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**DETERMINATION OF ANTIFUNGAL EFFECT OF SAMADERA INDICA LEAF EXTRACT AGAINST VAGINAL CANDIDIASIS**

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**Abstract**

The study of plants is an alternative to other forms of drug discovery. Because, according to World Health Organization, these would be the best sources for obtaining a wide variety of drugs. Samadera indica is a bitter plant widely distributed throughout India. The leaves of Samadera indica was taken for investigation because of its abundance and used by the local folklore practioners and tribal peoples in India to treat various diseases such as vata, kapha, arthritis, constipation, and skin diseases like leprosy and pruritus. In the current world population, incidence of infection is increasing tremendously. Vaginal candidiasis is the most common gynecologic problem for which women seek medical help. The current study aims to determine the effectiveness of Samadera indica as an antifungal agent against vaginal candidiasis.

**Keywords:** *Vaginal candidiasis, Samadera indica, Antifungal activity, Phytochemical Screening, Zone of inhibition.*

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**Introduction**

Man from the very beginning has been aware of the problems of life and for a very long time has been taking care of their health through diet and drugs. Plants were used extensively for cures and general wellbeing (1). The study of plants as an alternative to other forms of drug discovery has attracted great attention because, according to the World Health Organization, these would be the best sources for obtaining a wide variety of drugs and could benefit large Population (2)

.Fungal infections or mycoses are the great neglected disease of medical history. Historical sources and contemporary epidemiological investigations show that fungal infections were ubiquitous in human and animal populations (3). The incidence of fungal infections has increased significantly, so contributing to morbidity and mortality.

Antifungal drugs, which retain many side effects. *Candida* species are major human fungal pathogens that cause both mucosal and deep tissue infections (2). Among the fungal infections in human beings, candidal infections are predominantly reported. More than 200 species of *Candida* have been reported (4). More than 200 species of *Candida* have been reported (4). In healthy human beings, *Candida* is considered as a commensal, and its scope to produce either superficial or systemic infections depends on the host immune system and various risk factors. Vaginal candidiasis is the most common gynecologic problem for which women seek medical help. Women between the ages of 18- 50, 75% have at least once experienced symptomatic vaginal candidiasis, which usually presents as soreness, burning urination, itching and abnormal curd-like vaginal discharge(5).

Pregnancy was the commonest factor (55%) followed by usage of broad- spectrum antibiotics (8%). Other risk factors were use of oral contraceptive pills, estrogen therapy, diabetes mellitus, tuberculosis prior history of genital infections, consumption of corticosteroids or immunosuppressive drugs and frequent intercourse, changing vaginal flora can increase the risk of vaginal candidiasis.

It is perceived that *Candida albicans* presented for vaginal candidiasis cases of 70–90%, with a recent surfacing of non-*albicans* species (6). The leaves of the plant *Samadera indica* commonly known as Niepa Bark Tree (Karinghota in Malayalam) with botanical name *Quassia indica* belongs to the family Simaroubaceae was taken for investigation because of its abundance and locally used by the folklore practitioners and tribal peoples in India to treat various diseases (7). The plant parts such as bark, leaves, fruits and seeds are employed in ayurvedic preparations. *Samadera indica* had antitumor, antioxidant, antiviral, anthelmintic and anti-malarial activities, growth regulating activities and antimicrobial activities (8). It was used to treat diseases such as vata, kapha, arthritis, constipation, and skin diseases like leprosy and pruritus (9).

## **Materials and Methods**

### **Plant Material**

The fresh *Samadera indica* leaves were collected from Perumpalam in Alappuzha district, Kerala during May 2019.



**Figure 1: fresh *Samadera indica* leaves**

### **Preparation of Crude Extract**

The Collected leaves were cleaned, separated and crushed. 25g of crushed leaves were soaked in three different solvents such as Ethyl acetate (150m.), Chloroform (150ml), Chloroform (150ml) and N-butanol. Then the solvents were maintained in room temperature and kept in a Rotary shaker for 72 hours with intermediate shaking The process was done to break the plant cell wall and to release phytochemicals. After 72 hours, the mixture was filtered through Whatman filter paper no: 1 and the crude extract of the filtrate were obtained by evaporating the solvents for next 7 days(10).

