methyl paraben, propyl paraben and glycerine were added and kept for overnight. Take the leaf extract of and bark extract of *Acacia nilotica* in propylene glycol which was then added in polymer dispersion. Remaining quantity of water was then added and neutralized to pH 7 with triethanolamine by constant stirring [14].

Table 1: Control batch formulation of herbal gels

Ingredients	Quantity
Carbopol 940	1.0 gm
Propylene glycol	10 ml
Methyl paraben (0.5 %)	0.2 ml
Propyl paraben (0.2 %)	0.1 ml
Glycerin	1 ml
Triethanolamine (to maintain pH)	q.s.
Distilled water	100 ml

Table 2: Development of Herbal gel formulations

Ingredients	F1	F2	F3
Ocimum sanctum Extract	0.5 gm	1.0 gm	1.5 gm
Acacia nilotica Extract	0.5 gm	1.0 gm	1.5 gm
Carbopol 940	1.0 gm	1.0 gm	1.0 gm
Propylene glycol	10 ml	10 ml	10 ml
Methyl paraben (0.5 %)	0.2 ml	0.2 ml	0.2 ml
Propyl paraben (0.2 %)	0.1 ml	0.1 ml	0.1 ml
Glycerine	1 ml	1 ml	1 ml
Triethanolamine (to adjust pH)	q.s.	q.s.	q.s.
Distilled water	100 ml	100 ml	100 ml

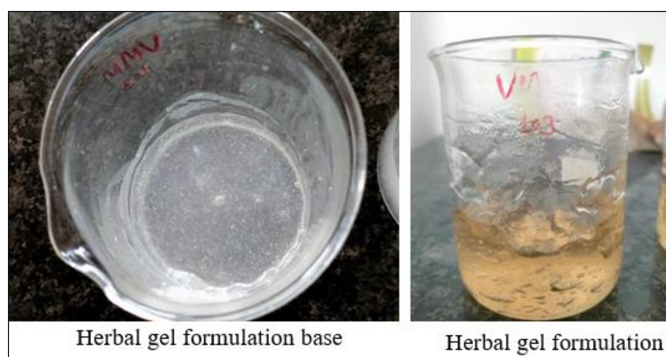

Fig 3: Herbal gel formulation of plant extract

Evaluation of Herbal gels

- **Physical evaluation:** All the formulated herbal gels were checked for color and homogeneity by visual observation.
- **pH:** The pH of all the formulated herbal gels was measured by using digital pH meter [15].
- **Viscosity:** Viscosity of herbal gels was determined by using Brookfield rotational viscometer at 100 rpm using spindle no.64 [15].

- **Spreadability:** The spreadability of gel formulations was determined by measuring the spreading diameter of 1g of gel between two horizontal plates [16].
- **Antibacterial activity:** The antibacterial screening of herbal gels was done by disc diffusion method.
- The gels were tested against bacterial agents namely, *Staphylococcus aureus* and *Aspergillus niger.* A loopful of the pure bacterial culture was suspended in nutrient broth and incubated for 24 hours. Nutrient agar media was sterilized and poured into petri plates. After solidification, 0.1ml of the inoculum was spread over the agar evenly using a rod. 6mm diameter cavity was prepared and formulated gel is placed in the cavity. A standard antibiotic was used as the control. The inoculated plates are incubated for 24 hours. Later, the zone of inhibition around the disc was measured and recorded [17].
- Antimicrobial activity refers to the ability of a substance to inhibit the growth or kill microorganisms, including bacteria, viruses, fungi, and protozoa. Antimicrobial agents can be classified based on their spectrum of activity, mechanism of action, and origin.

- **Antibacterial Agents**: These substances target bacteria and can be further classified based on their mechanism of action:
- **Cell Wall Inhibitors**: Drugs like penicillins and cephalosporins interfere with bacterial cell wall synthesis, leading to cell lysis and death.
- **Protein Synthesis Inhibitors**: Antibiotics like macrolides, tetracyclines, and aminoglycosides target bacterial ribosomes, disrupting protein synthesis.
- **DNA Gyrase Inhibitors**: Fluoroquinolones inhibit the bacterial enzyme DNA gyrase, interfering with DNA replication and leading to cell death.
- **Metabolic Inhibitors**: Drugs like sulfonamides and trimethoprim block essential metabolic pathways in bacteria, preventing their growth.

