

Project Report

FORMULATION AND EVALUATION OF ANTIBACTERIAL HERBAL EMULSIFYING GEL

Submitted to

ATMIYA UNIVERSITY



by

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CERTIFICATE

This is to certify that MS.ANUJA BARASARA Enrollment No. 200501002 has successfully completed project on “FORMULATION AND EVALUATION OF ANTIBACTERIAL EMULSIFYING HERBAL GEL” as part of curriculum of B.pharm Semester - VIII in the subject Project Work during the academic year 2023 -24.

BS
12/04/2024

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BS
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Declaration

hereby declare the Work is presented in the project report entitled **FORMULATION AND EVALUATION OG ANTIBACTERIAL HERAL EMULSIFYING GEL**

It is an authentic record of work carried out by us during the studying period of semester 8 at and under the guidance of Atmiya University, Rajkot, and is being submitted for partial fulfillment of the requirement for the award of a bachelor's degree in B.pharm. This is not submitted anywhere else for the award of any other degree/diploma.

ANUJA BARASARA

Title : **FORMULATION AND EVALUATION OG
ANTIBACTERIAL HERAL EMULSIFYING GEL**

Enrollment no.: 200501002

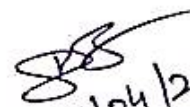
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Abstract:

- **Background:** *Tridax Procumbens* Linn ,Clove oil and Neem seed are well known for its traditional use as Ayurveda in folk medicine all over the world. These plant have many pharmacological activities like hepatoprotective activity, anti-inflammatory activity, wound healing, anti-diabetic activity, hypotensive effect, immunomodulating property, promote the growth of hair, prevent falling of hair and anti-microbial activity.
- **Aim:** Development and evaluation of antibacterial herbal Emulsifying gel.
- **Methodology:** After authentication of the plant, extract was prepared from the leaves of *Tridax procumbens* and essential oil like neem seed oil and clove oil. And dosage form is prepared and were tested against some standard strain of Bacteria like *Streptococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *P. Vulgaris*.

- bacteria like *Streptococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *P. Vulgaris*
Result: The plant extract of *Tridax procumbens*, clove oil and Neem seed oil responds against
- **Conclusion:** Prepared formulation have good antibacterial activity. It may be also prevent bacterial infection.

Rational:

Nowadays, most of drug are resist to bacteria toward antibiotics. So the alternative treatment are investigated and adopted. So we try to formulate a new formulation.

As far as dosage form is a matter of concern, gel become a good option. Gel has several advantages over the other dosage forms. Gel is semisolid system consisting of dispersions of small or large molecules in an aqueous liquid vehicle rendered jelly like by the addition of gelling agent. Advantage of gel dosage form over other dosage forms are less irritant, softening the skin and easy to removal.

Objective :

- To formulate a herbal gel using Tridax procumbens extract, clove oil and Neem seed oil.
- To evaluate physicochemical parameters of the prepared herbal formulation.
- To evaluate antibacterial activity of the prepared herbal formulation.

Introduction:

Traditional medicinal knowledge and its use for finding active chemical structures for medicine is necessary to have co-operative efforts between modern and traditional health workers and researchers. In developing countries, traditional medicine occupies a central place among rural communities but enough information is not available about the chemical composition and real biological possibilities of most of the plants traditionally in use.¹⁻²

For countries plants have been used for both nutritional and medicinal purposes. In conventional medicine is not cheap and a large population of the people depends on traditional medicine for their healthcare needs. Over the years, these herbal drugs have been shown to be effective. Many plants and their parts are used for the treatment of various diseases in different parts of the world and are being screened for antimicrobial activities and the result obtained from these scientific studies have aided in the rationalization of medical use of these parts.²⁻³⁻⁴

Infection of the skin and soft tissues are frequent ailments that cause significant morbidity. Microbes like bacteria and fungi frequently work together to cause this infection. Using topical antimicrobials is a desirable method of treating superficial skin infections. Topical therapy has minimal to no systemic side effect while delivering a high concentration of a medicine to the targeted location.⁵⁻⁶

Resistance in micro-organisms to many antimicrobials has resulted in morbidity and mortality from treatment failure and increased health care costs and increasing capability of microbes to develop multidrug resistance has encourage search for new, safe and effective bioactive agents of herbal origin. It has been reported that *Tridax procumbens* medicinal plants have been used in the treatment against different diseases.⁷

1.LITARATURE REVIEW OF PLANT :

1.1TRIDAX PROCUMBENS

India's rice fields are home to the common weed *Tridax procumbens*. It is a plant that is generally referred to as "coat button" and is present all over India. Historically, *Tridax procumbens* leaf juice has been applied topically to treat wounds. The plant's leaf juice, which has antimicrobial properties, can be used to treat fresh wounds, stop bleeding also as a hair tonic.⁸

Distribution:

- The plant is native of tropical America and naturalized in tropical Asia, Africa, Australia and India.⁹
- It *Tridax procumbens* is an annual or perennial herbaceous weed found in tropical and subtropical areas of the world, growing mainly during the rainy season at meadows, croplands, disturbed areas, lawns, roadside or settled areas. This medicinal herb shows a typical feature of a beneficial weed.¹⁰⁻¹¹⁻¹²



[Fig. 1 *Tridax procumbens*]

1.1.1 Morphological structure:

Leaves:

leaves are opposite, simple, carried by a petiole, 1 to 2 cm long. They are thick, soft and dark green. The lamina is oval to lanceolate, 2 to 6 cm long and 2 to 4 cm wide, base attenuate in the corner and with strongly and irregularly serrated margin. Both sides are hispid, with tuberculate based bristles. Wedge shaped base leaf, shortly petioled, hairy on both surfaces.



