

## Inhibitory Effect of Mucoadhesive Gingival Patch of Mangosteen Peel Extract Against Periodonto Pathogen Bacteria

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### Abstract

Chronic periodontitis is inflammation of periodontal tissue that develops slowly, mainly caused by *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. Mangosteen peel extract (*Garcinia mangostana*) is known to inhibit the growth of anaerobic Gram-negative bacteria so that it can be used as an alternative antimicrobial treatment for chronic periodontitis. Mucoadhesive gingival patch can be used as a topical drug administration through the mucosa and nanoparticles allowing drug ingredients to be delivered more quickly and on target. The purpose of this study is to prove the ability of mucoadhesive gingival patch mangosteen peel extract in inhibiting the growth of bacteria that cause chronic periodontitis. *P. gingivalis* and *F. nucleatum* were given gingival patch mucoadhesive mangosteen peel extract, mucoadhesive gingival patch nanoparticle mangosteen peel extract, doxycycline mucoadhesive gingival patch with menthol and without menthol, mucoadhesive gingival patch without extract, and mangosteen peel extract with 4 repetitions at a dose of 100 mg and 200 mg using the well diffusion method. Mangosteen gingival patch of mangosteen peel extract showed significant differences with a gingival patch of mucoadhesive nanoparticle mangosteen peel extract but not significant with mangosteen peel extract. Mucoadhesive gingival patch were not significant with doxycycline mucoadhesive gingival patch with or without menthol in studies of *P.gingivalis* and *F. nucleatum* at a dose of 100 mg. Nanoparticles gingival mucoadhesive patch mangosteen peel extract 200 mg against *F. nucleatum* showed significant differences with mucoadhesive gingival patch of mangosteen peel extract ( $p = 0.014$ ) and doxycycline mucoadhesive gingival patch with menthol ( $p = 0$ ) but not significant with mangosteen peel extract ( $p = 0.064$ ) and doxycycline mucoadhesive gingival patch without menthol ( $p = 0.934$ ). Mucoadhesive gingival patch mangosteen peel extract can inhibit the growth of bacteria that cause chronic periodontitis namely *P. gingivalis* and *F. nucleatum*.

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### Introduction

Periodontal disease in Indonesia has a prevalence of 96.58% and is often found in the form of gingivitis and periodontitis.<sup>1</sup> Chronic periodontitis is associated with the accumulation of plaque and calculus in the oral cavity with increasing Gram-negative bacteria on subgingival biofilms such as *Porphyromonas gingivalis* (*P.gingivalis*) and *Fusobacterium*

*nucleatum* (*F.nucleatum*).<sup>2</sup> Mechanical therapy such as scaling and root planing sometimes requires antibacterial administration to make treatment more effective.<sup>3</sup> Mangosteen peel (*Garcinia mangostana*) can be an alternative antimicrobial ingredient because it contains active compounds such as saponins, flavonoids, tannins, and xantons. The topical extract of mangosteen peel is known to reduce pocket depth after scaling and root planning.<sup>4,5</sup> *Garcinia mangostana* skin has also been shown to have inhibitory properties against *P. gingivalis*<sup>4,6</sup> and *F.nucleatum*.<sup>7</sup>

The administration of the drug through the mucosa (mucoadhesive), one of its forms is a patch. The mucoadhesive patch is flexible when applied so it does not interfere with the physiological functions of the mouth, does not

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dissolve easily in saliva, the medicinal ingredients contained are more easily absorbed by the body and are on target because they are administered locally.<sup>8,9</sup> Drug formulations are also developing along with the discovery of nanoparticle technology that can penetrate the space between cells and cell walls and is flexible to be combined with other technologies so that the active ingredients contained are faster and easier on target.<sup>10</sup>

The previous research by Shantiningsih<sup>11</sup> and Fitriyah<sup>12</sup> proves that the administration of drugs and herbal ingredients in the form of a mucoadhesive gingival patch gives good results. However, studies of mucoadhesive gingival patches containing mangosteen peel extract, including those in the form of nanoparticles, have not been tested yet for the growth of bacteria that cause chronic periodontitis, especially *P. gingivalis* and *F. nucleatum*.

### Materials and methods

This research is an in vitro experimental laboratory study with a total of 22 groups consisting of negative control groups in the form of mucoadhesive gingival patch (K-1) and mangosteen peel extract (K-2), positive control in the form of doxycycline mucoadhesive gingival patch (Doxicor) with the addition of menthol (K+1) and without the addition of menthol (K+2), and the treatment group namely mangosteen peel extract gingival patch (P1) and nanoparticle gingival patch mangosteen peel extract (P2) with 4 repetitions. Each group, except the mucoadhesive gingival patch (K-1), was tested at doses of 100 mg and 200 mg. Preliminary research was carried out using a 50 mg mangosteen peel extract patch.

