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Antibacterial, antioxidant and anti-inflammatory activities of red, brown, and black sorghum (*Sorghum bicolor*) cultivated in a dry land area

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Abstract. *Mukkun L, Arung ET, Ismayati M, Kleden YL, Pakan PD, Nguru ESO, Naibaho NM. 2024. Antibacterial, antioxidant and anti-inflammatory activities of red, brown, and black sorghum (*Sorghum bicolor*) cultivated in a dry land area. Biodiversitas 25: 2560-2569.* In an effort to find natural sources for skin treatment, several varieties of sorghum (*Sorghum bicolor* L. Moench) were evaluated. Sorghum is one of the most important food crops, it is mainly grown in semi-arid areas, because of its ability to tolerate both drought and low soil fertility. This study aimed to evaluate the antibacterial, antioxidant, and anti-inflammation properties of seven types of colored sorghums. Two species of bacteria, *Propionibacterium acnes* and *Staphylococcus epidermis*, were used to evaluate the antibacterial properties of sorghum extracts. The results exhibited that sorghum has potent antibacterial activity against *P. acnes*, ranging from very strong to strong, while moderate to high against *S. epidermis*. The red Sumba exhibited very strong antibacterial properties against *P. acnes* and *S. epidermis*. The brown Sumba showed the highest level of antioxidant activity among other types of sorghum, measured at 82.21%. The brown Sabu had high anti-inflammation activity with an IC_{50} value of 70.92 µg/mL. The phenolic compounds found in colored was 57.69 to 64.12% Gallic Acid Equivalent (GAE), dry weight. Six major bioactive compounds were detected using GC-MS, including methyl ester of hexadecanoic acid, n-hexadecanoic acid, methyl ester of 11,14-octadecadienoic acid, 9,12-octadecadienoic acid (Z, Z)-, methyl ester of 4-octadecenoic acid, cis-13,16-docasadienoic acid, aspidospermidin-17-ol, and 1-acetyl-19,21-epoxy-15,16-dimethoxy. Further studies are needed to investigate the effect of compounds extracted from colored sorghums as a commercial anti-acne in the future.

Keywords: Anti-acne, anti-inflammation, antioxidant, colored sorghum, dry land, *Propionibacterium acnes*, *Staphylococcus epidermis*

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is an important food crop that is grown mainly in dry climates, such as in Asia, Africa, and other semi-arid areas. Sorghum production in Indonesia is still low, ranging from only 4,000 to 6,000 tons by 2020. East Nusa Tenggara (ENT) province produces most of the sorghum, accounting for 78% of the total national production in 2020 (Mulyawanti et al. 2023). According to the central government program, a plantation of sorghum at ENT province will be expanded from 4,335 ha in 2022 to 40,000 ha in 2024, with a productivity of 4 tons/ha (Susanti 2024). Hossain et al. (2022) recognize the sorghum crop for its ability to tolerate drought and thrive in soil with low fertility. ENT province has low rainfall (<1000 mm per year), a short rainy season (3-4 months per year), and a long dry season (8-9 months per year) (Mudita 2013).

From a dietary perspective, sorghum is known to contain significant amounts of carbohydrates, particularly Resistant Starch (RS) and soluble dietary fiber (SDS), which have been associated with beneficial effects on human health. This contributes to a lower glycemic index and a decreased risk of developing chronic diseases, such as type 2 diabetes and obesity (Poquette et al. 2014; Hossain et al. 2022). Sorghum contains high concentrations of flavonoids and phenolic acids, making them the most prevalent phenolic chemicals in the plant (Lee et al. 2014; Dia et al. 2016; Mohamed et al. 2022). The phenolic compounds present in sorghum, including phenolic acids, flavonoids, and proanthocyanins, enhance gut microbiota and exhibit a wide range of biological activities, including anti-inflammatory, antioxidant, antithrombotic, and antidiabetic qualities. Pigmented sorghum, due to its rich polyphenol content and high antioxidant properties, can treat neurodegenerative diseases, such as Alzheimer's

disease (Rezaee et al. 2021). The presence of phenolic compounds in colored sorghum means that it has the potential to be used as an anti-microbial, anti-oxidant, and anti-inflammatory. The phenol compounds in colored sorghums are known to have antioxidant (Kumari et al. 2021), anti-microbial (Vora et al. 2018), anti-inflammatory (Arulselvan et al. 2016), anti-cancer (Smolensky et al. 2018; Castro-Jácome et al. 2021), and anti-diabetic (Chung et al. 2011; Park et al. 2012) properties.

