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A COMPARATIVE EVALUATION OF THE EFFICACY OF GARLIC AND PROPOLIS EXTRACTS AGAINST CANDIDA ALBICANS WITH AMPHOTERICIN-B AS CONTROL - AN IN-VITRO STUDY.

Dental Science

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ABSTRACT

Introduction: -Oral candidiasis is an infection of oral cavity caused by an over growth of candida species. The proportions of yeast in the periodontal pockets are similar to some of periodontal bacteria, thus suggesting the possible role of Candida species in pathogenesis of periodontal pocket. The plant extract such as garlic and propolis, contain bioactive components which act against these organisms with no or less side effects than by the conventional antibiotics.

Objectives: - To evaluate the efficacy of garlic and propolis extracts against candida albicans and compare it with Amphotericin-B as control at 3 different concentrations

Methodology: - Subgingival plaque samples were collected and selectively cultivated for candida albicans. The antimicrobial activity of propolis and garlic was assessed and compared with Amphotericin-B.

Conclusion: Garlic extract can be used as a potent agent in the eradication of candida albicans in chronic periodontitis patient.

KEYWORDS

Candida albicans, Garlic, Propolis, Amphotericin-B.

INTRODUCTION:

Oral Candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of Candida species, the commonest being Candida Albicans.[1] The main reservoir of *Candida* spp. is believed to be the buccal mucosa and these microorganisms can co-aggregate with bacteria in subgingival biofilm and adhere to epithelial cells.[2] Those interactions are associated with the capacity of *Candida* spp. to invade gingival connective tissue, and may be important in the microbial colonization that contributes to progression of oral alterations caused by diabetes mellitus, some medications, and immunosuppressive diseases such as AIDS.

An increase in the antifungal resistance of Candida Albicans isolates in periodontal pockets suggesting that the oral cavity could be a reservoir of resistant yeast to antifungal agents. The fungi is colonized at mucosal surfaces and therefore more endogenous infections occur in this area. Drug resistance of this fungi, have led to discovering new medicinal plant and natural materials with fewer side effects.[3,4] Through studies using electron microscopy showed that garlic and its bioactive compounds destroy the candidal cell membrane integrity, resulting in the escape of much of the cytoplasm by formation of pits and eventual cellular collapse.

Garlic (Allium Sativum), is a species from the family of Alliaceae and has been used as a medicine since ancient times and has long been known to have antibacterial, antifungal and antiviral properties. An individual component of garlic, allicin identified to inhibit the growth of fungi through the inhibition of succinate dehydrogenase.[5]

Allicin is not present in whole garlic, but is produced instantaneously when garlic cloves are crushed or damaged. The enzyme Allinase and the compound Allin appear to be present in separate compartments in whole garlic cloves.[6] However, when cloves are injured, the enzyme acts on allin to produce Allicin. This mode of reaction is believed to protect the garlic cloves from insects and fungi. Allicin reacts with free thiol groups in thiol-containing molecules through thiol-sulfide exchange, thus inactivating a variety of enzymes and affecting bacteria, fungi, parasites and viruses. Because Allicin affects multiple enzymes in each target organism, the frequency of resistance to allicin is extremely low.

Allicin in its pure form was found to exhibit (Ankri and Mirelman, 1999):[7]

(I) Anti-bacterial activity against a wide range of gram-negative and gram-positive bacteria, including multidrug resistant entero toxicogenic strains of Escherichia coli;
(ii) Anti-fungal activity, particularly against C. albicans
(iii) Anti-parasitic activity, including some major human intestinal protozoan parasites such as Entamoeba histolytica and Giardia lamblia
(iv) Anti-viral activity.

Propolis, a natural antibiotic, is a resinous yellow-brown to dark-brown substance that honey bees collect from buds, sap flow, shrubs and other botanic sources. The pharmacologically active molecules in the propolis are flavanoids and phenolic acids and their esters.[8] Propolis has a degree of anti-microbial action against fungi such as Candida Albicans and some bacteria including a range of oral micro-organisms and viruses and may be as effective as Acyclovir against

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Herpes Simplex virus.

Sub-gingival irrigation with Propolis extract as an adjunct to periodontal treatment may also be more effective than scaling and root-planing alone.[8] Propolis is a subject of recent dentistry research, since there is some evidence that its antimicrobial properties may actively protect against oral disease. Aim of the study was to evaluate the efficacy of garlic and propolis extracts in 3 different concentration against candida albicans and compare it with Amphotericin-B as control.

OBJECTIVES:

- To evaluate the efficacy of 3 different concentrations of Propolis and garlic extracts on Candida Albicans (10%, 20%, and 30%). And compare with amphotericin – B 10mg.

