

Formulation and Evaluation of Herbal Emulgel from Coccinia Grandis Linn Fruit Extract for Fungal Dermatological Infection.

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ABSTRACT

Herbs have been prized for their healing abilities and today we still rely on the curative properties of plants. After nearly two centuries of inexorable decline in the use of herbal medicines, Herbs, which have always been the principal form of medicine in developing countries, are once again gaining popularity throughout the developed world. Emulgel is the unique combination of gels and emulsions, these possess several merits over conventional dosage forms like creams and ointments like thixotropic, nongreasy, non-adhesive, etc. These are safe, effective and also economical in nature. The present review on the recent scientific advances related to the development and evaluation of emulgel formulations with plant-based drugs and related products. Coccinia is a medicinal plant that is used in ancient times for relieving insect bite itching and swelling. The aim of the present research work was to evaluate the potential and develop future scope of emulgel formulation for enhancing the topical delivery of coccinia grandis fruit extract.

Key words: coccinia grandis linn fruit, herbal emulgel, hydroalcoholic extract, antifungal activity.

I. INTRODUCTION

Man's existence on earth has been made possible only because of the vital role played by the plant kingdom in sustaining his life. Plants were being used as a source of medicine from the times immemorial as they were easily accessible as well as inexpensive, plants have provided the basis for the oldest medicinal systems of human history. The earliest mention of medicinal use of plant is found in Rigveda.^[1,2]

It is great to the credit of the people of India that they have been gifted with larger number of medicinal plants than the natives of any country on the face of earth. Natural products have served

as an important source of drugs since ancient times and about half of the useful drugs today are derived from natural sources, India is being sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicine. This country is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world. It is generally estimated that over 6000 plants in India are used in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the world countries.^[3,4]

The effective use of automated procedures and data bases in the isolation, identification and biological profiling of bioactive compounds from natural sources will be a best guarantee to the continued discovery of novel chemotypes from nature.^[5,6] Natural products research continues to explore a variety of lead structure, which may be used as template for the development of new drugs by the pharmaceutical industry, while microbial products have been the mainstay of industrial natural product discovery.^[7]

Emulgel is a combination of emulsion and gel, which is a new approach for topical delivery of drugs. It has a double control release like emulsion and gel.^[8] Gel is new class of formulation, it releases the drug faster in comparison ointment, cream and lotion. Incorporation of drug in emulgel formulation is suitable to treat skin disorders. Topical application of therapeutic agents provides various advantages over the other route of administration. The presence of a gelling agent in the aqueous phase converts a classical emulsion into an emulgel. Within the major group of semisolid preparations, like use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations.^[9] Emulgels have several complimentary properties for dermatological use such as being thixotropic, greaseless, easily spreadable, easily removable,

emollient, non-staining, long shelf life, bio-friendly, transparent and pleasing appearance^[10] Herbal medicine is still the main stay, about 75-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents. Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades, advances in phytochemistry and in identification of plant compounds, effective against certain diseases have renewed the interest in herbal medicines.^[11] *Coccinia grandis* belongs to the family Cucurbitaceae, is a growing wild throughout India and also cultivated in various parts of India. It is commonly known as kundru. The whole plant is traditionally used for various medicinal purposes. Leaves of this plant are used in Indian folk medicine for treatment of number of ailments including diabetes, wounds, ulcers, inflammation. Scientific investigation of crude extract of *Coccinia grandis* have showed that it possesses hepatoprotective^[12,13] antioxidant^[14,15] anti-inflammatory anti-diabetic^[16,17] hypolipidemic^[18] anti-bacterial^[19,20] and antitussive activities^[21,22]. The aim of the present study was to formulate Emulgel by using hydroalcoholic extract of *coccinia grandis* fruit and evaluate for antifungal potential.^[23]

II. PLANT PROFILE



fig.1 *Coccinia grandis* linn

2.1 Scientific classification

- Kingdom: - Plantae
- Clade: -Angiosperms
- Order: -Cucurbitales
- Family: -Cucurbitaceae
- Genus: -*Coccinia*
- Species: -*C. grandis*

2.2 Binomial Name

Coccinia grandis

2.3 Synonyms

Ivy gourd(Eng)
Kundru (Hindi)
Tondli(Marathi)
Dondakaya (Telagu)

2.4 Description

Coccinia grandis plants from Asia have white flowers, while plants from Africa and the Arab Peninsula have pale-yellow flowers. The white flowered form is a noxious weed in Hawaii, many tropical islands and Western Australia, and is an important target of biological control research. The yellow-flowered form was long considered a separate species called as *Coccinia schimperi* Naudin, but in 2015 this name was synonymized as *Coccinia grandis*^[21].

