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Black To Pink Gel: The Antibacterial Potency Of Curcuma Domestica And Moringa Oleifera Extract Gel Against P.Gingivalis (A Pilot Study)

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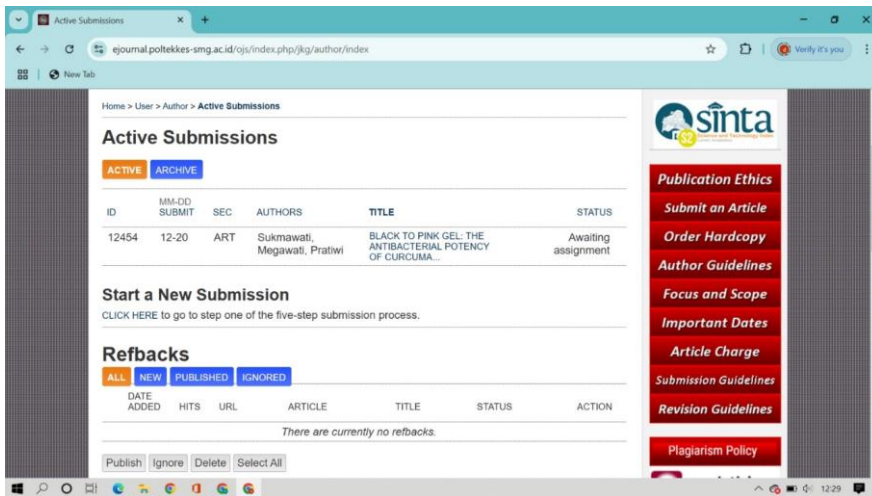
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COLLABORATION

Black To Pink Gel: The Antibacterial Potency Of Curcuma Domestica And Moringa Oleifera Extract Gel Against P.Gingivalis (A Pilot Study)

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ABSTRACT

Periodontitis continues to be a debilitating problem when improperly treated. This condition is associated with certain periodontopathological bacteria, including Porphyromonas gingivalis. Chlorhexidine, while proven effective against bacteria in the oral cavity, carries unwanted side effects. Curcuma domestica and Moringa oleifera are domestic herbs that possess several therapeutic effects. This study aims to investigate the antibacterial effect of the combined two herbs in extract gel. This experimental study used three combination formulas of C. domestica: M. oleifera (0.1%:2%, 0.5%:4%, 0.9%:6%) as the test group and chlorhexidine as the positive control group. The antibacterial inhibition disc method was used to determine the antibacterial activity in each group. The statistical analysis was carried out with Kruskal-Wallis and continued with the Mann-Whitney test. In this study, extract gel combination of Curcuma domestica and Moringa oleifera at concentrations of (0.1%:2%; 0.5%:4%; 0.9%:6%) can inhibit the growth of Porphyromonas gingivalis with average inhibition zone diameters of 12.50 mm, 14.49 mm and 16.66 mm, respectively. The inhibition zone falls in the strong category. The control group showed a mean inhibition zone of 20.96, which is classified as a very strong inhibition zone. Although there was a significant p-value between the test and control groups, the results suggested the potential of the extract gel as an alternative for treating periodontitis with lesser side effects.

Keywords: Curcuma domestica; Moringa oleifera; Porphyromonas gingivalis

Introduction

Globally, prevalence of periodontal disease reaches 20-50% [1]. This indicates that periodontal disease is still a dental and oral health problem that needs further attention. Periodontitis is a multifactorial disease which caused by the presence of pathogenic bacteria, the inflammatory response, host immunity, and is influenced by the environment and systemic condition [2].

Porphyromonas gingivalis the main bacteria that plays a role in the pathogenesis of periodontal disease. Porphyromonas gingivalis is part of the red complex bacteria, along with Tannerella forsythia and Treponema denticola [3]. It has virulence

factors that can trigger periodontal inflammation and invade periodontal tissue along with the increasing number of bacteria in subgingival plaque [4]. Its virulence factors include lipopolysaccharide (LPS), gingipain, fimbriae and capsules, which responsible for the occurrence of dysbiosis oral cavity microbes, biofilm formation and coaggregation [3], [5].

