DEVELOPMENT AND CHARACTERIZATION OF GREEN TEA LOADED GEL FOR THE TREATMENT OF ACNE

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ABSTRACT

Green tea is found to be rich in polyphenols having antibacterial, antioxidant properties. The aim of the present study was to investigate the potential of gel formulation for topical delivery of green tea in the treatment of acne. The leaves were subjected to sequential extraction using Soxhlet apparatus. High performance thin layer chromatography quantified catechin (0.458 %w/w) and High Resolution Gas Chromatography Mass Spectroscopy identified terpinen-4-ol (1.39%), main components responsible for anti-acne activity. The antimicrobial activity of extracts and GTME (methanolic extract of green tea) loaded gel was measured by disc diffusion methods against Staphylococcus aureus, Staphylococcus epidermidis and Propionibacterium acnes indicating antimicrobial potential of GTME loaded gel in the treatment of acne.

Key Words: Green tea extract; antibacterial; anti-oxidant; GC-MS; HPTLC.

INTRODUCTION

In our ancient systems of medicine, i.e., Ayurvedic, Homeopathy and Naturopathy, herbal remedies have been used to treat various skin infections. Acne vulgaris which is a cutaneous multifactorial disorder of pilosebaceous unit characterized by formation of seborrhoea, comedone, inflammatory lesions and presence of bacteria (Leyden, 2001). In clinical management of acne, large number of antibiotics and chemotherapeutic agents are available in the global market as topical or systemic treatment modalities but herbal remedies are gaining attention now a days over existing formulations because synthetic chemicals cause undesirable side effects and development of drug resistance (Bassett et al., 1990). Although
number of anti-acne formulations such as tablets, capsule and semisolids like creams, gels, ointments and lotions are commercially available but to surmount limitations and for patient compliance single-phase gel formulation is ideal for acne as it spreads easily and leaves no oiliness (Allen et al., 1999). Recently, natural products with antimicrobial potential have been explored by researchers with increasing interest. One of the natural products which have gained lot of attention is green tea as it possesses remarkable anti-oxidative, anti-inflammatory, anti-microbial, anti-tumour, and anti-ageing potential (Cowan, 1999) which can be attributed to the presence of the simplest compounds called catechins. Tea catechins are a group of flavonoids called flavan-3-ols (Harold & Graham, 1992) which are colourless and water-soluble compounds comprising of (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (Higdon & Frei, 2003). All these catechins act synergistically and exhibit higher antimicrobial activity and efficient free radical scavengers. Green tea extract irreversibly damages the bacterial cytoplasmic membrane and inhibits the adhesion of bacteria to cell membranes. Though, lot of available literature reveals the composition of \textit{C. sinensis}, its antioxidant, antibacterial and anti-inflammatory potential benefits, it still lacks research concerning the utilization of these extracts in the treatment of acne. The objective is to develop and characterize green tea extract and loaded gel with good viscosity and spreadability resulting improved patient compliance with reduction of side effects.

**EXPERIMENTAL**

**Materials**

Fresh green tea leaves were collected from Tata Tea Estate plantations at Munnar, Kerala and authenticated (voucher no: NISCAIR/RHM/consult/2008-09/978/09). Carbopol®940 and catechin were procured from Hi-media and Sigma Aldrich Chemicals respectively. All the chemicals used in the study were of analytical grade.

**Chemical profiling of green tea extracts using GC- MS analysis**

Green tea leaves were subjected to soxhlet extraction for 12 h using nonpolar solvent: petroleum ether, polar aprotic: dichloromethane and polar protic solvent: methanol and three sequential extracts: GTPE (petroleum ether extract), GTDCM (dichloromethane extract), GTME (methanolic extract) were stored at 4°C for GC- MS analysis. For analysis of volatile components Agilent 6890N gas chromatograph was connected to a 5975B mass-selective detector. The chromatographic separation was done on a capillary column of fused silica HP-
5 MS (30 m × 0.25 mm; 0.25 µm film thickness). 1 µl of each extract was injected in the split mode (1:50) with injector temperature at 280 °C. The oven temperature was programmed, starting from 70 °C (1 min) at 25 °C /min to 150 °C (0 min), at 3 °C /min to 200 °C (1 min) and at 8°C /min to a final temperature of 280 °C (3 min). Helium was used as carrier gas. The detection was performed in EI mode with ionization energy of 70 eV, source at 230 °C and quadruple at 150 °C (Nand et al., 2011). Relative percentage amounts of the separated compounds were calculated automatically from the peak areas of the total ion chromatogram. The identity of the components was assigned by comparison of their retention time and spectral data with the corresponding data from NIST’05 library (Adams, 1989).