2.5 Habitat

Coccinia grandis (Ivy gourd) is occasionally cultivated as a garden vegetable in the tropical and sub-tropical regions of the world. It is believed to be native to central Africa, India, and Asia. Its long history of usage, cultivation, and transportation by people has obscured its base. It is a common weed in South East Asia. It is considered a valuable wild vegetable by the indigenous people of southeast Asia and India^[22].

2.6 Part Used

Fruit

2.7 Traditional Uses

The whole plant is traditionally used for various medicinal purposes and leaves are used in Indian folk medicine for treatment of number of ailments including diabetes, wounds, ulcers, inflammation, in eruptions of skin, fever, asthma and cough. Scientific investigations have shown that the crude extract possesses hepatoprotective, antioxidant, anti-inflammatory, anti-nociceptive, anti-diabetic, hypolipidemic, antibacterial and antitussive activities. Though the plant has been reported for many biological activities. The present investigation was therefore, taken up to establish identity of fresh and dried leaves morphologically microscopically and physiochemically for the standardization of the drug.^[23]

III. MATERIAL AND METHODS

Plant material:

Collection and Drying

Fresh Fruits of *Coccinia grandis* linn were collected from the fields of LohegaonDist-Pune, Maharashtra India. cleaned and cut into small pieces of and dried at a room temperature. The dried fruits were coarsely powdered in grinder. Large difference in particle size of crude drug results in long extraction time as the coarse particles increases the extraction time and fine may form bed,so the powdered material was sieved through 60 to 120 mesh to remove fines and larger particles and the powder was subjected for further study.



fig.2 Collection of fruitsfig.3 Fresh fruits

3.2. Pharmacognostic Study of Fruits

3.2.1 Macroscopy

Size: 25-60 mm long,15-35mm in diameter.

Shape: Broadly ovate, Subpentagonal to orbicular in shape.

Color: Green with White stripes

Odor: Characteristics

Taste: Characteristics

3.2.2 Microscopy

Thin transverse section of middle part of the fresh fruit was taken stained with Phloroglucinol+Conc.Hcl (1:1), observed under 10X, and45X.

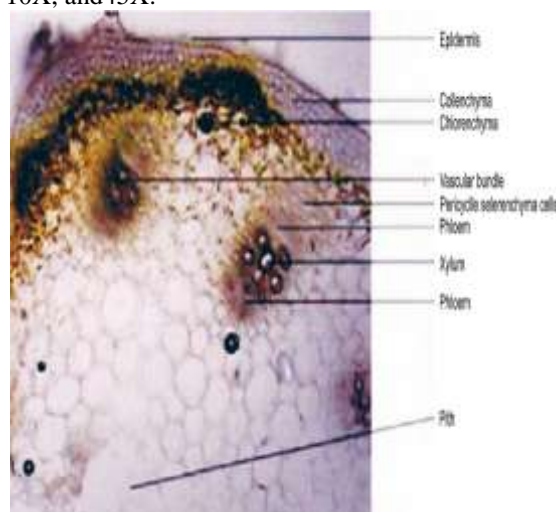


fig.4

3.2.3. Microscopic studies of Powder characteristics

The microscopic examination of powdered fruit material was performed to detect and to establish various peculiar microscopic characters in order to differentiate between the adulterated and the substituted powdered or intact leaves supply. Slides of powdered leaf material was prepared using formalin, glycerine and water (8:1:1 v/v/V) and were thus embedded and seen under microscope on different magnifications at 10x, 40x, and 100x after staining with phloroglucinol andconc HCL [24]

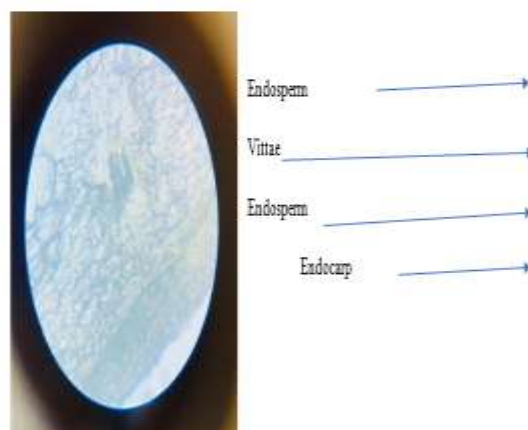


Fig.5 Powder Characteristics

3.3 Determination of Loss on Drying

Fresh fruit (5 gm) was taken without preliminary drying and was placed on a tarred evaporating dish and dried at 105°C for six hours and weighed.

