

# Effect of Essential Oil Mouthwash on Halitosis

RALUCA SABAU<sup>1</sup>, ALINA ORMENISAN<sup>1</sup>, ADRIANA MONEA<sup>1</sup>, MELINDA SZEKELY<sup>1</sup>, ADINA COSARCA<sup>1</sup>, TUDOR PETRU IONESCU<sup>2</sup>, GABRIELA BERESESCU<sup>1\*</sup>, CAMELIA ELENA DALAI<sup>3</sup>

<sup>1</sup> University of Medicine and Pharmacy Targu Mures, Faculty of Dental Medicine, 38 Gh. Marinescu, 540139, Targu Mures, Romania

<sup>2</sup> itu Maiorescu University of Bucharest, Faculty of Dental Medicine, 67A Gh. Petrascu, 031593, Bucharest, Romania

<sup>3</sup> University of Oradea, Faculty of Medicine and Pharmacy, 10 Piata 1 Decembrie Str., 410073, Oradea, Romania

*Halitosis is an embarrassing symptom with a significant social impact. Periodontal disease, tongue coating, interdental food impaction, and dental cavities are the predominant causative factors. The aim of this study is to evaluate the effect of essential oil mouthwash on halitosis. 30 patients aged between 16-25 were enrolled in this single-blind, parallel-group study. Informed consent was obtained. The following parameters were recorded: gingival index, plaque index, organoleptic breath assessment, and BANA test from tongue coating samples prior to treatment with essential oil mouthwash (group 1) or a placebo (group 2) at baseline and 28 days after the start of treatment. Histological examinations were performed from gingival tissue. There were significant differences ( $p < 0.05$ ) in plaque and gingival index after treatment with essential oil mouthwash compared to the placebo. The results provide a statistically significantly greater level of efficacy in controlling established plaque and gingival index after use of essential oil mouthwash. Therefore, it can be recommended in halitosis therapy.*

**Keywords:** halitosis, essential oil, histology plaque index, organoleptic breath, BANA test

Halitosis or bad breath is an embarrassing symptoms affecting millions of people all over the world. Each year professionals have struggled to deal this aspects, most of the time they came at a lost [1].

The term halitosis comes from Latine *halitus osis* meaning the pathologically modified air breath out [2]. The symptoms as a multifactorial ethology, however the most important cause is considered to be the decomposition of organic residues by the oral microorganisms. The first study on halitosis appeared in 1874 and in 1934 osmoscop was created that is an instrument which measures the density of small within the oral cavity in a semi-quantitative and subjective manner. In the 70s the main causes of halitosis was discovered as representing the sulphuric volatile components (VSC) and in the year to come other instruments were devised by means of which direct identifications of VSC was possible (hydrogen-sulphuric, methan-ethiol and dimethyl-sulphuric).

The organoleptical method is another way of measuring halitosis. It is a highly subjective method with very good quality results, but very poor quantity-wise since it mainly depends on the olphactiv acuity of the examiner. This is the main reason why objective instruments had to be developed, at the moment halimeter and BANA test being the most widely used [1].

Whenever halitosis is diagnosed it is necessary to be differentiating it from false halitosis (halitophobia) or a transitional halitosis caused by diet or dehydration. Bad breath can also be cause by NMY or respiratory diseases, digestive diseases such as GER or NS or hepatic cirosis. However, 90% of the halitosis cases have the oral cavity as starting point [3].

Halitosis can be caused by the pathology of the oral cavity such as dental caries, cauting tongue, expose dental pulp, food impaction, periodontal diseases, alterations or oral cancers. The main factor in halitosis is bacterian as Cocci and Gram-negative anaerob bacily have VSC as final metabolic products [1].

Halitosis is a condition with a high social impact, eighteen million people suffering from it over to million dollars being spent annually on products attempting to disguise the embarrassing small [2].

