IV DISCUSSION

India is one of the 12 richest mega biodiversity countries of the World contributing to about 2 biodiversity hot spots, 5 Centres of Plant Diversity (CPD) of ICUN and several Biodiversity Reserves, National Parks, etc. Biodiversity has an economic value, with local, regional, national and international implications and apart from this it has an intrinsic value, which is academic / scientific, that is unrelated to direct human use. Traditionally local communities worldwide are extremely knowledgeable about local plants and other natural resources, on which they are so immediately dependent. Much of this knowledge is lost as traditional cultures get eroded and ethnobotanists should play a major role in restoring the disappearing knowledge and returning it to local communities. India has about 18,000 species of Angiosperms, of which about 2,500 are considered as important sources of medicinal and aromatic chemical compounds and an equal number of other groups of plants like the algae, fungi, lichens, bryophytes, pteridophytes and gymnosperms also have great therapeutic and industrial potential which has not been realized. Drugs of plant origin contributes about 40 percent in the Indian pharmacopoeia (Ayensu, 1986) which recognizes only 3 percent of these species reported in conventional systems. 540 plant species of plants are in use in
different formulations in India (Kapoor and Mitra (1979). It is estimated that in the rich world, 25 percent of all medicinal drugs are based on plants and their derivatives (Principe, 1991) and in the poor world it is closer to 75 percent. India was one of the pioneers in the development and practices of well-documented indigenous systems of medicine, particularly, Ayurveda, Siddha and Unani. Compared to the South American, African and Chinese medicinal plants that have been subjected at present to a more serious and well recognized scientific study, the modern scientific attention paid to the Indian medicinal plants has been very inadequate and does not bring out their true potential. Modern phytochemical and pharmacological studies have confirmed the therapeutic potential of plants reported in traditional systems, yet these systems are not popular now. In the context of providing efficient and inexpensive medicine to the masses, to build up public confidence in indigenous medicine and for consolidating and protecting this invaluable heritage, it is necessary that the indigenous system is evaluated in the light of current concepts and modern research. This effort will make the Indian traditional medicine acceptable country wide, at all social and economic levels, which stands as the first step in making Indian systems internationally recognized and accepted. Even though from very ancient times there has been description of several plants as having antifungal property, only a few Indian plants have gained reputation in the treatment of fungal infections and that too only a very few systematic studies have been made so far to establish their efficacy. Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. New sources, especially plant sources, are also being investigated. Second, the public is becoming increasingly aware of problems with the overprescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical
care. A multitude of plant compounds (often of unreliable purity) is readily available over-the-counter from herbal suppliers and natural-food stores, and self medication with these substances is commonplace. The use of plant extracts, as well as other alternative forms of medical treatments, is enjoying great popularity in the late 1990s.

Invasive candidiasis is a life-threatening infection in the immunocompromised host such as bone marrow and organ transplant recipients, in patients receiving intensive chemotherapy regimens and in AIDS patients. Existing anti-candida drugs are often highly toxic, and drug-resistant infections are becoming more common. Although the first agent with antifungal activity, griseofulvin, was followed in 1960 by that of Amphotericin B (Gupta et al., 1994), which is still the "gold standard" for the treatment of severe systemic mycoses (Georgopapadakou and Walsh, 1996). Out of the several groups of antifungal agents (Jagdish Chander, 2002), in the United States, only 10 antifungal drugs are currently approved by the Food and Drug Administration (FDA) for the therapy of fungal infections, especially systemic. The fungicidal polyene drugs such as Amphotericin B act by binding to sterols in the plasma membrane, disrupting membrane function and is the most effective antifungal drug, but it is more toxic and is less tolerated by the body than the azoles (White et al., 1998). Most fungi are susceptible to Amphotericin B, which remains the empiric treatment of choice for invasive fungal infections. The fungicidal antifungal drug Amphotericin B has in vitro and in vivo activity against yeasts (Candida spp., Cryptococcus neoformans), molds (Aspergillus spp., Zygomyces, dematiaceous fungi), and dimorphic fungi (White et al., 1998). Regardless of which Amphotericin B formulation (conventional or lipid) is used, clinical experience and personal preference of the treating physician often determine the approach to administration of the drug, especially since few studies have carefully
addressed the issues. Hence Amphotericin B has been used as a positive control for in vitro and in vivo studies.