Periodontitis is a disease of the supporting tissues which is characterized by damage to the alveolar bone and loss of attachment [6]. Clinical symptoms that can be found include inflammation of the gums, presence of periodontal pockets, bleeding on probing and changes in the shape of the gums, tooth mobility, pathological migration and loss of alveolar bone on radiographic examination

[2], [7]. Periodontitis treatment can be done only mechanically (scaling and root planing-SRP) or accompanied by adjuvant therapy both locally and systemically. One of the adjuvant therapies that is often used is chlorhexidine.

Chlorhexidine (CHX) is the gold standard in periodontal treatment which has bacteriostatic and bactericidal properties against microbes that cause periodontitis [8]. This material is an antimicrobial and antiseptic agent so that it is necessary to pay attention to the frequency and duration of use to reduce the side effects that might arise. Side effects that can arise include dry mouth, taste disturbances, discoloration of the tongue, burning sensation, desquamation of the oral mucosa and stains on the teeth [8], [9]. Therefore, pharmacotherapeutic treatment is needed as an adjuvant therapy that is safe for long-term use and has the potential to inhibit microbes that cause periodontitis. The application of herbal ingredients is one of the effective and efficient alternative treatments.

Turmeric and Moringa leaves are herbal plants that have many benefits. Turmeric (*Curcuma domestica*) has various compounds such as alkaloids, flavonoids, curcumin, essential oils, quercetin, saponins, tannins and terpenoids. Curcumin and essential oils have been shown to have anti-inflammatory properties. In addition, the curcuminoid compound group has antibacterial properties, anticonvulsant, analgesic, antidiarrheal, antipyretic and antitumor [10].

Moringa leaves (*Moringa oleifera*) have a variety of active ingredients that can be used in several treatments. These active ingredients include tannins, steroids, terpenoids, flavonoids, saponins, anthraquinones and alkaloids. These compounds have strong antibacterial power with a working mechanism that damages bacterial cell membranes [11].

Sawant T, et al. revealed that a gel containing 4% *Moringa oleifera* used as an addition to SRP for topical application showed similar results to the use of 1% CHX gel in reducing the Plaque Index, Gingival Index, and Papillary Bleeding Index so that it can be used routinely as an alternative herbal non-surgical periodontal therapy [12]. Another study conducted by Irawati, et al. showed that methanol extract of *Moringa oleifera* seeds with concentrations of 2.5%, 5%, 10%, 15%, 20%, and 15% had antibacterial activity against *Porphyromonas gingivalis* bacteria [13]. A study showed that *Moringa oleifera* leaf extract with concentrations of 40% and 80% was effective in inhibiting *Porphyromonas gingivalis* bacteria [14]. Research conducted by Bomdyal, et al. showed that

the Minimum Inhibitory Concentration of curcumin in turmeric against *P. gingivalis* and *P. intermedia* bacteria was 100 µg/ml or 0.01% [15].

This study aims to test the effectiveness of turmeric gel (*Curcuma domestica*) and moringa leaves (*Moringa oleifera*) in various concentrations on the growth of *Porphyromonas gingivalis* bacteria.

Research methods

This research was conducted at the Semarang College of Pharmaceutical Sciences as a place for making gel and at the Laboratory of the Faculty of Dentistry, Airlangga University, Surabaya for antibacterial testing. Ethical clearance obtained from RSGM Unimus Health Research Ethics Commission with no.004/RSGM.KEPK/PE/2024.

Gel extract turmeric and moringa leaves made by mixing the gelling agent with extract *C.domestica* and *M.oleifera* according to the gel preparation formulated in Table 1.

Table 1.

Material	K (-)	Concentration		
		F1	F2	F3
Turmeric Extract	-	0.1%	0.5%	0.9%
Moringa Extract	-	2%	4%	6%
Carbopol	1%	1%	1%	1%
Propylene Glycol	15%	15%	15%	15%
TEA	3%	3%	3%	3%
Nipagin	0.1%	0.1%	0.1%	0.1%
Propyl Paraben	0.02%	0.02%	0.02%	0.02%
EDTA	0.03%	0.03%	0.03%	0.03%
Ethanol	0.1%	0.1%	0.1%	0.1%
Aquadest	100 ml	100 ml	100 ml	100 ml

Gel Formulation [10], [16], [17].

