International Journal Dental and Medical Sciences Research
Volume 3, Issue 1, Jan-Feb 2021 pp 86-91 www.ijdmsrjournal.com ISSN: 2582-6018

In Vitro Comparitive Evaluation of the Properties of a Tissue Conditioner with Melaleuca Oil As an Antifungal Agent

Dr Supriya Nandi, Dr. Nandeeshwar D.B.
Student, Bapuji Dental College and Hospital, Davangere
Professor and HOD, Bapuji Dental College and Hospital, Davangere
Date of Submission: 25-11-2020

Date of Submission: 15-12-2020 Date of Acceptance: 30-12-2020

ABSTRACT– Water Absorption and leaching of plasticizer contents were evaluated in Visco gel tissue conditioner (Dentsply Detrey GmbH, Konstanz, Germany) after immersing in distilled water for a period of 7 days. Test specimens for two groups were prepared using a custom fabricated metal die measuring 50mm in diameter and 0.5mm thickness. Melaleuca oil was added in group 2 (test). All Specimens were weighed prior to start of the study and denoted as **(W1)**. The specimens were immersed in distilled water 7 days[3] and weighed again after removal from the distilled water and denoted as **(W2)**. Finally, specimens were subjected to desorption using desiccator and weighed again and denoted as **(W3)**. Water Sorption and Solubility were further evaluated based on the differences of their weight.

Results: Water Sorption and Solubility of group 2 (test) were significantly lower than group 1 (control). **P value<0.001**

Conclusion: There is a significant decrease in the Water sorption and Solubility value of the tissue conditioner with incorporation of Melaleuca alternifolia oil (30%w/w).

Key Words: Denture stomatitis, Melaleuca Altrernifolia oil, Dynamic viscoelasticity,
Tissue conditioner

I. INTRODUCTION

The success of removable complete or partial denture depends on esthetics, comfort, and function. In an edentulous individual with a complete or a partial denture prosthesis the masticatory load and functional stresses are transmitted to the bone through mucoperiosteum. These functional stresses lead to chronic soreness, pathologic changes to oral tissues, and subsequent bone loss resulting in loss of accurate adaptation of the denture to the underlying tissues.

Denture stomatitis is the most common form of oral candidiasis prevalent in almost 11% to 70% of complete denture wearers, that exists as a localized physical condition where there is inflammation of oral mucous membrane beneath the denture. In about 50% - 98% of cases, Candida albicans species is the most prevalent one. It usually occurs in elderly individuals who constantly wear complete or removable partial dentures, ill fitted dentures, debilitated patients and in those who do not maintain cleanliness in oral cavity[1].

Tissue conditioners are distinctively used as an adjunct in conditioning the denture bearing tissues to a more nourishing state and is frequently used in obturators and protection of surgical areas. The softness and flexibility of these materials, as a result of their physical and chemical layouts, present the opportunity to safeguard the supporting tissues from functional and parafunctional tension.However, fungal proliferation in tissue conditioners is further equated with constant irritation of oral mucosa and due to lack of antifungal properties, these materials are associated with penetration of Candida albicans and other fungi making it cumbersome for its usage[2]

Candida albicans has been established as the primary pathogenic microorganism regularly isolated from patients suffering from denture stomatitis. C. albicans is a normal commensal organism of the oral cavity, but can acquire pathogenicity in cases of immunodeficiency and/or chronic local irritation.

Antifungal therapy should be initiated if fungal organisms are identified. Topical therapy is the first line of treatment. Triazole antifungal drugs (fluconazole and itraconazole) provide important tools for the treatment of denture stomatitis. Because the success of topical application of drugs in the oral cavity may be compromised by lack of patient compliance, antifungal agents can be incorporated in tissue conditioners. The indiscriminate use of antifungal agents has led to the emergence of resistant Candida strains. In recent years, use of many Phyto therapeutic agents have come up to be used as antifungal agents that can be effectively incorporated into these materials

International Journal Dental and Medical Sciences Research
Volume 3, Issue 1, Jan-Feb 2021 pp 86-91 www.ijdmsrjournal.com ISSN: 2582-6018

and have proven to be an efficient way of combating these dentures associated problems. Many herbal formulations including melaleuca alternifolia oil have been established as potent antifungal agents that can be used safely.[3]