[Fig. 2 *Tridax procumbens* leaves]



Stem & root:

Stem is cylindrical, hispid, branched, sparsely, hairy, rooting at nodes. The plant stem is ascending 30-50 cm height and covered with multi cellular hairs of 1 mm; tuberculation at the base. The root is a strong taproot system.



[Fig. 3 root of *Tridax procumbens*]



[Fig. 4 stem of *Tridax procumbens*]

Flowers:

The plant blossoms resemble daisies. The tubular flower has 3 toothed ray florets and white or yellow blossoms with a yellow center. It has two different flowers types: disc florets with basal placentation and ray florets. The flowers can occasionally have 3 lobes and lengthy, pendulous crowns. Black, 2.0-2.5 mm long, narrowly obconical achenes with feathery pappus. All year long this is flowering.



[Fig. 5 flower of *Tridax procumbens*]

Fruit:

Fruit is a conical hard achene covered with stiff hairs and having a feathery. Fruit is 3.5 mm high, pubescent and brown to black at maturity. At one end it has plume like white pappus.

Seed:

The plant seeds have pendulous embryo, endosperm is absent.¹³⁻¹⁴



[Fig. 6 seed of *Tridax procumbens*]

1.1.2 Taxonomic classification:¹⁵

- Kingdom: Plantae
- Sub-kingdom: Tracheobionta
- Division: Spermatophyta
- Sub-division: Magnoliophyta
- Class: Magnoliopsida
- Sub-class: Asteridae
- Order: Asterales
- Family: Asteraceae
- Genus: *Tridax*
- Species: *procumbens*

1.1.3 Vernacular names: ¹⁶⁻¹⁷⁻¹⁸

Region / Country	Common name
English	Coat button, <i>Tridax</i> daisy
Gujrati	Ghaburi
Hindi	Ghamra
Sanskrit	Jayanti Veda
Latin	<i>Tridax procumbens</i> Linn.
Tamil	Thata poodu
French	Herbecaille
Chinese	Kotobukigiku
Bengali	Tridhana

[Table 1 vernacular name of *Tridax procumbens*]

1.1.4 Chemical constituent:

- Flavonoids, alkaloids, carotenoids, hydroxycinnamates, lignans, benzoic acid derivatives, phytosterols, tannins, terpenoids, crude protein, crude fiber, soluble carbohydrates and calcium oxide are said to be present in the leaf and other parts of *T. procumbens* L. There have also been reports of fumaric acid, beta sitosterol, and the pentacyclic triterpenoid oleanolic acid.¹⁹
- In floral extracts, luteolin, glucoluteolin, quercetin and isoquercetin have all been reported. 2-6 dihydroxyacetophenone, 2-O-D-glucopyranoside, echioidinin,

pinostrobin, dihydroechioidinin, tectochrysin- 5-glucoside, methyl salicylate glucoside, and pinostrobin are a few of the other phytochemicals that are abundant in *T. procumbens*. Skullcapflavone-2-methylether, 5,7,8-trimethoxyflavone, androechin, 5,7,2-trimethoxyflavone, echioidin, 5,7-dimethoxyflavone and andrographidine.²⁰⁻²¹

Flavonoids:

- Twenty-three flavonoids have been found to be present in *T. procumbens*, with a total content of about 65 g/kg, according to a recent study. Around 17.59% and 26.3%, respectively, are made up of kaempferol, catechin, and its derivatives (-)-epicatechin, (+)-catechin, (-)-eigallocatechin, (+)-gallocatechin, (-)-epigallocatechin-3-gallate (EGCE) and (-)-epigallocatechin-3-gallate. Biochanin, apigenin, naringenin, daidzein, quercetin, butein, robinetin, baicalein, nobiletin, genistin, ellagic acid, luteolin, myricetin, baicalin, isorhamnetin and silymarin make up the remaining 56.1% of the flavonoids.²²
- The flavonoids found in *T. procumbens* are well known for mediating pharmacological properties such as free radical scavenging, anti-inflammatory, anti-allergic, anti-platelet aggregation, antimicrobial, anti-ulcer, antiviral, anticancer and antihepatotoxicity.²³
- The entire plant was used to identify two new flavones, 8,3-dihydroxy-3,7,4-trimethoxy-6-O-Dglucopyranosylflavone and 6,8,3-trihydroxy-3,7,4-trimethoxyflavone as well as four previously identified compounds having antioxidant activity, puerarin, esculetin, oleanolic acid, and betulinic acid.²⁴

Tannin:

- Tannin are water soluble polyphenols that are present in plants naturally. Tannin possess antimicrobial, anticarcinogenic and antimutagenic qualities, which may be related to their antioxidant capacities.²⁵
- Several research have described the presence of tannin in *T. procumbens*.²⁶⁻²⁷
- Acetone water or chloroform water showed the presence of tannin in leaf extracts of *T. procumbens*.²⁸
- *T. procumbens* buds and pedicle both contain tannin.²⁹

other constituent:

- Carotenoids are fat soluble pigments found in the leaves that have three main functions in a plant: light harvesting, protection from photooxidative damage, and pigmentation to attract insects. Carotenoids have been postulated to prevent damage to DNA by oxidative stress.³⁰⁻³¹
- Alkaloid - In a phytochemical screening analysis, using aqueous extraction of the leaves, thirty-nine alkaloids were present, mainly Akuamidine (73.91%) and voacangine (22.33%).²⁹

1.1.5 Mechanism of action of various phytochemicals:⁹

Phytochemical	Activity	Mechanism of action
Flavonoids	Anti-microbial activity	Complex with cell wall, binds to adhesins
Tannin		Binds to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption
Alkaloids		Intercalates into cell wall and DNA of parasites
Terpenoids		Membrane disruption
Quinones		Binds to adhesins, complex with cell wall, inactivates enzyme

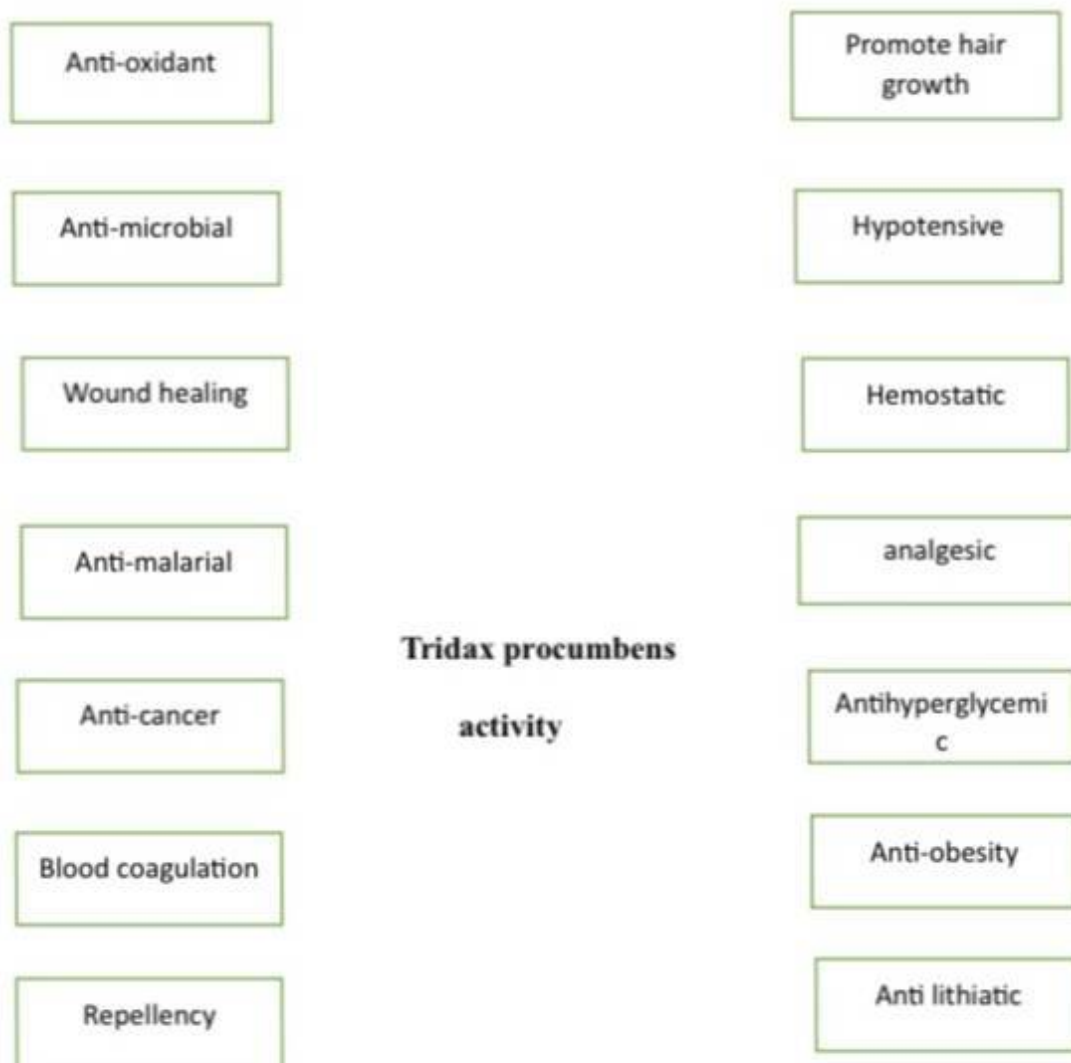
table 2 Mechanism of action of phytochemical

1.1.6 Pharmacological activities:

- *Tridax procumbens* has a wide range of potential medicinal properties, including antimicrobial, antioxidant, antibiotic, wound healing, insecticidal and anti-inflammatory properties as well as the ability to treat diarrhoea and dysentery. Leaf juice is applied to new wounds to heal them. Help treat hair problem and stop bleeding. *Tridax procumbens* is mostly used in India as an anticoagulant, antifungal, and insect repellent for the treatment of wounds. Leaf extracts were used in traditional medicine to treat infectious skin conditions. Along with treating gastritis and heartburn, it is a well-known medication for liver problems or for its

hepatoprotective properties. Using *Tridax procumbens* as a bio absorbent, hazardous Cr(VI) is removed from industrial effluent.³²

