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Antimicrobial Activity of Ficus Benghalensis and Vachellia Nilotica Against Denture Plaque Candida Albicans

Chika Wilfred

Department of Pharmacology, Singhania University, Rajasthan, India

ABSTRACT

Aim and objectives: Aim of the study was to evaluate the antimicrobial effect of the Ficus benghalensis and Vachellia nilotica against denture plaque Candida albicans.
Objectives of the study were: To evaluate the antimicrobial activity of the plant extract against isolated strains of Candida albicans from denture plaque. To compare the above antimicrobial activity with the standard strain of Candida albicans.

Key words: Herbal extracts, Anti-fungal activity, V Nilotica, F Benghalensis, C Albicans, Denture plaque.

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Corresponding author: Chika Wilfred
e-mail ✉ :@wilfredchika
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INTRODUCTION

One of the most important issues that denture wearers come across is the accumulation of plaque over the denture surface. The predominant organism which is present in this denture plaque is Candida albicans which takes advantage of the immuno-suppressed state of the patients and at the same time poses a significant threat to the patient's overall health by causing diseases like denture stomatitis, oral thrush including an increased risk of carcinogenesis [1]. Even though, several effective antifungal agents are available for the treatment of oral candida infections, the failure is possible because isolates of Candida albicans may exhibit intrinsic resistance to the drug therapy. Various anti-microbial alternatives are essentially required, in order to cope with the prevalent problem of antimicrobial resistance and to inhibit the disease which may be anti-bacterial, anti-fungal or anti-viral, as they are all known to suppress the infection by different modes of action [2,3].

India is considered to be a rich emporium of medicinal plants, mainly used in preventive and curative medicine. The use of natural products for the control of fungal diseases is considered to be an effective alternative to synthetic fungicides due to their low negative impact, reduced cost, and adverse reactions towards plant preparations compared to modern conventional pharmaceuticals. A variety of laboratory methods can be used to evaluate the in-vitro antimicrobial activity of an extract or a pure compound. Disk-diffusion and broth or agar dilution methods are amongst the most popular methods used today. Various other methods are specifically applicable for antifungal testing, for instance, the poisoned food technique[4].

According to the World Health Organization (WHO), there are nearly 20000 plant species used for therapeutic purposes. These plants have been known to increase the power of the defence of the body, support the functions of organs and/or accelerate recovery, thus having a positive effect on the functions of specific tissues and organs in the body. Scientists have been conducting various kinds of studies to identify the therapeutic agents responsible for the medicinal effects of these plants. Some plants are proven to be more effective than drugs due to the synergy of their active ingredients, to have preventive effects which, in turn, stimulate the regulatory action of the immune system of the body, and in turn prepare the body for a possible response against the external agents[5]. Thus, these plant extracts can be incorporated into various dental materials and products such as denture cleaning solution, immersion solutions for denture wearers, dentifrices (toothpaste) and mouthwashes, dental materials, pastes (cream or gel) that could be applied over the dentures etc,

It is also a known fact that medicinal plants are known to be the best and the safest source to obtain a variety of drugs. Though the pharmaceutical industries have produced several antibiotics in the last few decades, resistance to these drugs has been increasing substantially, which is a cause of prime concern as it results in high mortality amongst the patients with suppressed immunity and with multi-resistant bacterial strains. The problem of microbial resistance is growing and the attitude of people towards the use of antimicrobial drugs in the future is questionable. Therefore, there is an urgent need to ameliorate this problem by not only controlling the use of antibiotics but also by conducting

researches to better understand the genetic mechanisms of resistance and to continue studies to develop appropriate and efficient antimicrobial drugs for the treatment of the patient [6].

In a study, it was noted that around 80% of individuals from developing countries utilise traditional medicine with compounds derived from medicinal plants. Therefore, such plants need to be studied to better understand their properties, safety and efficiency. Recently, herbal products have been thoroughly researched for their potential in preventing oral diseases, particularly plaque-related diseases, such as dental caries. It is well-known that various natural plant extracts exhibit anti-microbial activity which could be utilized to prevent the growth of various microorganisms but the antimicrobial activity of many plant species like Ficus benghalensis and Vachellia nilotica lacks sufficient research data that could help its incorporation in future medical and dental patient management. Considering my close association with nature from childhood, there has always been a motivating force to use my passion with profession for bringing out an innovation in the field of dentistry to benefit the society.

MATERIAL AND METHOD

The methodology envisages randomized experimental study research design which is centrally concerned with carrying out research that is high in causal (internal) validity. Basically, the study has been conducted in the controlled conditions of the laboratory environment for 2-months duration in the State Forest Research Institute (SFRI), Chennai, Tamilnadu.

Inclusion criteria:

Patients wearing dentures (complete/partial) having signs and symptoms of oral Candida infection and are willing to participate in the study.

