

## Formulation & Evaluation of Myrrh Toothpaste

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Received: November 02, 2021  
Accepted for publication: March 07, 2022

### ABSTRACT

**Objectives:** To formulate and measure the cytotoxicity level of organic myrrh toothpaste. **Materials and Methods:** Myrrh extract was prepared by the freeze drying process. Toothpaste was formulated by mixing specified amounts of myrrh extract, hydroxypropyl methylcellulose (HPMC) polymer, sodium lauryl sulphate (SLS), mint, and sucralose with deionized distilled water. MTT test was performed using concentrations of 100, 200, 300 and 400 mg/ml to assess the effect of myrrh paste on the gingival fibroblasts viability at intervals of 24 and 48hrs. The results were analysed by using SPSS version 27. **Results:** The formulated myrrh toothpaste has a homogeneous consistency as the extracted myrrh successfully dissolved completely with the other components. Cell viability test showed that myrrh paste concentrations of 100 - 300mg/ml were effective in maintaining the rate of fibroblasts growth after 24 and 48 hours as compared to the control samples. Cell growth rate was suppressed in the test samples treated with paste concentration of 400mg/ml. The results of the study imply that 300mg/ml is the safe and optimal concentration for fibroblasts growth, whereas concentrations  $\geq 400$ mg/ml are intolerable and might be suppressing the proliferation of fibroblasts. **Conclusion:** Myrrh toothpaste concentration at 300mg/ml is the safe and optimal concentration for fibroblasts growth as depicted in the results, suggesting that at the optimal formulation, myrrh extract may not be toxic to the soft tissues and myrrh toothpaste may be useful for oral health care. Further clinical investigations are recommended to obtain the clinical efficacy data of the organic myrrh toothpaste.

**Key words:** Myrrh, toothpaste, cytotoxicity, cell viability

## INTRODUCTION

Decay of teeth and gingival problems are commonly occurring and negatively affecting the quality of life of population (Griffin, Jones, Brunson, Griffin, & Bailey, 2012). Despite the improvement in oral health measures and usage of fluoridated toothpaste, oral health problems incidence does not stop yet (Aoun, Darwiche, Al Hayek, & Doumit, 2018). Hence, there is a high demand for innovative efforts to develop new products which may help in reducing the intensity of oral problems (Jepsen et al., 2017; Philip, Suneja, & Walsh, 2018).

Various oral health care products containing herbal ingredients are available in the market. Randomised controlled trials have shown that herbal oral care products are relatively superior to non-herbal products (Janakiram, Venkitachalam, Fontelo, Iafolla, & Dye, 2020). To formulate a herbal oral care product, the active ingredient must be safe, non-toxic, effective and well known to the public (Kharaeva et al., 2020).

Myrrh is a resinous-exudate that is obtained from the stem of different species of *Commiphora* trees (Khalil, Fikry, & Salama, 2020). The genus *Commiphora* includes over 150 species which are mainly found in Eastern Africa, Arabia, and India. The name 'myrrh' comes from the ancient Arabic and Hebrew word 'mur', which means bitterness, due to the bitter and acrid taste of the resin (Dafni & Böck, 2019). It was reported that myrrh possesses many medicinal properties and has been used in traditional medicines against a variety of diseases including ulcerative colitis, fever, ailments of the gallbladder, skin infections, dysmenorrhea, amenorrhea, tumours, chest ailments and in burn treatments (Ahamad, Al-Ghadeer, Ali, Qamar, & Aljarboa, 2017). It has also been used for several decades in the perfume and incense industries. Myrrh is commercially available in many forms such as oil, resin and gums.

Previous investigations showed that myrrh is effective in oral wound repair within certain concentrations (Al-Mobeeriek, 2011). Myrrh has been shown to have many advantages. It acts as an antimicrobial and antifungal healing tonic and stimulant; it also has carminative, anti-catarthal, expectorant, diaphoretic, vulnerary, local antiseptic, immune stimulant, circulatory stimulant, anti-inflammatory, antiseptic, deodorizing, and astringent properties (Buckley & Evershed, 2001). Myrrh belongs to the family of sesquiterpenes which contain molecules have the same action mechanism as cannabinoids, a chemical that can alter neurotransmitters in the brain and improve memory impairment (Baral et al., 2015; Xu et al., 2011).