Melaleuca oil, commonly known as tea tree oil, is an essential oil, extract of the Australian plant Melaleuca alternifolia, and has proved to have beneficial medical properties when applied topically, including antibacterial, antiviral, antifungal, and antiseptic properties. Melaleuca alternifolia is effective topically because of its lipophilic nature, facilitating skin penetration. Its mechanism of action against C. albicans has been found to be its ability to inhibit formation of the germ tubes and alter the membrane characteristics and function.[4]

In concentration of 30%, when incorporated in tissue conditioner, it has proved to be an effective antifungal that showed significantly high mean inhibition diameter for a period of seven days.[3]

However, these soft liners exhibit multiple clinical failures characterized by loss of adhesion to denture base, surface, and/or bulk deterioration, accumulation of debris and plaque, loss of resilience and fungal or microbial accumulation and many of these problems result from the increased water sorption and solubility when dentures are soaked in saliva during use or kept in water or aqueous disinfecting solution during storage.[5]

Hence the present study is intended to Evaluate and Compare the Water Sorption and Water Solubility of a tissue conditioner after incorporation of Melaleuca Oil as an antifungal agent.

II. MATERIALS AND METHODS:

Source of Data:

All the materials were collected / purchased through scientific chemical / dental suppliers and specimens were prepared in the Department of Prosthodontics and Crown & Bridge, Bapuji Dental College & Hospital, Davangere and data were obtained from laboratory-based studies.

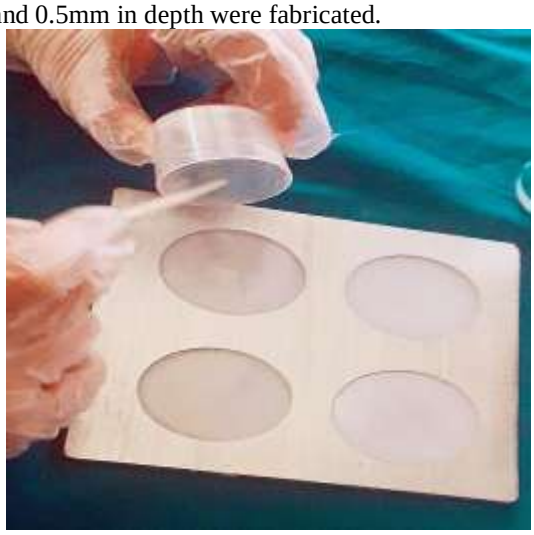
Materials used:

1. Tissue Conditioner - Visco gel(Dentsply Detrey GmbH, Konstanz, Germany).
2. Pure Melaleuca Oil.

A total of 30 samples of tissue conditioners were fabricated, that was further divided into Two groups consisting 15 test specimens each.

A. **Preparation of the framework**:

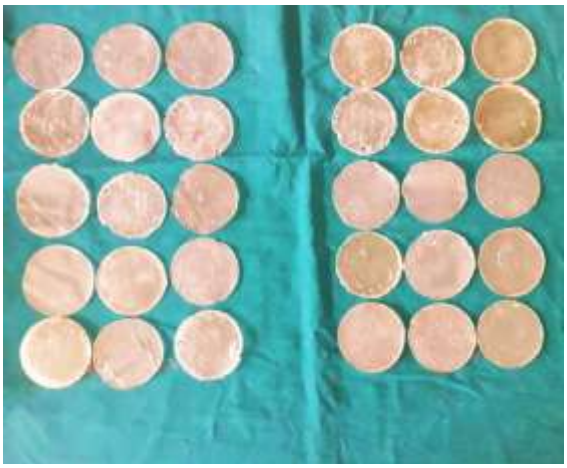
Stainless steel blocks were fabricated with shallow well cut into them, measuring 50mm in diameter and 0.5mm in depth were fabricated.



(Preparation of study Samples using standardized metal mold).

B. Preparation of the specimen:

The study comprised of 30 disc-shaped specimens of Visco gel tissue conditioner Specimens were divided into two groups of 15 specimens each based on whether Melaleuca Alternifolia oil was incorporated into tissue conditioner or not.



