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RESEARCH ARTICLE

ANTIFUNGAL ACTIVITY OF ETHANOLIC EXTRACTS OF NEEM TWIG AND BANYAN ROOT AGAINST CLINICAL ISOLATES OF CANDIDA SPECIES

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ABSTRACT

Aim: To determine and compare the antifungal activity of ethanolic extracts of Neem twig and Banyan root against clinical isolates of Candida albicans.
Materials and Methods: A total of 20 non repetitive clinical isolates of Candidaspecies were collected from different samples of immunocompromised individuals attending Saveetha Medical college, Thandalam. Characterized by carbohydrate fermentation and assimilation tests and confirmed further using HiChromagar. The ethanolic extracts of Neem and Banyan were prepared. The sensitivity of Candida to the extracts was tested.
Results: Of the 20 clinical isolates of Candidaspp, 6/20 (30%) were from oral thrush, 5/20 (25%) from urine, 3/20 (15%) from sputum, 3/20 (15%) from vaginal swab, 2/20 (10%) from ear swab and one (5%) from wound swab. The banyan extract seemed to have greater antifungal effect than neem.
Conclusion: Banyan was found to have greater effect than neem. Voriconazole as a standard has a greater sensitivity in comparison with the other two herbal extracts.

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INTRODUCTION

Antimicrobial compounds are known to be present in the extracts of different tissues of some medicinal plants (Akpata and Akinrimisi, 1977). Emeruwa (1982) showed that antibacterial substances were appeared to be protein in nature (Emeruwa, 1982). Marston *et al*. (1993) isolated xanthones from the root of Ficus which possess antifungal activity (Abebe, 2003). Different parts of Azadarchtaindica, a common tree plant in Nigeria, are used medicinally (Adeserrano, 1982). A study was made here to compare the antimicrobial spectrum of the extracts of A. indicato those of griseofulvin, erythromycin and ampicillin which are conventional antibiotics used against some pathogenic fungi and bacteria. The Candida species, particularly Candida albicans, which are commensal in the human oral cavity, can become pathogenic and cause oral mucosal infections under immunocompromised or certain disease conditions. (Premkumar *et al*., 2014; SamrithiYuvaraj *et al*., 2016) Patients with diabetic mellitus (DM) as well as Denture wearers are predisposed to having an increased density of candidal growth in the oral cavity. (Sardi *et al*., 2013) Although Candida albicans is by far the most common cause of candidal infection, the incidence of candidiasis caused by other species, such as, C. glabrata, C. tropicalis, C. krusei, and C. dubliniensis has also increased. (Pfaller *et al*., 2007) These latter species tend to be less susceptible to commonly used antifungal agents such as fluconazole, and it has been suggested that this may account for their emergence as significant pathogens. (Jeddy *et al*., 2012) Nevertheless, a reduced antifungal susceptibility in the non-albicans species and a correlation with routine fluconazole prophylactic use is suggested. (Bassetti *et al*., 2009) Intrinsic and emerging resistance to azoles is a major challenge for therapeutic management and prophylactic strategies. (Leroy *et al*., 2009) Due to increased dental health awareness among the public and the continuous media publicity, there has been an increase in the proportion of people using modern facilities for cleaning their teeth; however, in many rural areas, people still depend on natural materials to clean their teeth. Chewing sticks, which are even now being widely used, are effective for cleaning the teeth, costs little, possess various medicinal properties, and are easily available in the rural areas of the developing countries. Studies done to compare the oral health status of people using chewing sticks with that of people using toothbrushes show that the oral hygiene was comparable between the two groups. (Elangovan *et al*., 2012; Shetty *et al*., 2010) The use of extracts of neem andbanyan can prove to be useful gents for cleaningthe teeth and being naturalcauseremarkably less side effects.

Ficus compound showed significant antioxidant effects which may be attributed to their polyphenolic Nature, the bark of Ficusbengalensis decreased fasting blood sugar, anti-tumor

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activity, Anthelmintic activity, Anti-inflammatory, Anti stress and anti allergic, Antidiarrhoeal, Antidiabetic and Ameliorative, Anti-inflammatory, Hypolipidemic, analgesic & antipyretic, Wound healing, Anti-Ulcer. the different concentrations (25, 50and 75 mg/ml) of different extracts showed sustained activity. In case of Candidaalbicans, low concentration showed low activity (25 mg/ml) and high concentration showed high activity (75 mg/kg). So we can say the Ficusbengalensis leaf extract have antimicrobial activity in a concentration manner. (Singh and Rao, 2012) The present study was designed to evaluate and compare the antimicrobial action against Candida using ethanolic extracts of two chewing sticks, namely neem and banyan, that are commonly used in South India.