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All formula components were weighed. Carbopol 940 was soaked in water overnight to swell. The gel base (mass 1) was prepared by gradually neutralizing the formula using triethanolamine while stirring using a homogenizer. Methyl paraben was dissolved in propylene glycol (mass 2). Masses 1 and 2 were mixed until homogeneous using a homogenizer (mass 3). Turmeric rhizome and moringa leaf extracts were added to mass 3, followed by the remaining distilled water. The mixture was homogenized using a homogenizer at optimal speed and duration. All stages were carried out on each formula F1, F2 and F3. Furthermore, a series of gel tests were carried out consisting of organoleptic tests, homogeneity tests, adhesion tests, spreadability tests and pH tests.

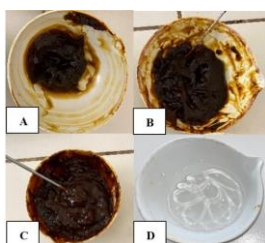


Figure 1. *C.domestica:M.oleifera* Gel. Gel A (0.1%:2% extract), Gel B (0.5%:4% extract), Gel C (0.9%:6% extract), Gel D (0% extract or empty gel without active ingredients)

Porphyromonas gingivalis bacteria were taken from pure culture strains using one colony of loops, then 0.5 ml of liquid Brain Heart Infusion (BHI) was added and incubated for 24 hours at 37° C. The suspension was diluted with sterile distilled water to a certain concentration according to the Mc Farland 0.5 standard, which is 1×10^8 CFU/ml.

Porphyromonas gingivalis ATCC 3327 bacterial samples in several petri dishes with each concentration of 0.1%: 2%, 0.5%: 4% and 0.9%: 6% will be repeated the same. The number of repetitions is calculated using the Federer formula and 5 repetitions are obtained so that sample 25 samples were used.

Antibacterial testing using the disc diffusion method. The surface of the MHA media was smeared with a suspension of *Porphyromonas gingivalis* bacteria with a sterile ose using the spreading technique. The disc paper was given 10 μ l of a combination gel of *C. domestica*: *M. oleifera* (0.1%: 2%, 0.5%: 4% 0.9%: 6%) as the treatment group, blank gel as the control negative And chlorhexidine gluconate 0.2% as a positive control. The disc paper was placed on the surface MHA media with space between the discs to prevent overlapping in the inhibition zone formed. Then, Mueller Hinton agar was incubated for 24 hours at a temperature of 37°C. Observation and measurement of the diameter of the clear zone around the disc paper were measured using a sliding caliper in millimeters. Data analysis using Kruskal-Wallis to prove the presence of a bacterial inhibition zone, followed by a Post Hoc test using the Mann Whitney method to determine significance mean difference between groups.

Results and Discussion

Antibacterial activity test of extract gel *Curcuma domestica* and *Moringa oleifera* on the growth of *Porphyromonas gingivalis* bacteria was carried out using the disc diffusion method. The parameter used was the diameter of the clear zone formed around the disc paper. The diameter of the clear zone indicates the strength of the active antibacterial ingredient that acts as an antibacterial. If the diameter of the clear zone increases, the antibacterial activity is greater. Table 2 shows that each group of *Curcuma domestica* and *Moringa oleifera* extract gel concentrations can form a clear zone.

Table 2.