(Samples Group 1 and Group 2 prepared for the study)

Group 1 specimen preparation: This served as the control group. 15 specimens of Visco gel conditioner were prepared according to manufacturer's recommended powder liquid ratio in a measuring jar and mixedfor 30 seconds until a homogenous mixture was obtained and poured into stainless steel mold placed on the glass plate and the powder-liquid mixture was allowed to set.

International Journal Dental and Medical Sciences Research
Volume 3, Issue 1, Jan-Feb 2021 pp 86-91 www.ijdmsrjournal.com ISSN: 2582-6018

Group 2 specimen preparation: This served as the test group and comprised of 15 specimens.
100 ml of tissue conditioning liquid was measured and mixed with 30 ml of Melaleuca Alternifolia oil using micropipette and poured into the sterile glass beaker containing the above premeasured conditioning liquid of the tissue conditioner. Individual weights of the test and control groups were determined using an Analytical weighing scale until a constant mass was achieved and grouped asinitial mass of specimens. After the initial mass **(W1)** is determined,

(Analytical balance used in the study for weighing the study samples)

the specimens were immersed in distilled water in sealed containers at 37^0 C for 7 days.[3]

(Samples immersed in distilled water for a period of 7 days for 'Water Sorption' and 'Solubility' test).

After the immersion period was completed, excess water was removed by blotting with absorbent papers until the specimen was free of visible moisture. Then the specimens were allowed to air dry for 15 seconds and weighed again after removal from the distilled water **(W2)**.Finally, specimens were subjected to desorption using desiccator (figure 5) and weighed again in an analytical balance with a precision of 0.001g at regular intervals until a constant mass **(W3)** is obtained.


(Desiccator unit used in the study for removing excess moisture from study samples)

Water Sorption and Solubility was calculated in micrograms per cubic millimetre using the following equations:

WATER SORPTION: $WS = W2-W3/V$.
SOLUBILITY: $S = W1-W3/V$.

Where;

- **W1** is the constant initial specimen mass of test specimens after first desorption.
- **W2** is the mass of test specimen after immersion in water.
- **W3** is the mass of test specimen after second desorption process.
- **V** is the specimen volume ($981.75mm^3$)[1].

III. RESULTS

The Water Sorption and Water Solubility values obtained from various groups were tabulated and analysed for statistical significance. Normality tests were applied to test the normality of the data. Solubility variable followed normal distribution hence Parametric Test (Independent Sample T Test) were applied. Water Sorption variable did not

International Journal Dental and Medical Sciences Research
Volume 3, Issue 1, Jan-Feb 2021 pp 86-91 www.ijdmsrjournal.com ISSN: 2582-6018

follow normal distribution hence non parametric test (Man Whitney U Test) were applied.
"P" values:
• P < 0.05 – Significant
• P < 0.005 – Highly significant
• P < 0.0005 – Very highly significant
• P > 0.05 – Nonsignificant.

Water Sorption of group 2 (75.97) was significantly lower than group 1 (100.02). Water Solubility of group 2 (2.34) was significantly lower than group 1 (11.4).
The difference was **highly statistically significant** for Solubility.

TABLE 1: Showing mean initial weight of group 1 and group 2 samples after first desiccation before subjecting for 'Water Sorption' and 'Solubility' tests
(W1 measured in grams)

Mean weight of group 1 samples (gm)	Mean weight of group 2 samples (gm)
2.918	2.962

TABLE 2: Showing mean weight of group 1 and group 2 samples after immersion in distilled water for 7 days subjected for measuring 'Water Sorption'
(W2 measured in grams)

Mean weight of group 1 samples (gm)	Mean weight of group 2 samples (gm)
3.013	3.040

TABLE 3: Showing weight of group 1 and group 2 samplesafter second Desiccation subjected for measuring 'Solubility' (W3 measured in grams)

Mean weight of group 1 samples (gm)	Mean weight of group 2 samples (gm)
2.908	2.960

TABLE 4: Showing Descriptive Inferential Statistics for difference in 'Water Sorption' and 'Solubility'.

