IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 22, Issue 8 Ser.9 (August. 2023), PP 55-68
www.iosrjournals.org

Evaluation Of The Peel Bond Strength And Antifungal Property Of Soft Tissue Conditioners After Addition Of Oregano Oil And Grape Seed Oil- An "In Vitro Study"

ChandniShrivastava MDS a ,AnkitPachori MDS b , Sandeep Shrivastava MDS c ,Brajendra Singh Tomar MDS d Abhishek Jain MDS a , NileshMukka MDS e

a Assistant Professor, Department of Prosthodontics, Crown & Bridge and implantologyRishiraj College of Dental Sciences& Research Centre, Bhopal Madhya Pradesh
b Assistant Professor, Department of Pedodontics and Preventive dentistry , Rishiraj College of Dental Sciences& Research Centre, Bhopal Madhya Pradesh.
c Assistant Professor, Department of Orthodontics and DentofacialOrthopaedics Daswani Dental College, Kota, Rajasthan
d Associate Professor, Department of Prosthodontics, Crown & Bridge and implantologyRishiraj College of Dental Sciences& Research Centre, Bhopal Madhya Pradesh
e PG Resident, Dept of Prosthodontics, Crowns n Bridges District Hospital , Betul, Madhya Pradesh.
Corresponding author:
Dr. Chandni Shrivastava
Assistant Professor
Department of Prosthodontics, crown & bridge and implantology, Rishiraj College of Dental Sciences & Research Centre Bhopal Madhya Pradesh

Abstract:
Resilientmaterialshavebeenroutinely usedwiththepurposeofrecoveringtissuesthatare incontactwiththedenturebase.Thesematerials partiallyabsorbchewingloadonthedentureduring function,thusreducingtheenergytransmittedtothe associatedparaprosthetic tissues.However,these materialsareeasilydegradableandsusceptibleto microbialcolonization,whichmaycausedifferent degrees of denture stomatitis. Toprolongtheclinicallongevityofresilient materialsandreduceplaqueaccumulation, incorporationofantimicrobialagentsintothese materialshasbeenproposed. Theincorporationofdrugsintopolymericmaterials, includingtissueconditionersandresilientliners, mayaffecttheirproperties.Recentlytherehavebeenmanyresearchfocusedtowards theantimicrobialpropertiesofherbalproducts.

Aim of the study:
The aim of this study was to evaluate the peel bond strength and antifungal property of soft tissue conditioners after addition of oregano oil and grape seed oil.

Material and methods*:*
The peel bond strength of heat cure acrylic resin and soft tissue conditioners was evaluated, using universal testing machine on day 1 and after 7 days and Antimicrobial test was evaluated by using thezonesofinhibition(lengthandwidth) method withthe helpofVerniercallipersandtheareawascalculated on day 1 and after 7 days.

Result: *From the result of 20% grape seed oil and 60% the present study, it was evident that Peel Bond Strength had significant difference between Control group , Study group I and Study group II on day 1. There was no significant difference for the Peel bond Strength among Control Group and Study Group II on 7th Day. However there was significant difference for the Peel bond Strength of Study Group I on 7th Day. There was significant difference between the 3 groups on the antifungal properties on day 1 however the antifungal property of organo oil is more effective than grape seed oil. However there was no significant difference among the antifungal property of organo oil and grape seed oil on 7th Day.*

Date of Submission: 21-08-2023 Date of Acceptance: 31-08-2023

I. Introduction:

Softdenturelinersmaybedefinedassoft polymerswhichmaybeappliedtothefittingor mucosalsurfaceofadentureforthepurposeofreducingandmoreevenlydistributingthe occlusalloadontheunderlyingmucosaltissues. Theuseofsoftdenturelinershasincreased inrecentyearsinanefforttoachievesome measureofredistributionandreductionoflocally damagingocclusalforcesofdenturesonthe underlyingoraltissues[1]. Thesematerialscanbe classifiedasprovisionalordefinitiveaccordingtotheircompositionofeithersiliconerubberor acrylicresin,andtheycanbeeitherchemicallyor heatpolymerized.

Theoralcandidiasisknownasdenturestomatitis isrelatedtotheuseofremovabledenturesandis consideredthemostcommonorallesionobserved (65%)[2] inpatientswearingremovabledentures. Althoughtheetiologyofdenturestomatitisis multifactorial,infectionby*Candida* species,especially *C.albicans* isconsideredthemainetiologicfactor. Localfactorsassociatedwiththedentureareralso relatedtothispathologysuchas:presenceof biofilm[3,4],localtraumacausedbydentures[5], xerostomia[3],continuoususeofthedenturesand alteration in salivary pH[6].

Differenttreatmentsfordenturestomatitis areavailableandmayincludetopicalantifungal andsystemictherapy,carewithoralhygiene, denturecleaninganddisinfectionprocedures[7], replacementof olddentures, elimination of anatomic irregularities, re-establishmentofatraumaticocclusion,andnutritional restitution[8].Furthermore, inordertoprotectandpreservetheintegrityof themucosalepithelium,patientsshouldsleep withoutthedentures[9].Thechoiceofatreatmentor association of more than one treatmentisan aspect tobeindividuallyconsidered.Re- infectionofthe treatedoralmucosamayoccurinuptotwoweeks post-treatment,andisattributedtothesurvival of*Candida*species.duetoinsufficientconcentration oftheantifungalagentonthedenturesurfaces[7]. Therefore,itiscrucialtoadoptmethodsthatreduce orpreferablyeliminatethemicroorganismsfrom denture surfaces.

Inaddition,resilientmaterialshavebeenroutinely usedwiththepurposeofrecoveringtissuesthatare incontactwiththedenturebase[8].Thesematerials partiallyabsorbchewingloadonthedentureduring function,thusreducingtheenergytransmittedtothe associatedparaprosthetic tissues[9].However,these materialsareeasilydegradableandsusceptibleto microbialcolonization[10],whichmaycausedifferent degrees of denture stomatitis.

Toprolongtheclinicallongevityofresilient materialsandreduceplaqueaccumulation, incorporationofantimicrobialagentsintothese materialshasbeenproposed[11].Thiscombination maybealogicaltherapyinthetreatmentof denturestomatitisbecauseofseveralfactors:[1] ·reducingthe trauma causedby the internalsurface ofremovabledentures;[2].eliminatingcontactof thecontaminatedsurfacewiththeoraltissuesand consequently,interruptingthecycleofre-infection, and[3].actionofantimicrobialagentsincorporated intothematerialontheinfectedtissues[11].In thiscontext,denturestomatitismaybetreated beforefabricatingnewdentures,inarelatively shortperiod.Thereasonisattributedtotheir gradualdegradationandhardening,soitshould nottakelongerthantwoweeks,whichisaperiod similartotheonerequiredforthetreatmentwith conventional topical antifungal drugs[12,13].