#### The making of mangosteen peel extract

Mangosteen was obtained from the Blitar region in East Java. Making mangosteen peel extract is done by drying and smoothing mangosteen peel. Mangosteen peel powder is then macerated with 96% ethanol in a ratio of 1:2 and then filtered with filter paper. The liquid is evaporated with a Rotary Vacuum Evaporator then freeze-drying is done until it forms a powder.<sup>7</sup>

#### The making of nanoparticle mangosteen peel extract

Making nanoparticles mangosteen peel extract is done by weighing 2 grams of

mangosteen peel extract in a 100 ml beaker then dissolved in 96% ethanol: water (70:30) and mixed with 2% chitosan solution and diluted with distilled water up to 1000 ml. The mixture was gradually added 700 ml of 0.1% Na-TPP solution while stirring at 12,500 rpm. The nanoparticles are then separated by centrifugation and the centrifugation precipitate is freeze drying to make a powder.<sup>13</sup>

#### The making of gingival mucoadhesive patch

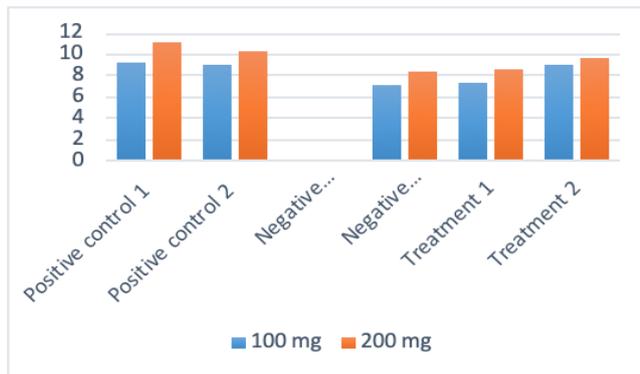
Patch are made by sprinkling 1.5-gram of CMC-Na into 30 ml of water then allowed to stand until swell and stir until it becomes a gel mass. 60.3 grams of hot water are added gradually to the gel base and this mixture called mixture I. Mixture II is made by dissolving 0.5 gram menthol with 96% ethanol and then mangosteen or doxycycline peel extract is added and stirred until dissolved. 2.5 gram propylene glycol is then added and stirred. Mixture II was then added to the mixture I and stirred until homogeneous. The preparations were then weighed using 70 grams of analytical scales and put in a petri dish and dried in an oven at 45°C.<sup>11</sup>

Patch were tested for bacterial inhibition by the diffusion method while mangosteen peel extract was tested by diffusion method using a paper disk. The inhibition zone is obtained by measuring the diameter of the clear zone that forms around the patch and paper disk using calipers in mm. This study was approved by the Ethics Commission of the Faculty of Dentistry Airlangga University Surabaya-Indonesia with number 448/HRECC.FODM/VII/2019 and used the Kolmogorov-Smirnov test to find out whether the data were normally distributed, the homogeneity variant test with the Levine test ( $\alpha > 0, 05$ ), as well as the ANOVA statistical test, in the SPSS 2.0 program.

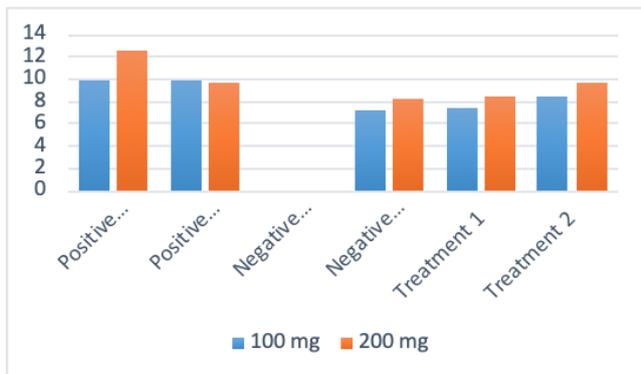
### Results

Measurement of inhibition zone diameter was made by using calipers. The mean diameter of the inhibitory zone of the mucoadhesive gingival patch of mangosteen peel extract approaches the mean diameter of the inhibitory zone of mangosteen peel extract. The mean mucoadhesive gingival patch of mangosteen peel nanoparticle extract approached the mean diameter of the doxycycline gingival patch inhibitory zone. Mucoadhesive gingival patch (K-

1) do not produce any inhibitory zones.



**Figure 1.** Graph of mean ± SD of inhibition zone diameter to *P.gingivalis*.



**Figure 2.** Graph of mean ± SD of inhibition zone diameter to *F.nucleatum*.

The normality test using the Shapiro-Wilk test obtained data with normal distribution ( $P > 0.05$ ) and then followed by the Levene test ( $P > 0.05$ ) showed homogeneous research data. ANOVA test with post hoc Tukey HSD was used to compare the results of each group. The results of research on *F.nucleatum* dose of 100 mg which have data not normally distributed ( $P < 0.05$ ) used the *Kruskal-Wallis* test.