### **Phytochemical Screening**

The extracts were subjected to various tests to determine the presences of phytoconstituents like proteins, flavonoids, alkaloids, saponins, phenolic compounds, and carbohydrates (11,12).

### **Collection of Test Organism**

Samples obtained from 60 female patients with age between 15-50 in Uzhavoor Government Hospital Kottayam, was collected by wet swab. Each vaginal swab was examined microscopically and processed for fungal culture. The medium used was sabouraud dextrose agar medium. The identification of candida species was done by morphological and physiological methods such as culture on CHROMagar Candida media and sugar assimilation test with Hicandida identification (13).

### **In-vitro Antifungal Activity**

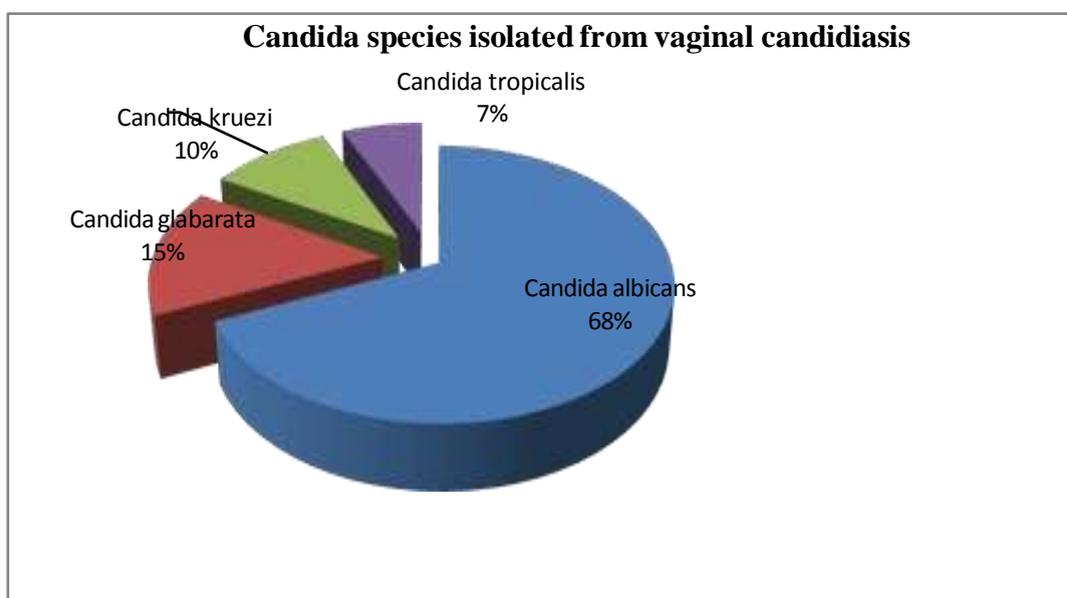
A suspension was prepared by picking 5 to 6 colonies from the SDA culture plate of ~ 1mm diameter from a 24 hours old culture of candida species. Colonies were then inoculated in 5 ml of sterile saline, and its turbidity was adjusted to 0.5 McFarland standard visually. Muller-Hinton agar, 2% Glucose with methylene blue agar surface was streaked to make a lawn of the isolate (14). Then, a well with diameter of 6 mm is punched aseptically with a tip. Desired extract of concentrations of 25 $\mu$ l, 50 $\mu$ l, 100  $\mu$ l introduced in to the well. Then it was allowed to diffuse at room temperature for 2 hours (15). The plates were then incubated in the upright position at 35<sup>0</sup> c - 37<sup>0</sup> c for 24 hours.

