



FORMULATION AND EVALUATION OF ANTISEPTIC SPRAY

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Title: An complete study on antiseptic spray for skin bacterial infection using cinnamon ,clove, orange peel and jasmine extract.

ABSTRACT

Topical spray are dosage forms in which polymeric solution of drug is sprayed over the intact skin so as to get a sustained release of pain from polymeric matrix. Some natural substances of plant origin have good antimicrobial properties and have been used as antimicrobial agent. The aims of present investigation were to assay antibacterial properties of crude extract of cinnamon stick and clove. Clove are rich in phenolic compound mainly eugenol which exhibit antioxidant, anti-inflammatory, antimicrobial, antifungal and wound healing properties. Topical film forming antiseptic spray of clove and cinnamon were developed for antiseptic application.

Keywords : skin, cinnamon extract , clove extract , Orange peel extract, Jasmin oil, topical delivery, antiseptic.

1: INTRODUCTION

Topical sprays are dosage forms in which polymeric solution of drug is sprayed over the intact skin so as to get a sustained release of pain from the polymeric matrix. The drug is present in saturated form in the polymer matrix. As the organic solvent vehicle evaporates, Slowly the drug diffuses through the polymer Matrix and passes from the skin barrier.^[1]

Some natural substances of plant origin have good antimicrobial properties and have been used as seasonings for centuries. Spices and aromatic vegetable materials have long been used in food not only for their flavor and fragrance qualities and appetizing effects but also for their preservative and medicinal properties. The aims of the present investigation, therefore, were to assay antibacterial properties of crude extract of cinnamon stick (Burmanni) against five common pathogenic bacteria; to identify and determine major bioactive components in the crude extract contributing to its antibacterial properties.^[2]

Cloves are rich in phenolic compounds, mainly eugenol, which exhibit antioxidant, anti-inflammatory, antimicrobial, antifungal, and wound-healing properties. Clove is a spice that is rich in bioactive compounds, which are responsible for its numerous health benefits. The main bioactive compounds found in cloves are eugenol acetyl eugenol, and Caryophyllaceous. The bioactive compounds found in cloves have led to the development of novel pharmaceuticals and nutraceuticals. ^[3]

3: INGREDIENT FOR ANTISEPTIC USES

3.1: Jasmine oil

Scientific name: Jasminum sambac



Family: Oleaceae

Chemical constituents:



Jasminum sambac contains dotriacontanoic acid, dotriacontanol, oleanolic acid, daucosterol, hesperidin, and [+]-jasminoids A, B, C, D in its Roots.

Leaves contains flavonoids such as rutin, quercetin and isoquercetin, flavonoids rhamnoglycosides as well as α -amyrin and β -sitosterol. A novel plant cysteine-rich peptide family named jasmintides were isolated from this plant.

Use

1. Traditionally *Jasminum sambac* has been used to treat dysmenorrhoea, amenorrhoea, ringworm, leprosy, skin diseases and also as an analgesic, antidepressant, anti-inflammatory, antiseptic, aphrodisiac, sedative, expectorant.
2. It is widely cultivated for its attractive and sweet fragrant flowers.
3. It is used in gardens as an ornamental plant.
4. Other commercially important species grown for the perfumer industry are *Jasminum officinalis*, *Jasminum grandiflorum*, and *Jasminum auriculatum*.

Jasmine extract

Method 1: Maceration

- Take 350 gm of *Jasminum sambac* (jasmine flowers) .
- Menstruum is poured in a vessel till the flowers are completely dipped in it (Ethyl alcohol).
- Keep it for 8 days in a glass vessel ^[4]

3.2:Cinnamon powder extract

Scientific name :

Cinnamomum zeylanicum,

Synonyms:

Dalchini,
Ceylon Cinnamon,
Cinnamon bark.

Family:

Lauraceae

Biological source:

Cinnamon consists of dried bark, freed from the outer cork and from the underlying parenchyma, from the shoots growing on the cut stumps of *Cinnamomum zeylanicum* Nees.



Cinnamon oil

- Colour: Yellow to reddish in colour.
- Specific gravity: 1.00 to 1.030.
- Optical rotation: 0 to - 2.
- Refractive index: 1.562 to 1.582.

