Research Article

Development and Evaluation of Antibacterial Herbal Paste

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ABSTRACT

The goal of this project is to use natural plant-based ingredients to create and thoroughly evaluate an herbal paste with potent antibacterial properties. The declining efficacy of traditional antibiotics has heightened the hunt for alternative therapies in the face of the growing worldwide problem of antimicrobial resistance (AMR). This has sparked a renewed interest in natural products and traditional medicine. Herbal remedies, which have been used for a long time in many cultures, are now being investigated scientifically to ensure their safety and effectiveness. New, sustainable, and less hazardous antibacterial alternatives are desperately needed as harmful microorganisms grow more resistant to synthetic antibiotics. The abundance of bioactive components found in plant-based formulations, including alkaloids, flavonoids, tannins, saponins, and essential oils, many of which have strong antimicrobial properties, is making them attractive substitutes. The purpose of this study is to create an herbal paste formulation using extracts of *Syzygium aromaticum* (Clove), *Curcuma longa* (Turmeric), and *Azadirachta indica* (Neem) having antibacterial activities. This herbal paste formulation has been tested against a variety of common Gram-positive and Gram-negative bacterial strains, as well as evaluated for organoleptic properties, Rhelogical properties, pH and stability. The study's goal is to create a powerful, affordable, and eco-friendly antibacterial product that promotes the development of phytomedicine and provides a safe, natural substitute for topical antibiotics.

Keywords: Herbal; Paste; Antimicrobial; Antibiotics; Antibacterial.

INTRODUCTION

Using whole or processed plant materials to create and prepare medicinal products is known as herbal formulation. These products are made to deliver the medicinal benefits of herbs in ways that are effective, stable, and easy to use. Enhancing the practicality, safety, and absorption of herbal remedies while preserving the potency of their active ingredients is the goal. The selection of appropriate herbs, the extraction method, the ingredient combination in multi-herb formulations, the stability of the product, and the application method are some of the factors that affect how effective an herbal formulation is. To guarantee effectiveness and dependability, a successful formulation frequently combines conventional wisdom with contemporary scientific techniques.

Plants have been essential to human health for thousands of years, serving as the foundation of conventional medical practices such as Ayurveda, Unani and Traditional Chinese Medicine. These long-standing customs have depended on botanical treatments and herbal mixtures to treat a variety of ailments, from minor infections to chronic diseases. Due to growing interest in complementary and alternative therapies worldwide, the study of medicinal plants has recently moved from cultural tradition to scientific inquiry. Developments in ethnopharmacology, which studies the traditional use of plants for medicinal purposes, and phytochemistry, which allows the identification and extraction of plant-based compounds, have greatly accelerated this change.

With over 80% of the world's population depending on herbal remedies for their primary healthcare needs, the World Health Organization has emphasized the vital role that medicinal plants play in global healthcare[1]. Traditional medicine's strong cultural roots are just one factor contributing to this dependence; other factors include natural remedies' accessibility, affordability, compatibility with the human body, and generally lower incidence of side effects when compared to synthetic pharmaceuticals. Herbal remedies are frequently essential therapeutic solutions, especially in settings with limited resources. The concerning rise in antibiotic resistance is one of the main causes of the renewed interest in plant-based medicine around the world. The emergence of multi-drug-resistant (MDR) bacterial strains as a result of the extensive abuse and overuse of antibiotics poses a serious a public health challenge. This has led to an urgent need for novel, safe, and efficient antimicrobial compounds. Alkaloids, flavonoids, tannins, terpenoids, glycosides, and phenolic compounds are examples of secondary metabolites from medicinal plants that are receiving a lot of attention because of their potent antimicrobial, antioxidant, anti-inflammatory, analgesic, and wound-healing qualities[2]. The capacity of plants to generate bioactive compounds makes them a natural and sustainable source of medicinal substances. The emergence of bacteria resistant to antibiotics has become a major global health concern in recent years. Synthetic antibiotics are overused and misused, which has decreased their effectiveness and increased their side effects. Researchers and the pharmaceutical industry are therefore becoming more interested in finding safer, plant-based