Results of Antibacterial Inhibition Zone Measurement of *Curcuma domestica* and *Moringa oleifera* extract gel against *Porphyromonas gingivalis* bacteria

Replicati on	F1 (0.1%:2%)	F2 (0.5%:4%)	F3 (0.9%:6%)	K(+)	K(-)
1	12.60 mm	14.40 mm	16.80 mm	21.00 mm	0 mm

2	12.40 mm	14.55 mm	16.75 mm	21.05 mm	0 mm
3	12.35 mm	14.35 mm	16.55 mm	20.95 mm	0 mm
4	12.55 mm	14.55 mm	16.60 mm	20.80 mm	0 mm
5	12.60 mm	14.60 mm	16.60 mm	21.00 mm	0 mm
Average	12.50 mm	14.49 mm	16.66 mm	20.96 mm	0 mm

Positive control : chlorhexidine gluconate 0.2%
 Negative control : Empty gel

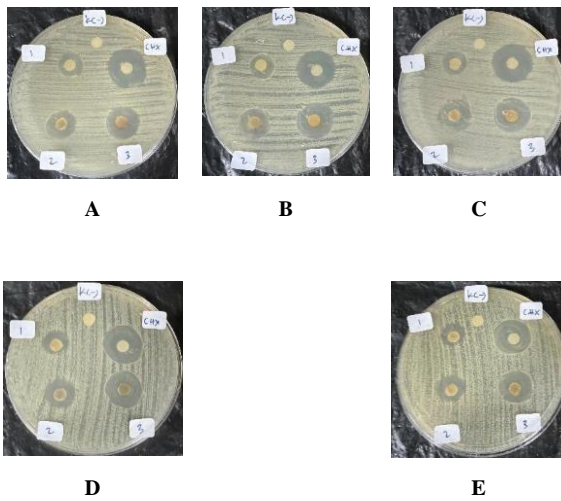


Figure 2. Inhibition zone of *Curcuma domestica* and *Moringa oleifera* gel formed against *Porphyromonas gingivalis* bacteria (A) First repetition (B) Second repetition (C) Third repetition (D) Fourth repetition (E) Fifth repetition.

Table 3. Kruskal Wallis Test Results

Group	P	Sig 0.05	Information
F1 (0.1%:2%)			
F2 (0.5%:4%)			
F3 (0.9%:6%)	0.00	P<0.05	Significant Difference
K (+)			
K (-)			

Table 4. Mann Whitney Test Results

Test group	I	II	III	IV	V
I	-	0.009*	0.009*	0.009*	0.005*

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II	0.009*	-	0.009*	0.009*	0.005*
III	0.009*	0.009*	-	0.009*	0.005*
IV	0.009*	0.009*	0.009*	-	0.005*
V	0.005*	0.005*	0.005*	0.005*	-

Information :

* : *p value* < 0.05

I : Extract gel *C.domestica*: *M.oleifera* (0.1%:2%)

II : Extract gel *C.domestica*: *M.oleifera* (0.5%:4%)

III : Extract gel *C.domestica*: *M.oleifera* (0.9%:6%)

IV : Positive control (chlorhexidine gluconate 0.2%)

V : Negative control (Empty gel without active ingredients)

In the treatment group, the lowest average clear zone diameter value was obtained in gel A with a concentration of *C.domestica* : *M.oleifera* (0.1%:2%) which is 12.50 mm while the highest value of the average diameter of the inhibition zone was obtained in gel C with a concentration of *C.domestica* : *M.oleifera* (0.9%:6%) which is 20.96 mm. The bacterial inhibition effect has been classified by Davis and Stout into four categories, a clear zone diameter of less than 5 mm indicates weak antibacterial activity, a clear zone diameter of 5 to 10 mm indicates moderate antibacterial activity, a clear zone diameter of 10 to 20 mm indicates strong antibacterial activity and a clear zone diameter of 20 mm or more indicates very strong antibacterial activity [18].

In this study, gels A, B and C had a strong antibacterial inhibitory response category with the average diameter of the inhibition zone was 12.50 mm, 14.49 mm and 16.66 mm respectively. These results indicate that the diameter of the inhibition zone of the *C.domestica* and *M.oleifera* extract gels was smaller when compared to the positive control, namely 0.2% chlorhexidine, which was 20.96 mm, which was included in the very strong category.

This is because chlorhexidine is positively charged which can damage the negatively charged bacterial cell membrane. The positive charge of chlorhexidine causes significant affinity with the negatively charged bacterial cell wall with components containing phosphate and sulfate groups [19]. Chlorhexidine will attach to the cell wall, disrupting the osmotic balance and causing cytoplasmic leakage. If there is stability or an increase in the concentration of chlorhexidine, then chlorhexidine will enter the cell and destroy the cell wall, resulting in lysis and cell death [20]. Chlorhexidine with high concentrations can provide a bactericidal effect, while chlorhexidine with low concentrations can provide a bacteriostatic effect.