The aim of this study is to extend research on the effect of various mouthwashes on halitosis and also on periodontal parameters, since most of the time halitosis is connected to periodontal disease. The paper also aims at evidencing the possibilities of using BANA test as means of diagnosis in halitosis and of early stages of periodontal disease.

## Experimental part

30 patients aged between 16-25 were enrolled in the study. All of them had corresponding oral hygiene with more than 10% of the site showed plaque index 1 and gingival index 1, all of them with halitosis. None of the patients had used antibiotics for the previous four weeks, were not subjected to orthodontic treatment and did not showed any systemic diseases at the moment of the investigations. None of the patients was smoker or presenting scrotal tongue. Informed consent was obtained after clear explanation of the stages of the study and its aims. The 30 patients were randomly divided in 2 groups of 15 subjects: control group and test group.

The oral cavity was assessed by the same calibrated examiner mainly focusing on the periodontal parameters. These parameters were measured before treatment and 28 days after the start of it. Each tooth was examined for presence or absence of bacterian plaque and gingival bleeding. The vestibular site of the first upper molars, lingual site of the first lower molars and vestibular site of the left and right upper central incisors were examined.

The presence or absence of plaque was evaluated with plaque index (O'Leary et al.1972) [5]. Gingival inflammation was assessed with gingival index (Loe and Silness, 1963) [6]. Halitosis was subjectively assessed by the subjects and objectively by the examiner both organoleptically and using BANA tests. Breath odour examinations were carried out objectively and subjectively.

\* email: gabriela.beresescu@gmail.com; Phone: 0745134184

The patients was instructed not to use onion, garlic, spicy food, alcohol, or smoke 48 h prior to examination, and not to use chewing gum, mouthwash or any other substance that could disguise halitosis prior to examination.

Objective examination (ORGO) was carried out by the same calibrated dental examiner so that the subjective perceptions would not modified the values in the test. The examiner had a normal sense of smell and did not intake alcohol, coffee, tea or cigarettes prior to examination. Perfumed cosmetics were avoided. The examination took place in the dental medicine surgery and the subjects were instructed to keep the mouth completed close for 3 min while breathing through the nose only. After 3 min they were intrusted to slowly release the air through the mouth and 10 cm from the nose of the examiner who graded the smell on a scale from 0 to 5.

Subjective examination (ORGS) was carried out by the patients before 28 days after the treatment. Each patient was instructed to keep the mouth completed close for 3 min, after which to lick their wrist after drying to grade the odour on a scale from 0 to 5 [7]:

Scor 0 – no odour. Odour not detectable.

Scor 1 – slight odour. Slight detectable odour, but not characteristic to halitosis.

Scor 2 – detectable odour. Detectable odour close to halitosis.

Scor 3 – moderate odour. Detectable odour easily recognisable, characteristic for halitosis.

Scor 4 – strong odour. Strong odour that can be tolerated by the examiner.

Scor 5 – severe odour. Sever odour that cannot be tolerated by the examiner.

BANA tests(N-benzoyl-DL-arginine naphthylamide), describe by Loesche et al. [8], is a rapid test for evaluation of non-sufuros compounds. BANA tests were used to evidence the three bacterian species, *Porphyromonas gingivalis*, *Tannarella forsythia*, *Treponema denticola*, which can be felt responsible for halitosis because of their proteolotic activity. These microorganisms release an enzyme able to hydrolyze the synthetic peptide benzoyl-DL-arginine-naphthylamide (BANA) on the test band. When either or all three species are present on the sample from the dorsal side of the tongue, BANA is hydrolyze, the test reads positive and the index became blue. The bluer the color, the higher the bacterian concentration. As BANA test are sensitive to light and humidity, they will be taken out the box only prior to use and the box be seal back. The upper part of the band provides place for name and data examination.