Chemical constituents

- Cinnamon bark contains volatile oils (0.5 to 1 percent), phlobatannins (1.2 percent), mucilage, calcium oxalate, starch and mannitol (responsible for sweetish taste).
- The essential oil (5 to 20 ml/kg) is composed of phenylpropane derivatives. Cinnamon oil mainly contains cinnamaldehyde (60 to 70 percent), eugenol (5 to 10 percent), benzaldehyde, cuminaldehyde and other terpenes such as phellandrene, pinene, cymene, caryophyllene.

Chemical test: To a drop of volatile oil add a drop of ferric chloride solution, a pale green colour develops (cinnamaldehyde produces brown colour and eugenol gives blue colour which results in the formation of pale green colour).

Uses:

The drug is used as aromatic stimulant, antibacterial, antifungal, antiseptic, carminative, stomachic and astringent. Commercially, it is also used as spice, condiment, in candy preparation, dentrifices and perfumery. Cinnamon oil is used in urinary infection and food technology. Cinnamon oil and cinnamaldehyde are irritating to skin and mucous membranes.^[5]

3.3: Clove powder extract

Scientific name : *Syzygium aromaticum*

Synonym :

Caryophyllus aromaticus L.
Eugenia aromatica (L.) Baill.
Eugenia caryophyllata Thunb.
Eugenia caryophyllus (Spreng.) Bullock & S.G.Harris

Biological source : aromatic flower buds of a tree *Syzygium aromaticum*.





Family: Myrtaceae ^[6]

Chemical constituent

Volatile oil (15-20%) eugenol 70-90%, Tannin 10-13% eugenol acetate
Resin, Isoeugenol, Caryophyllinmethyl and dimethyl furfural, Chromone.

Uses:

Carminative Stimulant
Antiseptic Aromatic
Flavouring agent Dental analgesic ^[7]

3.4: Orange peel extract :

Scientific name : Citrus reticulata

Synonyms : Orange cortex, Bigarade orange, Seville orange, china Orange,
Bitter orange peel

Biological source : Orange Peel is consists of fresh and dried outer part of the pericarp of citrus aurantium Linn.

Family : Rutaceae

Chemical constitution :



Limanene (90%) Citral (4%)
Vitamin C Pectin
Hesperidine

Chemical test

Shinoda test

A small quantity of test residue is dissolved in 5 ml of ethanol (95% v/v) and treated with few drop of conc. Hcl and 0.5 g of magnesium metal, pink or red colour is developed^[8]

Uses :

Stomachic
Aromatic
Carminative
Flavouring agent
Bitter Tonic ^[9]

3.5: Ethyl alcohol

Synonym : ethanol

Family : alcohol

Source: grains, barley, wheat

Ethanol is a plant fermentation by-product which is natural and it can also be produced through the hydration of ethylene. Ethanol is an important industrial chemical; it is used as a solvent, in the synthesis of other organic chemicals, and as an additive to automotive gasoline.

Ethanol Formula

Ethanol molecular formula is C₂H₆O, which means it has two carbon atoms and one oxygen atom. The structural formula for ethanol, C₂H₅OH, provides a bit additional information, indicating that the two-carbon chain has a hydroxyl group (-OH) at the end.

Alcohol-based hand sanitizer contains a certain percentage of the active ingredient ethyl alcohol (also known as ethanol) or isopropyl alcohol. The Centres for Disease Control and Prevention (CDC) note that hand sanitizers should contain at least 60% Trusted Source ethanol. Ethanol is a colourless, clear liquid with a strong taste. ^[10]

4: FORMULATION :

