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## **IN VITRO EVALUATION OF ANTIFUNGAL ACTIVITY OF COMBINATION OF METHANOL EXTRACT OF *TERMINALIA CHEBULA* FRUIT AND AMPHOTERICIN B AGAINST *CANDIDA ALBICANS***

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### **ABSTRACT:**

*Terminalia chebula* Retz. belongs to the family "Combretaceae", is a valuable medicinal plant. *T. chebula* showed a number of medicinal activities due to the presence of a large number of different types of phytochemicals. Amphotericin B is a very useful antibiotic for fungal infections. This present study has been done to evaluate the interaction between methanol extract of the fruits of *T. chebula* and Amphotericin B on their antifungal activity against *Candida albicans*. The results of this study showed that there is an increased activity in case of combination of plant extract and antibiotic. This Combination therapy could be a new way to treat diseases which are caused by *Candida albicans* (MTCC 183).

**KEY WORD:** *Terminalia chebula*, *Amphotericin B*, *Candida albicans*, *Combination therapy*.

### **INTRODUCTION:**

Various microorganisms are developing resistance against different antibiotics due to many reasons. One strategy to fight such resistance problem is to use combination therapy. The combination can be of different plant extract, plant extracts with standard antibiotics or antibiotics with some chemicals <sup>1</sup>. The combination of antibiotics and plant extracts is a novel concept and it could be effective <sup>2</sup>. Studies on use of plant extracts and antibiotics against various microbes have been reported.

*Candida albicans* is a dimorphic fungus that causes severe opportunistic infections in humans <sup>3</sup>. It is common in soil, organic debris and in humans where it occurs as a saprophyte in the nasopharynx and feces. It may cause clinically significant infections, such as thrush in infants, skin infections in diabetic patients and sepsis in immuno-compromised patients <sup>4</sup>.

*Terminalia chebula* Retz. belongs to the family "Combretaceae" and it is commonly known as black myrobalan <sup>5</sup>. It is native to India, China, Malaysia, Vietnam, Sri Lanka, Pakistan, and Tibet <sup>6</sup>. It is also called the 'King of Medicine' in Tibet and is always listed at the top of the list of 'Ayurvedic Materia Medica' due to its extraordinary power of healing <sup>7</sup>. *T. chebula* exhibit various biological activities including antibacterial <sup>8</sup>, antifungal <sup>9</sup>, antimalarial <sup>9</sup>, antiviral <sup>8</sup>, antidiabetic<sup>10</sup>, antioxidant <sup>11</sup>, anticarcinogenic <sup>12</sup>, and antiulcerogenic <sup>13</sup>.

Amphotericin B (AmB) is a significant agent in the management of serious systemic fungal infections <sup>14</sup>.

## **MATERIALS AND METHOD:**

### **Procurement of the plant material**

The fruits of *T. chebula* were collected from the local market and sample was authenticated at the Department of Botany, Sheth M.N. college of Science, Patan, North Gujarat, India.

### **Preparation of Methanol extract**

The dried fruits of *T. chebula* were powdered properly to get a coarse powder. The powder was stored in airtight glass container before extraction. 15 g of dried powder was extracted with 150 ml of Methanol using magnetic stirrers for 24 hours at room temperature. Then, extract was filtered through Whatmann filter paper No-1. Filtrate was concentrated to obtain the dry extracts and it was stored at 4°C until use.

### **Antifungal assay**

In vitro antifungal activity of the crude extracts was studied against *C. albicans* (MTCC 183) by the agar well diffusion method. Potato Dextrose Agar was poured into Petri dish. *C. albicans* was grown in Potato Dextrose Broth at 27 °C for 48 h. After getting the turbidity equal to 0.5 McFarland standards, inoculum was aseptically introduced on to the surface of sterile agar plates and sterilized cotton swabs were used for even distribution of the inoculum. Wells were prepared in the agar plates using a sterile cork borer of 8.0 mm diameter. The plant extract and antibiotic drug were dissolved in dimethyl-sulfoxide (DMSO) to get desired concentration. The wells were filled with plant extract (50µl) and antibiotic drug (50µl). The plates are incubated at 27 °C for 48 hours and then zone of inhibition was measured. In case of combination of plant extract and antibiotic, equal volume (25µl) of each was added in the well and zone of inhibition was measured.

### **RESULTS AND DISCUSSION:**

*T. chebula* showed a various medicinal activities due to the presence of a large number of different types of phytochemicals. *T. chebula* has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine <sup>15</sup>. The dried ripe fruit of *T. chebula* is used extensively in the indigenous system of medicine for its antitussive, cardi tonic, diuretic, homeostatic, and laxative activities <sup>16</sup>.

Amphotericin B (AmB) is an antifungal polyene antibiotic obtained from a strain of *Streptomyces nodosus*. Amphotericin B shows a high order of in vitro activity against many species of fungi. While *Candida albicans* is generally quite susceptible to Amphotericin B, non-*albicans* species may be less susceptible <sup>17</sup>.

Numbers of antimicrobials are obtained from the different plant and they have extensive therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials <sup>18</sup>.

The widespread use and misuse of antibiotics has led to the emergence and spread of resistant strains <sup>19,20</sup>. The ability of plant extracts to act synergistically with antibiotics could be a new approach to solve the problem of bacterial resistance and less susceptible bacteria or other resistant microorganisms <sup>21</sup>.

The antifungal screening of plant extract and antifungal antibiotic were carried out individually and combination effect was also screened in this research work. Plant extract had not showed inhibition at concentration of 10 mg/ml and 30 mg/ml but at the same concentration, antibiotic showed susceptibility against *C. albicans* (Table No-1). When plant extract combined with the antibiotic, zone of inhibition had been increased significantly as shown in Table No- 2. The antifungal activity is also increased in combination no 3. The enhancement of antifungal activity in case of combination of plant extract and antibiotic could be explained by the presence of biologically active compounds which are present in the plant extract. Thus, combination of *T. chebula* extract with Amphotericin B could be beneficial to increase antifungal activity against *Candida albicans*

### **CONCLUSION:**

The extract of fruit *T. chebula* exhibited potentials of synergy in combination with antibiotic against *Candida albicans*. This combination could be a new choice for treatment of various diseases caused by *Candida albicans*. It is required to carry out more research on isolation and identification of plant constituents which are responsible for enhanced antimicrobial activity.

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**Table 1: The results of the antifungal screening of methanol extract of *T. chebula* and Amphotericin B against *C. albicans* (MTCC 183)**

No.	Conc. Of Plant Extract (A)	Zone of Inhibition	Conc. Of Antibiotic (B)	Zone of Inhibition
1.	10 mg/ml	0 mm	50 µg/ml	5 mm
2.	30 mg/ml	0 mm	30 µg/ml	3 mm
3.	50 mg/ml	3 mm	10 µg/ml	2 mm

**Table 2: The results of the combined effect of methanol extract of *T. chebula* and Amphotericin B against *C. albicans* (MTCC 183)**

No.	Conc. Of Plant Extract (A)	Conc. Of Antibiotic (B)	Zone of Inhibition (A + B)
1.	10 mg/ml	50 µg/ml	17 mm
2.	30 mg/ml	30 µg/ml	12 mm
3.	50 mg/ml	10 µg/ml	6 mm