2.0 REVIEW OF LITERATURE

Opportunistic fungal pathogen

More than 40 million people worldwide suffer from HIV infection/AIDS and about 3 million people die every year from this disease, according to the December 2005 report from the Joint United Nations Programme on HIV/AIDS and the World Health Organization (http://www.unaids.org/en/). Although still a fatal disease in many developing countries, AIDS has become a chronic disorder in developed countries following the introduction of highly active antiretroviral therapy (HAART) in the mid-1990s. However, neuropathological conditions are still present in approximately 70% to 90% of AIDS patients (Del Valle et al., 2006). Considered an AIDS-defining condition (Abadi J et al., 1999, Mamidi A et al., 2002, Wright D et al., 1997), Cryptococcal meningitis is the most common fungal infection of the central nervous system (CNS) and the third most frequent neurological complication in AIDS patients.

Taxonomy

- Kingdom: Fungi
- Phylum: Basidiomycota
- Subphylum: Basidiomycotina
- Order: Sporidiales
- Family: Sporidiobolaceae
- Genus: Filobasidiella (Cryptococcus)
- Species: Filobasidiella neoformans (Cryptococcus neoformans)
**Discovery of Cryptococcus**

In 1984, the roman born Italian medical scientist Sanfelleie isolated an encapsulated yeast like fungus from peach juices (Sanfelice, 1894), which he named *Sacchromyces neoformans*. He produced pseudo-tumoral, sarcoma like lymphoadenic lesions in experimentally infected animals after intra peritoneal inoculation with this yeast like fungus. The most fascinating phenomenon of capsule production by the fungus in man and animal causing a penicillin tumor like alteration of tissue prompted Sanfelice to propose the species epithet ‘neoformans’. Subsequently he isolated yeast from a lymph node of an ox that died from carcinoma of the liver (Sanfelice, 1894) and he considered this yeast to be a little different from that of *S. neoformans* and named *S. lithogens* because of its tendency to produce calcification in tissues it was recently relegated to a synonym of *C. neoformans*. At about the same time in humans, (Busse, 1894 and Buschke, 1895) separately reported the isolation of the same fungus from a sarcoma like lesions of the tibia and other lesions in a 31 year old woman, and the fungus was named as *S. hominis* (Busser 1895).

In France Curtis, (1895) described a yeast species to be agent causing myxomatous tumors an inguinal gelatinous time factor and a lumbar ulcerated abscess in a young Frenchman. After a benign surgical intervention, the patient was discharged from the hospital and died ten month later with meningitis manifestation without other details (Curtis, 1895). Curtis named the yeast megalococcos mycoides and reported preliminary experimental dated on mice, rats and guinea pigs reproducing tumor like lesions in the following year Curtis who knew the publications of Sanfelice and Busse, gave a detailed description of the capsulated yeast with round cells and surprising elongated, oval and bacilliform yeasts and he empherised the absence of fermentation at 37° C (Curtis, 1895).
Curtis obtained experimental lethal infection in cats inoculated subcutaneous with enormous tumour like lesions as well as involvement of the lungs, spleen and kidneys. Similar lesions were obtained in mice, guinea pigs and dogs. Granulomatous cellular reaction or the absence of reaction similar to those observed in the patient were described. Curtis named this strain *Saccharomyces subcutaneous tumefaciens*, considering it to be distinct from the *S. neoformans* of sanfelice and the *S. hominis*. Busse, because of the morphological differences and its great affinity for the subcutaneous tissues of experimental animals.

In France the botanist and mycologist, Vuillemin (1901) examined several culture and he did not find the ascospore characteristics of the genus *Saccharomyces* and placed it in the genus *Cryptococcus* the generic name created for yeasts without endospores by Kutzing (1833). Therefore, Vuillemin reclassified the yeasts isolated by Bussed and Curtis in the genus Cryptococcus and the isolated of Sanfelice (1894) as *C. neoformans* confirmed by Fell *et al.* (1989). Several cases were reported especially in Europe and the disease was confused with blastomycosis and considered to be an European blastomycosis.

