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Formulation and Evaluation of Herbal Nail Polish with Antibacterial Activity

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ABSTRACT

The current study was to formulate & evaluate Herbal Nail Polish with antibacterial property. Natural nail polish refers to nail enamel that is free of many of the chemicals commonly found in regular nail polish. Natural nail polish is free of formaldehyde, toluene, artificial colors; xylene and phthalates, all of which are common ingredients in regular nail polish. Plant based active ingredient is the main component of the nail polish which makes it most safest than the synthetic ones. Clove is an ancient spice, which is believed to be originated in the first century, before Christ. The first clue about clove's fragrance was given by the ancient Chinese (207B.C. to 220 A.D.). Clove (Syzygium aromaticum) is the largest rubric of Myrtaceae family. Cloves contain perceptible quantities of unpredictable oil painting (used for flavouring foods and medicinals), which is substantially confined in upstanding corridor of shops. Clove shows antibacterial property. The formulation was tested for antibacterial activity against test organism staphylococcus aureus (S. aures) and Klebsiella pneumoniae (K. pneumoniae) using disk diffusion method. The minimum inhibition concentration determines the lowest concentration of an antibacterial agent that prevents the visible growth of microorganism. Gram-negative bacteria (K. pneumoniae) and Gram- positive bacteria (S. aures) were used throughout the study. Both Gram - positive and Gram - negative strains were cultured using nutrient broth. The result shows zone of inhibition was 15 mm against s.aureus and 15 mm against E.coli and herbal formulation shows 13 mm against s.aureus and 13 mm against E.coli. The study reveals that the developed nail polish with herbal active ingredient was comparatively better than other formulations because of its antimicrobial, antiviral, anaesthetic, analgesic and antioxidant activity when applied on skin.

Keywords: Antibacterial, nail polish, clove, E-coli.

INTRODUCTION:

The nail is the most prominent skin accessory. It continues to expand throughout one's life. The size and shape of the nails vary from Cutlet to Cutlet and toe to toe. Both epithelium and connective kerchief factors make up the nail outfit. The matrix epithelium is in charge of producing the nail plate. Establishment attachment is interceded by the nail bed epithelium. The nail serves as defensive ordnance and increases the perceptivity of the tip of the croquette. The distal interphalangeal joint's nail outfit, fingertip, tendons, and ligaments produce a performing unit that cannot be viewed singly. Cutlet and toe nails are formed of a thick protective caste known as α- keratin [1,2]. A nail is a cornucopia- suchlike envelope covering the interior aspect of the terminal phalanges of galettes and toes in humans, utmost on- mortal primates, and a numerous other mammals. Nails are similar to claws, which are set up on numerous other brutes. nails and toenails are made of a tough protein called keratin, as are brutes' hooves and cornucopias. The mammalian nail, claw, and hoof are all samples of unguis(plural ungues)[3].

Nails is the protective structures used for centuries as survival tools, have over the times come an accessory for beautification. The mortal nails not only protective and cosmetic part, but also considered as necessary pathway for the drug delivery, especially in nail conditions like psoriasis or Onychomycosis[4]. These nail conditions are considerably spread in the each over the population. Although the architecture and composition of the nail plate severely limits penetration of drugs and in addition to that only a bit of topical drug penetrates across the nail, oral antidotes are accompanied by the systemic side goods and the drug relations. For the perfect treatment of the nail complaint the applied active drug must weep through the thick keratinized nail plate and reach in the deeper layers, the nail bed and the nail matrix[5].

The shy disquisition and knowledge regarding the parcels of keratinized nail plate, the nail bed and the nail matrix caused a lower focus on the ungula system. The wanton structure of the nail plate is responsible for the penetration of the drug across it. It's hard enough the penetration becomes delicate, only a bit of topical drug penetrates across it. Hence the effective remedial attention is n't

achieved. The nail plate may appear abnormal as a result of dropped radiance. It's due to the involvement of nail bed, reduction of blood force, physical or chemical features of nail bed. As results variety of conditions occurs. These conditions can be cured by the achieving asked remedial attention of the drug by nail drug delivery system [6]. The challenges of the drug delivery to the nail, with the lack of the understanding of both the barricade parcels of the nail and phrasings to achieve the enhanced ungula delivery confining the effectiveness of topical treatments for nail conditions. And also suffer from low case compliance due to the long treatment periods up to 4 - 8 months which are demanded. Still, are being oral phrasings generally not only contain large pilules of active ingredients but also bear long treatment and creating the eventuality for systemic poison especially in the liver. Thus, developing further effective styles for nail drug delivery is an important ideal for the pharmaceutical sedulity [7].