Genetic variables and environmental factors in the growth location govern the quantity and quality of these phenolic compounds, which in turn influence the color of sorghum seeds (Zhang et al. 2015; Shen et al. 2018). Research findings revealed the presence of a minimum of 30 sorghum varieties on mainland Timor, with the seed's colors ranging from white/creamy, to black, red, and brown (Mukkun et al. 2018). White sorghums are used as primary ingredients for food and flour production, whereas colored sorghums are often employed as animal feed. This is because colored sorghums possess a flavor that is less preferred by humans due to the presence of tannin (Espitia-Hernández et al. 2022; Mohamed et al. 2022).

The excessive growth of *Propionibacterium acnes* and *Staphylococcus epidermis*, commensal skin bacterium, has been associated with the progression of *Acne vulgaris* (Wang et al. 2014). Prior research has established a correlation between oxidative stress and skin conditions, specifically inflammation (Kardeh et al. 2019). Reyna-Reyna et al. (2023) discovered that sorghum extract had a strong inhibitory effect on *Escherichia coli*. Chen et al. (2022) discovered that polyphenol extract derived from colored sorghum exhibited antibacterial properties against *Staphylococcus aureus*, *E. coli*, *Listeria* spp., and *Salmonella* spp. Lee et al. (2020) investigated six commercial red sorghum varieties and found that the stronger anti-inflammatory soluble phenolic fraction in red sorghum was correlated with its higher Total Phenolic Compound (TPC), Total Flavonoid Content (TFC), and radical scavenging. Today's growing consumer interest in natural cosmetics is a great opportunity to develop colored sorghum as an anti-acne ingredient. Acne infections are increasingly being treated organically, instead of with chemicals (Faccio 2020).

No previous research has been conducted to determine the antibacterial, antioxidant, and anti-inflammatory properties of colored sorghum grown in the arid region of ENT province. This study aimed to analyze the potential of red, brown, and black sorghum extracts grown in ENT Province dry areas to be used as an antimicrobial, antioxidant, and anti-inflammatory. These findings are an important opportunity to develop colored sorghums as an environmentally friendly cosmetic material.

MATERIALS AND METHODS

Sample collection

The research material utilized in this study consisted of pigmented local sorghums gathered from farmers working in the East Sumba, East Flores, and Sabu Raijua Districts of EST Province. Colored sorghums were grown by farmers in the dry land of ENT and had not previously been tested for their antimicrobial, antioxidant, and anti-inflammatory properties. Sorghum seeds undergo harvesting, followed by chopping, and subsequent exposure to sunlight until the moisture content was reduced to a range of 12-15 percent. The physical appearance of seven types of pigmented sorghum is presented in Figure 1. The mean weight of 100 consecutive seeds was 1.82 g for black Flotim (01), 2.91 g for black Sabu (02), and 2.67 g for red Sabu (03). The red Sumba sample (04) weighed 2.86 g, whereas the average weights of the brown Sumba (05), black Sumba (06), and brown Sabu (07) were 2.01 g, 1.87 g, and 2.27 g, respectively.

Figure 1. Pigmented sorghums from left to right: Black Flotim (01), Black Sabu (02), Red Sabu (03), Red Sumba (04), Brown Sumba (05), Black Sumba (06), Brown Sabu (07)

Sample preparation and extraction

The grain samples were ground prior to their examination in the laboratory. The extraction procedure used a solution consisting of 1% hydrochloric acid (HCl) and methanol in a volumetric ratio of 1:1. The seed powder was then macerated for 72 h at room temperature in a solution containing 50/50 (v/v) 1% chloric acid and methanol, with constant stirring using a magnetic stirrer GCMS analysis (Mukkun et al. 2021; Ola et al. 2021). For further analysis, about 31,87; 33,57; 34,90; 30,81; 26,17; 25,18; 28,20 g of samples were used. The samples were extracted in a solution consisting of 96% ethanol for a duration of 5 x 24 hours with constant steady shaking. The filtrates obtained from maceration were filtered using a Whatman paper. Then, resulting filtrate was concentrated at a temperature of 40°C using a rotary evaporator (Eyela Aspirator A-3S, Japan) at 250 rpm. Following evaporation, obtained extract was dried in a Memmert-type oven (Model, Memmert) at a temperature of 40°C until the weight reached stable state. The crude extracts were stored in vials at a temperature of -20°C (Eyela Cool Ace CA-1111, Japan) for subsequent analysis.

