susceptibility of bacteria and fungi in vitro (Odds, 1994). The anti-fungal drugs were used for the evaluation of MIC in a traditional culture media most suited for the microorganism for growth and enzyme production. On this line of research, a lot of work is going on all over the world today. For the test of the in vitro susceptibility of clinical yeast isolates, Pfaller (1991) had developed an improved micro-dilution method with improved reproducibility. However, the action of antimycotics whether fungicidal or merely fungistatic is another significant factor in the study of physiology of medically important fungi.

A number of new synthetic anti-fungal agents have been developed for clinical use in both topical and systematic administration. Griseofulvin is one of the anti-fungal agent extracted from the mycelial homogenates of *Penicillium griseofulvum* by Oxford, Raistrick and Simonart (1939). Then in 1946, the pure form was extracted from metabolic product of the culture filtrates of *Penicillium janczewskii* (Brian, Curtis and Hemming, 1946). Gentles (1950) first described the fungicidal action of this drug against experimental ringworm in guinea pigs and then it was used as an orally active anti-fungal drug against the ringworm in man by Williams et al (1958).

It has been observed that griseofulvin acts as an inhibitor in the metaphase of mitotic cell division (Dekker, 1984). Again, it causes “Curling” of hyphae, a major growth abnormality in sensitive fungi which is associated with its fungistatic effect and its fungicidal effect results from rupture of the cell wall (Bossche, 1995).

However, the mode of action of this drug is manifold. The insensitiveness of *C. albicans* to griseofulvin has been reported by several workers (Scholar & Polak, 1984; Ryley, 1990; Bossche, 1995). It has been also observed that there is no uptake of tubulin binding drug by *C. albicans*. It may depend on the variability of the fungal species, which, in turn, is determined by the various internal and external factors.

The best-known polyene compounds applied in clinical purposes internationally, are Nystatin, Amphotericin B, Candidicidin, Natamycin etc of which Nystatin was first ever anti-fungal agent to be used therapeutically for Candidiasis (Odds, 1988). It was discovered by Hazen & Brown in (1950) and was extracted from the filamentous bacteria belonging to *Staphylococcus* species or various substituted derivatives of the natural compounds (Odds, 1988). Here, in this study, Nystatin showed better fungistatic property than griseofulvin which stands contradictory to the previous observations made by several investigators, worked in this area (Athar & Winner, 1971; Athar & Swaroop, 1976; Boyer, 1976; Hamra & Pankiewicz, 1977;
Holbrook and Kippax, 1979; Segula et al, 1980; Nobre et al, 1981; Bergan & Vangdal, 1983; Odds & Abott, 1984; Polonelli & Morace, 1984; Guaschino et al, 1986; Swain, 1997). Although the MIC for the growth of the strains of *C. albicans* was more or less similar and in both the pattern and percentage of growth was more or less similar, yet the production of enzyme percentage was much higher in griseofulvin than nystatin (fig.). This suggested that the growth of the isolates was not completely stopped but was merely suppressed by the application of these antibiotic drugs. However, the major role of anti-fungal is to damage the cell membrane as a result of which its permeability is altered. Therefore the effect is fungistatic rather than fungicidal (Odds, 1988).

The other main family of anti-fungal agents are the azoles, whose efficacy against clinical yeast isolates has been studied by several workers (Rogers & Galagiani, 1986; Bodey, 1988; Bossche et al, 1989; Burke, 1989; Galgiani, 1990; Grant & Clissold, 1990; Hay, 1990; Arzeni et al, 1991; Pougam, 1991; Roberts, 1991; Speller & Warnock, 1991; Ragli, 1992; Graninger et al, 1993; Otcenasek & Bucheta, 1993; Odds, 1995). In the year 1940, Holt took interest in the antimicrobial azole while working on benzimidazoles. It has been observed that azoles are activated at neutral pH or above neutrality or in other words, the inhibition of Candidiasis is marked when the azoles are unprotonated (Minagowa et al, 1983; Beggs & Hughes, 1986). Again Odds (1988) described that the physiological status of fungal cells also affect the inhibitory action of azoles on *Candida* species. It has been observed that the inhibitory effect of the drug not only depends on the mode of action of azole groups but also the kind of azole used. The primary target of the azoles is the haemprotein that co-catalyses 14 L-demethylation of lanosterol—a cytochrome P₄₅₀. Differences in the extent of inhibition of fungal and mammalian cytochrome P₄₅₀ accounts for the selective toxicity of azoles for fungi. A second aspect profoundly affecting the overall inhibitory action of azoles on *Candida* is the physiological status of the fungal cells (Odds, 1988).