Selection of excipients and preparation of GTME gel

For topical preparation of active green tea extract (GTME) number of viscosity builders like methyl cellulose, hydroxy propyl methyl cellulose, sodium carboxy methyl cellulose, polyvinyl alcohol and carbopol®940 and cosolvents like polysorbate 80, propylene glycol were checked in different proportions for their spreadability and miscibility with GTME. Propylene glycol was selected as it was chemically stable and does not support microbial growth. Optimized GTME was mixed with hydrated Carbopol®940 (propylene glycol (30%) and distilled water) by stirring with double bladed mixer at 200 rpm for 10 min to form stable gel formulation. Exact composition of the herbal gels (Patent application reference no: 688/DEL/2012) is not disclosed (Nand et al., 2012). The GTME gel was then transferred into clear glass vials and gel base served as negative control.

Characterization of extracts and GTME gel

Determination of antimicrobial activity

Disc Diffusion Assay: The absorbance of the cultures was taken at 625 nm and should be 0.08 to 0.13 for 1-2x10⁵ cfu/ml and concentration was equilibrated according to 0.5 McFarland standards and diluted to 5x10⁴ cfu/ml and 100 µl was plated on nutrient agar (Baur et al., 1966). The sterile Whatman filter paper discs impregnated with 20µl of aqueous GTME (1%w/v), GTME gel and respective negative control were used. Clindamycin (10 µg/disc) served as positive control and exhibited a zone of inhibition of 21.17±0.14 against S. epidermidis. The discs were used on agar plates and incubated at 37 °C for 24hrs. Zone of inhibitions in the plates against bacterial strains greater than 7mm were recorded.
Viscosity, pH, and spreadability

GTME gel formulations were diluted with distilled water (1:10) and pH was measured using Lab India pH meter (model Pico). Viscosity of herbal gel formulations was determined using Brookfield Viscometer (Brookfield Engineering Laboratories, USA) with spindle # C 50-1 having a speed of 50 rpm. Spreadability (g.cm/sec) is expressed in terms of time taken in seconds by two slides to slip off from the gel placed between them, under certain load (Patel et al., 2002). The standardized weight tied on the upper plate was 20 g and length of the glass slide was 6 cm and spreadability was calculated.

High performance thin layer chromatography (HPTLC) for catechin estimation

GTME and GTME gel were analyzed for catechin content by spotting 5 µl on pre-coated TLC plates of silica gel 60GF254 of 10 x10 cm size (Merck KGA, Germany). Chromatogram was developed in Camag Twin Trough glass chamber of 10 x 10 cm with stainless steel lid equilibrated with optimized mobile phase. Application rate was maintained at 10 µl /min using Linomate-V applicator (automatic TLC applicator, Camag, Switzerland) (Sethi, 1996). Chromatographic plates were air-dried, observed under UV chamber (Camag UV chamber-3, model no. 022.9120) and scanned using densitometer at 254 nm (Camag TLC Scanner-3, model No. 027.6480). Rf values and the percentage of the phytoconstituents present in GTME and GTME gel were recorded.

RESULTS

Extraction yield of GTME was higher (9.55%w/w) in comparison to GTPE (1.38% w/w) and GTDCM (3.05% w/w) extracts and also indicated presence of flavonoids and terpenes in phytochemical screening. The results of our findings are shown in Tables 1-2. In Table 1, GCMS profiling of green tea extracts indicating percentages of compounds is indicated.

