



## Formulation and optimization of synthetic polymer based herbal emulgel for anti-microbial activity

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### Abstract

The aim of the present research was to develop and evaluate the anti-microbial emulgel by using three different types of synthetic polymers. The active moiety selected for formulation was the seeds of carica papaya fruit. The formulation was developed by performing extraction process; Soxhlet extraction by with ethanol and water. The seeds of carica papaya are reported to have anti-microbial property. The skin friendly i.e., topical formulation was selected for development. Emulgel was prepared by incorporating different polymers and then evaluating each of them for best results. Basic evaluation parameters such as organoleptic parameters, viscosity, consistency, pH, homogeneity has been done, that demonstrate the results as per the reference and standard articles. Along with that, qualitative and quantitative tests of carica seeds has also been performed, which indicates that the selected plant material is safe for usage. Finally, the most imp test, the anti-microbial test has been performed for determining the efficacy of prepared emulgel. Hence, it can be concluded that the prepared emulgel is safe and best for topical use as an anti-microbial.

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### Introduction

Herbal or plant-based remedies have been used to prevent and cure illnesses from old times and many more elements of these natural origins are still to be discovered [1]. It has led researchers to discover newer chemicals from herbal sources that can be used to cure a variety of viral illnesses [2]. Many medicinal plants, according to research, contain antibacterial, antioxidant, and anti-inflammatory qualities that have led the door for the treatment of many infectious illnesses and have significant societal advantages. The current state of infectious diseases reveals that the incidence of new and re-emerging infectious illnesses has increased alarmingly. Other major worry is the emergence of resistance to antibiotics in therapeutic settings [3]. As a result, there is an immediate requirement to produce a natural composition

that can combat the bacteria that cause skin disorders [4]. Carica Papaya, a member of Caricaceae family, is a nutritious hub and is available throughout the year. In clinical trials, the fruit have been asserted for its anti-fertility, anti-hypertensive, hypolipidemic, uteronic hypoglycemic, anti-coagulant and enzyme efficiency [5]. The bio efficacy of Carica papaya is predominantly to its active phytoconstituents- papain, an enzyme contained in the fruit and stem latex, vitamins, such as vitamin- C and B complex, minerals such as- calcium, phosphorus, and iron, polysaccharides, alkaloids, saponins, flavonoids, and phenolic acids [6].

### Materials and methods

#### Collection of Plant material

The collected seeds were subjected to surface cleaning by rinsing the seeds with sterile water, in order to remove dust particles, present on them. The seeds were allowed to drying in a dark place at room temperature for few days. The dried seeds were ground in electric chopper to get fine powder form for further use.

### Chemicals

Carbopol 940, triethanolamine, petroleum ether, ethanol, propylene glycol, etc and Chemicals were ordered from nearby market.

### Preparation of plant extracts [7]

The prepared powder of Carica papaya seeds was subjected to Soxhlet extraction using distilled water (aqueous extract), acetone, chloroform and ethanol. Each 5 grams of dried, powder of seeds was filled separately in the thimble and extracted successively with 60ml of solvents using a Soxhlet extractor for three hours. After solvent evaporation, each of these solvent extracts was weighed and preserved in room temperature until further use.

### Phytochemical screening

Qualitative and quantitative tests had been performed on the extracts to identify the constituents by following the guidelines given by Sachin *et. al* (2013), and Dewi *et. al* (2019).

### Preparation of gel

To the 150ml water, 1% w/w Carbopol 940 was added and dispersed uniformly, ensuring no lumps. A 0.5 N NaOH solution was added drop wise, until a gel was formed. The prepared gel was weighed and stored in air-tight containers [8].

### Formulation of Powdered Carica Papaya Seeds emulgel [9]

- For oil phase 0.5ml of span 20 and 0.01g of Powdered Carica Papaya seeds extract was taken in 4.5ml liquid paraffin.
- For aqueous phase, 0.5ml of Tween 20 was taken in a purified water.
- Both the phases were heated at 60-70°C temp.
- The prepared oily phase was added to the aqueous phase with continuous stirring. This form the emulsion.
- The prepared emulsion was added to the gel in 1:1 wt. ratio.
- For the consistency of the product, 0.5N NaOH was also added.

### Evaluation Parameters:

#### Optimization of emulgel [10]:

The prepared gel formulations were visually examined for their texture, color, clarity and existence of particle.

