COCCINIA GRANDIS FRUIT EXTRACT GEL FOR THE TREATMENT OF MOUTH ULCER ALONG WITH ASSOCIATED WOUND AND INFLAMMATION

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Abstract: Culinary utility of the Coccinia grandis (Cucurbitaceae) indicates the use of fruits in the form of different recepies. Coccinia grandis (Cucurbitaceae) is a perennial plant popularly known as kundru, Tondlee, Ivy gourd, Bimbi and Scarlet gourd etc. This herb is used in folk medicine in the form of intact fruits for healing oral ulcers. So the present work was done with an objective to develop a gel formulation from hydroalcoholic extract of coccinia grandis fruit for the treatment of ulcer and for healing of associated wound and inflammation. Molecular docking study was carried out for lupeol and taraxerone, the chemical constituents contained in the fruits. The dock score values were found to be -51.83 and -35.87 respectively for antiulcer activity. For wound healing activity the values found were -27.25 and -27.17 respectively for the two components, whereas the dock score values obtained for the anti-inflammatory activities were -58.6632 and -58. respectively for lupeol and taraxerone. The negative dock score value shows better affinity to bind receptor site. Application of hydroalcoholic extract gel of (20%w/w) for antiulcer and wound healing activities caused a significant reduction in ulcer and wound area when compared with the untreated controls and marketed formulation. Topical application of gel at 20%w/w concentration showed significant reduction in carrageenan induced rat paw edema. Studies carried out for Antiulcer, Wound Healing and Anti-Inflammatory activities showed promising results proving the standardized utilization of traditional herb having culinary applicability. Moreover the data was also supported by the results of computational studies of major constituents responsible for the said activities. The approach in the present study was fruitful to prove the prospectives of hydroalcoholic extract gel of Coccinia grandis fruit as the effective treatment for ulcer and healing associated with wound and inflammation.

Keywords: Antiulcer, Anti-inflammatory, Coccinia grandis, Culinary herb, High Throughput Screening, Molecular docking, Wound healing.

1 INTRODUCTION

Coccinia grandis (Linn) Voigt belonging to family cucurbitaceae, commonly known as “Ivy gourd, and synonym Coccinia grandis var. wightiana M poem Greb (www.theplant list.org) scarlet gourd” (English), “Kanduri, kundru (Hindi) and “Bimbi, tundika” (Sanskrit) is distributed throughout the hotter parts of India. In the literature, Coccinia grandis is discribed to have wide range of medicinal applications. Cucurbitacins comprise of a group of tetracyclic triterpenoids found in the Cucurbitaceae family. Plants containing cucurbitacins are found to possess anti-inflammatory, antipyretic, analgesic, anti-tumor and anti microbial, antiulcer, wound healing), antifungal, antidiabetic activities. Indian system of traditional knowledge i.e. Ayurveda is well known for its effective herbal treatments. The plant Coccinia grandis has been widely used in traditional Indian medicinal system (Ayurvedic, Unani, Siddha) since very ancient time. Traditional data base also reports many formulations containing different parts of plant of Coccinia grandis (Linn) Voigt as the medicinal component. Coccinia includes 29
additional species and they are found only in tropical Africa.

This herb is used in folk medicine in the form of intact fruits for healing oral ulcers. But the standardized approach to check the applicability through molecular docking studies and then to validate the predicted activities was not observed in the literature till date so the aim of the present research work was to use the standard approach to prove the utility of the herbal medicine for the treatment of Mouth Ulcer along with associated wound and inflammation.

2 MATERIALS AND METHOD

2.1 Excipients

Polyethyleneoxide were obtained from ColorCon Ltd, Goa India. Methyl paraben, Mannitol and Glycerin were obtained from Thomson bekar.

2.2 Drug and Extract

Lupeol were obtained from Natural Remedies, Bangalore. Hydroalcoholic extract of *Coccinia grandis* Fruit Extract were obtained from Green Chem Herbals, Bangalore.

2.3 Animals

A total healthy wistar rats weighing 150-250g of either sex, bred locally in the animal house of BKC MET’s IOP, Nashik were selected for the antiulcer and wound healing studies and also used for primary skin irritation. Swiss albino mice (26-35gm) were used in Anti-inflammatory experiments test.