In the U.S.A, Stoddard and Cutler (1916) described the clinical aspects of the disease. Ignoring the European literature, the investigators mistook the mucoid capsule of the yeast observed in the USA among European immigrants for histolysis of host tissue and therefore named the fungus *Torula histolytica* without any proof of enzymatic hydrolysis (Drouhet and Segretain, 1950) and the disease was known for a long time as Torulospy or Torula meningitis, until the turns *C. neoformans* and Cryptococcosis became widely used in medical mycology owing to the efforts of Benham (1935).
Benham clearly differentiated Cryptococcosis from the blastomycosis especially from North American blastomycosis by studying more than 40 species of Cryptococcus species including original cultures from Sanfelice, Busse and Curtis, for her dissertation on yeast identification.

**Infection cycle – Humans**

Cryptococcosis is not contagious. There is no person to person or animal to person transmission. However, two unusual cases of person to person transmission of cryptococcosis have been reported, in a recipient of a corneal transplant from a donor with cryptococcosis developed localized cutaneous cryptococcosis after accidentally inoculating himself with blood from a patient Cryptococceamia (Glaser and Gardern, 1985). The general infection cycle of this pathogen is given in Plate 1.

**Pathogenesis**

Generally the organism enters the host by the respiratory route in the form of dehydrated haploid yeast or basidiospores as an infectious propagate. After sometime in the lungs, the organism spreads haematogenously to extra-pulmonary tissues. Since C. neoformans has a special predilection for the CNS, most frequently diagnosed form of the disease is Cryptococcosis of CNS. The reason for predilection of Cryptococcus for the CNS has not been explained but it is believed that the organisms probably encounters less cellular (Phagocytic) response there and it has been theorized that selective nutritional factors for they yeast are present in the CNS. Entrance of the organism through the skin and nasopharyngeal mucosa is possible but is also considered extremely rare.
**Pulmonary Cryptococcosis**

Pulmonary Cryptococcosis includes patchy pneumonitis, single or multiple nodules, lobar consolidation and large mass lesions mimicking carcinoma. Caritation and pleural effusions are less common. The course of pulmonary Cryptococcosis is also variable. In patients with normal host defenses, it resolves spontaneously and in contrast, pulmonary Cryptococcal disease in immunocompromised hosts tends to be progressive and severe. Patients have not symptoms or a minority may experience cough, sputum production, weight loss or low grade fever (Joseph *et al.*, 1993; Mulanovich *et al.*, 1995).

**CNS Cryptococcosis**

Meningitis, usually subacute or chronic in nature, is the most common manifestation of CNS disease. Cryptococcosis appears to be less common in patients with AIDS than in other patient groups. Because upto 20% of patients with Cryptococcosis have no sign or symptoms of focal neurological disease or increased intracranial pressure. Meningoencephalitis is an uncommon form, is a fulminate, rapidly developing infection, often leading to coma and death within a short time.

The usual progression of symptoms are fever, headache, stiff neck and disorientation are accompanied by spinal fluid that is typically clear, increased opening pressure, presence of mononuclear cells, elevated proteins and normal or reduced chloride and sugar.

Complications of CNS Cryptococcosis include hydrocephalus, visual disturbances including blindness, hearing loss, other cranial palsies, ataxia seizures and dementia. The mortality rate ranges from 15-30% and most deaths occur within the first several weeks of illness (Dismukes, 1994; Powderly, 1993).
Miscellaneous forms

The skin, skeletal system and prostate gland follow in frequency of involvement after the lungs and CNS. Cryptococcal infection involves skin about 10% in patients with AIDS and it may be the first sign of infection. (Handa et al., 1996). Cryptococcal skin lesions can be highly variable in appearance and include papules, pustules, nodules, ulcers and draining sinuses. Osteomyelitis is more common than septic arthritis. The prostate gland appears to be an important reservoir of infection, which may serve as a source of relapse after completion of apparently successful primary therapy (Dismukes, 1994).

![Infection cycle of Cryptococcus](image)

Plate 1. Infection cycle of *Cryptococcus* (environment and host). (Lin and Heitman, 2006)

**Animals Cryptococcosis**

Naturally acquired Cryptococcosis has also been recorded in animals such as cattle’s, horses, dogs, culls, frogs, ferrets, monkeys, goats, pigs, birds, mice (Carter and Young, 1950; Emmons, 1952; Sacquet et al., 1959) and even in reptiles.
Environmental reservoir of *Cryptococcus* species.