Wells containing the same volume of Ethyl acetate, Chloroform, N- Butanol served as negative controls while standard antifungal discs of Clotrimazole 20mg and Amphotericin

B 20mg were used as the positive controls. After incubation, the zones of inhibition were measured in mm.

### Results and Discussion

Out of sixty samples the most common identified species of *Candida* were, *Candida albicans* (41) followed by *Candida glabrarata* (9), *Candida kruezi* (6), *Candida tropicalis* (4).



**Figure 2: *Candida* species isolated from vaginal candidiasis**

## 1. Phytochemical screening

The phytochemical screening of the *Samadera indica* leaf extracts shows the presence of proteins, flavonoids, alkaloids and phenolic compounds.

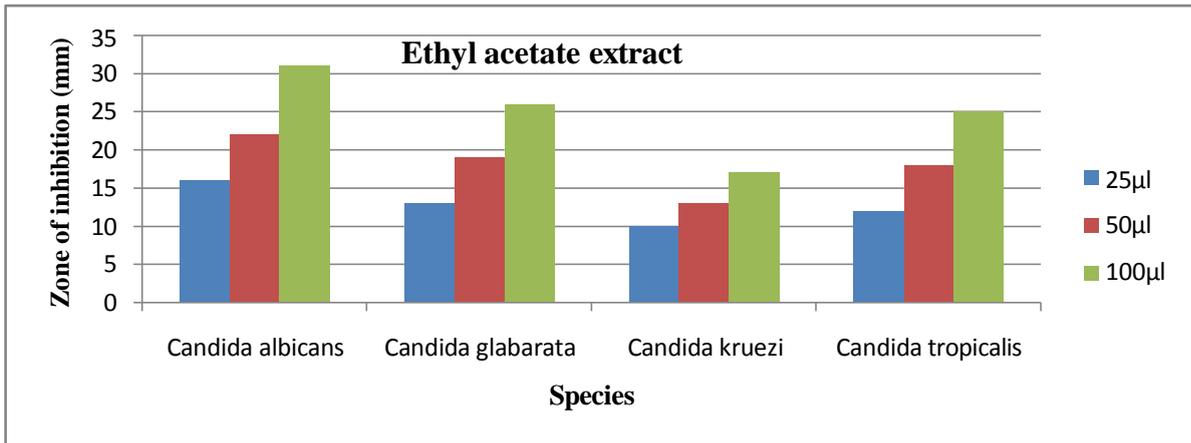
Phytoconstituents	Ethyl acetate extract	Chloroform extract	N-Butanol extract
Proteins	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+
Saponins	+	-	-
Phenolic Compounds	+	+	+
Carbohydrates	+	-	-