They were housed under controlled conditions of temperature (23 ± 2) °C, humidity (50 ±5) °C and 10-14 hours of light and dark cycles. The study was conducted after obtaining the approval from Institutional Animal Ethical Committee. (Reg. No.1344/AC/10/CPCSEA/IAEC Project Approval No.MET/BKC/IOP/IAEC2014-15Pr.05) as per the Indian CPCSEA guidelines.

3. EXPERIMENTATION

3.1 High Throughput Screenng

Molecular Docking Study of chemical constituent of *Coccinia grandis* for antiulcer, wound healing and anti-inflammatory activities.

Computational study for lupeol and taraxerone were carried out using Vlife MDS software. The results are mentioned in table 2 and figures 1-6.

Hardware and Software

All Docking studies and conformational analysis were performed using the Molecular Design Suite (VLife MDS software package, version 4.3; from VLifé Sciences, Pune, India)

Structure Conformation Generation

Structures of compounds were sketched using the 2D structure draw application (Vlife2D draw) and converted to 3D structures. All the structures were minimized and optimized with the AMBER method taking the root mean square gradient (RMS) of 0.01 kcal/molÅ° and the iteration limit to 10,000. Conformers for each structure were generated using Monte Carlo by applying AMBER force field method and least energy conformer was selected for further study.

Preparation of protein

The PDB structures [2PRG, 2MKG, 2J67, 3CFC, 4QDH] were downloaded (www.rcsb.org/pdb/home) and energy minimization of the protein complex was performed. All the bound water molecules, ligands, and cofactors were removed (preprocess) from the proteins which were taken in .pdb format. The tool neutralized the side chains that were not close to the binding cavity and did not participate in salt bridges. This step was then followed by restrained minimization of co-crystallized complex, which reoriented side-chain hydroxyl groups and alleviated potential steric clashes. The complex obtained was minimized using AMBER force field. The minimization was terminated after either completion of 5,000 steps or after the energy gradient converged below 0.05 kcal/mol.

Preparation of ligands

Structures of the LUPEOL and TARAXERONE ligands were sketched using built VLife2D draw taken in.mol2 format which converts it into 3D structure and perform a geometry
Clinical evaluation *Coccinia grandis* Fruit Extract Gel for the treatment of Mouth Ulcer

**Fig. 1:** (a) Hydrophobic Interaction and (b) Vander Waal Interaction of Lupeol with Active Site of Receptor antiulcer activity

**Fig. 2:** (a) Hydrophobic Interaction and (b) Vander Waal Interaction of Taraxerone with Active Site of Receptor antiulcer activity

**Fig. 3:** (a) Hydrophobic Interaction and (b) Vander Waal Interaction of Lupeol with Active Site of Receptor Wound healing activity
Fig. 4: (a) Hydrophobic Interaction and (b) Vander Waal Interaction of Taraxerone with Active Site of Receptor for wound healing activity

Fig. 5: (a) Hydrophobic Interaction and (b) Vander Waal Interaction of Lupeol with Active Site of Receptor Anti-inflammatory Activity

Fig. 6: (a) Hydrophobic Interaction and (b) Vander Waal Interaction of Taraxerone with Active Site of Receptor Anti-inflammatory Activity
minimization of the ligands. AMBER Force Fields with default settings were used for the ligand minimization.

Docking methodology

Docking study was performed on VLifeMDS version 4.3 on Lenovo computer, i3 processor with XP operating system. The Grip ligand docking with approximated a systematic search of positions, orientations, and conformations of the ligand in the enzyme binding pocket via a series of hierarchical filters. The minimum dock score of example may not be exactly reproducible. However changing the different input parameters in Grip Parameters dialog box (like Number of Generations, Translation, Rotation limits etc.) can result in dock scoring energies within desired range and improvement in the orientation of docked ligand as close to that of co-crystallized ligand as possible.

Interpretation of docking studies

[PDB ID: 2PRG, 2MKG, 2J67, 3CFC, 4QDH]. Docking studies were carried out and scoring functions, their binding affinities and orientation of designed compounds having blocking property of active site of the receptor were calculated and interpreted.

3.2 Estimation of drug content in Hydroalcoholic extract of Coccinia grandis

3.2.1 Preparation of standard solution

Lupeol (5mg) was taken and dissolved in 10 ml of Phosphate buffer 6.8 pH (Stock solution 1). From this stock solution, dilutions were prepared using micropipette and diluted with 10 ml Water: Ethanol (70:30) mixture [stock solution-2]. The dilutions prepared were of the concentration range 10, 20, 30, 40, 50, 100 mcg/ml respectively. The absorbance was measured at 255 nm against water: ethanol as blank.