Theincorporationofantimicrobialagentsinto resilientmaterialshasshowntobeeffectiveand feasiblebothin*invitro*and*invivo*studies[11-13]. Despitethese therapeuticadvantages,the incorporationofdrugsintopolymericmaterials, includingtissueconditionersandresilientliners, mayaffecttheirproperties.Recentlytherehavebeenmanyresearchfocusedtowards theantimicrobialpropertiesofherbalproducts.Grape(Vitisviniferea)isoneofthemostpalatablyediblefruits,grownall overtheworldandisconsideredtohavemanynutritional andmedicinalproperties.Ithasbeenreportedthatgrape containsalargeamountofphenoliccompounds,especiallytheseedwhichisconsideredtocontain60%-70%ofthe totalphenoliccontentofthefruit,comprisingofmonomeric phenoliccompoundssuchas(+)-cathechins,(-)-epicatechinand(-)-epicatechin-3-o-gallate, anddimeric,trimericand tetramericprocyanidins[14]

Grape seedextract obtainedfromgrapesgrown in Hasandede, EmirandKalecikKarasiwinecultivarsinTurkeyshowed concentrationsof2.5%-5%exhibitedthemostinhibitoryeffect againstawidevarietyofmicroorganismsincludingE.coli,K. pneumoniae,andS.aureus[15].Asimilargrapeseed extract product IH636wastestedagainst21strainsofgrampositiveandgramnegativecocciwhichshowedgrampositivecoccitobemore

susceptible,especiallyS.aureus. Inpresenceof1mg/mL,99%inhibitionwasreportedwithnofurtherbacterialrecovery[16]. Completeinhibitionof43clinicalstrainsofMethicillin resistantS.aureuswasnotedatconcentrationof3mg/mLcrudegrapeseedproanthrocyanideextract[17].

A feasible approach to limiting the transmission of these pathogens is to use essential oils as alternative agents or topical agents. Oregano, one kind of labiate Origanum plant that has been known for a long time as a popular remedy, is a very versatile plant. It was reported that Origanumcompactum, Origanumminutiflorum, and Origanummajorana exhibit antifungal activity, antibacterial activity, and antimicrobial activity, respectively[18]

Fortheresilientliner toadequatelyperformitsfunctionofrecovering thetissuesinjuredbytrauma,itshouldremain bondedtotheacrylicbaseoftheremovable denture[19]. Peelingoftheresilientmaterialfromthe denturebasehasbeenreportedasthecauseof clinicalfailureandthebondbetweentheresilient materialsandthedenturebaseacrylicresinshas beentheobjectofpreviousinvestigations[20,21].Thus, theaimofthisstudywastoevaluatetheeffect oftheadditionofantimicrobialagentsoregano oil and grape seed oil and toevaluate its effectonitspeelbond strengthtoadenturebaseacrylicresin.The hypothesisinvestigatedinthisstudywasthatthe additionofantimicrobialagentstoaresilientliner wouldresultinchangesinthepeelbondstrength to a denture base acrylic resin.

Aim of the study:

The aim of this study was to evaluate the peel bond strength and antifungal property of soft tissue conditioners after addition of oregano oil and grape seed oil.

Objectives of the study:

To determine the Peel bond strength of the soft tissue conditioners after incorporation of oregano oil 60 vol % after 1 day and 7 days, to determine the Peel bond strength of the soft tissue conditioners after incorporation of grape seed oil 20 vol % after 1 day and 7 days, to assess the antifungal property of soft tissue conditioners after incorporation of oregano oil 60 vol % after 1 day and 7 days, to assess the antifungal property of soft tissue conditioners after incorporation of grape seed oil 20 vol % after 1 day and 7 days.

II. Materials and Method:

Preparation of Test Specimens for Peel Bond Strength

Each specimen consists of three parts.

A rectangular acrylic base, central metallic ring for confinement of tissue conditioners and upper circular lid made up of acrylic with a metallic bolt in the centre, which will cover the tissue conditioner. (Fig. 1)

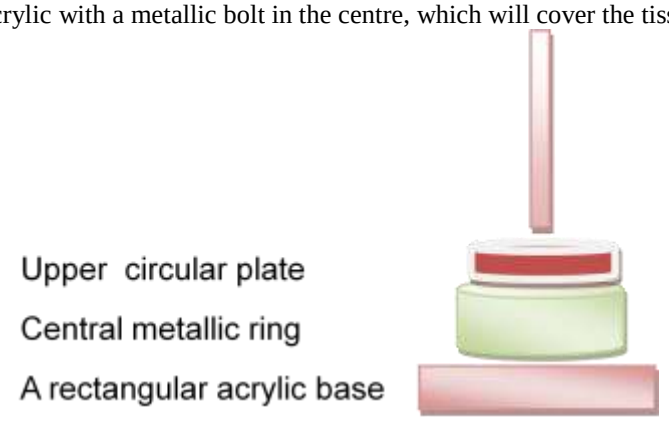


Figure 1:- Specimens for Peel Bond Strenth

Rectangular acrylic base

Specimens consist of lower rectangular block measuring50x50x5mmofheat-curingacrylictrevalon(DentsplyInd.e Com.Ltda.,Petrópolis,RJ,Brazil)weremade.For thispurpose,modeling waxmatrixesmeasuring 50x50x5 mm were molded. Each rectangular block were perforated , so that the tissue conditioners can flow through these holes and provide mechanical interlocking between acrylic and tissue conditioners(Fig2).

Figure 2:- Square acrylic base

Central Metallic Ring:

A standardized metal ring of diameter 35mm and 3 mm height was machined for confinement of soft tissue conditioners above the acrylic block (Fig 3)

Figure 3:- Central Metallic Ring

Upper Circular Plate (Fig 4):

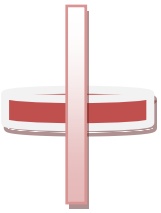


Figure 4:- Upper Circular Plate

Table 1 Material:

SNO.	Code	Materials	Manufacturer	Lot No.
1	V	Viscogel	DentsplyDeTreyGnbH, Konstanz, Germany.	1805000667
2	OO	Oregano oil	Suyash Herbs PvtLtd,Surat,Gujarat,India	161116/38/V-60/045
3	GO	Grape seed oil	Suyash Herbs PvtLtd,Surat,Gujarat,India	161116/38/V-60/045
4	DB	Trevalon Denture Base Resin	Dentsply Gurgoan ,India Pvt Ltd	122606
5	CA	Candida Albicans (ATCC24433)	Kothari pathology lab, Bhopal	00282691
6	A	Sabouraud Dextrose Agar	Sisco research laboratories pvt.Ltd.Mumbai	SD170412

Preparation of the Control group

Thirty specimens of control group were prepared. Each specimen consist of three parts: square acrylic base , central metallic ring for confinement of tissue conditioners and upper circular lid made up of acrylic with a metallic bolt in the centre, which will cover the tissue conditioner.

Preparation of Square Acrylic Base

For preparation of square acrylic base modeling wax of 50mm sides and 5mm height were prepared. These wax blocks were used to create uniform mold space. Type III gypsum was mixed using rubber bowl and a spatula according to manufactures instruction and then poured in the metal flask . Before the initial set the wax squares was placed in a metal flask. Once the setting reaction was over, separating media was applied and the counter pouring was done. After the setting of the stone, dewaxing is done and the flasks were opened. Thus the Square shaped cavities that were used as matrixes for the fabrication of heat cured acrylics resin specimens are formed.

