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Activity, Formulation and Effectiveness of Black Rice Extract (*Oryza sativa* L) Gel against *Staphylococcus aures* and *Escherichia coli* Bacteria

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Abstract: Black rice (*Oryza sativa* L.) contains chemical compounds, namely ferulic acid, -oryzanol, amino acids, tannins, phenolics, and anthocyanins which have potential as antibacterial agents. This study aims to formulate an antibacterial gel preparation from black rice extract with an extract concentration of 10% and to test the antibacterial effectiveness of the gel preparation against *Staphylococcus aureus* and *Escherichia coli* bacteria. Black rice extract was obtained by maceration using 70% ethanol solvent: 3% citric acid. The extract obtained was then formulated in a gel dosage form. The results showed that black rice extract could be formulated as an antibacterial gel preparation that met the organoleptic, homogeneity, pH, spreadability, and adhesion requirements. In the results of the antibacterial effectiveness test, there is a clear zone which represents the ability to inhibit the growth of the tested bacteria by the gel. Black rice extract can be formulated in a gel dosage form with an extract concentration of 10% and a physically stable formula is obtained in formula III with a carbopol concentration of 2%. The average diameter of the antibacterial gel FIII preparation of black rice extract in inhibiting *Staphylococcus aureus* bacteria was classified as strong, namely 11.17 ± 0.34 mm, while inhibition of *Escherichia coli* bacteria was also relatively strong, namely 12.20 ± 0.46 mm.

Keywords: Antibacterial; Black rice; Gel; *Staphylococcus aureus*; *Escherichia coli*.

Introduction

In general, rice is divided into two types, namely pigmented rice (red, black and brown rice) and non-pigmented rice (white rice) (Mangiri et al.,2016). Based on its pigmentation, black rice has high bioactive compounds including tocopherols, ferulic acid, -oryzanol, amino acids, flavones, tannins and phenolic compounds (Chakuton et al., 2012). In addition, black rice also has constituent components that are responsible for the formation of pigments, namely anthocyanins. Research on the bioactive compounds contained in pigmented rice shows that black rice contains the highest anthocyanin compared to other pigmented rice. Anthocyanins belong to a class of flavonoid derivatives that have antibacterial activity by inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting bacterial metabolism (Zahroh and Agustini, 2021).

Escherichia coli bacteria are gram negative bacteria are generally known to occur normally in the human gastrointestinal tract. The prevalence of mortality caused by diarrhea is 3.04% (Fariani and Advinda, 2021). While the *Staphylococcus aureus* bacteria is one of the

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bacteria that causes skin infections to eye infections. Currently, *Staphylococcus aureus* is becoming a very serious problem due to the increased resistance of this bacterium to various types of antibiotics. (Mahmudah *et al*., 2013).

Gels are semi-solid preparations consisting of suspensions made up of small inorganic particles or large organic molecules penetrated by a liquid. Gel preparations have advantages such as having high viscosity and adhesion so they do not flow easily on the surface of the skin, having thixotropic properties so that they are easily spread evenly when smeared, do not leave marks, only form a thin layer like a film when used, are easily washed off with water, and are able to penetrate further than creams, it is very good for use on hairy areas and is preferred cosmetically, gels can melt when in contact with the skin and form a layer and their absorption on the skin is better than creams (Rosida *et al*,, 2018).

The use of black rice extract in gel dosage form as an antibacterial has never been reported, so this study tested the activity, formulation and effectiveness of black rice extract gel as an antibacterial.

Method

Tools and Materials

The tools used in this study were autoclaves, cotton swabs, petri dishes, Erlenmeyer (Pyrex®), Measuring cups (Pyrex®), Incubators (Mammet®), Calipers, Laminar Air Flow (LAF) (Nuaire®), Spirit lamps , Circular ose, Oven (Mummert®), Tweezers, Test Tube (Pyrex®), Analytical balance, Mortar and pestle.