The antibacterial screening of herbal gels was done by disc diffusion method. The gels were tested against bacterial agents namely, *Staphylococcus aureus.* A loopful of the pure bacterial culture was suspended in nutrient broth and incubated for 24 hours. Nutrient agar media was sterilized and poured into petri plates. After solidification, 0.1ml of the inoculum was spread over the agar evenly using a rod. 6mm diameter cavity was prepared and formulated gel is placed in the cavity. A standard antibiotic was used as the control. The inoculated plates are incubated for 24 hours. Later, the zone of inhibition around the disc was measured and recorded [33].

Antimicrobial activity *in vitro* techniques:
- Diffusion method
  - Agar disk diffusion method
  - Anti-microbial gradient method
- Other diffusion method
- Agar well diffusion method
- Agar plug diffusion method
- Cross streak method Poisoned food method
- Thin-layer chromatography (TLC)–bioautography
- Agar diffusion
- Direct bioautography
- Agar overlay bioassay
- Dilution methods

Broth dilution method
Agar dilution method
- Time-kill test (time-kill curve)
- ATP bioluminescence assay
- Flow cytofluorometric method

Agar well diffusion methods
Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 mL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

Principal
This method is based on the principle that antibiotic-impregnated disk, placed on agar previously inoculated with the test bacterium, pick-up moisture and the antibiotic diffuse radially outward through the agar medium producing an antibiotic concentration gradient. The concentration of the antibiotic at the edge of the disk is high and gradually diminishes as the distance from the disk increases to a point where it is no longer inhibitory for the organism, which then grows freely. A clear zone or ring is formed around an antibiotic disk after incubation if the agent inhibits bacterial growth.

Media
The disk diffusion method is performed using Mueller-Hinton Agar (MHA), which is the best medium for routine susceptibility tests because it has good reproducibility, low in sulfonamide, trimethoprim, and tetracycline inhibitors, and gives satisfactory growth of most bacterial pathogens. The inoculum for the disk diffusion method is prepared using a suitable broth such as tryptic soy broth. This medium is prepared according to manufacturer's instructions, dispensed in tubes at 4-5 ml and sterilized. Sterile 0.9% salt solution may also be used. Media are supplemented with 1-2% sodium chloride (NaCl) if intended for marine organisms.

Material and method: Material
Nutrient agar: This is the solidifying agent derived from seaweed.
Nutrient broth: Nutrient broth or specific ingredients depending on the type of medium required (e.g., blood agar, MacConkey agar).
Distilled water. Nutrient broth Balance machine Autoclave
Biological safety cabinet Incubator
Cotton swab stick Millimetre scale

Preparation of agar medium
Procedure
- Measure out the appropriate amount of agar powder according to the desired concentration. Typically, agar is used at a concentration of around 1.5% to 2% (w/v) for solid agar medium.
- Measure out the appropriate amount of nutrient broth or

other ingredients required for the specific

- medium. The amount may vary depending on the type of medium being prepared.
- Add distilled water to a clean flask or beaker. The volume of water will depend on the final volume of
- agar medium needed.
- Heat the water to near boiling while stirring to dissolve the agar powder completely. Avoid prolonged
- heating to prevent evaporation.
- Once the agar powder is completely dissolved, add the nutrient broth or other ingredients to the agar
- solution. Stir well to ensure uniform mixing.
- Adjust the pH of the medium if necessary. The optimal pH for most microbial growth is around 7.0,
- but it may vary depending on the microorganism being cultured.
- After the ingredients are thoroughly mixed, distribute the medium into containers (petri dishes, tubes,
- or bottles) as needed for storage and use.
- Sterilize the medium by autoclaving at 121 °C (250°F) for 15-20 minutes. Ensure that the containers
- are tightly sealed to prevent contamination during sterilization.
- After sterilization, allow the medium to cool sufficiently to solidify before use. Agar solidifies at
- temperatures below 45 °C (113°F) but remains molten at temperatures above this.
- Once cooled and solidified, the agar medium is ready for use in culturing microorganisms. Store any
- unused medium in a cool, dry place.
- It's essential to maintain proper aseptic techniques throughout the preparation process to prevent contamination of the agar medium with unwanted microorganisms. Additionally, follow any specific protocols or recipes required for preparing specialized agar media, such as selective or differential media [34, 35].

