Indonesian Kepel Fruit (*Stelechocarpus burahol*) as Oral Deodorant

H.S. Darusman, M. Rahminiwati, S. Sadiah, B. Batubara, L.K. Darusman and T. Mitsunaga

1Department of Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University, Indonesia
2Biopharmaca Research Center, Bogor Agricultural University, Jl Taman Kencana No. 3 Bogor, 16151, Indonesia
3Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, IPB Darmaga Campus, Bogor, Indonesia
4Faculty of Applied Biological Science, Gifu University, 1-1 Yanagido, Gifu, 501-1193, Japan

Corresponding Author: L.K. Darusman, Biopharmaca Research Center, Bogor Agricultural University, JI Taman Kencana No. 3 Bogor, 16151, Indonesia Tel: +62-251-8373561 Fax: +62-251-8347325

ABSTRACT

Kepel (*Stelechocarpus burahol*) fruit has been known to have efficacy as an oral deodorant in Java Island, Indonesia for a long time. The research aimed on Indonesian Herbal Kepel as deodorant agent for reducing fecal odorants which are ammonia, trimethyl-amine and phenol. Pharmacological screening test was conducted by measuring the adsorbent activities of Kepel's fruit (pulp, seed and peel) to odorants which can be emitted from feces, such as ammonia (NH₃) and methyl-mercaptan (CH₃SH). To confirm its ability as an oral deodorant, *in vivo* test was performed by applying it orally on rats. Kepel's fruits pulp powder was regarded as the best absorbent agent (adsorbed 62.96 and 77.78% for NH₃ and CH₃SH, respectively). All fecal odorant on the 3rd and 7th day oral application result showed a significant decrease (p<0.05). Beside the odorant adsorption, it also activates the probiotic *Bifidobacter* sp. by increasing its population. In conclusion, Kepel had a potential pharmacological activity as an oral deodorant through its adsorbent function and probiotic activation.

Key words: Kepel (*Stelechocarpus burahol*), oral deodorants, prebiotics, adsorbent, fecal odorants

INTRODUCTION

As a large and diverse country, Indonesia is inherited by enormous natural resources which are beneficial for food, renewable energy, medicine and cosmetics. Several studies on herbs for medicine which emphasized on strategic usage, such as antioxidant agent, cytotoxic activities and geriatric diseases, was already published by Artanti et al. (2006), Lotulung et al. (2008), Setyowati et al. (2008) and Iswantini et al. (2009). Regarding the cosmetic purposes, Batubara et al. (2010a) published the potential use of Indonesian natural product for whitening agent. As a part of cosmetic herbal, Kepel (*Stelechocarpus burahol*) is an Indonesian medicinal plant which empirically famous, especially in the Yogyakarta Palace. This tree is a symbol of unity and integrity of mental and physical properties of living organism. Kepel fruit is traditionally known as an oral deodorant or body odor remover especially for princess in some palace in Java Island (Heriyanto and Garsetiasih, 2005; Tisnadjaja et al., 2005). By consuming Kepels fruits, people at middle Java,
Indonesia convinced that their feces and urine odor will be lowered and brought a pleasant fragrance, such as violet fragrance. The empirical information of Kepel as an oral deodorant was regarded as a highly beneficial for use as a pharmacological compound to reduce the fecal and urine odorants which is dominated by Ammonia (NH3) (Kim and Park, 2008). To confirm above empirical knowledge of Indonesian people, in vivo and in vitro test of Kepel fruit as oral deodorant was performed.