substitutes. Due to their effectiveness in treating wounds and infections, low side effects, and compatibility with human skin, herbal formulations especially those intended for topical application are gaining popularity. Pastes stand out among other herbal delivery methods due to their simple preparation, ease of use, and therapeutic effect. To treat cuts, burns, wounds, and infections, these preparations are usually created by mixing powdered herbs or plant extracts with bases like water, oils, or gels and applied directly to the afflicted skin areas. In treating bacterial skin infections, several herbal pastes have shown significant antibacterial activity against both Gram-positive and Gram-negative pathogens[3]. Using medicinal plants that have long been known for their antimicrobial qualities, the main goal of this study is to create and assess an antibacterial herbal paste. It looks at the paste's stability, antibacterial efficacy, and physical characteristics. It is expected that the findings will promote the development of herbal medicine and offer a strong substitute for traditional topical antibiotic therapies. Topical pastes are among the many herbal preparations that have found widespread use in both contemporary herbal applications and traditional healing. Active plant ingredients can be directly applied to the afflicted skin or wound site thanks to these semi-solid formulations. There are several advantages to this localized strategy, including improved targeted action, decreased systemic exposure, direct delivery of antimicrobial agents to the site, and accelerated healing through the formation of a moist, barrier-protecting barrier against contaminants. These pastes work especially well for treating skin disorders like inflammation, burns, rashes, cuts, abrasions, and infections. Three well-known medicinal plants neem (*Azadirachta indica*), turmeric (*Curcuma longa*), and clove (*Syzygium aromaticum*) will be used in this study to create and evaluate an antibacterial herbal paste. These plants were picked because of their long history of use in both traditional medicine and contemporary science, as well as their proven antimicrobial and wound-healing properties.

***Azadirachta indica* (Neem):** Known as the "village pharmacy" in India, neem has a long history in folk and Ayurvedic medicine. It has potent anti-inflammatory, antifungal, antibacterial, and immune-modulating qualities. (Subapriya *et al*, 2005; Biswas *et al*, 2002) Its efficacy in treating a range of skin conditions, including eczema, acne, wounds, and infections, is attributed to compounds like quercetin, azadirachtin, nimbin, and nimbidin[4,5,6].

***Curcuma longa* (turmeric):** This golden root is well known for its strong antibacterial, anti-inflammatory, and antioxidant properties and is frequently used as a spice and medication. Its main bioactive ingredient, curcumin, is a polyphenol that has anti-inflammatory, anti-microbial, and collagen-promoting properties, making it particularly useful for wound care[7,8].

***Syzygium aromaticum* (clove):** Eugenol, a bioactive compound with potent antibacterial, antifungal, analgesic, and anesthetic qualities, is found in cloves, which are widely used in cooking and dental care. (Chaieb *et al*, 2007; Cowan *et al*, 1999) It is a useful component of antimicrobial herbal products due to its demonstrated effectiveness against a variety of Gram-positive and Gram-negative bacteria[2,9,10].

Techniques for Extraction of crude extract from Plants

The extraction method used to separate bioactive compounds has a significant impact on how effective herbal formulations are. An essential procedure that concentrates the desired phytochemicals while removing extraneous ingredients is extraction. Depending on the type of plant and the desired constituents, a variety of conventional and contemporary techniques are used[11,12].

Maceration

One simple and popular technique is maceration. It entails soaking powdered plant material for 24 to 72 h at room temperature in an appropriate solvent, such as water, ethanol, methanol, or a hydroalcoholic mixture. After the soaking time, the extract is filtered and concentrated, and the mixture is periodically stirred. This method works especially well for heat-sensitive compounds[11,13].

Decoction

Boiling tougher plant parts, like roots, bark, or seeds, in water for a predetermined amount of time (usually 15 to 30 min) is known as decoction. The extract is obtained by filtering the mixture after it has been boiled. Although this age-old technique, which is frequently employed in Ayurveda, works well for removing ingredients from thick plant materials, it may destroy elements that are heat-sensitive[12,14].

