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Research article

A comparative evaluation of antimicrobial properties and four physical properties of heat-cure resin incorporated with Azadirachta indica and Melaleuca alternifolia: An in-vitro study

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ABSTRACT

In this in-vitro study, the researchers evaluated the effects of incorporating Azadirachta indica (neem) and Melaleuca alternifolia (TTO) into heat-cure resin on both its antimicrobial and physical properties. They specifically examined the resin's ability to combat Candida albicans and Staphylococcus aureus, two common pathogens, as well as its surface hardness, flexural strength, impact strength, and tensile strength. The results of the study revealed that the inclusion of neem and TTO in the resin led to enhanced antimicrobial properties against C. albicans and S. aureus. Additionally, the surface hardness of the resin increased when TTO was added, although no significant change was observed with neem. However, the study also found that the flexural strength, impact strength, and tensile strength of the resin decreased when either neem or TTO was incorporated. Interestingly, neem exhibited superior antimicrobial activity compared to TTO. Despite the positive impact on antimicrobial properties, the addition of neem and TTO resulted in a trade-off, as it led to a reduction in the resin's mechanical strength. These findings suggest that while neem and TTO can improve the antimicrobial efficacy of heat-cure resin, their incorporation can compromise its mechanical properties. Therefore, further research is needed to fully understand the clinical implications of these results and to explore potential applications in dental or medical settings.

INTRODUCTION

Polymethyl methacrylate (PMMA) is widely used in dentistry in general and prosthodontics in particular for different purposes such as partial dentures, full dentures, surgical and interim obturators, splints, implant prosthesis etc. Although dentistry has developed new materials and techniques used in rehabilitation of completely edentulous patients, poly methyl methacrylate resins have dominated the denture base market for over 80 years now since their introduction by Walter Wright and Vernon brothers in Philadelphia 1937[1]. PMMA is of two types namely heat cure and self-cure. Heat cure resins are used for making permanent prosthesis. Self-cure acrylic resin has been used in the fabrication of interim removable prosthesis, in the repair of broken denture bases, and for the fabrication of implant-supported fixed interim prostheses and maxillofacial prostheses [2]. However, PMMA resins have certain disadvantages, such as porosity, water sorption, solubility, deposition and formation of biofilm on the surface of PMMA resins [3]. It is known that denture bases may act as a reservoir of microorganisms and contribute to oral diseases and tissue damage. The intaglio surface of the denture it may serve as a breeding ground for opportunistic oral fungi. Epidemiological studies report that approximately 70% of removable denture wearers suffer from denture stomatitis (DS). Candida albicans (C.albicans) adhesion and biofilm formation are regarded as essential prerequisites for denture stomatitis. Poor care and maintenance of the prosthesis gives way to the adherence of microbial cells on the denture surface by means of biofilm formation, and thus denture stomatitis [4]. Another critical aspect of poor oral hygiene is the possible dissemination of pathogens from denture biofilm in immunosuppressed patients, which can make way to systemic infections [5]. The presence of biofilm on dentures can have severe consequences. It has been associated with other disorders like malodor, aspiration pneumonia, infectious endocarditis, gastrointestinal infection, and chronic obstructive pulmonary disease. Systemic factor including diabetes mellitus, AIDS, neoplastic diseases, chemotherapy, radiotherapy and broad spectrum antibiotic therapy further aggravates the lesions. Patients with an acquired defect after maxillectomy or mandiblectomy procedure are expected to lack oral hygiene due to limited mouth opening. The immunocompromised state of such patients makes them more susceptible for opportunistic infections, to counteract these infections, addition of external agents can be made to the material used for making the prosthesis.

