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Formulation and Evaluation of Polyherbal Antimicrobial Cream Using Betel Leaf Extract

Sri Sowmya Veeravalli[1], Bhaskara Raju Vatchavai[2], Jhansi Lakhsmi Polisetti[3], Sri Lakshmi Prasanna Guttikonda[4], Navya Satya Sri Palepu[5], Lakshmi Naga Chandana Karri[6], Naga Satya Krishna Gudupu[7]

[1]*Assistant Professor, Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, Andhra Pradesh, India*
[2]*Principal , Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, Andhra Pradesh, India*
[3, 4, 5, 6, 7]*Student, Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, Andhra Pradesh, India*

Abstract: ***Creams were topically applied semisolid compositions. Formulations with two or more herbs are known as polyherbal formulations. Piper betel, a plant with therapeutic properties and used in Asian nations like India and Nepal (betel leaf juice is associated with diuretic characteristics), was used to make anti-microbial cream. The synergistic therapeutic potential of polyherbal formulations and their lower side effects as compared to synthetic medicines have drawn a lot of interest in recent years. The present investigation's objective was to establish a stable w/o herbal cream using liquid paraffin as the oily phase, neem, tulsi, and turmeric extract as the aqueous phase, and constant stirring to create a homogenous polyherbal cream. A range of pharmaceutical properties, including viscosity, spreadability, rheology, electrical conductivity, pH, and stability, were assessed for the cream. The antimicrobial effectiveness of polyherbal creams is the main topic of this study, with an emphasis on formulation creation, assessment, and standardization. The maceration process was used to extract the dried plant materials of neem, tulsi, betel leaf, and turmeric. Cream compositions may be employed as antibacterials to treat a range of bacterial diseases.***

Keywords: piper betle, neem, tulsi, turmeric, polyherbal cream.

I. INTRODUCTION

Herbal medicine, also known as botanical medicine or phytomedicine, is the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medical purposes.[1] The evergreen perennial dioecious creeper Piper betel L. (Piperaceae) is also referred to as "Paan" or "Nagvalli."[2] The betel leaf is a perennial, evergreen creeper with a glossy, white, heart-shaped catkin. Tropical and subtropical areas of the world are home to the majority of the Piper genus (Piperaceae).[3] Herbal resources are natural assets for pharmaceutical intermediates, nutritional supplements, nutraceuticals, traditional medicine, folk medicine, modern medicine, and chemical precursors for synthetic medications.(4) 1,8-cineole, cadinene, camphene, caryophyllene, limonene, pinene, chavicol, 4-allyl-pyrocatechol, carvacrol, safrole, eugenol, and chavibetol are the terpenoids and phenylpropanoids found in Piper betel.[5] The tree Azadirachta indica, which belongs to the Maliaceae family and is commonly referred to as the neem tree, has been utilized in traditional medicine from ancient times.[6] It has been discovered that A. indica exhibits antibacterial action against both Gram-positive and Gram-negative bacteria, including Escherichia coli (E. coli) and Enterococcus species.[7] The leaves, seeds, and dried roots of tulsi are the portions that are typically used. Ocimum tenuiflorum, also referred to as Ocimum sanctum, Holy basil, or tulsi is a fragrant member of the Lamiaceae family of plants. that is indigenous to the Indian Subcontinent and widely grown in the tropical regions of Southeast Asia.[8] About 70% eugenol, 20% methyl eugenol, and 0.7% volatile oil components are found in Tulsi leaves.[9] Turmeric is made from the rhizomes of Curcuma longa, a member of the Zingiberaceae family. Curcuminoids, which are lipophilic, polyphenolic carotenoids, give curcuma its dark orange hue.[10] Yellow curcuminoids, which have antioxidative and anti-inflammatory qualities, and volatile oil, which has carminative and antifungal characteristics, are the sources of the rhizomes' medicinal applications.

II. MATERIALS AND METHODS

A. Collection and Extraction Procedures

1) Betel Leaf Extraction

1. After being newly dried for seven days at room temperature, the leaves were milled into a powder. Acetone was used to cold macerate PBL for 72 hours at room temperature, with sporadic stirring. 500 ml of acetone with 100 g of powdered PBL for 72 hours.
2. Collection of samples: After being gathered at the neighbourhood market, the betel leaf was dried at room temperature, pounded in a pestle and mortar, and kept in an airtight container.[11].

