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Cyclodextrin nanoparticles incorporated fluconazole and medicianal plant extracts preparation for the improved anti fungal activity against human pathogenic fungi

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Abstract: Fungal resistance to drugs in the azole class tends to occur gradually over the course of prolonged drug therapy, resulting in clinical failure in immunocompromised patients such as patients with advanced HIV receiving treatment for thrush or esophageal *Candida* infection. Development of novel anti fungal drugs with the improved efficacy is an important emerging area of medicine. Evolution of improved antifungal activity of chemotherapeutic conventional antifungal drugs and various medicinal plants with cyclodextrin nanoparticles against human pathogen fungi *Candida albicans* and *Trichophyton rubrum* was carried out in the present study. Cyclodextrin nanoparticles were synthesised by ionic gelation methods and the synthesised nanoparticle was further loaded with fluconazole, methanol and chloroform plant extracts. Distinct effect on mycelial growth, biomass was recorded in the nano-drug conjugate. The present study would suggest the possible utilization used of chitosan coated cyclodextrin incorporated with fluconazole and plant extract's as an effective antifungal drug against for human pathogenic fungi.
Keywords.. Cyclodextrin nanoparticles, *Candida albicans, Trichophyton rubrum*, fluconazole,plant extracts.

Introduction

Human beings are often infected by microorganisms such as bacteria, molds, yeasts, and viruses present in their living environments. Because of the emergence and increase in the number of multiple antibiotic-resistant microorganisms and the continuing emphasis on health-care costs, many scientists have researched methods to develop new effective antimicrobial agents that overcome the resistances of these microorganisms and are also cost-effective. Such problems and needs have led to resurgence in the use of silver-based antiseptics that may be linked to a broad-spectrum activity and considerably lower propensity to induce microbial resistance compared with those of antibiotics[1,2,3].Among the different microorganism, fungi are the major candidature known to cause a wide range of fungal infection[4].Many of the conventional anti fungal drugs are fail to treat the fungal infection because the unusual drug resistance mechanism developed the organism.Hence the development of novel drugs against fungal organism is an emerging field in the area of medicine.

Nanotechnology is significant on account of its pre-eminence upon the comprehension, use, and control of matter at magnitudes of a minute scale, akin to approaching atomic levels, with which to manufacture new substances, instruments, and frameworks[5].Nanoparticles possess exceptional physical and chemical properties, which lead to rapid commercialization. Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging, sensing, targeted drug delivery, gene delivery Systems and artificial implants. Hence, nanosized organic and inorganic particles are catching increasing attention in medical applications due to their amenability to biological functionalization[6]. In present situation,

metallic and non metallic nanoparticles are in great use in the medicinal, pharmaceutical, agricultural industry and in water purification[7].Taken together, nanoparticles as a highly safe compound may be considered for combination therapy against pathogenic microorganism due to its potential synergistic effect with important antibiotics. These nanoparticles exhibit tolerable monodispersity and in the case of particles synthesized extracellularly, exhibit excellent long term stability[8].

Cyclodextrins have a wide range of applications in different areas of drug delivery and pharmaceutical industry due to their complexation ability and other versatile characteristics[9].The most common pharmaceutical application of cyclodextrin is to enhance the solubility, stability, safety and bioavailability of drug molecules. The objective of this contribution is to focus on the potential use of chemically modified cyclodextrins as high-performance drug carriers in drug delivery systems with emphasis on the more recent developments[10].Thus cyclodextrins, because of their continuing ability to find several novel applications in drug delivery, are expected to solve many problems associated with the delivery of different novel drugs through different delivery routes[11].In the present study, chitosan stabilized cyclodextrin nanoparticles incorporated fluconazole with the medicinal plant extracts was evaluated against *C.albicans* and *T.rubrum*

Experimental

Preparation of cyclodextrin nanoparticles- fluconazole conjugate

Cyclodextrin nanoparticle incorporated drug conjugate was synthesised by modified method of Adriana Trapani, *et al* [12]. 0.5 % of cyclodextrin was mixed with equal volume of chitosan .The mixture was stirred under magnetic stirrer at room temperature by adding drop-drop of sodium tripolyphosphate. Commercial available fluconazole (1.0mg) was added (1ml) to the mixture and the preparation was kept under magnetic stirrer and the slurry thus obtained was freeze dried, used for further studies. Characterization of the nano drug conjugate was studied by scanning electron microscopy (Carl zeiss Germany equipped with energy dispersion spectroscopy.