	K-2	K+1	K+2	P1	P2
K-2		0,00*	0,00*	1,00	0,00*
K+1	0,00*		0,738	0,00*	0,715
K+2	0,00*	0,738		0,00*	1,00
P1	1,00	0,00*	0,00*		0,00*
P2	0,00*	0,715	1,00	0,00*	

**Table 1.** P-value of Tukey HSD test for *P.gingivalis* group with 100 mg of mangosteen peel extract and doxycycline.

	K-2	K+1	K+2	P1	P2
K-2		.0,00*	0,00*	1,00	0,013*
K+1	0,00*		0,127	0,00*	0,003*
K+2	0,00*	0,127		0,00*	0,289
P1	1,00	0,00*	0,00*		0,018*
P2	0,013*	0,003*	0,289	0,018*	

**Table 2.** P-value of Tukey HSD test for *P.gingivalis* group with 200 mg of mangosteen peel extract and doxycycline.

	K-2	K+1	K+2	P1	P2
K-2		0,00*	0,00*	0,998	0,002*
K+1	0,00*		0,914	0,00*	0,007*
K+2	0,00*	0,914		0,00*	0,036*
P1	0,998	0,00*	0,00*		0,00*
P2	0,002*	0,007*	0,036*	0,00*	

**Table 3.** P-value of Tukey HSD test for *F.nucleatum* group with 100 mg of mangosteen peel extract and doxycycline.

	K-2	K+1	K+2	P1	P2
K-2		0,00*	0,015*	0,926	0,064
K+1	0,00*		0,00*	0,00*	0,00*
K+2	0,015*	0,00*		0,003*	0,934
P1	0,926	0,00*	0,003*		0,014*
P2	0,064	0,00*	0,934	0,014*	

**Table 4.** P-value of Tukey HSD test for *F.nucleatum* group with 200 mg mangosteen peel extract and doxycycline.

The gingival patch of mucoadhesive mangosteen peel extract showed significant differences with gingival patch of nanoparticle mucoadhesive mangosteen peel extract but not significant with mangosteen peel extract. Nanoparticle mucoadhesive gingival patch were not significant with doxycycline mucoadhesive gingival patch with or without menthol in studies of *P.gingivalis* and *F.nucleatum* at a dose of 100 mg. The nanoparticle mucoadhesive gingival patch of mangosteen peel extracts 200 mg against *F.nucleatum* showed significant differences with the mucoadhesive gingival patch of mangosteen peel extract and doxycycline mucoadhesive gingival patch with menthol but not significant with mangosteen peel extract and doxycycline mucoadhesive gingival patch without menthol.

## Discussion

Based on the research result, the administration of mucoadhesive gingival patch does not cause inhibition zones which means that the mucoadhesive gingival patch has no antibacterial ability. The mean diameter of the inhibitory zone of mangosteen peel extract was almost the same as the diameter in the group of gingival patch of mucoadhesive mangosteen peel extract which meant the patch was able to deliver the contents of mangosteen peel extract well. Bacteria that are exposed to the mucoadhesive gingival patch of mangosteen peel extract will be influenced by the content of mangosteen peel extract which is saponin that can increase the permeability of the bacterial membrane resulting in hemolysis, tannins and xanthenes that inhibit bacterial replication, and flavonoids that interfere with bacterial metabolic processes.<sup>6,14</sup> The study of inhibition of mucoadhesive gingival patch mangosteen peel extract using the diffusion method also allows the antibacterial ability of mangosteen peel extract contained in the mucoadhesive gingival patch to diffuse directly into the bacterial growth media, not just on the surface of the growth media.

The results showed that the mucoadhesive gingival patch of mangosteen peel extract nanoparticles had an average inhibition zone diameter that was greater than the mucoadhesive gingival patch of mangosteen peel extract and nearly approached the positive control group. This can be explained by the mucoadhesive gingival patch of mangosteen peel extract nanoparticles used in this study was made by the ionic gelation method into polymeric nanoparticles using chitosan and 2% acetic acid solvent. Chitosan and Na-TPP solutions were added in the process of making mangosteen peel extract nanoparticles as a cross-link agent. Nanoparticles will be formed spontaneously through the mechanism of electrostatic interaction between amines from chitosan and the negative charge of polyanion, Na-TPP in stirring magnetic stirrers at room temperature.<sup>15</sup> The content of chitosan in chitosan nanoparticles also functions as a penetration enhancer by opening tight junctions to the epithelium when applied in vivo.<sup>16</sup> Chitosan nanoparticles also help in maximizing the antibacterial ability of mangosteen peel extract with 3 different mechanisms, namely the interaction of positive

charge on the chitosan molecule with a negative charge on the bacterial cell membrane resulting in changes in the permeability properties of the cell membrane that makes the bacterial cell osmotic pressure unbalanced and ultimately inhibits the growth of the bacteria. The second mechanism is the hydrolysis of peptidoglycan bacterial cell walls due to electrostatic interactions so that intracellular electrolytes such as potassium ions and molecules such as proteins and nucleic acids exit the cell. The third mechanism is the ability of metal chitosan to form chelation that blocks the flow of nutrients from bacteria so that bacterial growth is inhibited.<sup>17</sup>