Agar well diffusion method

The agar well diffusion method, also known as the agar disk diffusion method or Kirby-Bauer method, is a widely used technique in microbiology for assessing the antimicrobial activity of various substances, such as antibiotics, plant extracts, or synthetic compounds. Here's an overview of the procedure:

Materials and Reagents

- Agar plates: Prepared with a suitable growth medium (e.g., Mueller-Hinton agar for bacteria).
- Microorganisms: Test strains of bacteria or fungi.
- Sterile swabs or inoculating loops.
- Antimicrobial agent: This could be an plant extract
- Sterile forceps.
- Sterile cork borer or pipette tip: Used to create wells in the agar. Incubator [46].

Procedure

- Prepare agar plates by pouring the sterile agar medium into petri dishes and allowing it to solidify.
- Streak the surface of the agar plates with a standardized inoculum of the test microorganism using a sterile
- swab or inoculating loop. Ensure even distribution of the inoculum.
- Allow the inoculum to dry on the agar surface for a few minutes to prevent excess moisture.
- Using sterile forceps, place the antimicrobial agent onto the surface of the inoculated agar plates. This could be in the form of antibiotic disks or small volumes of liquid solutions (e.g., plant extracts).
- If using a cork borer or pipette tip, create wells in the agar around the antimicrobial agent. Wells should be evenly spaced and sufficiently away from the edge of the plate to prevent overlap of inhibition zones.
- Incubate the plates inverted (agar side up) in an incubator at the appropriate temperature for the test microorganism. Incubation time and temperature vary depending on the microorganism being tested (e.g.,
- 37 °C for bacteria, 25-30 °C for fungi).
- After the incubation period, examine the plates for zones of inhibition around the wells containing the
- antimicrobial agent. Measure the diameter of the zones using a ruler or caliper.
- Interpret the results based on the diameter of the inhibition zones and compare them with established
- standards or breakpoints for the specific microorganism and antimicrobial agent being tested [37].

The agar well diffusion method provides a qualitative assessment of the antimicrobial activity of the test agent against the target microorganism. It's important to note that factors such as inoculum density, incubation conditions, and the diffusion rate of the antimicrobial agent can influence the results and should be standardized for accurate interpretation [38].

Results and Discussion

Extraction of Powders:

Table 4: Extractive values of Tulsi and Babul

Sample	Extraction method	Solvent used	Wt. of sample	Extraction value (%w/w)
Ocimum sanctum	Maceration extraction	Ethanol	40 gm	10% w/w
Acacia nilotica	Maceration extraction	Ethanol	40 gm	10% w/w

The results of physical parameters of formulated herbal gels like colour, homogeneity, pH, viscosity and spredability were shown in below Table 3. The spredibility of formulated herbal gels is shown in below Fig 2