The present study involving ethnodiocated method evaluated the anticandidal activity of some commonly used plants mentioned in traditional knowledge systems of India (Siddha, Ayurveda, Unani and other health care systems of India) by adopting systematic procedures. About 31 plant species and a herbal preparation were tried against the fungus Candida albicans. Agar disk diffusion assay was followed (Kirby-Baue method), which is generally followed for bacteria and most of the fungi like Candida albicans (Rex et al., 2001). As a screening tool, the disk diffusion procedure correlated well with the NCCLS reference broth macrodilution method (Kirkpatrick et al., 1998). For Amphotericin B, there was almost complete agreement (98%) between visual and spectrophotometric readings, even when the strictest endpoints (AA-0 and Spec-95) were used (Lozano-Chiu et al., 1999) and hence visual determination of the MIC was followed here. Negative control shows the growth of the inoculated organism Candida albicans, i.e., there is no contamination of other organisms; positive control shows remarkable zone of inhibition, meaning that the Amphotericin B was effective and the strains used were susceptible to this antifungal agent. Among the treatments, acetone extract of Calotropis giganta of 1000ppm concentration (6 - 6.6 mm (zone of inhibition)), acetone extract of Zingiber officinale of 1000ppm concentration (6.3 – 6.5 mm), alcohol extract of Leucas aspera 1000ppm concentration (6 - 6.1 mm) and Herbal formulation of 100ppm concentration (6.6 – 6.67 mm) were all found to be greater than 5mm zone of inhibition i.e., they are effective in controlling the growth of fungus. From Table II it was evident that herbal preparation has more of phenolics and tannins, acetone extract of Zingiber officinale has alkaloids, emodins which are absent in others, acetone extract of Calotropis giganta, alcohol extract of Leucas aspera and acetone extract of Zingiber officinale has steroids in common while
acetone extract of *Zingiber officinale* and herbal preparation has flavanoids and tannins in common. From these it is evident that phenolics, tannins, flavanoids and steroids may play a vital role in anticandida activity.

Previous work, as indicated below, also shows that these chemicals are effective as anti-microbial agents. Caffeic acid, a phenolic acid is effective against viruses, bacteria and fungi (Duke, 1985). Catechol and pyrogallol, both hydroxylated phenols, are toxic to microorganisms. Eugenol (found in clove oil) is an effective fungistatic compound. (Duke, 1985).

Flavanoids are synthesized by plants in response to microbial infection (Dixon *et al.*, 1983). Catechins (in teas), inhibited *in vitro Vibrio cholerae O1, Streptococcus mutans, Shigella*, and other bacteria and microorganisms. The catechins inactivated cholera toxin in *Vibrio* and inhibited isolated bacterial glucosyltransferases in *S. mutans*, possibly due to complexing activities described for quinones. This latter activity was borne out *in vivo* tests of conventional rats. When the rats were fed with a diet containing 0.1% tea catechins, fissure caries (caused by *S. mutans*) was reduced by 40%. Phloretin, found in certain serovars of apples, may have activity against a variety of microorganisms.

Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins. Scalbert (1991) reviewed the antimicrobial properties of tannins. He listed 33 studies, which had documented the inhibitory activities of tannins up to that point. According to these studies, tannins can be toxic to filamentous fungi, yeasts, and bacteria.

The herbal formulation prepared by us showed greater anti-candida activity when compared to the other effective combinations and hence it was preferred over others for further investigation. Purification of herbal formulation has not been attempted since the individual components are not effective against the fungus like that in their combination and similar to several other traditional
preparation it would be due to synergistic effect (antifungal activity). The next stage is providing the drug in the form it could be used more effectively. A vehicle (base or carrier) chosen properly in the case of herbal preparation by mixing it in white petroleum jelly base (1mg/10mg & 1mg/50mg). This formulation was used for further analysis. The formulation was in ointment form. Ointments are best used to lubricate, especially if applied over hydrated skin; they are preferred for lesions with thick crusts, lichenification, or heaped-up scales, and may be less irritating than a cream on some eroded or open lesions (Panda, 2000). Although messier to use, ointments are usually more potent than creams for effectively delivering a drug to the skin.