- *Tridax procumbens* have shown many activities:¹⁵



[Fig 7 *Tridax procumbens*

Antimicrobial activity:

- It has been discovered that ethyl alcohol extracted *Tridax procumbens* leaf works best as an antibacterial agent against *pseudomonas vulgaris*. The ethanolic extract had excellent results. Antimicrobial action against non-fermenting, gram-negative, drug-resistant bacteria due to the presence of phytoconstituents such as flavonoids and tannins, *pseudomonas* isolated from nosocomial infections may have various different modes of action, including the inhibition of DNA gyrase, the suppression of cytoplasmic membrane function and the inhibition of energy metabolism.³³

- The secondary metabolites have excellent therapeutic potential and fewer negative effects than synthetic antibacterial medicines, which are common.³⁴

Anti-bacterial activity:

- The complete plant parts of *Tridax procumbens* have been found to exhibit antimicrobial action against diverse bacterial species in a previous research investigation. To extract juice that is administered twice daily for 4-5 days to treat cuts and wounds, an entire plant is squeezed between the palms of hands. Only *Pseudomonas* was resistant to the antimicrobial effects of plant's whole extract. Using the disc diffusion assay, *Aeruginosa*. Two bacterial strains used in the test were gram positive is *Bacillus subtilis* and gram-negative bacteria is *Escherichia coli*.³⁵

Anti-fungal activity:

- To assess the antifungal activity of the plant decoction, the disc diffusion method was applied to two fungus strains, *Aspergillus Niger* and *Candida albicans*. Minimum inhibitory and minimum fungicidal concentrations were used to measure total activity. Alkaloids decoction had no effect on either of the test fungus, while flavonoids decoction had the maximum efficacy against *Aspergillus niger*.³⁶

1.1.7 Traditional use:

- *Tridax procumbens* is a common weed and a blooming plant with a number of therapeutic benefits. It has historically been utilized in India as a wound healing agent, anti-coagulant and anti-microbial. Boils and blisters can also be treated with it. This healing herb is frequently employed as a folk remedy for conditions including ulcers and hair loss. Its leaf decoctions had a reputation for ethnic medications are used to treat infection skin problems. Because the plant decoctions have a hepatoprotective effect, it is a well-known ayurvedic remedy for liver problems. In addition, the extracts are utilized to treat gastritis and heartburn.
- It is frequently used to wounds to reduce haemorrhage caused by cuts bruises, and wounds. The herb is also used to treat severe diarrhoea and dysentery, as well as excessive blood pressure and blood sugar levels.
- It can be used to stop hair from falling out and it encourages hair growth. The herb is also used to treat respiratory conditions. It possesses strong anti-insect and immune-modulating properties.

- Rural medical professionals and indigenous peoples in west Africa and the tropical region of the world utilize the plant's leaves as a treatment for conjunctivitis.
- Moreover, this medicinal plant was employed in the ethnic medical system to treat liver problems and jaundice.
- Ethanol decoctions of *Tridax procumbens* were also used for curing kidney stone diseases.

1.2 Clove oil :

Clove is mainly used in Ayurvedics. It is usually known as “lavang”. Clove is mainly used in Ayurvedics. It is usually known as “lavang”. Clove (*Syzygium aromaticum*) a precious spice, it is a member of Myrtaceae. Clove is mainly used for preparation of food. Clove oil is used for antimicrobial, antiviral, anti-inflammatory, anti-diabetics and antioxidant properties Clove is mainly used for preparation of food. Clove oil is used for antimicrobial, antiviral, anti-inflammatory, anti-diabetics and antioxidant properties.³⁷



[Fig 8. Clove oil]

2.1.1 Taxonomic classification:³⁸ [table 3 Taxonomic classification of clove]

Kingdom	<i>Plantae</i> - Plants
Subkingdom	<i>Tracheobionta</i> - Vascular plants
Superdivision	<i>Spermatophyta</i> - Seed plants
Division	<i>Magnoliophyta</i> - Flowering plants
Class	<i>Magnoliopsida</i> - Dicotyledons
Subclass	<i>Rosidae</i>
Order	<i>Myrtales</i>
Family	<i>Myrtaceae</i> Juss. - Myrtle familyP
Genus	<i>Syzygium</i> P. Br. ex Gaertn. - syzygiumP
Species	<i>Syzygium aromaticum</i>

1.2.2 Vernacular names of clove :³⁹ [table 4 Vernacular names of clove]

Languages	Names used
Bengali	Lavanga
Gujarati	Lavang
Hindi	Laung, Laung, Lavang
Kannada	Lavanga, Karambu, Daevakusuma
Malayalam	Grampu, Karampu, Karayampu
Marathi	Luvang
Oriya	Labanga
Punjabi	Laung
Sanskrit	Bhadrasriya, Lavanga, Varala, Haricandana, Devakusuma
Tamil	Kirampu, Kiraambu, Grambu
Telugu	Devakusumamu, Lavangamu, Lavangalu
Urdu	Laung, Loung

