

## Effect of *Burahol* [*Stelechocarpus burahol* (Blume) Hook.f. & Thomson] fruits extract mouthwash on mouth bad deodorization

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*Burahol* [*Stelechocarpus burahol* (Blume) Hook.f. & Thomson] fruit is well known as food and used traditionally as bad deodorization. The objective of this study was to examine mouth bad deodorization activity of *burahol* mouthwash. Firstly, methyl mercaptan capturing activity of the *burahol* fruit extracts was evaluated using gas chromatography. The antibacterial activity against *Porphyromonas gingivalis*, a volatile sulfide compound (VSC)-producing Gram-negative anaerobic bacterium was tested. The mouthwash mouth bad deodorization activity was performed with cross over design in healthy subjects, randomized controlled trial. Subjects were treated by the *burahol* mouth wash, as positive control was used commercial mouth wash containing green tea extract. The *burahol* fruits extract captured 97.5% methyl mercaptan. The *burahol* mouthwash demonstrated no antibacterial activity against *P. gingivalis*. The treatment of the *burahol* mouthwash on the subjects reduced the volatile sulfur compounds (VSCs) concentration. The *burahol* mouthwash mouth bad deodorization activity is not different statistically compared with that positive control ( $P < 0.05$ ). This study showed that *S. burahol* fruits demonstrated the mouth bad odor reducing activity.

**Keywords:** *Burahol*, Mouth bad odor, *Stelechocarpus burahol*, Volatile sulfur compounds (VSCs).

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*Burahol* [*Stelechocarpus burahol* (Blume) Hook.f. & Thomson] is distributed from Java and Malaya. The leaves of *burahol* have been traditionally used in Indonesia as anti-gout, diuretic, and renal inflammation<sup>1,2</sup>. The fruits were traditionally used by royal family in Central Java, especially in Yogyakarta Palace for removal bad odor and oral deodorant<sup>2</sup>. The previous study reported that *burahol* fruit can eliminate methyl mercaptan and ammonia in feces *in vitro* and *in vivo*<sup>3</sup>. Oral malodor, also called halitosis or bad breath, is a common problem in human. Mouth odor mostly originated from food metabolism by Gram-negative oral anaerobic bacteria. Volatile sulfur compounds (VSCs) such as hydrogen sulfide, methyl mercaptan and dimethyl sulfide are the major contributor to halitosis<sup>4</sup>. Several studies have proven some food and botanical extracts are used on the reduction effects on oral malodor<sup>5-8</sup>. This research studied the standardized ethanolic extract of

*burahol* fruits and prepared as mouthwash. The antibacterial activity of the mouthwash was tested against *Porphyromonas gingivalis*, *in vitro* methyl mercaptan capturing activity was determined by gas chromatography, and to confirm the mouth bad deodorization activity, the mouthwash was examined in healthy volunteers.

### Materials and methods

#### Plant material

*Burahol* fruits were obtained from Magelang, Central Java, Indonesia. The materials were determined by Herbarium Bogorienses, Cibinong, Indonesia. The voucher specimen was deposited in Herbarium of Pharmacognosy Laboratory, Faculty of Pharmacy, Universitas Indonesia.

#### Preparation of extracts

Fresh fruits of *burahol* were ground and macerated using 96 % ethanol for 24 hrs, and the maceration was repeated two times. The extract was filtered and was

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concentrated under reduce pressure using rotary vacuum evaporator at 50 °C, to give ethanolic extract. The extract was dispersed in warm water, and then partitioned successively with n-hexane, ethyl acetate, and *butanol*. The organic layers were concentrated under reduce pressure to give hexane, ethyl acetate and butanol extract. The aqueous layer was dried using freeze-dryer, and then dissolved subsequently with methanol and water. The extracts were concentrated using rotary vacuum evaporator and freeze-dryer, respectively, to methanol and water extracts.

### Characterization of the ethanolic extract

Determination of standard parameters for the extract was performed through the standard official procedure<sup>9</sup>. It was including organoleptic appearance, water content, total ash, water-soluble extractive, ethanol soluble extractive, and loss on drying. Preliminary phytochemical analysis was performed through standard official procedure. Total phenol and flavonoid phenol content were also determined.

