Formulation And Evaluation of Pharmaceutical Aqueous Gel of Citrus Aurantium Dulcis (Orange) Peels Extract for Mouth Ulcer Treatment

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Abstract: The objectives of present investigation were to formulate and evaluate herbal gel for mouth ulcer treatment of dried powdered citrus aurantium dulcis (orange) peels extract. Herbal gel was prepared by using different concentration of powdered citrus aurantium dulcis and carbopol 934, propylene glycol as a gel base. Formulations were evaluated for various physical parameters. The formulated gel was transparent, homogeneous and had PH ranges from 7 to 7.5.Also this formulation showed acceptable rheological behavior with applicable spread ability and extrudability properties. Antimicrobial studies of gel showed excellent efficacy against staphylococcus aureus and Escherichia coli. From the experimental evidence of in-vitrostudies it was observed that powdered contain flavonoids so it showed significant antioxidantand antimicrobial activity. This herbal aqueous gel formulation was stable, safe and effective for the treatment of mouth ulcer.

Keywords: Mouth ulcer gel, Orange, Antimicrobial activity, Antiulcer activity, citrus aurantium dulcis.

1. INTRODUCTION:

Mouth ulcers are yellowish to whitish depression with red margination in mucus lining of mouth cavity which was characterized by pain and inflammation. On the basis of clinical status, there are three groups of mouth ulcer patients: major, minor and herpitiform. The diagnosis was based on clinical signs due to the unknown or often misunderstood Etiology. **[Jain NK et al .2020**]. Mouth ulcer is also recognized as canker sore –painful lesions so as to develop in mouth at base of yourgum. This mouth ulcer causes various problems during drinking, eating and talking uncomfortable. The Treatment includes antibiotic or analgesic gel formulation, semi-solid formulation and antiseptic mouthwash, such as chlorhexidin mouthwash or povidone iodine mouthwash. **[Madaan V .et al 2022]** Generally gels are containing synthetic as well as semi synthetic active agents which have some disadvantages like irritation, staining on the teeth, and burning sensation only it is due to the presence of alcohol content and other organic compounds. The present study deals with use of herbal powdered orange peel in the treatment of mouth ulcer in pharmaceutical gel. **[Shaikh S et al., 2018]**

Citrus, genus belonging to family Rutaceae includes a variety of species of diverse sizes and forms, usually known as lemons, oranges, limes, citrons, mandarins and grapefruits. This is one of the central horticultural crops and had a universal agricultural production with 100 million tons per year. Previously, Citrus plants were linked by herbal medicine in many Asian countries such as china, Japan, and Korea etc. This citrus plant is in recent years commercialized for their fruits along with juice, or used as additives in several industries. Other than it contain rich in vitamin C and vitamins B, The Citrus fruits also contain minerals, macronutrients likely lipids, carbohydrates, crude proteins, dietary fibers and phenolic compounds with important health-promoting properties. Alternatively, the citrus species containing essential oils are widely used in pharmaceutical, food, beverages, perfumes, and cosmetic industries. **[Maksoud S, 2021]**

Antioxidants have been commonly used as food additives so it provides protection against oxidative degradation of foods. Thire are some commonly used synthetic antioxidants are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), Propyl gallate (PG), and butylated hydroquinone. But, these synthetic antioxidants have also some side effects such as liver damage to carcinogenesis. That's why; there is a need for isolation and characterization of natural antioxidant which having less or no side effects, in that condition foods or medicinal materials in order to replace synthetic antioxidants. The significance of aromatic plants as natural antioxidants has been well traditional. **(karoui IJ and Marzou B, 2013)**

2. MATERIAL AND METHOD

Collection of Plant material:

Orange fruit were collected from the local area and then the peels powder was dried at room temperature in shade. The peels powder prepared for extraction purpose with ethanol. In this study Carbomer 934, Triethanolamine, methyl paraben, propyl paraben and ethanol chemicals was used.

