Journal of Positive School Psychology http://journalppw.com
2022, Vol. 6, No. 3, 3966 – 3974

Antifungal activity of Ficus benghalensis and Vachellia nilotica against denture plaque Candida albicans-an invitro study

Krishanga Srivastava [1] , Jacob Mathew Philip [2] , Helen Mary Abraham [2] , C. J.Venkatakrishnan [2]

1. BDS student, Department of Prosthodontics,Tagore Dental College and Hospital, Chennai

2. Faculty, Department of Prosthodontics, Tagore Dental College and Hospital, Chennai

Abstract

Background-

Denture plaque Candida are opportunistic fungi that can cause various oral disease in certain conditions. The purpose of this study is to evaluate the antifungal effect of extracts of Ficus benghalensis and Vachellia nilotica against denture plaque Candida albicans to observe if their herbal extracts can potentially be used in the management of oral candidiasis.

Materials and Methods-

Antifungal activity was assessed using above mentioned plant extracts against strains of C. albicans isolated from 50 samples of denture plaque using 2 concentrations (5mg/ml and 10 mg/ml) in a liquid medium based anti-fungal assays.

Results-

The alcoholic and aqueous extracts of V.nilotica and the aqueous extract of F.benghalensis exhibited anti-candidal activity against 46 samples out of the 50 samples of C. albicans in both concentrations. Extracts prepared with other solvents did not demonstrate anti-candidal activity.

Conclusion-

The alcoholic and aqueous extracts of V.nilotica and F.benghalensis have demonstrated anticandidal property and can be incorporated into various denture hygiene products to prevent candida associated denture stomatitis.

Keywords- Herbal extracts, Antifungal activity, V. nilotica, F. benghalensis, C. albicans

Introduction

One of the most common findings seen in acrylic denture wearers is the accumulation of plaque over the denture surface[i]. This accumulated plaque can pose a significant threat to the patient's overall health by causing diseases like denture stomatitis and oral thrush with an increased risk of carcinogenesis.

Denture stomatitis is characterized by inflammation and erythema of the oral mucosal

areas covered by the denture.[ii] The predominant organism which is present in this denture plaque is *Candida albicans,*[iii] that takes advantage of the immune-compromised state of the system to cause various diseases.[iv]

Even though, several effective antifungal agents are available for the treatment of oral Candida infections,[v] failure in treatment is increasingly seen due to possible development of resistance to drug therapy by the organism.[vi] which is a cause of prime concern as it results in high morbidity amongst the patients with suppressed immunity and multi-resistant bacterial strains[vii] Thus, alternative anti-fungal agents are therefore required, in order to cope with this problem of antifungal resistance to prevent disease.[viii, ix, x]

According to the World Health Organization, there are nearly 20000 plant species[xi] used for therapeutic purposes. These plants have been known to increase the immunity of the body, support the functions of organs and/or accelerate recovery. They al0s0o help in stimulating the regulatory action of the immune system of the body, and in turn prepare the body for a possible response against the microbial agents[xii]. Thus, these plant extracts can be incorporated into various dental materials and products that could be applied over the dentures to prevent candida associated denture stomatitis.

It is well-known that various natural plant extracts exhibit anti-microbial activity which could be utilized to prevent the growth of various microorganisms[xiii], but the antifungal activity of many plant species like *F.benghalensi s*and *V.nilotica*[xiv] lacks sufficient research data that could promote its application in disease prevention. Therefore, the aim of the present study was to evaluate the antifungal effect of herbal extracts of *F.benghalensis* and *V.nilotica* against denture plaque *C. albicans.*

Material and methods

The methodology envisages randomized experimental study research design which is centrally concerned with carrying out research that is high in causal (internal) validity and has been conducted in the controlled conditions of the laboratory environment.

