Oil Pulling Mouthwash as a Natural Alternative to Chemical Mouthwashes: An Exploratory Study

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Contents

1. Study Objective

The objective of this study is to assess the efficacy of oral rinse products (“Study Formulations”) in human subjects for controlling oral malodor.

2. Identity of Investigational Product(s)

Breath Freshening Oral Rinse

3. Overall Study Design and Plan — Description

Prior to the start of the study, the study will be approved by Advarra Institutional Review Board. This is a randomized, double-blinded clinical trial. Sixty (60) adult volunteers, men and women, with oral malodor of at least 7.0 ± 0.5 on a hedonic scale will be enrolled. At baseline, a hedonic breath evaluation will be conducted by three blinded and calibrated organoleptic judges. Sixty subjects (30/Group A and 30/Group B) that meet the continuance criteria will be randomly assigned (Computer Generated randomization able) to group A that will receive the active product and Group B that will receive the placebo product. The product will be distributed at the baseline visit. The study duration will be three weeks consisting of three visits including, baseline, 12-hour visit and three-weeks.

A. Screening

Prior to the baseline exam, a sufficient number of subjects will be asked to sign an informed consent and pre-screened with oral malodor of at least 7.0 ± 0.5 on a hedonic scale to determine their potential enrollment into this study.

The subjects who will be invited to participate for the baseline visit were will be instructed to refrain from the following:

- intake of alcohol,
- foods containing sulfur compounds such as garlic or onion,
- scented cosmetics,
- smoking or using any tobacco products 12 hours prior to the visit,
- medicated lozenges, mints, sweets or gum that contain antimicrobial agents, including but not limited to, Xylitol, Essential Oils, Cetylpyridinium chloride, Chlorine Dioxide and Zinc 24 hours prior to the visit.
- Antibiotics
- Any other mouthwash

B. Visit 1(Baseline)

During pre-screening, the subjects will be asked to read and sign duplicate copies of the informed consent form which will be witnessed by site staff. Subjects will be given a signed copy of the informed consent form and the other copy is maintained as site source documentation.
Medical history will be updated on site source documents. Demographic information and study entry criteria will be obtained and documented on the appropriate case report form (CRF). A comprehensive oral examination to evaluate the oral and peri-oral regions, including hard and soft tissues, will be performed by a California licensed dentist, followed by oral malodor evaluation. Up to 66 qualified subjects with a range of 7.0 ± 0.5 on a hedonic scale will be randomly assigned to a treatment group. Subjects will be given a study kit containing their assigned test products. Products will be dispensed in a protected area that will ensure blinding of the organoleptic judges to the identity of the test products. Subjects will be instructed to use 10 of the mouthwash for two minutes and once per day. Subjects will be provided with both verbal and written instructions on product usage and perform their first treatment under the supervision of study personnel to ensure proper usage of the product. They will take their products home and will be instructed to use them that evening and once daily for the remainder of the study. Subjects will be reminded to bring their treatment kit to their next visits.

General comments, if any, will be recorded on the appropriate CRF.

C. Visit 2 (12 Hours)

Subjects returned to the site with the Group A/Group B kit provided to Subjects will return to the site with the Group A/Group B kit provided to them at the baseline visit. The subjects will be instructed to use the mouthwash 12 hours prior to their visit, and they will be reminded to follow the same instructions as outlined in the Screening section.

Continuance criteria will be assessed. A comprehensive oral examination to evaluate the oral and perioral regions, including hard and soft tissues, will be performed by a California licensed dentist, followed by organoleptic evaluation.

Any General Comments or Adverse Events will be recorded on the appropriate Clinical Report Forms -CRFs.
wash 12 hours prior to their visit, and they were reminded to follow the same instructions as outlined in the Screening section.

Continuance criteria were assessed. A comprehensive oral examination to evaluate the oral and perioral regions, including hard and soft tissues, was performed, followed by organoleptic evaluation.

