Formulation and Evaluation of Anti-bacterial Herbal gel Containing Terminalia catappa Extract

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ABSTRACT

Terminalia catappa linn has been mentioned in the Indian system of medicine as natural remedy for the treatment of various ailments and infectious diseases. Based on the traditional use, the aim of present study was to formulate and evaluate antibacterial herbal gel containing hydroalcoholic extract of fruit & leaves of Terminalia catappa. The topical gel was prepared by using carbopol-940 (1%w/v), leaves & fruit extract, HPMC, PG, methyl paraben, triethanolamine, ascorbic acid, amaranth colour and required amount of water. Designed formulation was evaluated for physical appearance, homogeneity, rheological studies, pH, viscosity, spread ability and washability. The formulation was also evaluated for their antibacterial potential by disc plate method against bacterial strains, the herbal gel formulation was observed to possess antibacterial potential.

Keywords: Terminalia catappa, antibacterial gel, Carbopol 940.

INTRODUCTION

Since ancient era to modern system of medicines, plants are the major and important part of the medicine. Herbal medicines plays important role in health services. Around one fourth population of world are relay on traditional herbal medicine, for the primary health care, especially on plant drugs1. Folklore information suggested antimicrobial potential of certain Indian medicinal plants. Very few reports are available on inhibitory potential against certain pathogens. Hence, proper scientific evidence are required to discover the potential of medicinal plants. As the herbal practitioners dispense their own recipes, there is need to design and develop new formulation with diverse chemical nature, novel mechanism of action and most important resistance free medicine2. In the present study, Indian almond also called as tropical almond botanically equated as Terminalia catappa Linn. was used to explored scientific evidence for microbial inhibition. The plant is tall deciduous erect tree reaching 25-40m with an upright symmetrical crown and horizontal branches naturally distributed throughout India. Near the end of the twigs, leaves are crowned and alternate. Leaf blades are big and thick having smoother margins. Matured leaves are shiny above and pubescent below and dark green in colour. While new or younger leaves are with soft covering of hairs. After maturation and before falling in the winter they turned to yellow, red and purple shades. Fruits are 2 inches or more long and 1 inch across, fleshy fibrous pulp surrounding the large seed which is edible and tasted somehow sweet and same as almond. Full-sized fruits are green and turn red, brown, or yellow at maturity2. Traditionally, leaves, bark and fruits were used as medicine in various diseases and ailments. It also has nutritional value, used as source of Vit. C & E and dietary minerals4.

Leaf was documented to possesses antioxidant5, hepatoprotective6, antidiabetic7, and anti inflammatory8 activities. While bark and fruits possess an anti-diarrheic, antipyretic hemostatic9, analgesic and anti-inflammatory potential10. The gel is semisolid system of at least two transparent phases interpenetrating into one another.

Gels that contain water are called hydrogels, while those that contain an organic liquid are called organogels. Hydrogels are the mixture of water and cellulosic derivatives11.

MATERIALS AND METHODS

Collection of plant materials

The fruits and leaves of Terminalia cattapa Linn were collected from college campus of Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra and authenticated from Department of Botany, RTMNU, Nagpur.

Preparation of extract from leaves

Fresh leaves of Terminalia catappa were air dried and then crushed by using mechanical blender to obtain a coarse powder. 150 g of powder plant was macerated in 600 ml of ethanol for 72 hr at room temperature, and then filtered into a beaker using funnel and whatman filter paper No.1 (125mm). The filtrate was concentrated by evaporation in...
water bath at a temperature of 50°C to obtain the crude extract.

**Preparation of extract from fruit**

Analytical grade reagent and chemical were used. The fresh fruits were collected from the Kamla Nehru College of Pharmacy from Butibori, Nagpur. The fruit were cleaned with water and pericarp and mesocarp were separated from the whole fruit. The pericarp and mesocarp which were only used for further study and were then cut into small pieces of about 1sq centimetre. These pieces were divided into three parts of 50 g each and these were macerated for 24 hr separately with 100 ml of solution 70% ethanol and 30% water. The extract was evaporated at room temperature to nearly 1/3rd of the original volume to get concentrated extract. These extracts were then preserved in tightly closed glass container and stored away from direct sunlight for 48 hr till use.

**Preparation of gel**

Carbopol 940, HPMC (3 different concentrations) was dissolved slowly with stirring in 60ml of demineralized water for 1 h to avoid agglomeration. Then propylene glycol solution, methyl paraben, ascorbic acid, amaranth colour was added and mixed well. Then triethanolamine was added dropwise to adjust pH by stirring the solution until clear consistent gel was formed. Three different gel were prepared using the formulae given in Table 1 and the viscosity was determined.

**Table 1: Formulation of gel base (all the quantities are in %)**

| Formulation              | F1 | F2 | F3 |
|-------------------------|----|----|----|
| Fruit extract           | 1  | -  | 1  |
| Leaves extract          | -  | 1  | 1  |
| Carbopol -940           | 1  | 1  | 1  |
| HPMC                    | 1  | 1  | 1  |
| Propylene glycol        | 4  | 4  | 4  |
| Methyl paraben          | 0.2| 0.2| 0.2|
| Ascorbic acid           | 1  | 1  | 1  |
| Triethanolmine          | q.s.| q.s.| q.s.|
| Amaranth colour         | q.s.| q.s.| q.s.|
| Water                   | q.s.| q.s.| q.s.|

**Agar well diffusion method**

Sufficient quantity of nutrient agar was taken, and water was added to make up the volume.