### Total phenolic content

The total phenolic content of the extract was determined by using the Folin-Ciocalteu's reagent as described by Hossain *et al.*, 2013 with some modification. Briefly, 100 µl extract solution in ethanol was added 3 ml distilled water, mixed thoroughly with Folin-Ciocalteu reagent (100 µl) for 1 min. Sodium carbonate (300 µL, 15 %) was added, and the volume was adjusted to 5 mL with distilled water. The mixture was allowed to stand for 2 hrs, and the absorbance was measured at 760 nm at Shimadzu UV-Vis spectrophotometer (Japan). The total phenolic content was calculated from the calibration curve, and was expressed as mg gallic acid equivalents (GAE)/gm of samples<sup>10</sup>.

### Total flavonoid content

The total flavonoid content (TFC) of the extract was determined using the aluminium chloride assay through colorimetric method with modification<sup>11</sup>. Briefly, 50 µL of extract solutions were mixed with 2mL of distilled water, then was added 0.15 mL of 5 % sodium nitrite solution; and 0.15 ml of 10 % AlCl<sub>3</sub> solution and incubated for 5 min. After incubation, the mixture was allowed 6 min, followed by the addition of 2 ml of 5 % NaOH solution and volume was made upto 5ml with distilled water. The mixture

was allowed for 15 min, and the absorbance was measured at 510 nm. The TFC was expressed in mg of quercetin equivalents per gram of extract. All the measurements were carried out three times<sup>12</sup>.

### Methyl mercaptan capturing activity

Methyl mercaptan is a one of VSCs component that has been associated with halitosis. The analysis *in vitro* methyl mercaptan capturing activity was performed according to the method of Negishi *et al.* (2004)<sup>7</sup>. Briefly, apple powder (10 mg) and 100 mg samples were mixed thoroughly in 2.0 mL water, then was added 100 µL methyl mercaptan suspension (0.1 %), and was shaken for 3 min. The residue of methyl mercaptan was determined with GC-14B (Shimadzu, Japan) equipped with an FID detector (250 °C) and a glass column (PPE 5 ring 10 %, 3.2 mm x 3.1 m). Volume headspace gas was 5 ml, and Helium gas (60 ml/min) was used as a carrier gas.

### Preparation of *burahol* fruit extract mouthwash

The ingredients of mouthwash were purchased from local supplier. The mouthwash formulation consists of the *burahol* fruit extract, menthol, malic acid, sodium benzoate, isomalt, sorbitol, glycerine and water. Final concentration of the extract in the mouthwash was 3.2 %.

### Antibacterial activity

The antimicrobial activity included the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) with dilution method. The mouthwash containing the *burahol* extract and commercial mouthwash containing green tea extract (positive control) were diluted in various concentration with Brucella broth media. The samples were added to Brucella broth media containing bacteria, *Porphyromonas gingivalis* ATCC 3327. The mixture was incubated anaerobically at 37 °C for 72 hrs. The MIC was recorded as the lowest of the sample to inhibit growth of the bacteria. To confirm the MBC, after incubation the samples were transferred to plate of blood Brucella agar + kanamycin.

### Mouth bad deodorization

The study was approved by Ethics Committee of Faculty of Dentistry, Universitas Indonesia (No: 92/Ethical Clearance/FKG UI/IX/2013). A crossover design, randomized controlled trial, conducted at the healthy students of Faculty of Dentistry, Universitas

Indonesia. The experiment was performed on subjects ( $n = 30$ ) were selected students which comply with inclusion criteria.

### Study protocol

The study protocol was performed according to the method Fukui *et al.* (2008)<sup>13</sup>, with some modifications. A crossover experiment was carried out: tooth brushing, breakfast, positive control, and mouthwash of *burahol* fruit extract. Three days' washout period was employed.

As baseline organoleptic test score, and the VSCs (hydrogen sulfide, methyl mercaptan and dimethyl sulfide) level was determined using GC-SCS (Oralchroma®, Japan) at 5AM after weak without tooth brushing and mouthwash. After breakfast at 7 AM, the subjects were instructed to take 10 mL of *burahol* fruit extract mouthwash or commercial mouthwash containing green tea extract for 30 seconds. At 9 AM, organoleptic score and the level of VSCs were determined using GC-SCS. The subjects were instructed to take meal at 12 AM, and then have the positive control or mouthwash of *burahol* fruit extract. The level of VSCs and organoleptic score were measured at 3 PM. Organoleptic test scores were estimated by 1 evaluator using scale 0-5 (0, absence of odor; 1, questionable odor; 2, slight malodor; 3, moderate malodor; 4, strong malodor; 5, severe malodor)<sup>14</sup>.