Structure of the human nail:

The human nail consists following parts;

1) Nail matrix or the root of the nail.

2) Nail plate.

3) Nail bed.

4) Hyponychium.

5) Eponychium or cuticle.

6) Paronychium.

7) Lunula.

1. **Nail matrix or the root of the nail**: The nail matrix is the area where your cutlet nails and toenails start to grow. The matrix creates new skin cells, which pushes out the old, dead skin cells to make your nails, as the result, injuries to the nail bed or diseases that affect the matrix can affect your nail growth. The root of the fingernail is also known as germinal matrix. The edge of germinal matrix is seen as the white, crescent shaped (a thin, twisted shape like half- moon) structure called the lunula. This portion of the nail is behind the fingernail and extends several millimeters into the cutlet. The root of fingernail produces the utmost of the volume of the nail and nail bed. This portion of the nail does not have any melanocytes, or melanin producing cells.

2. **Nail plate**: The part that we call the nail is technically known as the “nail plate”. The nail plate is substantially made of a hard substance called as the keratin. It's about partial millimeter thick and slightly twisted. The nail is attached to the nail bed. The nail bed is skin with a subcaste of the epidermis and a subcaste of the dermis. The epidermis of the nail bed is attached to the nail plate via grooves called as matrix ridges.

3. **Nail bed**: the nail bed is part of the nail matrix called as the sterile matrix. It's the pinkish coloured soft towel underneath nail plate. It extends from the edge of the germinal matrix (lunula) to the hyponychium. It's a thin, soft,non-cornfield epithelium, connected with the frontal subcaste of the nail plate and underpinning papillary dermis and contains the blood vessels, jitters, and melanocytes, or melanin- producing cells. As the nail is produced by the root, it streams down on with the nail bed, which adds material to the under face of the nail making it thicker.

4. **Hyponychium**: The hyponychium is the skin just under the free edges of your nail. It's located just beyond the distal end of nail bed and near the fingertips, but occasionally the hyponychium can over grow and come thicker. As the hedge from origins and debris, the hyponychium stops external substances from getting under nail.[8]

5. **Eponychium or cuticle**: The eponychium is the thickened subcaste of the skin at the base of the fingernails and toenails.it can also be called as the medium or proximal nail fold. The eponychium differs from the cuticle, the eponychium comprises live skin cells whereas the cuticle is dead skin cells.

6. **Paronychium**: The perionychium is the skin that overlies the nail plate on its sides. It's also called as the paronychial edge. The perionychium is the point of hangnails, ingrown nails, and an infection of the skin called paronychia. Paronychia is an infection of the towel conterminous to a nail, most frequently a fingernail.

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7. **Lunula**: The lunula is the visible portion of the distal nail matrix that extends beyond the proximal nailfold.it is white, partial-moon shaped (wind like structure), appears by week 14 of gravidity, and has unique histological features. The lunula has a primary structural part in defining the free edges of the distal nail plate[9,10].

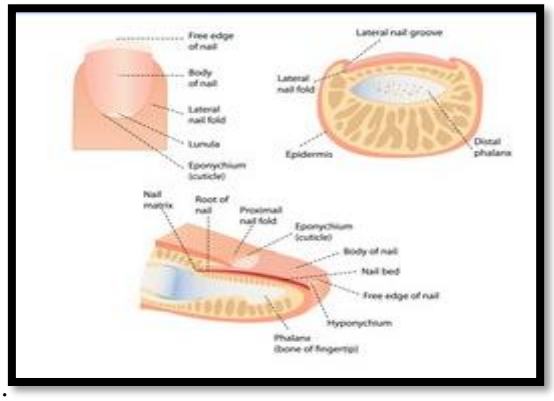

Fig 1: Structure of human nail

Functions of Nail

- Protect the soft tissues of the fingers and toes from injury.
- Enhance sensation.
- Used for cutting and scraping.
- Support the tips of fingers and toes.
- Precise movement of fingertips. [11,12]
- Fingernails are used for scratching and grooming and are an efficient natural weapon.
- Without nails on the fingertips, it is impossible to clutch and hold the things accurately or correctly.
- The nail acts as a counterforce when the end of the finger is come into contact with object and then it enhancing the sensitivity of the fingertip, even though there are no nerve endings in the nail itself.
- The nails also increase to the aesthetic appearance of the hand and foot [13,14].

