

study and evaluation of the oxidative, inflammatory, and microbial activity of anthocyanin pigment  
in purple rice

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## **Study and Evaluation of the Oxidative, Inflammatory, and Microbial Activity of Anthocyanin Pigment in Purple Rice**

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### **Abstract:**

Samples are collected, which represents taking varieties of purple sticky and non-glutinous rice, where the samples are extracted using an ethanol solution employing high-performance liquid chromatography and (TPC), (TFC), (the content of flavonoids, antioxidants, and antibacterial) against food-borne diseases. The extract contains high levels of High cyanidin 3-O-cyanidin 3-O-glucoside. In addition, by studying their activity of antioxidants through a test ABTS test (524.26 4.63, mg L-ascorbic-ascorbic/g extract), oxidative stress (IC<sub>50</sub> = 19.70 0.31 g/mL), radical (IC<sub>50</sub> = 11.20 0.25 g/mL), nitric oxide (IC<sub>50</sub> = 17.12 0.56 g/ml), a nitric oxide inhibition action (IC<sub>50</sub> = 18.32 0.82 g/mL), and in Monocyte lymphocytes from animals. Furthermore, KGLP shown antibacterial efficacy towards pathogenic organisms such as Staphylococci, E. coli, Salmonella, and Vibrio. Their findings suggest that Thai sticky violet wheat-grown highlands might be a valuable biodiversity source of bioactive compounds, pro-government, and antimicrobials suitable for usage as a natural active drug component in healthy ingredients and nutritional solutions.

**Keywords:**rice violet; anthocyanin, ABTS, TPC, TFC, E. coli, Salmonella, LC<sub>50</sub>,

### **Introduction:**

Oxidative stress is atoms that have one maybe more unattached electrons in the outermost valence and are highly reactive to biomolecules such as lipid, Genetic material, or peptides. [1]Oxidizing agents harm physiological functioning, as well as generate substances such as metals and poisonous chemicals [2]. Reactive Oxygen Types and Volatile Nitrogen Types Diabetic, atherosclerotic & inflammation illnesses, brain conditions, tumors, as well as other illnesses are all examples of chronic diseases [3]. Antibodies are thought to be extremely efficient in controlling either ROS or RNS activities [4]. It plays critical functions in a variety of biochemical functions by initiating change chemicals such as hydrogen peroxide, peroxides, oxidants soluble result in producing fringe groups, and nitric oxide. According to the potential development of radicals to combat the radicals existing in the system of the living thing, including such glutathione, ubiquinone, superoxide, catalase, and so on, or from food and pharmaceutical applications [3-5]. Oxidation found in plants it is mostly made up of polyphenols, flavonoids, carotenoids, and vitamins [6]. Antioxidant compounds abound in traditional medicines. Therefore, chemicals generated by diverse bacteria are exploited as a biological action. Nutritional and Pharmacological Substances (APIs) to Optimize Health of individuals [7-8], Vitamins, generally, pro agents, anti-microbial agents, and pro agents are examples [9], Rice has more proteins, vitamins, elements, and beneficial compounds than other grains. Rice is

comparable. Therefore, the concentration of bioactive metabolites varies by location. [10] Varietal and varietal Violet rice is regarded as a healthy meal, particularly in Thailand. It is regarded as a rich potent antioxidant and health-promoting chemicals [11]Anthocyanin, tocopherols, tocopherols are all antioxidants. These areautomobiles. It has been shown to regulate and mitigate disorders in public health, including oxidationincluding cancer, through a range of biochemical actions. [Purple rice is believed to have superior nutritional and health advantages versus colored rice. fixed Cambodia, like other Asian nations, grows and consumes huge amounts of food. Resources of antioxidants for research and development refer to biological constituents and physiological activities [12-13].The goal of this research is to demonstrate that Thai blue rice is a naturally derived source of natural antioxidants for preventing disease. The objective is to move from roots that were oxidizing to the end goal of some other antioxidants. Analysis results from anthocyanin and their composition, as well as doing cell-based studies on anti-inflammatory and antibacterial action versus food-borne pathogens.