The material used in this research is Aquadest, Bacteria *Staphylococcus aureus* and *Escherichia coli*, Black Rice, DMSO 10 %, Mueller Hinton Agar (MHA), 0.9 % NaCl, Handsanitizer gel, Carbopol 940, Propylene glycol, Triethanolamine (TEA).

Sample Processing

Collection of black rice (*Oryza sativa* L.) samples obtained from Nanggala District, Tana Toraja Regency, South Sulawesi. Black rice samples were washed with running water and dried in an oven at 50°C. The black rice was crushed using a mortar to reduce the particle size which was then sieved with a 10 mesh sieve to equalize the particle size. Furthermore, the powder is stored in a dry place, tightly closed and protected from sunlight and ready for extraction.

Preparation of black rice extract (Oryza sativa L.)

Black rice powder is weighed as much as 500 grams. Then the powder is put into the maceration vessel. Then the sample was added and solvent was added with a ratio of 1:3, 1.275 mL of 70% ethanol and 225 mL of 3% citric acid, with a ratio of 85:15. Then stored for 3 x 24 hours with occasional stirring. The results of maceration are filtered using filter paper. Furthermore, the macerated samples were filtered again and combined with the first filtrate. Then the combined filtrate is evaporated using a fan for 3-5 days. The sample obtained a thick extract.

Preparation of Mueller Hinton Agar (MHA) Medium

Mueller Hinton Agar (MHA) was weighed as much as 38 grams put into Erlenmeyer, dissolved with distilled water and heated until all ingredients dissolved. Then the volume was sufficient with distilled water up to 1000 mL. Erlenmeyer closed with cotton wrapped in sterile gauze. Sterilize the media in the autoclave at 121°C for 15 minutes (Akinduti *et al*., 2022).

Preparation and rejuvenation of Test Bacteria

The bacteria used were isolates of *Staphylococcus aureus* bacteria and *Escherichia coli*. The test bacterial stocks of *Staphylococcus aureus* and *Escherichia coli* were taken as much as one ose, streaked aseptically on slanted media in a test tube containing media, covered with cotton and placed at an angle of 30-45° in the incubator and incubated for 1x24 hours at 37°C After incubation, the bacteria can be used as test microbes (Akinduti *et al.*, 2022).

Preparation of Test Bacterial Suspension

The test bacteria were taken as much as one ose mixed into 5 mL of 0.9% NaCl then homogenized. The bacterial suspension was then equalized for its turbidity with 0.5 McFarland standard solution.

Black Rice Extract Activity Test

Black rice extract activity was tested with variations in extract concentrations namely 1%, 3%, 5%, 7%, and 10%. Then prepared 24 petri dishes for replication in each comparison and poured 10 mL of MHA media into each petri dish until it solidified, then the test bacterial suspension was spread evenly on each petri dish and then placed disc paper that had been soaked with the respective extract solution. – each sample with a concentration of 10% with a certain calculation. And the positive control used tetracycline paper disks and the negative control 10% DMSO. After testing, all plates were incubated at 37^{o}C for 24 hours. The diameter of the clear zone formed around the disc paper was observed in each comparison and then measured with a caliper. The clear zone was the inhibition zone (McMurray *et al.*, 2020). Design of Black Rice Extract Gel Preparation Formula.

Table 1. Black Rice Ethanol Extract Gel Formula

Ingredient	Formulation (%)			
	I	II	III	IV
Black rice extract	10	10	10	
Carbopol® 940	0,5	1	2	2
TEA	15	15	15	15
Propilenglikol	10	10	10	10
DMDM Hydantoin	0.1	0.1	0.1	0.1
Aquadest	ad 100	ad 100	ad 100	ad 100

Preparation of Gel Preparations

The way of making gel is carbopol dispersed in 1x24 hours aquadest until a thick and clear solution is formed, then add TEA little by little until a stable gel mass is formed. The black rice extract was dissolved using proplenglykol, then added to the base, stirring until homogeneous. After that the gel is stored in a closed container.