The oral deodorant has several pharmacological activities to reduce several chemical compounds which regarded as the odorant. The odorant emission can be reduced by it pharmacological properties as an adsorbent which assessed by in vitro and in vivo methods. The pharmacological properties of an adsorbent from natural compound were performed by Seedher and Sidhu (2007) on green tea leaves to chemical pollutants. Mosavi et al. (2005) emphasized the odor substances which are typically used for adsorbent activities, such as sulfidrol (H2S) compounds. On the other hand, Bozkurt (2005) published the zeolith usage for reducing NH3 as an odorant in a farming system. Furthermore, the information were generated on this research by characterizing the odorants as NH3 and other sulfidrol group such as Methyl Mercaptan (CH3SH). By in vitro test, a deodorant agent will be assessed by reducing the odorant emission, such as NH3 and CH3SH. in vivo test of deodorant agent will assessed the reduction of fecal odorants such as NH3, Tri-Methyl Amine (TMA) and bacterial intestinal decomposer substances (phenol, scatole, indole and crescol) (Yamakoshi et al., 2001).

Regarding the fecal odorants also derived from decomposer substance which is produced by the intestinal bacteria, mostly the Enterobacteriaceae, then an oral deodorant will be beneficial as a probiotic or prebiotic agent. The probiotic or prebiotic can reduce the population of several pathogen bacteria in intestinal tract as well as the Enterobacteria and increase adsorption of nutritious compounds (Ogueke et al., 2010). By enhancing the probiotic activity, the population of odorant-producing bacteria will be reduced and finally the odorant itself will be decreased in feces (Yamakoshi et al., 2001).

This research aimed on Kepel’s pharmacological activity as an oral deodorant by in vitro and in vivo test for reducing fecal odorants which are ammonia, methyl-mercaptan, tri-methyl-amine and phenol. Furthermore, an oral deodorant was also determined by its activity in promoting the number of probiotic, such as Bifidobacteria.

MATERIALS AND METHODS

Samples: Kepel (Stelechocarpus burahol, annonaceae) fruit was collected on January 2010 and obtained from local cultivation located on Matesih village, Karanganyar city, Middle Java Province, Indonesia. The sample was identified by Herbarium Bogoriense, Cibinong, Indonesia and deposited in Biopharmaca Research Center, Bogor Agricultural University. Furthermore the fruit was sliced, dried, powdered and resulted as a fruit’s pulp, peel and seed powder. The powders were the main samples which were used to determine the deodorant activities by in vitro and in vivo techniques.

Animals: Eighteen male Sprague Dowley rat (Rattus rattus) were used as laboratory animals for in vivo test. The animals were divided into three groups which are control, samples and prebiotics group. The animal were kept on individual cage and fed by commercial food in 10% proportion amount from its body weight which contain 20% of protein. The animals were daily observed by veterinarian and technician to ensure its welfare according to the 3 R’s (Replacement, Reduction and Refinement) and 5 F’s (Freedom) animal welfare principles.
Deodorant activity

In vitro test: The in vitro was developed to describe the oral deodorant mechanism as adsorbent agent. To determine the adsorption activities, all fruit's powder are exposed to NH₃ and CH₃SH odorants. Kitagawa precision pump and detection tubes for NH₃ and CH₃SH were used to quantify precisely the amount of odorants emission and the adsorption by the samples. The odorants and samples were arranged in separated desiccators (Fig. 1) which connected by a three way-tubing connections. Odorants on liquid form was placed at desiccators A for 10 min to allow the evaporation; meanwhile the samples were on desiccators B. After 10 min, the three way tubes were opened and the odorants gas flowed from desiccators A to B for fifteen min. The odorants were quantified by Kitagawa precision pump and detection tubes which attached on the desiccators B.

The amount of whole odorant gas was obtained by measuring its concentration without any sample placed on desiccators B while the adsorbed one obtained by placing a sample on desiccators B. The whole odorant and the adsorbed one were symbolized by C and S, respectively. The value of odorant-adsorption activities was termed by deodorant activities. The deodorant activity was calculated by a percentage value which obtained from deduction of C from S, divided by C value and time a hundred percent. The highest deodorant activities which produced by one of the Kepel's fruits sample was used for in vivo test.