Preparation of plant extracts incorporated cyclodextrin nanoparticles

Collection of plant materials

Aloe vera (Aloe vera), Tulsi (Ocimum tenuiflorum), Eucalyptus (Eucalyptus globus), Cascara or Golden Shower (Cassia fistula) leaves were collected, washed in distilled water and allowed to shade dry, the dried material was homogenized in domestic mixture into fine powder, stored in plastic container at room temperature

Preparation of crude extracts

Crude extract of the respective plant materials was carried out by modified method\ of Wilson [13] .The dried powder of respective plant materials (50g) was soaked separately with 250ml of methanol & chloroform in 500ml conical flask for 48 hrs at room temperature without shaking. The solvent was filter through (Whatman filter paper no.1) & concentrated on rotary vacuum evaporator. Concentrated crude extract was reconstituted in dimethyl sulfoxide (DMSO) to make stock solution of 10, 25, 50, 75&100g/ml & stored at 20*C until used.

Preparation of cyclodextrin nanoparticles incorporated plant extracts

Respective plant extract incorporated CDs+CS+F formulation was prepared by mixing equal volume of CDs+CS+F suspension with 1ml of methanol and chloroform of respective plant extract separately,kept under stirring for two hours.Homogenous substance thus obtained was dried at hot air oven at 45^0C and the dried material was used for further studies

Evaluation of anti fungal activity

Anti fungal activity was studied against human pathogenic fungi *Candida albicans* and *Trichophyton rubrum*. Both the fungi were obtained from Madurai Medical College and the respective fungal strains were maintained on sabouraud agar slant. Well diffusion assay was used for anti fungal activity against *C.albicans* and food poisoning technique was adopted against *T.rubrum*.

24 hour culture of *Candida albicans* was swabbed on SDA plate.. Well were made using cork porer and 100µl of respective plant material incorporated formulation and free fluoconazole was added into the wells. The seeded plates were incubated at 35*C 24-48hrs. After the incubation the zone of inhibition was measured.

In the case of *T.rubrum*, anti fungal activity was determined by food poisoning technique. 0.1 ml of the respective plant extract incorporated cyclodextrin nnaoparticle suspension was added to the 100 ml of sterile molten SDA media, mixed well and poured into sterile petri plates, allowed to solidify.After solidification, fungal plug (1cm ^{2}) of *T.rubrum* was kept on the media. Seeded plates were incubated at 35 C and mycelial growth was measured. Improved activity was calculated by the following formula

Improved efficacy =

CDs+CS (Nano-particle) – Zone of inhibition in plant extract treatment X100
CDs+CS (Nano-particle)