One of the advantages of myrrh is that, like many other herbal and botanical products, it is safe to humans. Myrrh has been approved by the U.S. Food and Drug Administration (FDA) (Alhejaili et al., 2019). Moreover, myrrh when ingested may benefit the systemic system. Myrrh can help in reducing cholesterol and triglycerides (Nohr, Rasmussen, & Straand, 2009). The extract of myrrh is also used as a digestive aid drug as it attenuates oxidative and inflammatory processes in acetic acid-induced ulcerative colitis (Fatani et al., 2016).

It is expected that the myrrh toothpaste used in the current study may help to reduce gingival inflammation. Myrrh as an antimicrobial agent, has been used to treat the gingival inflammation as well as stimulate the production of white blood cells (Lisa, Carac, Barbu, & Robu, 2017). A study done on *C. albicans*, a causative microorganism of candidiasis, showed that myrrh effectively inhibited the growth of these microorganisms (Orchard & van Vuuren, 2017).

Aside from being traditionally used to treat gingivitis (Shin et al., 2019), myrrh has also been used to treat oral ulceration. Previous studies on patients with Behcet's disease, myrrh was shown to induce a complete relief of pain and remarkable ulcer resolution, within a week of treatment (Albishri, 2017). It was also reported that myrrh can stimulate plasma cell production and induce angiogenesis, hence it is potentially useful in the induction of wound healing and repair (Russo et al., 2005; Young, Liu, Butler, Cohn, & Galli, 1987).

Existing oral care compositions lack an effective natural component for the maintenance and treatment of bleeding or swollen gums as well as for reducing tooth decay. Furthermore, it is apparent that oral care medicaments using natural products or compositions contribute to lower cost of production and attract the

attention of users/individuals and attain their satisfaction. Though there are numerous choices of natural ingredients, it appears that there is only a limited number of natural products that are suitable to be used in oral care, particularly gingival care and tooth decay.

The aim of the study was to formulate a toothpaste containing myrrh extract and to assess the toothpaste effect on the viability of gingival fibroblasts after 24 and 48hrs.

## MATERIALS AND METHODS

### Extraction of Myrrh

Myrrh resin (ATQANA Enterprise 2276514-P) was crushed into powder with pestle and mortar. Myrrh powder was then soaked in deionised distilled water (at a ratio of 100g myrrh to 500ml of water) at room temperature for 48hrs. Then, the watery myrrh was filtered to obtain the purified myrrh solution. Following the filtration process, the myrrh solution was placed inside centrifuge tubes and underwent a freeze-drying process for 72 hours. The freeze-dried myrrh was kept in the fridge to be used in the preparation of myrrh toothpaste.

### Preparation of Myrrh Toothpaste

Ingredients: Polymer, baking soda, xylitol, sodium lauryl sulphate (SLS) and myrrh extract (Fig. 1).

Procedure:

Sixty-five grams of baking soda were poured into a bowl. Five tablespoons of xylitol were added into the bowl. Thirty-two 32 grams of HPMC were gradually added into the mixture. Two teaspoons of myrrh extract and a few drops of sodium lauryl sulphate were poured into the mixture. All the ingredients were mixed well until the desired texture was achieved.



Figure 1: Myrrh toothpaste ingredients

## Cytotoxicity Test

### Effect of myrrh on gingival fibroblasts growth

Human Gingival Fibroblast Cells (ScienCells Research Laboratories, Inc., California, USA) were cultured in 96-well microplate by referring to the equation/calculation mentioned above. 50µl of complete FM and cells ( $2.5 \times 10^4$  cells/well) were added to each 96-well microplate. The cells were incubated at 37°C, 5% CO<sub>2</sub>, 95% O<sub>2</sub> for 24 hours. Confluent cells were then taken from the incubator and viewed under an inverted microscope to check the percentage of the confluent cells. The medium in the 96-well microplate containing confluent cells was

removed by a micropipette.