Exclusion criteria:

- Patients who do not wear dentures.
- Patients who do not have signs and symptoms of oral candida infection.
- Patients who are not willing to participate in the study

Collection of the Sample:

- Included patients of the study are briefed about the procedure and informed about the study along with collection of the demographic data and overall health status after obtaining consent from them.
- Then using a cotton swab, a swab is taken from their denture containing the accumulated matter called denture plaque.
- This Swab is then dropped in the transport medium.
- Then the swab is cultured.
- Isolation of Pathogens
- Isolation of pathogens is done by culturing in sabouraud dextrose agar (SDA).
- Confirmatory test like germ tube test is performed.
- Assessment of Anti-microbial activity of Plant Extract.
- Antimicrobial activity of the extract on pathogen is assessed using 2 concentrations of the plant extracts (5 and 10). Preparation of working solution of extracts is done as follows
- Plant extracts were taken from the storage and checked if they dissolved in DMSO solution.
- After they showed a positive dissolution in DMSO, the extracts were weighed.
- The plant extract weighed to 30mg
- By adding DMSO to the dried plant extract 30mg/ml concentration was obtained
- This solution was transferred to 96 well plate using a micropipette
- Anti-fungal assay reaction was set-up in 200 µL 96-well plates
- This reaction was set up for 50 patient samples using 2 concentrations along with standard strain sample
- Results were recorded after 3 days of incubation.

RESULTS

The presence of anti-fungal activity of extract is indicated by clearing of solution (after 24-48 hrs.) due to lysis of fungal cells and subsequent inhibition of growth. Absence of clear solution indicates that the extract does not possess anti-fungal activity (Anti-fungal activity using liquid medium for fungal growth). Tables 1 and 2 show the results obtained from the liquid medium based anti-fungal assay for 5mg/ml and 10mg/ml respectively in respect of the 2 plant extracts (Table 1-2).

Table1: 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

Table2: Results obtained from liquid medium based anti-fungal assay (10 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

Table3: Results obtained from liquid medium based anti-fungal assay (10 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

The extracts of under-mentioned plant samples against the given solvents exhibited anti-fungal activity in all patients' Candida albicans samples except for sample numbers 19, 26, 27 and 31.

- N-hexane Vachellia nilotica (5 and 10 mg / mL)
- Water Vachellia nilotica (5 and 10 mg / mL)
- Water Ficus benghalensis (5 and 10 mg / mL)

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

Medicinal properties of the plants is known to the mankind since generations and use of plant extracts for medicinal purpose in all civilizations and cultures has been playing a major role in health care systems all over the world. Various parts of the plants like seeds, leaf, flower, bark etc have been used for antiseptic, antispasmodic, antibacterial activities. Plant extracts do find application in various dental problems and few herbal extracts have been used in antimicrobial studies. The 2 species viz. Ficus benghalensis and Vachellia nilotica were selected for the study on ground of limited availability of the data and research of the plants in question against denture plaque Candida albicans. Solvent extraction meth od was considered quite economical and easy to use for the empirical study and all the selected solvents fell in the standard category.

The selection of the Candida sample adopting both inclusion and exclusion criteria was significant in the study as it would reduce the occurrence of bias and also would cover all categories of patient which is crucial for such a study. The selection of sabouraud dextrose agar (SDA) for culturing and isolation of pathogen was considered as the same provides a conducive medium for the growth of fungus. Antimicrobial activity of the extract on pathogen has been assessed using 2 concentrations of the plant extracts viz. 5mg/ml and 10mg/ml mainly for establishing broad threshold value in the growth of the

fungus and also knowing the results quickly on the 'presence/absence' data.

The presence of anti-fungal activity of extract is indicated by clearing of solution after 2-4 days due to lysis of fungal cells and subsequent inhibition of growth. Several techniques of fungal cell wall disintegration exist which also includes ultrasound disintegration, bead mill homogenization, application of chemicals, and osmotic shock. The release of proteins from fungal cells and the movement of cytosolic enzyme alongwith glucose-6-phosphate dehydrogenase, in the plant extracts are assayed which determines the efficacy of anti-microbial activity. The mechanical method of disintegration of yeast provides for the quick results and absence of clear solution indicates that the extract does not possess anti-fungal activity. Almost 92% of the Candida samples (46 out of 50) exhibited presence of anti-fungal in both 5mg/ml and 10mg/ml concentrations with water and n-hexane as the solvents in respect of Vachellia nilotica and only water in respect of Ficus benghalensis indicting that the 2 selected plants could be utilized in the dental treatment.

The above results from the plant part i.e. tree twigs show anti-fungal properties in both the selected species and we may now consider other parts of the plant for having more efficacious results. Similarly, other tree associates belonging to the same plant family also could be considered in the development of various therapeutic ameliorative alternatives which are not only economical but also easily available.

CONCLUSION

Candida albicans are frequently encountered among denture wearers and combating them is extremely important and this can be successfully done through herbal antimicrobial agents. Also, the problem of resistant strain due to the indiscriminate use of various drugs can be overcome by the use of natural plant extracts.

There is considerable evidence that plant extracts have the potential to be developed into agents that can be used for various preventive or treatment therapies for oral diseases which help to reduce the overall burden of oral diseases. However, the use of herbal products in dentistry has seen a positive upsurge and nowadays these are used extensively in treatment of gingival inflammation, as antiplaque agents and as antiseptics, antioxidant, antimicrobial, antifungals, and analgesics. These natural products contribute in effective microbial plaque control in gingivitis and periodontitis and thereby strengthen the immune system. With the above study, it can be concluded that the plant extracts of Vachellia nilotica and Ficus benghalensis have antimicrobial property and can be incorporated into various dental products and be used to improve the overall health. By incorporating these plant extracts in the daily routine of denture wearers, we can combat Candida associated denture stomatitis.

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