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2) Preparation of Plant Extracts

Betel leaf methanol, aqueous extract, and acetone extract were made using the procedures. Three beakers were filled with 20 grams of finely powdered betel leaf powder, 80 millilitres of acetone, 80 millilitres of methanol, and 80 millilitres of water. After soaking them for 72 hours, the solution was carefully filtered through muslin cloth into a sterile 100 ml conical flask. The filtrates were then refrigerated at 4° C until needed. [12].



3) Neem Extraction

Gathering and preparing leaves of neem (Azadirachta indica). Neem trees were the source of the neem (A. indica) leaves. After that, the leaves were cleaned for five minutes under running water to get rid of any dust particles that had adhered to their surface. Ten days of room temperature drying were given to the leaves. After that, the grinded dry leaves were made into a powder using a dry blender for a more effective and efficient organic extraction.

4) Extraction of Secondary Metabolites

The dried leaves of A. indica were crushed into a powder. After soaking 50 g of each sample in acetone and chloroform in a closed 500 ml conical flask, the samples were left at room temperature for a full day. The extraction procedure was carried out twice (for two days). The extracts were then concentrated at low pressure using a rotary evaporator at 40° Antibacterial 45° C after being filtered via Whatman 1 filter paper. For later use, the dried extracts were stored at 4 °C in the refrigerator. The concentration of 100 m was used for the agar disc-diffusion experiment [13].

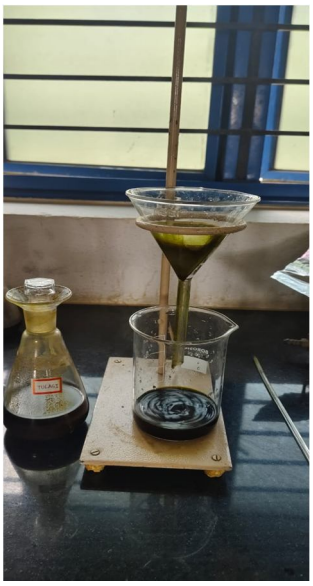


5) Tulsi Extraction

They gathered the leaves. Stem fragments, debris, and dust were eliminated, cleaned three times in sterile distilled water, allowed to dry in the shade for a full day, and then ground into a uniform powder using a mechanical grinder (HL juicer mixer grinder, Philips, Hyderabad, India). For later use and analysis, all of the dried leaf powders were kept in storage at -20°C.

6) Preparation of Various Extracts

Each species of tulsi, including Camphor, Krishna, Lavanga, and Vishnu, had its dried leaf powder (100 g) extracted in 500 mL of acetone, water, dichloromethane, ethanol, hexane, or methanol for 24 hours. The powder was then stored at room temperature in a shaker (Julbo shaking water bath, SW23, Julbo GmbH, Germany). A rotary evaporator (Hanaper, HS-2001NS, Hanshin Scientific Co., TTL Technologies Pt. Ltd. Bangalore, India) was used to separate and concentrate the supernatants. After that, the extracts were dried till the residue was collected using a digital water bath (NBS, New Bio Science Co., Mysuru, Karnataka, India). Until they were needed, all 24 extracts were stored in airtight bottles.



7) Turmeric Extraction

Plant Extract Preparation To get rid of the raw smell, the rhizomes were gently cleaned with clean water and steamed for ten minutes. The material was ground into a powder and passed through a sieve with a nominal mesh size of 2 mm in diameter after being dried for 24 hours at 50°C in a hot air oven. After dissolving 20 g of Curcuma longa in 100 ml of acetone, the mixture was let to soak for a whole day. Whatman's filter paper number one was used to filter the mixture, which was then stored for analysis.



III. PHYTOCHEMICALS TESTS

1) Betel leaf:(Piper betel)

Betel leaf consists of eugenol, chavicol, safrole, alkaloids, tannins and saponins.

Test for alkaloids:

Mayer's test: a few drops of Mayer's reagent were applied to one millilitre of the filtrate. Alkaloids are indicated by a cream-colored precipitate. [14]

Test for Tannins:

Ferric chloride test: 2 millilitres of each extract solution were gradually treated with a 5% ferric chloride solution. The colour blue was achieved. The blue colour changes to olive green when more ferric chloride solution is added. It verifies that tannins are present.[15]

2) Turmeric: (Curcuma longa)

Turmeric consists of curcuminoids, flavonoids, saponins, volatile oils.