Preparation of Upper Circular Lid

For preparation of Upper lid a metallic ring with 28mm diameter and 6mm height is used . Modeling wax is poured in the metallic ring and thus the circular wax pattern are obtained . These circular wax pattern were used to create uniform mold space. Type III gypsum was mixed using rubber bowl and a spatula according to manufactures instruction and then poured in the metal flask . Before the initial set the wax ring was placed in a metal flask once the setting reaction was over, separating media was applied and the counter pouring was done After the setting of the stone, dewaxing is done and the flasks were opened. Thus the circular shaped cavities that were used as matrixes for the fabrication of heat cured acrylics resin specimens are formed.

Packingofthespecimen:

Heat polymerized (TREVALON) acrylic rsins were mixed in the ratio 3:1 by volume such that the monomer thoroughly wet the polymers partices. HeatcurePMMAresinwasmixedaccordingtomanufacturers instructionsandpackedintothemouldspacesindoughstageandflasks were closed.

Benchcuring:

TheflaskassemblywasplacedintoHydraulicpress,andpressure wasappliedincrementallysothattheresindoughcanflowevenly throughoutthemouldspace.Someamountofexcessmaterial,Flashwas displacedeccentrically.Theapplicationofpressurewascontinueduntil the major portionsofthe flaskclosed approximatedeachother. The flasks were benchcured for30 minutes.

Processingofthespecimens

Thespecimenswereprocessedat74 ^{0}Cforapproximately2hoursfollowedbyincreasingthetemperatureofwate rbathto100 ^{0}Candthenprocessedfor1hourmore.Followingthecompletionofpolymerizationcycle,thedentureflaskswe recooledslowlytotheroomtemperature.Subsequently, the flasks were immersedincool tapwater for 15 minutes.

FinishingandPolishingofthespecimens

Thespecimenswerethenretrievedfromtheflasksandtheflashof acrylicresin,remainingstoneandnodulesofacrylicwereremovedusing acryliccarbideburs.Thebordersofthesampleswerethenfinishedand smoothened.Sandpapersof100,300 and500gradewereusedforthe finalfinishing.Usingawetragwheel,dippedinpumicethespecimens werepolishedandthenbuffedwithaclean,soft,drybrushwheel.

SurfacePreparationtoReceivethe Soft Liner

After this, specimens were submitted to surfacepreparationtoreceivethe soft liner.Oneofthespecimensurfaces of upper circular lid wasabraded automaticallyinapolishingmachineusing#600 silicacarbideabrasivepaper(Versharp).Theabradedsurfacewas cleanedwithdetergentfor20s,washedunder runningwater,anddried. While the lower square shaped specimenwas perforated with stainless steel round bur to create perforation which will help in mechanical entrapment of tissue conditions with the acrylic base.

Tissue conditioners was mixed according to the manufactures instruction and then tissue conditioner was confinedin a hollowstainlesssteelcircularmoldwith internal measurementsof 30 mm diameter and 3 mm height. Thissetwascoveredwith upper circular diskandkeptunderfingerpressureduring theresilientlinerpolymerizationtimerecommended bythemanufacturer.Theexcessesof themodifiedresilientlinerwereeliminatedandthe specimenwasremovedfromthemold.Therelined specimenswerethenstoredindistilledwaterat 37°C for 24 h prior to the peel test.

Preparation of the Test group specimens

Thirty specimens of each test group were prepared. The 60% of organo oil and 20% grape seed oil was mixedin each experimentalgroup. They weremanuallymixedwithresilient liningpowderwithaspatula,untilahomogenous mixturewas obtained.Theresilientlining liquidwasaddedtothismixtureandthematerial wasmixedinaccordancetothemanufacturer's instructions. Themodified material was insertedintothehollowcircular ringwith squareheat-curingacrylicresinprepared fortherelining procedure. Thissetwascoveredwith upper acrylic lidandkeptunderfingerpressureduring theresilientlinerpolymerizationtimerecommended bythemanufacturer.Theexcessesof

themodifiedresilientlinerwereeliminatedandthe specimenwasremovedfromthemold.Therelined specimenswerethenstoredindistilledwaterat 37°C for 24 h prior to the peel test.

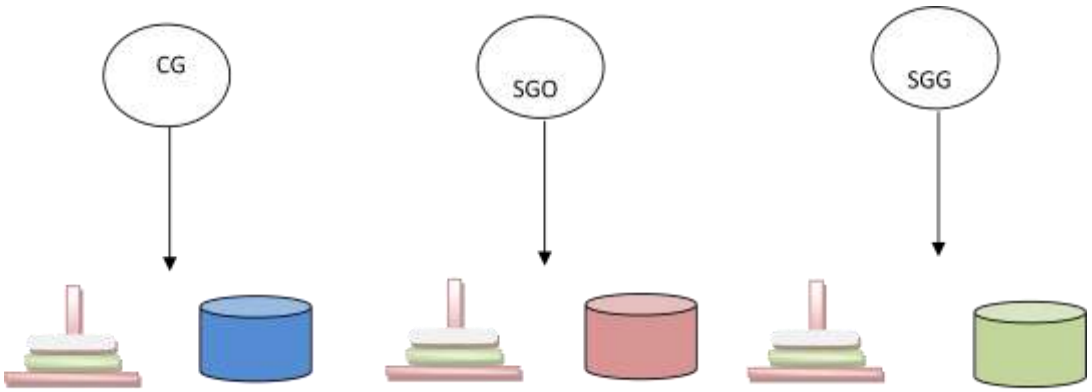
Grouping of the specimens

90 specimen of heatcure acrylic resin and tissue conditioners were made and divided into 3 groups of 30 each. Control group I (CG). Study group II with oregano oil (SGO) and Study group III with grapeseed oil (SGG). All specimens prepared were tested for peel bond strength and antifungal properties.

Group I: Control Group (CG 30 specimens).
Group II: Study Group with oregano oil (SGO 30 specimens).
Group I: Study Group with inorganic oil (SGG 30 specimens).