The drying was continued until two successive reading matches each other or the difference between two successive weighing after drying for 30 minutes in a desiccator, showed not more than 0.01 g difference.

Weighed and calculated the loss on drying in terms of percent w/w.^[25]

Observation

Weights of powdered sample taken = 5 gm

Weights of powdered sample After drying = 0.49 gm

Result:

The percentage of loss on drying = 9.2 % w/w

3.4 Determination of ash Value

Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards. Ash value is used to determine quality and purity of crude drug. Ash value contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. sometimes, inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects.^[26]

Procedure:

2 gm of the air dried powdered drug was taken in a crucible and ignited in the furnace at 150°C for 45 minutes and weighed same procedure was followed for 4 times till constant weight of the ash, was formed weight of the ash was taken and calculated the percentage of total ash with reference to the air dried sample of the crude drug.

Before ignition of powder:

- Weight of empty silica crucible = 15.78 gm
- Weight of powder = 2gm
- Weight of silica crucible + powder = 17.78gm

After ignition of powder:

- Weight of silica crucible + ash = 16.27 gm
- Total ash = 0.49 gm

Result

The percentage of total ash obtained = 24.5 % w/w

3.5. Extraction Methodology

Material and Methods

Preparation of the plant extract

Powder sample of fruit coccinia grandis measuring 100 gm was extracted using 500 ml of hydroalcoholic solvent by maceration method after filtration the extract was concentrated using a rotatory vacuum evaporator and the semidried extract were dried kept in air tight

3.6 Percentage yield of Extract

It indicates the approximate measures of the chemical constituent plant. All the values were taken in triplicate and the mean average was taken. 50 gm of the powdered drug was taken in a weighing bottle and transfer it in 500 ml graduated flask filled with the solvent (90 % alcohol).

Flask was closed and set aside for 24 hours, shaking frequently. (Maceration) extract was filtered into a 50 ml cylinder. When sufficient filtrate has collected, 100 ml. of the filtrate was taken and evaporated to dryness on a water-bath and complete the drying in an oven at 100° C. it was cooled in a desiccator and weighed^[27] percentage w/w of extract was calculated with reference to the air-dried drug.

- Weight of powdered taken. = 50gms
- Weight of Extract obtained = 2.5gms

Result

Percentage yield of hydro-alcoholic extract was found to be 5.0 % w/w.

3.7 Preliminary Phytochemical Screening

1. Test for Steroids^[28]

a) Salkowski test

One ml of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish brown colour exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

b) Liebermann test

2 mg of the residue few ml of acetic anhydride was added and gently heated. The contents of the test tube were cooled and 2 ml of concentrated sulphuric acid was added from the side of the test tube. Development of blue colour gave the evidence for presence of sterols.

c) Liebermann-Burchard test

10 mg extract was dissolved in 1ml of chloroform and 1ml of acetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid

from the sides of the test tube. Formation of reddish violet colour at the junction indicates the presence of steroids.

2. Test for Saponins^[29]

a) Foam formation test

1ml solution of the extract was diluted with distilled water to 20 ml And shaken in a graduated cylinder for 15 minutes. The development of stable foam indicates the presence of saponins.

3. Test for Alkaloids^[30]

a) Dragendorff's test

01 ml dilute hydrochloric acid and 0.1 ml Dragendorff's reagent was added in 2 ml of extracts in test tube. Formation of orange brown precipitate indicates the presence of alkaloids.

b) Mayer's test

02 ml of extract was taken in test tube. 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff precipitate indicates the presence of alkaloids.

c) Wagner's test

2ml of extract was treated with 0.2 ml dilute hydrochloric acid and 0.1 ml of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

4. Test for Glycoside^[31]

a) Modified Borntrager's test

The extract was treated with ferric chloride solution and heated on Boiling water bath for 5 min. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half its volume of ammonia solution. The formation of rose pink or cherry red colour in the ammoniacal layer indicates the presence of anthraquinones.

b) Million's test

The extract was treated with 2 ml of Million's reagent. The formation of white precipitate, which turns to red upon heating indicates the presence of proteins and amino acids,

5. Test for Tannins^[32]

a) Ferric Chloride test

Five ml of extract solution was allowed to react with 1 ml of 5 per cent ferric chloride solution. Greenish black colouration indicates the presence of tannins.

b) Lead Acetate test

5 ml of extract was treated with 1 ml of 10 per cent aqueous lead acetate solution. Development of yellow coloured precipitate indicates the presence of tannins.

c) Potassium Dichromate test

Five ml of extract was treated with 1 ml of 10 per cent of aqueous potassium dichromate solution. Formation of yellowish-brown precipitate suggests the presence of tannins.