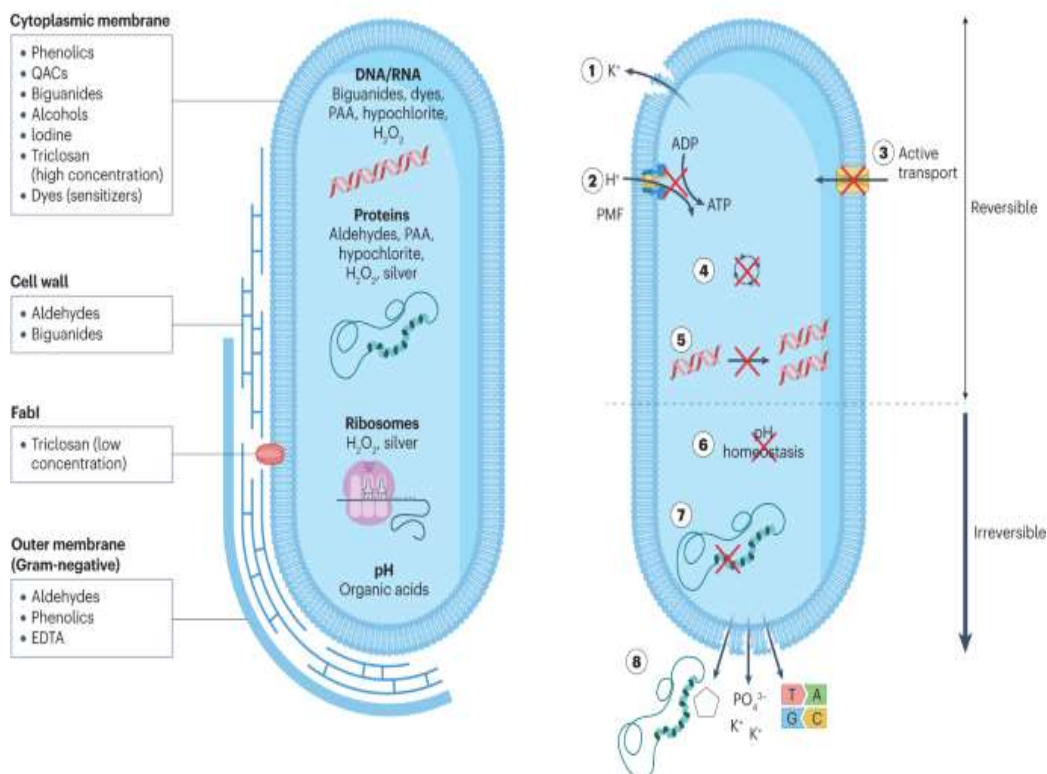
Sr. No.	Ingredients	Quantity %
1)	Jasmine oil	40%
2)	Orange oil	8%
3)	Ethyl alcohol	36%
4)	Cinnamon extract	8%
5)	Clowe extract	8%

Formula of spray

5: METHODOLOGY:

- Take 40 % of jasmine oil & 8% of orange oil.
- Add 36% ethyl alcohol.
- Add 8% clove extract to it and add 8% cinnamon extract to it.
- Fill it into suitable containers for use. ^[11]

6.MECHANISM OF ACTION



(General antiseptics activity)

The mechanisms of action of phenolic compounds on bacterial cell have been partially attributed to damage to the bacterial membrane, inhibition of virulence factors such as enzymes and toxins, and suppression of bacterial biofilm formation.

The antibacterial mechanism of eugenol against *S. Aureus* was probably related to the damage of cell wall and membrane, the inhibition on biofilm formation, the oxidative stress-mediated apoptosis and the disruption of DNA synthesis.^[12] Cinnamaldehyde exerts antibacterial effects by destroying the structure of cell membranes.

7. ADVANTAGES OF ANTISEPTIC SPRAY

7.1: Instant and targeted effect :

One of the most significant advantages of using sprays over oils, cream and other formulation is the speed at which they provide relief. Sprays are designed to deliver their active ingredients in a fine mist, allowing for quick absorption and immediate effect