Soxhlet Extraction

A common continuous hot extraction method in lab settings is Soxhlet extraction. This method involves heating, evaporating, and then repeatedly condensing a solvent over plant material in a thimble inside a Soxhlet apparatus. To accomplish effective extraction, this cyclical process lasts for several hours. Petroleum ether, methanol, and ethanol are examples of common solvents. Both polar and non-polar compounds can be effectively isolated using this method[11,15,16].

Cold Percolation

In cold percolation, a solvent is allowed to slowly percolate through a percolator filled with powdered plant material while being pulled by gravity. Drop by drop, the extract is gathered gradually. This technique is widely used in pharmaceutical preparations and is perfect for maintaining thermolabile (heat-sensitive) ingredients[13,17].

Ultrasonic Extraction (Ultrasound-Assisted Extraction)

By breaking down plant cell walls with ultrasonic waves, this contemporary extraction technique increases solvent penetration and extraction efficiency. It drastically cuts down on extraction time, uses less solvent, and works especially well for protecting sensitive phytochemicals[11,16,18].

Microwave-Assisted Extraction

By heating the plant material and the solvent with microwave energy, this technique improves the release of active compounds. Because it can efficiently extract bioactive in a short amount of time, this quick and energy-efficient method is frequently used in contemporary phytochemical research[14,16,19].

Selection of the Extraction Method

The target phytochemicals' solubility, the plant material's sensitivity to heat, and the intended use of the finished paste will all be taken into consideration when choosing the extraction method for this project. For herbal pastes, hydroalcoholic maceration or decoction is typically preferred because these techniques are easy to use and efficient in separating polar and somewhat non-polar substances [12,17].

OBJECTIVES

- To develop Herbal paste formulations with crude extracts of the root of *Curcuma longa,* leaves of *Azadirachta indica* and flower buds of *Syzygium aromaticum*
- To evaluate herbal paste formulations for Organoleptic properties, Rheological properties, pH and stability. Also, assessing the better herbal paste formulation for antibacterial effectiveness against specific pathogenic bacteria, such as Gram-positive and Gram-negative strains, which are frequently linked to skin infections and delayed wound healing.

MATERIALS AND METHODS

Figure 1: Selected Medicinal Herbs

(a) Turmeric roots (*Curcuma longa*); (b) Clove flower buds (*Syzygium aromaticum*); (c) Neem leaves (*Azadirachta indica*)

a) *Curcuma longa* [Part used- Root; Family- Zingiberaceae): The perennial herbaceous plant turmeric is a member of the ginger family. Since ancient times, its tuberous underground stems, or rhizomes, have been used as an aromatic stimulant in traditional medicine, as well as a spice and textile dye. It is now widely grown throughout the Indian Ocean region, having originated in southern India and Indonesia. The rhizomes are distinguished by their vivid orange-yellow pigment, mildly bitter, warm flavor, and peppery aroma[20].

b) *Syzygium aromaticum* [Part used- Flower buds; Family- Myrtaceae]: For centuries, people have utilized the highly prized spice clove for a variety of medicinal and food preservation applications. It originated in Indonesia and is currently grown in a number of places across the world, including the Brazilian state of Bahia. Clove is useful for the food, cosmetic, pharmaceutical, and agricultural industries because it is one of the most abundant natural sources of phenolic compounds, including gallic acid, eugenol, and eugenol acetate. Archaeological findings at the ancient port of Mantai demonstrate that cloves were already traded in Ceylon (present-day Sri Lanka) by the tenth and twelfth centuries. Because it imported cloves from Southeast Asia and traded them with India, Sri Lanka was a major player in the Indian Ocean spice trade[21,22].

c) *Azadirachta indica* [Part used- leaves; Family- Meliaceae]: A fast-growing member of the Meliaceae family of mahogany trees, neem is prized for its valuable timber, organic pesticides, and medicinal qualities. It is thought to have originated in the arid regions of South Asia and the Indian subcontinent, but it has also spread to many South and Central American nations, parts of Africa, and the Caribbean. Neem has long been used in Ayurvedic and folk medicine. It is also used in cosmetics and is essential to organic farming methods. Neem trees have round, pretty crowns and deeply furrowed bark, and they can reach heights of 15 to 30 meters (49 to 98 feet). Although they may shed during extreme droughts, their compound leaves are typically evergreen and have toothed leaflets. Clusters of tiny, fragrant white flowers, either male or bisexual, are produced by the tree at the leaf axils. A smooth, yellow-green drupe with a sweet pulp is its fruit[23].