Test for Phenol:

Ferric Chloride Test: The test extract was mixed with four drops of an alcoholic FeCl3 solution. The presence of phenol is indicated by its bluish-black appearance. [16].

Test for Flavonoid: A 50% methanol solution (1.5 ml) was added to four millilitres of extract solution. After heating the mixture, metal magnesium was added. When five to six drops of strong hydrochloric acid were added to this solution, the flavonoids turned red and the flavones turned orange.

Test for Saponins:

After shaking 0.2 g of the extract with 5 ml of distilled water, it was brought to a boil. The presence of saponins is indicated by frothing, which appears as a creamy mass of tiny bubbles [17].

3) NEEM (Azadirachta indica)

Neem consists of flavonoids, Tannins, Saponins Fatty acids, limonoids.

Test for flavonoids:

Alkaline reagent:

When two millilitres of NaOH were added to two millilitres of plant extract, a yellow hue developed that turned colourless when diluted. Acid was added to show the presence of flavonoids.

Test for terpenoids:

Salkowski Test:

To create a layer, 5 ml of the extract was combined with 2 ml of chloroform and concentrated sulfuric acid. Terpenoids are indicated by a reddish-brown tint. [18].

Test for saponins:

In a test tube, the plant sample (0.5 g) was combined with 5 ml of distilled water. After being vortexed, the mixture produced a stable, long-lasting foam. Three drops of olive oil were added to the foam, which was then vortexed to create an emulsion.

Test for terpenoids:

A 0.5 g plant sample was dissolved in 1 ml of chloro form, followed by the addition of 1 ml of acetic anhydride and 2 ml of concentrated H_2SO_4. A reddish violet colour was seen to form.

Test for tannins:

In a test tube, 0.5 g of ground neem leaves were cooked in 10 ml of water and then filtered. After adding a few drops of 0.1% ferric chloride, the solution was checked for a blue-black or brownish green colour.

Test for cardiac glycosides:

keller-kiliani test:

After dissolving a 0.5 g sample of neem leaves in 5 ml of water, 2 ml of glacial acetic acid solution with one drop of ferric chloride solution was added. One millilitre of pure H_2SO_4 was placed underneath this. The presence of cardenolides' deoxy sugar properties was shown by a brown ring at the contact. In the acetic acid layer, a greenish ring may emerge slightly above the brown ring and progressively expand across this layer, while a violet ring may appear underneath the brown ring. [19]

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4) TULASI (Ocimum tenuiflorum)

Tulasi consists of Flavonoids, Tannins, Alkaloids, Saponins, Phenolic compounds, Volatile oils.

Test for flavonoids:

Ammonia test: One millilitre of the extract was put in a test tube with a few drops of a 1% NH3 solution. The presence of flavonoids was indicated by a yellow hue. Sodium hydroxide test: One millilitre of the extract was mixed with a few drops of a 20% NaOH solution. The extract's yellow colour changes to a colourless solution upon the addition of HCl, indicating the presence of flavonoids.

Test for alkaloids:

Dragendorff test:

A few drops of Dragendorff reagent were added to 1 millilitre of the extract. The test is positive when a noticeable yellow precipitate forms. Wagner test: One millilitre of extract was mixed with a few drops of Wagner's reagent on the edge of the test tube. The test is confirmed as positive by a reddish-brown precipitate.

Mayer test:

In a steam bath, 1 ml of the extract and 5 ml of 1% HCL were mixed. After filtering the resultant solution, a few drops of Mayer's reagent were added to one millilitre of the filtrate. When Mayer's reagent was added, the extract filtrate's turbidity was interpreted as proof that alkaloids were present. [20]

Test For Alkaloids:

Wagner's test:

In 2 millilitres of methanol, 20 milligrams of turmeric were added. Add a few drops of 1% HCl to it. After that, the mixture was heated, cooled, and held in steam. drops of the reagent Wagner. We looked for precipitation or turbidity in the sample. In a test tube, 20 mg of turmeric were dissolved in 1 millilitre of distilled water. One to three drops of ferric chloride were then added to the mixture to test for tannins. After then, the mixture's colour was checked for blue or green.

Test For Saponins:

Foam test:

After dissolving 40 mg of turmeric in 5 ml of distilled water, the mixture was rapidly agitated until a stable, long-lasting froth was produced. After adding three drops of olive oil to the foam and giving it a good shake, the emulsion was checked.