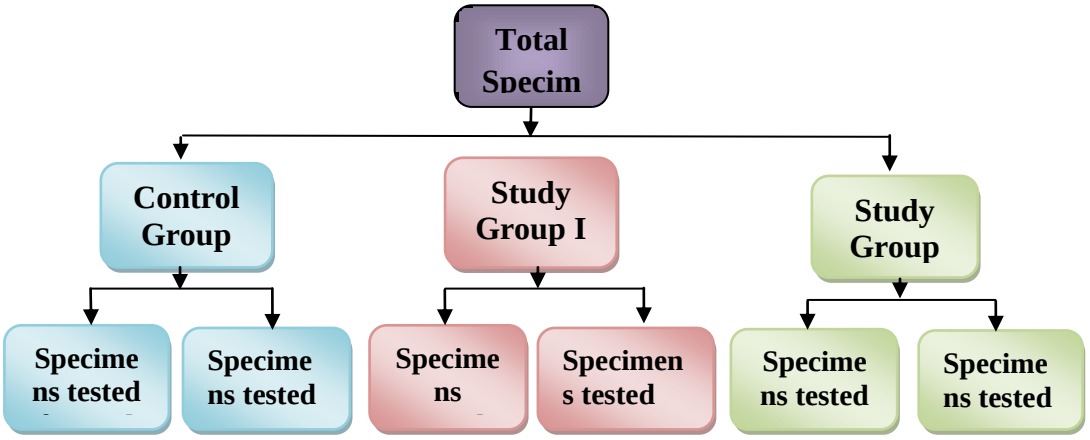
Table-II: Grouping of specimens

Group I (CG)	Tissue conditioners
Group II (SGO)	Tissue conditioners + oregano oil
Group III (SGG)	Tissue conditioners + grape seed oil



Schematic representation of grouping of samples

The specimens were serially numbered in each group, and each specimen was measured.



Flowchart showing grouping of samples

Measurement of Peel Bond Strength

Auniversaltestingmachine(Instron)was usedtoperformthepeelingbondstrengthtestof therelinedtestspecimensatanangleof180°.A portionofmodifiedresilientmaterialnotbonded totheresinbase(65mm)wasattachedupwards andfixedontothetophookoftheequipmentat 20mmfromtheadhesivebondareaofthetest specimen.Theotherun-relinedportionofthe heat-curingacrylicresinwasfixedontothebottom hookoftheequipmentatthesamedistance fromtheadhesivebondarea.Eachtestspecimen wassubmittedtotensiontopromotepeelingofthe modifiedresilientlinerfromtheheat-curingacrylic resinbaseataspeedof10mm/minuntilfailure occurred

Bondfailureswerevisuallyobservedand classifiedintothreecategories:adhesive,when peelingoccurredbetweenthemodifiedresilient liner and the denture base acrylic resin; cohesive,

whentherewastearing(ruptureofthe resilient linerwithintheareabondedtothedenturebase)or snapping(resilientmaterialhadstretchedandthen rupturedawayfromthebondedarea)withinthe modifiedresilientliner;andmixed,whenregions withtwotypesoffailurewereobservedonthe surface of the denture base material[13,17].

Theresultsofrupture forcewereinitially obtainedinNandtransformedintopeelingbond strengthinMPaandthensubmittedtoone-way ANOVA at a significant level of 5%.

MeasuringtheAntifungalActivity:

TheAgarDiffusionmethod

Agardisk-diffusiontestingdevelopedin1940[Heatley],isthe officialmethodusedinmanyclinicalmicrobiologylaboratoriesfor routineantimicrobialsusceptibilitytesting.Nowadays,manyaccepted andapprovedstandardsarepublishedbytheClinicalandLaboratory StandardsInstitute(CLSI)forbacteriaandyeaststesting[CLSI,2004,2012].

TestOrganism

*Candidaalbicans*strain(ATCC66026)wasusedastest organism.Thestockwasgrownandmaintainedinnutrientbroth/agar andCandidaagar (HimediaLabs, Mumbai). Forallthetestspecimen, andcontroltheantimicrobialassaywasperformed intriplicate.

Procedure

i. 1mlsuspensionofthe24hoursoldactivelygrowingpureculture of*Candidaalbicans*wasaseptically pouredintoeachPetridish .
ii. Approximately30mlofmeltedandcooledCandidaagarmedium wasthenpouredtoeachplateandthecontentsweregentlymixed and allowed tosettle.
iii. .Theacryliclestblockswerethengentlyplacedoverthesolidified medium.
iv. Theplateswereappropriatelylabelledandincubatedat32^{O}Cfor 24hours.
v. Thezonesofinhibition(lengthandwidth)weremeasuredwiththe helpofVerniercallipersandtheareawascalculated(LX W) after one day and one week.

III. Observation and result:

The peel bond strength of heat cure acrylic resin and soft tissue conditioners was evaluated, using universal testing machine on day 1 and after 7 days .

Table III: Peel bond strength (MPa) of Test Groups on 1st Day

S No.	Control group (MPa)	Study group I (MPa)	Study group II (MPa)
1	0.60	0.12	0.23
2	0.47	0.20	0.24
3	0.70	0.18	0.12
4	0.58	0.16	0.28
5	0.56	0.22	0.26
6	0.56	0.24	0.30
7	0.54	0.20	0.24
8	0.62	0.18	0.22
9	0.54	0.19	0.24
10	0.56	0.16	0.26
11	0.58	0.12	0.28
12	0.60	0.14	0.24
13	0.62	0.18	0.28
14	0.66	0.10	0.32
15	0.62	0.13	0.24

Table IV: Peel bond strength (MPa) of Test Groups on 7th Day

S No.	Control group (MPa)	Study group I (MPa)	Study group II (MPa)
1	0.58	0.56	0.30
2	0.49	0.44	0.32
3	0.55	0.48	0.24
4	0.57	0.58	0.28
5	0.53	0.40	0.26

6	0.53	0.53	0.27
7	0.46	0.56	0.32
8	0.60	0.50	0.28
9	0.58	0.58	0.30
10	0.52	0.52	0.24
11	0.60	0.45	0.29
12	0.59	0.62	0.31
13	0.65	0.52	0.28
14	0.62	0.45	0.29
15	0.59	0.39	0.32

Antimicrobial test was evaluated by using thezonesofinhibition(lengthandwidth) methodwiththe helpofVerniercallipersandtheareawascalculated on day 1 and after 7 days.

Table V: Zone of Inhibition of Test Groups on 1st Day

S No.	Control group (mm)	Study group I (mm)	Study group II (mm)
1	0	34	14
2	0	31	11
3	0	32	13
4	0	30	13
5	0	34	11
6	0	33	12
7	0	35	10
8	0	32	13
9	0	31	9
10	0	33	11
11	0	34	13
12	0	35	12
13	0	32	11
14	0	33	10
15	0	35	12

Table VI: Zone of Inhibition of Test Groups on 7thDay

S No.	Control group (mm)	Study group I (mm)	Study group II (mm)
1	0	23	3
2	0	21	3
3	0	19	5
4	0	18	3
5	0	22	4
6	0	21	5
7	0	24	3
8	0	23	5
9	0	21	2
10	0	20	4
11	0	16	6
12	0	16	5
13	0	20	4
14	0	21	4
15	0	19	3

The Average Value of peel bond strenth and minimum zone of inhibition of control Group, study group I and study group II on day 1 and day 7 are shown in Table III, Table IV, Table V,and Table VI.

The data were collected from all quantitative studies of heat cure acrylic resin Trevalon and soft tissue conditioner Viscogel were compared with 60% vol. by weight organo oil and 20% vol. by weight grape seed oil using two-way analysis of variance (ANOVA) statistical. A significance level of 0.05 was used for all tests.

Table V depicts the comparison between mean values of control group, study group I and study group II. There is significant difference between the 3 groups based on peel bond strength on 1st day (0.04) between the study group I and II and antimicrobial activity with (0.0001) . For peel bond on 7th day, there is a significant difference between study group I and study group II but for peel bond strength on day 1 the significance is between control and study group I.