*C. neoformans* was isolated in nature first from peach juice (Sanfelice, 1894) then from milk, soil and pigeon excreta (Emmons, 1951 and 1955; Ajello, 1958). Although pigeon droppings commonly colonized with *C. neoformans*, pigeons do not appear to become sick due to Cryptococcosis, perhaps because their high body temperature, is determined to growth of the organisms (Abou-Gabal and Atia, 1978)

Since Emmon’s original reports, *C.neoformans* has been isolated from soil, pigeon excreta and sites contaminated by pigeon excreta in various parts of the world e.g. U.S.A (Littman and Schneierson, 1959), Australia(Frey *et al.*, 1964) England (Randhawa *et al.*, 1965), Swedan (Bergman, 1963), Nigeria(Gugnani and Nijok-obi, 1973) Thailand (Balankura, 1974) and India (Gugnani *et al.*,1976).

It has been isolated from other avian sp; including canaries chickens, parrots, shylaks, sparrows, starlings, doves and turtle (Abou-gadal and Atra, 1978; Bauwens *et al.*, 1986). The reason for the high frequency of *C. neoformans* in avian excreta is not clear but may be related to the ability of the fungi to assimilate xanthine, urea, uric acid and creatinine, all of which are abundant in the droppings (Littman and Walten, 1968).

The concentration of *C.neoformans* in pigeon droppings often exceed $10^6$ viable organism/gram and most investigators have encountered little difficulty in isolating *C. neoformans* directly from pigeon excreta or samples contaminated by such excreta (Littman and Schneierson, 1959; Emmons, 1960; Muchmore *et al.*, 1963; Procknow *et al.*, 1965; Littman and Borok, 1968).

In contrast to the situation with pigeon droppings, a lower of percentage of soil samples were positive for *C. neoformans* and the concentration of organisms
in soil tend to be less (Ajello, 1958). Probably the soil forms an inhospitable environment for *C. neoformans*. In support of this anaerobic condition, high temperature, decreased humidity, direct sunshine, low PH and the presence of soil amoeba and other microbes have all been shown to be detrimental to the survival of *C. neoformans* in soil (Bunting *et al.*, 1979; Ruiz *et al.*, 1982).

The experimental findings (Bunting *et al.*, 1979) indicate that Acanthamoeba polyphage in the trophozoite stage actively ingests and kills of the cells of *C. neoformans* and some of the surviving cells of *C. neoformans* developed into colonies containing pseudohyphae. These pseudohyphae forms may be a biological “escape batch” and that the soil amoeba may be an important biological coated mechanism in nature. The studies of (Ruiz *et al.*, 1982) suggest that many soil organisms like *Pseudomonas aeruginosa*, *Bacillus subtilis*, trophozoites, mites and sow bugs that occur in pigeon droppings may be a complex of biological factors that influence *C. neoformans* persistence, reproduction, morphology and distribution in nature.

Staib (1963) reported that pigeon dropping provide an excellent culture medium for *C. neoformans*. Valta and Yee (1968) failed to isolate *C. neoformans* from chicken droppings and they suggested that this could be done to their high alkalinity and the presence of low molecular weight, thermostable, growth suppressing substance in this material. Littman and Borok (1968) reported that *C. neoformans* multiplied rapidly in an extract from sterile pigeon droppings and remained viable in this material for many months.

*C. neoformans* is occasionally isolated from various non-avian sources, including fruits, vegetables, dairy products and the digestive tract of the cockroach (Pal and Mehrotra, 1984; Swinne *et al.*, 1986). Dead and decaying vegetation could also form a suitable substrata for this fungus (Staib *et al.*, 1972).
The *C. neoformans* that was isolated from bird excreta or soil (contaminated with bird excreta) throughout the world belong to var *neoformans* (Muchmore *et al.*, 1980; Mishra *et al.*, 1981; Kwon-chung and Bennett, 1984).