Variables	**Group**	**n**	**Mean**	**Median (Interquartile range)**	**Std. Deviation**	**P Value**
Water sorption (WS)	1	15	100.02	100.02(100.01,100.03)	0.02	**<0.001****
	2	15	75.97	77.05(76.01,78.10)	4.64	
Solubility (S)	1	15	11.40	11.5(10.08,13.02)	1.44	**<0.001****
	2	15	2.34	2.08 (1.8, 2.8)	0.9	

****HIGHLY SIGNIFICANT**

TABLE 5: Showing tests of Normality

Tests of Normality							
Variables	Group	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	n	Sig.	Statistic	n	Sig.
Water Sorption (WS)	1	0.331	15	0.000	0.580	15	0.000
	2	0.303	15	0.001	0.806	15	0.004
Solubility (S)	1	0.156	15	0.200*	0.964	15	0.769
	2	0.159	15	0.200*	0.928	15	0.257

International Journal Dental and Medical Sciences Research
Volume 3, Issue 1, Jan-Feb 2021 pp 86-91 www.ijdmsrjournal.com ISSN: 2582-6018

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

IV. DISCUSSION

One of the most common sequelae of long-term denture wearing is chronic atrophic candidiasis, also known as denture stomatitis (DS) or denture sore mouth.

Clinically, appearance varies from discrete areas of pinpoint inflammation often associated with the ducts of the palatal mucous glands, to a more commonly observed vivid inflammation of the area coincident with that covered by the upper denture.

Due to denture trauma, poor denture hygiene, dietary factors, xerostomia, continuous wear of denture without removal,chronic illnesses, and a compromised immune system.[6] Despite the multifactorialetiology, Candida albicans has been established as the primary pathogenicmicroorganism regularly isolated from patients suffering from denture stomatitis and antifungal therapy should be initiated once C. Albicans is isolated from denturestomatitis lesion.

However, inherent with this indiscriminate use of antifungal agents, either alone or as incorporated in tissue conditioners, is the emergence of resistant Candida strains. So alternative to conventional antifungal agent is the need for the day. It has been shown that certain herbal medicines known as phytotherapeutic agents have antifungal, antibacterial and antiviral activity. Amongst those herbal formulations, melaleuca alternifolia oil has shown potent antifungal activity and it is safe to use without any side effects. It was found out that Tea Tree Oil exhibited maximum antifungal activity while Evening Primrose had the lowest antifungal property.[7]

Tissue conditioners are amorphous polymers, formed by mixing of a polymer powder and a liquid plasticizer. Alcohol acts as an accelerator for plasticizer penetration into the polymer to produce a clinically acceptable gelation time. However, EtOH leaches into water, resulting in loss of viscoelastic properties over time. As it is regarded that efficacy & clinical success of the denture tissue conditioner materials is determined by their viscoelastic property and durability, thus incorporation of any antifungal agent must not have any deleterious effect on these properties.

Therefore,the present study aimed to Evaluate and Compare the 'Water Sorption' and 'Solubility' of a Tissue conditioner (Visco gel) before and after incorporation of M. Alternifolia as an 'antifungal agent'. **Graham et al** reported that tissue conditioners continue to flow for 7 days and suggested that they are clinically effective throughout this period.[7] The study time parameter of 7 days was therefore decided upon for the study

Result from the studyrevealed a significant difference between values of 'Water Sorption' of the samples of Visco gel tissue conditioner without Melaleuca oil as antifungal agent (Group 1) with a mean of 100.01 µg/mm^3 versus samples of Visco gel tissue conditioner with Melaleuca oil as antifungal agent (Group 2) with a mean of 78.03 µg/mm^3, exhibiting a lower value in Group 2 as compared to Group 1.

Similarly, the values of 'Solubility' of the samples of Visco gel tissue conditioner without Melaleuca oil as antifungal agent (Group 1), with a mean of 11.2 µg/mm^3 was much higher compared to the samples of Visco gel tissue conditioner with Melaleuca oil as antifungal agent (Group 2), with a mean of 2.38 µg/mm^3. This indicated a reduced amount Solubility in Group 2 as compared to Group 1. The p values obtained were less than 0.001 indicating the study is Highly Significant.