Procurement of Plants Materials

- Rhizomes of *Curcuma longa* were purchased from local market located in the city Rohtak, Haryana. Plant material Specimen was authenticated by Dr. S.S. Yadav,

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- Flower buds of *Syzygium aromaticum* were purchased from local market located in the city Rohtak, Haryana. Plant material Specimen was authenticated by Dr. S.S. Yadav, Assistant Professor, Department of Botany, M. D. University, Rohtak, Haryana, India.
- Leaves of *Azadirachta indica* were purchased from local market located in the city Rohtak, Haryana. Plant material Specimen was authenticated by Dr. S.S. Yadav, Assistant Professor, Department of Botany, M. D. University, Rohtak, Haryana, India.

Processing of Plants Materials

- Dried leaves of *Azadirachta indica* were powdered by using grinder. Then, powdered material was macerated with solvent mixture of Ethanol: Water (80:20 v/v) for 3 days with intermittent stirring. After this, solution was filtered with filter paper and filtrate was dried with rota vapour and dried crude extract was collected weighed.
- Dried flower buds of *Syzygium aromaticum* were powdered by using grinder. Then, powdered material was macerated with solvent chloroform for 3 days with intermittent stirring. After this, solution was filtered with filter paper and filtrate was dried with rota vapour and dried crude extract was collected weighed.
- Dried rhizomes of *Curcuma longa* were kept in boiled water for a period of 30 minutes. After this, Solution was filtered with filter paper and filtrate was dried with rota vapour and dried crude extract was collected and weighed.

Phytochemical Screening

Procedure[24,25,26]

A qualitative evaluation called phytochemical screening is used to find the bioactive ingredients in plant extracts. These organic substances are essential to the therapeutic efficacy of herbal remedies. The precise steps and formats for the results used in different phytochemical tests are described below.

Carbohydrates

Molisch's Test: Gently apply concentrated sulfuric acid down the test tube's side after combining two drops of Molisch's reagent with the plant extract. The interface displays a purple or violet ring confirming the presence of carbohydrates.

Benedict's Test: Mix the extract with 2 ml of Benedict's reagent, then place the mixture in a boiling water bath for five minutes. Formation of a green, yellow, or red precipitate indicates the presence of carbohydrates.

Iodine Test: Mix the extract with a few drops of iodine solution. The presence of starch is indicated by a blue-black coloring.

Glycosides

Borntrager's Test: Bring the extract to a boil with diluted hydrochloric acid, let it cool, and then strain it. After separating the chloroform layer and shaking the filtrate with chloroform, add ammonia. The ammoniacal layer turns pink to red suggesting the presence of glycosides.

Keller-Killiani Test: Carefully apply concentrated sulfuric acid along the test tube's side after adding glacial acetic acid containing ferric chloride to the extract. Where the layers meet, a reddish-brown ring appears confirming the presence of glycosides.

Alkaloids

Dragendorff's Test: Mix the extract with Dragendorff's reagent directly. A reddish-brown precipitate's appearance is a positive indication.

Mayer's Test: Mix the extract with Mayer's reagent. The development of a creamy white precipitate is a positive indication.

Wagner's Test: Mix the plant extract with Wagner's reagent. The formation of a reddish-brown precipitate is a positive indication.

Terpenoids

The Salkowski Test: It involves mixing the extract with chloroform and then cautiously adding concentrated sulfuric acid along the test tube's side. The intersection of the two layers takes on a reddish-brown color.

