ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 3, Issue – 4, July - 2020, Page No. : 14 - 20

Evaluation of Anti-Fungal Activity of two medicinal plants against Candida Albicans: An In- Vitro study.

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Citation of this Article: Zeel Vipul Somani, Vilas Patel, Sareen Duseja, Harekrishna Raval, Gaurav Vaishnav, Manali Vipul Somani,“ Evaluation of Anti-Fungal Activity of two medicinal plants against Candida Albicans: An In- Vitro study.”, IJDSIR- July - 2020, Vol. – 3, Issue -4, P. No. 14 -20.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Purpose: to explore newer natural antimicrobial substances and evaluate the antifungal property of Vitex negundo and Euphorbia hirta against Candida albicans

Material method: Leaf extracts of both V. negundo and E. hirta at 30µl concentration were cultured in Sabourad Dextrose Agar plate with 0.1ml of C. albicans isolated from oral cavity and incubated at 37ºC for 72 hrs. Result: Significant zone of inhibition was found around both the plant extracts implying their effective antifungal activity

Conclusion: Considering the economical condition of elderly population and oral hygiene maintenance in geriatric population in India, these cost-effective and time-tested herbs have potential to be used as mouthwash and denture cleanser.

Keywords: Euphorbia hirta, Vitex negundo, antifungal, Candida albicans

Introduction

Human oral cavity comprises of oral microbiome comprising of complex and diverse bacterial communities which may or may not be pathogenic in nature. The

pathogen Candida albicans is a common component of oral bacterial flora and is present in >60% of healthy adult population. Although the presence of yeast is not, per se, indicative of oral infection, some local and systemic factors could be conversion from a commensal to a parasite form, releasing the yeast from biologic competition with the bacteria and allowing conversion into pathogens.

Glass RT et al.[1] reported the presence of over 900 varieties of aerobic and anaerobic bacteria in the biofilm of dentures. Another study also reported the frequent occurrence of stomatitis in patients using dentures, where, Candida albicans (C. albicans), the main causative bacteria of stomatitis, was found in approximately 86% of these patients.[2]

Due to increased microbial resistance to antibiotics, toxic and harmful effects of few common antimicrobial agents, there is a continuous need for alternative therapies which are affordable, non toxic and effective, such as medicinal plants. Denture cleansing is crucial to maintain the service ability of the denture, because of aesthetic concerns and for prevention of denture related stomatitis. Adequate denture hygiene is believed to be the most effective preventive and curative treatment for the pathogens

An ideal denture cleanser has to be biocompatible, bactericidal and fungicidal, harmless to the structure of denture, should effectively remove organic and inorganic deposits and should be easy to use effectively

Denture cleansers can be oxidizing agents (alkaline perborates), reducing solutions (sodium hypochlorite), effervescent agents (perborates and carbonates), chelating agents (ethylenediaminetetraacetic acid), detergents (sodium polyphosphate), enzymes (protease, amylase), and disinfectants (glutaraldehyde).[3] The most commonly used among these are alkaline peroxides.[4] However, studies have shown that denture cleansers which are currently used have deteriorating effects on the physical properties of denture base resins. Of late, there has been an increased resistance shown against these chemical agents by pathogenic microorganisms, thus giving center stage to plant extracts as novel antimicrobial and antifungal agents.[5]

Newer drug research targeting fungal species are the most neglected ones, this can be evident from the fact that “gold standard drug” for antifungal therapy remains the same since 1956. Very few antifungal agents are known till now, and their continuous and indiscriminate use has led to the development of resistance by fungal species, some show ineffectiveness toward fungal disease.[6]

In this study we have investigated the antifungal activity of extracts of V. negundo(Fig.1) and E. hirta (Fig.2) in order to explore possibility for newer antimicrobial substances against C. Albicans.

Fig. 1: Vitex negundo

Fig 2: Euphorbia hirta

Hence this study was conducted with the null hypothesis that the leaf extracts of Euphorbia hirta and Vitax negundo will have similar antifungal activity as of control group chlorhexidine.