Of the various azoles now being used internationally for the treatment of cutaneous, systemic and mucocutaneous infections caused by *C. albicans*, only five widely proven ones were tested such as Clotrimazole, Ketoconazole, Tinidazole, Itraconazole and Fluconazole. Moreover, these anti-fungals were tested for being used as orally, topically and topically-orally respectively by the patient.

Very little is known about the mode of action of Clotrimazole. However, it is strikingly similar to those of polyenes. Odds, (1988) showed high efficacy of Clotrimazole against superficial *Candida* infections particularly those of skin, mouth
and genitalia. It has been found that the drug is only used topically and is applicable to both dermatophytes and gram positive bacteria apart from yeasts (Sharma et al, 1993). The anti-fungals are being taken by the patients orally, topically or topically-orally. However, the growth and enzymatic activity of the isolates was best checked by Clotrimazole whose MIC value was determined to be 2 - 3 $\mu g/ml$. This finding was supported by Odds (1988) which showed similar results and also demonstrated the high efficacy of Clotrimazole against superficial Candida infections. It has been observed that relatively high concentration potentiality damage the fungal cell wall and plasma-lemma making them preferable to aminoacids, phosphates, intracellular macromolecular synthesis (Emmons et al, 1977). It also effected suppression of active concentration in lymphocytes (Odds & Webster, 1988).

Ketoconazole, another azole drug was first synthesized in 1976 but has been recently introduced in India (Sharma et al, 1991). For the treatment of chronic mucocutaneous Candida infections, this antifungal drug has been used internationally (Hay, 1980; Drouhet and Dupont, 1980; Hay et al, 1980; Petersen et al, 1980; Notling and Fegeler, 1987). It has been studied that this drug helps in the accumulation of peroxidase by interfering in the synthesis of oxidase and peroxidase enzymes (Sharma et al, 1991). It also inhibits in the synthesis of sterol in the mycotic cell membrane by inhibiting the demethylation of lanosterol which is a precursor of ergosterol. Due to all the above reasons, the drug was recommended for being generally used orally, topically and both orally and topically.

Fluconazole, another effective triazole derivative, has been proved to be effective in treating Candidiasis (Richardson et al, 1985; Humphrey et al, 1985; Lyman, 1986; Saag and Dismukes, 1988; Liss and Letourneau, 1989) and a variety of fungi and yeasts including Cryptococcus neoformans (Dixon & Walsh, 1991). Liss and Letourneau, (1989) made an in vivo study of fungal specificity of fluconazole which was proved to be meaningful because any drug-induced change in composition of the bacterial flora can favour opportunistic mycotic infection and disease (Sutter, 1983). Thus fungal specificity ensures targeted therapy against the causative pathogen and does not alter or diminish protective microbial species (Liss and Letourneau, 1989). Being the first member of a new sub-class of synthetic triazole, it was selected here for determining its properties. It has been observed that the in vitro activity of fluconazole is very difficult to assess and its M.Cs are among the hardest to detect meaningfully.
The fungal specificity of fluconazole depends solely on the culture medium in which it is supplemented to inhibit the growth and enzymatic activity of the fungus. However, in the present study, MIC value of fluconazole was determined to be 30 μg/ml in which the growth of the isolates was completely checked, though complete inhibition of enzyme activity was found at 40-45 μg/ml. These results proved that the strains of *C. albicans* are more resistant to fluconazole than ketoconazole, tinidazole, itraconazole and clotrimazole here at Rourkela. Although several reports favour the present finding (Odds, 1980) while several others stands contradictory which suggest that among the azoles, fluconazole has been proved to be highly effective in treating mucocutaneous Candidiasis and is much better tolerated than amphotericin B (Liss & Letourneau, 1989; Mathews, 1994). Besides the traditional azoles like clotrimazole, fluconazole, ketoconazole etc. two other azoles such as itraconazole and tinidazole were used here to know their anti-fungal effect. These synthetic anti-fungal drugs have been probably used for the first time in the determination of MIC of *C. albicans* isolates. The retarding effects of itraconazole on the growth as well as extracellular extrusion of protease are probably new.

Unfortunately, long term applications of some azoles have been shown to produce carcinogenic effects (Polak & Hartman, 1991). For example, long term use of azoles leads to nausea, vomiting, persistent diarrhoea and elevated effect of liver functions, gynaecomastia in males and gastro-intestine reaction with some patients (Shephard *et al.*, 1985). It has been also reported that certain strains are drug resistant (Ryley *et al.*, 1984; Smith *et al.*, 1936; Warnock *et al.*, 1988; Person *et al.*, 1991 and Molina *et al.*, 1992).

Inspite of all the drawbacks, the contribution of antifungal chemotherapy to the medical science is tremendous. However, it is clear that, a broad spectrum of fungicidal drug is required which are safe enough to be used prophylactically over a long period of time to very ill patients. It should be observed when it induces resistance.