Phenols

Ferric Chloride Test: Mix the extract with two to three drops of a 5% ferric chloride solution. The development of a deep blue, green, or purple hue is a positive indication.

Flavonoids

Test for Sodium Hydroxide: Mix 10% sodium hydroxide into the extract and look for any yellowing. Add diluted hydrochloric acid after that. The yellow hue goes away and turns colorless. This suggests the presence of flavonoids.

Lead Acetate Test: Combine the extract with a 10% lead acetate solution. A precipitate turns yellow.

Preparation of Herbal Paste Formulations

Herbal Paste Formulations were prepared by mixing various ingredients in step-wise manner. Dried crude extract of all three plant materials was added after preparation of paste. Crude extracts were mixed with the paste with continuous agitation until they were properly mixed.

Evaluation of Herbal Paste Formulations

a) Organoleptic Properties

Homogeneity: Visual inspection was used to evaluate the herbal paste's homogeneity and consistency.

After Feel: To assess the herbal paste's absorption characteristics, texture during application, and interaction

with the skin's surface, the process entailed applying it directly to the skin.

Ease of Removal: Clean, dry skin was covered evenly with the herbal paste, which was then left to sit for a predetermined amount of time. Either plain water or a moist cotton swab with light wiping motions were used for removal.

Color: Herbal paste Formulations were checked visually for color.

Odor: Odor of the Herbal paste Formulations were examined by sensing the smell of the formulations.

Irritancy: A tiny amount of the herbal paste was applied to the hand's lateral region and left undisturbed for a full day in order to gauge skin irritancy. At regular intervals, the area was checked for any obvious symptoms of irritation, like redness, swelling, or itching. Following the 24 h period, the area was reassessed for any delayed skin reactions and the paste was carefully removed.

b) Rheological Properties

Viscosity: A Brookfield digital viscometer (LV DV–II Ultras programmable Remoter, USA) was used to measure the viscosity of the prepared herbal paste. Spindles 1 and 2 were rotated at 25 rpm for one minute in order to perform the assessment at 30 °C.

Extrudability: It was checked by applying pressure at the crimp end of the collapsible tube after filling it with herbal paste formulations with a closed cap for a period of 10 s. Extruded herbal paste formulation was weighed.

Spreadability: It was noted by placing the herbal paste formulations between the two glass slides and keeping a weight of 100 g on the upper slide for a period of 5 min using a formula as:

$S = m \times L/T$

Where S- Spreadability

m- Mass in gram placed on the upper glass slide

L- Length of the herbal paste formulation achieved after applying a pressure for a specific time

T- Time period used for the spreading of the formulation

c) Ph: pH of Herbal paste formulations was determined using Digital pH meter.

d) Stability: Stability testing of Herbal paste formulations was performed at $45^0C \pm 2^0C$; 70± 5 % RH for a period of 4 weeks as per ICH guidelines.

Antibacterial Activity

The agar well diffusion method was used to assess the antibacterial activity of polyherbal extract on Mueller-Hinton agar. Bacterial culture of *Bacillus subtilis* and *Escherichia coli* were transferred to the agar plates using spread plate method. Total of three wells were prepared on each agar plate. Concentration of herbal extract was added to the one of the well on agar plate containing bacterial culture. One well was used for Standard antibacterial substance (Gentamycin sulphate) and other one for blank (containing solvent). Following a 24 h incubation period at 37°C, the antibacterial activity of the herbal formulation was assessed by measuring the diameter of zone of inhibition around the well containing formulation and compared with the diameter of zone of inhibition around the well containing Standard substance.

RESULTS AND DISCUSSION

All three crude extracts were obtained and weighed using Digital Electronic Balance. Percentage of yield of all extracts is mentioned in the Table 1.

