ABSTRACT

Topical agents are widely used to treat skin conditions. As the development of bacterial resistance to antibiotics and controversy regarding the use of topical antiseptics persist, man turned to his prehistory and found literally thousands of phytochemicals, which inhibit different types of microorganisms. The specific aim of the project is to prepare and evaluate a single compound preparation or a magic bullet to treat commonly infecting skin pathogens. The first step was the selection of plant parts according to long history of use, references in Ayurvedic literature, discussion with Ayurvedic experts and 5 plants were identified [Neem leaves (Azadiracta indica A.Juss), Garlic bulbs (Allium sativum Linn.), Turmeric rhizomes (Curcuma longa Linn.), Dantappala leaves (Wrightia tinctoria Roxb.), Kanikonna leaves (Cassia fistula Linn.)]. All plants were collected randomly from Tropical - Humid regions of Kerala, India, under the guidance of an expert from Indian herbs. All the specimens were identified, voucher specimens were prepared and stored for future use. Plant materials were dried in the dark at room temperature and quality control tests were carried out for plants as per WHO-Guidelines and quality samples were selected for extraction. Three solvents of increasing polarity were selected and a total of fifteen crude extracts were prepared from five different plants used in traditional medicine of India, using different solvents (water, methanol and pet. ethar) by soxhlet extraction procedure. These extracts were subjected to antimicrobial assy by well diffusion method to detect potential antimicrobial activity against S. aureus, P. aeruginosa, E. coli and C. albicans, some common human skin infecting pathogens. The result showed that methanol extracts of Cassia fistula leaf posses strong in-vitro antibacterial activity. The bioassay guided purification of the crude methanol extract of Cassia fistula leaf resulted in the isolation and identification of chrysophanol (1,8-dihydroxyl-3-methylanthaquinon), as the potent metabolite responsible for the antimicrobial activity. UV spectroscopic method has been developed for the estimation of chrysophanol in pure form and in gel formulations. Chrysophanol was estimated at 225nm in methanol medium. Beer’s law was obeyed in the concentration range of 1–10 μg/ml ($r^2 = 0.998$). The method was tested and validated for various parameters according to ICH guidelines. The Limit of detection & Limit of quantification were found to be 0.57μg/ml & 1.72μg/ml, respectively. The results demonstrated that the
procedure is accurate, precise and reproducible (relative standard deviation < 1 %). In order to utilize the potent antibacterial property of Chrysophanol (Minimum inhibitory concentration 1-4µg/ml, Minimum bactericidal concentration 2-4µg/ml) it was formulated into topical gel. The partition of drug between skin and the hydrogel matrix was considered to play an important role in the permeation process. The effect of three levels of carbopol and three different permeation enhancers on chrysophanol permeability was determined in-vitro. Each formulation were characterized in terms of viscosity, pH, extrudability, spreadability, homogeneity, drug content and drug release studies. The pharmacokinetic study reveals that the drug release is controlled by both diffusion and relaxation processes. The release exponent for the formulations ranged from 0.934-1.0. A linear relationship (r=0.941-0.985) was observed between the amount released and square root of time. The gel consisting of 1% carbopol as gel base and 15% Dimethylformamide (C12) showed superior physicochemical and permeability properties and it was ranked best. Skin irritation and stability study of C12 formulation shows that it is stable and will not produce any irritation. Comparative Pharmacodynamic evaluation of gels revealed that formulation C12 has high degree of anti-bacterial and wound healing activity that can be compared with that of marketed formulation (p<0.05).

Keywords: gel, Chrysophanol, herbal