MATERIALS AND METHODS

One patient who had reported to Dept. of Periodontics with Chronic Periodontitis was selected. Patient was informed and written consent obtained prior to collection of samples.

Criteria for patient selection

Inclusion criteria

Systemically healthy individual with age ranging between 36-55 years, Patient with no history of recent (6 months) periodontal therapy.

Exclusion criteria

Smokers, alcoholics, medically compromised patients, Pregnant or lactating women, Patients who were on sedatives, tranquillizers or antidepressants, and those who were on immunosuppressive medication**.**

Microbial sampling

Subgingival plaque sample was collected using a sterile Gracey curette from periodontal pocket of a chronic periodontitis patient. Sample was immediately carried to the microbiology lab in *Saline* medium within a period of 10minutes the resulting suspension was vortexed for 15 seconds and adjusted to match that of 0.5 McFarland and inoculated into the Sabouraud's dextrose agar culture plate.

Preparation of culture media [preparation of yeast nitrogen base (ynb), preparation of sabouraud's dextrose agar (sda) media, preparation of roswell park memorial institute medium (rpmi) broth], preparation of 10%, 20% and 30% garlic ~~extract~~ and propolis extract concentrations, preparation of Amphotericin-B 10 % concentration were done according to CLSI guidelines.

Upon sampling, the culture plates were placed inside the incubator for duration of 48 hours at temperature of 37°Celsius. After a period of 48 hours the candidal growth over the culture plate were noted by observing the candidal colonies. The obtained candidal growth was isolated further to get individual colonies of *Candida* using RPMI medium to get a pure culture. Once the plates were obtained a lawn culture and a radial culture from the primary culture was made by swabbing and radially streaking the candidal colonies from primary culture and was inoculated over the RPMI medium.

Inoculum was prepared according to CLSI Guidelines by picking colonies from 48 hr culture plates. The colonies were suspended in 5 mL of sterile saline. The resulting suspension was vortexed for 15 seconds and its turbidity was measured by turbidometry and adjusted to match that of 0.5 McFarland. A working suspension was made by a 1:50 dilution followed by a 1:20 dilution of the stock suspension with RPMI broth to obtain a final inoculums 5×10^5cells/ml.

Antifungal susceptibility testing was performed in 96 well flat-bottom plates containing 100µL RPMI broth in each well in triplicates. 100µL of different extract concentrations were added to the respective wells. 100µL of the working inoculum suspension which was prepared according to the forementioned procedure was also added to the wells in triplicates. 10mg concentration of the standard antifungal drug Amphotericin-B was used as positive control. Inoculum without any drug was used as a growth control. Sterility, growth and negative controls (solvents only) were also included. The plates were incubated at 37°C for 48hours. The antimicrobial action was measured by comparing the turbidity of the growth control with the turbidity of the tested extracts at time periods of 24hrs & 48hrs.

Statistical analysis

The mean and S.D are calculated for all the 3 concentrations at each intervals and subjected for statistical analysis by keeping p – value < 0.05 as statistically significant. For intra-group comparison of variable 'student paired t – test' is used and for comparison between the group repeated test of 'ANNOVA' is used. Data was analyzed using statistical software SPSS. All experiments involved in the present study were performed in triplicates. Confidence interval of 95% was considered with *p*-value < 0.05 is considered to be statistically significant, < 0.001 as highly significant and > 0.05 is considered as non-significant.

RESULTS:

The turbidity level of plain Garlic, Propolis and Amphotericin-B, at different concentration were evaluated at the beginning of the study.There was appreciable inhibition of growth by reduction in turbidity in all triplicate well with 10% Garlic extract for 24hrs and 48hrs.When the turbidometric values were calculated, negligible candidal growth at turbidometric value 0.0006±0.001 S.D was observed, and at 20 & 30% Garlic concentration, the candidal growth seen was zero, indicating that Garlic at higher concentration was able to completely eliminate Candida albicans at both 24hrs and 48hrs (Table - 1). In control broth, the growth in the wells without any active drug showed an increase in turbidity, which was in the range of 0.05±0.03 S.D.At 10% concentration, Propolis was not able to inhibit the growth of *Candida albicans*, both at 24 and 48hrs time periods. At 20% had turbidometric values which was similar to growth control. However, Propolis at 30% inhibited candidal growth at 24hrs. But, this inhibitory action was lost in the time period from 24hrs to 48hrs (Table – 2&3).The standard drug Amphotericin-B also gave readings similar to the results obtained with Garlic extract.

When 10% concentration of both Garlic and Propolis was compared with Amphotericin-B at 24hrs, there was a statistically significant reduction in the turbidity values for Garlic. However, the inhibition of Candidal growth with Garlic when compared with Amphotericin-B was not statistically significant, indicating that Garlic at 10% concentration was as effective as standard antifungal drug Amphotericin-B at 24hrs duration .When the same was compared at 48hrs, there was statistically significant difference with Propolis and the plain inoculum. But, the reduction in turbidity of Amphotericin-B was less compared to Garlic indicating that it was equally effective with Amphotericin-B after 48hrs of culturing (Table – 2 & 3).