Anti-bacterial assay

An anti-bacterial assay was conducted using the agar well-disk diffusion method as described by Arung et al. (2021) and Kusuma et al. (2020). Seven varieties of sorghum, namely, black Flotim, black Sabu, red Sabu, red Sumba, brown Sumba, black Sumba, and brown Sabu were utilized for antibacterial test. The extracts were tested at concentrations of 62.5 ppm, 125 ppm, 250 ppm, 500 ppm, and 1000 ppm.

Bacterial strains, namely *P. acnes* and *S. epidermis* were cultured on nutrient agar. Sterile media aliquots of 20 mL were placed onto petri dishes and then allowed to solidify. The media plates were inoculated with a microbial suspension of 20 µL, which was evenly dispersed across the surface of plates. A sterile cork borer was used to create a well with a diameter of 7 millimeters. Subsequently, a volume of 20 µL of acetone solution, comprising extracts ranging from 65.5 to 1.000 ppm, was added to the well. A concentration of 10 µL/well of chloramphenicol was employed as a positive control. The plates were subjected to incubation under dark conditions at a temperature of 32°C for 24 hours. The zones of inhibition around the well was measured in millimeters. Activity Index (AI) was calculated by dividing the mean inhibition zone for the test sample by the mean inhibition zone for the standard drug.

Antioxidant assay

The antioxidant properties of sorghum extracts were assessed using DPPH method. A solution of 1 nM DPPH was prepared by weighing 19.7 mg 1,1-diphenyl-2-picrylhydrazyl, and subsequently dissolved in 50 mL of dimethylsulfoxide (DMSO). Antioxidant activity was measured by weighing 1 mg of extract sample, followed by adding 4 mL of 1 nM DPPH solution and then homogenized. A spectrophotometer UV-Vis (UV mini-1240, Shimadzu) was used to measure the absorbance of liquids at a wavelength of 517 nm. The positive control or standard was Vitamin C at 100 µg/mL (Punia et al. 2021).

Total phenolic content

The Total Phenolic Content (TPC) of sorghum extract was measured using colorimetric Folin Ciocalteu technique (Khoddami et al. 2017), with modifications. As per the test, 250 microliters of a 10% (v/v) F-C reagent was added to 250 microliters of a sample at a concentration of 2 mg/mL. The mixture was then vortexed and incubated for six minutes in the dark. Then add 2.5 mL of 7% Na_2CO_3 and keep at room temperature in the dark for 90 minutes. Finally, the absorbance of bluish color solution was measured at a wavelength of 760 nm with a UV/visible spectrophotometer (UVmini-1240, Shimadzu).

Membrane stabilization activity as an anti-inflammatory test

Anti-inflammatory activity testing was carried out by stabilizing a red blood cell-membrane according to the procedure of Naibaho et al. (2023) in vitro. 1 mL of phosphate buffer, 2 mL of hyposaline, and 0.5 mL of Human Red Blood Cell (HRBC) solution were combined with positive control, control, and various amounts of extract (6.25-100 µg/mL). Indomethacin (100 µg/mL) was used as a positive control. After an incubation period of 30 minutes at 37°C, all test solutions were centrifuged for ten minutes at 5,000 rpm. After decantation of the supernatant fluid, hemoglobin concentration was measured using a spectrophotometer (UVmini-1240, Shimadzu) at 560 nm. The percentage of membrane stability was calculated using the following formula, with blood controls showing 100% lysis or 0% stability = 100 - (treatment absorbance - control drug absorbance) × 100. For each sample, the maximum inhibitory concentration (IC_{50}) was determined by plotting the concentration against the percentage inhibition of hemolysis.

