INTRODUCTION

In humans, the largest organ of the integumentary system (organ system that protects the body from damage) is the skin, the outer covering of the body. The skin is one of the most important parts of the body because it interfaces with the environment and is the first line of defense from external factors like extreme temperature, Ultra Violet radiation, allergic chemicals and pathogens. "Your skin is the fingerprint of what is going on inside your body, and all skin conditions, from psoriasis to acne to aging, are the manifestations of your body's internal needs, including its nutritional needs," says Dr. Georgiana Donadio, founder of the National Institute of Whole Health.

The skin of the human body is colonized by a diverse milieu of microorganisms, most of which are harmless or even beneficial to their host. Colonization is driven by the ecology of the skin surface, which is highly variable depending on topographical location, endogenous host factors and exogenous environmental factors. Dermatophytes ('skin plants') are a common label for a group of three types of fungi that commonly cause skin disease in animals and humans. They are: anamorphic (asexual or imperfect fungi) genera *Microsporum*, *Epidermophyton* and *Trichophyton* with a total of about 40 species and the teleomorphic (sexually reproducing fungi) genus *Arthroderma,* of the Ascomycota. Candidiasis or thrush is also due to fungal infection (mycosis) of any of the *Candida* species (all yeasts), of which *Candida albicans* is the most common. Candidiasis is also technically known

as candidosis, moniliasis, and oidiomycosis. Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases.

The currently available antifungal agents for the treatment of systemic mycosis include polyene antibiotics (Amphotericin B), fluoropyrimidine (Flucytosine), Nystatin and azole group of drugs (Ketoconazole, Fluconazole, Itraconazole). But the development of resistance in the targeted organisms and the cause of minor to major side effects to the patients are the problems in the treatment of fungal diseases. Side effects such as hepatotoxicity, bone-marrow depression and gastrointestinal problems in flucytosine (Benson & Nahata, 1988) and mild gastrointestinal problems in fluconazole have been reported (Goa & Barradell, 1995).

Due to the above problems, searching of novel drug is going on continuously throughout the world. The CDRI, Lucknow, India, evaluated approximately 2,000 plant species for several biological activities, including antibacterial, antidiabetic, antifertility, antifungal, antihypercholesteremic, anti-inflammatory, antitumor, cardiovascular, central nervous-system depressant, cytotoxicity, diuretic, and others (Dhar *et al.* 1968). To date no biologically active drugs for human use have arisen from that program, even though a large number of known and novel bioactive compounds were isolated from the plants (Rastogi & Dhawan, 1982). The failure may be due to several reasons, including random screening of plants for bioactive compounds. Perhaps the first company in the United States to investigate plants strictly

through the ethnomedical approach was Shaman Pharmaceuticals in South San Francisco, California (Oubre *et al.* 1997). Their approach was to send botanists and physicians teams to tropical areas to assess firsthand the use of plants by traditional healers and to collect interesting plants and assess them for validity in the Shaman laboratories. Initial interest was directed toward antifungal and antiviral agents (Ubillas *et al.* 1994) and several active compounds were discovered but failed in clinical trials. The reason for the failure of isolated bioactive compounds in clinical trials may be due to the absence of additional compounds, which are present in ethnomedicinal plants, for synergistic action. So, the searching of new drugs with group of compounds, as in Ayurveda and Siddha, will be more successful when compared to the isolated single compound. The ultimate aim of drug formulation is to deliver the active pharmaceutical ingredient through biological membranes to the right target in the right concentration during a defined time interval. An effective antimicrobial solution comprises at least one alcohol, at least one antimicrobial agent and at least one chelator and/or anticoagulant (Raad, 2005).

