PHENOTYPIC AND GENOTYPIC IDENTIFICATION OF CHITIN PRODUCING FUNGI AND STUDY OF THEIR PHARMACEUTICAL APPLICATION

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ABSTRACT

Objectives: Current days, the production and utilization of natural biodegradable polymers have wide application in pharmaceutical and biological fields. The aim of the present study is to isolate and identify chitin polymer from *Aspergillus japonicus* Sanjo College of Pharmaceutical Studies (SCOPS-A1) and to study their role in pharmaceutical applications.

Methods: *Aspergillus japonicus* SCOPS - A1 was isolated from *Phyllanthus niruri* plant leaf from Palakkad, Kerala and it was identified by fungal universal primers ITS4 & ITS5 at ITS region of rDNA (NCBI Accession numbers MG833000, MG833001). The chitin was isolated from *Aspergillus japonicus* SCOPS - A1 by submerged culture using potato dextrose broth. 200 mg/g-1 (milligrams of chitin polymer/gram of dry biomass) of chitin polymer was extracted from 15-days mycelia by alkali - acid treatment. Extracted Chitin was characterized by Fourier transform infrared spectroscopy (FTIR), and X-Ray Diffraction (XRD). Chitin-Almotriptan malate microspheres were prepared by water in-oil-in-oil double emulsion method.

Results: To highlight Chitin pharmaceutical application, Chitin-Almotriptan malate microspheres were prepared by water-in-oil-in-oil double emulsion method which is used for migraine treatment. Infrared spectroscopy analysis conform the absence of drug and polymer interaction. **Conclusion:** In this study we conclude that *Aspergillus japonicus* SCOPS - A1 produces Chitin and it can be used as the choice of polymer for Pharmaceutical formulation.

Keywords: *Aspergillus japonicus* SCOPS - A1, Chitin, Microspheres, FTIR.

INTRODUCTION

Aspergillus species are saprophytic, ubiquitous fungi that can be found worldwide, mainly present in plants, water, soil, air and food. Chitin is a polymer of N-acetyl-D-glucosamine monomer units having β -1-4 glycosidic linkage, most copious renewable natural resources. Chitin mainly present in crustaceans and also the main cell wall component in fungi. Chitin is a non-toxic, biodegradable, biocompatible and mucoadhesive natural polymer. Developing a better drug delivery system will decrease pharmacological toxicity, enhance sensitivity and specificity of the drug. Microspheres are carrier mediated drug delivery formulation which acts as an important role in novel drug delivery system. Microspheres are free flowing, spherical and monolithic matrix type component. [1] Almotriptan is a second-generation 5-HT (1B/1D) agonist that exhibits vascular selectivity for meningeal arteries and has efficacy for the treatment of acute migraine. Almotriptan notably reduces blood flow through extra cerebral cranial vessels and increases cerebral blood flow.

Figure 1 Different type of fungi growth on *Phyllanthus niruri* plant extract surface

RESULTS AND DISCUSSION

Morphological and Molecular Identification

Figure 2

Figure 3

Molecular Identification

Table 1 Top five hits upon BLASTn analysis

Gene Bank Accession No.	Description	Max score	Query cover	Query coverage	E value	Identity (%)
KY848666.1	<i>Aspergillus japonicus</i> isolate SF1	933	933	100%	0.0	100%
KY086235.1	<i>Aspergillus</i> sp. isolate JSM 06261661	933	933	100%	0.0	100%
KX011594.1	<i>Aspergillus aculeatus</i> strain IM31	933	933	100%	0.0	100%
KJ439214.1	<i>Aspergillus japonicus</i> isolate V42-355	933	933	100%	0.0	100%
KJ192202.1	<i>Aspergillus</i> cf. <i>japonicus</i> C15-68	933	933	100%	0.0	100%

Growth kinetics

Figure 4 Growth kinetics of *Aspergillus japonicus* SCOPS - A1

Isolation and characterization of Chitin

Figure 5 FTIR Spectrum of commercial Chitin

Figure 6 FTIR Spectrum of Chitin extracted from *Aspergillus japonicus* SCOPS - A1

Table 2 : Calibration Curve of AlmotriptanMalate

Concentration (μ g/ml)	Absorbance (nm)
0	0
2	0.2166
4	0.4085
6	0.6155
8	0.8098
10	1.0031

Figure 7 Calibration curve of Almotriptan malate

Compatibility Studies of drug & excipients

Figure 8 FTIR of Almotriptan Malate

Figure 9 FTIR of Almotriptan maleate + Chitin (polymer)

Microspheres

Figure 10 Almotriptan maleate + Chitin (polymer) microspheres (SEM)

Figure 11 In-vitro studies of Chitin microspheres for Almotriptan maleate

In-vitro study of Chitin microspheres for Almotriptan maleate drug delivery

Table 3 Determination of In-vitro release profile

Time(hr)	F1	F2
1	11.88	19.93
2	15.66	22.15
3	18.9	26.58
4	20.52	31.01
5	26.96	38.76
6	35.64	49.29
7	46.44	61.47
8	58.86	75.32

Drug release kinetics of Microspheres formulation

Figure 12 Drug release kinetics

DISCUSSION

Almotriptan malate is a typical second-generation 5-HT (1B/1D) agonist mainly used in the treatment of migraine. In order to increase the drug release, an attempt was made to formulate Almotriptan malate in the form of microspheres. *Aspergillus japonicus* SCOPS - A1 Chitin was used as polymer blend for the preparation of microspheres. Based on physicochemical parameters and in-vitro release studies, formulation F2 with Chitin polymer was considered to be the best formulation.

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