Any General Comments or AEs were recorded on the appropriate CRFs.

D. Visit 3 (Three Weeks)

Subjects returned to the site with the Group A/Group B kit provided to them at the baseline visit. The site visually assessed the product to look for product use compliance. The subjects used the mouthwash for three weeks as instructed. Subjects will return to the site with the Group A/Group B kit provided to them at the baseline visit. The site will visually assess the product to look for product use compliance. The subjects will use the mouthwash for three weeks as instructed.

Continuance criteria will be assessed. A comprehensive oral examination to evaluate the oral and perioral regions, including hard and soft tissues, will be performed by California licensed dentist, followed by organoleptic evaluation.

Any General Comments or AEs will be recorded on the appropriate CRFs. Subject Accountability will be completed, and subjects will be dismissed from the study.

E. Subject Accountability

A Subject Accountability form was completed for each subject. All 60 subjects completed the study.

F. General Comments and Adverse Event Recording

There were no Adverse Events during this study.

4. Inclusion Criteria

In order to be included in the study, each subject must:

- Provide written informed consent prior to participation and be given a signed copy of the informed consent form.
- Be able to follow verbal and/or written instructions, perform oral hygiene procedures and return to the test facility for specified Study

examinations.

- Be between the ages of 18 and 75 years of age, male or female and racially diverse population will be included.
- Have normal oral mucosa.
- Be in good general health as determined by medical history and clinical judgment that no severe or debilitating disease exists that would impede participation in the study.
- Had an average organoleptic score of at least 7.0 ± 0.5 on a hedonic scale.

5. Exclusion Criteria

Subjects will be excluded if any of the following were present:

- Pregnant or nursing per subject report.
- Diagnosis of Xerostomia, including medication induced Xerostomia.
- Any oral or extra-oral piercing that would interfere with the ability to perform study procedures and/or clinical assessments in the mouth.
- Fixed or removable oral appliances, such as orthodontic brackets or retainer, partial or complete dentures.
- Had advanced periodontal disease or excessive gingival recession, per Investigator/Examiner discretion.
- A known allergy or sensitivity to products planned for use in this study.
- Unwillingness to abstain from all other oral hygiene products other than those prescribed for the duration of the study.
- Heavy deposits of calculus, either supragingival and/or subgingival, per Investigator/Examiner discretion.
- Had a history of severe transmissible infectious disease (hepatitis, HIV, tuberculosis).
- Had a medical or dental condition that would be unduly affected by participation in this study, per Investigator’s discretion.
- Any other condition that the Principal Investigator would consider interfering with the study.
- COVID-19 Positive
- Smokers.
- Medications, such as antibiotics and antivirals two weeks prior to participation in the study

6. Identity of Investigational Product(s)

Ingredients: The mouthwash contains MCT oil, Peppermint oil,Spearmint oil, Clove oil, Tea tree oil, Cardamon oil, Oregano oil, Fennel oil, Vitamin E, Vitamin D, Vitamin K2 & stevia. This mouthwash is in the market.

7. Product Usage

Subjects will be instructed to rinse their mouth with 10ml mouth wash once daily for two minutes. In addition, they will be instructed to continue with their normal oral hygiene practices, including tooth brushing and flossing but will be instructed not to use any other oral rinses or mouthwashes except the study mouthwash. Subjects will use an ADA approved toothpaste and toothbrush for their oral hygiene that will be provided to them during the course of the study. The subjects will also be instructed not to use any other non-study related products such as breath mints, lozenge, gums, etc. as well as refrain from elective dental procedures during the study period.