Plate 2. *Mating types and life cycle of the yeast.* (Lin and Heitman, 2006).

**Biology of the yeast:**

Biological variants in this yeast are identifiable based on mating, serology and molecular differences. A varietable revolution in the knowledge of *C. neoformans* was the discovery of its sexual reproduction by Kwon-Chung (1975), who proposed the genus Filobasidiella to accommodate this basidiomycete. *C. neoformans* is most commonly isolated as budding yeast from patients and the environment (Casadevall *et al.*, 1998). However, as a heterothallic basidiomycete, it can undergo a dimorphic transition to a filamentous growth form by two distinct differentiation pathways: mating and monokaryotic fruiting
(Plate 2). *C. neoformans* mating was discovered three decades ago and involves fusion of haploid cells of opposite mating types, “a” and “α” to produce dikaryotic filaments, which eventually leads to the formation of a basidium in which meiosis occurs to produce four chains of readily aerosolized basidiospores (Kwon-Chung, 1975 and 1976).

Shadomy and Utz (1966) and later Shadomy (1970) had reported clamp connections in hyphae forming isolates of *C. neoformans*. Formation of the *F. neoformans* teleomorph was observed when crosses were made between two mating types of serotype A or D isolates (Kwon-chung, 1975 and 1976a) where as the *F. bacillispora* teleomorph was observed when similar crosses were made among serotype B and C isolates Kwon-chung, 1976b). Kwon chung (1976b) concluded that there were two varieties of *F. neoformans*, *F. neoformans* var *neoformans* corresponding to the sexual state, *C. neoformans* var *neoformans* with serotypes A and D, and *F. neoformans* var *bacillispora* corresponding to the asexual state of *C. neoformans* var *gattii* with the serotypes B and C.

Although *C. neoformans* is heterothallic and assumes a basidiomycetous state, upon mating in the laboratory isolates of the two compatible mating types from the same clinical or environmental sources have rarely been found. This indicates the fungus primarily reproduces asexually and only rarely generates genetically distinct clones within given population (Kwonchung and Bennett, 1978).

The existence of self fertile isolates that produces a complete sexual state in the apparent absence of cross mating has been also reported (Phaff and Fell, 1970; Erke, 1976; Kwon-chung, 1977).
Cryptococcus can undergo intervarietal (serotype A×serotype D) and interspecies (B×D, C×D) matings, but the viability of the basidiospores is reduced and many diploid and aneuploid progeny are generated from these matings, indicating that genomic divergence impairs meiosis (Lengeler et al., 2001). It has long been observed that clinical and environmental isolates are predominately of the α-mating type (>98%–99.9% average) and that mating type is a virulence factor. ‘α’ strain of C. neoformans var. neoformans are more virulent than congenic ‘a’ strains in certain genetic backgrounds (Kwon-Chung, et al., 1978 and 1992). ‘α’ strains of C. neoformans var. grubii are also more likely to penetrate the CNS during coinfection with congenic ‘a’ strains via the pulmonary route of inoculation (Nielsen et al., 2005).

C. neoformans var. neoformans strains undergo monokaryotic fruiting, producing filaments and basidiospores under laboratory conditions. Although monokaryotic fruiting was thought to be strictly haploid, mitotic, and asexual, fruiting was recently recognized to be a modified form of sexual reproduction occurring between strains of the same mating type (Lin et al., 2005) Monokaryotic fruiting is predominantly observed in ‘α’ strains (Wickes et al., 1996) and future research should address which α-specific genes confer this advantage to ‘α’ strains, whether monokaryotic fruiting occurs in C. neoformans var. grubii or C. gattii, and when and where this developmental cascade occurs in nature, needs to be studied.