The dispersion was heated to boiling was sterilized in autoclave at 121°C. Then the solution was transferred to petridish and allowed to cool and solidify. Bacterial dispersion was spread on the agar surface. The wells were bored and solutions of standard antibiotic and gels containing extracts were added to the wells. The solutions were allowed to diffuse through agar and then incubated at 37°C for 24 hrs and zone of inhibition were observed.

**Evaluation of final gel**

**Visual examination**

The prepared gel formulae were inspected visually for their colour, appearance, texture.

**Determination of pH**

Weighed 50 gm of each gel formulation were transferred in 10 ml of the beaker and pH was measured by using the digital pH meter.

**Viscosity estimation**

The viscosity of gel was determined by using a Brookfield viscometer DVII model with a T-Bar spindle in combination with a helipath stand.

**Spread ability**

In this method, slip and drag characteristic of gel involves. Formulated gel (2g) was placed on the ground slide under study. The formulated gel placed (sandwich like) on this slide and another glass slides for 5min to expel air and to provide a uniform film of the gel between slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80g with the help of string attached to the hook and the time (sec) required by the top slide to cover a distance of 7.5cm was noted. A short interval indicated better spread ability.

**Formula was used to calculate spread ability**

\[ S = M \times L / T \]

Where,

- \( S \) = Spread ability
- \( M \) = Weight in the pan (tied to the upper slide)
- \( L \) = Length moved by the glass slide

**Extrudability**

The gel formulation was filled in standard capped collapsible aluminium tubes and sealed by crimping to the end. The weight of tubes was recorded and the tubes were placed between two glass slides and were clamped. 500gm was placed over the slides and then the cap was removed. The amount of extruded gel was collected and weighed. The percent of extruded gel calculated as 1- When it is greater than 90%, then extrudability is excellent. 2- When it is greater than 80% then extrudability is good. 3- When it is 70% then extrudability is fair.

**Irritancy Test**

The gel was applied on left hand dorsal side surface of 1sq.cm and observed in equal intervals up to 24hrs for irritancy, redness and edema.

**Homogeneity**

The developed gel was tested for homogeneity by visual inspection they were tested for their appearance with no lump.
**Grittiness**

All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope. Hence, obviously the gel preparation fulfills the requirement of freedom from particulate matter and form grittiness as desired for any topical preparation.

**RESULTS AND DISCUSSION**

The herbal gel was prepared and subjected to evaluation of various parameters. The gel was amaranth red in colour (Fig 1) with a translucent appearance. The pH was constant throughout the study to about 6.98 and the gel did not produce any irritation upon application to the skin. Extrudability was excellent and the gel also showed good spreadability. The initial viscosities were recorded at 25°C. The gel was found to be stable under normal conditions of stability. All evaluation data was shown in table 2.

**Table 2: Evaluation parameters of the formulated herbal gel**

| Parameters          | Observation       | F1    | F2    | F3    |
|---------------------|-------------------|-------|-------|-------|
| Visible appearance  |                   |       |       |       |
| Colour              | Reddish brown     | Reddish brown | Reddish brown | Reddish brown |
| Odour               | Characteristics fruity | Characteristics fruity | Characteristics fruity | Characteristics fruity |
| Appearance          | Viscous           | Viscous | Viscous | Viscous |
| Texture             | Smooth            | Smooth | Smooth | Smooth |
| pH                  | 6.98              | 7.01   | 6.98   |       |
| Viscosity (cp)      | 2800              | 8250   | 35000  |       |
| Spreadability (gm. cm./sec) | 14.5 | 13.5 | 13.9 |
| Extrudability       | Excellent         | Excellent | Excellent |       |
| Irritancy           | Absent            | Absent | Absent | Absent |
| Homogeneity         | Homogenous        | Homogenous | Homogenous | Homogenous |
| Grittiness          | Absent            | Absent | Absent | Absent |

**Antibacterial activity of gel**

Nutrient agar plates using agar well diffusion method with ciprofloxacin disc (10μg/ml) at the centre which served as a positive control. Zones of inhibition was measured for all the contents and activity was compared. Shown in figure 2 and table 3.

**Figure 2: Result of antibacterial activity of T. catappa gel containing fruit and leaves extract**

**Table 3: Zone of inhibition of leaf extract, fruit extract, gel and standard drug.**

| Bacteria          | Diameter of Zone of Inhibition in mm |
|-------------------|--------------------------------------|
| Escherichia-coli  |                                       |
| fruit extract     | 7mm                                  |
| leaves extract    | 15mm                                 |
| Standard (ciprofloxacin) | 22mm     |
| Gel (fruit +leaves extract) | 20mm     |
CONCLUSION

From the above observation, it has been revealed that herbal gels of plant *Terminalia catappa* leaves & fruit extract formulated using carbopol 940 and HPMC as polymer with other constituents and the evaluation of physical parameters shown satisfactory results. For the bacterial inhibitory potential, the gel formulation revealed more inhibitory potency than individual leaves extract and fruit extract in comparison to standard drug ciprofloxacin against tested bacteria. Hence, from the overall observations, it was finally concluded that the herbal gel containing leaves and fruit extract of *Terminalia catappa* have significant antibacterial potential and hence will safe and effective in microbial infection.

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