Preparation of the plant extract:

The herbal extracts were prepared from twigs of *V.nilotica* and *F.benghalensis* after being certified by a botanist. One kilogram of twigs of each species was collected from Guindy National Park, Chennai. The collected samples were washed with water to remove dust and foreign particles and spread over a clean surface and subjected to shed drying for 7 days. Once the samples were dried, they were fine powdered using a grinding machine. Solvent extraction was performed by adding 10 grams of powder to 100 ml of solvent in a conical flask. Following solvents were used for extraction: Ethanol, N-hexane, Chloroform, Methanol, Acetone, Isopropyl alcohol and Water. The above solution was covered with an air tight lid and is kept in a shaker cum combiner for 24 hours at 25^0C.The resultant solution is then filtered using Whattman's filter paper into a dark glass beaker, stored in a refrigerator and used for further assays.

Fig 1: a-b (left to right) Shed drying of twig samples of Ficus benghalensis and Vachellia nilotica

Fig 6 (top to bottom & left to right) Ficus bengalensis samples with following solvents: a. ethanol b. methanol c. isopropyl alcohol d. n- hexane e. acetone f. chloroform g. water.

Fig 7 (top to bottom & left to right) Vachellia nilotica samples with following solvents: a. ethanol b. methanol c. isopropyl alcohol d. n- hexane e. acetone f. chloroform g. water..

1. Obtaining plaque sample:

Denture plaque was collected from 50 acrylic partial or complete denture wearers having signs and symptoms of oral Candida infection and were willing to participate in the study after obtaining written informed consent. The clinical segment of the present study was approved by the IEC of TDCH (IEC/TDCH/025/2019)

Non denture wearers, smokers, alcoholics, pan chewers, pregnant and lactating women and individuals with systemic diseases were excluded from the study.

The protocol of the plaque sampling involved the use of a cotton swab which was streaked on to the denture surface, immediately dropped into the transport medium and transferred to the mycology laboratory for further assays.

2. Isolation of *C. albicans*:

Isolation of the fungus was done by culturing in sabouraud dextrose agar (SDA) and using the germ tube test for confirmation,

Assessment of Anti-fungal activity of the herbal extract:

Antifungal activity of the extract on candida was assessed using 2 concentrations of the plant extracts (5 mg/ml and 10 mg/ml) using DMSO after determination of the minimal inhibitory concentration. The anti-fungal assay reaction was set-up in micro-titre plates for 50 candida samples, along with a standard strain of the fungus in a suspension adjusted to match the turbidity of a 0.5 McFarland standard according to CLSI guidelines (i.e., optical density = 0.12~0.15 at 530 nm, corresponding to 1~5 × 10^6 colony forming units (CFU)/ml)[xv] and sterile SDA broth. Results were recorded after 2 days of incubation

.

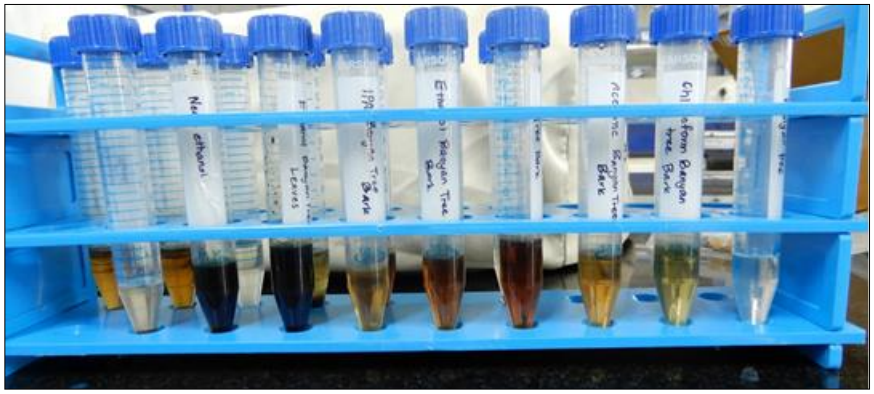

Fig.12. Preparation of working solution of extracts of 30 mg/mL concentration by dissolving suitable amount of dried extract in DMSO

Results

The presence of anti-fungal activity of the test extract is indicated by clearing of solution due to lyses of fungal cells and subsequent inhibition of growth. Absence of clear solution indicates that the extract does not possess anti-fungal activity. Tables 1 and 2 demonstrate the results obtained from the liquid medium based anti-fungal assay for 5mg/ml and 10mg/ml respectively for the 2 plant extracts.