Serotypes and antigenic composition

Based on capsular agglutination reactions (Belay et al., 1996), there are five serotypes: A, B, C, D, and AD hybrid (hybrids between serotypes A and D). On the basis of biochemical tests, such as the ability to use glycine as the sole carbon and nitrogen source, resistance to canavanine, EDTA resistant urease, and
the morphology of the sexual state (such as the shape and texture of basidiospores), *C. neoformans* was originally accepted to include two varieties: var. *neoformans* (serotypes A, D, and the AD hybrid) and var. *gattii* (serotypes B and C) (Levitz, 1991). More recently, *C. neoformans* var. *gattii* has been recognized to be a separate species, *Cryptococcus gattii* (Kwon-Chung *et al.*, 2002). In addition to the previously observed phenotypic differences, molecular studies and genome sequences have detected significant genetic variations between serotypes A and D, and recently serotype A has been distinguished as a new variety, var. *grubii* (Franzot *et al.*, 1999). Currently, this organism is classified into two varieties and a sibling species: *C. neoformans* var. *neoformans* (D), *C. neoformans* var. *grubii* (A), and *C. gattii* (B/C).

**Molecular Types**

*C. neoformans* has been further subdivided into nine distinct molecular types (Plate 3) on the basis of DNA sequence polymorphisms detected by PCR fingerprinting, RAPD, amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), and multilocus sequence typing (MLST) analyses (Litvintseva *et al.*, 2005, Meyer *et al.*, 2003 and Sorrell *et al.*, 1996). Serotype A isolates produce VNI, VNII, or VNB (so far unique to Botswana) patterns; AD hybrids produce a VNIII pattern; and serotype D isolates produce a VNIV pattern. Serotype B defines VGI, VGII, and VGIII patterns, while serotype C yields predominantly a VGIII or VGIV pattern (Meyer *et al.*, 1999) (Plate 3). The boundary between serotypes and molecular types is not distinguishable in all cases, particularly in *C. gattii*. As a basidiomycete with a bipolar mating system, *C. neoformans* contains only one *MAT* locus, and it occurs in two idiomorphic forms, corresponding to the two opposite mating types, ‘a’ or ‘α’. Mating type can be determined by mating assays or molecular analysis.
Matings are scored for filamentation and basidiospore formation. Often PCR-amplified serotype- and mating type-specific products are used for mating type confirmation in cases of self-filamentous strains, sterile strains, or diploid or aneuploid isolates.

Plate 3: Evolution of the *C. neoformans* species complex (Lin and Heitman, 2006).

**Virulence factors**

The capsule formation is one of the two best known virulence factors of *C. neoformans* for man and animals. Drewhet *et al.*, (1950) studied the chemical composition of the capsular polysaccharide of *C. neoformans* which led to an important identification of Glucuronoxylomannan (GXM) by paper chromatography. They showed that GXM is a virulence factor related to the dimensions of the polysaccharide capsule of natural factorials (smooth, weak by capsulated yeasts) and M (mucoid, broadly capsulated yeasts) colonies. The GXM inhibits the migration of leucocytes and phagocytosis as showed by Drouhet and Segretain (1951).
It has been demonstrated that capsule deficient mutants that little or no virulence in mice (Bulmer et al., 1967; Kozel and Cazin, 1971). The degree of encapsulation in vitro however has never been shown to correlate with the degree of *C. neoformans* virulence in vivo (Dykstra et al., 1977). Although previous studies on the role of encapsulation in the virulence of *C. neoformans* were convincing they did not explain why *C. neoformans* is the only pathogen in the genus *Cryptococcus* in which, all members are normally encapsulated.

The formation of melanin pigment is yet another virulence factor. The enzyme responsible for the pigment formation was identified as a membrane bound phenoloxidase enzyme (Polacheck et al., 1982; Polacheck and Kwon chung 1988).

Phenoloxidase enables *C. neoformans* to synthesis melanin from certain catecholamine precursors. Phenoloxidase activity and melanin production have been postulated to contribute to the propensity for *C. neoformans* to invade the CNS, an area rich in catecholamine (Rhodes et al., 1982).

The end product of the phenoloxidase activity was implicated in certain aspects of the virulence and its experimental pathology on animals studied (Kwonchung et al., 1982, Rhodes et al., 1982, Polacheck et al., 1990, Kwonchung, 1992). Huffnagle et al., (1996) has shown in animal experiments that a low melanin producing the strains induced production of tumour necrosis factor (TNF) followed by inflammation and resolution of the infection but a high melanin producing strain did not elicit TNF until a late stage and the infection progressed and become disseminated. More ever the pigmented cells are less susceptible to free radicals killing, suggesting that the melanin like pigments protects against oxidants produced by host affector cells and the pigmented cells are also less susceptible the antifungal agent and Amphotericin B and this may contribute to
persistence of infection in human (Wang et al., 1995). Kwonchung and Rhodes (1986) showed in experimental Cryptococciosis in animals, the virulence of C. neoformans is highly dependent upon combination of two factors, the capsule formation and the production of melanin at 37° C.