The mean values for peel bond strength of the control group, study Group I and study group II on day 1 are: 0.5873, 0.1680 and 0.2473 respectively. The standard deviation of the above mentioned values of control group, study group I and study group II are: 0.054, 0.04 and 0.4 respectively. (Table VII) There is significant difference between the three groups based on peel bond strength on 1st day (0.013).

The mean values for peel bond strength of the control group, study Group I and study group II on day 7 are: 0.5640, 0.505 and 0.2867 respectively. The standard deviation of the above mentioned values of control group, study group I and study group II are: 0.050, 0.06 and 0.026 respectively. (Table VII) There is significant difference between the control group and study group1 (0.013)on day 7 however there is no significant difference between the study groups based on peel bond strength on 7 day (0.00).

The mean values for zone of inhibition of a control group, study Group I and study group II on day 1 are: 0, 32.93 mm and 20.26 mm respectively. The standard deviation of the above-mentioned values of control group, study group I and study group II are: 0.0, 1.57 and 2.37 respectively. Results are shown in Table VIII. This table depicts the comparison between mean values of control group, study group I and study group II. There is significant difference between the three groups based on antimicrobial activity on 1 day (less than 0.0001).

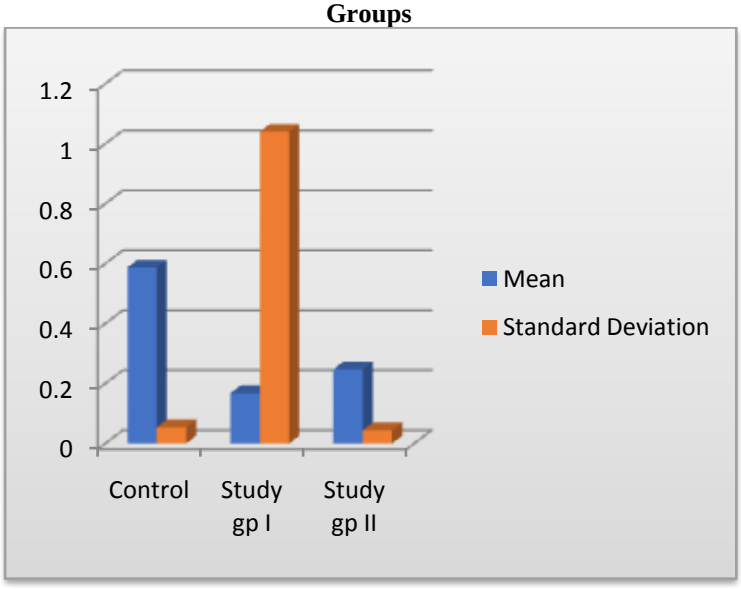
The mean values for zone of inhibition of a control group, study Group I and study group on day 7 are: 0, 11.66 mm and 3.93 mm respectively. The standard deviation of the above-mentioned values of control group, study group I and study group II are: 0, 1.39 and 0.1.09 respectively. Results are shown in Table VIII. This table depicts the comparison between mean values of control group, study group I and study group II. There is significant difference between the three groups based on antimicrobial activity on 7 day (0.0001).

Table VII: Comparing Peel Bond Strength on Day 1 and Day 7 among the 3 Groups

		N	Mean	Std. Deviation	P value
Control	Day 1	15	0.5873	0.054	0.001
	Day 7	15	0.5640	0.050	
Study Gp I	Day 1	15	0.1680	0.04	0.013
	Day 7	15	0.505	0.069	
Study Gp II	Day 1	15	0.2473	0.044	0.002
	Day 7	15	0.2867	0.026	

There was no significant difference seen by storage of sample on day1 and day 7 on peel bond strength of control and studygp II .However here was significant difference seen by storage of sample on day1 and day 7 on peel bond strength of study gp I.

Graph 1Comparing Mean Value and Standard Deviation of Peel Bond Strength on Day 1 among the 3 Groups



Graph 2 Comparing Mean Value and Standard Deviation of Peel Bond Strength on Day 7 among the 3 Groups

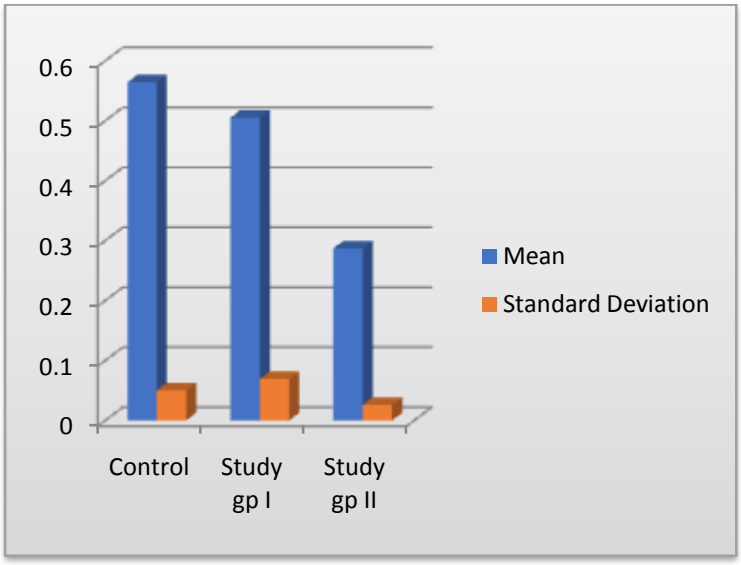
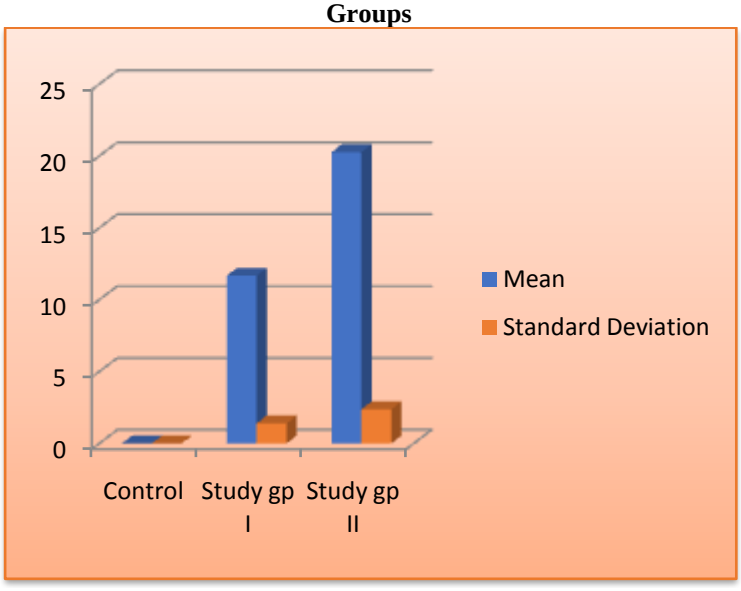