Phytochemical Screening

Outcomes of phytochemical screening of all three plants extracts are mentioned in Table 2. Several important Phytochemical classes were found in *Curcuma longa*, *Azadirachta indica*, and *Syzygium aromaticum* after phytochemical screening. While phenols were found to be absent, *Curcuma longa* tested positive for flavonoids, alkaloids, terpenoids, glycosides, and carbohydrates. *Azadirachta indica* tested negative for flavonoids but positive for phenols, alkaloids, terpenoids, glycosides, and carbohydrates. Carbohydrates, glycosides, alkaloids, terpenoids, and flavonoids were all detected in *Syzygium aromaticum*, but phenols were not. Common elements found in all three extracts included terpenoids, alkaloids, glycosides, and carbohydrates. Their distinct therapeutic actions may be influenced by variations in the phenol and flavonoid content.

Preparation of Herbal Paste formulations

Two herbal paste formulations were prepared by varying composition of ingredients. Dried crude extracts were added after preparation of homogenous pastes. Extracts were mixed with continuous agitation until they were uniformly mixed. Composition of both herbal paste formulations (F1 and F2) are mentioned in the Table 3.

Table 1: Percentage of Yield of crude extracts of plants

Sr. No.	Plants Material	Solvent used	% of Yield
1.	*Azadirachta indica*	Ethanol: Water (80:20 v/v)	0.5
2.	*Syzygium aromaticum*	Chloroform	1.35
3.	*Curcuma longa*	Water	4.0

v/v: Volume by volume

Table 2: Outcomes of Phytochemical Screening

Sr. No.	Phytochemical Class	Outcomes of Phytochemical Screening		
		Extract of *Curcuma longa*	Extract of *Azadirachta indica*	Extract of *Syzygium aromaticum*
1.	Carbohydrates	+	+	+
2.	Glycosides	+	+	+
3.	Alkaloids	+	+	+
4.	Terpenoids	+	+	+
5.	Phenols	-	+	-
6.	Flavonoids	+	-	+

+: Present; -: Absent

Photographs of tests of phytochemical screening of all three extracts of plants are shown in Figure 2.

Figure 2: Phytochemical Screening of crude extracts (a) Rhizomes of *Curcuma longa*; (b) Flower buds of *Syzygium aromaticum;* (c) Leaves of *Azadirachta indica*

Table 3: Composition of Herbal Paste Formulations

Ingredients	F1 (% w/w)	F2 (% w/w)
Sodium Carboxymethyl cellulose	9.0	10.0
Curcuma longa Extract	1.0	1.0
Syzygium aromaticum Extract	1.0	1.0
Azadirachta indica Extract	0.5	0.5
Methyl Paraben	0.05	0.05
Rose water	q.s.	q.s.
Water	q.s.	q.s.

w/w: weight by weight; q.s.: quantity sufficient

Photographs of Herbal paste formulations (F1 and F2) prepared according the formula mentioned in Table 3 are shown in Figure 3.

(a) (b)

Figure 3: Herbal Paste formulations; (a) F1 Formulation; (b) F2 Formulation

Evaluation of Herbal Paste Formulations

Outcomes of organoleptic properties, Rheological properties, pH and stability testing of both Herbal paste formulations F1 and F2 are shown in Table 4.

a) Organoleptic Properties

Homogeneity: Homogeneity of both formulations of paste are found to be very good to uniform.

After Feel: The herbal pastes were absorbed quickly after application, leaving the skin feeling smooth and soft.

Ease of Removal: Using either plain water or a moist cotton swab, herbal paste formulations were easily removed from the skin's surface.

Color: The herbal pastes are having brown to dark-brownish color.

Odor: Both formulations are having a pleasant odor.

Irritancy: No erythema and oedema observed after application of paste formulations.

b) Rheological Properties

Viscosity: Viscosities of Herbal paste formulations F1 and F2 were observed 3852 cps and 3841 cps respectively.

Spreadability: Spreadability of Herbal paste formulations F1 and F2 were found 9.2 cm and 9.4 cm respectively.

Extrudability: Extrudability of Herbal paste formulations were observed very good to excellent.

c) pH: pH values of formulations are in the range of 7.4-7.5.

d) Stability: Both formulations are observed stable after testing for a period of 4 weeks at $45^0C \pm 2^0C$; 70± 5 % RH.

Formulation F2 is better than Formulation F1 in terms of Organoleptic and Rheological properties. So, it was selected for assessing antibacterial activity.