**Laboratory Diagnosis**

Direct microscopic examination of the samples or cultures is the easiest method for diagnosis in the lab. The encapsulated yeast exhibit capsules under negative staining. These cells are culturable on routine mycological or bacteriological media with characteristic colony morphology. Special growth properties distinguish this yeast from other common yeast such as Candida spp. For instance, unlike other species of Cryptococcus – the pathogen grow well on 37 ºC. They also grow well on media carrying o - diphenol producing brown to black pigment due to synthesis of melanin and laccase (Shaw and Kabica, 1972). For e.g, their growth on Staib’s bird seed agar is a routine laboratory test. Urease test, lack of nitrogen utilization, proline utilization and lack of fermentation of sugars are other distinguishing biochemical test. Differential reaction on CGB and GCP media help distinguish between the serotypes. (Shadomy et al., 1987).

Many of these features also help pathogen survive adverse conditions. For instances capsules protect these cells from desiccation. (Casadevall et al., 2003).

Culture in the laboratory and molecular tests are routinely used for diagnosis. But sensitivity and selectivity in some samples results in indeterminate results. Many of the tests also take time to confirm, for instance culture for three days. Hence search for robust, reliable assays that can be done in a short time is the main purpose of several ongoing studies. In this context, we evaluated FTIR spectroscopy for diagnosis of this disease.
Indian studies

The first case of Cryptococcosis in India was reported by Balakrishna Rao and Lialauwala, 1952), subsequently a number of isolated case reports on Cryptococcosis have appeared from various parts of the country (Sinha and Barua, 1960; Basu Mallik et al., 1961; Subramanian et al., 1965; Aikal et al., 1967). However, some studies with larger series started appearing only after 1985 from different centers (Talwar and Meera, 1986; Banerjee et al., 1995; Khanna et al., 1996). But in depth prevalence study and characterization of clinical and environmental isolates have not been done.

Pal et al. (1990) isolated C. neoformans from environmental sources, all of which were var neoformans. Pal et al., (1991) reported that the ‘α’ mating type occurs more frequently in both clinical and environmental isolates. Padhye et al., (1993) reported that of 18 clinical isolates, 15 belonged to var neoformans and 3 belonged to var gattii. It was the first documented record of the var gattii from Eucalyptus camaldulensis in India, (serotype B).

FTIR Spectroscopy

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic and inorganic chemists. Simply it is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies IR radiation. Using various sampling accessories, IR spectrometers can accept a wide range of sample type such as gases, liquids, and solids. Thus IR spectroscopy is an important and popular tool for structure elucidation and compound identification (Germlich, 1999).
**IR frequency range and spectrum**

Infrared radiation spans a section of the electromagnetic spectrum having wave numbers from roughly 13,000 to 10 cm⁻¹, or wavelength from 0.78 to 1000 µm. It is bound by the red end of the visible region at high frequencies and the microwave region at low frequencies (Cooper *et al.*, 1995).

IR absorption positions are generally presented as either wave numbers or wave lengths. Wave number defines the number of waves per unit length. Thus, wave numbers are directly proportional to frequency, as well as the energy of the IR absorption. The wave number unit (cm⁻¹, reciprocal centimeter) is more commonly used in modern IR instruments that are linear in the cm⁻¹ scale.

Wave numbers and wavelengths can be interconverted using the following equation:

\[
V \text{ (in cm}^{-1}) = \frac{1}{\lambda \text{ (in µm)}} \times 10^4
\]

IR absorption information is generally presented in the form of a spectrum with wavelength or wave number as the X-axis and absorption intensity or percent transmittance as the Y-axis. Transmittance, T, is the ratio of radiant power transmitted by the sample (I) to the radiant power incident on the sample (I₀). Absorbance (A) is the logarithm to the base 10 of the transmittance (T).