Some attempts to change base material properties have included the incorporation of anti-infectious agents, nystatin, miconazole, ketoconazole, fluconazole, itraconazole, chlorhexidine, triclosan, titanium dioxide, and zeolites which would undergo gradual release in the oral cavity [6]. In the last years more attention has directed toward the incorporation of natural extracts into acrylic resins to provide antibacterial properties. Among these neem is one of the commonly available source and TTO in recent times has gained its popularity as anti- fungal agent, immunity enhancing, antidiabetic, anti-inflammatory, antifungal, antimalarial, antibacterial, antiviral and anti-carcinogenic properties **(Figure 1)** [2,7]. The importance of studying the antimicrobial effects of neem powder and tea tree oil in heat cure and self-cure acrylic resin material is based on its versatility in clinical applications that require the use of this resin. One of the main advantages of the self-cleansing acrylic resin material is homogeneous distribution of the drug and prevention of development of resistance to antifungal therapy in immunocompromised patients, patients undergoing radiotherapy etc. Utilization of it, aims to avoid or at least decrease the microbial colonization over the acrylic resin denture base material, increasing oral health status and improving life quality. Clinically, production of a denture base with an antimicrobial effect would be a remarkable achievement.The purpose of the present study was to evaluate antimicrobial property of self cure resin incorporated with neem powder and TTO. This study was performed to observe the changes in flexural strength, tensile strength, impact strength and surface hardness of resin incorporated with neem and TTO. The null hypothesis is that addition of these agents to PMMA will not affect its physical properties.

MATERIALS AND METHODS

Material preparation:

A total of thirty heat cure samples were fabricated and divided into three groups **H1:** Heat cure resin without any additives; **H2:** 2.5 g of pre-weighed neem powder was mixed to 1000 g PMMA; **H3:** 1.5ml of 100 % TTO was mixed with 100ml monomer (n = 10). A control group without incorporation of agents and the other two incorporated with neem (2.5%) and TTO (15%) respectively **(Table 1).**

Fabrication and testing of the samples:

For the study a precise, a two way open metal mould was custom fabricated to make test specimens. The molds were of dimensions 60mm x 10 mm x 2 mm for tensile strength and another of dimension 80mm x 4mm x 10mm for impact strength and flexural strength, according to ISO specification 20795-1:2013,. **(Figure 2,3).**Thirty wax patterns were obtained by pouring molten wax into the metal mold which was placed on a flat surface following the application of petroleum jelly to the mold space .The wax patterns were retrieved once it was set. Only the patterns which were devoid of voids and inaccuracies were selected. The wax patterns were then invested in a dental flask. Once the plaster was completely set, dewaxing was done in a dewaxing unit at 100° Celsius for 5 mins. The wax was removed by flushing away with boiling water. The flask was allowed to dry and cool at room temperature. Then separating media applied on all surfaces and allowed to dry.

Ten molds each were packed with H1, H2 and H3 material in the ratio of 1:3 (monomer and polymer) as per the manufactures instructions. Both the monomer and polymer were mixed with a clean dry wax spatula and the packing was done in the dough stage. The specimens were bench cured for 1 hr and polymerized by short curing cycle at 74°C for 2 hrs, followed by 1 hr of terminal boiling. After processing, the flasks were bench cooled and then deflasking was done. The excess resin on the specimens was trimmed with acrylic trimmers. Specimens were finished with various grits of sand paper, followed by polishing with pumice. Thickness, length and width of each specimen was verified using Vernier caliper and were tested for the following properties.

For testing anti-microbial activity:

Disk shaped specimen was fabricated of thickness 3mm for antimicrobial property testing The samples of each group were tested for Antimicrobial activity using Kirby Bauer disk diffusion method. Specimen disk was placed on Mueller Hinton agar plates containing lawn cultures of C.albicans and S.aureus. It was incubated at 37°