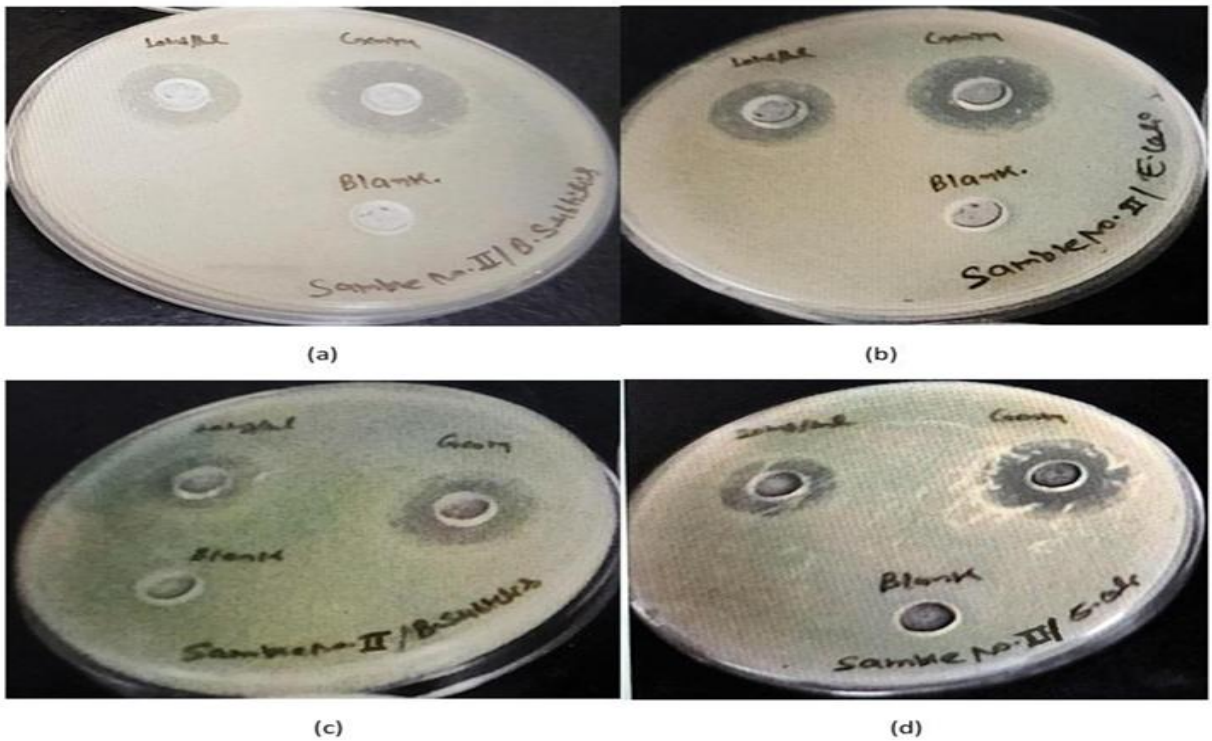
Antibacterial Activity

Antibacterial activity of herbal paste formulation (F2) was performed by agar well diffusion method. Bacterial cultures of *Bacillus subtilis* and *Escherichia coli* were used for ascertaining the antibacterial activity of Herbal paste formulation. Standard antibiotic (Gentamycin) was utilized as a positive control for comparison of zone of inhibition of herbal paste formulation with the standard. Dilution of Gentamycin of 10 µg/ml was prepared from stock (1mg/ml in water). Concentrations of Herbal paste formulation were kept 10mg/ml and 20 mg/ml in water. Water was used as a Negative control (Blank). Diameter of zone of inhibition of Herbal paste formulation and controls are shown in Figure 4.