The samples from the dorsal site of the tongue was taken on a cotton swab then place on the marked lower part of the BANA band. After placing it, the upper part of the test which is pink-orange is dampened with distilled water by means of sterile swab. Care should be taken not to used too much water since it can dilute the reactive and induce a false negative response. The test is then folded on the dotted area so that the side containing the reactive will came in contact with the side with the sample from the tongue. The folded test is next introduced in a incubator in 55°C for 5 min, after which is it taken out, cut on the dotted line and the upper part of the test, with the reactive is examined. The results were logged in accordance with the blue zones as per manufactures instruction. The card contains three codes: Negative – no color changes; Slight positive – light blue dots randomly spread on the band; Positive – dark blue patches on light brown test band.

The biopsy cores collected were submitted for histological examination. The histological examination

was carried out according to a specific protocol: fixation in Lille neutral formaldehyde for 5 days, dehydration in consecutive solutions of alcohol, immersion in xylene, inclusion in paraffin, sectioning, staining with Hematoxiline-Eosine and examination under optic microscope at different magnifications.

The control mouthwash was distilled water for placebo, and the test mouthwash had the following recipe: distilled water, essential oil of lemon, essential oil of meant, Natrium dicarbonate .

After examination of the subjects, registering the organoleptic scores and the BANA test results, the patients were examined and instructed on the correct techniques of the brushing and the cleaning the mouth. The subjects received the same type of tooth paste and the same amount of mouthwash according to the group: control group – placebo mouthwash and test group – test mouthwash. The mouthwash was used twice daily for 28 days.

## Results and discussions

Halitosis represents a condition largely spread all over the word and numerous attempts have been made to eliminate it. The present paper evidences the effect of essential oil mouthwash on halitosis, plaque index, bleeding on probing index, bacterian concentration and organoleptic examination carried out by both examiner and subject.

The initial results showed significant changes after treatment, with a dramatic difference in the test group regarding the plaque index, bleeding on probing, gingival index, BANA test results and organoleptic examinations values.

The plaque index showed significantly higher changes in the test group as compared with the control group. Prior the treatment, the control group (fig.1) had an average plaque index in all subjects of 1.333, and after treatment it decreased to 1.211, which represents merely 9.25% ( $p < 0.05$ ). The difference could be due to the higher attention to oral hygiene during the study. The test group (fig.2) showed a decrease in the plaque index of 31.5%, from 1.388 before treatment to 0.966 after the treatment ( $p < 0.05$ ).

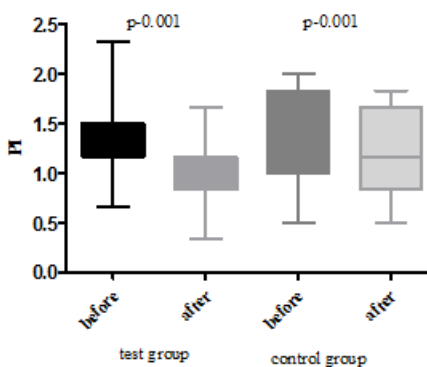


Fig. 1. Plaque index before and after treatment in both group (test group and control group)

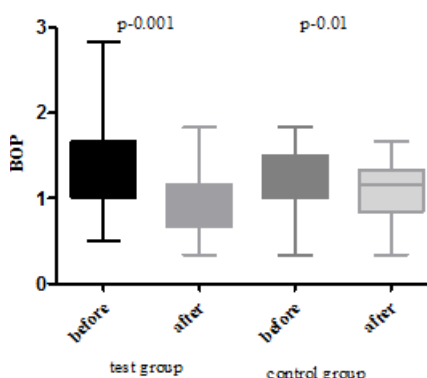


Fig. 2. BOP before and after treatment in both groups (test group and control group)

Bleeding on probing showed positive results after treatment in the control group with a difference of 6.02% compared to baseline and a difference of 34.49% compared to the baseline in the test group. Our observations demonstrate the adjuvant effect of antimicrobial mouthwash with essential oils combined with correct brushing on adequate oral health with PI and BOP of 0 or 1 (fig 2).