Table VIII: Comparing antimicrobial Activity on Day 1 and Day 7 among the 3 Groups

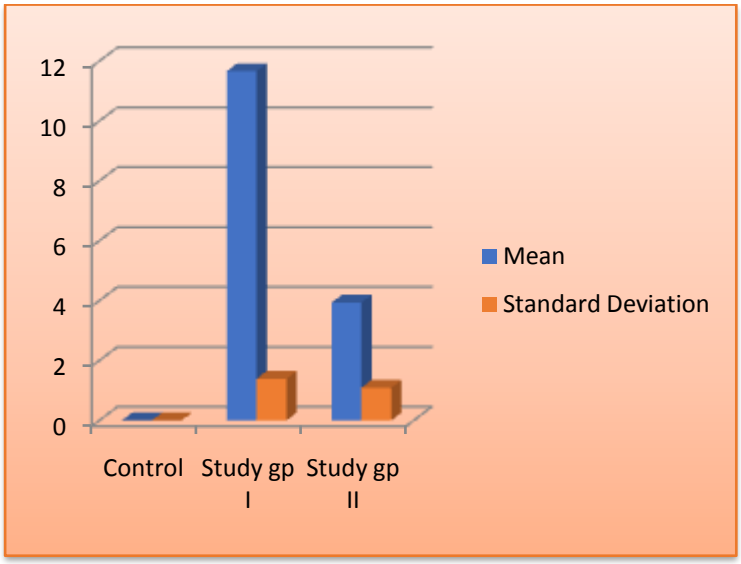
		N	Mean	Std. Deviation	P value
Control	Day 1	15	0	0	0.0001
	Day 7	15	0	0	
Study gp I	Day 1	15	32.93	1.57	0.0001
	Day 7	15	11.66	1.39	
Study gp II	Day 1	15	20.26	2.37	0.0001
	Day 7	15	3.93	1.09	

Among 3 groups, the mean value for zone of inhibition of both study group is statistically significant with the control group i.e. 0.0001 . Moreover the mean value for zone of inhibition among the two study group is also statistically singnificant i.e. less than 0.0001.

Graph 3Comparing Mean Value and Standard Deviation of antimicrobial activity on Day 1 among the 3 Groups



Graph 4 Comparing Mean Value and Standard Deviation of antimicrobial activity on Day 7 among the 3 Groups



IV. Discussion:

Tissue conditioners are routinely used to improve the fit and function of an ill-fitting denture prior to its replacement. When applied to the fitting surface of the denture absorb the impact of mastication and distribute the force widely, thereby reducing the mucosal pain.[1-4.]However, these materials are easily degradable and susceptible to microbial colonization[14]**,** Fungal growth on tissue conditioners is known to cause irritation of the oral mucosa, [5-8] and is due to the lack of antifungal activity.Which may cause different degrees of denture stomatitis. Denture stomatitis is related to the use of removable dentures and is considered the most common oral lesion observed (65%) [39] in patients wearing removable dentures. Although the etiology of denture stomatitis is multifactorial, infection by *Candida* spp., especially *C.albicans*, is considered the main etiologic factor. Different treatments for denture stomatitis are available and may include topical antifungal and systemic therapy, care with oral hygiene, denture cleaning and disinfection procedures[18], replacement of old dentures, elimination of anatomic irregularities, re-establishment of atraumatic occlusion, and nutritional restitution.

Thorough denture cleansing with daily brushing and cleaning is necessary.[14] Topical application of antifungal agents has not been encouraged, as the medication gets washed away by the saliva, leaving an insufficient concentration at the site of action.[11, 15, 16] Systemic administration requires large doses of drugs with a serious risk of side effects.[11] To overcome these disadvantages, anti-fungal agents have been incorporated into the denture liners. This combination may be a logical therapy in the treatment of denture stomatitis. The incorporation of antimicrobial agents into resilient materials has shown to be effective and feasible both in *invitro* and *invivo* studies**.**[6,20]Despite these therapeutic advantages, the incorporation of drugs into polymeric materials, including tissue conditioners and resilient liners, may affect their properties.

In the recent past, use of phytotherapeutic agents has increased because of the overuse and misuse of antibiotics, which leads to side effects and the emergence of resistant bacterial strains. This is the only method in which the failure proceeds at controlled section of force throughout the bond area.[22]

In the peel bond strength test, the stress is confined to a line restricted to the end of the bond, and is considered the most clinically representative of the failure modes.[51]The peeling test simulates the lining procedure more precisely, with a uniform and constant distribution of load.[52] The ability of the material to resist tearing is of practical importance. In clinical use, including the cleaning and disinfection procedures, the soft materials are submitted to conditions that start the tearing process. Adequate bonding between denture base resin and soft material is therefore essential. Clinical failure of these materials is frequently attributed to the rupture of this bond and the measurements of this bond are clinically relevant. Reduced bond between the soft liner and the denture base resineffectivelynegates any other property considered adequate for this material.[22]

In this study the peel bond strength of acrylic based soft liner, Viscogel was determined on 1 day and 7 day of stored samples in water at room temperature. However this study showed that with the incorporation of antifungal agents the peel bond strength of both study group I (0.168 MPa) and study group II (0.24MPa) was less than the control group(0.58MPa). Viscogel is a self curing , two component resilient methacrylate. The powder of Viscogel is a polyethyl methacrylate, and liquid consist of ester based plasticizer and ethyl alcohol.mixing of powder and liquid results in the polymer chain entanglement and formation of a coherent gel.[42]

On 1st day Peel bond strength of control group was high (0.58MPA) , as no bonding agents are needed to achieve a bond with acrylics resins. The results of the present study are supported by Emmer et al[38]who examined the bond strength of soft denture liner to denture base resin. They explained that these material debond immediately after elastic deformation with little stretching or plastic deformation. These failures (debonding) were internal (cohesive), which indicated that these materials are brittle, strong and bonded strongly to the denture base. The adhesive strength was higher than the cohesive strength.

Incorporation of antifungal agents resulted in the decreased peel bond strength of both study group I (0.168 MPa) and study group II (0.24MPa). Antimicrobial agents assessed, oregano oil and grape seed oil, they are insoluble in monomers and plasticizers. the essential oil of oregano oil is composed primarily of monoterpenoids and various compound among which thymol and carvacol ranging to over 80% while others to lesser content. Thymol is extremely soluble in ethyl alcohol which is the major component of liquid of soft lines. Thus, they could not interfere with the polymerization process of these materials. This possibly explains the decreased peel bond of study group I as compared to Study group II. Moreover, their physical presence within the polymer matrix could interrupt the structure of the polymerized materials21. In the study conducted by Addy and Handely[23] in 1981 also showed that change in material properties may be consistent with the incorporation pattern of the medication into the polymer matrix. In a previous study[22]conducted by Waters and Jagger also confirms that a tissue conditioner modified by the addition of nystatin showed cohesive strength values less than to those of the control group.