Table 4: Outcomes of Organoleptic, Rheological Properties, Ph and stability

Properties	F1	F2
Homogeneity	Very Good	Uniform
After Feel	Very Good	Excellent
Color	Brown	Dark Brown
Odor	Pleasant	Pleasant
Irritancy	No	No
pH	7.4	7.5
Viscosity (cps)	3852	3841
Spreadability (cm)	9.2	9.4
Extrudability	Very Good	Excellent
Stability (4 weeks)	Stable	Stable

F1: Herbal Paste Formulation 1; F2: Herbal Paste Formulation 2



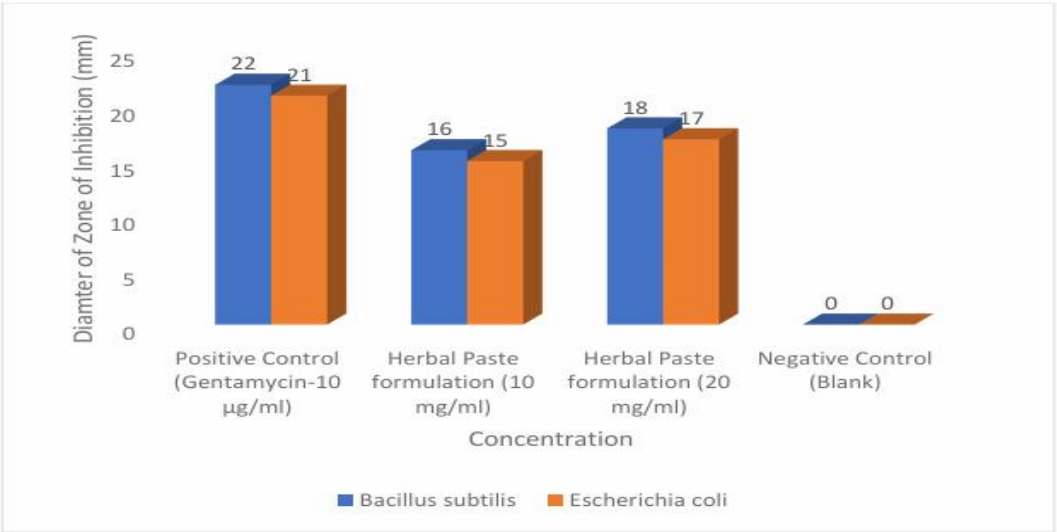


Figure 4: Diameter of Zone of Inhibition of Herbal Paste Formulation and controls

It has been observed from the above Figure 4 that approximately 40 mg/ml of Herbal paste formulation will be equivalent to the Positive control (Gentamycin-10 µg/ml) concentration. Herbal paste formulation shows very good antibacterial activity against G (+) *Bacillus subtilis* and G (-) *Escherichia coli* bacterial cultures.

Using extracts of root of *Curcuma longa*, leaves of *Azadirachta indica* and fruits of *Syzygium aromaticum*, the herbal paste demonstrated beneficial properties for topical application. The therapeutic potential of the formulation was highlighted by phytochemical screening, which confirmed the presence of classes of phytochemicals with antimicrobial, anti-inflammatory, and wound-healing properties. On organoleptic evaluation, the Herbal Paste Formulation (F2) is uniform with semi-solid consistency having dark brown color and pleasant odor, all contribute to its high user acceptability. Moderate viscosity, good spreadability, and ease of extrusion were confirmed by rheological evaluation. Formulation is found stable on stability testing for a period of 4 weeks. Antibacterial activity of the herbal paste formulation confirms that the formulation can be used for topical application for preventive and healing effects in various skin conditions.

CONCLUSION

Herbal paste formulations were developed and evaluated for organoleptic properties, Rheological properties, pH and Stability. Better formulation was assessed for antibacterial potential against *Bacillus subtilis* and *Escherichia coli* bacterial strains. Herbal paste formulation (F2) exhibits antibacterial properties, offering a promising natural alternative for topical application. It is a convenient, affordable, and safe substitute for synthetic topical treatments.

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ETHICAL STATEMENT

This study did not involve any animal experimentation in compliance with ethical research practices.

ABBREVIATION

AMR: Antimicrobial resistance

MDR: Multi-drug-resistant

WHO: World Health Organization

ICH: International Conference on Harmonization

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