Individual bioactive compounds identification by GCMS

About 1 µL of sample extracts (1%, weight/volume) were subjected to Gas Chromatography-Mass Spectrometry (GC/MS). The screening metabolites were analyzed using a GCMS-QP2010 Ultra (Shimadzu, Japan), equipped with a SH-Rxi-5Sil MS Cap. column (30 m, 0.25 mm, 0.25 µm) and a flame ionization detector using helium as the carrier gas. The injection temperature was 250°C and the split ratio was 50:1. The temperature profile for Gas Chromatography (GC) analysis was as follows: 1 min at 50°C, increasing until 140°C (rate 20°C/min), 3 min 140-280°C (10°C/min). The pressure was 100.1 kPa, column flow was 1.69 mL/min. Peaks were identified using library NIST 2017 (Ajani et al. 2021).

Statistical analysis

The data were analyzed by ANOVA, using SPSS IBM 23, followed by Tukey's Least Significant Difference (LSD) tests at $P<0.05$ level to determine the differences among the different varieties of sorghum. A normality test of the data was performed using a Shapiro-Wilk test at a significant difference of 0.05.

RESULTS AND DISCUSSION

Antibacterial activity of sorghum extract against *Propionibacterium acnes* and *Staphylococcus epidermis*

The results of antibacterial assays of pigmented sorghum extract against *P. acnes* bacteria are presented in Tables 1 and 2, whereas the findings against *S. epidermis* bacteria are shown in Tables 3 and 4. The results of statistical test ($p<0.05$) indicated that sorghum types with varying seed skin colors and extract concentrations had significant different inhibitory capacities against the growth of *P. acnes* and *S. epidermis*. At a concentration of 1000 µg/mL, inhibition index of sorghum extract against *P. acnes* bacteria ranged from 16.56 to 21.33 mm (Table 1). Similarly, against *S. epidermis*, the inhibition index varied from 9.22 to 17.00 mm (Table 3). The antimicrobial capacities of extracts can be divided into four categories based on the diameter of the inhibition index. Substances with a diameter greater than 22 mm have extremely strong antibacterial properties, strong if the diameter is between 11 and 20 mm, moderate if the diameter is between 6 and 10 mm, and weak if the diameter is less than or equal to 5 mm (Davis and Stout 1971). The results exhibited potent antibacterial activity against *P. acnes*, ranging from very strong to strong, while against *S. epidermis*, the extracts indicate a moderate to high antimicrobial effect. The red-seeded sorghum from Sumba exhibited very strong antimicrobial properties against *P. acnes*. In line with these results, red sorghum was also capable to inhibit the growth of *S. epidermis* bacteria, being in a strong category.

Table 1. Mean surface area inhibition of sorghum extracts against *Propionibacterium acnes*

Sorghum types	Zone of inhibition (mm)					
	CP	1000 ppm	500 ppm	250 ppm	125 ppm	62.5 ppm
Black Flotim (01)	28.00 ^{e}	18.67 ^{d}	15.33 ^{bc}	14.22^{bc}	11.56^{b}	10.00 ^{a}
Black Sabu (02)	28.67 ^{d}	17.11 ^{c}	13.00 ^{b}	11.22 ^{b}	9.44 ^{b}	0.00 ^{a}
Red Sabu (03)	28.00 ^{e}	20.22 ^{d}	15.89 ^{c}	13.11 ^{c}	10.67 ^{bc}	6.00 ^{a}
Red Sumba (04)	28.89 ^{e}	21.22 ^{d}	19.33 ^{bcd}	20.67 ^{abc}	18.89 ^{ab}	17.11 ^{a}
Brown Sumba (05)	27.22 ^{b}	19.56 ^{a}	17.89 ^{a}	19.00 ^{a}	18.89 ^{a}	16.56 ^{a}
Black Sumba (06)	28.44 ^{d}	18.00 ^{c}	14.56 ^{bc}	12.56 ^{a}	11.11 ^{a}	10.89 ^{a}
Brown Sabu (07)	27.89 ^{e}	16.56 ^{d}	14.00 ^{cd}	12.22 ^{bc}	10.11 ^{b}	0 ^{a}

Note: CP: Chloramphenicol. Numbers on the same line followed by different letters, significantly different at a level of $p<0.05$

Table 2. Mean percentage zone of inhibition of sorghum extract against *Propionibacterium acnes*

