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Formulation and Evaluation of Polyherbal gel from plant extract of *Azadirachta indica*, *Ocimum sanctum*, and *Acacia nilotica*

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Abstract : This study addresses the need by focusing on the current rising threat of antimicrobial resistance which demands novel therapeutic agents especially from natural sources. A polyherbal gel which is utilized by plant extracts of Neem (*Azadirachta Indica*), Tulsi (*Ocimum Sanctum*) and Babul (*Acacia Nilotica*). We evaluate the extracts individually, in combination and within formulated gels. Our preliminary in-vitro assessment involves disk diffusion method to measure growth inhibition zones against various microorganism. Additionally, we characterize physical properties of formulated gel using standard procedures.
Our findings revealed that the gel formulation containing equal concentration (1:1:1) of all extracts exhibited the most significant antimicrobial activity, through it was less potent then Gentamicine®. The synergistic action of these plant extracts presents a promising alternative to synthetic antibiotics actively contributing to the fight against antimicrobial resistance and fostering sustainable healthcare solutions.

Keywords: *Azadirachta indica, Ocimum sanctum, Acacia nilotica, Anti-microbial activity, Polyherbal gel.*

I. INTRODUCTION

The rise of antibiotic-resistant bacteria has spurred a search for novel drug source and accessible antimicrobial solutions. This is particularly important for rural populations, who frequently suffer from microbial infections. As a result, there's a growing interest in natural options like herbal drugs, which are increasingly being developed into convenient dosage forms, including herbal gels.[1] An antimicrobial substance can either kill or stop the growth of bacteria. This ability is crucial for preventing diseases and skin infections in the human body.[2] Recognizing that herbal drugs are increasingly formulated as gels, this research focused on creating an herbal gel from extracts of holy basil Tulsi (*Ocimum sanctum*), Neem (*Azadirachta indica*), and Babul (*Acacia nilotica*). The primary objectives were to investigate the extracts antimicrobial activity against prevalent microorganisms, and to evaluate the resulting gel's stability and phytochemical profile. Plant offers a diverse array of bioactive principles positioning them as valuable sources for both antimicrobial agents and a wide rage of pharmaceuticals. An antiseptic gel works by destroying or inhibiting bacterial growth. Earlier research has shown that medicinal plants are highly effective in wound care, speeding up healing while minimizing patient pain, discomfort, and scarring.[3]

Plant Profile

Fig 1: Neem (*Azadirachta indica*)

The Neem tree (*Azadirachta indica*), a tropical evergreen from the Meliaceae family, is a versatile plant with origins in India and Burma. It has since adapted well to West Africa and is extensively cultivated in Nigeria for both its aesthetic appeal like ornaments and its medicinal properties. Over 135 compounds have been identified from different parts of the neem tree, and notably, its leaves have shown considerable effectiveness against a variety of bacterial and fungal strains.[4,5]

Fig 2: Tulsi (*Ocimum sanctum)*

Tulsi (*Ocimum sanctum*) stands out as a highly valued and holistic medicinal plant, deeply rooted in India's traditional medicine for centuries. Often titled as the 'Queen of Herbs' and Mother of Medicine of Nature, Tulsi boasts a remarkable beneficial medicinal properties. This important herb is widely cultivated across India and other parts of South-East Asia.[6]

Fig 3: Babul (*Vachellia nilotica*)

Acacia nilotica (also known as *Vachellia nilotica* Wild) is a genus of shrubs and trees within the Mimosoideae subfamily of the Fabaceae (or Leguminosae) family. For decades, it has been traditionally used to treat a wide range of ailments, including diarrhea, dysentery, leprosy, cancers, ulcers, burns, boils, wound ulcers, and diabetes. Various parts of the plant also combat inflammation, ophthalmia, hemorrhoids, bleeding piles, and leukoderma.[7]

2. Material and Methods

Material

Test Microorganisms

The microorganisms were obtained from Microbiology Laboratory, of School of pharmacy under YBN University Ranchi. Bacteria: Staphylococcus aureus, and Fungi: Aspergillus niger.

Collection of Plant Material

For this in-vitro experiment, fresh Tulsi and Neem leaves were sourced from local courtyards, while Babul bark was acquired from a vendor in the local market. To ensure accuracy, the plants underwent identification and authentication at the Herbarium of the Department of Agriculture, YBN University, Ranchi (JH), with specimens retained for future reference**.**

Methods

Preparation of Extract

First, the leaves were detached from their stems and thoroughly washed with clean water. Next, they were allowed to air-dry for seven days until completely moisture-free. Finally, the dried leaves were individually powdered using an electric grinder until a uniform consistency.[8] After collecting *Acacia nilotica* bark from a local market, it underwent a seven-day drying process. Following the same process and the bark was individually powdered.