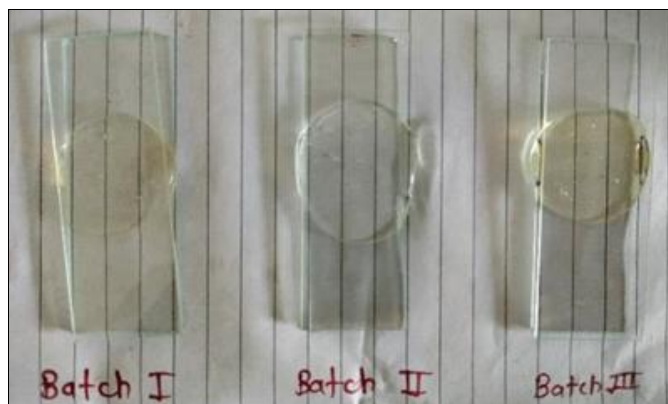

Fig 4: Spreadability of formulated herbal gels

Table 3: Results of physical parameters of all formulated herbal gels

Formulation Code	Colour	Homogeneity	pH	Viscosity (cp)	Spreadability (mm)
F1	Light Yellowish	Homogeneous	6.8/±0.03	3615 ±0.11	16.15/±0.005
F2	Yellowish	Homogeneous	7.0 /± 0.03	3714 ±0.21	15.40/±0.005
F3	Dark Yellowish	Homogeneous	7.1 /± 0.03	4137 ±0.43	15.39/±0.005

Fig 4: Batches of Polyherbal Gel

The results of antibacterial activity of all formulated herbal gels against some pathogenic microorganisms is shown in below Table 4 and the results of zone of inhibition of all formulated herbal gels against the pathogens is represented graphically in below Fig 3

Table 4: Zone of Inhibition

Micro-organism culture	Zone of inhibition of Herbal gels (mm)			
	Standard drug Gentamicin (0.80) [C]	F1 [A] (Conc. %- 10)	F2 [B] (Conc. %- 5)	F3 [D] (Conc. %- 100)
S. aureus	10	8	No zone	20

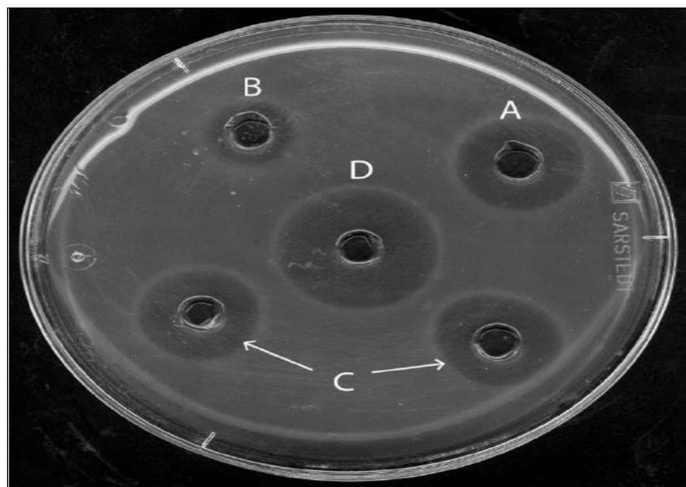

Fig 5: Antibacterial activity of formulated herbal gels

Conclusion

The data show that the leaves extract of *Ocimum sanctum* and bark extract of *Acacia nilotica* GEL had a 95%- 100% inhibitory effect on the development of bacteria in agar-diffusion tests and a 100% inhibitory effect on fungi in atmospheric-diffusion trials. In conclusion, the research on the "Formulation and Evaluation of Antimicrobial Polyherbal Gel by Utilizing Plant Extracts" underscores the potential of herbal medicine in addressing microbial infections. Through systematic formulation and rigorous evaluation, the study has demonstrated the efficacy and safety of the polyherbal gel against a spectrum of pathogens, including bacteria and fungi. The incorporation of various plant extracts known for their antimicrobial properties has yielded a synergistic effect, enhancing the overall effectiveness of the gel. Moreover, the gel's favorable characteristics such as stability, skin compatibility, and ease of application make it a promising candidate for topical antimicrobial therapy. The findings of this research contribute to the growing body of evidence supporting the use of herbal remedies in modern medicine. By harnessing the therapeutic potential of natural plant compounds, the polyherbal gel offers a sustainable and potentially cost-effective alternative to conventional antimicrobial agents. However, further studies are warranted to optimize the formulation, elucidate the mechanisms of action, and evaluate the long-term safety and efficacy of the polyherbal gel in clinical settings. Additionally, considerations regarding standardization, quality control, and scalability should be addressed to ensure the reproducibility and commercial viability of the product. Overall, the formulation and evaluation of the antimicrobial polyherbal gel represent a significant step forward in the development of herbal-based therapies for microbial infections, with the potential to complement existing treatment modalities and address emerging challenges in antimicrobial resistance.

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