Sorghum types	Zone of inhibition (%)					
	CP	1000 ppm	500 ppm	250 ppm	125 ppm	62.5 ppm
Black Flotim (01)	100.00^{e}	66.80^{d}	54.78^{c}	50.96^{b}	41.31^{ab}	35.82^{a}
Black Sabu (02)	100.00^{e}	59.63^{d}	45.33^{c}	39.10^{bc}	32.92^{ab}	0.00^{a}
Red Sabu (03)	100.00	72.28	56.83	46.80	38.11	21.09
Red Sumba (04)	100.00	73.51	66.82	71.52	65.36	59.06
Brown Sumba (05)	100.00b	71.88^{a}	65.74^{a}	69.83^{a}	69.37^{a}	60.82^{a}
Black Sumba (06)	100.00c	63.32^{b}	51.12^{b}	44.10^{ab}	39.03^{ab}	38.33^{a}
Brown Sabu (07)	100.00^{d}	59.50^{c}	53.52^{bc}	43,52^{b}	36,28^{b}	0,00^{a}

Note: CP: Chloramphenicol. Numbers on the same line followed by different letters, significantly different at a level of $p<0.05$

Table 3. Mean surface area inhibition of sorghum extracts against *Staphylococcus epidermis*

Sorghum types	Zone of inhibition (mm)					
	CP	1.000 ppm	500 ppm	250 ppm	125 ppm	62.5 ppm
Black Flotim (01)	23.56^{d}	10.89^{c}	10.11^{c}	8.22^{b}	0.00^{a}	0.00^{a}
Black Sabu (02)	22.00^{c}	9.22^{b}	8.89^{b}	7.89^{b}	0.00^{a}	0.00^{a}
Red Sabu (03)	21.44^{e}	13.56^{d}	10.78^{c}	7.56^{b}	0.00^{a}	0.00^{a}
Red Sumba (04)	22.44^{d}	17.00^{c}	16.22^{bc}	16.11^{bc}	14.78^{b}	10.00^{a}
Brown Sumba (05)	22.78^{c}	14.00^{b}	13.44^{b}	13.89^{b}	13.00^{b}	11.44^{a}
Black Sumba (06)	22.78^{d}	11.11^{c}	8.45^{b}	7.89^{b}	7.56^{b}	0^{a}
Brown Sabu (07)	22.78^{d}	10.67^{c}	9.00^{c}	6.67^{b}	0.00^{a}	0.00^{a}

Note: CP: Chloramphenicol. Numbers on the same line followed by different letters, significantly different at a level of $p<0.05$

Table 4. Mean percentage zone of inhibition of sorghum extract against *Staphylococcus epidermis*

Sorghum types	Zone of inhibition (%)					
	CP	1000 ppm	500 ppm	250 ppm	125 ppm	62.5 ppm
Black Flotim (01)	100.00^{d}	46.25^{c}	42.98^{c}	34.98^{b}	0.00^{a}	0.00^{a}
Black Sabu (02)	100.00^{c}	42.11^{b}	40.52^{b}	35.83^{b}	0.00^{a}	0.00^{a}
Red Sabu (03)	100.00	63.29	50.14	35.28	0.00	0.00
Red Sumba (04)	100.00^{d}	75.70^{c}	72.28^{c}	71.80^{bc}	65.82^{b}	44.58^{a}
Brown Sumba (05)	100.00^{c}	61.50^{b}	59.06^{b}	60.95^{b}	57.03^{b}	50.15^{a}
Black Sumba (06)	100.00^{d}	48.79^{c}	37.09^{b}	34.63^{b}	33.20^{b}	0.00^{a}
Brown Sabu (07)	100.00^{e}	46.98^{d}	39.59^{c}	29.43^{b}	0.00^{a}	0.00^{a}

Note: CP: Chloramphenicol. Numbers on the same line followed by different letters, significantly different at a level of $p<0.05$