In vivo test: The in vivo test was directed to discover the efficacy of Kepel's fruit as an oral deodorant regarding its empirical. These studies adopted the in vivo methods from a published paper by Yamakoshi et al. (2001). The in vivo tests of the sample and control group were assessed to analyze certain parameters which are the amount of feces odorant such as ammonia and trimethyl amine and also intestine decomposed products such as phenol compound. The in vivo test of the prebiotics group was assessed by comparing the amount of fecal Bifidobacteria on sample group to a prebiotics group which received an oral ingestion of prebiotics.

The Kepel's dose used an extrapolation dose which adopted from the empirical dose in human by using an extrapolation table from Laurence and Bacharach (1964). The determined dose on human which was 100 gram/human, will be 13 mg kg⁻¹ body weight on rat.

The feces were collected before the treatment, regarded as Day zero (D0) for the baseline data and followed on the third and seventh day which are D3 and D7. The fecal ammonia, trimethyl amine and phenol were measured on the D0, D3 and D7 while the probiotic was only on D7. The

![Fig. 1: NH₃ and CH₃SH adsorption measurements](image)

182
measurement of the fecal odorants followed the SNI 2354.8:2009, Indonesian standard methods for quantification of total-volatile nitrogen and trimethyl-amine (BSN, 2009).

The result was compared to the control group by statistical methods to obtain the significance difference. The significances were categorized based on day of application odorants and fecal odorants type. Significances on day of application category were determined to predict the appropriate activity time of Kepel’s pulp fruit powder as an oral deodorant while fecal odorants category will stressed on its activity to a specified odorant.

**Prebiotics activity**

*In vitro test:* One of the most probiotics bacteria which used as an indicator for its activity and function as oral deodorant is bifid bacteria (*Bifidobacter bifidus*) (Petschow *et al.*, 1999). The activity is indicated by the increasing or decreasing number of its population which quantified as colony forming unit per gram (CFU g⁻¹). The activity was conducted in two methods which are in *vitro* and *in vivo* methods, adapted from Yamakoshi *et al.* (2001). Principally *in vitro* method was assessed by calculating its population after the samples addition to Bifidobacteria isolate while the *in vivo* methods will be on laboratory animal feces, after an oral digestion of the sample.

*In vivo test:* The *in vivo* test of prebiotics activity was performed by quantifying its amount on fecal samples. The feces which were used for the *in vivo* test came from the D7 feces of control, sample and probiotic group.

**Statistical analysis:** The results were analyzed by Statistical Package for the Social Sciences (SPSS v17.0, Chicago, IL). Descriptive statistics were used to characterize and compare groups. The groups were compared by t-tests and ANOVA for quantitative variables. Significance results were indicated by p value less than five percent (p<0.05).

**RESULTS**

**Deodorant activity**

*In vitro test:* All of Kepel’s fruit parts (peel, pulp and seed) showed activities to reduce the odorant emission *in vitro* which are above 50 and 70% for NH₃ and CH₃SH, respectively. The highest deodorant activity was founded on Kepel’s fruit pulp which had an adsorption activity in average to ammonia and methyl-mercaptan emission about 62.96 and 77.78%, respectively (Table 1). The most significant in term of percentage was performed by fruit pulp on CH₃SH odorant which valued 77.78% emission reduction.

*In vivo test:* The highest adsorbent activity by *in vitro* test was performed by Kepel’s fruit pulp powder and furthermore it was used for *in vivo* test. *In vivo* result mainly assessed the fecal odorant concentration after 24 h of Kepel’s oral application and the fecal odorants are NH₃, TMA and phenol. The results were presented in Table 2. From Table 2, the result described a significant lowered fecal odorants concentrations from sample group compare to the control group. The most significant result in term of lowering fecal odorants was performed by Kepel’s fruit pulp oral consumption on NH₃ concentration after 7 days (D7) application where the average concentration was 10 times lower than control group. The fecal NH₃ concentration after 7 days oral application was 29.46±12.43 mg/100 g fecal while the control groups values was 318.89±123.76.
Table 1: Odorant adsorption activities to ammonia and methyl-mercaptan