Nail Polish

Nail polish is one of the primary forms of nail beautification. Also known as nail varnish, enamel, or lacquer, nail polish has three main types. The first is the basecoat, which serves the purpose of creating a smooth and uniform layer upon which the pigmented nail polish may be applied. The second is the pigmented nail polish, itself. The third is the aftercoat, which is applied on top of the pigmented nail polish to provide fortification against chipping as well as an added sheen. Nail polish often contains the following components: a film former, a plasticizer, a thermoplastic resin, a solvent-extender, pigment, and possibly a suspending agent. The film former is often composed of nitrocellulose. The plasticizer, often dibutyl phthalate, serves to enhance adhesion and provides flexibility. The thermoplastic resin, often toluene sulfonamide-formaldehyde, improves adhesion, hardening, and gloss. The solvent extenders allow the components of the nail varnish to remain in a liquid form, and compounds used are usually ethyl acetate, isopropyl alcohol, butyl acetate, or toluene. Finally, the pigment component may be highly variable and may include elements such as iron oxides, color lakes, and mica. The pigment may be organic or inorganic, although inorganic compounds must have low heavy-metal content [15].

Downsides of synthetic nail polish

- Thins natural nails and makes them weaker.
- Chemicals irritate the skin around the nails; they're absorbed into the skin and blood sluice.
- Synthetic colors used, causing neurotoxic and carcinogenic effect.
- Readily dries on skin and makes it dehydrated.
- Frequently causes antipathetic responses.
- Chemicals like toluene, formaldehyde, dibutyl phthalate are toxico the terrain.
- poisonous effect on body

✓ Toluene – inconvenience to skin and causes frazzle, confusion, memory loss.

✓ Formaldehyde – a known carcinogen.

✓ Camphor – cause yellowing of nail by stripping off its nutrients [16].

Benefits of herbal nail polish

Thicker, stronger, more durable nails

- Brittle free nails.
- Chip- resistant nails.
- Moisturized, doused nails
- Break resistant nails[17,18].

Plant Profile

Biological source

Clove(Syzygium aromaticum), a precious spice, is a member of the Myrtaceae family which has been employed for centuries as a food preservative and medicine because of its antimicrobial and antioxidant parcels. Syzygium is the largest rubric of Myrtaceae family, comprising about 1200 to 1800 species of unfolding shops, which are considerably distributed in tropical and tropical areas of Asia, Africa, Madagascar, and throughout Pacific and Oceanic regions. Cloves contain distinguishable amounts of changeable oil painting oil(used for flavouring foods and medicinals), which is mainly confined in upstanding corridor of shops. Clove is known by different conversational names in different languages. It's known as qaranful(Arabic), Karamfil(Bulgarian), Ding xiang(Chinese), Kruidnagel(Danish), Garifalo(Greek), Mikhaki(Georgian), NelkeN(German), Szegfu(Hungarian), Cengkeh(Indonesian), Choji(Japanese), Jeong Hyang(Korean), Krustnaglinas(Latvian), Lwang(Nepalese), Cravo da India(Portuguese), Mikhak(Persian), Kala(Pashto), Gvozdika(Russian), Clavo(Spanish)[19,20,21].

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Fig 2: Clove (Syzygium aromaticum)

Taxonomical Classification:

Kingdom	Plantae
Division	Tracheophyta
Class	Magnolipsida
Order	Myrtales
Family	Myrtaceae
Genus	Syzygium
Species	Aromaticum

History:

Clove is an ancient spice, which is believed to be originated in the first century, before Christ. The first clue about clove's fragrance was given by the ancient Chinese (207B.C. to 220 A.D.). At that time, a Chinese Physician wrote that court visitors were required to hold a clove in their mouth to prevent the emperor from visitor's bad breath. Cloves were traded to Europe by the Arabs in the 4th century A.D. The origin and source of clove was a mystery, until the discovery of Indonesia or Moluccas Island, by the Portuguese, in the 16th century [22].