Table-1. Results obtained from liquid medium based anti-fungal assay (5 mg/mL):

S. N	Solvent used for extraction	Ficus benghalensis	Vachellia nilotica
1.	Ethanol	Absence of antifungal activity	Absence of antifungal activity
2.	Methanol	Absence of antifungal activity	Absence of antifungal activity
3.	Chloroform	Absence of antifungal activity	Absence of antifungal activity
4.	N-Hexane	Absence of antifungal activity	**Presence of antifungal activity**
5.	Isopropyl alcohol	Absence of antifungal activity	Absence of antifungal activity
6.	Acetone	Absence of antifungal activity	Absence of antifungal activity
7.	Water	**Presence of antifungal activity**	**Presence of antifungal activity**

Table-2. Results obtained from liquid medium based anti-fungal assay (10 mg/mL):

S. N	Solvent used for extraction	Ficus benghalensis	Vachellianilotica
1.	Ethanol	Absence of antifungal activity	Absence of antifungal activity
2.	Methanol	Absence of antifungal activity	Absence of antifungal activity
3.	Chloroform	Absence of antifungal activity	Absence of antifungal activity
4.	N-Hexane	Absence of antifungal activity	**Presence of antifungal activity**
5.	Isopropyl alcohol	Absence of antifungal activity	Absence of antifungal activity

6.	Acetone	Absence of antifungal activity	Absence of antifungal activity
7.	Water	**Presence of antifungal activity**	**Presence of antifungal activity**

The n- hexane and aqueous extracts of *V.nilotica* and the aqueous extract of *F.benghalensis* exhibited anti-candidal activity against 46 samples of *C. albicans* in both concentrations. The candida isolated from samples 19, 26, 27 and 31 showed resistance to the herbal extracts. Extracts prepared with other solvents did not demonstrate anti-candidal activity.

It was noted that N-hexane and aqueous extracts of *F.benghalensis* and aqueous extract of *V.nilotica* showed antifungal activity to standard strain of *C. albicans* at concentrations of 5mg/dl and 10mg/dl which was similar to the results obtained for the 46 strains of *C. albicans* isolated from the patient denture plaque samples.

Discussion

The demonstration of antimicrobial activity of extracts of *F.benghalensis* and *V.nilotica* indicates the presence of phytochemicals possessing antimicrobial activity[xvi]. The antimicrobial activity of both plants is perhaps due to the effect of such phytochemicals either actingalone or synergistically.

Bark, leaves and fruits of *F. benghalensis* has been used because of their anti-microbial, anti-oxidant, anti-inflammatory, anticancer, anti-diabetic properties. It is also used in the treatment dysentery, diarrhoea, asthama, ulcers or other skin diseases.[xvii] Studies suggests anti-inflammatory, anti-diabetic, hypolipidaemia, anti-helminthic, anti-allergic, wound healing and anti-stress properties of bark extracts by ethanolic, methanolic, aqueous and petroleum extraction. In rural India aerial roots of *F. benghalensis* has been used to boost immune system and various other medicinal purposes.[xviii]

Phytochemical analysis has shown the presence of steroids, saponins,carbohydrates, flavanoids, amino acids and tannin like phytoconstituents in the extracts of Ficus benghalensis.[xix] The antimicrobial efficacy of the plant may be attributed to single or combined effect of these chemicals. In a previous study, glucoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitostirolalpha-D-glucose and meso-inositol have been isolated from the bark of *F.benghalensis.*[xx]It may be speculated that these may be responsible for its antifungal activity. The role of these constituents in eliciting antimicrobial properties requires further detailed phytochemical investigation.

The tender twig of *V.nilotica* is used as a toothbrush in south-east Africa and the Indian subcontinent.[xxi] Its anticandidal efficacy and phytochemical composition were hithertoundocumented. The present study reveals that the aqueous extract of *V. nilotica*demonstrates antifungal activity against *C. albicans.* This is being reported for the first time in literature.