Evaluation of Extract Gels

Organoleptic test

Organoleptic tests were carried out to see the physical appearance of the preparations by observing the shape, color and aroma of the preparations that had been made (M Campolo *et al.*, 2022).

Homogeneity Test

The homogeneity test was carried out by smearing the gel on a transparent glass where the preparation was taken in 3 parts, namely the top, middle and bottom. Homogeneity is indicated by the absence of coarse grains (Sarukh *et al.*, 2019).

pH test

The pH of the gel preparation was measured using a pH meter, dipped into the diluted gel sample, let stand for a while and the results were adjusted to the standard skin pH, namely in the interval of 4.5-6.5 (Sarukh *et al.*, 2019).

Viscosity Test

Viscosity measurements were made by placing a number of samples in a Brookfield Viscometer at 3 rpm (rotations per minute) using "spindle" no.64. The spindle is dipped in the gel preparation that has been made (M Campolo *et al.*, 2022).

Spreadability Test

The spreadability test was carried out by weighing 0.5 gram of gel and then placing it on a glass and overlapping it with another transparent ballast. Then let it stand for 1 minute and measure the diameter (Merchyta Winarjo *et al.*, 2017)

Stickiness Test

The adhesion test was carried out by weighing a sample of 0.25 grams placed between 2 glass objects, then pressing it with a 1 kg load above it and leaving it for 5 minutes. After that the glass object was placed on the tool and the weight weighing 80 grams was released, then the time was recorded until the glass object was released (Merchyta Winarjo *et al.*, 2017).

Antibacterial Effectiveness Test of Black Rice Extract Gel Preparations

Testing the effectiveness of Black Rice (*Oryza sativa* L.) extract gel preparation on the growth of *Staphylococcus aureus* and *Escherichia coli* was carried out using the agar diffusion method (well method) using MHA medium. MHA medium was poured into a sterile petri dish as much as 5 ml for the base layer and then allowed to solidify (base layer).

After the medium has solidified, 3 reservoirs (wells) are placed on the surface of the base layer of the medium, which are spaced so that the observer areas do not grow together, then 15 ml of medium which has been inoculated with bacterial suspension for the second layer (seed layer) is added. aseptically from a petri dish, put in a stable gel preparation (1% concentration). Hand sanitizer gel (positive control) and gel base (negative control). Then incubated at 37^{o}C for 1×24 hours. The clear zone was observed to form and the diameter of the inhibition area was measured with a caliper. This treatment was carried out 3 times and the average were taken.

Result and Discussion

This research was carried out using black rice samples from Nanggala District, Tana Toraja Regency, South Sulawesi. Black rice powder was macerated with 70% ethanol with the addition of 3% citric acid then allowed to stand for 24 hours. The maceration method is used because it is easy and does not need heating so that natural materials are less likely to be damaged or decomposed and the maceration method takes a long time and the silence during maceration allows many compounds to be extracted. The use of ethanol and citric acid solvents in black rice produces the most intense red color due to protonation of anthocyanins in an ethanol solution which produces flavilium cations at low pH (Kim *et al.*, 2019). The results obtained from the extraction are the characteristic odor of the extract and the purple-black color.