Methodology

Plant collection: The fresh plant leaves of Vitex Negundo and Euphoria Hirta were harvested from the campus of Veer Narmad South Gujarat University located in Surat, Gujarat, India. The taxonomic identity of the plant was confirmed by the Botanist of the Department of Rural Studies, of the same University. The plant materials were washed under tap water and leaves were separated. The separated parts were air dried in shade and then in hot air oven at 55ºC. The samples were then ground using an electric blender and stored in clean labeled airtight bottles.

For the study, extracts were divided into four groups

Group 1: Distilled water (negative control)

Group 2: Chlorhexidine (positive control)

Group 3: Vitex negundo (leaf extract)

Group 4: Euphorbia hirta (leaf extract)

Preparation of extract: The finely ground powder was used for preparation of leaf extract with ethanol and distilled water. A hundred grams of powder of each plant was extracted by maceration in ethanol (500 mL). The mixture was filtered through clean muslin cloth followed by filtration with Whatman No.1 filter paper. The mixture was allowed to dry at room temperature till all the ethanol content was evaporated.

Preparation of culture: Petri plates of Sabouraud Dextrose Agar (SDA) were prepared and 0.1 ml of diluted culture was poured in each plate. The plates were allowed to cool and solidify for 30min at room temperature.

Agar well diffusion method:4 Wells (6mm diameter) were made in each of these plates using sterile cork borer. 30µl of each i.e. distilled water, V. Negundo, E. Hirta and chlorhexidiene were filled in each well using micropipette.

Determination of antifungal activity : The plates were allowed to stand for 30 min for prediffusion of the extract to occur and then incubated at 37 ºC for 48 hours for yeast and the zones of inhibition (including the diameter of disk) were measured to the nearest mm. The mean of triplicate results was calculated.

Result

For each of the medicinal extracts, the results were analyzed at the end of 48 hours using mean Zone of Inhibition (ZOI) and were compared with chlorhexidine as the positive control group. Although both the plant extract showed results that were equivalent to that of chlorhexidine, but, amongst those, V. negundo showed better result compared to both followed by chlorhexidine and E. hirta respectively. There was no zone of inhibition seen around distilled water which showed growth of C. albicans around it.

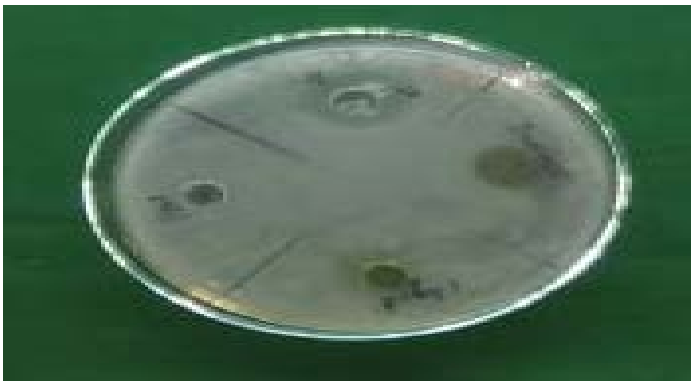
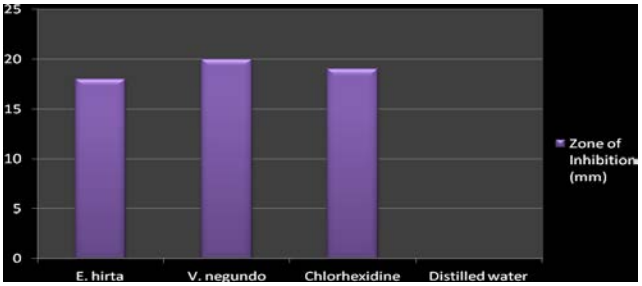


Figure 3: Fig 3: Zone of inhibition in triplicated cultured plates



Graph 1: mean zone of inhibition

H_0: There is not significant mean difference in Zone of inhibition mm across samples

H_1: There is a significant mean difference in Zone of inhibition mm across samples

To test above mentioned hypothesis ONE WAY ANOVA is applied with post hoc analysis.

Descriptives

Zone of inhibition mm

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Sample 1	10	18.0000	1.15470	.36515	17.1740	18.8260	16.00	20.00
Sample 2	10	20.0000	1.82574	.57735	18.6939	21.3061	17.00	22.00
Sample 3	10	19.0000	1.56347	.49441	17.8816	20.1184	17.00	22.00
Total	30	19.0000	1.70193	.31073	18.3645	19.6355	16.00	22.00

ANOVA

Zone of inhibition mm

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20.000	2	10.000	4.219	.025
Within Groups	64.000	27	2.370		
Total	84.000	29			

Caclulated value of F-test is 4.219 and its associated significance value if 0.025 which is less than 0.05; hence at 95% confidence level Null hypothesis got rejected. This means there is a significant mean difference in Zone of inhibition mm across samples at 95% confidence level.