## 2. Results and Discussion: -

### 2.1. Anthocyanin Levels:

Anthocyanin is a source of anti-inflammatory and oxidative stress, which is the anthocyanin pigment found in the outer shells of grains, where the concentration of the purple-colored anthocyanin in this research, as well as cyanidin glucoside, peonidin -glucoside as shown in the table1 below.C3G, P3G, and D3G are the abbreviations for C3G, P3G, and D3G, respectively. According to research, paddy that appears persistently black has higher levels of pigment [17-18]. The nutritive benefits of northern Thai violet rice grown at different altitudes were studied. In the highlands, someGrain varieties create grains with increased color and production.

In the lowlands, only a few plants had polymeric anthocyanin concentrations [19]. Anthocyanin content was calculated in 25 ricerecommendations from 25 different rice cultivars. The outcomes the largest anthocyanin discovered is cyanidinglucoside (82.3 percent) [17-25].C3G and P3G levels including whole grains KGPEK, KGDSK, and KHN were found to be 89and 33, 50 and 49, and 135 and 22 mg/100 g dried materialsequally [20–22]. Furthermore, C3G and P3G levels in KGLP and KHN wheat grainwere observed to be 2277 and 792 mg/100 g tests, correspondingly [24-26].According to research, the anthocyanin content of violet rice increases exponentially. So, while most violet rice types are conventional rice throughout regions and growth [27-28]. Shows Table 1 the anthocyanin level in violet rice extracts.

**Table 1 the anthocyanin level in violet rice extracts.**

Samples	Cyanidin 3-O-Glucoside (mg/g Extract)	Peonidin 3-O-Glucoside (mg/g extract)	Delphinidin 3-O- Glucoside (mg/g Extract)
KGLP	55.26 ± 0.71 <sup>a</sup>	14.24 ± 0.46 <sup>b</sup>	1.95 ± 0.13 <sup>a</sup>
KGPEK	52.20 ± 0.89 <sup>b</sup>	15.91 ± 0.47 <sup>a</sup>	1.55 ± 0.12 <sup>b</sup>
KGDSK	29.62 ± 0.80 <sup>c</sup>	10.48 ± 0.38 <sup>c</sup>	ND
KND	28.91 ± 0.74 <sup>cd</sup>	10.70 ± 0.40 <sup>c</sup>	ND
KHN	27.04 ± 0.68 <sup>d</sup>	8.54 ± 0.31 <sup>d</sup>	ND

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## 2.2. Total Phenolic and Total Flavonoid Content:

Grain is high in polyphenolic compounds. Hyperpigmented wheat includes solely phenolic acids, but colored rice includes more compounds than polyphenols [13]. In Table 2. Vital, TPC and TFC levels differed amongst 5 rice varieties. KGLP has been demonstrated, TPC and TFC had considerably greater ( $p < 0.05$ ) GAE/g isolate ( $595.53 \pm 7.36$  mg GAE/g extracts and  $379.35 \pm 4.26$  mg QE/g extraction) than KGPEK, which had  $570.49 \pm 6.53$  mg GAE/g extract of TPC and  $340.24 \pm 3.64$  mg QAE/g remove of TFC. Although KHN has the least TPC while KGDSK has the highest TFC.