### Statistics analysis

The data were presented in terms of mean and standard deviation. The statistical significance was evaluated by Wilcoxon and paired t-test. Differences at  $p$  value of less than 0.05 was considered statistically significant.

## Results

### Characterization of the extract

The results of physicochemical identification showed that the extract still rich in water (21.7 %). Total ash was 5.05 %. The residue of solvent was very low in this study the residue of ethanol was under the limit of quantitation. The phytochemical screening the extract was detected containing: steroid, terpenoid, saponin flavonoid and tannin. In this study, the total phenolic compounds and total flavonoids in the ethanolic extracts of *burahol* fruits are 39.71 mg/gm GAE and 30.53 mg/gm QCTE, respectively.

### Antibacterial activity

The *burahol* fruit extract mouth wash showed MIC of 50 % (v/v), but did not show MBC until the highest concentration. Whereas mouthwash containing green tea extract has showed antibacterial activity with MIC and MBC at 6.25 % (v/v). Based on these results *burahol* fruit extract mouthwash did not demonstrate antibacterial activity against *P. gingivalis*.

### Methyl mercaptan capturing activity

Deodorization activities of ethanolic extract of the *burahol* fruits and the fractions of the extract are shown in the Fig. 1. The ethanolic extract was very effective in removing methyl mercaptan, whereas butanol and ethylacetate extract demonstrated strong activity to eliminate of methyl mercaptan, at 250 ppm, these extracts can capture 96.07 % and 72.02 % methyl mercaptan, respectively.

### Mouth bad deodorization

The results of organoleptic score evaluation before and after treatment with the *burahol* mouthwash and positive control are presented in Fig. 2. Treatment of *burahol* mouth wash and positive control reduced significantly mouth bad odor before treatment and 2hrs after lunch, based on organoleptic score ( $p < 0.001$ ). This evaluation is a subjective procedure,

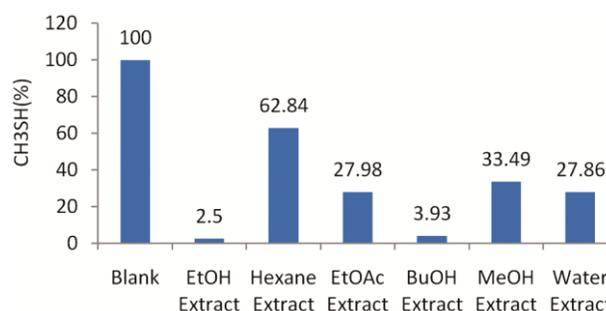


Fig. 1—Methyl mercaptan capturing activity of extracts from *Burahol* fruits.

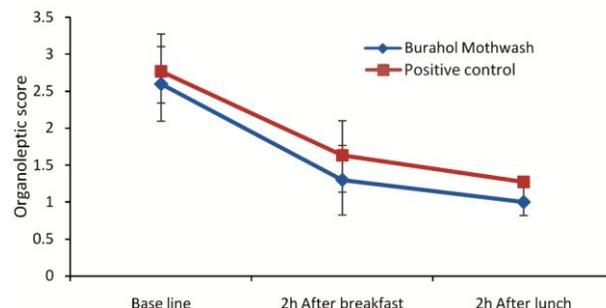


Fig.2—Organoleptic score before and after treatment with mouthwash containing *Burahol* fruits extract and positive control.

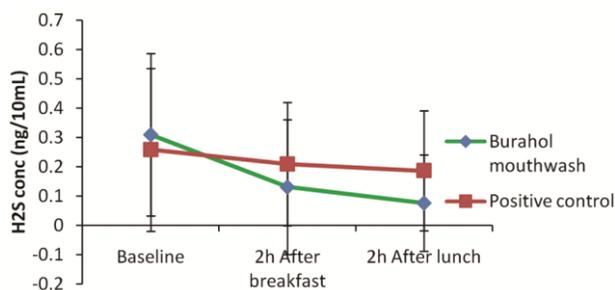


Fig.3—Effect of mouthwash containing *Burahol* and positive on hydrogen sulfide level.