1.2.3 Chemical Constituents of clove oil :⁴⁰[table 5 chemical constituents of *Syzygium aromaticum*]

No.	Eugenol	β -Caryophyllene	α -Humulene	Eugenyl acetate
1.	87 ^a	3.56 ^a	0.40 ^a	8.01 ^a
2.	49.71 ^b	18.94 ^b	-	-
3.	72.4 ^b	12.61 ^b	1.56 ^b	9.59 ^b
4.	47.6 ^b	35.4 ^b	-	13.4 ^b
5.	89.2	-	-	8.6
6.	27.1	8.7	1.1	-
7.	76.8	17.4	2.1	1.2
8.	81.13 ^a	3.45 ^a	0.38 ^a	11.60 ^a
9.	66.37 ^a	15.38 ^a	1.97 ^a	12.99 ^a
10.	72.34 ^a	12.51 ^a	2.34 ^a	5.34 ^a

1.2.4 Pharmacological activity of Clove oil:

1.2.4.1 Antibacterial activity :

Numerous investigations have proven clove's strong antibacterial properties. cloves contain a number of components that contribute to their inhibitory action, primarily eugenol, eugenyl acetate, and β -caryophyllene, 2-heptanone, methyl salicylate, α -humulene, acetyl-eugenol, iso-eugenol, methyl-eugenol, and phenyl propanoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnetin, myricetin, gallic acid, ellagic acid and oleanolic acid .

Similarly, clove oil was shown to be effective against gram negative bacteria (*Y. enterocolitica*, *S. choleraesuis*, *P. aeruginosa*), as well as gram positive bacteria (*S. aureus*, *B. cereus*, *E. faecalis*, and *L. monocytogenes*) that are carried by food.⁴¹

1.2.4.2 Antifungal activity :

Clove oil and eugenol have been shown in numerous studies to exhibit antifungal activity against filamentous fungi and yeasts, including several foodborne fungal species and human pathogenic fungi. In animal models, eugenol and clove oil have also been investigated as antifungal agents. Carvacrol and eugenol, two of clove's phenolic components, are known to have fungicidal properties, including the ability to combat fungi isolated from onychomycosis because the spores and micelles were lysed, chromatographic examination revealed that eugenol was the primary component responsible for the antifungal activity. Another study described a comparable mode of action for the disruption and distortion of macromolecules caused by eugenol.

Clove oil and eugenol were found to have a wide range of fungicidal activity against *Candida*, *Aspergillus*, and dermatophytes. The mechanism of action was linked to cytoplasmic membrane lesions.

Their hydrophobicity, which causes them to partition into the lipid bilayer of the cell membrane and change permeability, thereby contributing to the activity by allowing cell contents to seep out.⁴¹

1.2.5 Traditional use of Clove oil :

Cloves can be used in cooking and medicine, among other applications. A useful culinary spice, cloves can be used to salad dressings, herbal teas, soups, onions, and tomatoes. In addition, meat products, biscuits, chewing gum, pickles, spicy fruits, chocolates, soft drinks, puddings, sandwiches, pastries, and sweets are all flavored with it. To add essence to toothpaste, soaps, fragrances, and medications, volatile oil is employed. In Indonesia, a unique type of cigarette known as "Kretek" is made by combining tobacco and cloves in a 1:2 ratio.

With its ability to combat bacteria, clove is a common ingredient in mouthwashes, dental creams, throat sprays, and tooth pastes. Moreover, it relieves painful gums. Dental cavities are temporarily filled with a mixture of zinc oxide and eugenol, the principal bioactive component of cloves. Flavonoids are the reason clove oil has anti-inflammatory qualities. For the aromatherapy of rheumatism and arthritis, pure clove oil is employed. Skin disorders can be treated using a paste made of clove powder and honey. Bite and cut healing is accelerated by using a paste made of water and clove powder. Many digestive issues, such as dyspepsia, nausea, flatulence, and loose motion, can be treated with cloves. Clove oil strengthens the body's defenses against invasive microorganism⁴²

1.3 Neem seed oil:

- The versatile medicinal plant neem (*Azadirachta indica*) is the source of a number of chemical structures and biological effects. Many studies have already been conducted in the past to comprehend the chemistry and therapeutic applications of various components of neem for industrial and therapeutic usage. The plant *Azadirachta indica* is now a source of numerous medicinal compounds utilized in conventional medicine⁴³⁻⁴⁴
- The neem seed oil is obtained from the seed kernels of the neem tree and the process of obtaining it is fairly simple process⁴⁵



[Fig. 9 Neem seed]

1.3.1 Taxonomic classification:⁴⁶

- Order: Rurales
- Suborder: Rutinae
- Family: Meliaceae
- Subfamily: Melioidene
- Tribe: Melieae
- Genus: Azadirachta
- Species: Indica

1.3.2 Chemical Constituents: [Table 6 chemical constituent of Neem seed oil]

- More than 135 compound have been isolated from neem.⁴⁷ Following constituents shown in neem seed oil⁴⁸

Sr no.	Compound Name	Biological activity
1	Nimbidin	Anti-inflammatory, Antiarrhritie, Antipuretic, Antibacterial, Antifungal, Spermicidal
2	Sodium nimbidate	Anti-inflammatory
3	Azadirachtin	Antimalarial
4	Nimbin	Spermicidal
5	Nimbolide	Antimalarial, Antibacterial
6	Gedunin	Antimalarial, Antibacterial
7	Mahmoodin	Antibacterial

2.LITARATURE REVIEW OF DISEASE DUE TO BACTERIA :