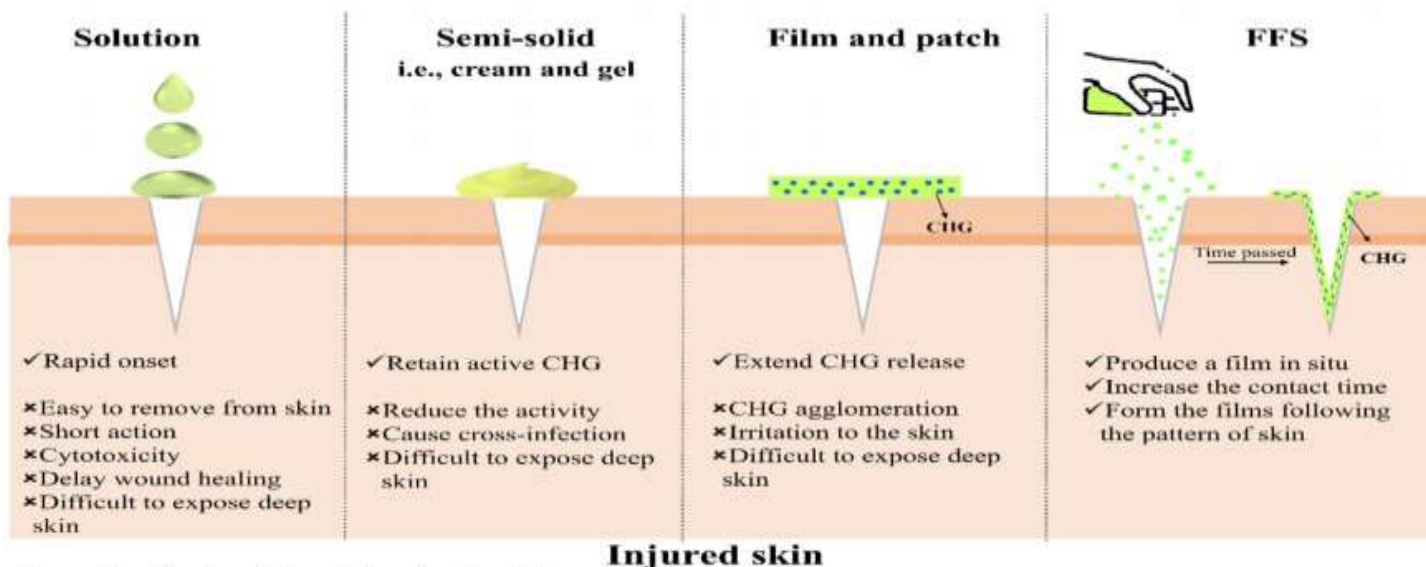
7.2: Non-greasy and non-sticky:

Antiseptic cream often leave a greasy or sticky residue on the skin, which can be inconvenient and uncomfortable. In contrast, sprays offer a non-greasy and non-sticky application. The evaporates quickly upon contact with the skin, leaving no residue behind.

7.3: Hygienic and no cross-contamination:

One significant concern with creams and oils is the potential for cross-contamination. When multiple individuals use the same container or when hands come into direct contact with the product, there is a risk of spreading bacteria or other contaminants. This issue is effectively mitigated with sprays, as the product is dispensed in a controlled manner and does not require physical contact.

7.4: Enhanced absorption and penetration:



Presenting the benefits and drawbacks of dosage forms

✓ = benefits and ✗ = drawbacks

Sprays offer a unique advantage in terms of absorption and penetration into the skin. The fine mist allows the active ingredients to be evenly distributed across the affected area, ensuring thorough coverage.

FFS is film forming spray ^[13]

8. DISADVANTAGES OF ANTISEPTIC SPRAY

When using antiseptics at home, a person should follow all safety instructions on the bottle. Using antiseptics with too high a concentration may cause irritation or chemical burns on the skin.

Over-the-counter antiseptics spray are not suitable for long-term use.

People should avoid using antiseptics on:

- Large wounds and burns
- Areas where a foreign object is stuck in the skin
- Animal bites and scratches
- Eye infections



9. USES OF ANTISEPTIC SPRAY

Preventing infections on the skin, particularly for cuts, scrapes, or minor burns. Dry hand-washing, which healthcare workers may do between different procedures or patients. Cleaning the skin before a medical procedure, such as a blood draw or surgery. For helpful in traveling for hand sanitation. [14]

10. APPLICATION OF ANTISEPTIC SPRAY

10.1: In topical antibacterial treatment

To clean contaminated cuts, wounds, abrasions, burns and bite's for prevent acute infections and any big skin disease. For skin infection, Skin burn for prevention of further bacterial infection

11. EVALUATION TEST

11.1. Physicochemical characteristics

11.1.1. Pressure test:

Each container placed in an upright position and actuator pressed for remove liquid from the dip tube. The actuator removed and pressure gauge at place of actuators. The gauge pressed to actuate the valve and the pressure exerted by valve was noted for each aerosol container with the help of pressure gauge. That is 25 – 30 psi.

11.1.2. Irritancy test:

Skin irritation studies were carried out using wistar rats as animal model. The optimized formulation was sprayed on the pre shaved skin and reactions if any erythema and edema were scored after 7 days.

Spray a formulation on normal skin for check irrigation.

11.1.3. pH

Make spray dilute solution, take 1ml formulation dilute it for 10 ml shake and add on pH paper drop by drop and observe a change of pH paper color. It is slightly acidic.

pH meter calibrated using two buffers (pH 4 and pH 7) for calibration. The tip of the probe after rinsing with water was dipped in to samples. The meter was allowed to equilibrate and then pH noted.