However with the storage of the samples in water for 7 days have resulted in increase of peel bond strength of controlled group (0.58MPA) and study group I

When immersed in soaking solutions or placed in the oral cavity, soft denture liners undergo two processes: leaching out of plasticizers and other soluble materials, and sorption of water or salivary components. The fluctuation between these two processes affects the properties of the denture liner material.[42] Conflicting results regarding bond strength following storage in water at room temperature. While some studies reported increase in bond strength [23, 24, 25, 26] on account of less lengthening and increased rigidity of the material others reported decrease in bond strength[27, 28].The present showed the increased in the strength on storage which could have been due to the swelling and stress buildup at the bond interface or of the changed viscoelastic properties of the resilient lining material, which renders the material stiffer and transmits the external loads to the bond site. In our study, the peel bond strength of all the material increases on storage in water which was in agreement with the previous studies.

It is speculated that the incorporation of an antifungal agent in a short-term denture liner may be beneficial. Douglas and Walker (1973) had the idea of combining the therapeutic effects of a tissue conditioner and an antifungal agent. This had the advantages that the action of the drug was prolonged, the cost was low and tissue recovery from trauma was encouraged.[29]

In this study, the tissue conditioners did not show any antifungal activity when tested there is absolutely no zone of inhibition (0 mm) of *C. albicans*seen in control group. This result confirms with the results of a study conducted by Kanathila et al[30] and previous study by Thomas et al,[31] who observed that Viscogel alone was completely inert and, therefore, would not be beneficial without antifungal agents in the treatment of denture stomatitis. Also, in an *in vitro* study conducted by Chow et al[32] to know the efficacy of antifungal agents in tissue conditioners.

in inhibiting *C. albicans*, samples containing only tissue conditioners did not exhibit significant fungicidal activity as compared to combinations of antifungal agents plus tissue conditioners.[31]

In the present study, on Day 1 study group I showed the highest zone of inhibition (32.93mm) i.e. antifungal activity while the study group II showed the least zone of inhibition (20.26mm). This observation is due to the fact that oregano oil is more potent antifungal agent than grape seed oil. The possible explanation for which is that Oregano oil contains carvacrol, 4-terpineol, and thymol, with wide variations in their relative percent ages, depending on geologic and climatic conditions. As high as 81.93%of carvacrol in oregano oil has been previously reported.[33] Both thymol and carvacrol impart significant antifungal properties to the Oregano oil.[34] Previously, superior antifungal activity was observed for the oil compared to thymol and carvacrol.[35] The Oregano oil's anti-microbial action was found to be due to the inhibition of germination and filament formation, which are essential for tissue invasion.[36] However the Phenolic contents found in grape seed are partially hydrophobic, and are considered to interact with the bacterial cell wall and lipopolysaccharide interfaces by decreasing membrane stability. [38]

However on Day 7 both study group I shows decrease in zone of inhibition (11.66mm) and study group II (3.93mm) i.e. antifungal activity decreases with time. The Minimal Inhibiting Zone diameter was found to reduce with time, which may be due to the leaching of the essential oil from the tissue conditioner.[37]

For the potency of antifungal property among the two study group, study group I is more antifungal compared to study group II which is due of the fact that the surface roughness of tissue conditioners with

oregano oil was less than that for the study group II. Low surface roughness coupled with the antifungal activity of oregano oil resulted in reduced adhesion of *Candidaalbicans*.[41]

Further research into the incorporation of modifiers to improve these materials is warranted. It is assumed that such modification will have some effect on their other properties. In addition, clinical studies should be conducted to establish correlation with laboratory findings. Finally, the search for more acceptable material.

V. Summary:

Tissue conditioners are resilient materials that are often used for conditioning the denture bearing mucosa sored by ill fitting dentures. Addition of antimicrobial agents into the base material might produce adverse effects on the physical, mechanical properties, biocompatibility etc. The storage of the material may further deteriorate the properties of the material, increasing the chances of adherence of these microorganisms, affecting the health of the tissue. In this *in-vitro* study, the Peel Bond Strength and Antifungal property of soft tissue conditioners after addition of oregano oil and grape seed oil was compared with control group.

From the result of 20% grape seed oil and 60% the present study, it was evident that Peel Bond Strength had significant difference between Control group , Study group I and Study group II on day 1. There was no significant difference for the Peel bond Strength among Control Group and Study Group II on 7^{th} Day. However there was significant difference for the Peel bond Strength of Study Group I on 7^{th} Day. There was significant difference between the 3 groups on the antifungal properties on day 1 however the antifungal property of organo oil is more effective than grape seed oil. However there was no significant difference among the antifungal property of organo oil and grape seed oil on 7^{th} Day.

VI. Conclusion:

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

1. The Peel bond strength of Control group is more than Study group I and Stdy group II irrespective of time period.
2. The Peel bond strength of control group and study group II decreases with time however the Peel bond strength of Study group I increases with time.
3. There was significant difference regarding the effectiveness of antifungal properties of control group and Study group I and Study group II after 1 day.
4. Maximum antifungal activity was shown by Study group I followed by Study group II

References:

[1]. Dm. The Effectiveness, In Vitro, Of Miconazole And Keteconazole Combined With Tissue Conditioners In Inhibiting The Growth Of Candida Albicans. J Oral Rehab 1985;12:177-82.
[2]. ZegarelliDj.FungalInfectionsOfTheOralCavity. OtolaryngolClin North Am. 1993;26:1069-89.
[3]. BantingDw,GreenhornPa,McminnJg.EffectivenessOfA TopicalAntifungalRegimenForTheTreatmentOfOralCandidiasisIn Older,ChronicallyIll,Institutionalized,Adults.JCanDentAssoc. 1995;61:199-200,203-5.
[4]. MontagnerH,MontagnerF,BraunKo,PeresPe,GomesBp.InVitroAntifungalActionOfDifferentSubstancesOverMicrowaved-Cured Acrylic Resins. J Appl Oral Sci. 2009;17:432-5.
[5]. LombardiT,Budtz-Jörgense. TreatmentOf denture Induced Stomatitis: AReview.EurJProsthodontRestorDent.1993;2:17-22.
[6]. TorresSr,PeixotoCb,CaldasDm,SilvaEb,AkitiT,NucciM, EtAl. RelationshipBetween salivary flowRatesAndCandida counts In SubjectsWith Xerostomia.OralSurgOralMed OralPatholOral RadiolEndod. 2002;93:149-54.
[7]. Aldana l,MarkerVa,KolstadR,IacopinoAm.EffectsOf candida TreatmentRegimensOnThePhysicalPropertiesOfDentureResins. Int J Prosthodont. 1994;7:473-8.
[8]. CarterGm,KerrMa,ShepherdMg.TheRational managementOf OralCandidosisAssociatedWithDentures.NZDentJ.1986;82:81-4.
[9]. UrbanVm,Seóרs,GianniniM,ArraisCa.Superficial DistributionAndIdentificationOfAntifungal/AntimicrobialAgents OnAModifiedTissueConditionerBySem-EdsMicroanalysis:A Preliminary Study. J Prosthodont. 2009;18:603-10.
[10]. MccabeJf,CarrickTe,KamoharaH.AdhesiveBondStrength AndComplianceForDentureSoftLiningMaterials.Biomaterials. 2002;23:1347-52.
[11]. MäkiläE,Hopsu-HavuVk.MycoticGrowthAndSoftDenture Lining Materials. ActaOdontol Scand. 1977;35:197-205.
[12]. SchneidTr.AnInVitroAnalysisOfASustainedReleaseSystem ForTheTreatmentOfDentureStomatitis.SpecCareDentist. 1992;12:245-50.
[13]. TruhlarMr, Shay K, SohnleP. Use Of ANew Assay Technique ForQuantificationOfAntifungalActivityOfNystatinIncorporatedIn Denture Liners. J Prosthet Dent. 1994;71:517-24.
[14]. Shrestha B Et Al .Asian Pac J Trop Biomed 2012; 2(10): 822-825
[15]. Baydar Ng, Sagdic O, Ozkan G, Cetin S. Determination Of Antibacterial Effects And Total Phenolic Contents Of Grape (Vitisvinifera L.) Seed Extracts: Int J Food SciTechnol2006. 41: 799-804.
[16]. Kao Tt, TuHc, Chang Wn, Chen Bh, Shi Yy, Chang Tc, Et Al. Grape Seed Extract Inhibits The Growth And Pathogenicity Of Staphylococcus AureusBy Interfering With DihydrofolateReductase Activity And Folate-Mediated One-Carbon Metabolism. Int J FoodMicrobiol2010. 141:17-27.
[17]. Al-Habib A, Al-Saleh E, Safer Am, Afzal M. Bactericidal Effect Of Grape Seed Extract On Methicillin-Resistant Staphylococcus Aureus(Mrsa). J ToxicolSci2010. 35:357-364.
[18]. Si H , Hu J, Liu Z And Zeng Z-L . Antibacterial Effect Of Oregano Essential Oil Alone And In Combination With Antibiotics Against Extended-Spectrum B-Lactamase Producing Escherichia Coli, FemsImmunol Med Microbiol 53 (2008) 190–194.
[19]. BradenM,WrightPs,ParkerS.SoftLiningMaterials-AReview. Eur J ProsthodontRestor Dent. 1995;3:163-74.