On comparison at 20% concentration Garlic and Propolis with Amphotericin-B and plain inoculum at 24hrs, it was observed that there was complete inhibition of growth of *Candida albicans* with Garlic. There was significant reduction in turbidity of Amphotericin-B, whereas for Propolis, there was almost zero inhibition of growth of *Candida,* indicating that Propolis was not an effective anifungal agent even at 20% concentration. The results were same at 48hrs also (Table - 2 & 3).

When comparing 30% concentration of Garlic and Propolis with Amphotericin-B at 24hrs, there was no statistically significant difference between the turbidometric values of Garlic, Propolis and Amphotericin-B, indicating that all the 3 drugs were successful in eliminating *Candida albicans* at 24hrs duration. Same was true at 48hrs, except, for Propolis, which failed to show any inhibition of growth of *Candida albicans* at 48hrs duration, indicating that Propolis is an effective anti-fungal agent only at higher concentration (30%) at 24hrs duration and it lost its anti-candidal effect after 24hrs of inoculation. (Table - 2 & 3).

Table 1: Intragroup comparision of anticandidal action at differernt concentration at 24 & 48 hrs duration.

	Concentration	Duration	Mean	Std. Deviation	t Value	Significance
Garlic	10 %	24 Hours	0.000667	0.0011547	0.822	0.457 NS
		48 Hours	0.004000	0.0069282		
	20%	24 Hours	0.000000	0.0000000	—	—
		48 Hours	0.000000	0.0000000		
	30%	24 Hours	0.000000	0.0000000	—	—
		48 Hours	0.000000	0.0000000		

Propolis	10%	24 Hours	0.056000	0.0052915	1.522	0.203 NS
		48 Hours	0.098667	0.0482735		
	20%	24 Hours	0.082333	0.0087369	1.474	0.214 NS
		48 Hours	0.133333	0.0592818		
	30%	24 Hours	0.003333	0.0023094	6.393	0.003 S
		48 Hours	0.131000	0.0345109		
Amphotericin-B	10%	24 Hours	0.002000	0.0017321	1.265	0.275 NS
		48 Hours	0.000667	0.0005774		
Inoculum		24 Hours	0.059333	0.0396274	1.904	0.130 NS
		48 Hours	0.110000	0.0235160		

Table – 2: Intergroup comparision of anticandidal action at different conc. For 24 hrs duration.

	Mean	Std. Deviation	F Value	P Value and Significance
Garlic – 10%	0.00066	0.0011547	7.936	0.009 S
Propolis - 10%	0.0560	0.0052915		
Amphotericin B	0.0020	0.00173		
Inoculum	0.0593	0.03963		
Garlic – 20%	0.000000	0.0000000	12.471	0.002 S
Propolis – 20%	0.082333	0.0087369		
Amphotericin B	0.002000	0.0017321		
Inoculum	0.059333	0.0396274		
Garlic – 30%	0.000000	0.0000000	6.309	0.017 S
Propolis- 30%	0.003333	0.0023094		
Amphotericin B	0.002000	0.0017321		
Inoculum	0.059333	0.0396274		

Table 3: Intragroup comparision of anticandidal action at different concentration at 24 & 48 hrs duration

	Mean	Std. Deviation	F Value	P Value and Significance
Garlic – 10%	0.004000	0.0069282	14.291	0.001 HS
Propolis – 10%	0.098667	0.0482735		
Amphotericin B	0.000667	0.0005774		
Inoculum	0.110000	0.0235160		
Garlic – 20%	0.000000	0.0000000	14.745	0.001 HS
Propolis – 20%	0.133333	0.0592818		
Amphotericin B	0.000667	0.0005774		
Inoculum	0.110000	0.0235160		
Garlic – 30%	0.000000	0.0000000	33.619	< 0.001 HS
Propolis – 30%	0.131000	0.0345109		
Amphotericin B	0.000667	0.0005774		
Inoculum	0.110000	0.0235160		

DISCUSSION:

The oral cavity contains diverse microbial species and *Candida* is an important commensal in the oral cavity. The increasing incidence of HIV/AIDS, use of therapeutic immunosuppressive agents, widespread use of broad spectrum antibiotics and the increasing prevalence of lifestyle conditions like diabetes has led to an increase in the incidence of opportunistic infections like Oral Candidiasis, caused by an overgrowth of commensal *Candida* spp. *C.albicans*, and to a lesser extent *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. pseudotropicalis*, and *C. guilliermondi* are the causative agents of this disease[9].