The IR region is commonly divided in three smaller areas -

<table>
<thead>
<tr>
<th></th>
<th>Near IR</th>
<th>Mid IR</th>
<th>Far IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave number (cm⁻¹)</td>
<td>1300-4000</td>
<td>4000-200</td>
<td>200-10</td>
</tr>
<tr>
<td>Wave length (µm)</td>
<td>0.78-2.5</td>
<td>2.5-50</td>
<td>50-1000</td>
</tr>
</tbody>
</table>

With the diagnostic region being 4000-200 cm⁻¹
**Swietenia mahagoni**

Members of Meliaceae or neem family have long been known to possess antifungal activity. Neem and its extracts have been used to control agricultural pests and fungal phytopathogens. Investigations to study the efficacy of these extracts to inhibit *C. neoformans* are however lacking.

**Antifungal activity of extracts from Swietenia mahagoni**

**Scientific name** - *Swietenia mahagoni*

**Common name** - Telugu (mahogany chettu), Tamil (mahogany ciminukku), Malayalam (cheria mahogany), English (mahogany), Hindi (mahogany).

**Classification** - Based upon - Bentham and Hooker (1862), Pandey (2001)

- Division  - Phanerogams
- Class  - Dicotyledones
- Sub class  - Polypetalae
- Series  - Disciflorae
- Order  - Geraniales
- Family  - Meliaceae
- Genus  - Swietenia
Plate 4: *Swietenia mahagoni*

This tree species grows to a height of 40 – 50’ feet. Parts of the tree have been used in traditional medicine.

**The genus *Swietenia***

This genus is found only in the neotropics (Navarrol *et al.*, 2004) and consists of three species: *Swietenia mahagoni* Jacq., *Swietenia macrophylla* King, and *Swietenia humilis* Zucc.; and two natural hybrids. One of these is a product of a cross between *S. macrophylla* and *S. humilis*; this is found in the areas of the distribution range in which the two species overlap; the other is a cross between *S. macrophylla* and *S. mahogani*, named *S. xaubrevilleana* which has been found close to plantations of the two species (Pennington *et al.*, 1981).
Traditional usage of *Swietenia mahagoni*

<table>
<thead>
<tr>
<th>Part</th>
<th>Country</th>
<th>Condition</th>
<th>Treatment</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>Mexico</td>
<td>Decoctions or Infusions of ground seed.</td>
<td>Antihelminthic, cure of ameobiasis, chest pain, cough.</td>
<td>(Rosabel et al., 1993).</td>
</tr>
<tr>
<td>Seed</td>
<td>Indonesia</td>
<td>Decoctions.</td>
<td>Hypertension, Diabetes, Malaria.</td>
<td>(Kadota et al., 1990).</td>
</tr>
<tr>
<td>Bark</td>
<td>Indonesia</td>
<td>-</td>
<td>Antipyretic, Tonic, astringent.</td>
<td>(Blume, 1998).</td>
</tr>
<tr>
<td>Bark</td>
<td>Godhavari</td>
<td>Grinded bark juice and water.</td>
<td>Paradum (releasing of sperm in after mating).</td>
<td>(Blume, 1998).</td>
</tr>
</tbody>
</table>

Biological activity of *Swietenia*

Compounds isolated from *Swietenia mahagoni* cotyledons swietemahonin-A, swietemahonin-E, 3-O-acetylswietenolide and 6-O-acetylswietenolide belong to the class of tetranortriterpenoids exhibiting antagonistic effect on platelet activating factor (PAF), (Kadota et al., 1991).

Humilinolide-A, humilinolide-B, humilinolide-C, humilinolide-D compounds belong to the class of limnoids. They were isolated from air dried and pulverized plant material of *Swietenia humilis* and showed antifungal, antiviral, and bacteriocidal activity. It has also anti-feedant property, which on testing showed growth inhibition on 3rd instar larva of *Tenebril molitor* and some growth regulatory effect on the radical growth of *Amaranthus hypochondriacus* and *Echinocholoa crusgalli* (Rosabel et al., 1993).
6-acetyl swietenine, 6-acetyl-3-tigloylswietenilide is a limnoid isolated from *S. mahagoni*. They showed antifungal activity against the groundnut rust of *Puccinia arachidis* (Govindachari *et al.*, 1999).