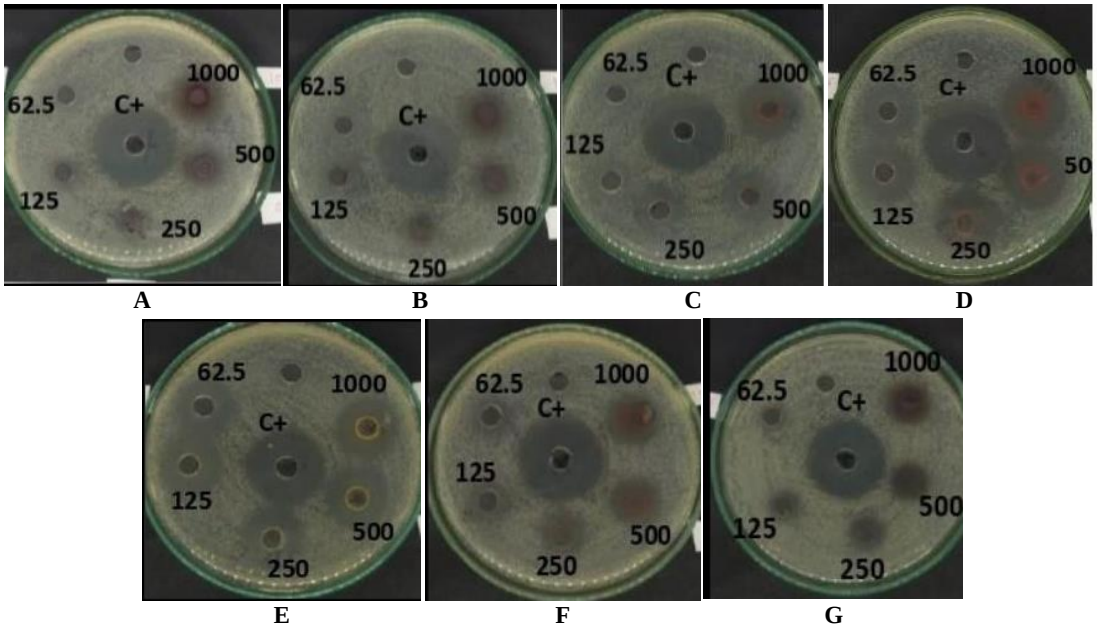

Figure 2. Zone inhibition of sorghum extract against *Propionibacterium acnes* bacteria. A. Black Flotim (01), B. Black Sabu (02), C. Red Sabu (03), D. Red Sumba (04), E. Brown Sumba (05), F. Black Sumba (06), G. Brown Sabu (07)

Acne is most common between puberty (16-18 years) and 30 years of age. It is caused by *P. acnes*, which lives inside the pilosebaceous follicles adjacent to the sebaceous glands. Although the trigger of acne is unknown, excess sebum production by the glands or follicle blockage causes excess growth of *P. acnes*, which results in inflammation of the skin (Arung et al. 2017; Sparavigna et al. 2015). Epidemiological studies state that acne has no capability to attack humans (Sparavigna et al. 2015). The efficacy of sorghum extract in terms of inhibiting the growth of *P. acnes* was assessed using *in vitro* test (Figure 2). This involved determining the percentage of inhibition for each sample extract and comparing it to the commercial antibiotic chloramphenicol at a concentration of 20 µg/mL. The results indicate that the average inhibitory effect of sorghum extract, at a concentration of 1.000 µg/mL, against *P. acnes* varied between 59.50 and 73.51% when compared to the positive control (Table 2). The findings of this study suggest that colored sorghums extract have the potential to be used as cosmetic and medical products, thereby boosting the economic worth of sorghums. Reyna-Reyna et al. (2023) conducted similar research and reported that sorghum extract had a strong inhibitory effect on *E. coli* bacteria. Chen et al. (2022) discovered that polyphenol extract derived from colored sorghum exhibited antibacterial properties against *S. aureus*, *E. coli*, *Listeria* spp., and *Salmonella* spp. Other studies have found that effectiveness of sorghums against *E. coli* bacteria was determined by the presence of tannic acid (Ofosu et al. 2021; Meena et al. 2022). The presence of polyphenols in sorghum extract increased the conductivity of cell suspensions by disrupting the integrity of the cell membrane, causing the release of cell electrolytes. Indications of changes in the morphology and internal composition of bacteria were observed (Chen et al. 2022).

There is evidence that colored seed sorghums contain beneficial bioactive compounds, including antimicrobial, antioxidant, anti-inflammatory, anticancer, and antidiabetic properties (Khoddami et al. 2017; Smolensky et al. 2018; Choi et al. 2019). In comparison to vitamin C, antioxidant activity of colored sorghum varied between 30.58 and 92.80%, with red sorghum from Sumba (91.94%) and Sabu (92.8%) exhibiting the maximum levels. The dry-weight Gallic Acid Equivalents (GAE) exhibited significant total phenol content, varied between 57.69 and 64.54%. The presence of polyphenol compounds in sorghums may be one of the contributing factors to its exceptional health-promoting attributes. The antimicrobial activity of sorghum extract against *S. aureus* bacteria was assessed by means of polyphenol compounds and additional secondary metabolite compounds (Kil et al. 2009).