Almost 92% of the Candida samples collected from the patients dentures in the present study exhibited susceptibility to the herbal extracts at both 5mg/ml and 10mg/ml concentrations with water and n-hexane as the solvents for *V.nilotica* and water as the solvent for *F.benghalensis*. Alternative methods to determine the phytochemical activity include colorimetric or fluorescent-based techniques which has not been performed in the present study. Flow cytometry technique has been defined as an effective tool for assaying the vulnerability of different

microorganisms[xxii] and can also be performed as an alternative technique to demonstrate in vitro anti fungal activities of various herbal extracts.

There is considerable evidence that herbal extracts have the potential to be developed into agents that can be used for various preventive or therapeutic modalities for human diseases. However, the use of herbal products in dentistry has seen a recent positive upsurge and are being used extensively as antiplaque agents, antiseptics, antioxidants, antimicrobials, antifungals, and analgesics. These natural products contribute in effective microbial plaque control and prevent gingivitis, periodontitis and denture stomatitis. Herbal extracts have been documented to prevent the adhesion of microbes to denture acrylic surfaces. Herbal extracts that prevent the adhesion of *C. albicans* to denture surfaces may prevent onset of denture stomatitis.

The present study reveals that the certain plant extracts contain antifungal activity, which can be further developed as a potential phytomedicine against denture plaques microbes and can be used effectively in the management of denture stomatitis. Screening for identifying the solvent potency can further help in establishment of lead molecule that could possibly be utilized in drug development against plaque pathogens. The limitations of the present study are based on the fact that we used crude plant extracts. Further research should focus on the use of specific phytochemicals from the extract which could be incorporated into denture cleansers and disinfectants which could successfully be employed for the prevention of denture stomatitis. If fruitful results are obtained such products could serve the purpose and could be efficient alternatives to chemical denture cleansers that could pose the threat of several adverse effects to the user.

Conclusion

C. albicans are normal commensals encountered among denture wearers.Combating them is extremely important among immunocompromised denture wearers. The adverse effects of antifungal drugs and the emerging trend of drug resistance has led to the search of alternative antifungal agents. Herbal antimicrobial agents are suitable alternatives which do not have adverse effects and may be used to treat drug resistant organisms.

With the results of this study, it can be concluded that herbal extracts of *V.nilotica* and *F.benghalensis* have antimicrobial property and can be incorporated intodenture hygiene products such as denture dentifrices, mouth washes and denture cleaning solutionsto combatcandida associated denture stomatitis.

References

[i]Srinivasan, M., Delavy, J., Schimmel, M., Duong, S., Zekry, D., Trombert, V., Gold, G., & Müller, F. (2019). Prevalence of oral hygiene tools amongst hospitalised elders: A cross-sectional survey. *Gerodontology*, *36*(2), 125–133. https://doi.org/10.1111/ger.12388

[ii]Gendreau, L., & Loewy, Z. G. (2011). Epidemiology and etiology of denture stomatitis. *Journal of prosthodontics : official journal of the American College of Prosthodontists*, *20*(4), 251–260. https://doi.org/10.1111/j.1532-849X.2011.00698.x.

[iii] Budtz-Jörgensen E. (1978). Clinical aspects of Candida infection in denture wearers. *Journal of the American Dental Association (1939)*, *96*(3), 474–479. https://doi.org/10.14219/jada.archive.1978.0088

[iv]Ramirez-Garcia, A., Rementeria, A., Aguirre-Urizar, J. M., Moragues, M. D., Antoran, A., Pellon, A., Abad-Diaz-de-Cerio, A., & Hernando, F. L. (2016). Candida albicans and cancer: Can this yeast induce cancer development or progression?. *Critical reviews in microbiology*, *42*(2), 181–193. https://doi.org/10.3109/1040841X.2014.913004.

[v] Al-hebshi, N., Al-haroni, M., & Skaug, N. (2006). In vitro antimicrobial and resistance-modifying activities of aqueous crude khat extracts against oral microorganisms. *Archives of oral biology*, *51*(3), 183–188. https://doi.org/10.1016/j.archoralbio.2005.08.001

[vi]al-Bagieh, N. H., Idowu, A., & Salako, N. O. (1994). Effect of aqueous extract of miswak on the in vitro growth of Candida albicans. *Microbios*, *80*(323), 107–113.