3.2.2 Preparation of test solution of Coccinia grandis extract

Content estimation of extract was done by the following procedure: Stock solution (1000g/ml) was prepared in 6.8 pH phosphate buffer, then working dilutions (50g/ml) was prepared using water:ethanol (70:30) as a solvent ratio in triplicate. The absorbances of resulting dilution were measured at 255 nm 1 max using Uv-visible spectrophotometer (Shimadzu, 1800) against respective solvent blank. Mean of absorbance 50g/ml were put in Equation of standard Lupeol Y=0.0035x+0.0248 and the content of lupeol in hydroalcoholic extract of coccinia grandis fruit extract was calculated. 1 Max of Lupeol in water:ethanol (70:30) and calibration curve are shown in figure. 7

3.3 Evaluation of gel

The results are mentioned in table 3

3.3.1 Physical Examination

The optimized gel formulations were inspected visually for their physical appearance.

3.3.2 Measurement of pH

The pH of optimized formulations was determined by using calibrated pH meter (Systronic pH meter). Gel (1gm) weighed and taken in 50 ml beaker, 10ml of water was added to it and the gel was disperse properly. pH was determined in triplicate.

3.3.4 Viscosity

The viscosity of optimized oral gel formulation was determined at room temperature using a Brook field viscometer. The viscosity was measured by using Spindle No. 2012)
3.3.5 Spreadability

One of the criteria for gel to meet the ideal properties is that it should possess good spreadability. The spreadability of the optimized formulation was determined using an apparatus described in the literature. This apparatus was fabricated in our laboratory. The apparatus consisted of two glass slides (7.5×2.5 cm), one of which was
fixed onto the wooden board and the other was movable, tied to a thread which passed over a pulley carrying a weight. One gm of formulation was placed between the glass slides. 100 gm weight was allowed to rest on the upper slide for 1 to 2 minutes to expel the entrapped air between the two slides and to provide a uniform film of the formulation. The weight was removed and the top slide was subjected to a pull obtained by attaching 20 gm weight over the pulley. The time required for moving slide to travel premarked distance i.e. 10 cm was noted. The readings obtained were indications of relative spreadability of different formulations. It is term expressed to denote the extent of area to which gel readily spreads on application to skin or affected part. The therapeutic efficacy of a formulation also depends upon its spreading value. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from oral gel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability.

3.3.6 Content Estimation

Optimized gel formulation (50 mg), containing approximately 10 mg of drug (lupeol) for 20% gel was taken in a 100ml volumetric flask and diluted with Phosphate buffer (6.8pH) and shaken to dissolve. From this stock solution 1ml was taken and diluted to 10 ml Water: Ethanol (70:30). The content of the drug was estimated spectrophotometrically by using standard curve lupeol plotted at 255 nm.

3.3.7 Permeability studies

In vitro diffusion was carried out by Lab Fabricated diffusion cell. A glass cylinder with both ends open, 10 cm height, 3.7 cm outer diameter and 3.1 cm inner diameter was used as diffusion cell. An egg membrane (soaked in phosphate buffer 24 hours before use) was fixed to one end of the cylinder with the aid of an adhesive to result as a diffusion cell. One gm of gel was taken in the cell (donor compartment) and cell was immersed in a beaker containing 100 ml of phosphate buffer pH 6.8 as receptor compartment. The entire surface of the cell was in contact with the receptor compartment which was agitated using magnetic stirrer and a temperature of 37±1°C was maintained.

Samples (5 ml) were withdrawn from the receptor compartment at 10 min interval of time over a period 90 min with same amount replaced to maintain sink condition. From 5ml withdrawn samples solution, 1ml sample was again withdrawn, then diluted upto 10 ml with Water:ethanol (70:30) and was analyzed at 255 nm against blank water:ethanol(70:30) using UV Spectrophotometer. Drug Released at various time intervals was calculated.

3.3.8 Primary skin irritation test

This study was carried out on healthy wistar rats of either sex. The animals were divided into three groups i.e. control, test and standard each group containing 3 rats. The back skin of area was shaved before one day of starting the study. Formalin was used as standard. The study was carried out for 2 days. At the end of study, the animals were observed for any skin irritation like erythema or rashes.

3.4. Antiulcer and wound healing activity studies of gel

3.4.1 Formulations:

The optimized batches from 10%w/w and 20% w/w gel were selected. The gel was prepared by incorporating 10g and 20g of hydroalcoholic extract of Coccinia grandis fruit respectively into 100g of gel.