Majority of oral *Candidal* infections are treated with topical applications of polyenes, azoles and DNA analogues in the form of mouth washes, oral suspensions, pastilles, creams, and lozenges. Apart from causing oral candidiasis the fungi of these species are also known to cause systemic diseases, Fungal infections particularly those involving *Candida* have been found to be the 4th leading cause of hematogenous infections. Systemic antifungal therapy is preferred in invasive *Candidal* infections & severely immunocompromised patients. Antifungal drugs, as local or systemic agents are known to have severe adverse effects like hepatotoxicity ,nephrotoxicity, gastrointestinal discomfort, cardiotoxicity and thrombophlebitis. Extensive use of these antifungal drugs has also led to the emergence of drug-resistant strains of *Candida*[10].

Antifungal resistance in *Candida* spp. may be due to different mechanisms such as:

(I) Reduced drug intracellular accumulation,

(ii) Decreased target affinity/ processivity for the drug, and

(iii) Counteraction of the drug effect.

Polyene antifungal agents such as Amphotericin B represented the standard of therapy for the systemic fungal infections until the discovery of the azoles. There is an association between polyene susceptibility and the presence of sterols in the plasma membrane of the cells. All organisms susceptible to polyenes, e.g., yeasts, algae, and protozoa, contain sterols in their outer membrane, while resistant organisms do not. Despite more than 30 years of clinical use, resistance to polyene antibiotics, such as amphotericin B and nystatin, is rare, with resistant isolates being confined mostly to the less common species of Candida, such as C. lusitaniae, C. glabrata, and C. guilliermondii.

Alternative treatment modalities such as the use of natural products, synthetic agents or polymeric materials have been studied. Plants resist the continuous attack of microorganisms by producing numerous secondary metabolites and as part of an ongoing process of defense are able to develop newer and faster antimicrobials.

In this study we have compared the anti-candidal activity of Garlic and Propolis with Amphotericin-B as control. Till date this is the first study comparing the efficacy of commercially available products of Garlic and Propolis against *Candida Albicans*. In the present study the anti-candidal efficacy of both Garlic and Propolis was noted after 24hrs and 48hrs. It was found that the efficacy of Garlic was similar to the standard drug Amphotericin-B as anti-fungal agent and the inhibition of *candidal* growth seen was almost 100% for both 20% and 30% concentrations at both 24hrs and 48hrs durations. However, Propolis was not able to inhibit the *candidal* growth with 10% and 20% concentrations at both 24hrs and 48hrs. But showed inhibition at 30% concentration at 24hrs duration.[11]

The major growth inhibitory component in garlic extract is believed to be allicin (diallyl thiosulfinate). Because garlic extract inhibits growth, it was expected that all macromolecular synthesis would be inhibited. The extent of protein and nucleic acid synthesis inhibition indeed paralleled growth inhibition, but lipid synthesis was completely blocked.[12] Garlic has been reported to affect the lipid constituents of the outer surface of C. albicans.[13] In addition to the probable difference in lipid content in the yeast cell, the presence of the rigid cell wall also provides an additional barrier. The cell wall of *Candida albicans* also possesses a high proportion of carbohydrate that may lack the thiol groups shown to be targets for allicin. Consequently, a higher concentration of garlic may be required before the cell itself accommodates an inhibitory concentration of garlic. Therefore, in the present study, 10% concentration of Garlic showed less growth inhibition when compared to 20% and 30% concentrations. The loss of cytosolic components, as demonstrated by electron microscopy[13], may be explained by the attack of the garlic components on the cell membrane targets. It is evident that much of the cell membrane integrity is damaged, resulting in the escape of much of the cytoplasm, formation of pits and eventual cellular collapse. As there were no previous studies which tested the efficacy of commercially available products of Garlic and Propolis against Candida Albicans, direct comparison was not possible.

Future Prospects:

As the present study is an in-vitro study, further in-vivo studies can be considered with large sample size to prove the clinical efficacy of these commercially available products. Commercially available products of Garlic and Propolis against major periodontopathic bacterias like Aggregatibacter actinomycetemcomitans, Porphyromonas Gingivalis, Tannerella forsythia should be tested.

CONCLUSION

The results of present study shows significant beneficial activity of Garlic extract at 3 different concentrations and different durations against the candida albicans when compared to that of the commonly used drug Amphotericin-B. Though Garlic and Propolis extract were evaluated at even higher concentration than the control drug, Amphotericin-B, Garlic can be considered as effective and alternative antifungal agent because the anti-candidal activity was observed even at lowest concentration (10%) which showed statistically significant results at 24hrs and 48hrs. However Propolis failed to prove its anti-candidal activity when compared to Amphotericin-B.

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