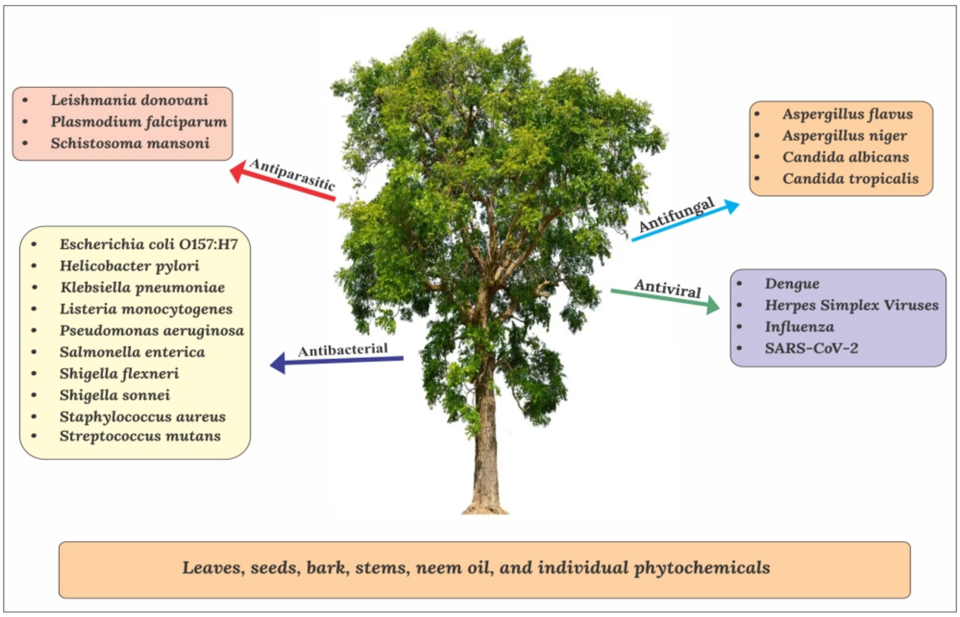

Figure 1: Targets for antimicrobial action found in the neem tree

centigrade for 24 hours to check the zone of inhibition **(Figure:4).**

For flexural strength:
A Sample of dimension (80mm x 4mm x 10mm) from each group will bemeasured using 3 point bend test in a universal testing machine. The apparatus is made to exert force until a bend is seen.

For testing tensile strength:
Precise rectangular two way open metal mold was custom fabricated in stainless steel to make test specimens according to the ANSI/ADA Specification of dimensions60mm x 10 mm x 2 mm. A sample of each group was tested using the universal testing machine. The apparatus was made to exert tensile force at a crosshead speed of 5 mm/minute until the specimen fractured, and the load at the time of fracture was recorded. The same procedure was repeated for all the specimens.

For impact strength:
A sample of each group was measured using impact tester .The rectangularsample of dimension (80mm x 4mm x 10mm) was inserted in the anvil to receive the blow of moving mass. For plastic type material like the acrylics the pendulum apparatus imparting the load is half disc shape. Pendulum swings down gaining energy from gravitational force and the height from which it is released. It hit on to the specimen and the specimen broke .Forces are calculated and the impact strength of the material was calculated.

For testing surface hardness:
The sample of tensile strength was used for surface hardness testing. A sample of each group was tested under Vickers hardness tester. Three measurements was recorded on different areas of each specimen and an average of these three readings was recorded.
The values collected were tabulated and was subjected to one way analysis of variance (ANOVA) and Independent sample t test procedures.

RESULTS

The results showed that incorporation of the antimicrobial agents (neem & TTO) in heat cure resin showed significantly increased antimicrobial activity against both *C.albicans* and *S.aureus*($P \leq 0.05$). The mean flexural strength decreased (H2=58.00,H3=47.60) and tensile strength decreased (H2=94.84MPa, H3=77.01MPa), mean impact strength decreased (H2=28.74 MPa, H3=30.59 MPa).The mean surface hardness increased following the addition of antimicrobial agents (H2=24.34 N/mm^2, H3=25.20 N/mm^2).On comparison between the groups incorporated with the agents, neem showed better antimicrobial activity than TTO.