After several pilot trials of cell viability tests, it is decided to use the following myrrh concentrations of 100, 200, 300 and 400 mg/ml to assess the effect of the paste on the viability of gingival fibroblasts in the test samples. Placebo samples contain polymer only were used for treating the control samples. 100µl of each myrrh concentration were added into the divided wells. The cells were then incubated with myrrh for another 24 hours. At the end of the incubation period, the entire medium was removed from the wells using a micropipette followed by wells washing with 30µl of PBS. 100µl fibroblast medium was then added for each well. MTT cell proliferation assay was implemented according to the method described by Yazawa et al; (2005). 15µl of [3- [4,5, dimethylthiazol-2-yl]-5-[3-caboxymethoxy-phenyl]-2-[4-sulfophenyl]-2H-tetrazolium (MTT) dye solution (Cell Titer 96 Non-radioactive Cell Proliferation Assay kit, Promega, Madison, WI, USA) was added into each well followed by incubation for 4 hours. After incubation, 100µl of the stop solution was added to each well. The experiments were performed in triplicate. The optical cell density was checked using a microplate reader (BioTek, Winooski, VT, USA) (Plate 3.5) at a wavelength of 570nm. The cell cultures were assessed for their optical density at intervals of 24 and 48hrs after treatment. Percentage of cell viability was determined by using the following equation:

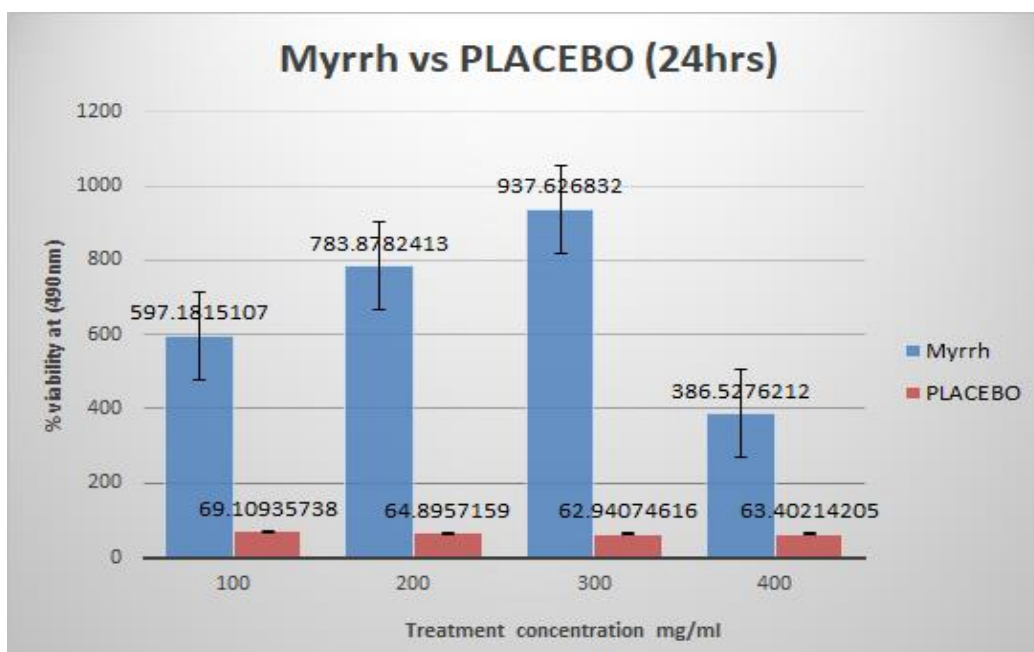
$$\text{Cell viability} = \frac{(\text{Sample} - \text{blank media})}{(\text{Control cell} - \text{blank media})} \times 100$$

IBM SPSS Statistics 27 was used to analyse the data obtained. The readings were calculated as mean values ± S.D. Outcomes of the research were subjected to Kruskal-Wallis test, to compare the number of cell growth between different concentrations of myrrh (100mg, 200mg, 300mg and 400mg), and also a Mann-Whitney test, to evaluate whether the differences between the test and control groups. *P* value of less than 0.05 were considered to be significant.