The poly-herbal hydro-ethanol extract was prepared from the previously obtained three different plant material powders. This extraction was carried out using the maceration method.[9] First, 100 grams of finely powdered *Ocimum sanctum*, *Azadirachta indica*, and *Acacia nilotica* were prepared for extraction. Each powder was then dissolved in adequate solution of ethanol and distilled water (H2O) in a ratio of 60:40. The mixture was covered with aluminum foil and allowed to macerate for 72 hours. Finally, it was filtered using Whatman filter paper to obtain a clear filtrate. Filtrate is now evaporated their excess solvents and make them dry by using water bath (temp not more than 30℃).

Formulation and Evaluation of Gel

The placebo gel (control formulation) was prepared by first dispersing Carbopol 940 in distilled water. Subsequently, methyl paraben, propyl paraben, and glycerin were introduced, and the mixture was allowed to stand overnight. Finally, the remaining water was added, and the formulation was continuously stirred and neutralized to pH 7 with triethanolamine.[10] The control batch formulation is shown in Table 1.

Table 1: Control Batch Formulation

Ingredients	Quantity
Carbopol 940	1.0 gm
Propylene glycol	0.2 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

Development of Poly-herbal Gel

The gel formulation began by dispersing Carbopol 940 in distilled water, followed by the addition of methyl paraben, propyl paraben, and glycerin this base was left overnight. Separately, the extracts of *Azadirachta indica* leaves, *Ocimum sanctum* leaves, and *Acacia nilotica* bark were dissolved in propylene glycol before being added to the polymer dispersion. The remaining water was then introduced, and the mixture was continuously stirred for 10 minutes and adjusted to pH 7 using triethanolamine.[10] The development of different concentration of formulations is shown in Table 2 and fig 3.

Table 2: Development of herbal gel formulations

Ingredients	F1	F2	F3	F4
Ocimum sanctum Extract	1.0 gm	-	-	1.0 gm
Azadirachta indica Extract	-	1.0 gm	-	1.0 gm
Acacia nilotica Extract	-	-	1.0 gm	1.0 gm
Carbopol 940	1.0 gm	1.0 gm	1.0 gm	1.0 gm
Propylene glycol	10 ml	10 ml	10 ml	10 ml
Methyl paraben	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Propyl paraben	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Glycerin	1 ml	1 ml	1 ml	1 ml
Triethanolamine	q.s.	q.s.	q.s.	q.s.
Distilled water	100 ml	100 ml	100 ml	100 ml

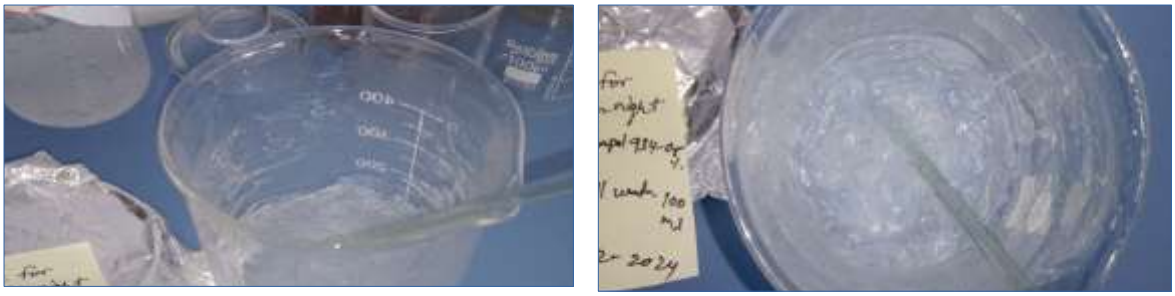

Fig 3: Development of poly-herbal gel

Evaluation of herbal gels

Physical evaluation: Color, Homogeneity by visual observation.
pH: The pH of all gels
Viscosity: Determined by Brook-field rotation viscometer at 100rpm.
Spreadablity: The spreadablity determined by 1gm of gel between 2 horizontal plates.