Test For Flavonoids:

Ferric chloride test:

In 1 millilitre of distilled water, 20 milligrams of turmeric were added. A diluted ammonia solution (0.5 ml) was added. Subsequently, sulfuric acid was added. Flavonoids were signified by the colour yellow. The yellow hue vanished when the solution was left to stand.

Test For Terpenoids:

Salkowski's test:

After dissolving 20 mg of turmeric in 1 millilitre of chloroform, 1 millilitre of strong sulfuric acid was added. Terpenoids were visible at the interface as a reddish-brown discoloration.

Test For Carbohydrates:

Fehling's test: Fehling's A and B solutions are heated with a few drops of extract. The presence of carbohydrates is indicated by the appearance of orange-red precipitate.

Test For Proteins:

Biuret's test:

To 2 ml of extract, add 2 ml of Biuret reagent. Warm it in a water bath and give it a good shake. Proteins are present when they appear red or violet in colour. [21]

IV. FORMULATION OF ANTI-BACTERIAL CREAM

INGREDIENTS	QUANTITY
Betel leaf extract	0.8g
Neem leaf extract	0.15g
Tulasi leaf extract	0.10g

Turmeric extract	0.15g
Stearic acid	1.0g
Cetyl alcohol	0.3g
Liquid paraffin	0.5g
Glycerin	0.5g
Propyl paraben	0.02g
Methyl paraben	0.002g
Flavoring agent	q. s

A. Betel leaf



Biological source: It is obtained from dried of leaves of Piper betel.
Family: Piperaceae
Constituents: - Terpinene, P-cymene, carvacrol, chavicol, allyl catechol, eugenol, estragole, oxalic and malic corrosives, and amino acids are all found in plants.
Uses of betel leaf: - Headache, Inadequate or blocked urine, Nervousness weakness, Throat pain [22].

B. Neem

Name: Azadirachta indica
Family: Maliaceae
Synonym: Neem, Margosa, Nimtree, or Indian lilac
Biological Source: A tree native to the Indian subcontinent and parts of Southeast Asia, belonging to the mahogany family (Maliaceae)
Biological Name: Neem
Chemical Constituents: Limonoids, flavonoids, alkaloids, and phenolic compounds



Uses:
Scabies, eczema, and other skin illnesses have traditionally been handled with neem. It is thought to contain antibacterial and anti-inflammatory qualities that can ease sensitive skin and encourage healing. Neem is useful in treating a variety of diseases, including those brought on by bacteria, viruses, and fungus, due to its demonstrated antiviral, antibacterial, and antifungal qualities [23].

C. Turmeric

Synonyms: Saffron Indian; Haldi (Hindi); Curcuma; Rhizome cur-Cumae.
Biological Source: The dried rhizome of Curcuma longa Linn. (syn.C. domestica Valeton) is known as turmeric.
Family: Zingiberaceae
Chemical Constituents: Curcumin

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Uses:
It has strong antifungal properties. Fungal cell membrane rupture and inhibition were two aspects of the antifungal action. Turmeric's curcumin has antifungal properties against Aspergillus species, lowering oxidative stress and inflammation [24].

D. Tulsi
Biological source: Tulsi consists of the fresh and dried leaves of Ocimum species like Ocimum sanctum L.
Family: Lamiaceae
Chemical Constituents:70% Eugenol, Caryophyllene, Volatile oil, essential oil.



Uses:
At a dosage of 0.2 mg/ml, tulsi leaves exhibited antifungal efficacy against clinically isolated dermatophyte. Nearly every Ocimum species produced an antifungal essential oil. Tulsi leaves have been shown to have a potent antifungal impact on Aspergillus species. antifungal activity in vitro against species of Candida [25].