#### Consistency of emulgel [11]:

To determine the consistency of the prepared gel, a small amount of gel was squeezed between the thumb and the index finger and the consistency of the gel was observed.

#### Homogeneity of emulgel [12]

All formulated gels were visually inspected for homogeneity after they were stored in the container. They were examined for their appearance and availability of any aggregates.

#### Determination of pH [13]

Accurately weighed 1.0g of various prepared gel and dispersed in 100ml purified water. The pH was measured by using digital pH meter. In order to ensure that the formulation can be used without the harm of skin irritancy, the pH of the preparation has been determined.

#### Spreadability [14]

0.5g gel was mounted within a circle of 1 cm diameter pre-marked on a glass plate of 20\*20cm and another glass plate was mounted over it. A mass of 100g was placed on the upper glass slide. The change in diameter due to the expansion of gel was reported.

#### Viscosity [15]

Viscosity of gel was carried out by using Brookfield Viscometer at 25°C, having spindle speed at 12rpm.

#### 1.1. In vitro test for anti-microbial study [16, 17, 18]

#### 1.2. Preparation of reagents and media

##### 1.2.1. Nutrient agar

Required volume of 2.8% nutrient agar was prepared using sterile water.

##### 1.2.2. Oxoid nutrient broth

Required volume of 3.5% of Oxoid Nutrient broth was prepared using sterile water. The nutrient broth was autoclaved at 121°C for 20 min.

##### 1.2.3. 50X Vogel-Bonner minimal medium (VB)

Required amount of VB was prepared in sterile water which contains 1% Magnesium sulphate, 10 % Citric acid monohydrate, 50% Potassium hydrogen phosphate (dibasic) and 17.5 % Sodium ammonium phosphate.

##### 1.2.4. 35% Glucose

35.0 g of glucose solution was prepared in one litre of sterile water and was stored in (2 - 8) °C until use.

##### 1.2.5. Basal Agar medium

Required volume of basal agar was prepared by adding 50X VB medium, 1.5% Bacto agar and 35% glucose in the ratio (1:18:1) using sterile water and mixed thoroughly. Basal agar was plated and stored in refrigerated condition.

##### 1.2.6. Top Agar medium

1% of bacto agar and 0.5% of sodium chloride was prepared and mixed well.

##### 1.2.7. Histidine-Biotin solution

0.5 mM of biotin and 0.5 mM of histidine was prepared and sterilized.

##### 1.2.8. Antibiotic

Required volume of Ampicillin (25 µg/ml) tri/tetrahydrate and tetracycline (2 µg/ml) solution was prepared, individually using sterile water.

1.2.9. **Crystal violet**

0.1% of crystal violet solution was prepared with water and stored at room temperature.

1.3. Test system details and preparation of tester strains, test item and controls

Table 4: Test system details

Species	Salmonella typhimurium strains
Strain	TA strain (TA98)
Source	Lyophilized discs of TA98 strain obtained from Krishgen Biosystems, Mumbai
Supplied by	KRISHGEN Biosystems No: 318/319, Shah & Nahar Ind. Premises, 3rd Floor Off. Dr. E. Moses Road, Worli, Mumbai Maharashtra – 400 018 Phone: 022-49198700 Email: info@krishgen.com
Storage conditions	4°C

1.4. Characteristics [19]

Salmonella typhimurium strains are histidine-dependent. Each strain contains a different type of mutation in the histidine operon. Frame shift and base-pair substitution defects are represented to identify mutagens of both the types. Additional genetic markers also serve to make the strains more sensitive to certain types of mutagens.

1.5. Preparation of tester strains [20]

Revival of Bacterial Stock Culture

- i) Lyophilized discs of TA98 strain were used to culture the bacterial strains.
- ii) Vial containing each strain was kept at room temperature in bio safety cabinet, to inoculate in Oxoid NB.
- iii) The Oxoid broth was incubated in a shaker water bath at 37°C for 12 h or overnight.
- iv) Following 12hours, the conical flask was stored at 2-8°C until use.
- v) 0.1 ml of Salmonella typhimurium strains such as TA98 was inoculated in 10 ml of Oxoid nutrient medium and incubated in a shaker water bath at 37°C for 12h with agitation at 70-80 rpm.
- vi) At the end of incubation, the cell viability of the bacterial strain was checked by measuring the optical density of the nutrient broth at 620 nm using a Microplate Reader (BIORAD).

vii) The optical density (OD) of all the bacterial strains was greater than 0.4 at 620 nm. Hence cultures were considered to have a viable cell count of  $\geq 1.5 \times 10^8$  cells/ml.

viii) Bacterial strain density was checked using the dilutions of the strains at different time points and the respective dilutions used were taken for the conduct of the study. The Bacterial strain growth curve report of the individual strain data will be appended in the raw data file of the study.