6. Test for Proteins^[33]

a) Biuret test

The extract was treated with 1 ml of 10 per cent sodium hydroxide solution and heated. A drop of 0.7 per cent copper sulphate solution was added to the above mixture. The formation of purplish violet colour indicates the presence of proteins.

b) Xanthoproteic test

A little test residue was taken in 2 ml of water and to it 5 ml of concentrated nitric acid was added. Formation of yellow colour indicates the presence of proteins.

c) Million's test

The extract was treated with 2 ml of million's reagent. The formation of white precipitate, which turns to red upon heating, indicates the presence of proteins and amino acids.

7. Test for Amino Acids^[34]

a) Ninhydrin test

The extract was treated with Ninhydrin reagent at pH range of 4-8 and boiled. Development of purple colour indicates the presence of amino acids.

8. Test for Carbohydrates^[35]

a) Molish test

Two ml of extract solution was treated with few drops of 15 per cent ethanolic alpha-naphthol solution in a test tube and 2 ml of concentrated sulphuric acid was added carefully along the sides of the test tubes. The formation of a reddish violet ring at the junction of two layers indicates the presence of carbohydrates

b) Barfoed's test

The test residue was dissolved in water and heated with a little quantity of Barfoed's reagent. Development of brick red precipitate

within wt.0 minutes shows the presence of monosaccharide.

9. Test for Reducing sugars ^[36]

a) Fehling's test

Five ml of extract solution was mixed with 5 ml of Fehling's solution equal mixture of Fehling's solution A&B & boiled. Formation of

brick red Precipitate indicates the presence of reducing sugars.

b) Benedict's test

Equal volumes of Benedict's reagent and extract in test tube boiled for min solution appeared green, yellow or red depending upon the amount of reducing sugar present



Fig: preliminary test (1)



Fig: preliminary test (2)

Table:1 Preliminary Phytochemical Screening

Chemical tests	Hydroalcoholic Extract	Benzene
Test for Steroids	+	-
Test for Saponins	+	-
Test for Alkaloid	+	+
Test for Glycosides	+	-
Test for Reducing sugars	-	+
Test for Tannins	+	+
Test for Flavonoids	+	-
Test for Amino acids	+	-
Test for Carbohydrates	+	-

3.8 Formulation of emulgel

Formulations with different quantity of ingredient were made as shown in Table 1. The gel portion of the emulgel was made by dissolving carbopol-934 in cold water with constant stirring at a moderate speed until uniform mixture was made. The pH was then adjusted to 6-6.5 using triethanolamine (TEA). Tween 80 was dissolved in distilled water to prepare the aqueous phase of the emulsion while for the preparation of the oil phase of the emulsion; span 80 was dissolved in liquid paraffin. To preserve the emulsion, methyl

parabene was dissolved in propylene glycol and the extract was dissolved in ethanol then both solutions were mixed with the aqueous phase. Both the aqueous and the oil phase were heated in a water bath at 70 °C separately. Then the oil phase was added drop wise to the aqueous phase with continuous stirring using homogenizer at speed of 3000 rpm for 10 min then cold to room temperature. At the end the gel and emulsion portions were mixed in 1:1 ratio with moderately stirring to prepare emulgel ^[37]

Table: 3

Sr.No.	Ingredient	Quantity Taken
1	Hydroalcoholic extract	5gm
2	Carbopol 934	0.75 gm
3	Liquid paraffin	2.5ml
4	Span 60	0.45ml
5	Tween 80	0.50ml
6	Propylene glycol	3.5ml
7	Methyl paraben	0.01gm
8	Distilled water	Up to q.s.