Phytochemical Screening

To characterize the extracts, a phytochemical screening was performed following standard procedures. This screening specifically looked for anthraquinones, alkaloids, glycosides, flavonoids, reducing sugars, saponins, pentose sugars, carbohydrates, and tannins.[11]

Evaluation of Antimicrobial Activity

We evaluated the antimicrobial activity of three extracts, both individually and in various combinations and concentrations, using the agar well diffusion method.[12] A marketed cream Gentamicin I.P. 0.03% w/w which is marketed brand name as "Gentlee Cream" a product of Atopic Laboratories Ltd. was used as the standard. The agar well diffusion method (also called the agar disk diffusion or Kirby-Bauer method) is a common microbiology technique. It's used to qualitatively assess the antimicrobial activity of substances like antibiotics, plant extracts, or synthetic compounds against specific microorganisms. For accurate results, it's crucial to standardize factors such as the amount of bacteria used, incubation conditions, and how quickly the antimicrobial agent diffuses.[13]

The gels were evaluated for their activity against Staphylococcus aureus and Aspergillus niger. To do this, a bacterial culture was prepared in nutrient broth and incubated. Sterilized nutrient agar was poured into Petri plates, and once solidified, 0.1ml of the bacterial inoculum was spread evenly. A 6mm cavity was then made in the agar, into which the formulated gel was placed. A standard antibiotic served as a control. After 24 hours of incubation, the zone of inhibition around each gel was measured and recorded.[14]

3. Result and Discussion

The results of physical parameters of formulated herbal gels like colour, homogeneity, pH, viscosity and spredibility were shown in table 3. It's possible that specific phytochemicals within the plant leaf extract give it its antimicrobial capabilities.[15] Our in vitro study revealed that hydro-ethanolic extracts from Tulsi, Neem leaves, and Babul bark exhibit concentration-dependent antimicrobial effects against both *Staphylococcus aureus* and *Aspergillus niger*. We found that zones of inhibition values for all different formulated extracts against the tested microorganisms is different in compared to prior studies,[16] which we attribute to variations in the extraction methods, the data is shown in table 5. A key finding was the absence of any antimicrobial activity from the ethanol negative control, indicating that the solvent itself doesn't possess antimicrobial properties. Given their significant antibacterial activity against *Staphylococcus aureus*, and on fungus *Aspergillus niger* as well these extracts show promise for treating skin conditions like impetigo ("school sores") and ecthyma (crusted ulcers) that are caused by bacterium.

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

Formulation	Colour	Homogeneity	pH	Viscosity (cp)	Spreadability (mm)
F1	Light Yellowish	Homogeneous	6.8/±0.03	3553±0.10	15.48/0.005
F2	Yellowish	Homogeneous	6.7/±0.03	3627±0.19	16.02/0.005
F3	Light Brownish	Homogeneous	6.9/±0.03	3815±0.50	15.40/0.005
F4	Brownish	Homogeneous	7.1/±0.03	4032±0.54	15.25/0.005

Table 5: Result of zones of inhibition (mm) of formulations

Micro-organisms	F1 (10 mg/ml)	F2 (10mg/ml)	F3 (10mg/ml)	F4 (30 mg/ml)	Gentamicin Std. Drug 3mg/ml	2% Ethanol
S. aureus	20.0±0.7	22.5±1.0	17.6±0.6	26.5±0.3	30.5±1.1	-
A. niger	18.5±1.0	19.6±0.4	12.0±1.1	24.0±0.5	28.8±0.5	-

4. Conclusion

The research on the "Formulation and evaluation of polyherbal gel by extracts of *Azadirachta indica*, *Ocimum sanctum*, and *Acacia nilotica*" highlights the significant antimicrobial potential of herbal medicine. Our data demonstrate that the leaves extract of *Ocimum sanctum*, *Azadirachta indica*, and the bark extract of *Acacia nilotica* GEL exhibited inhibitory effects. Specifically, we achieved 90-100% bacterial inhibition in agar diffusion tests and 95-100% fungal inhibition in atmospheric diffusion trials.

This study systematically formulated and rigorously evaluated a polyherbal gel, proving its efficacy and safety against both bacterial and fungal pathogens. The combination of various plant extracts, each known for its antimicrobial properties, created a synergistic effect, boosting the gel's overall effectiveness.

Furthermore, the gel's desirable characteristics, including its stability, skin compatibility, and ease of application, position it as a promising candidate for topical antimicrobial therapy. These findings significantly contribute to the expanding evidence supporting herbal remedies in modern medicine. By leveraging the therapeutic power of natural plant compounds, this polyherbal gel offers a sustainable and potentially cost-effective alternative to conventional antimicrobial agents.

Ultimately, the development and evaluation of this antimicrobial polyherbal gel mark a crucial advancement in herbal-based therapies for microbial infections. It holds the potential to complement existing treatment methods and help address the increasing challenge of antimicrobial resistance. Nevertheless, more research is required to fully understand their bio-availability and ensure their long-term stability.

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