[vii]Cohen M. L. (1992). Epidemiology of drug resistance: implications for a post-antimicrobial era. *Science (New York, N.Y.)*, *257*(5073), 1050–1055. https://doi.org/10.1126/science.257.5073.1050

[viii]., Aboaba O. O., ., Smith. I. S., & ., Olude. O.F (2006). Antibacterial effect of edible plant extract on *Escherichia coli* 0157:H7. *Pakistan Journal of Nutrition*, *5*(4), 325–327. https://doi.org/10.3923/pjn.2006.325.327

[ix]Almas K. (1999). The antimicrobial effects of extracts of Azadirachta indica (Neem) and Salvadora persica (Arak) chewing sticks. *Indian journal of dental research : official publication of Indian Society for Dental Research*, *10*(1), 23–26.

[x]Almas K. (2001). The antimicrobial effects of seven different types of Asian chewing sticks. *Odonto-stomatologie tropicale, Tropical dental journal*, *24*(96), 17–20.

[xi]Ozaslan, M., & Oguzkan, S. B. (2018). Use of Plant Extracts in Alternative Medicine. *Pakistan journal of biological sciences : PJBS*, *21*(1), 1–7. https://doi.org/10.3923/pjbs.2018.1.7

[xii]Cruz Martínez, C., Diaz Gómez, M., & Oh, M. S. (2017). Use of traditional herbal medicine as an alternative in dental treatment in Mexican dentistry: a review. *Pharmaceutical biology*, *55*(1), 1992–1998. https://doi.org/10.1080/13880209.2017.1347188

[xiii]Jebashree, H. S., Kingsley, S. J., Sathish, E. S., & Devapriya, D. (2011). Antimicrobial Activity of Few Medicinal Plants against Clinically Isolated Human Cariogenic Pathogens-An In Vitro Study. *ISRN dentistry*, *2011*, 541421. https://doi.org/10.5402/2011/541421

[xiv]Sharma, H., Yunus, G. Y., Agrawal, R., Kalra, M., Verma, S., & Bhattar, S. (2016). Antifungal efficacy of three medicinal plants *Glycyrrhiza glabra*, *Ficus religiosa*, and *Plantago major* against oral *Candida albicans*: A comparative analysis. *Indian journal of dental research : official publication of Indian Society for Dental Research*, *27*(4), 433–436. https://doi.org/10.4103/0970-9290.191895.

[xv]Lee, J. A., & Chee, H. Y. (2010). In Vitro Antifungal Activity of Equol against Candida albicans. *Mycobiology*, *38*(4), 328–330. https://doi.org/10.4489/MYCO.2010.38.4.328

[xvi]Aswar M, Aswar U, Wagh A, Watkar B, Vyas M, Gujar KN. Antimicrobial activity of Ficus benghalensis. Pharmacologyonline. 2008; 2:715-25

[xvii]Joseph B, Raj SJ: An overview- *Ficus benghalensis* International journal of Pharmaceutical Sciences Review 2011; 6: 21-24.

[xviii]Khan, T., Tatke, P., & Gabhe, S. Y. (2008). Immunological studies on the aerial roots of the Indian banyan. *Indian journal of pharmaceutical sciences*, *70*(3), 287–291. https://doi.org/10.4103/0250-474X.42970

[xix]Aswar et al. antimicrobial activity of ficus benghalensis. Pharmacologyonline2008; 2: 715-725

[xx]Subramanian, P. M., & Misra, G. S. (1978). Chemical constituents of Ficus bengalensis (part II). *Polish journal of pharmacology and pharmacy*, *30*(4), 559–562.

[xxi]Saurabh Rajvaidhya et al.A review on Acacia Arabica, an Indian medicinal plant. International Journal of Pharmaceutical Sciences and Research 2012; 3(7); 1995-2005

[xxii]Kirk, S. M., Callister, S. M., Lim, L. C., & Schell, R. F. (1997). Rapid susceptibility testing of Candida albicans by flow cytometry. *Journal of clinical microbiology*, *35*(2), 358–363. https://doi.org/10.1128/jcm.35.2.358-363.1997.