Research on *P.gingivalis* shows that there is no significant difference between the inhibitory ability of mangosteen peel extract and mucoadhesive gingival patch mangosteen peel extract but significant differences exist in the mucoadhesive gingival patch of mangosteen peel extract with nanoparticle mucoadhesive gingival patch of mangosteen peel extract. This can occur because mangosteen peel extract with small particle size is easier to diffuse into the bacterial cell wall<sup>18</sup>. The small size of the nanoparticles can also increase drug capacity in the drug-carrying system<sup>19</sup>. The ability to more easily enter the cell wall of the bacterium makes the nanoparticle extract of mangosteen peel more effective in giving an antibacterial effect. This is supported by the results of the analysis between the insignificant gingival patch of mucoadhesive nanoparticle mangosteen peel extract with doxycycline which means it is almost as good in giving an antibacterial effect on *P.gingivalis*.

Research on *F. nucleatum* also shows that there is no significant difference between the inhibitory ability of mangosteen peel extract and the mucoadhesive gingival patch of mangosteen peel extract. The nanoparticle mucoadhesive gingival patch of mangosteen peel extract gives a significant difference in results with the mucoadhesive gingival patch of mangosteen peel extract and the doxycycline mucoadhesive gingival patch except at a dose of 200 mg. The nanoparticle mucoadhesive gingival patch of mangosteen peel extract gave insignificant results with doxycycline mucoadhesive gingival patch without menthol and mangosteen peel extract. This can happen because, in a dose of 200 mg nanoparticle mucoadhesive gingival patch of mangosteen peel extract, mangosteen peel extract, and doxycycline mucoadhesive

gingival patch without menthol have almost the same antibacterial power against *F. nucleus*. Doxycycline works to inhibit bacterial growth by being bound to 30S subunits and 50S ribosomal so that binding of aminoacyl tRNA with mRNA is inhibited and ultimately inhibits bacterial protein synthesis<sup>20</sup>. Mechanism of doxycycline growth inhibition has a different path from mangosteen peel extract which inhibits by damaging bacterial cell walls.<sup>21</sup>

The doxycycline gingival patch of doxycycline uses doxycycline dose of 100 mg and 200 mg according to the dosage of doxycycline therapy in adults which is 200 mg on the first day of treatment and followed by a maintenance dose of 100 mg/day.<sup>20</sup> Mucoadhesive patches of gingiva are added by menthol which functions as enhancers, substances that help improve the bioavailability of drugs so that it can more easily penetrate the mucosa. Menthol can be used as a permeation enhancer because menthol is able to increase the solubility of medicinal ingredients and can improve drug diffusion by interfering with the sequence of intercellular lipid conformation in the mucosal bilayer layer.<sup>22</sup> Menthol which is a monocyclic terpene has a pleasant taste and odor and is safe, non-carcinogenic, effective and widely used as an enhancer in delivering transdermal drugs.<sup>23</sup> Menthol can be a penetration enhancer so it helps diffusion for hydrophobic or hydrophilic drugs through interactions with the lipid membrane.<sup>24</sup> Menthol essential oils are also known to be active against various microorganisms including Gram-positive and Gram-negative bacteria and fungi because they can integrate and disrupt the structure and function of bacterial membranes.<sup>25</sup> Based on the analysis of the results of research on giving menthol to the doxycycline mucoadhesive gingival patch showed a larger inhibition zone diameter than without menthol although the difference was not always significant.

## Conclusions

Mucoadhesive gingival patch mangosteen peel extract (*Garcinia mangostana*) can inhibit the growth of bacteria that cause chronic periodontitis namely *P. gingivalis* and *F. nucleatum*.

Further research needs to be done on:

1. Inhibitory ability of mangosteen peel extract

(*Garcinia mangostana*) against *F. nucleatum* with several different concentration.

2. Mucoadhesive patch of mangosteen peel extract (*Garcinia mangostana*) against other bacteria that cause chronic periodontitis.
3. Nanoparticle mucoadhesive gingival patch of mangosteen peel extract (*Garcinia mangostana*) with smaller nanoparticle sizes in *in vitro* and *in vivo*.

## Declaration of Interest

The authors report no conflict of interest.

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