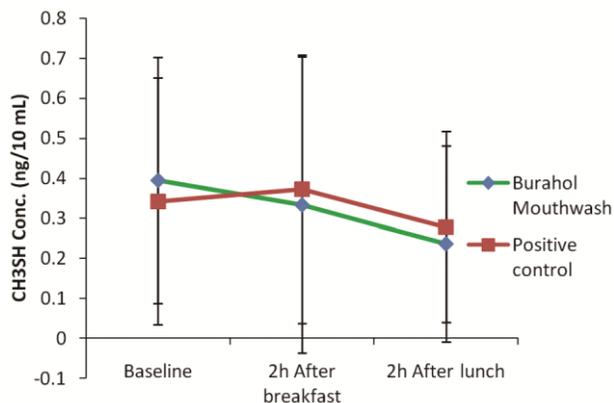


Fig. 4—Effect of mouthwash *Burahol* fruit extract and positive control on methyl mercaptan level.

but it can describe the human olfactory perception on mouth bad odor. In addition, the extract of *burahol* fruits have good odor, so it can give masking effect. However, the *burahol* mouth wash showed no significant different in mouth bad odor activity compared to that positive control ( $P < 0.05$ ).

The effect of *burahol* mouthwash and positive control on  $H_2S$  level is presented in Fig. 3. Treatment of *burahol* mouthwash 2hrs after breakfast and 2hrs after lunch reduced  $H_2S$  level if compared to base line, whereas positive control demonstrated low effect in reducing  $H_2S$  level ( $p < 0.05$ ). There was no significant difference effect between *burahol* mouthwash group and positive control group in reducing hydrogen sulfide. Fig. 4 shows *in vivo* evaluation of *burahol* mouthwash fruits extract and positive control on reducing methyl mercaptan level. Treatment of *burahol* mouthwash reduced significantly the level of methyl mercaptan ( $P < 0.05$ ). In this study, *burahol* mouthwash was observed higher activity than that of positive control on reducing methyl mercaptan level. However, these differences were not statistically significant.

Fig. 5 shows dimethyl sulfide level before and after treatment with *burahol* mouthwash. The

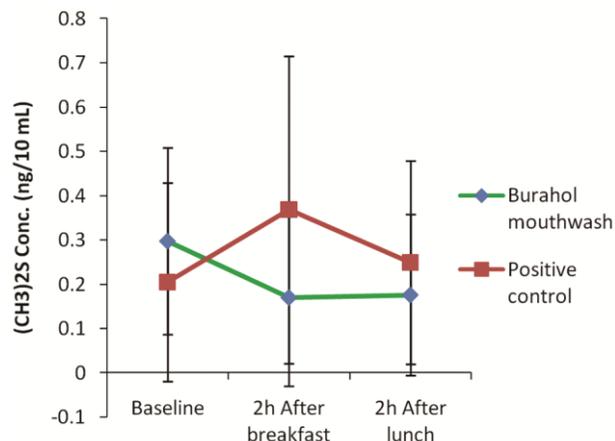


Fig. 5—Effect of *Burahol* mouthwash and positive control on dimethyl sulfide level

concentration of dimethyl sulfide was stable before after treatment with the *burahol* mouthwash. The similar phenomenon was shown by treatment of positive control. Treatment of *burahol* mouthwash and positive control did not show significant effect on reducing dimethyl sulfide level ( $p = 0.084$  and  $0.674$ , respectively).

## Discussion

*Stelechocarpus burahol* (Bl.) Hook. F& Th. belongs to Annonaceae family is indigenous to Java, Indonesia and very useful plant. The fruit is used as deodorant, whereas the leaves have various biological properties<sup>12</sup>. Currently, this plant is included in the rare and endangered plant. Because, the plant is less common cultivated in Java, due to does not have economic value. Maceration of *burahol* fruits gave thick brown with good odor. Total flavonoid content of the fruits extract was lower than that leaves extract of *burahol*<sup>15</sup>.