Table 1. GCMS profiling of green tea extracts.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RT</th>
<th>Percentage of compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GTPE</td>
</tr>
<tr>
<td>4-Cymene</td>
<td>7.86</td>
<td>3.11</td>
</tr>
<tr>
<td>2-Coumaranone</td>
<td>12.35</td>
<td>-</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>13.36</td>
<td>-</td>
</tr>
<tr>
<td>Coniferyl alcohol</td>
<td>31.57</td>
<td>-</td>
</tr>
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Table 2 represented disc diffusion assay for comparison of antimicrobial potential against three test strains *S. aureus, S. epidermidis* and *P. acnes*

### Table 2: Antimicrobial activity of green tea extracts and GTME gel

<table>
<thead>
<tr>
<th>Test strains</th>
<th>GTPE</th>
<th>GTDCM</th>
<th>GTME</th>
<th>GTME gel</th>
<th>Clindamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>7.5 ± 0.28</td>
<td>9.0 ± 0.06</td>
<td>4.4 ± 0.27</td>
<td>13.3 ± 0.11</td>
<td>14.94 ± 0.08</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
<td>7.4 ± 0.05</td>
<td>10.0 ± 0.06</td>
<td>7.8 ± 0.16</td>
<td>13.0 ± 0.15</td>
<td>18 ± 0.11</td>
</tr>
<tr>
<td><em>P. acnes</em></td>
<td>7.6 ± 0.18</td>
<td>7.8 ± 0.1</td>
<td>13.8 ± 0.2</td>
<td>10.9 ± 0.49</td>
<td>18 ± 0.05</td>
</tr>
</tbody>
</table>

Key: GTPE = Petroleum ether extract, GTDCM = Dichloro methane extract, GTME = Methanolic extract, zone of inhibition = (mm ± SEM)

Figure 1. HPTLC fingerprinting of GTME. (A-1) = catechin, (B-1) = GTME.
DISCUSSION

Sixteen compounds were identified using GC-MS method (Table 1) but terpinen-4-ol (1.32%), the principal component (Carson, & Riley, 1993) documented for anti-acne activity was found in GTDCM and GTME. In this study, disc diffusion assay was used as an in vitro model for comparison of antimicrobial potential and it was found that all the strains were sensitive but GTME indicated maximum zone of inhibition against S. epidermidis (17.8 ± 0.16 mm) when compared to S. aureus and P. acnes. It was then formulated as GTME gel and further screened for antibacterial activity and compared with placebo. Though the zone of inhibition of formulation was less in comparison to GTME but it has the potential against three test strains (Table 2) and probably due to its effect on dihydrofolate reductase, enzyme required for the synthesis of purines and pyrmidines by pathogens (Chung et al., 2003). In clinical management of acne, topical preparations are more effective and patient compliant. Therefore GTME gel was assessed for homogeneity, pH, viscosity and spreadability. The visual inspection indicated that there were no lumps in the gel and pH was found near to the pH value of skin (5.43±0.09) indicating its compatibility and unlikely to exert any pH effects on human skin. The intrinsic viscosity for GTME gel was (6702±55 cp) with spreadability value of (7.9±0.08 g.cm/sec). Green tea catechins have galloyl and gallic moieties in their structure which cause deteriorating effect on the lipid bilayer membrane and resulting in loss of cell structure and leads to cell death (Tsuchiya et al., 1996). Present study also confirmed the fact that green tea is a rich source of phenolics using validated HPTLC method for quantitative analysis. HPTLC analysis enabled monitoring of densitograms of GTME and its gel formulation at various wavelengths. Amongst phenolics, catechin (0.458 %w/w) was recorded at Rf = 0.22 (Figure 1) which is also probably responsible for antimicrobial activity (Kono et al., 1994).

CONCLUSIONS

Stable optimized GTME gel was assessed and it was found to possess antimicrobial potential against the test strains. The optimized gel formulation with optimum viscosity and spreadability contained carbopol as viscosity builder, propylene glycol as co-solvent and water for hydration. Further isolation and formulation of individual polyphenolic compounds and in vivo studies is needed to exploit the natural constituents for the treatment of acne vulgaris.
ACKNOWLEDGEMENTS
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REFERENCES