[20]. MachadoAl,BreedingLc,PuckettAd.EffectOfMicrowave DisinfectionOnTheHardnessAndAdhesionOfTwoResilientLiners. J Prosthet Dent. 2005;94:183-9.
[21]. MccabeJf,CarrickTe,KamoharaH.AdhesiveBondStrength AndComplianceForDentureSoftLiningMaterials.Biomaterials. 2002;23:1347-52.
[22]. Waters Mg, Jagger Rg. Mechanical Properties Of An Experimental Denture Soft Lining Material. J Dent. 1999;27:197-202.
[23]. Addy M, Handley R. The Effects Of The Incorporation Of Chlorhexidine Acetate On Some Physical Properties Of Polymerized And Plasticized Acrylics. J Oral Rehabil. 1981;8:155-63.
[24]. Aydin Ak, Terzioglu H, AkinayAe, Ulubayram K, Hasirci N. Bond Strength And Failure Analysis Of Lining Materials To Denture Resin. Dent Mater. 1999;15:211–218.
[25]. Craig Rg, Gibbons P. Properties Of Resilient Denture Liners. J Am Dent Assoc. 1961;63:382–390.
[26]. DootzEr, Koran A, Craig Rg. Physical Property Comparison Of 11 Soft Denture Lining Materials As A Function Of Accelerated Aging. J Prosthet Dent. 1993;69:114–119...
[27]. Leon Bl, Del BelcuryAa, Rodrigues Garcia Rc. Water Sorption, Solubility And Tensile Bond Strength Of Resilient Lining Materials Polymerized By Different Methods After Thermal Cycling. J Prosthet Dent. 2005;93:282–287.
[28]. Polyzois Gl. Adhesion Properties Of Resilient Lining Materials Bonded To Light-Cured Denture Resins. J Prosthet Dent. 1992;68:854–858.
[29]. .YaseminKo, Atilla S, Hale G. Effect Of Thermocycling On Tensile Bond Strength Of Six Silicone-Based, Resilient Denture Liners. J Prosthet Dent. 2003;89:303–310.
[30]. Urban Vm, SeóRs, Giannini M, Arrais Ca. Superficial Distribution And Identification Of Antifungal/Antimicrobial Agents On A Modified Tissue Conditioner By Sem-Eds Microanalysis: A Preliminary Study. J Prosthodont. 2009;18:603-10.
[31]. Kanathila H, Bhat Am, Krishna Pd. The Effectiveness Of Magnesium Oxide Combined With Tissue Conditioners In Inhibiting The Growth Of Candida Albicans: An In Vitro Study. Indian J Dent Res 2011;22:613.
[32]. Thomas Cj, Nutt Gm. The In Vitro Fungicidal Properties Of Viscogel, Alone And Combined With Nystatin And Amphotericin B.J Oral Rehabil 1978;5:162-72.
[33]. Chow Ck, MiiteaiDw, Lawrence Hp. Efficacy Of Antifungal Agents In Tissue Conditioners In Treating Candidiasis. Gerodontology 1999;16:110-18.
[34]. GiordaniR,RegliP,KaloustianJ,MikaïlC,AbouL,PortugalH.AntifungalEffectOfVariousEssentialOilsAgainstCandidaAl-Bicans.PotentiationOfAntifungalActionOfAmphotericinBByEssentialOilFromThymusVulgaris.PhytotherRes2004;18:990-5.
[35]. GuoN,LiuJ,WuX,BiX,MengR,WangX,EtAl.AntifungalActivityOfThymolAgainstClinicalIsolatesOfFluconazole-SensitiveAnd-ResistantCandidaAlbicans.JMedMicro-Biol2009;58:1074-9.
[36]. NostroA,BlancoAr,CannatelliMa,EneaV,FlaminiG,MorelliI,EtAl.SusceptibilityOfMethicillin-ResistantStaphylococciToOreganoEssentialOil,CarvacrolAndThymol.FemsMicrobiolLett2004;230:191-5.
[37]. ManoharV,IngramC,GrayJ,TalpurNa,EchardBw,BagchiD,EtAl.AntifungalActivitiesOfOriganumOilAgainstCandidaAl-Bicans.MolCellBiochem.2001;228:111-7.
[38]. Douglas Wh, Clarke Da.Physical And Mechanical Properties Of NystatinContaingDebture Liners. J Prosthet Dent. 1975;94:1428-34.
[39]. Kulak-Ozkan Y, Sertgoz A, Gedik H. Effect OfThermocycling On Tensile Bond Strength Of Six Silicone-Based, Resilient Denture LinersjProsthet Dent. 2003 Mar;89(3):303-10.
[40]. 56464562
[41]. Urban Vm Et Al. Effect Of The Association Of Nystatin With A Tissue Conditioner On Its Ultimate Tensile Strength. J Proghodont. 2006 Sept.- Oct.
[42]. Rodrigues S., Vidya S And Shetty T. Resilient Liners : A Review I Indian Prosthodont Soc. 2013 Sep : 13 (3) : 155-164