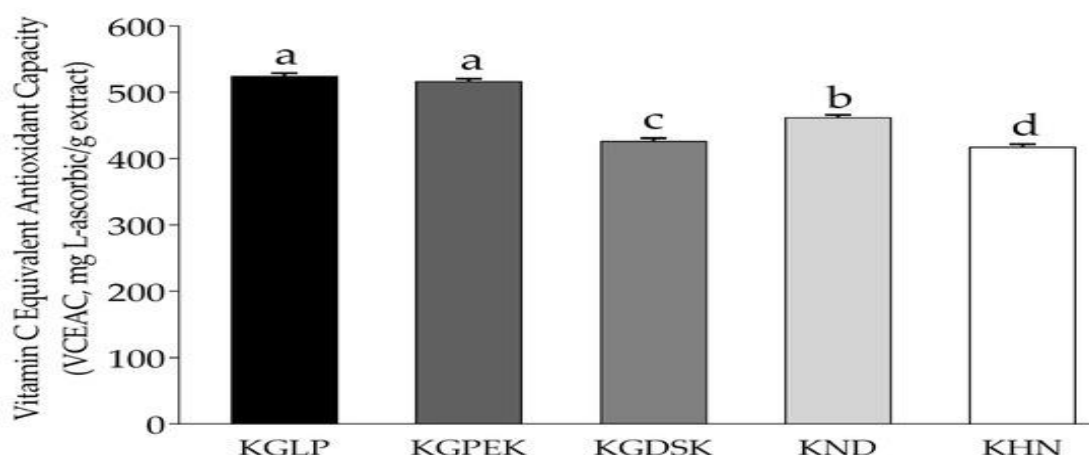
The findings coincide also with the total flavonoids identified in Highlanders rice isolates (KGLP and KGPEK) that have had more TFC than lowlands rice extracted (KGDSK, KGPEK). (KND and KHN) Furthermore, sticky rice isolates (KGLP, KGPEK, KGDSK, etc.) KND) has more TPC & TFC than non-glutinous rice extract (KHN). TPC, as well as TFC, was greater in KGLP than the one in KGDSK and KHN, which is consistent with the prior study [25,23] The study found Obtains of sticky violet rice bran TPC levels were greater in KGLP than in KHN, a non-glutinous violet modified rice extraction. TFC levels appear to be greater in KHN rice bran concentrate than in KGLP rice isolated Gallic acid was the most prevalent phenolic chemicals [29], whereas anthocyanin is now the most common flavonoid in colored rice. Furthermore, and flavonoids were shown to be the least prevalent, but eugenol was revealed to be the most prevalent flavone element in pigmented rice [30]. See Table 2. The value of the variable the phenolic compounds and flavonoids levels in pigmented grain separates. Integrating many avatars in almost the same game provides significant competition.

**Table 2. The value of the variable the phenolic compounds and flavonoids levels in pigmented grain separates.**

Samples	Total Phenolic Content (TPC) (mg GAE/g Extract)	Total Flavonoid Content (TFC) (mg QE/g Extract)
KGLP	$595.53 \pm 7.36^a$	$379.35 \pm 4.26^a$
KGPEK	$570.49 \pm 6.53^b$	$340.24 \pm 3.64^b$
KGDSK	$489.39 \pm 5.16^d$	$291.93 \pm 3.99^d$
KND	$533.91 \pm 5.54^c$	$323.21 \pm 4.74^c$
KHN	$451.81 \pm 4.85^e$	$286.40 \pm 3.82^d$

## 2.3. Test for Antioxidants:

ABTS evaluates the ascorbic acid content for a well-extracted microbiological analysis component [31]. Whereas Figure 1 indicated that KGLP and KGPEK had considerably greater radical action on  $ABTS \cdot +$ , Fig.2 revealed that substantially lower radical isolate. These findings are comparable with those of prior research by Pramai [25]. The activities stated by KGLP also have much greater  $ABTS \cdot +$  Total antioxidant activities than KGDSK & KHN. Show in figure 1.



**Fig. 1** Illustrates the oxygen radical absorbance ability of violet rice isolates against ABTS.

Material 2021, 26, x for peer riverTable 3 reveals that KGLP exhibited the strongest peroxidation and nitric oxide reduction ability, with  $I_{c50}$  of 19.70 0.31 and 17.12 0.56 (g/ml), respectively, and the results were not radically different for KGLP and KGPEK demonstrated considerably greater with  $I_{c50}$  values of 11.20 0.25 and 11.96 0.65 (g/mL), correspondingly. ABTS • + maximum removal was revealed to have a proportional connection utilizing  $r^2$  0.960, 0.935, 0.893, 0.826, and 0.916;  $P$  0.01, correspondingly. Such findings might imply that radicals ABTS • + and nitric oxide and supercharged ionic cleansing capabilities, as well as an inhibitory impact on lipid peroxidation in violet rice isolates, correlate with anthocyanin, TPC, and TFC. [32]. Arrived out a similar investigation. KGDSK and other sticky rice varieties from Thailand were tested. Show Table 3:  $I_{c50}$  for oxidative damage inhibition via nitric oxide all parameters are presented as an average absolute variation. Various letters within every approach imply a significant variation of less than 0.05.