Table 2. Inhibition Zone Extract

Bacterial	Inhibition zone diameter extract (mm)					
	3%	5%	7%	10%	C -	C +
S.aureus	6.4	6.65	8.57	11.95	0	28.11
E. coli	6.11	6.41	6.58	11.65	0	27.31

Based on activity testing on *Staphylococcus aureus* bacteria, the results obtained were the inhibition zone formed was measured using a caliper, the average inhibition diameter for 3% concentration was 6.24 mm, 5% concentration was 6.65 mm, 7% concentration was 8.57 mm, and 10% concentration of 11.95 mm. Inhibition at the four concentrations can be said to be in the very strong category while the positive control average inhibition diameter of 28.11 mm and 6 mm negative control there is no inhibition zone. Whereas in the activity test on Escherichia coli bacteria, the results obtained were the inhibition zone formed was measured using a caliper, the average inhibition diameter for 3% concentration was 6.11 mm, 5% concentration was 6.41 mm, 7% concentration of 6.58 mm, and 10% concentration of 11.65 mm. The inhibition power at these four concentrations can be said to be in the very strong category, while the positive control average inhibition diameter is 27.31 mm and the negative control is 6 mm. There is no inhibition zone in this study. as a negative control and tetracycline as a positive control. In this study, MHA medium was used because it has good nutritional content for most bacterial cultures. In addition, it is also neutral, so it does not affect the antibacterial test procedure. The choice of DMSO as a negative control was because according to Natheer et al, 2012 that the substance used as a negative control was a solvent used as a compound diluent. In this study DMSO was used as a solvent. So that DMSO is used as a negative control for the purpose of comparison that the solvent used as a diluent does not affect the results of the antibacterial test of the compound to be tested. Tetracycline was chosen as the positive control because tetracycline is a broad-spectrum antibiotic that can inhibit almost all gram-negative and positive bacteria. The antibacterial activity of the ethanol extract of tea leaves is due to the presence of secondary metabolites. The active compounds in tea leaves are catechins. Catechins have antibacterial properties which have a working mechanism of inhibiting fatty acid synthesis in bacteria and inhibiting the production of toxin metabolites in bacteria (Pattananandecha *et al.*, 2021).

This extract is then formulated into a gel preparation with an extract concentration of 10%. The reason for using an extract concentration of 10% is based on the results of activity tests conducted by previous studies, the inhibition power at a concentration of 10% is more optimal than other concentrations. From the results of the formulation, an evaluation of the physical stability of the gel preparations was carried out. The evaluation was carried out to obtain physically and chemically stable preparations, while the evaluation carried out was the physical stability of the gel including organoleptic examination, homogeneity examination, pH examination, viscosity test, spreadability test and adhesion test.

Table 3. Evaluation Gel

Parameter	F1	F2	F3	F4
Organoleptic				
- Colour				
- Scent	Purple	Purple	Purple	Clear
- Form	No smell	No smell	No smell	No smell
	Semi solid	Semi solid	Semi solid	Semi solid
Homogenity	homogeneous	homogeneous	homogeneous	homogeneous
pH	8.5	6.6	5.24	6.11
Viscosity	15	38.5	40.5	31
Spreading power	8.21 cm	7.57 cm	6.14 cm	5.6 cm
Stickiness	0.9 s	1 s	1.29 s	2.92 s

Organoleptic testing is a test based on the five senses. The organoleptic test aims to see the suitability of aroma, color and shape, which is as close as possible to the preparation specifications that have been determined during formulation. From the results of research F1, F2 and F3 the purple colored gel and the distinctive aroma of buni, this was due to the addition of 10% oryza sativa extract which resulted in the color of the gel being purple.

Homogeneity testing which aims to find out the active substances and additives are well mixed (homogeneous), which is characterized by the absence of visible coarse grains (Sarukh *et al.*, 2019). The homogeneity test results for all formulas produce a homogeneous mixture.

Examination of the pH of gel preparations aims to ensure that the pH of the gel matches the pH of the skin so that it does not cause irritation when used. The pH

test of the preparation was carried out using a pH meter. The results of measurements carried out on formula 3 are included in the skin pH range, where the skin pH is 4-6. Meanwhile, F1 and F2 are not included in the pH range.

Viscosity testing aims to determine the value of the viscosity of a substance. The higher the viscosity value, the higher the viscosity of the substance. In testing the viscosity of the gel, there was an increase in the viscosity of each formula. This refers to its physical stability. The smaller the change in the viscosity of the gel, the more stable the gel will be (Nurman *et al.*, 2019).