2.1 E.coli :

Escherichia coli is one of the most important agents of extraintestinal infections, with the potential to cause infection in almost any anatomical site. It can be grouped into pathotypes,

such as uropathogenic *E. coli* (UPEC), septicemia-associated *E. coli* (SePEC), skin and soft tissue infection (SSTI)-associated *E. coli*, neonatal-meningitis-causing *E. coli* (NMEC). Skin and soft tissue infections (SSTIs) are major bacterial infections, often self-limiting but in severe cases requiring hospitalization and parenteral antibiotic therapy.⁴⁷

2.2 Staphylococcus aureus :

Staphylococcus aureus is the most common pathogen involved in skin infections worldwide, regardless of the patient's age, the climate or geographical area. Localized *S. aureus* skin infections are either primary or secondary. A primary or "spontaneous" cutaneous infection is an infection occurring without preceding clinically evident lesions or secondary to a minimal skin lesion. These infections include impetigo, folliculitis, furuncles, and primary abscesses. Secondary skin infections are those occurring as a consequence of a pre-existing cutaneous lesion (usually incorrectly called "superinfections"). *Staphylococcus aureus* infections of the skin to consider this rich and varied clinical spectrum.⁴⁸

2.3 Bacillus subtilis :

A common Gram-positive aerobic environmental bacterium is *Bacillus cereus*. Cutaneous *B. cereus* skin infections are often associated with drug-induced neutropenia, haematological malignancies, and open wounds. The pathogen may enter non-traumatic instances through small skin abrasions on the hands and/or feet. Numerous kinds of soils, sediments, dust, and plants all contain *B. cereus*. This is important because it may be accessed by small cuts on the skin.⁴⁹

2.3 P.vulgaris :

A class of uncommon autoimmune bullous disorders affecting mucous membranes and the skin is known as pemphigus diseases. The goals of topical therapy for PV lesions are to lessen discomfort and stop subsequent infections. Usually, creams containing corticosteroids and/or antibiotics are used. Tacrolimus use has been documented, especially in relation to face lesions. Antiseptic solutions like chlorhexidine or potassium permanganate (1:10,000 or 1:20,000) may be used in really severe instances. For the oral mucosa, stronger gel corticosteroids such clobetasol dipropionate may be used. For recalcitrant skin lesions (such pemphigus vegetans), intralesional injections of triamcinolone acetonide (10 mg/mL) may be utilized.⁵⁰

Material Use in Formulation :

Equipment: Measuring cylinder, Beaker, Stirrer, Thermometer, white porcelain dish, water bath, weighing balance, pipette, mortar pestle,

Collection of material:

Tridax procumbens collected from the garden. Neem seed oil and gel base materials are used form laboratory ingredient.

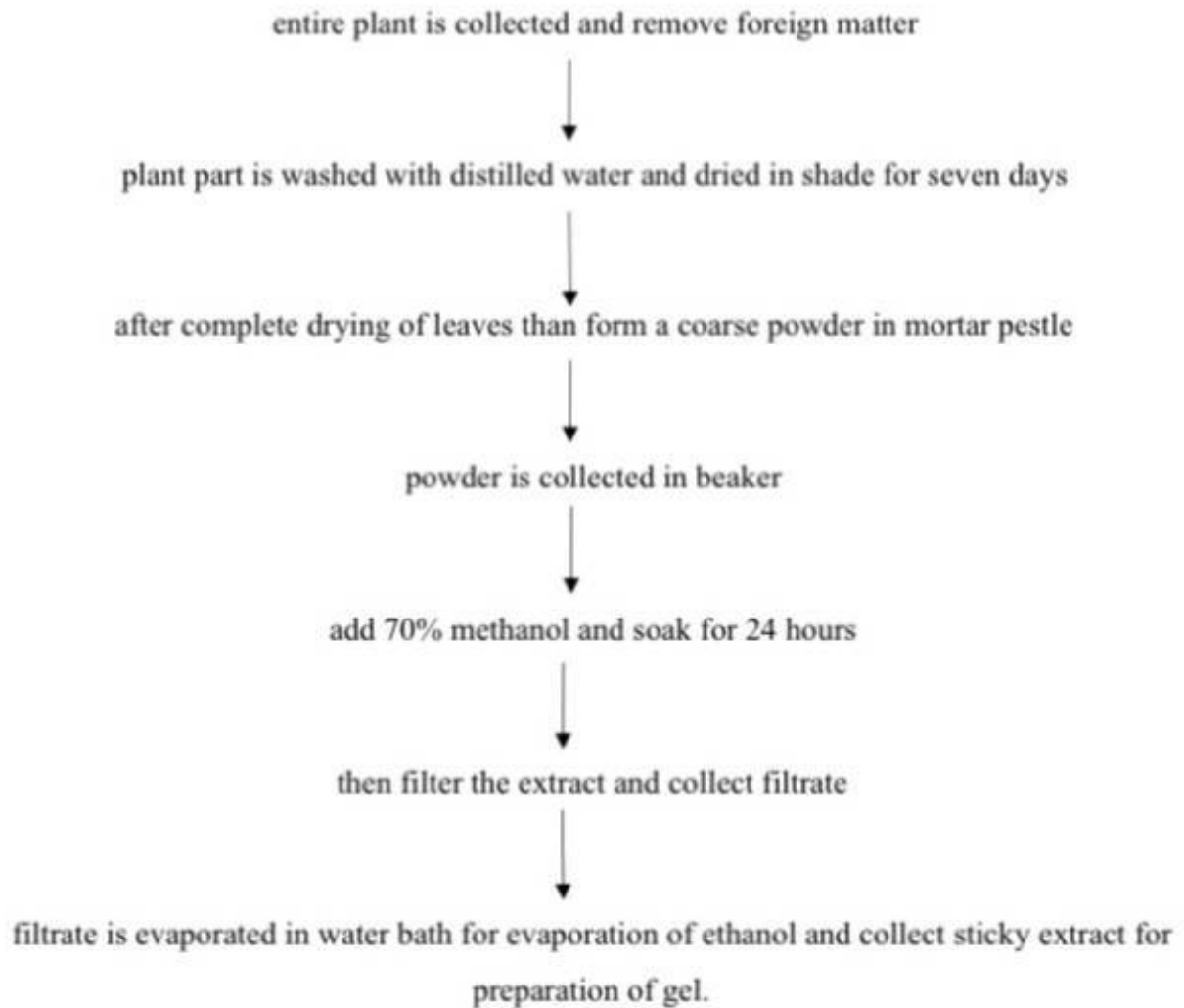
Ingredients:⁵¹ [Table 7 Ingredients]