DISCUSSION

Acrylic copolymers have a wide variety of applications in prosthetic dentistry as artificial teeth, denture base materials, facings in crown and bridge restorations, impression trays, temporary crowns, surgical, interim or definitive obturators for cleft palates, maxillectomy and mandibulectomy defects [8]. Heat cured acrylic material can be used for making the definitive or permanent prosthesis. However, consistent studies are going on to improve the antimicrobial property by

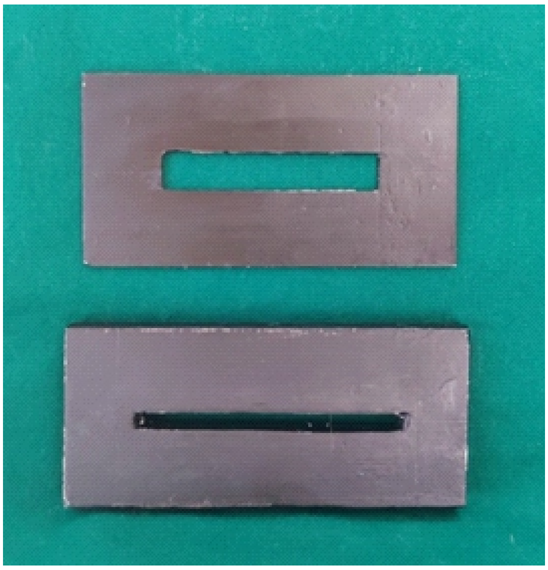

Figure 2: stainless Steel Moulds

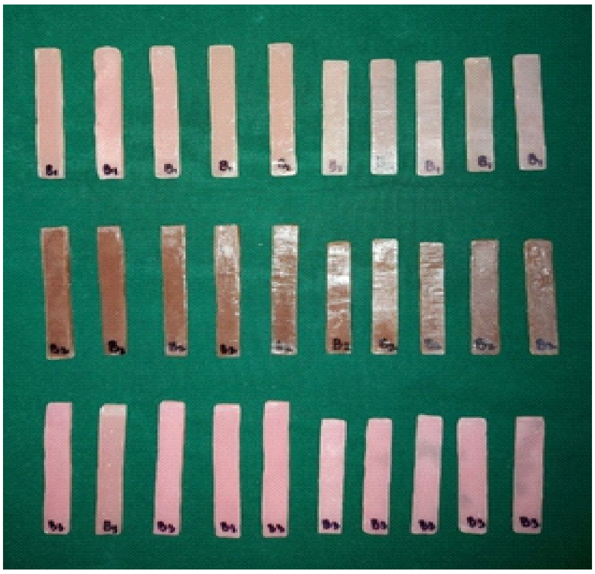

Figure 3: Heat Cure Specimen

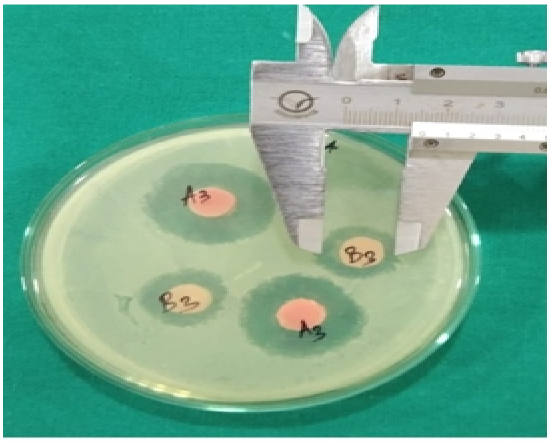

Figure 4: Zone of Inhibition

Table 1: Antimicrobial properties and four physical properties of heat-cure resin

	ANTI MICROBIAL (Staphylococcus aureus) [In mm]	ANTI MICROBIAL (Candida albicans) [In mm]	FLEXURAL STRENGTH (Mpa)	TENSILE STRENGTH (Mpa)	IMPACT STRENGTH (J)	SURFACE HARDNESS (N/mm^{2})
H1	0.00	0.00	89.40	106.52	30.56	24.24
H2	11.80	7.80	58.00	94.84	28.74	24.34
H3	11.20	6.60	47.60	77.01	30.59	25.20
	H2>H3>H1	H2>H3>H1	H1>H2>H3	H1>H2>H3	H1=H3>H2	H3>H2=H1

incorporating phytomedicines into the materials with and added advantage of not effecting the physical properties of the material.