11.2. Performance

11.2.1. Delivery rate of topical spray

The delivery rate of spray was evaluated according to USP procedure. Six aerosol containers are used. Each valve was actuated for 5 seconds at a temperature of 25 °C. The test was repeated three times for each container. The average delivery rate is 70%.

11.2.2. Drug content per actuation

Reproducibility of the dosage was determined as per USP. The average amount of active ingredient delivered through actuator per actuation was assayed, the amount delivered per actuation was determined. It is 0.9ml.

11.2.3. Spray pattern of topical spray

Spray formulation was sprayed onto absorbent paper for 2 seconds. The distance separating the container from the target was kept constant, at 5 cm. Spray pattern was evaluated by spraying the concentrate in horizontal position. Ovality ratios were determine is Dminis 4 cm and Dmax is 9 cm.

11.2.6. Leakage test of topical spray

Aerosol containers were selected and the date and time were recorded to the nearest half hour. Container was weighed to the nearest mg and recorded as W1. The containers were allowed to Stand in an upright position at a temperature of 25.0 ± 2.0 °C for not less than 3 days, before the second weight was recorded as W2. The leakage rate, in mg per year, of container was calculated using formula: $(W1 - W2) / (113-98) = 15$ is leakage rate

11.3. Biological characteristics

11.3.1. In vitro antibacterial activity of topical spray:



E.coli is one of the invasive bacteria found in most of the bacterial infections was used for studying in vitro bacterial activity of topical spray. Cup and plate assay method was used for determining the zone of inhibition for both placebo and formulated spray preparation. They were transferred from 0.9 % sodium chloride saline solution to nutrient agar broth in a sterile tube with the help of sterile inoculation loop for sub culturing.

Nutrient agar plates were prepared with appropriate turbidity by pouring plate method. Agar powder, sodium chloride, peptone and beef extract were used for susceptibility testing of E coli. A sterile inoculation loop is dipped in the bacterial suspension and the loop is streaked in at least three directions over the surface of the nutrient agar media to obtain uniform growth. A final sweep is made around the rim of the agar. The plates are allowed to dry for approximately five minutes. A sterile borer was used to create well in the centre of agar plate. The well in the centre of the plate was than filled with drug solution and compared with without test formulation as control. The plates were incubated within 15 minutes after, applying the disks and boring the well with drug solution. The temperature was kept $35^{\circ} \pm 2^{\circ}\text{C}$ for incubation and incubation time was 24 hours. After the overnight incubation, clearing zone around each of antibacterial solution in well was measured with the help of ruler. The diameter was measured and recorded in millimeters (mm).^[15]

11.4.Stability studies

The optimized formulation was stored for stability testing as per ICH guidelines for 1 month. The chemical stability of the formulation was assessed by estimation of the percent drug remaining in the formulation, drug release pattern and physical stability was evaluated by monitoring any change in flammability, pressure, density, pH, delivery rate, spray pattern, spray angle. Biological stability was determined by in vitro antibacterial activity of topical spray

12.RESULT AND DISCUSSION

The topical antiseptic spray were successfully prepared using clove extraction Cinnamon extraction orange peel extraction jasmine oil and using ethanolic solvent. A homogeneous film after actuation was obtained. This generated the fast thin film formation on the skin with dosage form satisfaction. The formulation inhibited the growth of E. Coli invitro and provide antiseptic activity in vivo. The spray formation is non- toxic to human skin cells. They having suitable pressure,pH and physicochemical characteristics to human skin. They non irritant to human skin having good performance through delivery rate drug content per actuation, spray pattern and good 6 month stability of formulation. Show a good result through invitro antibacterial study using nutrient agar plate method. Thus topical antiseptic spray may be a potential candidate for topical antiseptic application.

13.CONCLUSION

Developed formulations of clove & cinnamon evaluated for the physiochemical parameter pressure test, irritancy test. pH, were such as performance study delivery. Sate of topical stay. Drug content per actuation, spray pattern. Spray angle, biological characteristic, In vitro antibacterial activity study. And stability study.In pressure test study formulation give positive result giving the pressure is 25-30 psi, not irritancy causes by applying PH is adjust with Skin PH is (pH I 4-7). Performance characteristic of formulation is good such as delivery Rate, drug content, Spray angle, Minimum fill test. Good Spray pattern fills and leakage test.In vitro study of formulation shows antiseptic Results having 6 month stability of formulation. So it is Suitable for topical application not shows toxic and harmful Reaction on skin tissue.

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