Ingredients	Uses
Carbopol 940	Viscosity enhancer, gelling agent
Propylene glycol	Humectant
Methyl paraben	Preservative
Propyl paraben	Prevent growth of micro-organism
Glycerin	Natural Humectant, Softening agent
Tridax procumbens	Anti-microbial
Clove oil	Anti-bacterial, Anti-fungal
Neem seed oil	Anti-bacterial, Anti-fungal
Tween 80	Emulsifier
Triethanolamine	Thickening agent, maintain ph
Distilled water	Water base



[Fig. 10 material use in Formulation]

Extraction process:



Agar cup method :

Preparation of agar:

- Nutrient broth was mixed in 150 ml of water.
- Agar was added in nutrient broth and heat until solution become transparent with constant stirring.
- Media was autoclave for 15 mins.

Preparation of agar plat:

- Arrange the petridish on level surface.
- Sterilize the neck of the flask on a flame and pour 15-20 ml of medium into each dish and stand until media become solidified.
- Add bacterial culture media (E. coli, S. aureus, B. subtilis , p.vulgaris) with micropipette and spread over the plat.
- Then boring on agar media and filling the sample.
- Then plat put in incubator for 24 hrs.
- After 24 hr., the plat was observed and measure zone of inhibition.

Methodology:

Weight accurate Carbopol 940 and take $\frac{3}{4}$ quantity of water in beaker Place beaker over night , to soak the Carbopol 940 in water



Add this Carbopol 940 and water mixture into mortal pestle , slowly add one by one all aqueous soluble component with constant trituration



Add glycerin, Methyl paraben , propyl paraben ,Propylene glycol and Tridux procumbens extract one by one



Then add Tween 80 drop by drop



Then slowly add Neem seed oil and clove oil with constant trituration



Add one to Two drops of Triethanolamine



[Fig.20 Emulsifying g

Formulation of Emulsifying gel : [Table 8 Formulation]

Ingredient	F1	F2
Carbopol 940	0.2 gm	0.2 gm
Propylene glycol	2 ml	2 ml
Methyl paraben	0.04 ml	0.04 ml
Propyl paraben	0.02 ml	0.02 ml
Glycerin	0.2 ml	0.2 ml
Tridax procumbens	10 ml	10 ml
Clove oil	1 ml	1 ml
Neem seed oil	1-2 drops	1-2 drops
Tween 80	1-2 drops	1-2 drops
NaOH	-	1-2 drops
Triethanolamine	Quantity sufficient	-
Distilled water	20 ml	20 ml

Here, we prepared two different formulation using different proportion of ingredient ,and we find out that **F2** have good result compare to **F1**.

Ingredients	Final batch
Carbopol 940	1 gm
Propylene glycol	10 ml
Methyl paraben	0.04 ml
Propyl paraben	0.02 ml
Glycerin	1 ml
Tridax procumbens	10 ml
Clove oil	5 ml
Neem seed oil	1 ml
Tween 80	Few drops
Triethanolamine	Q.S
Distilled water	100 ml

Evaluation parameter :

1.Physical appearance:

Color is important for patient compliance. The prepared gels were inspected visually for clarity, colour, order and texture etc.

2. pH:

The pH of gel was determined using digital pH meter, 2 gm herbal gel was stirred in distilled water till uniform suspension is formed. The volume was made up to 40 ml and pH of the solution was measured.⁵²

3. skin irritation:

Mark the area on hand surface. Then gel was applied to the area and time was noted. Then its is checked for irritancy, erythema, edema checked for on interval of 24 hrs.⁵³

4. spreadability:

The spreadability of the gel formulations was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates after one min. The standard weight applied on the upper plate was 125 gm.⁵⁴

5. Antibacterial activity:

Preparation of agar:

- Nutrient broth was mixed in 150 ml of water.
- Agar was added in nutrient broth and heat until solution become transparent with constant stirring.
- Media was autoclave for 15 mins.