Preparation of herbal Gel:

Required amount of Carbopol 934 was dispersed in specified quantity of distilled water with continuous stirring. The given quantity of methyl paraben and propyl paraben with 5ml of distilled water was heat to dissolve by using water bath. Then mixed the above mixture to different concentration of Orange peel ethanolic extract, Finally this full mixture were mixed properly to the Carbopol 934 gel by continuous stirring and add triethanolamine drop wise to this formulation for maintaining the required pH (6.8-7). Make up the volume till 100 ml with distilled water; lastly add few drops of flavoring agent such as Peppermint oil. **(Das S, 2010).** The composition of herbal gel prepared from the powdered Orange peel coded as F1, F2, and F3 is tabulated in Table 1.

Table no 1: Composition of various gel formulations

Ingredients	F1	F2	F3
Orange peel extract	2%	1%	0.5%
Carbopol 934	2%	2%	2%
Methyl paraben	0.0015%	0.0015%	0.0015%
Propyl paraben	0.01%	0.01%	0.01%
Triethanolamine	q.s + pH 6.5-7	q.s + pH 6.5-7	q.s + pH 6.5-7
Peppermint oil	q.s	q.s	q.s
Distilled water	Up to 20ml	Up to 20ml	Up to 20ml

Evaluation of Herbal Gel:

Physical Appearance:

Physical parameters include appearance and colour was checked.

Measurement of Ph:

The pH of herbal gel formulations were evaluated by using digital pH meter. 1 gm of above gel was taken and dispersed in 10 ml of distilled water then keep aside for two hours. The determination of pH of formulation was carried out in three times and the average values are noted. **(Sanghvi NM, 1989**) The pH of gel formulation was reported in **table no 2**.

Homogeneity:

The prepared gel formulations were tested for homogeneity by visual inspection. The gel was tested for their presence and appearance of any aggregates **(Gupta A, 2010).** The homogeneity of gel formulation was reported in t**able no 2**

Spreadability:

It is determined by glass slide and wooden block apparatus. Weight about 20gm was added to the pan and the time was noted for upper slide to move to separate completely from the fixed slide. An excess amount of gel 2gm was placed on this ground slide, then sandwiched the gel between this slide and a further glass slide having the fixed ground slide (there is provided with the hook). A 1kg weighted was located on the top of the slides for 5 minutes to give a homogeneous film of the gel and air between the slides was removed. Remove off the excess of the gel, The top plate was pull with the help of spring attached to the hook, the time required by the top slide to cover a distance of 7.5cm was be noted. A short or less interval shows better Spreadability. **(Pawar DP, 2013**) The Spreadability of gel was determine by using the above formula and was reported in table no 2.

$S = M \times L / T$

Where,

S - Spreadability.

M -Weight in the pan which is tied to the upper slide.

L- Length moved by the glass slide.

T- Time taken to separate the slide completely each other. (Seconds)

Clarity:

The clarity of all batches was determined by visual inspection

Viscosity:

It was determined by using Brookfield viscometer (DV-III programmable Rheometer). **Extrudability:** The above gel formulations were filled in standard capped collapsible aluminium tubes and sealed to the finish. It was determined by pressing of the thumb.

Gel strength:

The 5gm Sample of each optimize batches was taken as well as 3.5gm weight was placed on the surface of gel. The penetration time was noted as shown in **table 3**. This Gel strength was determined by the time (seconds) required by the weight to penetrate in the gel.

Bioadhesive Strength:

This was used to measuring the force required to detach the formulation from cellophane membrane by using wooden block apparatus and the glass slides. 1 gm of formulated gel was taken on glass slide and wrapped with cellophane membrane; the movable glass slide was placed on fixed slide. Two minute contact time was required to ensure contact between membrane and formulations. The weight was added in pan until slides got detached**. (Jaiswal J, 2012).**

The bioadhesive force, expressed in dyne/cm2 was determined by given formula, which was reported in table 3.

Detachment stress = $m \cdot g/A$

Where,

m = Weight required to detach two glass slides from each other (gm).

g = Acceleration due to gravity (980 cm/s2).

A = Area of membrane exposed (cm2).

Stability study:

It was done with open and close containers. Here, the stability of gel at a room temperature for 1 month Stability study was calculated in table 3.