Since, the beginning of human civilization, plants have been accepted as one of the source of the medicine (Mukherjee *et al.*, 1992). The secondary metabolites produced by plants serve as an important source of pesticides, microbicides and pharmaceutical drugs (Balandrin *et al.*, 1985; Heisey *et al.*, 1991). Hundreds of plant species have been tested for anti-microbial properties (Hayes, 1947; Fong *et al.*, 1972; Dube *et al.*, 1989; Nair & Burke, 1990) but the vast majority have not yet been
adequately evaluated (Balandrin et al, 1985; Heisey et al, 1991). Plant produced compounds are a source of safer and more effective substitute for synthetically produced anti-microbial agents and hence are of great interest. The aim of this investigation was to discover plant products that inhibit the microbe C. albicans as it causes 90% of the infection in man that are difficult to be controlled effectively. The pharmaceutical arsenal presently available to control the species is limited. Hence, plant products that inhibit them without harming the host may have potential for use as therapeutic agents (Heisey et al, 1991).

Against pathogenic yeasts like C. albicans, garlic extract was found to be much effective than nystatin, gentian violet or methylene blue (Kubelic, 1970). In certain feed protected chicks, Candidiasis was induced by oral inoculation and that could be cured by providing them a ration containing 5% garlic (Frasad & Sharma, 1980). Since then, a few reports on the anti-mycotic and particularly anti-Candidiatic properties of plant extracts are available (Giesbrecht et al, 1987, 1988; Barnabar and Nagarajan, 1988; Belousova et al, 1989; Howary et al, 1991; Abd-El-Nabi, 1992).

Of the various Candidostatic plant extracts, seeds of Pongamia glabrata produce oil, which showed anti-candidial properties at 20-30 µl/ml concentration. Another oil extracted from the seeds of neem, Azadirachta indica, is an economically important medicinal plant and is a good source of anti-fungal compounds (Rastogi & Dhar, 1987). Venugopal et al (1994) studied the anti-dermatophytic activities of the aqueous and ethanolic extracts of Azadirachta leaves and used about 86 clinical isolates of Trichophyton and Microsporum species to determine the minimal inhibitory concentration. In the present study, the Candidostatic efficacy of Azadirachta was found to 40-50 µl/ml. Similarly, oil of Ricinus communis showed anti-candidial property at 60-70 µl/ml concentration, Eucalyptus at 90-100 µl/ml concentration. However, Curcuma oil did not show any remarkable inhibition of growth and enzyme production in either of the strains even at the concentration of 100 µl/ml. The order of the Candidostatic efficacy of the various oils was observed to be Pongamia > Azadirachta > Ricinus > Eucalyptus > Curcuma. However, complete inhibition by this two, Eucalyptus and Curcuma was not observed.

It has been observed that oil extracted from the seeds of P. glabrata, A. indica, R. communis oils have therapeutic value. The use of such oils might be preventing the organism to come in contact with water and thereby impairing the growth of the...
Pongamia oil contains Karanjin and Azadirachta oil contains azadirachtin and nimbidin and that of Ricinus possess predominantly ricinoleic acid which probably contribute to their antimycotic properties. The toxic nature of R. communis against Aspergillus sydowi has been studied by Mishra et al., (1988).

It has been observed that the extracts prepared from certain plants contain mixtures of compounds which by themselves have no effect but together may produce a cumulative effect (Krallish et al, 1991). Oil and its products are multicomponent mixtures composed mainly of hydrocarbons, which are poorly soluble or insoluble in water. The interaction of lipophilic hydrocarbons, with membrane lipids and proteins must affect the thickness and fluidity of the phospholipid bilayers, as well as the activity of membrane enzymes and transport proteins (Sikkema et al, 1995). This, in turn, must lead to a malfunction of cell membranes, in particular to leakage of protons and other intracellular ions. These changes in the structure and permeability of membranes may influence the energy state and the homeostasis of cells, leading eventually to a decrease in their viability (Fomchenkov et al, 1997).

Here, in the present investigation, antifungals and oils were tested but the MIC value of the oils were found much more in comparision to the antifungals. This could be due to the fact that the extracts have been obtained in crude form and need to be properly purified. However, oil extracted from Eucalyptus and Curcuma had no or negligible effect on the growth and enzyme production in the two strains of C. albicans. In all the cases, the mycelial form showed greater resistance to both anti-fungals and plant extracts than the yeast form which proved the higher pathogenicity of mycelial form. This has also been observed by several workers (Odds, 1979, 1988; Rippon, 1982; Rook et al, 1987).

This investigation demonstrated that compounds inhibitory to C. albicans are present in certain plants. The metabolites of the plants not only retard the growth of the fungal isolates here but inhibit the enzyme secretion also.