<table>
<thead>
<tr>
<th>Kepel’s fruit samples</th>
<th>Ammonia (NH₃)</th>
<th>Methylmercaptan (CH₃SH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulp</td>
<td>62.96±22.45</td>
<td>77.78±4.81</td>
</tr>
<tr>
<td>Peel</td>
<td>58.34±21.55</td>
<td>68.06±6.36</td>
</tr>
<tr>
<td>Seed</td>
<td>52.81±7.32</td>
<td>72.22±4.81</td>
</tr>
<tr>
<td>Overall averages</td>
<td>53.66</td>
<td>70.14</td>
</tr>
</tbody>
</table>

Table 2: In vivo deodorant activities to fecal odorants

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (Daily)</th>
<th>Collection day (D)</th>
<th>NH₃ (mg/100 g)</th>
<th>TMA (mg/100 g)</th>
<th>Phenol (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Distilled water</td>
<td>D0</td>
<td>313.47±102.58</td>
<td>20.46±8.30</td>
<td>0.51±0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D3</td>
<td>382.69±59.66</td>
<td>16.56±6.07</td>
<td>0.46±0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D7</td>
<td>313.89±123.76</td>
<td>9.12±1.67</td>
<td>0.55±0.09</td>
</tr>
<tr>
<td>Sample</td>
<td>Kepel’s pulp fruit</td>
<td>D0</td>
<td>316.23±86.13</td>
<td>19.90±4.150</td>
<td>0.45±0.03</td>
</tr>
<tr>
<td></td>
<td>13 mg g⁻¹ BB</td>
<td>D3</td>
<td>139.60±23.55*</td>
<td>7.06±1.72*</td>
<td>0.36±0.04*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D7</td>
<td>29.46±12.43*</td>
<td>4.36±0.87*</td>
<td>0.21±0.06*</td>
</tr>
</tbody>
</table>

*Indicate significant differences p<0.05

All of the fecal odorants were decreased significantly (over than 60 for NH₃, 64 for TMA, 20% for phenol decreased compared to control) on the day 3 of application and got less number (over than 90 for NH₃, 78 for TMA and 53% for phenol decreased compared to control) on day 7. The results showed that Kepel’s fruit pulp powder which was given by extrapolated human oral dose to rat, was effectively adsorb the fecal odorants after 3 days of application.

Prebiotics activity

**In vitro test:** The highest growth of Bifidobacteria from the in vitro test was performed by the fruit pulp powder. The result on Table 3 describes the comparison of Bifidobacteria population between control and Kepel’s part. It showed the potential pharmacological activity of Kepel’s fruit peel powder as a prebiotics agent. The Bifidobacteria’s population in control group was 5.8×10⁷ colony forming unit every gram feces (CFU g⁻¹) whereas the Kepel’s fruit pulp powder was 5.1×10⁸ CFU g⁻¹ feces. It showed that there was a raised one hundred times more than the control group (Table 3). The second most potential activity as a prebiotic was the seed which distinct a small number with the pulp part. The fruit pulp powder was furthermore used for prebiotics in vivo test.

**In vivo test:** The result was described in Table 4 which describes the comparison of Bifidobacteria’s population between Kepel’s fruit pulp, saline control and prebiotic control. The highest growth of fecal Bifidobacteria population founded on prebiotic control valued 4.8×10¹⁰ CFU g⁻¹ while the control group was 3.1×10⁸ CFU g⁻¹. The result indicated that the animal which are used in our studies were physiologically normal in its digestive system. In comparison to the solvent control, the Kepel’s fruit pulp powder demonstrated significantly a prebiotic activity which valued 3.0×10⁸ CFU g⁻¹. Therefore, it was increase 100,000 times more Bifidobacteria population compared to control.
Table 3: Kepel’s fruit activities to Bifidobacterium bifidus growth