To know among the samples are there any significant difference prevails in the mean value of Zone of inhibition mm; post hoc test is applied using LSD.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Zone of inhibition mm

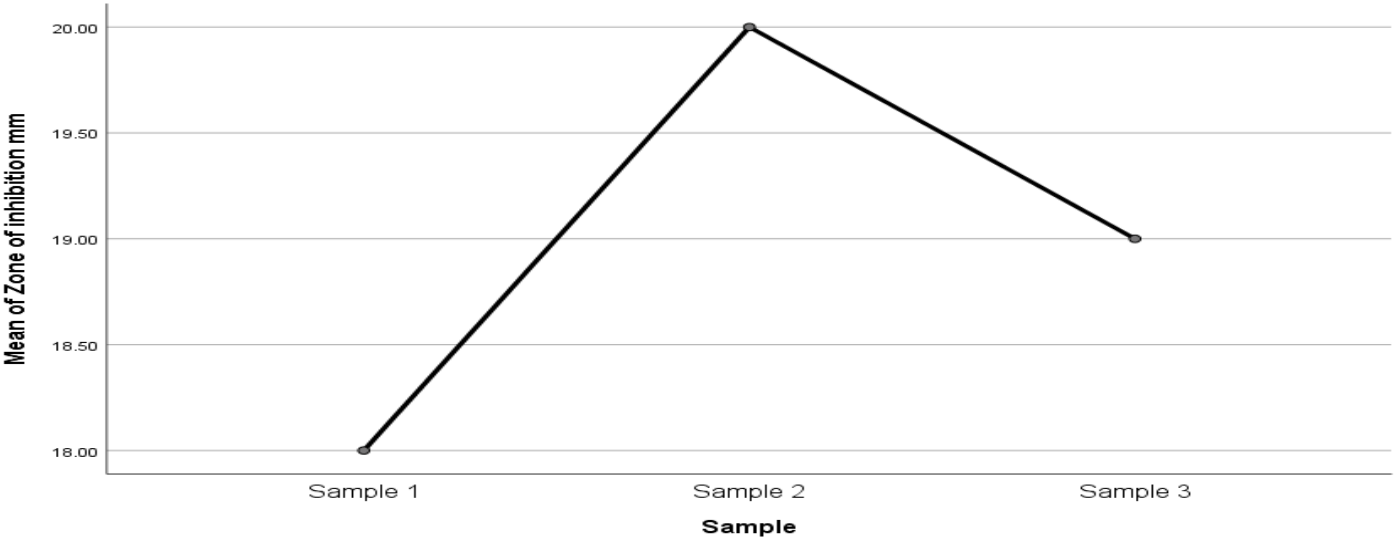
LSD

(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Sample 1	Sample 2	-2.00000*	.68853	.007	-3.4127	-.5873
	Sample 3	-1.00000	.68853	.158	-2.4127	.4127
Sample 2	Sample 1	2.00000*	.68853	.007	.5873	3.4127
	Sample 3	1.00000	.68853	.158	-.4127	2.4127
Sample 3	Sample 1	1.00000	.68853	.158	-.4127	2.4127
	Sample 2	-1.00000	.68853	.158	-2.4127	.4127

*. The mean difference is significant at the 0.05 level.

Result of post hoc test suggests that there is a significance mean difference in Zone of inhibition mm for Sample 1 with Sample 2 and vice versa (sig value is 0.007 < 0.05). But there is no statistically significant difference observed in mean value of Sample 1 and Sample 2 with Sample 3 (sig value is 0.158 > 0.05).

Means Plots



Discussion

With the increase in patient awareness on oral hygiene control, various studies have been conducted to develop various oral hygiene products. Special hygiene control or cleaning products are recommended for patients who use removable dental prostheses, for maintenance of denture hygiene.