3.4.2 Marketed formulation:

Oracep gel and Soframycin Cream (FramycetinSulphate IP) - 1% w/w were selected as antiulcer and wound healing activity standards.

3.4.3 Treatment schedule:

Optimized batches from 10% w/w and 20% w/w gel applied twice a day to experimental animal until they were cured.

3.4.4 Study Protocol:

3 Groups were used. Each group consisted of 3 animals as refered in table 1
3.4.5 Burn Wound:

Partial thickness burn ulcer and burn wound were inflicted on animal by heating glass rod on burner; the glass rod was placed on the lower jaw of the animal covering an area upto (40 mm²) in circular area and for wounds hot molten wax was poured at 80 °C temp. The wax was poured on the shaven back of the animal using a glass rod area upto (40 mm²) in circular area. The wax was allowed to remain on the skin till it got solidified. Immediately after injury and subsequent days, gel was applied topically.

3.4.6 Measurement of ulcer and wound area:

The ulcer and wound were traced on camera on the day of subsequently on alternate 3 days until healing was complete. Changes in the ulcer and wound area were calculated giving an indication of the rate of ulcer and wound contraction. The number of days required for falling of the wound was determined as period of epithelization. The appearance of wound healing on day - 0, 3, 6, and 9, days is shown in photographs. Table no.4 & 5 and figures 8,9,10 for antiulcer activity and

\[ \text{% Wound Contraction} = \frac{(\text{Healed Area})}{(\text{Total Area})} \times 100 \]

3.4.7 Statistical analysis

The results are presented as mean ± standard deviation, when applicable. Results for antiulcer and wound activity groups were compared with a paired Student–Newman–Keuls test carried out by using INSTAT software. Considering values of p ≤ 0.05 statistically significant.Anti-inflammatory ANOVA

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<tr>
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<td>Control</td>
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<td>Test (10% w/w)</td>
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<td>Test (20%w/w)</td>
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<td>Standard (Marketd.)</td>
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Values are mean ±SD of 3 readings

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<th>Table 5. Ulcer contraction area</th>
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Table 6. Wound area

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<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Epithelization time (In days)</th>
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<td>Test (20%w/w)</td>
<td>43.43±18.17</td>
<td>31.98±18.86</td>
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<td>Standard (Marketd.)</td>
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Values are mean ±SD of 3 reading

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<th>Table 7. Wound contraction area</th>
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Clinical evaluation *Coccinia grandis* Fruit Extract Gel for the treatment of Mouth Ulcer

generated by INSTAT software. For 20% w/w gel the P value was found to be 0.0001, considered extremely significant by using Dunnett Multiple Comparisons Test.

### 3.5 Anti-inflammatory study of gel

**Carrageenan induced mice paw edema**

Oral gel containing *Coccinia grandis* was evaluated for anti-inflammatory activity topically using carrageenan induced Swiss albino mice paw edema method. Swiss albino mice (30-40 g) were randomly distributed into three groups of three animals each. The first group served as a control (no application of any gel), second group served as the standard (Voltaren Diclofenac sodium gel topically), while the third group received topical (oral) gel from optimized batch of (20% w/w gel) formulation applied to right hind paw. After 1 hr 0.1 ml of 1% w/v suspension of carrageenan (Sigma Aldrich) was injected into the sub-plantar region of the right hind paw to all the three groups. The paw volumes were measured using vernier caliper every hour till 5 hr after carrageenan injection, and mean increase in paw volumes were noted. The control group received only distilled water. The paw thickness was measured at 30 mins intervals for 5 hr. The results are mentioned in Tables 8, 9 and 10.

4 RESULTS

4.1 Preliminary evaluation

4.1.1 Molecular docking study of chemical constituent of *Coccinia grandis* fruit result are mentioned in Table 2 and fig. 1-6 Lupeol and Taraxerone

4.1.2 Content Estimation of extract

Calculated using straight line equation(Fig 7). Lupeol was found 10μg/ml in 50μg/ml of extract considering the concentration of lupeol in extract.
Fig. 9: Observation of Standard and Control animal for antiulcer activity
**Clinical evaluation** *Coccinia grandis* Fruit Extract Gel for the treatment of Mouth Ulcer

**TEST:** (a) 10% w/w gel application

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(b) 20% w/w gel application

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*Fig.10:* Observation of 10 and 20% gel application for antiulcer activity
So the 1 gram of Hydroalcoholic extract of *Coccinia grandis* fruit extract was found contain 200mg lupeol.