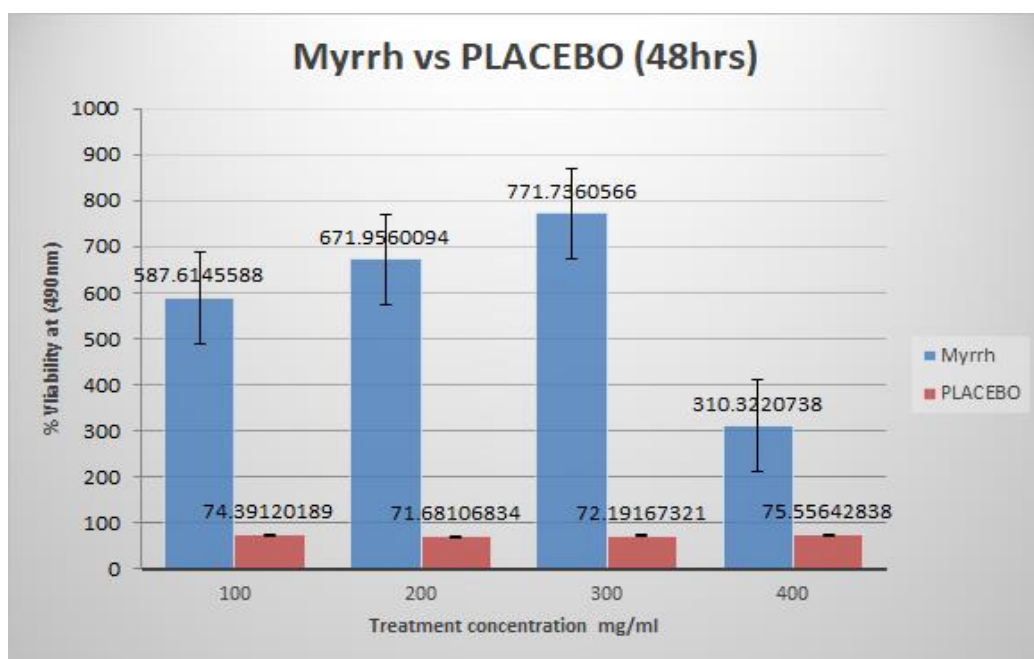
## RESULTS

The processed myrrh toothpaste had a homogeneous consistency as the extracted myrrh successfully dissolved completely with the other components. It had a suitable flowability and did not flow unless it was squeezed from the tube container.

The MTT test was performed in triplicate. The results of tests are shown in Graphs 1 and 2 for 24 and 48hrs, respectively. Based on the graphs, the results depict that myrrh pastes at 100, 200 and 300 mg/ml concentrations enhanced the growth of gingival fibroblasts after treatment for 24 and 48 hours. However, by increasing the concentration above 300mg/ml, the cells showed less growth ability. Results also showed that the highest level of cell growth at 24 hours (cell viability=938%) and 48 hours (cell viability=772%) were obtained in the samples treated with a 300 mg/ml concentration of myrrh paste.



Graph 1: MTT cytotoxicity test of myrrh toothpaste vs placebo after 24hr.



Graph 2: MTT cytotoxicity test of myrrh toothpaste vs placebo after 48hr.

Table 1, 2 and 3 show that myrrh paste has significantly affected the level of cell growth, the H-value = 21.619,  $P = 0.0001$  after 24 hrs; while after 48 hrs the H-value = 21.600,  $P = 0.0001$ . Pairwise comparisons with adjusted  $P$ -value showed that there was a significant difference in the cell growth of samples treated with 300mg/ml myrrh paste as compared to the samples treated with 100mg/ml concentration after 24 and 48hrs ( $P = 0.020$ ). However, there were no significant differences in the cell growth of samples treated with myrrh concentration 100mg/ml as compared to 200mg/ml; and between samples treated with 200mg as compared to 300mg/ml myrrh concentration after 24hrs ( $P = 0.849$ ) and 48hrs ( $P = 0.850$ ).

**Table 1: MTT analysis of myrrh toothpaste after 24 and 48hrs.**

Variables	Myrrh Paste Concentration	n	Median (IQR)	X <sup>2</sup> Statistics (df) <sup>a</sup>	P-value
MTT 24 Hours	100mg	6	0.358 (0.086)	21.619 (3)	0.000
	200mg	6	0.464 (0.010)		
	300mg	6	0.551 (0.025)		
	400mg	6	0.229 (0.065)		
MTT 48 Hours	100mg	6	0.757 (0.043)	21.600 (3)	0.000
	200mg	6	0.855 (0.047)		
	300mg	6	0.983 (0.006)		
	400mg	6	0.374 (0.109)		