The spreading power test aims to determine the spreading power of the gel on the skin. In testing the spreadability of the gel, the results of the spreadability were obtained, namely in F1, F2 and F3 respectively, namely 8.21; 7.57; 6,14. The difference in spreadability is due to the higher concentration of carbopol, the smaller the resulting spreading area due to the increase in viscosity. Based on the results obtained in accordance with the literature which states that the good spread of the gel is between 5-7 cm.

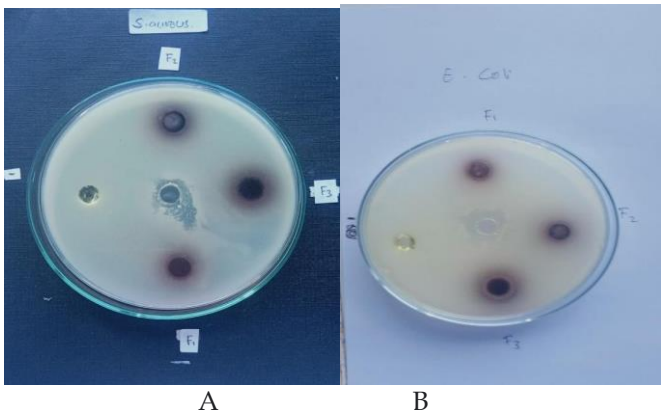
The adhesion test aims to determine the ability of the gel to stick to the skin. In the gel adhesion test, the results were obtained on F1, F2, F3, namely 0.90, 1.00, 1.29 seconds. The difference in base concentrations used has different results in the measurement of adhesion test by showing that the higher the concentration of carbopol, the longer the adhesive time of the resulting gel due to an increase in viscosity. Based on the results obtained from the literature that the adhesion requirement is more than 1 second (Bono, Anisuzzaman and Ding, 2014).

Testing the effectiveness of gel preparations with an extract concentration of 10% was carried out on Staphylococcus aureus and Escherichia coli using the well or scavenger method. The results of diameter measurements can be seen in the Table 4.

Table 4. Inhibition zone diameter

Bacterial	Inhibition zone diameter (mm)				
	F1	F2	F3	F4	C +
S. aureus	8.94 ±0.25	9.93 ±0.67	11.17± 0.341	0±0	14.16 ±1.26
E. coli	8.29 ±0.86	9.14 ±1.09	12.20 ±0.46	0±0	18.61 ±4.75

The positive control used in this study was a hand sanitizer gel on the market and the negative control used was a gel formula without extract (F4). In testing against *Staphylococcus aureus* and *Escherichia coli* in the gel formula an inhibition zone was formed, this shows that after the extract is in the formula in the gel dosage form it still has antibacterial power, where FIII is included in the strong antibacterial category.



A B

Figure 1. Zone of Inhibition on the Gel (A) *Staphylococcus aureus* (B) *Escherichia coli*

Conclusion

Based on the results of the study, it was concluded that: (1) Black rice extract (*Oryza sativa* L.) can be formulated in a gel dosage form with an extract concentration of 10% and a physically stable formula is obtained in formula III with a carbopol concentration of 2%; (2) Testing the effectiveness of black rice (*Oryza sativa* L. indica) gel preparation against *Staphylococcus aureus* and *Escherichia coli* resulted in an optimal inhibition zone in formula III with an average diameter of the strong category of inhibition zone against *Streptococcus aureus* 11.17 ± 0.34 mm, while *Escherichia coli* produced a relatively strong inhibition zone with an average inhibition zone diameter of 12.20 ± 0.46 mm. The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section

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Author Contributions
N.K formulation and evaluation of gel preparations; S.H microbiological testing; M.L analyzes data; M.R extraction.

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Conflicts of Interest
The authors declare no conflict of interest

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