Methylene chloride and methanol extracts of 20 Indonesian plants with ethno medical uses have been assessed for *in vitro* antibacterial and antifungal properties by disk diffusion method. Extracts of the six plants: *Terminalia catappa*, *Swietenia mahagoni Jacq.*, *Phyllanthus acuminatus*, *Ipomoea* spp., *Tylophora asthmatica* and *Hyptis brevipes* demonstrated high activity in this bioassay system (Goun *et al.*, 2003).

The peroxisome proliferator-activated receptor PPARγ agonistic activity of *S. mahagoni* extract at a concentration of 50 μg/L was approximately half that of 35.7 μg/L (0.1 μmol/L) of rosiglitazone (clinical drug). At the dose of 1000 mg/kg, *S. mahagoni* extract remarkably decreased the blood glucose concentration of *db/db* mice from (15.26±2.98) to (7.58±2.20) mmol/L, and reduced the blood glucose levels by 55.49% compared with the control group (*P*<0.01) (Dan-dan *et al.*, 2005).

Scientific investigation of the bioactivities of extracts of meliaceae members included antifungal, antiviral, antibacterial activities. Antifungal investigations usually were reported for plant pathogens while human pathogenic fungi have not been systematically investigated.
### Compounds isolated from *Swietenia*

<table>
<thead>
<tr>
<th>Name of the compound</th>
<th>Plant species (plant parts)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl anglonsate</td>
<td><em>S. mahagoni</em> (cotyledon)</td>
<td>(Govindachari et al., 1999)</td>
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<tr>
<td>Methyl 6-hydroxyangolensate</td>
<td>&quot;</td>
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<tr>
<td>3-o-acetylswietenolide</td>
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</tr>
<tr>
<td>Swietemahonin(A-G)</td>
<td>&quot;</td>
<td>&quot;</td>
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<tr>
<td>Swietemahonolide</td>
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<tr>
<td>6-O-acetyleswietenolide</td>
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<td>3-O-tigloyl-6-O-acylswietenolide</td>
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<tr>
<td>Swietenine</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Swietenine acetate</td>
<td>(ether soluble fraction of cotyledon)</td>
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<tr>
<td>Swietenolide</td>
<td>&quot;</td>
<td>&quot;</td>
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<tr>
<td>3, 6-O, O-diacetylswietenolide</td>
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<td>Proceranolide</td>
<td>&quot;</td>
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<tr>
<td>7-deacetoxy-7-oxogedunolide</td>
<td>&quot;</td>
<td>(Allan Ramlal et al., 1999).</td>
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<tr>
<td>3-O-tigloylswietenolide</td>
<td>&quot;</td>
<td>(Chatterjee et al., 1960).</td>
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<tr>
<td>Swietemahonin (A, E, F)</td>
<td>(amorphous mass obtained from the ether extract)</td>
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<tr>
<td>Mahonin</td>
<td>(oily fraction of ether extract of Cotyledon)</td>
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<td>Secomahonin</td>
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<td>Cyclomahogenol</td>
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<td><em>S. macrophylla</em></td>
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<td>Sietenolide</td>
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<tr>
<td>Adirobin</td>
<td>Seeds</td>
<td>Hisao et al., (1990)</td>
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<tr>
<td>2-hydroxy swietenine</td>
<td>&quot;</td>
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<td>6-deoxyswietenine</td>
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<td>Deacetylgedunin</td>
<td><em>S. aubrevilleana</em></td>
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<td>6-hydroxymethylangoleensate</td>
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<td>6-acetoxyhumulinolide</td>
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<td>Humulin B</td>
<td><em>S. humulis</em> (Seeds)</td>
<td>Rosabel et al., (1993)</td>
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<td>2-methyl-2hydroxy-3(30)-enate</td>
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<tr>
<td>2-methyle-3-tigloylloxymeriac-aenate</td>
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<td>Humulinolide (A, B, C, D)</td>
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