BANA test evidenced three anaerobe bacteriae acting on the periodontium and bad breath. In the control group the results of the test decreased by 4.38% while in the test group the decrease was more significant of 38.09% (fig 3).

Organolectical scores, both from the subjects and examiner, are subjective which explains the differences in assessing an odour of the same intensity.

After treatment, the patients assessment of their own halitosis changes, especially in the test group. Four patients in the control group considered their breath is less smelly after placebo with a difference of 9.52% before and after treatment. In the control group, 13 of the 15 patients reported an improvement of their breath after using the mouthwash with the difference 36.84% (fig 4).

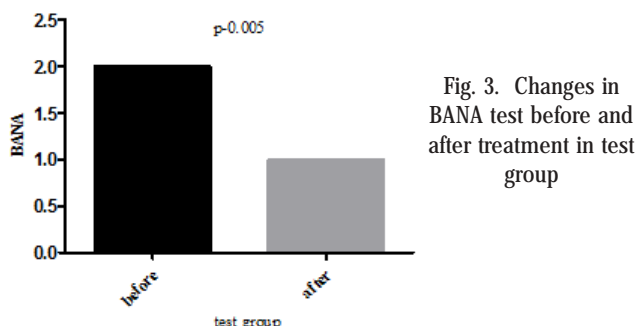


Fig. 3. Changes in BANA test before and after treatment in test group

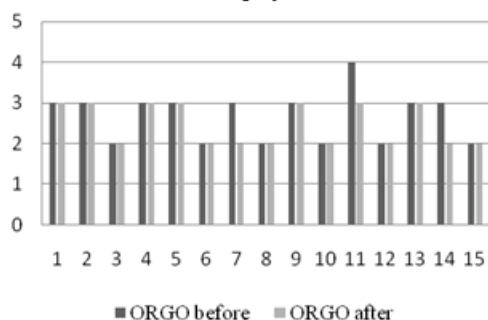


Fig. 4. Changes ORGO in the control group

As for the objective organoleptic examination it also showed an improvement in the subject's breath, more dramatic in the test group. After 28 days the scores in the control group decreased by 7.50% and in the test group by 45.95% (fig 5).

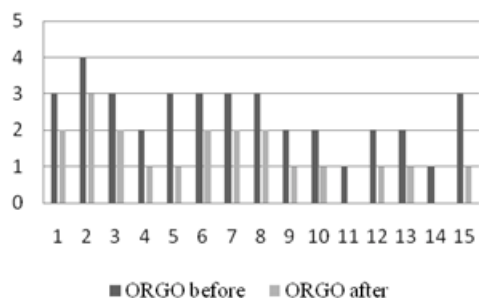


Fig. 5. Changes ORGO in the test group

The present study evaluated the effect of essential oil mouthwash on halitosis using de BANA test and organoleptic examination.

Halitosis is an embarrassing symptom with a significant social impact. Periodontal disease, tongue coating, interdental food impaction, dental cavities are the predominant causative factors.

Halitosis is brought about by the action of bacteria on food debris and shed epithelial cells, which turn releases volatile sulphur compounds.

BANA test is efficient in the periodontal diagnosis. The test detect the *red complex species* [8].

In the present study, BANA test showed significant associations in all periodontal parameters. Bleeding on probing, gingival and plaque index were found to be significant with BANA. Puscasu et al. [9] observed a statistical correlation with the severity of periodontal status and BANA. DeBoever [1] and Bosy demonstrated that tongue coating samples were positive for BANA test and the tongue coating of individuals with high

Asokan et al. [10] investigated the effect of oil pulling with sesame oil on halitosis and the microorganism responsible for it. Their study showed equally effective like chlorhexidine against halitosis. The finding of present study was similar regarding the beneficial effects of essential oil mouthwash on the clinical and microbiological parameters of periodontal inflammation.