**Table 3:  $I_{c50}$  for oxidative damage inhibition via nitric oxide all parameters are presented as an average absolute variation.**

Samples/ Positive Control	Lipid Peroxidation	Superoxide Anion	Nitric Oxide
KGLP	19.70 ± 0.31 c	11.20 ± 0.25 c	17.12 ± 0.56 c
KGPEK	21.45 ± 0.38 b	11.96 ± 0.65 c	18.81 ± 0.33 b
KGDSK	21.62 ± 0.37 b	14.78 ± 0.30 ab	19.38 ± 0.38 b
KND	25.00 ± 0.31 a	15.60 ± 0.45 a	22.31 ± 0.36 a
Cyanidin-3-O- glucoside	16.64 ± 0.38 d	9.55 ± 0.34 d	17.54 ± 0.30 c
L-ascorbic acid	ND	7.55 ± 0.31 e	ND
Curcumin	ND	ND	6.68 ± 0.28 e

TPC and TFC content as well as antimicrobial properties versus Nitric oxide, peroxide, researchers discovered that the proportion of TAC, TFC, and TPC linked with extraction and antioxidant effects. Phenols function as reductants, predators of free radicals, and oxygen generators. In contrast to a greater amount of flavonoids and polyphenols, colored grain variants demonstrated

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significant[33].Past research has shown that ant oxidative capabilities are sensitive to TPC and TFC in tests. The heritable traits of various varieties, storage temperature, and measurement methodologies are all major contributors to the wide range of powerful antioxidants [34].

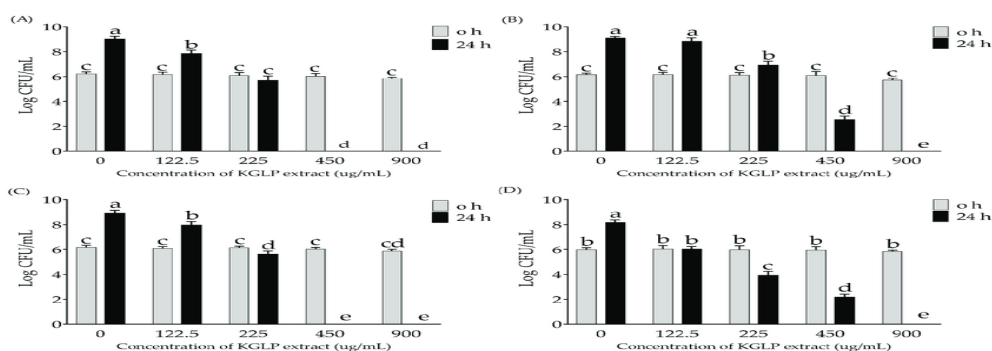
### 2.4. Anti-Inflammatory Activities Assay:

Purple rice's impact on iNOS generation in concurrent lipopolysaccharide (LPS)-interferonactivation Table4 illustrates this. KGLP inhibited both intracellular NO and the most (p 0.05), with IC50 values of 18.32 0.82 and 23.43 1.21 g/mL, respectively.

There was also a link discovered between TPC and peonidin 3-O-glucoside resistance. Impact of coupled LPS-IFN on NO and iNOS production cells as seen in Table S1.AR C3G and its metabolic products isolated from rice were found to inhibit the manufacturing of inflammatory cytokines such as tumor necrosis component, chemokines (NO and prostaglandin E2) expression in molecules [35]. It has been demonstrated that 9 flavonoids impede activity. It contains tumor necrosis factor B (NF-B), a transcription factor necessary for the iNOS pathway. It has also triggered the signal transducers and regulators [36].

### 2.5.Foodborne Pathogenic Organisms Antibacterial Activities:

Usually, diseases are carried through eating, which is also the major cause of various disorders and, as a consequence, an increase in mortality. The much more prevalent organisms that cause food contamination episodes are *S. aureus*, *E. coli*, *S. intestinal blockages*, (log CFU/ml) of viruses that were cultured at successive dosages throughout 24 hr. Derived using the KGLP method. The results are depicted in Fig. 2. The antimicrobial effect was shown to be related to the quantity of KGLP employed during the study. Alenteritis was significantly (p 0.05) decreased by 90 percent. KGLP at 900 mcg/mL completely reduced *E. coli* and *E. coli* even during the test.Purple rice isolation has been proven to be an antibiotic disaccharide that is capable of killing microbes such as *S. aureus*, *E. coli*, *S. enteritis's*, and *V. parahaemolyticus* [39-42].Gram-positive microorganisms are much more susceptible to anthocyanin impacts than *Staphylococci* microorganisms. [34]The findings demonstrated that violet grain extraction has the potential to be utilized as a potent antioxidant to suppress the development of bacteria species.