**Table 1: Phytochemical Screening**

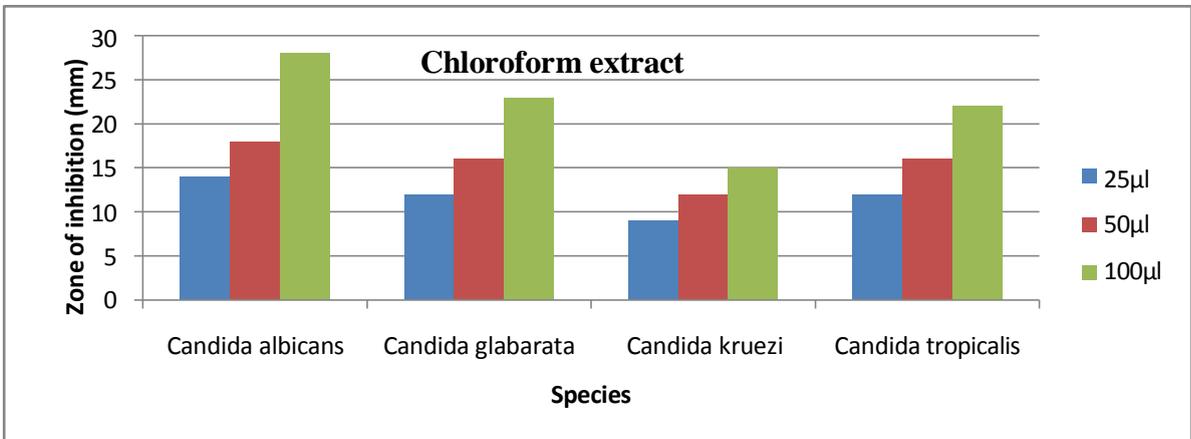
## 2. In-vitro Antifungal Activity

Species	Zone of inhibition (mm)								
	Ethyl acetate extract			Chloroform extract			N-Butanol extract		
	25µl	50µl	100µl	25µl	50µl	100µl	25µl	50µl	100µl
<i>Candida albicans</i>	16	22	31	14	18	28	14	17	29
<i>Candida glabarata</i>	13	19	26	12	16	23	12	16	24
<i>Candida kruezi</i>	10	13	17	9	12	15	9	13	15
<i>Candida tropicalis</i>	12	18	25	12	16	22	12	17	23

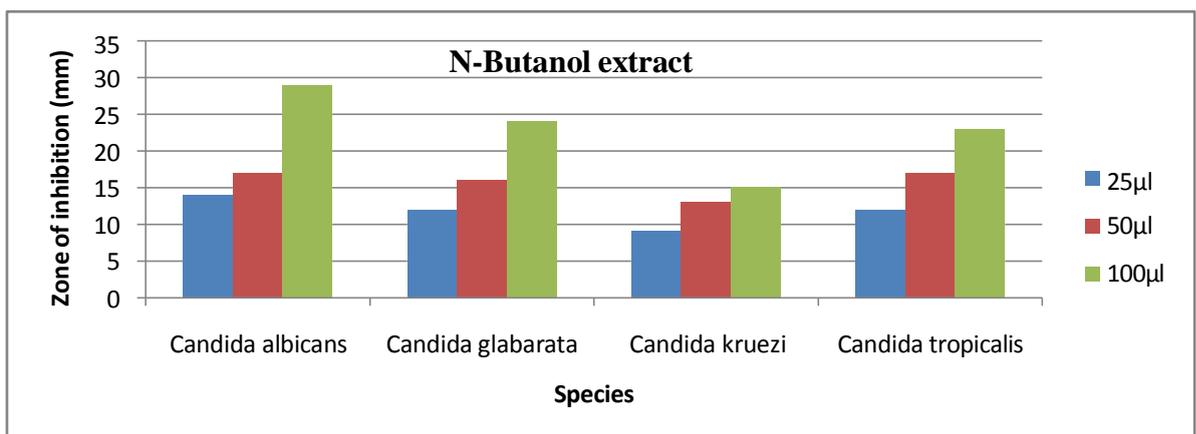
**Table 2: In-vitro Antifungal Activity**



**Figure 2: In-vitro Antifungal Activity of Ethyl acetate extract**



**Figure 3: In-vitro Antifungal Activity of Chloroform extract**



**Figure 4: In-vitro Antifungal Activity of N-Butanol extract**

## Conclusion

In the present study, the leaf extract of the plant *Samadera indica* from the family Simaroubaceae was examined. Phytochemical screening of the *Samadera indica* leaf extracts showed the presence of proteins, flavonoids, alkaloids and phenolic compounds. Out of sixty samples collected the most common identified species of *Candida* were, *Candida albicans* (68%) followed by *Candida glabrata* (15%), *Candida kruezi* (10%), *Candida tropicalis* (7%). The leaf extract of *Samadera indica* showed significant zone of inhibition against various species of *Candida*. Among the three extracts, Ethyl acetate extract possesses an increased antifungal activity. The presence of phenolic and flavonoid content revealed that the extract could have an antioxidant effect. Hence, we reached the conclusion that the extract can be an effective antifungal agent against vaginal candidiasis.

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