Result and Discussion

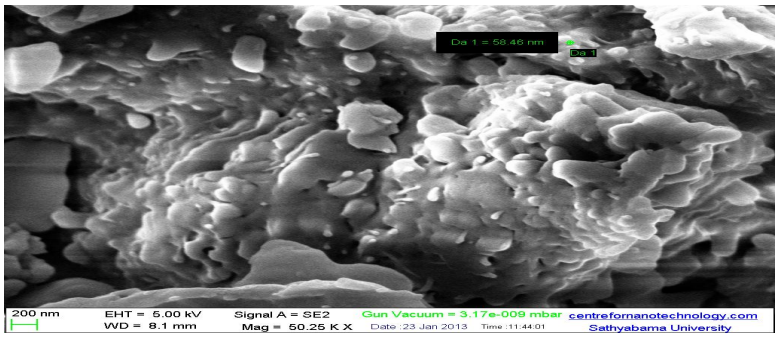


Figure 1.SEM image of CS-CDX Nps

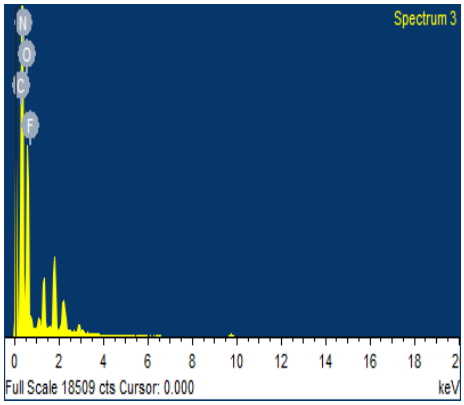


Figure 2.EDAX analysis of CS-CDX Nps

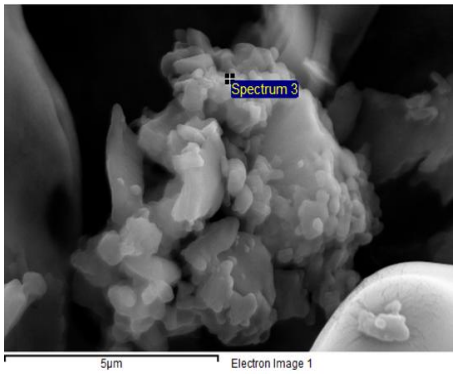


Figure 3.SEM image of CS-CDX-F

Chitosan coated cyclodextrin nanoparticles (CS-CD Np) synthesis was primarily confirmed by formation of white color precipitate. Particles morphology by scanning electron microscopy reveals spherical particles with the size range of 80-90 nm (Figure 1).Elemental composition of nano-conjugate was confirmed and show the percentage of carbon atomic is 57.32% with weight of 50.79% and all the other compostion are recorded (Figure 2). Synthesis and characterization of chitosan stabilized cyclodextrin nanoparticle) incorporated Fluconazole was primarily confirmed by dense milky white precipitate. Particle size was confirmed by SEM show sphere nanoparticle with size 117.0 nm with electron dense core cell (Figure 3). Further characterization was carried out with EDAX which reveals all the elemental components of nano drug conjugate. FTIR study also shows characteristic peak pattern (Figure 4,5).