8. Blinding and Labeling

The identity of the mouthwash was disguised. Subjects will receive a kit containing 3 bottles of mouthwash, an ADA approved toothpaste and toothbrush. Product use instructions will also be included in the kit.Safety Variables

9. Safety Variables

a. Safety Observations and/or Measurements

Safety will be assessed by the absence of irreversible side effects. All The study related documents will be locked up in the cabinets and only the staff involved with the study will have assess to it. No adverse events are expected they have been minimized by sterilization of dental instruments, surface disinfection, any discomfort associated with dental examination, any non physical risks and unknown risks.

b. Confidentiality of Records

The records of your participation in this study are confidential and these records are available only to the Investigator, the sponsoring company and possibly the U.S. Food and Drug Administration (FDA), the American Dental Association and Advarra Institutional Review Board. The results of this study may be published in a scientific journal and your initials and age, but not your name, may be used.

c. Oral Examination

Assessment of the oral soft tissue will be conducted by a licensed california dentist via a visual examination of the oral cavity and perioral area utilizing a standard dental light, dental mirror, and gauze.The structures examined will include the gingiva (free and attached), hard and soft palate, oropharynx/uvula, buccal mucosa, tongue, floor of the mouth, labial mucosa, mucobuccal/mucolabial folds, lips, and perioral area. Assessment of the oral hard tissues will be conducted via a visual examination of the dentition and restorations utilizing a standard dental light, dental mirror, and air syringe. All abnormal findings will be recorded and categorized by their location; hard tissue findings will be categorized as “other.”

10. Efficacy Variables

A panel of three trained odor judges will perform organoleptic judging at each visit. Odor judges will be calibrated and standardized using a range of standard odorants sufficient to reflect the different patterns of nose receptors. In order to create a reproducible assessment, subjects will be instructed to close their mouth and breathe through their nose for two minutes. After two minutes the subject will be instructed to count out loud (from 1- 20) while the subject is counting out loud the subject is exhaling through the mouth during this time all the odor judges one at a time assess the odor intensity at approximately 10 centimeters from the subject’s mouth.

Hedonic Malodor Evaluation by three calibrated organoleptic judges that were blind was performed utilizing a nine-point hedonic scale:

1 – Most Pleasant
2 – Very Pleasant
3 – Moderately Pleasant
4 – Slightly Pleasant

5 – Neither Pleasant nor Unpleasant
6 – Slightly Unpleasant
7 – Moderately Unpleasant
8 – Very Unpleasant
9 – Most Unpleasant.

11. Results

A total of 60 subjects, 30 subjects in Group A and 30 subjects in the Group B The mean age for Group A was 40 and the mean age for group B was 46. The subjects were instructed to use the mouthwash for 2 minutes, once a day, for 3 weeks.

Demographics

Mouhtwash	Female	Male	Total	pct F
A	16	13	29	55.20%
B	18	13	31	58.10%
total	34	26	60	56.70%
pct A	47.10%	50.00%	48.30%	
gender p value=1, Fisher exact test				

Table 1A – Gender: The gender of the 60 subjects was compared between Group A and B. The p-value for comparing gender between group A and B was computed using Fisher's exact test.