Searching of novel drug with the all required components, based on ethnomedicinal knowledge, will be of more success. In addition, one may look into the chemical ecology of plants to identify a particular chemical which may be useful to the plant and harmful to other organisms. Chemical ecology appears to be an attractive tool for identification of such compounds. In competitive ecosystems, it is generally accepted that many organisms thrive

because they produce secondary metabolites providing a selective advantage over competing organisms. By employing biorational criteria in selecting sources, potential drugs can be more effectively located. Therefore, biorationale predicts organisms encountering fungal competitors or pathogens will be a good source of fungistatic or fungicidal chemicals. Examples of such sources include antagonistic fungi, plants with fungal pathogens, and mycoparasites. Studies on antagonistic species will serve as a valuable complement to random, high-throughput screening for new bioactive compounds (Eckerman & Graham, 2000). The primitive vascular land plants, pteridophytes have developed several adaptive and antagonistic mechanisms to cope with the terrestrial environment particularly by the development of an important protective structure called 'cuticle' which is made up of lipophilic chemical compounds. Some plants synthesize several defensive chemical compounds in special structures called glandular trichomes. They produce large amounts of specialized (secondary) metabolites of diverse classes for a variety of adaptive processes, including defense against herbivores and microorganisms as well as in ion homeostasis. Because trichomes protrude from the epidermis they can often be easily separated from it and harvested for biochemical analysis (Schilmiller *et al.,* 2008). By extracting the oils and liquids from these structures, we can also benefit from their natural fungus prevention. Antibacterial, anticandidal, antiviral activities have been reported from the cuticular and extracuticular layers of plants (Nebojsa & Ilya, 2003; Paulraj, 2007; Olubunmi *et al.,* 2009).

Detailed studies on morphology, biochemistry and bioactivity on epidermal glands of six thelypteroid ferns (Paulraj, 2007) have shown the presence of variety of lipophilic chemical compounds. Bioactivity studies on the glandular extracts of these ferns showed antibacterial, anticandidal, insecticidal, piscicidal and larvicidal activities. The same extract enhanced seed germination. All these six ferns have antagonistic relation with their enemies like microbes and insects and mutualistic relation with angiosperms by enhancing germination of seeds of angiosperms which provides shelter for these ferns. The glandular fern *Cyclosorus interruptus* (Willd.) H. Ito is used as green manure, by local people, for banana cultivation in Kaniyakumari District, Tamil Nadu. Paulraj (2007) has confirmed the growth promoting effect and antimicrobial effect of this fern. Several kinds of lipid compounds including fatty acids are known to possess antibacterial, antimalarial and antifungal activity and they play an important role in defense mechanism. It is well known that lipid compounds and their derivatives like terpenoids and hydroxylated / oxygenated / halogenated fatty acids hold great potential as environmental friendly antifungal agents or leads for novel antifungal drugs (Pohl *et al.* 2011). With the available knowledge on the antifungal lipophilic compounds which are commonly present as defensive chemicals in epidermal glands of thelypteroid ferns, the present study has been aimed to make a thorough study on antifungal, particularly antidermatophytic, effects of epidermal glands of selected thelypteroid ferns. Thus the present study is nothing but the searching of medicines by a physician for more susceptible human skin from more resistant plant skin under the guidance of a botanist.

Main objectives of the present study:

- To make preliminary antifungal screening on epidermal gland extracts of selected thelypteroid ferns, *Amphineuron terminans* (Hook.) Holttum, *Christella parasitica* (L.) H. Lev. and *Cyclosorus interruptus* (Willd.) H. Ito and to select a potential antifungal fern.
- To evaluate antimicrobial efficacy of various macroscopical and microscopical parts of potential antifungal fern, selected from the preliminary screening, in order to find out the part(s) with maximum antimicrobial activity.
- To make preliminary separation of bioactive compounds, by sequential fractionation of the crude extract of potential antifungal fern; to make antifungal / antidermatophytic screening and to find out the Minimum Inhibitory Concentration for all the fractions of the crude extract.
- To make intensive anticandidal screening on clinical samples with the potential fraction of crude extract.
- To make comparative phytochemical screening by TLC, HPTLC, HPLC and FTIR analysis on crude extract and its fraction(s) in order to find out the chemical difference for the difference in antifungal activity.