MATERIALS AND METHODS

Candida isolates

A total of 20 non repetitive clinical isolates of *Candidaspecies* were collected from different samples of immunocompromised individuals attending Saveetha Medical college, Thandalam. They were characterized by carbohydrate fermentation and assimilation tests and confirmed. Isolates were preserved in semisolid Sabouraud chloramphenicol semi solid stock and stored at 4˚C until further use.

Characterization of *Candida* species

Candida species were further characterized by using Hichrom agar (Himedia, Mumbai).

Preparation of Hichrom agar

CHRO Magar *Candida* (HiMedia, Mumbai) was prepared following manufacturer's instructions. About 21.02 gram of HiChrome*Candida* differentiation agar base (modified) was suspended in 500 ml of distilled water. It was heated to boiling gently to dissolve the medium completely. Then it was allowed to cool to 50^{o}C and rehydrated (one vial) contents of Hichrome*Candida* selective supplement was added under aseptic precautions. It was mixed well and poured into petridishes.Isolates were identified on Hichrome agar based upon the characteristic color of the colony by subculturing from Sabouraud's chloramphenicol agar plates and the *Candida* Hichrome plates were incubated at 37^{o}C for 24- 48 hours. Based on colour produced by the isolates speciation have been made.

Candida species	Colour
C.albicans	Green
C.tropicalis	Blue
C.krusei	Pink dry colonies
C.kefyr	Pale
C.parapsilosis	Pale

Preparation of ethanolic extract of neem bark and banyan root extract

50g of dried powder of Neem bark and banyan root were taken in a separate container. To this 250 ml of ethanol was added and kept for 24 h with periodic shaking. Filtered and the filtrate was collected.

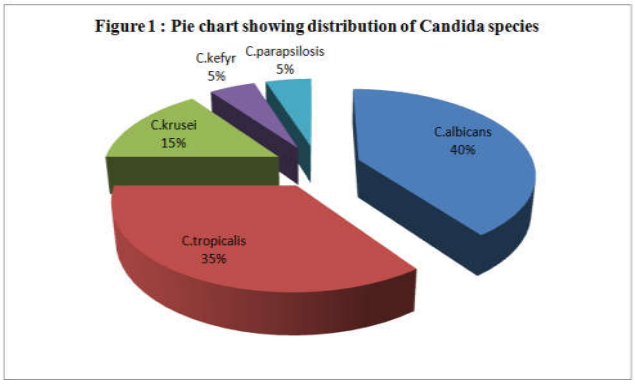
Agar diffusion method

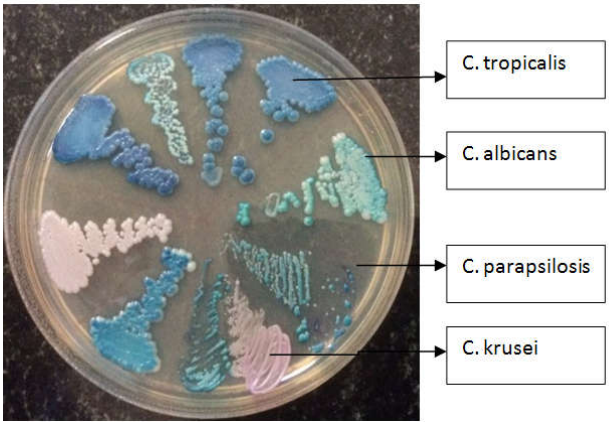
A sterile cotton swab were soaked into 0.5McFarland Standard Candida species isolates and were lawn cultured on sabouraud dextrose agar (SDA). 50mg/ml and 100mg/ml concentrations were prepared and each dilutions were been impregnated onto the sterile discs and air dried. These discs were placed onto the lawn cultured plates and incubated at 37°C for overnight. The sensitivity results were interpretated based on zone of inhibition of bacteria.

RESULTS

Characterization of *Candida* species by Hichrom *Candida* agar

A total of 20 clinical isolates of *Candida* spp were seeded on to Hichrom *Candida* agar and results were tabulated based on pigment production.