Preparation of agar plat:

- Arrange the petridish on level surface.
- Sterilize the neck of the flask on a flame and pour 15-20 ml of medium into each dish and stand until media become solidified.
- Add bacterial culture media (E. coli, S. aureus, B. subtilis , p.vulgaris) with micropipette and spread over the plat.
- Then boring on agar media and filling the sample.
- Then plat put in incubator for 24 hrs.
- After 24 hr., the plat was observed and measure zone of inhibition.

Result and discussion of prepared Emulsifying Gel :

1.physical appearance: *[Table 9 Physical appearance]*

parameter	F1	F 2
colour	White	White
odour	Characteristic	Characteristic
clarity	Yes	Yes
Texture	Smooth	Smooth
State	Semisolid	Semisolid

2. pH: *[Table 10 pH]*

The pH of the gel has been mostly found between 6 to 8

Sr no.	Formulation	pH
1.	F1	6.8
2.	F2	6.5

3. Skin irritancy: *[table 11 skin irritancy]*

The gel has been not shown redness of skin, erythema, edema, irritancy during the study.
The gel has been safe.

Sr no.	formulation	Irritancy	Erythema	Edema
1.	F1	No	No	No
2.	F2	No	No	No

4. spreadability: *[Table 12 Spreadability]*

Sr no.	Formulation	Evaluation
1.	F1	55 mm
2.	F	60 mm



6. Result of antibacterial activity of plant extract & oils on bacteria : : [Table 13 Antibacterial activity]

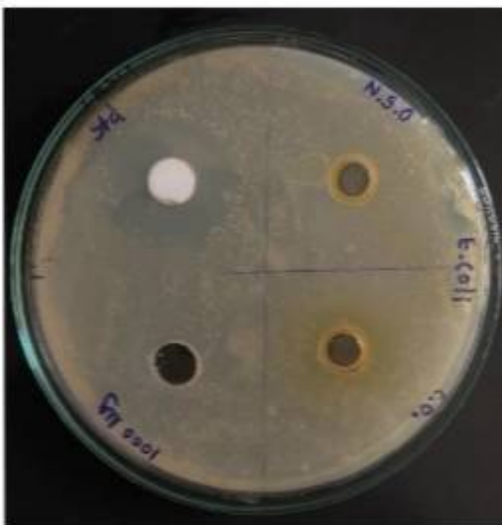
Bacteria	Std.(mm ± SD)	Clove oil(mm ± SD)	Neem seed oil	1000µg/ml (TP)
B. subtilis	15.66 ± 0.57	10.33 ± 0.57	-	-
S. aureus	15.66 ± 0.57	12 ± 0.86	-	-
E. coli	15.66 ± 0.57	10 ± 0	-	-
P. Vulgaris	12.33 ± 0.57	10 ± 0	-	-



[Fig. 12 Antibacterial activity of plant on p.valgarin]



[Fig. 13 Antibacterial activity of plant on B.subtilis]



[Fig. 14 Antibacterial activity of plant on E.coli]



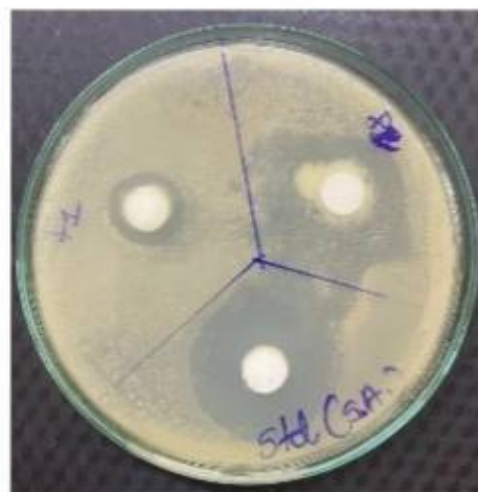
[Fig. 15 Antibacterial activity of plant on S.aureus]

7. Result of Antibacterial activity of F1 & F2 on bacteria : [Table 14 Antibacterial activity]

Species	F1 ,(mm ± SD)	F2.(mm ± SD)	Standard ,(mm ± SD)
B. subtilis	14.66 ± 0.76	14 ± 1	25.83 ± 0.7
S. aureus	14.66 ± 0.76	30.16 ± 0.2	32.83 ± 0.7
E. coli	14.66 ± 0.76	20.33 ± 0.5	31.33 ± 0.5
P. Vulgaris	13.83 ± 0.76	17.83 ± 0.7	29 ± 1



[Fig.22 Antibacterial activity of formulation on B.Subtitis]



[Fig.23 Antibacterial activity of formulation on S.aureus]



[Fig. 24 Antibacterial activity of formulation on E.coli]



[Fig.25 Antibacterial activity of formulation on p.vulgaris]

Here ,F2 give more Antibacterial activity compare to F1 .Maximum activity is against s.aureus.

Conclusion:

From the present investigation, it has been revealed that herbal emulsifying gels of plant *Tridax procumbens*, Clove oil and neem seed oil can be formulated using Carbopol 940 as polymer with other ingredients and the evaluation of physical parameters shown satisfactory results. From the antibacterial activity it was found that prepared herbal Emulsifying gel have good antibacterial activity ,it may be use to prevent bacterial infection. we can also go for fungal studies if we getting good result then we can also use it for antifungal activity . It should beneficial to the society for several bacterial and fungal infection.

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