<sup>a</sup> Kruskal-Wallis Test

**Table 2: Pairwise analysis of myrrh toothpaste concentrations after 24hrs.**

Pairwise Comparisons of Concentration (MTT24)					
Sample 1- Sample 2	Test Statistics	Std. Error	Std. Test Statistic	Sig.	Adj. Sig <sup>a</sup>
400mg-100mg	6.000	4.081	1.470	.141	.849
400mg-200mg	12.000	4.081	2.941	.003	.020
400mg-300mg	18.000	4.081	4.411	.000	.000
100mg-200mg	-6.000	4.081	-1.470	.141	.849
100mg-300mg	-12.000	4.081	-2.941	.003	.020
200mg-300mg	-6.000	4.081	-1.470	.141	.849

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050

<sup>a</sup> Significance values have been adjusted by the Bonferroni correction for multiple tests.

**Table 3: Pairwise analysis of myrrh toothpaste concentrations after 48hrs.**

Pairwise Comparisons of Concentration (MTT48)					
Sample 1- Sample 2	Test Statistics	Std. Error	Std. Test Statistic	Sig.	Adj. Sig <sup>a</sup>
400mg-100mg	6.000	4.082	1.470	.142	.850
400mg-200mg	12.000	4.082	2.939	.003	.020
400mg-300mg	18.000	4.082	4.409	.000	.000
100mg-200mg	-6.000	4.082	-1.470	.142	.850
100mg-300mg	-12.000	4.082	-2.939	.003	.020
200mg-300mg	-6.000	4.082	-1.470	.142	.850

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050

<sup>a</sup> Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 4 shows the result of the Mann-Whitney Test. The MTT assay readings of myrrh paste treated groups ( $Mdn_{24}=0.42685$ ,  $Mdn_{48}=0.798450$ ) were higher than the placebo groups ( $Mdn_{24}=0.045600$ ,  $Mdn_{48}=0.093650$ ) in both 24 and 48 hours. Mann-Whitney test indicated that this difference was statistically significant,  $U=0.00$ ,  $P = 0.001$ .

**Table 4: Comparing the variables of analysis between placebo and myrrh toothpaste groups.**

Variables		Median (IQR)	Z statistic <sup>a</sup>	P-value
MTT 24 hours	Myrrh (n=24)	0.42685000 (0.242675)	-5.939	0.000
	Placebo (n=24)	0.04560000 (0.046953)		
MTT 48 hours	Myrrh (n=24)	0.798450 (0.4326)	-5.939	0.000
	Placebo (n=24)	0.093650 (0.0082)		

<sup>a</sup> Mann-Whitney Test

## **DISCUSSION AND CONCLUSION**

In the current study, myrrh toothpaste was formulated following several trials on the ingredient's solubility and homogeneity. Myrrh toothpaste was subjected to cytotoxicity test to assess the effect of the paste on the viability of gingival fibroblasts. During the formulation, it was noticed that continuous stirring and nano size myrrh particles were necessary to ensure homogenous consistency of myrrh toothpaste. Very strict sterilization and isolation processes were required for the equipment, materials and chamber used in the paste formulation to prevent bacterial and fungal growth in the formulated paste.

The results of this study infer that myrrh paste at a concentration of 300 mg/ml is the optimal concentration as it induced the highest cell viability as compared to other concentrations used in the trials after 24 and 48hrs. Samples treated with 400 mg/ml concentration demonstrated less cell viability than 100, 200 and 300 mg/ml. This suggest that 400 mg/ml treated samples received an intolerable dose of myrrh paste concentration that significantly suppressed the growth rate of cells in these samples as compared to the other test samples. In the placebo samples, the cell viability remained almost constant in all concentration groups and was noticeably less than that in the test samples after 24 and 48 hrs.

The findings of this research can be utilized in future clinical investigations to ensure the safety and the effectiveness of the myrrh toothpaste in reducing plaque accumulation and subsequently gingival inflammation and caries development. The current investigation has shown that myrrh toothpaste is safe to be used at a certain adjustable concentration. Future clinical studies are recommended to evaluate the safety and effectiveness of myrrh toothpaste in animals and human and the possibilities of future usage of the myrrh-containing toothpaste in oral health care.

## **CONFLICT OF INTEREST**

The authors would like to declare that there is no conflict of interest.



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