The antimicrobial property among the heat cure experimental groups is of significance compared to the control group. On comparison between the experimental groups Neem incorporated (H2) and TTO incorporated heat cure resin (H3) are not significant. [H2=H3>H1] against *S.aureus*. On comparison between the experimental groups H2 is better than H3 against *C.albicans*. [H2>H3>H1]. Similar result was reported and reasoned that because neem includes the chemical constituents of alkaloids, flavonoids, terpenoids and tannins [9], it has the ability to prevent microbial infections specifically because of its antimicrobial biological activities [10]. The ability to create a complex with the bacterial cell walls can be seen in these chemicals, which demonstrates their antibacterial activity[9,10]. Additionally, azadirachtin is a bioactive metabolite of neem that also plays a role in neem's antibacterial potential via its inhibitory activity towards DNA topoisomerase enzyme II [11,12]. Similar action might be with *S.aureus* also. TTO acts within biological membranes, damaging their integrity and inhibiting the action of enzymes incorporated to increase membrane fluidity, with subsequent leakage of intracellular components [13]. According to Cox and Markham, chemical constituents are characteristically hydrophobic and will accumulate in the lipid-rich environments of cell membrane structures and cause structural and functional damage [14]. As the agents incorporated showed antimicrobial activity, the materials were checked for its effect on the physical properties of the heat cure acrylic resin.

The flexural strength is the mixture of compressive, tensile, and shear strength, which directly reflects the materials rigidity and its fracture resistance [15]. According to ISO, the flexural strength of heat polymerized PMMA should be higher than 65 MPa.It can be observed that there is decrease in flexural strength with B2,B3 groups compared to B1group. It can be due to non uniform distribution of the neem or TTO particles in the matrix, insufficient binding of composite particles in the resin matrix .Similar results was reported by Al-Harbi *et al.,* Karci *et al.,* Maitra *et al.,* Abdulwahhab, Sodagar *et al* [16-20]. It was noted in the study by Chander NG *et al.,* that the spatial distribution of particles and formation of multilayer coupling agents around the particles can reduce the availability of functional groups to react with the monomer.

The impact strength has decreased significantly in H2 as compared to the H1 and H3. The results revealed that the impact strength nearly the same after the addition of tea tree oil and this may due to the concentration of added oil which act as elastomer to PMMA [22]. Fernanda *et al.,* who stated that the addition of elastomer in smaller proportions (10% and 20%),the improvement in the impact strength was not evident [23].

The results of this study indicated that incorporation of neem and TTO significantly decreased the tensile strength of PMMA, which might be attributed to less neem or TTO particles per unit area of the PMMA matrix because of larger particle size. This may also enhance the chances of void formation from entrapped air and moisture and incomplete wetting of the particles by resin. Therefore the net effect of embedding particles was to weaken the polymer. Similar reasons were cited by Sehjpal and Sod, who reported that addition of silver, aluminum or copper powder to PMMA at a concentration of 25% by volume significantly decreased (by as much as 35%) the tensile strength of the acrylic resin polymer [24].

The test results shows that the surface hardness is increased significantly in the H3 as compared to the H2 and H1. The surface hardness is not significant between H1 and H2. [H3 > H2 = H1] may be due to reduced amount of residual monomer.

On the other hand, one of the problematic issues in incorporating these particles into acrylic resin is the lack of chemical bond between organic materials such as neem powder and TTO, with inorganic material such as PMMA. Accordingly, we can extrapolate that by finding out more appropriate substances as coupling agents between these particles and PMMA, it might be possible to decrease its deleterious effects on mechanical properties. It is very crucial to appropriately select the materials in order to construct the denture. Because of the right selection of denture material, the performance and life span of the prosthesis during its function in the oral cavity can be improved or extended [25].

CONCLUSION

Within the limitations of the study it can be concluded that:

- Incorporation of neem and TTO to heat cure resin shows antimicrobial property against *C.albicans* and *S.aureus*.
- The surface hardness increased with the incorporation of TTO
- The surface hardness showed no significant difference with incorporation of neem
- The flexural strength, impact strength and tensile strength decreased with the addition of the material.

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