In the 17th century A.D Cloves were introduced to Sri Lanka. In the 18th century Cloves were established in India by the East India Company. In European countries, there is a tradition to make "Pomanders" by studding oranges with clove buds, and to hang them around the homes, during Christmas, for decorative purposes and to spread fragrance.

Clove cultivation was almost entirely confined to Indonesia, and in the early 17th century the Dutch eradicated cloves on all islands except Amboina and Ternate in order to create scarcity and sustain high prices. In the latter half of the 18th century the French smuggled cloves from the East Indies to Indian Ocean islands and the New World, breaking the Dutch monopoly. In the early 21st century, Indonesia was the world's largest producer of cloves, followed by Madagascar, Tanzania[23].

Fig 3: Clove Flower

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Fig 4: Clove Bud

RESEARCH METHODOLOGY:

Preliminary Phytochemical analysis

This clove oil (marketed) was tested in order to find out the presence of active compounds by use of the following standard methods.

1) Test for Alkaloids:

a) Dragendorff's test: 2ml of Dragendorff's reagent was added to 1 ml of plant extract. Formation of orange brown indicated the presence of alkaloids.

b) Wagner's test: 1 ml extract was treated with Wagner's reagent; formation of brown reddish precipitate indicates Presence of alkaloids.

c) Mayer's Test: 1 ml of clove flower bud extract, 2 drops of Chloroform, and 2 drops of Mayer's reagent were added. A Positive alkaloid reaction resulted in the production of White deposits.

2) Test for Tannins:

a) Lead acetate test: To the filtrate, 5ml of 10% lead acetate solution was added. Formation of white precipitation indicates the presence of tannin.

b) Ferric chloride test: Five drops of a 5% ferric chloride solution were added to the filtrate. Formation of blue green color indicated the presence of tannin.

c) Gelatin test: To 500 µl of the filtrate, 1% gelatin solution was added. Formation of curdy white precipitate indicated the presence of tannin.

3) Test for Carbohydrates:

a) Molisch's test: 1 ml of plant extract was added to 0.4 ml of Molisch's reagent. Afterwards, 1 ml of conc. Sulphuric acid was added along the side of the test tube. A purple color indicates the presence of carbohydrates (starch).

b) Fehling's Test: Boiling and filtering 1 ml of plant extract with 2 ml of purified water. Then, 2 ml of Fehling's reagent were added to 2 ml of filtrate, which was then heated. Reddish brown precipitate indicates the presence of carbohydrate (glucose).

c) Benedict's test: 1 ml of plant extract and 1 ml of Benedict's reagent were heated for 5 minutes. The presence of carbohydrates (disaccharides) was shown by the formation of an orange precipitate.

4) Tests for flavonoids

Alkaline reagent test

Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. The yellow solution that turns colourless indicates the presence of flavonoids.

5) Test for Steroids

Salkowski Reaction: To 2 ml of extracts was added 2 ml of chloroform and 2ml of concentrated H2SO4 shake well. Chloroform layer shows greenish yellow fluorescence.

6) Test for Glycosides

Keller- Killani Test: 1 ml of glacial acetic acid containing traces of FeCl3 and 1ml of concentrated H2SO4 were added to the extracts carefully. Reddish- brown color is formed at the junction of two layer and the upper layer turns bluish green in presence of glycosides [24].

7) Test for Saponin

About 0.2 g of the extract shaken with 5ml of distilled water and then heated up to the boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins by forming 1cm layer of foam.

8) Test for lipids:

a) Solubility test: 1 ml of plant extract was evaporated to dried powder. Few drops of petroleum ether were poured into the test tube and shaken well. Complete dissolution of Extract identified the presence of lipids.

b) Glycerol test: To 1 ml of 1% CuSO4.5H2O solution, 5 drops of the plant extract were added and mixed thoroughly. Then it received 5 drops of a 10% sodium hydroxide solution. A clear blue solution was obtained which indicates the presence of glycerol.

c) Sudan III test: To 1ml of plant extract, few drops of Sudan III solution were added. Appearance of red color indicated the presence of lipids.