V. EVALUATION TESTS FOR ANTI-BACTERIAL CREAM

A. Spreadability
Two standard-sized glass slides were used to assess the spreadability of the prepared herbal cream. One slide was carefully filled with a predetermined amount of the formulation, and the other slide was placed on top of it such that the cream formed a thin layer that stretched 6.5 cm between them. The upper slide was then subjected to a 200 g weight to guarantee that the cream was uniformly compressed into a homogeneous layer. A few minutes later, the weight was taken off, and any extra formulation that had stuck to the slides was carefully scraped off. After that, the paired slides were positioned on a stand so that an applied load could cause the higher slide to move freely. The upper slide had a 20 g weight fastened to it, which allowed gravity to pull it down the lower slope. It was noted how long it took the upper slide to fully disengage and travel the 6.5 cm distance. The spreadability of the cream formulation was calculated by repeating the process three times and averaging the results. The formula for calculating spreadability was S = m. l/t Were S – Spreadability, m 20 g of weight is fastened to the upper slide. - The glass's length (6.5 cm), t - The duration in seconds [26].

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B. Irritancy Test

During irritation testing, cream exhibits no redness, irritation, edema, or inflammation. You can use these formulas on your skin without risk [27].

C. Patch Test

A piece of cloth or a funnel was used to apply one to three grams of the item to be tested to a sensitive area of the skin, such as the region behind the ears. The test cosmetic was applied to one square meter of skin. There were also control patches used. A 24-hour inspection is conducted at the patch site.

- ❖ Test for microbial growth in formulated creams:

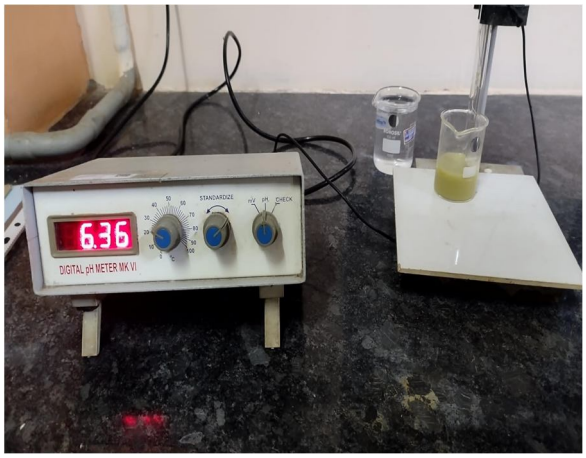
The prepared creams were infected using the streak plate method on agar medium plates, and a control was created by leaving out the cream. The plates were put in the incubator and kept there for a whole day at 37 °C. Plates were removed after the incubation period in order to compare the microbial growth to the control [28].

D. Viscosity

The Brookfield Viscometer (model DV-E) was used to measure the formulation's viscosity at 10 rpm with spindle number 64 [29].

E. Determination of pH

A standard buffer solution was used to calibrate the pH meter. After gathering around 2 g of each formulation in a beaker, the pH was measured. The pH values of the antimicrobial cream formulations ranged from 4.56 to 6.89, indicating a mild acidity. Since they fall within the typical pH range of the skin's surface (4.0 to 7.0), they can be applied to the skin without causing irritation. The skin's rather acidic composition aids in preventing the growth of dangerous microorganisms [28].





VI. INVITRO STUDIES (MICROBIAL TEST)

The microbial growth investigation employed nutrient agar and nutrient broth media. Using blank and sample Petri plates, the cream sample was aseptically placed onto the sample plates in a cross pattern, and microbial growth was monitored. antibacterial activity was evaluated against strains of E. coli and Staphylococcus aureus after 24, 48, and 72 hours, and it was discovered to show notable antibacterial activity.

Determination of the minimum inhibitory concentration (MIC) An anti-microbial agent's minimum inhibitory concentration (MIC) is the lowest concentration at which bacteria appear to be prevented from multiplying; the lower the concentration needed, the more effective the molecule is. The micro broth dilution method from the Clinical and Laboratory Standards Institute was used to determine the MIC. The concentration of the extract was measured at 0.1–2 mg/ml. 0.1 mL of a standardized inoculum (1–2 × 107 colony-forming unit/mL) was applied to each test tube. The tubes were aerobically incubated at 28°C for 48–72 hours. There were two controls for every test sample. By comparing the diluted extract with the control tubes and identifying the lowest concentration (maximum dilution) at which no microbial growth was seen, the minimum inhibitory concentration (MIC) was calculated [30].