Next to the mouse, the rat is the most extensively used experimental animal, particularly in the fields of nutrition, transplantation, immunology, genetics, cancer, pharmacology, physiology, neuroscience and aging research. Its larger size allows scientists to perform many procedures that can be accomplished in the mouse, only with difficulty. In vitro tissue culture systems derived from nonhuman sources were used by a few investigators to study the pathological processes in candidal infection much earlier than the introduction of the in vivo experimental animal models. Partridge (Partridge, 1959) was the earliest to confront this problem and used the chick chorioallantoic membrane to culture pathogenic fungi. Subsequently, Cawson (1973) used the same assay to evaluate the hyperplastic response of the ectoderm to candidal invasion while Hurley and Stanley (1969) experimented with cultured mucosal cells from the lingual dorsum of neonatal rats for the same purpose. They also assessed the yeast-induced cytopathic effect and the association between the growth phase of yeasts and the lethal effect on tissues. The effect of the MIC of an agent as determined under in vitro conditions that approximate the situation in vivo would be a better predictor of in vivo efficacy (Okeke et al., 2000).
Dermatophytoses, which constitute the majority of superficial fungal infections, are infections of keratinized tissues such as the stratum corneum, nail, and hair by dermatophytic fungi. Some agents with high levels of in vitro antidermatophytic activity show rather poor in vivo effects (Galgiani, 1987 and Rex et al., 1993). This difference in the in vivo and in vitro activities of some agents is due to the dependency of the in vivo action on the interaction of drug molecules with tissue components (Espinell-Ingroff et al., 1991 and Hawser et al., 1998). Thus, the efficacy of a topically applied antidermatophytic agent is influenced not only by its antifungal property but also by the ability of the drug molecules to penetrate the keratinized tissue (Mertin and Lippold, 1997).

Animals used in drug research are subjected to stringent standards of care beginning with the animal supplier. The Animal Welfare Act enacted in 1966 and amended in 1970 & 1976 contains provisions to ensure that animals intended for use in research receive humane care and treatment (Fort, 2002). In this case dermal wound was created and infected by fungus followed by treatment with positive, negative and drug of different concentrations. Generally used concentration of inoculum was not effective in disease manifestation and so inoculum concentration was increased and was primarily due to the fact that the MTCC strain used was human pathogen and difficult to induce disease in rat model. Antifungal agent (Amphotericin B) and the herbal treatment, both has a direct effect on the wound healing, while negative control shows initial healing but on the incidence of fungal infection, the wound area increases. The efficacy of a topically applied antidermatophytic agent is influenced not only by its antifungal property but also by the ability of the drug molecules to penetrate the dermal layer. The treatment was comparable to the positive control and was efficient in curing candidiasis.

The above result was further substantiated by the microscopic analysis of the infected wound. The utility of cytologic specimens in the diagnosis of...
infectious disease has been well established during the last 50 years. Cytology is not intended to replace microbiologic techniques, but should be viewed as a means of rapid initial recognition of an infectious process by using safe, cost-effective methods of specimen procurement. Material for cytologic evaluation may be procured by one of three mechanisms: exfoliation, abrasion, or aspiration (Hopper et al., 1992, Kinney, 1993, Koss et al., 1992, Stanley and Lowhagen, 1993). Most widely followed technique in the case of studying dermal infection is by collection of samples from it by means of manual abrasion technique. With rare exceptions, most fungi are diagnosed in cytology specimens by their morphologic rather than staining characteristics. This approach, combined with the proper clinical information, is quite accurate for the majority of fungal organisms encountered. The presence of hyphal and/or yeast structures begins a mental algorithm that uses size, shape, budding, and branching characteristics to narrow the differential diagnosis. Small budding yeasts (3 to 4 μm) and pseudohyphae are typically generically identified as *Candida* spp. Microscopic analysis reveal the presence or absence of fungus on the wound (Figs. 44 to 49). These figures shows the absence of fungal cells on the 0\(^{th}\) day, infection of fungus on 8\(^{th}\) day and absence of fungal cells on the 20\(^{th}\) day for positive control and treatments, while negative control shows an increase in number of fungal infection from 8\(^{th}\) to 20\(^{th}\) day. It was evident that treatment was very effective in controlling candidiasis in *in vivo* conditions also. It (graph 1 to 8) also throws light on the initial, middle and final stages of introduction of fungal inoculum, presence of fungal cells (manifestation of disease) and its cure (absence of fungal cells) and thereby giving a complete scenario of the disease manifestation and cure that mimics human system and thereby stand as a prelude to the clinical trial in human being.