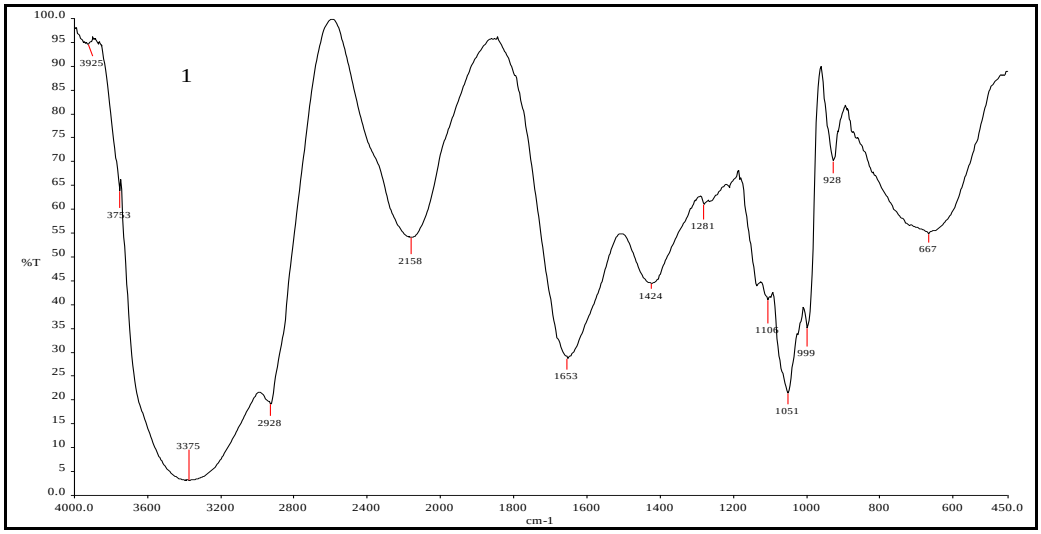


Figure 4.FTIR spectra of CS-CDX

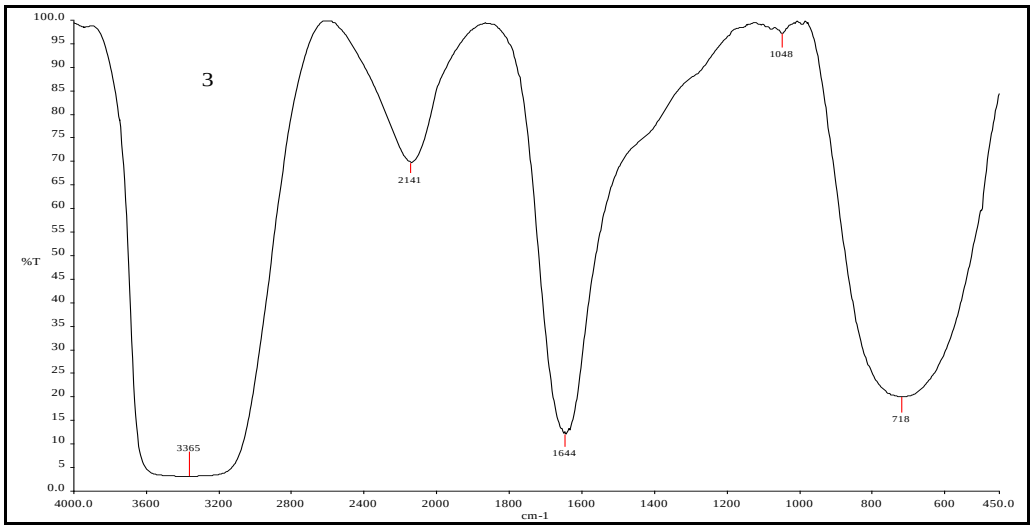
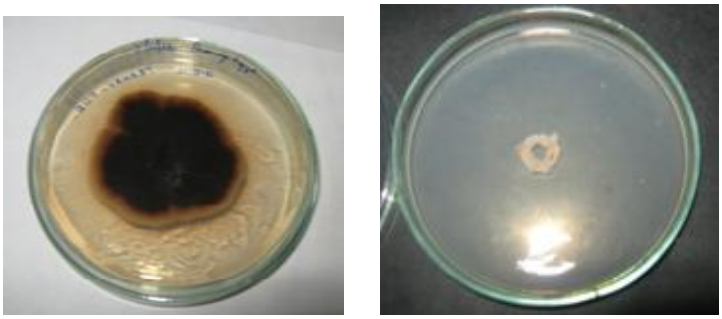


Figure 5.FTIR spectra of CS-CDX-F



Control **CS-CDX-F**

Figure 6.Mycelial growth of *Trichophyton* treated with CS-CDX-F

Antifungal susceptibility test with different concentration of free flucanalzole against *Trichophyton* and *C.albicans* shows all the tested concentration inhibited both the tested fungi. In the case of *Trichophyton*, maximum inhibition was observed in 100µg with 2.5cm of mycelia growth (Figure 6). 3.5, 4.0 and 4.2 cm mycelial growth was recorded at 50,25 and 10 µg concentration (Table 1).Free cyclodextrin and chitosan coated cyclodextrin nanoparticles did not show any activity. 12.0 mm of zone of inhibition was recorded in *C.albicans*.But CS-CDX incorporated fluconazole (CS-CDS-F) showed improved activity against both the tested fungi.In the case of *C.albicans*, 21.0mm of zone of inhibition was recorded.Similar improved activity was also observed in *Trichophyton* which revealed complete reduction of mycelia growth.