<table>
<thead>
<tr>
<th>Samples</th>
<th>Population of Bifidobacterium sp. (CFU g⁻¹, triplo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (NaCl 0.9%)</td>
<td>5.8×10⁷</td>
</tr>
<tr>
<td>Kepel’s fruit pulp powder</td>
<td>5.1×10⁷</td>
</tr>
<tr>
<td>Kepel’s fruit peel powder</td>
<td>1.3×10⁷</td>
</tr>
<tr>
<td>Kepel’s fruit seed powder</td>
<td>1.3×10⁷</td>
</tr>
</tbody>
</table>

Table 4: Bifidobacterium bifidus population on feces

<table>
<thead>
<tr>
<th>Treatment (group)</th>
<th>Population of Bifidobacterium sp. (CFU g⁻¹, triplo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent control</td>
<td>3.1×10⁵</td>
</tr>
<tr>
<td>Probiotic control</td>
<td>4.8×10⁵</td>
</tr>
<tr>
<td>Kepel’s fruit pulp powder</td>
<td>3.0×10⁵</td>
</tr>
</tbody>
</table>

DISCUSSION

Deodorant activity

In vitro test: Generally, all of the research’s results support the empirical usage of Kepel as deodorant oral by adapting the methods by Yamakoshi et al. (2001, 2002). So far, there were no published scientific studies on Kepel’s plant as an oral deodorant, therefore the article by Yamakoshi et al. (2001) was regarded as the most relevant reference. Compared to the article published by Yamakoshi et al. (2001) on grape seed as an oral deodorant, Kepel fruit also clearly demonstrated the similar oral deodorant mechanism or activity which is an adsorbent agent to odorants. The in vitro test result was beneficial to determine the most significant part of Kepel’s plants which can be used for in vivo test candidate.

The highest adsorption value which performed by Kepel’s pulp fruit can also be interpreted by its activity to reduce the odorant amount up to 42.04 and 22.22% left. The activity to reduce the odorant by adsorbing its emission demonstrated the pharmacological properties of Kepel’s fruit pulp as an adsorbent agent.

The molecular weight of ammonia was smaller than methyl-mercaptan which allows it to have a higher volatility. The higher volatility will caused a higher emission and fill all the ambient circumstances while methyl-mercaptan gas will be less emitted and tend to precipitate in the sample’s surface. Above condition can attributed to the differences of deodorant activity of the samples which caused a higher adsorption percentage of methyl-mercaptan in relative to ammonia.

The reasonably-high deodorant activity can be supported by its phytochemical properties such as tannin and flavonoids compounds. The phytochemical of Kepel's peel fruit was contain several phytochemical properties such as tannin and flavonoid (Batubara et al., 2010b). Phytochemical properties describe the secondary metabolites compound which potential as an active compound (Leboeuf et al., 1982). Flavonoid regarded as the derivative compound of flavon and it differentiate as anthocyanidine, proantocyanidine, flavonol, glyco-flavon, flavanon and isoflavon (Sunarni et al., 2007).

Since all the Kepels contain flavonoid and tannin, then the deodorant mechanism can be explained by one of its proantocyanidine compound. According to Yamakoshi et al. (2001), proantocyanidine has a potential pharmacological activity as an adsorbent from its procyanidine oligomer molecule. Procyanidine has a bigger molecular mass which possibly adsorb the odorant. Furthermore, the adsorbent activity of Kepel’s peel fruit was conducted by in vivo test to discover its mechanism as an adsorbent to fecal odorant.
**In vivo test:** The result of *in vivo* test was supported by Yamakoshi et al. (2001) published paper, since the fecal odorants were lowered and the Bifidobacteria were increased, after Kepel’s fruit pulp oral application. The significant result in lowering all of the fecal odorants demonstrated the efficacy of Kepel’s fruit as an intestinal adsorbent (Brander et al., 1991; Webster, 2001) or oral deodorant. The adsorbent activity came from of its phytochemical properties which are flavonoid and tannin, specifically the proanthocyanidine compound. The proanthocyanidine content a bigger oligomer named procyanidine which possibly adsorb the odorants (Yamakoshi et al., 2001).