**Results**

Table 4: Macroscopic Character

Characters	Results
Color	Green to black
Shape	Big oval or Pear shaped
Size	17-25 cm long; 15-20 cm diameter
Weight	0.5 to 20 lbs.
Apex	Acuminate to blunt
Surface	Smooth
Odor	Odorless

**Quantitative Tests**

Parameters	Results
Ash Value	5.22%
Water soluble Ash	6.30%
Acid insoluble Ash	00.80%
Water soluble extractive values	0.74%
% Moisture Loss	10.2%

**Qualitative Physiochemical Parameters**

Table 6: Qualitative Physiochemical Parameters

Sn.	Phytochemicals	Extracts	
		Ethanol	Aqueous
1	Carbohydrates	++	++
2	Proteins	--	--
3	Vitamin C	++	--
4	Steroids	--	++
5	Saponins	++	--
6	Flavonoid	++	--
7	Alkaloids	++	++
8	Tannins	++	--
9	Phenolic	++	--

Table 7: Evaluation Parameters

Parameters	Types of Formulations		
	F1	F2	F3
Texture	Smooth	Smooth	Smooth
Color	Light Brown	Light Brown	Dark Brown
Consistency	Good	Poor	Good
Clarity	Clear	Clear	Clear
Grittiness	Present	Absent	Absent
Homogeneity	Present	Present	Present

Table 8: Data of pH of Different Formulation

Sn.	Formulations	pH (Mean ± SD)
1	Carbopol 940 (F1)	7.4±0.1
2	Carbopol 934 (F2)	7.5±0.1
3	Xanthine gum (F3)	7.4±0.1

Table 9: Data of Consistency of Different Formulation

Sn.	Formulations	Consistency
1	Carbopol 940 (F1)	Soft
2	Carbopol 934 (F2)	Soft
3	Xanthane gum (F3)	Soft

Table 10: Data of Color of Different Formulation

Sn.	Formulations	Color
1	Carbopol 940 (F1)	Transparent
2	Carbopol 934 (F2)	Transparent
3	Xanthane gum (F3)	Transparent

Table 11: Data of Odor of Different Formulation

Sn.	Formulations	Odor
1	Carbopol 940 (F1)	None
2	Carbopol 934 (F2)	None
3	Xanthane gum (F3)	None

Table 12: Group Data of Spread ability of Different Formulation

Sn.	Formulations	Spread ability (Mean ± SD)
1	Carbopol 940 (F1)	48±1
2	Carbopol 934 (F2)	38±1
3	Xanthane gum (F3)	39.3±0.6

Table 13: Group Data of Homogeneity of Different Formulation

Sn.	Formulations	Homogeneity
1	Carbopol 940 (F1)	Homogenous
2	Carbopol 934 (F2)	Homogenous
3	Xanthane gum (F3)	Homogenous

Table 14: Group Data of Viscosity of Different Formulation

Sn.	Formulations	Viscosity (Mean ± SD)
1	Carbopol 940 (F1)	5121.7±1.5
2	Carbopol 934 (F2)	4309.7±0.6
3	Xanthane gum (F3)	945.3±1.5

Table 15: Group data of colonies

Concentration (mg/plate)	Formulations (Mean ± SD)		
	F1	F2	F3
NC (Sterile Water)	51.33±1.5	53±1.0	52.66±2.1
25	46±4.4	50.66±1.5	49.33±1.5
50	33.33±1.5	39±1.0	38±1.0
100	25±1.0	29±2.0	28.66±0.6

### Conclusion

This experiment was the scientific report that proves phytochemical studies and also observed that, from all the three formulations, the F1 formulation containing Carbopol 940 was best suited. The F1 formulation established greater spread ability and consistency when compared with other developed gels. The formulated F1 gel demonstrated good homogeneity, an excellent pH, minimal skin irritation and great stability. The maximum percentage of moisture loss was found to be 4.6% after 24 hrs. in F1 formulation. In vitro test for the anti-microbial properties were performed with salmonella typhi, strain TA 98. The colonies of the bacteria were counted and recorded for the analysis and finally 100mg/mL concentration showed the better result as compare to the other concentrations of the formulation.

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