Table 1.Mycelial radial growth (mm) of *Trichophyton* and zone of inhibition of *C.albicans* treated with free fluconazole

Concentration (µg/ml)	**Mycelial radial growth (cm) of *Trichophyton***	**zone of inhibition of *C.albicans* (cm)**
10	4.2	10.0
25	4.0	11.2
50	3.5	13.5
100	2.5	14.0
Control	4.7	0.0

Table 2. Mycelial radial growth (mm) of *Trichophyton* and zone of inhibition of *C.albicans* treated with chitosan coated cyclodextrin nanoparticles incorporated fluconazole (CS-CDX-F)

Treatment	**Mycelial growth (cm)**	**Zone of inhibition (mm)**
Free cyclodexrin nanoparticles	4.7	0.0
Free chitosan	4.7	0.0
Chitosan coated cyclodextrin nanoparticles incorporated fluoconazole (CS-CDX-F)	0.1	21.0

Table 3. Mycelial radial growth (mm) of *Trichophyton* and zone of inhibition of *C.albicans* treated with plant extracts incorporated with CS-CDX

Treatment	**Mycelial growth (cm)**	**Zone of inhibition (mm)**
Free Aloe vera methanol	4.7	7.0
Free tulsi methanol	4.7	10.0
Free Aloe vera chloroform	4.5	9.8
Free tulsi chloroform	4.3	8.0
CS-CDX-A-Methanol	3.2	16.0
CS-CDX- T-Methanol	2.1	18.0
CS-CDX-A-Chloroform	3.3	15.0
CS-CDX-T-Chloroform	2.4	17.6
Control	4.9	0.0

Anti fungal activity of individual plant extracts incorporated with chitosan coated cyclodextrin revealed improved activity against the both the tested fungi.A increase in 5-10mm of zone of inhibition and 10 -20 % mycelia growth reductions was recorded in respective plant extracts incorporated CS-CDX. But free plant extracts of all the tested plants revealed less inhibition towards the tested fungal organism.CS-CDX incorporated nethanolic extract of tulsi recorded maximum inhibitory activity against both the tested fungi. Zone of inhibition against *C.albicans* was found to be 18.0mm. Similar enhanced activity was observed in chloroform extract (Table 3) and 10.0mm was recorded in free tulsi extract. CS-CDX-T of both the extracts showed mycelial growth reduction of *Trichophyton*. CS-CDX-A extract of methanol and chloroform revealed 16.5mm and 15.0mm of zone of inhibition against *C.albicans* (Figure 7).Mycelial growth reduction was also

recorded in *Trichophyton*. Similar improved anti microbial activity of cyclodextrin nanoparticles incorporated antibiotics against pathogenic bacteria has been reported [14]. Bilensoy *et al* [15] reported enhanced anti cancer activity of pacitaxol incorporated cyclodextrin nanoparticles.Oral delivery of peptide glutamine via chitosan coated cyclodextrin drug delivery system has been studied by Adriana Trapani. Further study will helpful to formulate the formulated drug and plant products as the effective anti fungal agent against human pathogenic fungi

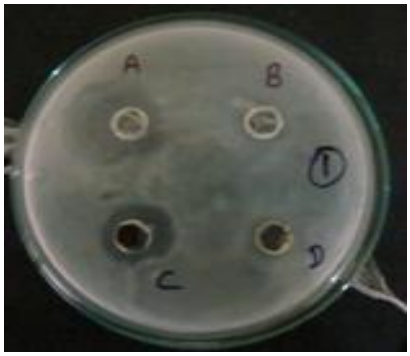


Figure 7.aZone of inhibition (mm) of CS-CDX incorporated A) Methanol of tulsi (c) Methanol of Aloe vera (b)Free Tulsi methanol (d) free Aloe vera chloroform

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