The proantocyanidine itself has chemical structure which allowed a binding with such an odorant such as NH₃ from its electrostatic side. The side was distributed in a manner for possible complex of proantocyanidine and the odorant. The deodorant activity of Kepel’s peel fruit by *in vitro* methods which describe it mechanism as an adsorbent was corresponding with the *in vivo* test result. It clearly demonstrate the correlation between the mechanism as adsorbent and it efficacy as an oral deodorant.

Since proanthocyanidine also promote vascular health (Corder et al., 2006), then Kepel has another potential pharmacological activities to promote animal or human health. Publication by Osorio et al. (2007) and Wiart (2007) stated other pharmacological activities of Kepel based on family taxon proximity. Ammonia (NH₃) as a physical hazard, plays an important role in health management, especially on poultry farm since it can be a predisposition factor to respiratory infections.

Based on our results, Kepel’s fruit pulp demonstrated pharmacological activities as an adsorbent to odorant which made it potentially beneficial not only as an oral deodorant and also as one of health management component in poultry or livestock farm. Its pharmacological activities as an adsorbent to ammonia brought a new perspective to reduce the biohazard through oral consumption.

**Prebiotics activity:** The *in vitro* test clearly described the prebiotic potential activities of Kepel’s fruits pulp and seed for further oral application. Based on the result, both *in vitro* and *in vivo* test of Kepel’s as prebiotics, support the publication by Yamakoshi et al. (2001) which described the prebiotic activity as a part of oral deodorant activity. Kepel fruit pulp increase the Bifidobacteria growth significantly as well as the grape seed’s extract which was conducted by Yamakoshi et al. (2001).

The reasonably high number of fecal Bifidobacter on Kepel fruit pulp powder group indicated the higher population in intestinal tract which also correlate with its pharmacological activities as prebiotics in reducing pathogens bacteria, anaerobic bacteria and Enterobacteriaceae (Hajati and Rezaei, 2010) as well as preventing inflammation on intestinal tract (Imaoka et al., 2008). The TMA is known as a gas-odorant which belongs to amine group and distinguished by the stink and fishy-odor (Kim and Park, 2008). Beside of TMA, the bacteria also produced others odorants, such as NH₃ itself hydrogen sulfide (H₂S) and intestinal decomposer products. By promoting the Bifidobacter, the number of the bacteria which causing the fecal odorant will be reduced and furthermore the fecal odorant concentration will be lowered significantly. Through, *in vitro* and *in vivo* test of adsorbent and prebiotics activities, Kepel showed a promising use for human medicine as oral deodorant and also in veterinary medicine for promoting better health by lowering NH₃ emission, especially in poultry farm (Ghiyasi et al., 2007; Hajati and Rezaei, 2010).
CONCLUSION

Kepel fruit pulp was scientifically proven as an oral deodorant through its potential pharmacological activity as fecal odorants adsorbent and prebiotics function by enhancing Bifidobacteria growth.

ACKNOWLEDGMENTS

The studies was deeply in debt with the people at Matesih, Karanganyar, Middle Java province for their indigenous knowledge in consuming kepel’s fruit as an oral deodorant. Biopharmaca Research Center for samples preparation, Laboratory of Natural Product Chemistry, Gifu University for providing the Kitagawa precision pump and gas detector, the meticulous technicians in fecal odorant analysis, prebiotics and whole in vivo studies at the Division of Analytical Chemistry, Division of Microbiology Health and Division of Pharmacology and Toxicology, IPB Bogor, respectively.

REFERENCES


Bozkurt, Y., 2006. The use of zeolite to improve housed beef cattle performance by reducing ammonia accumulation in small farm conditions. AJAVA 1: 60-64.