The probable reason for the significant reduction in the amount of 'Water Sorption' and 'Solubility' could be;

1. Because the Molecular weight of Melaleuca oil (716.4 g/mol)[8] is greater than that of the Di butyl phthalate (plasticizer) in the Tissue Conditioner (194.19 g/mol) which does not allow fluid from the surrounding media to get incorporated into the matrix of the Tissue Conditioner. Therefore, the absorption and release of the soluble components is reduced than its counterpart.
2. Also, the leaching of soluble components and fluid absorption is influenced by drug diffusion through channels and pores created in the polymer matrix. Since, the Melaleuca oil was uniformly spread on the surface of Visco Gel. This particle distribution pattern in the material may be responsible for the constant and effective drug release in sustained therapy that prevented leaching and absorption of soluble components.[8]

It can be stated that, addition of 'Melaleuca Alternifolia Oil' into the 'Tissue Conditioner' not only serves to be an effective antifungal agent, in addition it improves the properties of 'Tissue Conditioner'. Thus, this combination is an effective remedy for the

treatment of 'Denture Stomatitis' and can be suggested for clinical use as well.

V. LIMITATIONS OF THE STUDY

- As in vitro results cannot be extrapolated in vivo, further investigation is needed by conducting in vivo clinical trials.
- In addition, when in the mouth, denture resilient liners may be subjected to additional thermal stress, pH range, and occlusal load, which could lead to other patterns of 'Water Sorption' and 'Solubility' of these products.
- Only one brand of Tissue Conditioner was used in the study, thus the results obtained here may not apply to other Tissue Conditioners incorporated with the same concentration of Melaleuca oil.

VI. CONCLUSION

Melaleuca Oil is a potent antifungal agent with superior properties and addition of it to Tissue Conditioner enhances its viscoelastic properties for long term safe use.

VII. CLINICAL RELEVANCE

As this treatment option does not depend on patient compliance, it may be beneficial for the elderly patients with physical or mental disabilities, or in institutional settings where patients and staff cannot follow all recommended topical antifungal treatment instructions.

VIII. FUTURE RESEARCH PROSPECTIVE

Results of the present study can direct future research where in addition of a phytotherapeutic agent, Melaleuca oil not only acts as an effective antifungal agent but also enhanced the properties of Tissue Conditioner. It can be suggested to be possibly marketed commercially for dental therapeutic use. Moreover, it would be authenticating if such studies are performed in vivo that will hold great clinical significance.

BIBLIOGRAPHY

[1]. **Lima JF, Maciel JG, Arrais CA, Porto VC, Urban VM, Neppelenbroek KH**. Effect of incorporating antifungals on the water sorption and solubility of interim resilient liners for denture base relining. J Prosthet Dent. 2016;115(5):611-16.

[2]. **Winkler S.** (1987), Essentials of Complete denture prosthodontics, second edition: USA. Mosby year book 81-86.

[3]. **Sharma S, Hegde V**. Comparative evaluation of antifungal activity of melaleuca oil and fluconazole when incorporated in tissue conditioner: an in vitro study. J Prosthodont. 2014;23(5):367-73.

[4]. **Srivatstava A, Ginjupalli K, Perampalli NU, Bhat N, Ballal M**. Evaluation of the properties of a tissue conditioner containing origanum oil as an antifungal additive. J Prosthet Dent. 2013;110(4):313-19.

[5]. Council on Dental Materials. Revised ADA specification no 12 for denture base polymer. J Am Dent Assoc 1975;90:145- 54.

[6]. **Wilson J.** In vitro loss of alcohol from tissue conditioners. Int J Prosthodont. 1992;5(1):17-21.

[7]. **Graham BS, Jones DW, Sutow EJ**. Clinical implications of resilient denture lining material research. Part II: Gelation and flow properties of tissue conditioners.JProsthet Dent 1991;(65):413-8.

[8]. **Carson CF, Riley TV**. Antimicrobial activity of the major components of the essential oil of Melaleuca alternifolia. J ApplBacteriol 1995;(78):264-9.