**Fig. 2 the antibacterial effect of KGLP isolates against *E. coli*, *S. aureus* (A), *Escherichia coli* (B), *Escherichia coli* (C), and *Vibrio cholera* (D). The characteristics are stated for p 0.05, implementing various letters shows a substantial variation.**

## **Martial and methods: -**

### **3.1. Preparation of Purple Rice Extract:**

Plant-derived anthocyanin has effects that interact with microbial cell membranes via ionic bonding and water interactions. It is capable of isolating ions critical to the protein level and has always been removed and kept at -20°C until needed. Grain extracts were prepared using ethanol pH 2.0 1:10 for 120 min at 60 °C while shaken at 180 pm. Samples were concentrated and condensed and also placed before being recovered in the vacuum desiccator. Table 5 illustrates this.

### **3.2.HPLC Anthocyanin Determination:**

In the HPLC system, Symmetric Shields C-18 columns (4.6 x 250 mm) and a multi-wavelength to filter the data from columns yet again, a persistent logistic gradient was applied. The phase lasts between 0 and 30 minutes, with methanol concentrations ranging from 10% to 20%, after which the isolated anthocyanin is detected and evaluated at 520 nm.

### **3.3. Total Phenolic Contents Assessment:**

To analysis is being used to ascertain the density of the product's phenolic content. [45]. 100 L of the test (1 mg/mL) and the conventional answer was scaled back to 2 mL, utilizing freshwater well mixed with 100 ml of the reaction mixture, and 300 L of 20% (w/v) sodium chloride was added. The solution was incubated at 37 temperatures for 9 min at 37 °C before being measured for absorbance at 725 nm with a spectrometer with Ultraviolet wavelengths. The dry extract sample was obtained.

### **3.4. Determination of Total Flavonoid Content:**

#### **3.5. Antioxidant Assay: -**

The flavonoids used in the research were pure [46]. In a nutshell, one milliliter (1 mL) of the substance to be studied is collected. The material was expected to stand for 5 minutes before being tested. Then, 2 ml of NaOH extract was placed, yielding a final book of after ten minutes of mixing, the solution was diluted to 10 ml with ethanol and the absorption at 510 absorbance value was measured. Total flavonoid level the procedure has been used to compute it, and these outcomes are communicated flavonoid Contemporaries per g dry removal.

#### **3.5.1. Lipid Peroxidation Assay:**

Tris-HCl, a linoleic acid surfactant, Ascorbic acid, flavonoid, and the C3G test were used in reactive mixtures. In addition, ferrous sulfate was added and incubated for 1 at 37 °C than add trichloroacetic acid. A part of 500 l is added to the mixed solution, which is then warmed for ten min at 95 °C until chilling in frizz. The homogenate for fifteen min at 3500 rpm, collected, and the absorbance at 540 nm was determined. Activity that inhibits.

#### **3.5.2. Superoxide Anion Scavenging Activity Assay:**

It is made from a non-enzymatic substance. The phenazine meth sulfate-nicotinamide adenine dinucleotide complex is evaluated using nitro blue tetrazolium reduction. The experiment has been conducted in a 96-well plate with 200 l of phosphorus saline at pH 7.4 containing 1.5 NADH, 0.5 M

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NBT, 2.5 M EDTA, and different doses of quercetin test. I was in the midst of my menstrual cycle. It is administered to start the procedure, which is then kept at 37c. The resultant combination was then spectrophotometric analyzed at 560 nm to estimate

### 3.5.3. Test for Nitric Oxide Scavenging Ability:

The activity was assessed by the presence of sodium nitroprusside with oxygen, followed by nitrate ions from the Griess reaction. With a capacity of 200 liters, the reaction mixture comprises 800 liters of sodium nitroprusside and varying concentrations of chemicals such as quercetin, C3G, and curcumin. After heating the reaction mixture for 120 minutes at 37 °C, the sample solutions were mixed with the iodine solution. Absorption of the chromium carrier formed following nitrite diazotization was detected at 540 nm using Griess reagent, and the IC50 was estimated by comparing it to the positive control.