3.9 Antifungal Assay

Three fungal strains (*Aspergillus niger*, *Rhizopus*, *mucor*) These strains have been selected for the basis of its application purpose of further

formulation study. antifungal potential of emulgel was assessed in terms of zone of inhibition of bacterial growth. The results of the antifungal activities are presented in Table.2

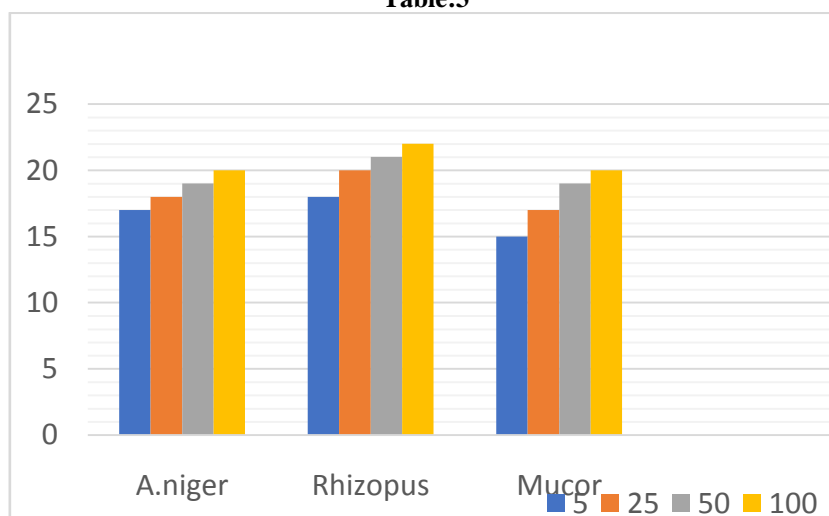
Table:2
Antifungal activities of EMULGEL of fruit coccinia grandis against fungal organism

Antifungal Activity (zone of inhibition)			
Concn. In ($\mu\text{g/ml}$)	A . Niger.	Zone of inhibition in mm Rhizopus	Mucor
5	17	18	15
25	18	20	17
50	19	21	19
100	20	22	20

Antifungal activities of the extracts increased linearly with increase in concentration of extracting ($\mu\text{g/ml}$). As compared with standard drugs, the results revealed that in the extracts for fungal activity, *Rhizopus* shows good result as

compare with *Aspergillus Niger* and *Mucor* The growth inhibition zone measured ranged from 11 to 20mm for all the sensitive bacteria, and ranged from 14 to 20mm for fungal strain.

Table:3



3.10. Evaluation of Topical Gel Formulation

A. Physical Evaluation:

Physical parameters such as color and appearance were checked.

B. Measurement of pH:

pH of the gel was measured by using Ph meter.

C. Viscosity;

Viscosity of gel was measured by using Brookfield viscometer with spindle.

D. Spreadability:

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at an end. By this method spreadability was nearest on the basis of slip and drag characteristics of gel. An excess of gel (about 2g) under study was placed on the ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A one kg weighted was placed on the top of the two sides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gm. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5cm be noted. A shorter interval indicate better spreadability. Spreadability was calculated using the following formula:

$$S = M \cdot L / T$$

Where,

S = Spreadability,

M = Weight in the pan (tied in the upper slide)

L = Length moved by the glass side

T = Time (in sec.) takes to separate the side completely each other.

E. Stability Study:

The stability study was performed as per ICH guidelines & The Formulated gel were filled in the collapsible tubes and stored at different temperatures and humidity conditions, viz 25°C/20% RH, 30°C/60% RH, 40°C/75% RH for a period of three months and studied for appearance, pH, and spread ability.

IV. RESULT AND DISCUSSION

Coccinia grandis Linn fruit under microscope T.S shows mesophyll cells, palisade cell. L.S. shows Xylem Phloem and Fibres, powder shows Fibres and Stomata. *Coccinia grandis* Linn fruit after drying calculation of loss of drying was to be obtained 21.6% w/w and powder under muffle furnace gives ash Value of 23%

w/w. *Coccinia grandis* Linn fruit after maceration extraction and constant stirring in water bath gives the product, that product was calculated 5% w/w to be obtained. *Coccinia grandis* Linn showing Preliminary Phytochemical screening Hydroalcoholic Extract shows presence of Steroids, alkaloids, Glycosides, Saponins, phenols, Tannins, Flavonoids. Benzene shows presence of reducing sugars, alkaloids, tannins are present.

V. CONCLUSION

Emulgel of *Coccinia grandis* linn fruit showed prominent anti-fungal activity against human pathogenic, thus *Coccinia grandis* linn fruit can be used as a potential antifungal drug against human pathogenic fungal infection. Lupeol and Taraxerone are measure constituents of Hydroalcoholic extract of *Coccinia grandis* fruit. In-vivo studies performed for activities like mouth ulcer, wound healing and anti-fungal showed the beneficial use of 20% w/w of hydroalcoholic extract of *Coccinia grandis* fruit for the said activities. It was also supported by molecular docking study carried out for Lupeol and Taraxerone which gives better Dock score for mentioned activity.

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