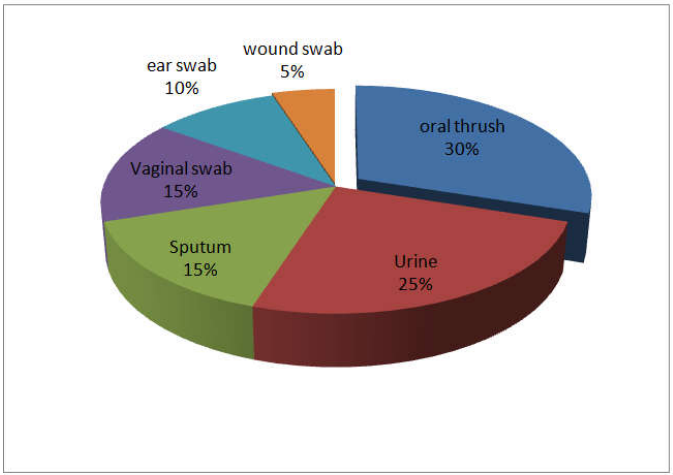




Representative picture showing *Candida* species on Hichrom *Candida* agar

Sample wise distribution of *Candida* species

Of the 20 clinical isolates of *Candida*spp, 6/20 (30%) were from oral thrush, 5/20 (25%) from urine, 3/20 (15%) from sputum, 3/20 (15%) from vaginal swab, 2/20 (10%) from ear swab and one (5%) from wound swab.



Pie chart showing the sample wise distribution of *Candida* species

Antibacterial activity of ethanolic extract of neem twig and banyan root against Candida species:

Isolates	Neem twig extract (50mg/ml) (mm)	Banyan root extract (50mg/ml)	Voriconzole
1	9	14	S
2	16	20	S
3	15	28	S
4	10	22	S
5	10	22	R
6	-	14	S
7	-	12	S
8	-	20	S
9	-	24	S
10	7	26	S
11	14	18	S
12	12	20	R
13	12	-	R
14	-	28	S
15	9	16	S
16	12	20	S
17	13	-	S
18	-	21	S
19	8	-	S
20	10	16	S

DISCUSSION

Ethanolic extracts of the chewing sticks to be tested were prepared at 10% concentrations. Normal saline and voricanazole was used as control. Culture plates of Candida albicans were prepared using pure cultures and the disc diffusion method was used. The results show that extracts of neem, and banyan have antimicrobial activity in descending order against candida and the activity was greater at higher concentrations. Banyanshowed greater antimicrobial activity against Candida than Neem. However Both banyan extract and saline showed no antimicrobial activity against the organisms tested in the study by Elangovan *et al*. The aqueous extracts of all plant parts did not exhibit any antimicrobial activity against the test organisms at the concentrations tested. The zones of inhibition produced by acetone and methanol extracts of the leaves against the organisms were not significant. However, they were quite significant for the bark. In the studies done by Wolinsky *et al*., Prasanth *et al*., and Khalid the antimicrobial activity of neem can be attributed to the presence of fluoride (1.0 µg/g) and silica; the former is known to exert an anticariogenic action, and the latter is an abrasive and prevents accumulation of plaque. (Wolinsky *et al*., 1996; Prasanth *et al*., 2007; Khalid, 1999) Alkaloids, known to exert an analgesic action, also contribute towards dental well-being.The presence of the alkaloid margosine, resins, gum, chloride, fluoride, silica, sulfur, tannins, oils, saponins, flavanoids, sterols, calcium, triterpenoids, phenolic compounds, carotenoids, steroids, valvenoids, ketones, and the tetranor triterpenoidazadirachtin confers various medicinal and antimicrobial properties to neem extract. (De and Ifeoma, 2002) Candida is the fourth most common cause of blood stream infection in hospitalized patients. (De and Ifeoma, 2002) Candidiasis is associated with intravenous lines and prosthetic lines are problematic, as they can act as substrates for biofilm production, the propensity of forming this is more in immuno suppressed patients. (Varshan *et al*., 2016) A total of 20 clinical isolates of Candida spp were obtained from different clinical conditions of different immuno compromised patients and were processed for its characterization followed by assessing the biofilm production. Of 20 isolates, 40% were C. albicans, 35% were C. tropicalis, 15% were C. krusei and each of 5% were C. kefyr and C. parapsilosis respectively. 5% of isolates were strong biofilm formers whereas, 65% were moderate biofilm producers in our Candida isolates. The present study reveals there was an increase in degree of isolation of non-albicans Candida spp from immuno compromised individuals. These isolates were moderately producing biofilm. Hence, prompt identification and detection of biofilm are essential in clinical setup where more number of Candida have been encountered.

Conclusion

Voriconazole as a standard has a greater sensitivity in comparison with the other two herbal extracts. When we compare the antifungal activity between these two,banyan root has a better sensitivity than Neem twig.

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