### 3.6. Anti-Inflammatory Activity Determination:

RAW 264.7 cultures were incubated in Cells that were grown Eagle mixture (DMEM) with 10% serum, 100 unit's/ml penicillin, and 100 microgram doses streptomycin. For 24hrs, cells were cultured in 24 well plates to test for anti-inflammatory activity. Sterile media with various amounts of Curcumin, quercetin, and C3G samples were administered to the cells. Introduced after 12 h of reproduction and maintained for 70 hours. The filtrates were gathered and analyzed for nitric oxide using the Griess reaction. The colonies are also deconstructed to produce cellular lysates, and the amount of iNOS produced is quantified. The mice ELISA for internal iNOS is available on the market. The molecule is generated also examined using Bradford reagent to measure cell survival, and the influence of the samples or standard solution on viable cells following stimulation is assessed. For 72 hours, LPS-IFN-in the condition of violet rice isolates was used as a cell growth assay. Show Table 4. IC50 for nitrite and iNOS reduction, all data are reported as average absolute deviation.

**Table 4. IC50 for nitrite and iNOS reduction, as average absolute deviation.**

Samples/ Positive Control	IC <sub>50</sub> (µg/mL)	
	Nitric Oxide	iNOS
KGLP	18.32 ± 0.82 <sup>d</sup>	23.43 ± 1.21 <sup>d</sup>
KGPEK	20.34 ± 0.98 <sup>cd</sup>	24.66 ± 0.87 <sup>c</sup>
KGDSK	24.50 ± 0.97 <sup>b</sup>	29.43 ± 0.98 <sup>ab</sup>
KND	22.54 ± 0.80 <sup>bc</sup>	27.94 ± 1.17 <sup>b</sup>
KHN	29.66 ± 0.91 <sup>a</sup>	31.74 ± 1.32 <sup>a</sup>
Quercetin	15.86 ± 0.67 <sup>e</sup>	20.61 ± 1.18 <sup>d</sup>
Curcumin	12.61 ± 0.74 <sup>f</sup>	14.70 ± 0.91 <sup>e</sup>
Cyanidin-3-glucoside	13.48 ± 0.85 <sup>ef</sup>	16.68 ± 0.92 <sup>e</sup>

### 3.7. Activity against Foodborne Pathogens:

DeterAnthocyanin was chosen for its anti-inflammatory and antioxidant characteristics, and also its microbiological activity. Broths Staphylococcus, Escherichia coli ATCC 25922, S. adapted to [39]

and Pelyuntha et al. [49] were used to dilute the material. 200 liters of each form of inoculation were mixed with various amounts of samples (1800 liters), yielding final levels sample was a combination of standard broth and the inspected material, which did not contain any pathogens, whereas the control sample was a combination of standard broth. Most of the test materials Cells for *Listeria monocytogenes* were counted on sterile soy medium at 0 and 24 hours, whereas cell lines for *L. monocytogenes* were counted were rotated at 180 cycles after being mixed with a standard soup and each analyzed microorganisms Antimicrobial Each minute for 24hrs at 37 degrees Celsius The mitochondria then sprang to life.

#### 4. Conclusions: -

The investigation overview covers purple rice types that are devoid of antioxidants and inflammation as a result of numerous tests, including (TPC, TFC) in which the accompanying variations are included. Grains such as KGLP and KGPEK are the best overall and perform much better than (kGpSK-KNP) and non-glutinous rice types (KHN). The findings of this study revealed a connection seen between components of the factory anti-inflammatory and antioxidants, with purple sticky rice (GLP-KGPEK) being one of the ideal scenarios since that contains a significant proportion of anthocyanin, as well as its energetic activity of TFC, TPC, ABTS•+ and its high performance against Free origins. Additionally, discovered KGLP- is one of the antiviral drugs conveyed by foods Bacterial infections such as Aureus, E. coli, and Pseudomonas Violet rice be some of the crops that improve nutritive value, nutraceuticals, and dietary items that are crucial to the individual's health.

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