4.1.3 Evaluation of gel

Composition and Evaluation parameter for Prepared Gel

4.1.4 Antiulcer, wound healing and anti-inflammatory activity studies of *Coccinia grandis* fruit gel

The effect of *Coccinia grandis* fruit extract administered orally and topically for antiulcer and wound healing in rats with burn wound model. Ulcer and wound contraction studies in *Coccinia grandis* hydroalcoholic extract revealed that 20 %w/w of hydroalcoholic extract have maximum antiulcer and wound healing activities.

**Antiulcer activity:** In this model, *coccinia grandis* gel treated animals for ulcer were found to epithelise in 9 days while the standard (marketed) and the untreated rat’s epithelise with 11 and 12 days respectively. On the 9th day, the percentage wound area reduction calculated for control rats, marketed rats and the extract treated rats 10% and 20% gel were 67.02%, 42.15% and 23.46% and 17.52% respectively. Result are shown in Table 4 & 5 and figures 8,9,10 for antiulcer activity and Table 6 & 7 and figures 11,12,13 for wound healing activity.

**Wound Healing Activity:** In this model, *coccinia grandis* gel treated animals for wound were found to epithelise in 7 days while the standard (marketed) and the untreated rat’s epithelise with 8 and 12 days respectively. On the 6th day, the percentage wound area reduction calculated for control rats, marketed rats and the extract treated rats 10% and 20% gel were 73.33%, 50.02% and 25.01% and 33.33 %respectively result are shown Table 6 & 7 and figures 11,12,13.

4.1.5 Statistical analysis

ANOVA for antiulcer and wound healing test of 20% oral gel followed by Student –Newman-keuls test, the P value <0.0001 is consider as extremely significant. ANOVA for 10%w/w oral gel is not significant. ANOVA for anti-inflammatory test of 20% gel followed by Dunnett test the P value is<0.0001 is consider as extremely significant using INSTAT software. 3.5

4.1.6 Anti-inflammatory Activity

The study reveals that the extract show a significant reduction in carageenan induced rat paw edema at the dose of 200 mg/kg body weight. ANOVA generated by INSTAT software indicates P value of 0.0001 which is considered extremely significant by using Dunnett Multiple Comparison test

5. DISCUSSION

Chemical Characterization studies reported in Literature indicate the presence of Lupeol and Taraxerone in Hydroalcoholic extract of *coccinia grandis* fruit. Computational studies using V-life MDS software justifies the permeable dock score of Lupeol and Taraxerone for mouth ulcer,Wound Healing and Anti-inflammatory activities. Equally their might be presence of other chemical constituents responsible for said activities but literature reports Lupeol and Taraxerone are the constituent measurably responsible for the performed activities which was successfull found with the help of in-vivo studies.

Normal

![Fig.11: Observation of Normal animals for wound healing activity](image-url)
Clinical evaluation *Coccinia grandis* Fruit Extract Gel for the treatment of Mouth Ulcer

**Standard** Soframycin Cream (Framycetin Sulphate IP) - 1% w/w.

![Fig.12: Observation of Standard and Control animals for wound healing activity](image_url)

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**Control**

![Fig.12: Observation of Standard and Control animals for wound healing activity](image_url)

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TEST

(a) Gel application of hydroalcoholic extract of *Cocciniagrandis* fruit (a) 10 %w/w Gel

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(b) 20 % w/w optimized Gel

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Clinical evaluation Coccinia grandis Fruit Extract Gel for the treatment of Mouth Ulcer

Literature survey reveals that the content of Lupeol in Coccinia grandis fruits obtained by sun drying method is found to be 382 μg/gram. Whereas in the present study the lupeol content of hydroalcoholic extract was found to be 200 mg/gram.

Present study focused on mouth ulcer, wound healing, and anti-inflammatory activities of hydroalcoholic extract of Coccinia grandis fruit. Wound healing and anti-ulcer activities were carried out by using suitable models. Different percentage of oral gel 10 and 20%w/w of hydroalcoholic extract of Coccinia grandis fruit were compared with standard drug Soframycin Cream for wound healing and Orasep gel for mouth ulcer activities and observed effective wound healing and antiulcer and anti-inflammatory dose was found to be 200 mg.

6. CONCLUSION

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-inflammatory 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 activities.

7. Acknowledgements

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References


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