Most of the commonly recommended mouthwash solutions are prepared from synthetic chemical components, and contain chlorhexidine as the antimicrobial component. Chlorhexidine, which is a cationic broad spectrum antimicrobial compound belongs to bis-biguanide family, which is known to inhibit the adhesion of microbes on the oral mucosa or the surface of dentures. However, the major drawback of chlorhexidine is that its long term use results in discoloration of the teeth and dentures. Also, patients with oral disease or elderly patients with impaired movement of the tongue or oral muscles may ingest these solutions during usage. Therefore, it needs to be prepared and handled carefully.[8]

In order to use natural extracts as mouthwash and denture cleaning solutions, their antibacterial and antifungal effects against major groups of bacteria that adhere to the mouth and dentures must be verified. The acidic environments resulting from the formation of organic acids during glucose metabolism promote the adhesion of C. albicans to the oral mucosa and dentures. Candida albicans is common opportunistic fungus associated with candidal infections. In conditions such as compromised host immune system, these C. albicans species and related species may overcome host immune response, can become pathogenic, causing systemic, vaginal, or oral candidiasis.

E. hirta is a very popular herb amongst practitioners of traditional medicine, widely used as a decoction or infusion to treat various ailments including intestinal parasites, diarrhoea, peptic ulcers, heartburn, vomiting, amoebic dysentery, asthma, bronchitis, hay fever, laryngeal spasms, emphysema, coughs, colds, kidney stones, menstrual problems, sterility and venereal diseases. Moreover, the plant is also used to treat

affections of the skin and mucous membranes, including warts, scabies, tinea, thrush, aphthae, fungal afflictions, measles, Guinea-worm and as an antiseptic to treat wounds, sores and conjunctivitis. The plant has a reputation as an analgesic to treat severe headache, toothache, rheumatism, colic and pains during pregnancy. It is used as an antidote and pain relief of scorpion stings and snakebites. The use of the latex to facilitate removal of thorns from the skin is common.[9] The sedative, anxiolytic, analgesic, antipyretic and anti-inflammatory properties of E. hirta have been reported in the literature.[10]

Vitex negundo used as medication within the indigenous system of medication, the leaves square measure the foremost potent for medicative use. It's used for treatment of eye-disease, toothache, inflammation, leucoderma, enlargement of the spleen, skin-ulcers, inflammation fever, autoimmune disorder, sexually transmitted disease and respiratory disease. They're conjointly used as tonics, vermifuge, lactagogue, agent, bactericide, antipyretic and antihistaminic agents, acute and sub-acute inflammation.[11, 12]

In this study, two medicinal plants extracts were tested against C. albicans so as to study their antifungal properties in order to verify its use as denture cleansing solution and mouth wash. Commonly used chlorhexidine was considered as positive control while distilled water was used as negative control group to evaluate the results of E. hirta and V. negundo.

The results showed a significant antifungal activity of both the samples by producing a zone of inhibition equivalent to that of chlorhexidine. The results of triplicated SDA plates showed average mean value of 21mm for V. negundo followed by 20mm for chlorhexidine and 18 mm for E. hirta.

Further studies are required to determine the minimum inhibitory concentration of medicinal plants at which they start showing their antifungal efficacy and measures to determine their side effects in humans, as this is an in vitro study.

Till now, only a few studies have been done on antifungal effects of Indian medicinal plants against oral C. albicans; it is better that the effect of these herbal extracts on other strains of oral Candida be studied, the bioactive compounds responsible for antifungal activities of these plants need to be identified and isolated, and it should also be taken into account that results obtained from in vitro assays may not necessarily be reproducible in vivo due to the metabolic processes in the test subjects.

Conclusion

Within limitations of this study, it can be concluded that both the leaves extract of V. negundo and E. hirta showed antifungal activity similar to that of commonly used mouth wash and denture cleansers containing chlorhexidine.

Further study needs to be done to assess its effect on denture base resins so as to validate its use as a denture cleanser.

Acknowledgement

Dr.Vipul J Somani, Professor and Head, Department of Rural Studies, Veer Narmad South Gujarat University, Surat, Gujarat, India for his contribution in taxonomic identification of plants.

Dr. Gaurav Shah, Professor and head, Department of Bioscience, Veer Narmad South Gujarat University, Surat, Gujarat, India for his guidance regarding the culture of plant extract.

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