Antimicrobial Activity:

Agar plate media was prepared by adding 28 g of a nutrient agar powder in 1 liter of distilled water heat the mixture and dissolve all components. The dissolved mixture is put in autoclave at 121^0 C for 15 min, allow cooling but not solidifying. Then inoculated the given microorganism in to nutrient agar medium and poured into plates allow until solidified. Then by using the agar well diffusion method, holes about 9 mm diameter in the same medium with a borer. The antimicrobial solution of Orange peel extract directly placed in the holes. The plates are incubated and reported in **table no 4** and shown in **figure 1. [Thawkar MM et al 2022, Jeurkar MM et al 2019, Jeurkar MM et al., 2022, Pandey A et al, 2011]**

In-Vitro Anti-Oxidant Study Of Powderedorange Peel Extract

DPPH Assay (1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity)**:**

The Orange peel extract was measured by DPPH assay for free radical scavenging activity. The powder was dissolved in methanol to prepared 0.1 mm solution and 1 ml of this solution to prepared different concentrations of test tube. Concentration up to 50 to 300 µg/ml. After 30 min, absorbance was measured at 517 nm. If Shows lesser absorbance of reactant mixture then it indicates superior free radical scavenging activity. The experiment was repeated for three readings, which was reported in **table no 5 (Blois MS, 1958) (Mathangi T and Prabhakaran P, 2013)**

3. RESULT AND DISCUSSION:

Formulations were evaluated for various physical parameters. The formulated gel (F3) was transparent, homogeneous and had PH ranges from 7 to 7.5. Also this formulation showed acceptable rheological behavior with applicable spread ability and extrudability properties. Antimicrobial studies of gel showed (figure no.1) excellent efficacy against staphylococcus aureus and Escherichia coli. From the experimental evidence of in-vitro studies it was observed that powdered contain flavonoids so it showed significant antioxidant and antimicrobial activity.

Table no 2: in-vitro evaluation parameters

Formulation	Physical appearance	pH	Homogeneity	Spread ability	Viscosity (Pa.S)	Extrudability
F1	Greenish	6.8±0.9	Good	5.70±0.1	3.174 ±0.01	Good
F2	Greenish	7±0.9	Good	5.86±0.15	3.073±0.049	Good
F3	Greenish	6.9±0.5	Good	6.52±0.05	2.334±0.012	Good

***Spread ability (gm.cm/sec)**

Table no 3: In-vitro evaluation parameters of gel

Formulation	Bioadhesive strength	Gelling Strength	Open Container	Closed Container
F1	4422.22 ± 18.82	42±0.75	Unstable	Stable
F2	3525.31 ± 31.09	36±0.07	Unstable	Stable
F3	2873.48 ± 18.25	27±0.5	Unstable	Stable

Table no 4: In-vitro Anti-microbial Activity

Formulation	Bacteria	
	Staphylococcus aureus	***Escherichia coli***
F1	14.8	14.1
F2	16.2	15.7
F3	18.3	16.8

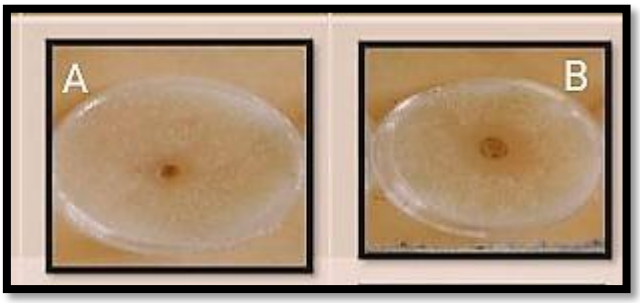

Figure no 1: Anti-microbial Activity of gel of citrus aurantium dulcis (F3)
A: *Staphylococcus aureus* and B:*Escherichia coli*

Table no 5: In vitro antioxidant study by DPPH assay

Concentration µg / ml	% Inhibition of Powdered Orange peel extract	Concentration µg / ml	% Inhibition Ascorbic acid
25	71.080±0.045	25	45.91±0.78
50	75.342±0.048	50	50.19±0.02
100	78.614±0.085	100	55.83±0.07
150	80.974±0.020	150	63.12±0.96
200	88.051±0.010	200	79.06±0.22

4. CONCLUSION:

In this research study, it was concluded that the formulation of herbal gel having significant, therapeutically effective and appropriate Antimicrobial activity. The developed herbal aqueous gel formulation was stable, safe, effective and suitable for the treatment of mouth ulcer.

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