In this study, gel *C. domestica* and *M.oleifera* have strong antibacterial activity against *Porphyromonas gingivalis* bacteria. Referring to the research conducted by Rudhra, et al. (2018) stated that the inhibition zone formed in 0.2% turmeric gel was 10.3 mm against *Porphyromonas gingivalis* bacteria so that it was included in the moderate category [21]. This proves that the combination of *C.domestica* and *M.oleifera* has greater potential in inhibiting *Porphyromonas gingivalis* bacteria.

Based on the data obtained, Gel C has a clear zone with the largest diameter compared to Gel A and Gel B. This study is in line with the study conducted by Wardani, et al (2022) regarding the effectiveness of the inhibitory power test of turmeric rhizome extract. *Curcuma domestica* against *Escherichia coli* bacteria with concentrations of 6.25%, 12.5%, 25%, 50%, and 100%, it was proven that the largest inhibition zone was found at a concentration of 100% [22]. This proves that the higher the concentration, the larger the clear zone formed. The increase in the diameter of the clear zone is due to the high secondary metabolite content in the high concentration group [11].

Curcuma domestica and *Moringa oleifera* contains several secondary metabolites that act as antibacterials, thus affecting the clear zone that is formed. *Curcuma domestica* contains curcumin compounds, essential oils, quercetin, flavonoids and alkaloids which have been proven effective in various dental problems, one of which is periodontal disease [23], [24]. *Moringa oleifera* also has active compounds that have antibacterial effects such as alkaloids, flavonoids, saponins, steroids, tannins, and terpenoids [11].

Curcumin and essential oils act as broad-spectrum antimicrobials against gram-negative and gram-positive bacteria, parasites, viruses and fungi. Curcumin has the ability to prevent bacterial

adhesion and inhibit quorum sensing (QS), which contributes significantly to the formation of biofilms [25]. Quercetin works on several strains that are resistant to several drugs. The hydroxyl group in quercetin acts as a mediator in the interaction of compounds with bacterial cells, causing cytoplasmic rupture [26]. Flavonoids can cause cell necrosis by damaging the bacterial cell wall. In addition, alkaloids can also cause cell necrosis due to disruption of enzyme activity caused by denatured proteins [27].

Active compounds *Moringa oleifera* have antibacterial activity with different working mechanisms in each compound. Saponins can cause cell rupture in bacteria caused by increased membrane permeability [28]. Steroids are active compounds that play their role by invading the lipid membrane, thus creating holes in liposomes [29]. Tannins act as inhibitors of DNA topoisomerase and reverse transcriptase enzymes which result in the absence of bacterial cells. Terpenoids have the ability to damage porins by forming strong polymer bonds on the outer membrane of the bacterial cell wall. This condition results in the inhibition or death of bacterial cells due to nutrient deficits and decreased cell wall permeability [30].

The type of bacteria can also affect the increase in the diameter of the clear zone. The cell walls of gram-negative bacteria are more complex than gram-positive, namely they have a peptidoglycan content of 5-10%, lipopolysaccharides and lipoproteins so that they have stronger resistance to antibacterial compounds [31], [32]. This is in line with previous research stating that the antibacterial activity or clear zone of yogurt starter against *E. coli* (gram negative) is lower compared to *S. aureus* (gram positive) [32].

Presearch others showed that the Minimum Inhibitory Concentration of curcumin in turmeric against *P. gingivalis* and *P. intermedia* bacteria was 100 µg/ml or 0.01% [15]. In this study, the concentration of curcumin used was above 0.01%, namely 0.1%, 0.5% and 0.9%.

Conclusion

Black to Pink Gel extract of *Curcuma domestica* and *Moringa oleifera* with concentration of *C. domestica* : *M. oleifera* (0.1%:2%, 0.5%:4%, 0.9%:6%) has strong antibacterial activity against *P. gingivalis* bacteria.

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