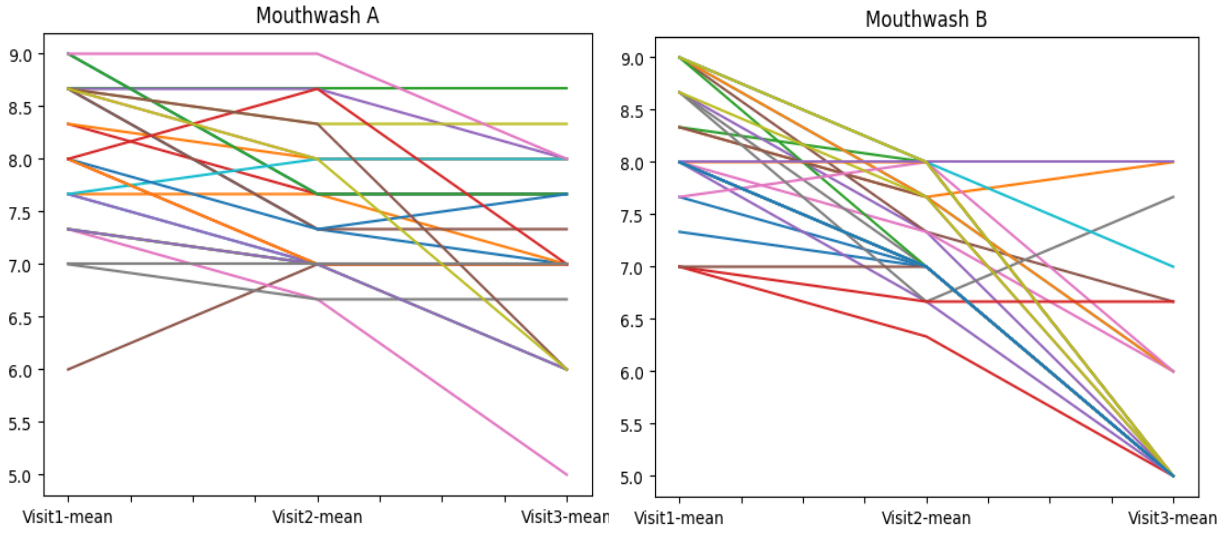
Mouhtwash	n	min	Q1	median	mean	Q3	max	SD
A	29	18	21	39	39.5	51	71	17.8
B	31	19	31.5	49	46.4	61.5	70	17.7
age p value = 0.1352, t-test								

Table 1B – Age: Ages of the 60 subjects were compared between the two mouthwash groups. The p-value for comparing age was computed with a t-test.

Mouthwash	Spanish/Hispanic /Latino	White (Caucasian)	Pacific Islander	African American	Other	Total
a	11	10	2	1	5	29
b	11	10	2	2	6	31
total	22	20	4	3	11	60
pct A	50.00%	50.00%	50.00%	33.30%	45.50%	48.30%
ethnicity p value = 1 Fisher exact test						

Table 1C – Ethnicity. The ethnicity of the 60 subjects was compared between groups A and B. The p-value for comparing ethnicity between group A and B was computed using Fisher's exact test.

Organoleptic Score Comparison between Two Groups



Graph 1 (left – Group A, and right – Group B): The mean score change from visit 1 across visits 2 and 3 was compared using a repeated measure (mixed) analysis of variance model.

Table 2 Mean Organoleptic Score Comparisons

Visit	Mouthwash A				Mouthwash B			
	n	mean	SD	SE	n	mean	SD	SE
1	29	8	0.76	0.14	31	8.2	0.62	0.111
2	29	7.57	0.67	0.125	31	7.31	0.49	0.089
3	29	7.16	0.82	0.152	31	5.58	0.97	0.174

Table 2: In Group A, the organoleptic score reduced from 8 to 7.57 after 12 hours and to 7.16 after 3 weeks. In Group B the organoleptic score reduced from 8.2 to 7.31 after 12 hours and to 5.58 after 3 weeks.

Table 3 Organoleptic score difference from visit 1 (baseline) using repeated ANOVA model.

Visit	Mouthwash A			Mouthwash B		
	n	mean	SD	n	mean	SD
2	29	-0.425	0.577	31	-0.892	0.567
3	29	-0.839	0.829	31	-2.624	1.067

A - B difference		
Visit	Mean diff	p value
2	0.467	0.0000
3	1.785	0.0000

Table 3. Significant reduction in organoleptic scores occurred in Group B after 12 hours and after 3 weeks ($p < 0.0$).

12. Conclusion

In conclusion, the active group (Group B) showed statistically significant reduction in oral malodor as compared to placebo group (Group A). According to statistical analysis, demographics such as age and ethnicity did not affect the organoleptic score difference between Group A and B. The organoleptic score between placebo and active groups demonstrates statistically significant reduction of oral malodor after 12 hours and three weeks. The p value using repeated ANOVA Model was p=0.0000.

There were no adverse events reported during the study. During the course of the study there were no abnormal conditions observed during oral exams conducted at each visit. Thus, there were no safety events experienced during the product usage for three weeks.