Other studies on antibacterial properties of the essential oil obtained similar results showing an improvement of 26% in the test group scores as compared with the control group scores [11].

The association of essential oil [12] with mouthwash has shown to be effective antiplaque agent. Ross et al. [13] showed that essential oils annihilate microorganisms in the presence of serum.

The results of our study provides a statistically significantly greater level of efficacy in controlling established halitosis, plaque and gingival index after use the essential oil mouthwash.

Ultimately, with more research in the future, it will be possible to arrive at better diagnostic tools along with improved treatments and treatment options.

## Conclusions

The evaluation of the results confirmed the work hypothesis that is the essential oil have a benefic clinical effect on halitosis and periodontal parameters. The present study contain practical implication and can be used whenever patients ask for help with their breath bad.

The present study improve by using a larger of number of patient and a longer a period of treatment and also by including antibacterial substances with benefic effect on oral halitosis.

**Acknowledgement:** This paper was partially sustained by the project contract number 912/2015 financed by S.C.OPTOMED SRL in collaboration with University of Medicine and Pharmacy of Targu Mures and by the project contract number 1262/2015 financed by SC ANDSER Medica SRL in collaboration with University of Medicine and Pharmacy of Targu Mures

## References

1. DE BOEVER EH, LOESCHE WJ: Assessing the contribution of anaerobic microflora of the tongue to oral malodor. J Am Dent Assoc. 1995; 126(10):1384-93.
2. ONGOLE R, SHENOY N: Halitosis: Much beyond oral malodor, Kathmandu University Medical Journal, 2010, Vol. 8, No. 2, Issue 30, 269-275.
3. MENINGAUD JP, BADOE, FAVRE E., BERTRAND JC: Halitosis in 1999. Rev Stomatol Chir Maxillofac, 1999:100:240-4.
4. ADA Council on Scientific Affairs. Oral malodor. J Am Dent Assoc. 2003; 134(2):209-14.
5. O'LEARY TJ, DRAKE, NAYLOR JE: The plaque control record, J Periodontol 43, 1972 38.

6. LOE H, SILNESS J: Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand*, 1963, 21: 533-551.
7. MIYAZAKI H, ARAO M, OKAMURA K, KAWAGUCHI Y, TOYOFUKU A, HOSHI K, YAEAGAKI K: Tentative classification of halitosis and its treatment needs, *Niigata Dent J*, 1999, 32: 7-11.
8. LOESCHE WJ, GIORDANO J, HUJOEL PP: The utility of the BANA test for monitoring anaerobic infections due to spirochetes (*Treponema denticola*) in periodontal disease, *J of Dental Research*, 1990, 69(10): 1696-1702.
9. PUSCASU CG, DUMITRIU AS, DUMITRIU HT: The significance of BANA test in diagnosis of certain forms of periodontal disease, *J of Oral Health and Dental Management*, 2006, 5(3): 31.
10. ASOKAN S, KIMAR S, EMMADI P, RAGHURAMAN R, SIVAKUMAR N: Effect of oil pulling on halitosis and microorganisms causing halitosis: A randomized controlled pilot trial, *J of Ind Soc of Pedod and Prev Den*, 2011, 2(29): 90-95.
11. CHARLES C: Increasing antiplaque/antigingivitis efficacy of an essential oil mouthrinse over time: an in vivo study, *General Dentistry* 2013, Jan/Feb; 23-28.
12. GALUSCAN A, JUMANCA D, VASILE L, PODARIU AC, ARDELEAN L, RUSU LC, Chemical Antibacterial Inhibitors used in Toothpaste, *REV. CHIM. (Bucharest)*, 2012, 63(7): 707-710.
13. ROSS NM, CHARLES CH, DILLS SS: Long-term effects of Listerine antiseptic on dental plaque and gingivitis. *J Clin Dent*, 1989, 1(4): 92-5

---

Manuscript received: 3.06.2016