9) Test for Resins:

In a dry test tube, to 0.5 ml of acetic acid and 2 drops of conc. Sulphuric acid were added. It was clear that resins were Present because a purple color that turned violet in about 10 Minutes.

10) Test for Triterpenes:

Few milligrams of plant extract residue were mixed with 5 ml of chloroform and warmed for 30 min at 40°C. Few drops of Conc. Sulphuric acid were added and mixed well. The Appearance of red color indicated the presence of Triterpenes.

11) Test for Anthraquinones:

2ml of each plant extract was shaken with 10ml benzene, and 5ml of 10% ammonia solution was added. The mixture was shaken in order to obtain the color of antraquinones. The Ammonical layer acquiring pink colour indicated the presence Of Anthraquinones.[25]

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Formulation Development:

Table 1: List of chemicals used and their property

Sr.no	Name of the ingredients	Property
1.	Clove oil	Antimicrobial
2.	Poly vinyl pyrrolidine	Film forming agent
3.	Acacia	Thickening agent
4.	Castor oil	Plasticizer
5.	Ethanol	Solvent
6.	Beetroot powder	Colouring agent

Formulation of nail polish:

Table 2: Formulation of nail polish

Sr. No	Ingredients	Quantity for 10 ml
1.	Clove oil	3ml
2.	Poly vinyl pyrrolidine	5gm
3.	Acacia	3gm
4.	Castor oil	1ml
5.	Ethanol	10ml
6.	Beetroot Powder	0.5gm

Procedure:

1. Accurately weigh the poly vinyl pyrrolidone (film forming agent) and transfer it into a mortar.
2. Add the ethanol (solvent) and triturate.
3. Add the acacia (thickening agent) into it to form a smooth paste.
4. Followed by the addition of castor oil (plasticizer) and clove oil (antimicrobial agent).
5. Then add beetroot powder as colorant.
6. Triturate until proper consistency is obtained.
7. Close the jar tightly and let the mixture sit for at least 24 hours to allow the herbs to infuse into the nail polish base.
8. Shake the jar gently once or twice to help distribute the ingredient.
9. Strain the nail polish mixture using a cheese cloth to remove any solid particle.
10. Pour it, into the nail polish bottle using the funnel.
11. Label the nail polish with the ingredients and date of manufacturing [26].

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Fig 5: Final Product

Evaluation parameters:

- **Physical evaluation**

The physical appearance of the formulation was checked visually.

- **Colour**

The colour of the formulation was checked out against white background.

- **Odour**

The odour of the nail polish was checked manually.

- **Consistency**

The consistency was checked by applying on nail.

- **pH**

pH of prepared herbal nail polish was measured by using digital pH meter.

- **Grittiness**

The product was checked for the presence of any gritty particle by applying it on the nail.

- **Drying time**

Apply the nail paint on nail. Explore the nail paint to air and determine the drying time of the nail paint. Optimum drying time obtained.

- **Smoothness**

This is the character of film. The film is applied to a surface of the nail paint then after the drying rub the nail surface and check the smoothness of nail polish.

➢ **Hardness**

Nail polish apply on surface of nail and then check the hardness of the nail paint by applying the pressure by hand and determine the hardness of nail paint.

➢ **Water resistance**

A continuous film is spread on the surface of the glass plate and dried and weighed. Then the plate is immersed in distilled water for 24 hours. The panel should then to be removed and dried and reweighed. The greater the increase in weight, the lesser the water resistance.

Antibacterial Activity Evaluation:

The formulation was tested for antibacterial activity against test organism staphylococcus aureus (S. aures) and Klebsiella pneumoniae (K. pneumoniae) using disk diffusion method. The minimum inhibition concentration determines the lowest concentration of an antibacterial agent that prevents the visible growth of microorganism.

➢ **Microbial organism:** Gram- negative bacteria (K. pneumoniae) and Gram- positive bacteria (S. aures) were used throughout the study. Both Gram - positive and Gram - negative strains were cultured using nutrient broth.