VII. CONCLUSION

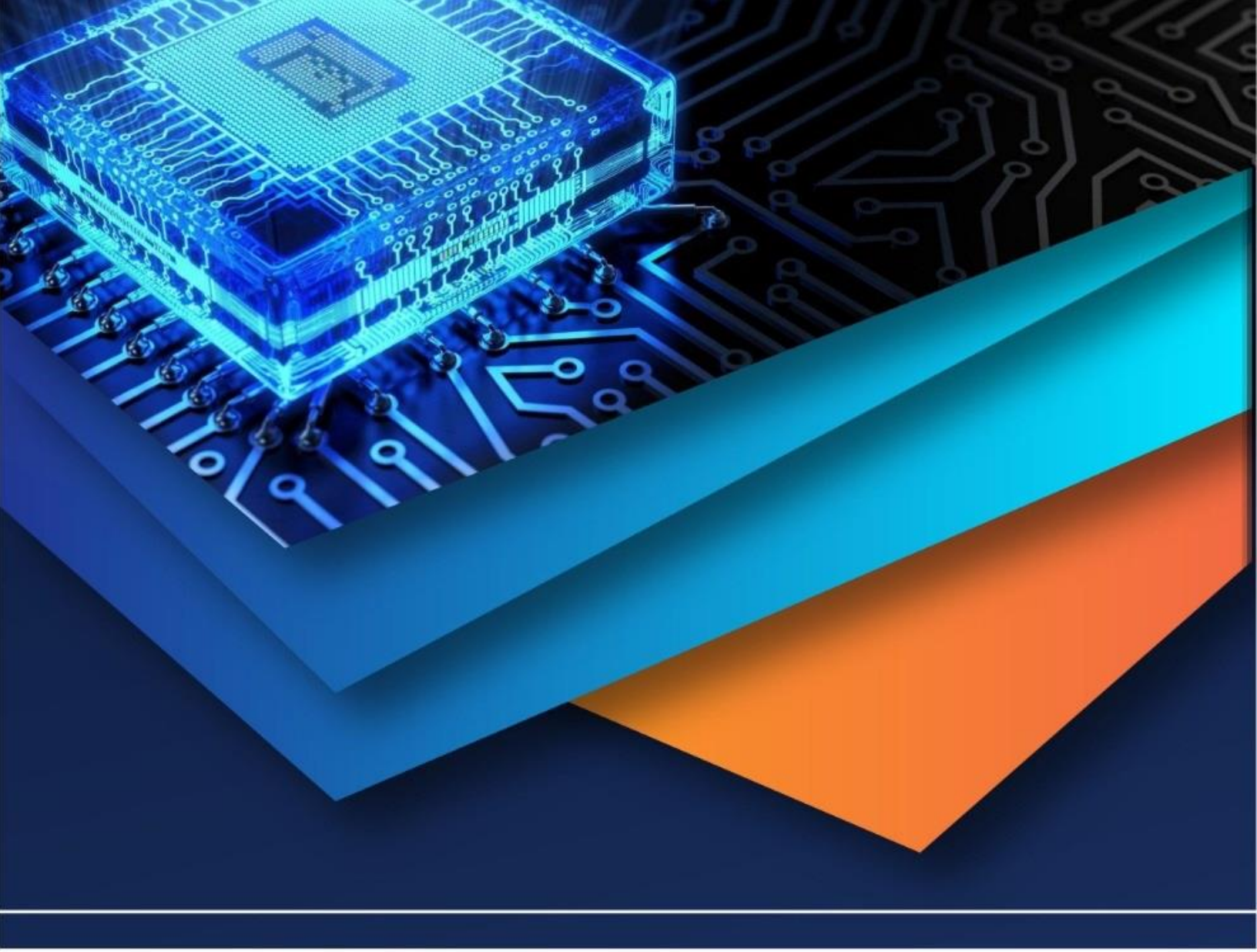
The present study focused on the formulation and evaluation of a polyherbal antimicrobial cream containing extracts of Piper betel, Azadirachta indica, Ocimum tenuiflorum, and Curcuma longa. These medicinal plants are well known for their antimicrobial, anti-inflammatory, and antioxidant properties, which make them suitable candidates for topical therapeutic formulations. The use of multiple herbal ingredients in a single formulation may enhance the overall biological activity due to synergistic interactions between phytoconstituents. The extraction procedures used in this study allowed the isolation of various phytochemical compounds from the plant materials. Preliminary phytochemical screening confirmed the presence of several biologically active constituents such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds. These compounds are widely reported in the literature for their antimicrobial activity. Flavonoids and phenolic compounds are known to inhibit microbial growth by damaging microbial cell membranes and interfering with essential metabolic processes.

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