The antibacterial activity assay of the mouthwash was conducted against *P. gingivalis*. This is anaerobic Gram-negative oral bacteria and play role on the degradation of food, especially amino acid containing sulfide to give VSCs such as methyl mercaptan and hydrogen sulfide<sup>16</sup>. One approach to reduce oral malodor is inhibition of anaerobic Gram-negative bacteria. Betel (*Piper betle*) leaves which are used to treat halitosis, can reduce the volatility of methyl mercaptan<sup>17</sup>. Phenolic compound, allyl pyrocatechol from betel leaves showed promising antibacterial activity against obligate oral anaerobes responsible for halitosis<sup>18</sup>. Other phenolic compounds, such as catechin and resveratrol from tea and licorice inhibit anaerobic oral bacteria and reduce malodor<sup>19</sup>.

In this study, *burahol* mouthwash no showed antibacterial activity.

Halitosis or oral malodor derives from the proteolytic activity of anaerobic Gram-negative oral bacteria<sup>20</sup>. These bacteria degrade amino acid containing sulfur such as cysteine, cystine, and methionine to produce volatile sulfur compounds (VSCs) mainly hydrogen sulfide (H<sub>2</sub>S), methyl mercaptan (CH<sub>3</sub>SH) and dimethyl sulfide ((CH<sub>3</sub>)<sub>2</sub>S). Although more than 200 volatile compounds are found in human breath, only VSCs have been found to have a good correlation between concentration and organoleptic scores<sup>13</sup>. No other compounds have been reported to be correlated with organoleptic values. Furthermore, VSCs involve very strong and unpleasant malodor. In this study, the parameter of mouth bad odor was evaluated based on organoleptic score and the VSCs level, such as methyl mercaptan, hydrogen sulfide, and dimethyl sulfide.

Phenolic compound, such as caffeoylquinic acid derivate demonstrated methyl mercaptan capturing activity through conjugation reaction<sup>7</sup>. Variiegatic acid isolated from *Boletus subveltipes* showed methyl mercaptan removal activity as high as that of (-)-epigallocatechingallate. This compound has been reported to demonstrate highest deodorization activity among tea catechins. Variiegatic acid was oxidized by polyphenol oxidase to form quinone compound and bind to methylmercaptan<sup>21</sup>. Takagaki *et al.* (2015) reported that peroxidase promotes oxidase of polyphenolic compound in *Rubus suavissimus* extract. Then, the oxidized polyphenolic compounds react with methyl mercaptan. This is confirmed by the deodorization activity of chewing gum containing *R. suavissimus* extract with peroxidase was greater than that only the extract<sup>22</sup>. In this study, addition of PPs accelerated the reaction if compared without PPs (data was not showed).

Organoleptic score evaluation is a subjective procedure, but it can describe the human olfactory perception on mouth bad odor. In addition, the extract of *burahol* fruits have good odor, so it can give masking effect. Halitosis is caused mainly by VSCs such as hydrogen sulfide, methyl mercaptan, and dimethyl sulfide. To measure bad odor objectively is via determination of odor-causing compounds concentration directly. Oralchroma ® is one method to determine the level of VSCs. This method is used instead of the GC-FPD procedure, or organoleptic measurement for the assessment of oral bad odor.

The accuracy and precision of this method are similar with GC-FPD<sup>23</sup>. In this study commercial mouthwash containing green tea extract was used as positive control. Green tea is important source of polyphenols, including epigallocatechingallate (EGCG). The previous study reported that green tea extract removed H<sub>2</sub>S<sup>24</sup>. Green tea extract was also reported very effective in reducing malodor because of its disinfectant and deodorant activities<sup>6</sup>. In this experiment treatment of *Burahol* mouthwash showed no significant different activity in reducing H<sub>2</sub>S level if compared with positive control. The oral deodorant activity of *burahol* fruits *in vitro* and *in vivo* have been reported<sup>3</sup>. The fruits were reported to absorb methyl mercaptan and ammonia in feces *in vitro* and volatile nitrogenous compound from gastrointestinal tract and feces. The fruit can reduce body bad odor through absorption of methyl mercaptan, trimethylamin and phenolic compounds by polymeric compounds. *Burahol* fruits also increased the population of *Bifidobacterium* sp in feces<sup>3</sup>. The results of this study confirmed traditional use of *burahol* fruit for mouth bad deodorization. Further research is needed to isolate the bioactive compound and to elucidate the mechanism of bioactive in elimination of mouth bad odor.

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