➢ **Medium:** Muller – Hinton agar plate, standard: Sparfloxacin

Procedure: Disk diffusion method

1. The bacteria staphylococcus aureus and K. pneumoniae was inoculated on the surface of Muller-Hinton agar media plate.
2. In each culture plate two discs were placed at 60° to each other.
3. The plates were shifted to incubator and incubated at 37°C for 24 hours.
4. After the incubation period the clear zone is measured in mm using a scale. The clear zone represented the zone of inhibition.

RESULTS AND DISCUSSION:

Phytochemical screening:

Table depicts results of screening of different solvent extracts of plant for various phytochemical constituents. It shows presence of alkaloids, tannins, flavonoids, carbohydrates, steroids etc.

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Table 3: Result of Phytochemical Screening

Chemical Constituents	Test	Result
ALKALOIDS	Dragendorff's test	+
	Mayer test	+
	Wagner's test	+
TANNINS	Lead acetate test	+
	Ferric chloride test	_
	Gelatine test	_
CARBOHYDRATES	Molish's test	+
	Fehling test	+
	Benedict test	+
FLAVONOIDS	Alkaline reagent test	+
STERIODS	Salkowski test	+
GLYCOSIDES	Keller – Killani test	+
SAPONIN	Froth formation test	+
ANTHRAQUINONE	Hydroxyanthraquinone test	_
LIPID	Solubility test	_
	Glycerol test	_
	Sudan test	_

Note: 1. Positive (+) sign indicates the test is passed.

2. Negative (-) sign indicates the test is failed.

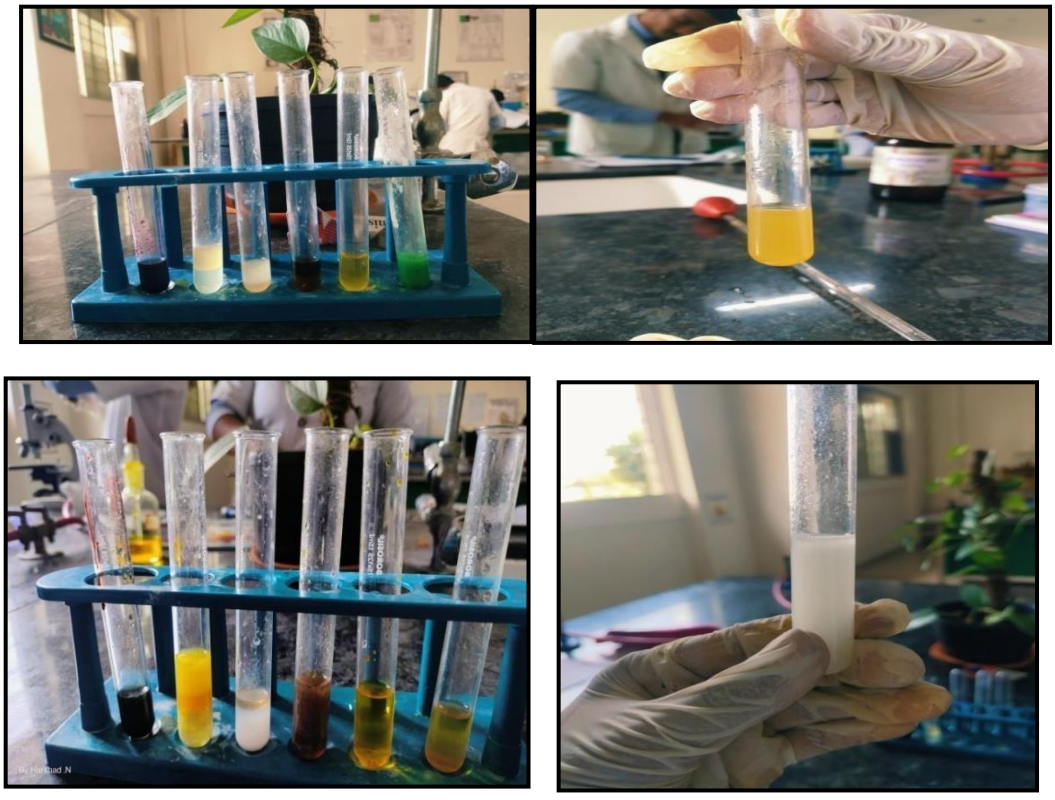

Fig 6: Phytochemical Screening

Physical evaluation:

Table 4: Physical Evaluation of product

Parameter	Observation
Colour	Reddish pink
Odour	Milder
Consistency	Viscous
pH	5.2
Grittiness	Nil
Smoothness of film	Free from foreign particles
Hardness	Sufficient hard

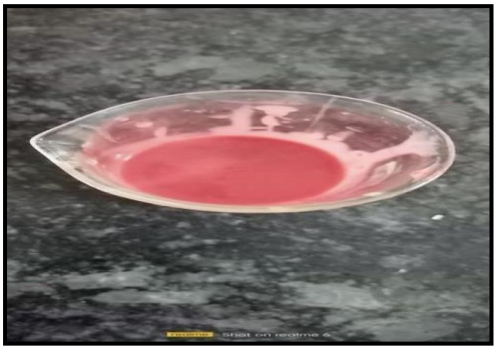

Fig 7: Color

Fig 8: Water Resistance

Fig 9: Drying time

Antimicrobial evaluation:

Table 5: Average zone of inhibition of formulation against test bacteria

Samples	*S. aureus*	*K. pneumonia*
Sparfloxacin	15 mm	15mm
Formulation	13 mm	13 mm

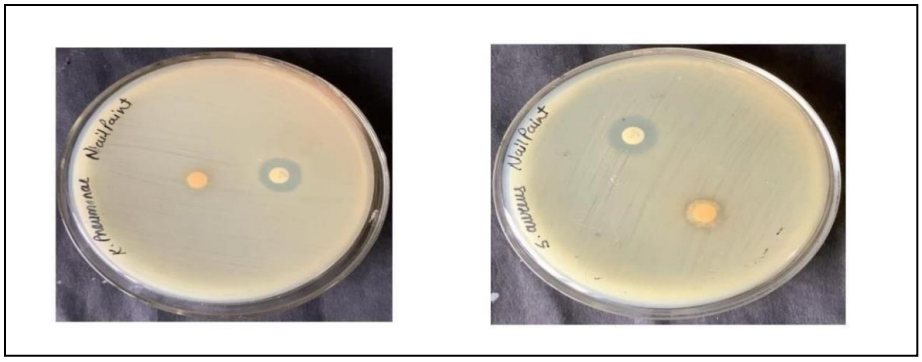

Fig 10: Antibacterial property of formulation against *S. aureus* and *K. pneumonia*

Zone of inhibition and interpretation:

Table 6: Zone of inhibition and interpretation

Sr.no	Zone of inhibition	Interpretation
1	<10	Inactive
2	10-13	Partially active
3	14-19	Active
4	>19	Very active

CONCLUSION:

The study reveals that the developed nail polish with herbal active ingredient was comparatively better than other formulations because of its antimicrobial, antiviral, anaesthetic, analgesic and antioxidant activity when applied on skin. Our study was mainly focussed on the antibacterial activity using bacteria (S. aureus and E. coli) in which the formulation shows highest activity against. Based on physical evaluation of formulated nail polish, showed good appearance, favourable pH, consistency, drying time and smoothness. Due to its antibacterial activity clove can be considered as a significant natural source of antibacterial. Clove, being a natural product, can offer more safety to people and environment, and is considered to be less of risk for resistance development by pathogenic microorganism. Formulation of nail polish with herbal active ingredient makes it more effective over synthetic products. Even-though formulation needs further studies for effective benefits.

The nail polish can cause negative health effects. The several ingredients use in nail polish has been connected to cancer, heart problems, reproductive abnormalities, thyroid disorders and allergies. They can cause an even higher chance to connect to cancer in many other diseases. Nitrocellulose is a film-forming polymer that is the main ingredient in most nail polish. Nail polish consists of a film-forming polymer dissolved in a volatile organic solvent; nail polish has a vast environment result on our environment. The U.S EPA considers nail polish to be house held dangers waste because of the toxic chemicals flock within that bottle of glint and shine.

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