The drug development team's scientists may account for much of the scientific expertise, but the roles of the research team overlap to form a
scientifically sound, medically astute cohesive group (Cato et al., 2002). Ideas for clinical trials usually come from researchers. After researchers test new therapies or procedures in the laboratory and in animal studies, the treatments with the most promising laboratory results are moved into clinical trials. During a trial, more and more information is gained about a new treatment, its risks and how well it may or may not work. Of course, plants have been used for centuries to treat infections and other illnesses in humans in aboriginal groups, but controlled clinical studies are scarce. In some cases, traditional healers working together with trained scientists have begun keeping records of the safety and effectiveness of phytochemical treatments, but these are generally uncontrolled and unrandomized studies.

Clinical trials that are well-designed and well-executed are the best treatment approach for eligible participants to: Play an active role in their own health care, Gain access to new research treatments before they are widely available, Obtain expert medical care at leading health care facilities during the trial and Help others by contributing to medical research. (http://www.clinicaltrials.gov/ct/info/resources)

There are risks to clinical trials: There may be unpleasant, serious or even life-threatening side effects to treatment, The treatment may not be effective for the participant, The protocol may require more of their time and attention than would a non-protocol treatment, including trips to the study site, more treatments, hospital stays or complex dosage requirements.

Since, the herbal formulation tested here is plant based there is less possibility of side effects (already safety in animal models are established) and effectiveness was also evaluated in in vitro and in vivo conditions, this clinical trial was undertaken. Phase I trial (20-80 persons) of treatment trial reveals that the effectiveness of a herbal drug against Tineasis and Candidiasis. The diagnosis of Candida infection relied on direct inspection of freshly obtained specimens for
the presence of organisms microscopically consistent with *Candida* spp and the recovery of *Candida* spp in cultures of tissues. Direct microscopic examination of specimens not only provided semi-quantitative estimates of the amount of *Candida* spp, but also supplemented culture results (Girishkumar *et al.*, 1999). The clinical trial process depends on the kind of trial being conducted. The clinical trial team includes doctors and nurses as well as social workers and other healthcare professionals. They check the health of the participant at the beginning of the trial, give specific instructions for participating in the trial, monitor the participant carefully during the trial, and stay in touch after the trial is completed.

Apart from the ethical dilemmas associated with experimentation on live humans, humans are notoriously dissimilar in terms of their dietary and social habits, immune status, etc (Samaranayake and Samaranayake, 2001). These factors, plus the racial, ethnic, and cross-cultural variations in human demographics, add to the confounding matrix of factors influencing the etiology and pathogenesis of diseases such as candidiasis, where the invading organism is not a true parasite but an opportunistic pathogen. Hence, in theory at least, the development of an ideal animal model for superficial candidiasis would provide a standardized tool, which can be controlled and manipulated to derive universally comparable data on the etiopathology, diagnosis, and management of the disease process. Out of 70 participants, 5 were control, 22 were infected by Tineasis and 43 were infected by candidiasis. The results (Table - II, Table - III and Table - IV) reveals that out of 15 observed trials for Tineasis, 10 were cured and 5 uncured (1-diabetic) i.e., 66.7% (10 out of 15) were cured and was significant value. Out of 38 observed trials for Candidiasis, 30 were cured and 8 uncured (1-pregnant and 1-lactating), here 78.9% were cured and was very significant result that too for a fungal infection. Clinical trial represents a vital stage in the development process a stage that is no less daunting than the preclinical research stage. The data obtained from the first time human phase I studies and initial
safety evaluations, can make or break the entire developmental program for a drug candidate. To date, few randomized clinical trials of plant antimicrobials have been performed. Giron et al, 1988 compared an extract of *Solanum nigrescens* with nystatin, both given as intravaginal suppositories, in women with confirmed *C. albicans* vaginitis. The extract proved to be as efficacious as nystatin. In 1996 the long-recognized effects of cranberry juice on urinary tract infections were studied in a group of elderly women.

In this study, superficial candidiasis was cured completely by herbal ointment (treatment) and the results were comparable to that of Amphotericin B (positive control), time taken for cure was very less (15-30 days) with respect to fungal infection and there was no observed side effects or uneasiness felt by the participants taking part in this clinical trial. Hence this herbal drug could be used as an efficient anti candida agent for treating superficial candidiasis after undergoing the phase II and phase III trials.