Antioxidant activity of colored sorghum cultivated in dry land in East Nusa Tenggara

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a rapid and accurate method for evaluating the antioxidant capacity of plant extracts. This method is based on DPPH discoloration in the presence of antioxidants in the tested sample. A color change from purple to yellow indicates strong antioxidant ability of the sample (Vora et al. 2018). The antioxidant activity of the analyzed sorghum ranged from 41.41 to 82.21%, as shown in Table 5. Brown sorghum from Sumba exhibited the highest (82.21%) level of antioxidant activity among other types of sorghum. Other varieties included red sorghum from Sumba (69.23%), brown sorghum from Sabu (66.74%), black sorghum from Flotim (56.13%), and Sabu (52.94%). The antioxidant activity of selected sorghums was classified as high to extremely high. A DPPH radical inhibition percentage exceeding 90% signifies a remarkably high level of antioxidant activity. A range of 50-90% indicates a high level of antioxidants, while 20-50% suggests a moderate level. A percentage

below 20% indicates low antioxidant activity, and a 0% signifies either the absence of any antioxidant activity or the absence of DPPH radical suppression (Basri and Saefudin 2020). These results are similar to the study by Desta et al. (2023), which showed that sorghums with different colored seeds have different antioxidant effects, with an average antioxidant activity of 48%. Hong et al. (2020) also found that black sorghum extracts have the ability to inhibit DPPH production appeared to exceed 90% at 100 µg gallic acid eq/mL. The present study showed that pigmented sorghum extracts exhibited remarkable DPPH radical activity. This DPPH radical activity was positively correlated with total polyphenol content (Table 5), with values ranging from 57.69-64.12% GAE dry weight. Previous studies have demonstrated that condensed tannins in sorghum have elevated antioxidant activity. Sorghum with pigmented testa appeared to have a greater antioxidant activity than other varieties because of its high level of condensed tannins (Hong et al. 2020; Dykes et al. 2013).

The IC_{50} of the examined sorghum extract varied between 3.19 ppm and 6.32 ppm (Table 5). IC_{50} is the minimum concentration of the sample solution needed to block 50% of DPPH free radicals. IC_{50} values are obtained by calculating the linear regression equation obtained by describing the concentrations of the tested solution and the percentage of the DPPH scavenger as the parameters of antioxidant activity, with the concentration of the solution tested (ppm) as the X-axis and the proportion of the scavenger as the Y-axis. As for the group of antioxidants based on IC_{50}, it is (1) very strong (<50 µg/mL), (2) strong (50-100 µg/mL), (3) moderate (101-150 µg/mL), and (4) weak (151-200 µg) (Shahidi and Ambigaipalan 2015; Surjanto et al. 2019). In Table 5, data indicates that red and brown sorghum extracts exhibited the highest antioxidant ability, with an IC_{50} of 3.19 µg/mL; however, there was no significant different when compared to other sorghum types.

Total Phenol Content (TPC) of colored seed sorghums

The phenol compounds found in colored sorghum (Table 6) was in the range of 57.69-64.12% GAE (dry weight). The results of statistical analysis was not significant between all types of sorghum analyzed. Black sorghum from Sumba and Flotim exhibited the highest total polyphenol content in comparison to other varieties. Polyphenols are a class of organic chemicals that occur naturally in plants. Polyphenol molecules have advantageous effects on health due to their antioxidant, anti-inflammatory, anticancer, antidiabetic, and other degenerative disease capabilities (Shahidi and Ambigaipalan 2015; Smolensky et al. 2018; Arung et al. 2021; Pontieri et al. 2021).

Sorghum contains important phenolic chemicals, including protocatechuic, p-hydroxybenzoic, caffeic, p-coumaric, and ferulic acid, which are present in both free and bound forms. Cinnamic and vanillic acids are also present in both free and bound forms, but only in certain kinds of sorghum. The polyphenol content of sorghum is mostly found in the seed's skin, which is made up of phenolic acid, flavonoids, and tannins (Dykes et al. 2013). The way these chemicals are combined depends on the plant's genes (Dykes and Rooney 2006, 2007). The color of sorghum seeds is determined and controlled by the R and Y genes and is categorized into four categories: white, yellow, red, and black. Black sorghum, originally red in color, undergoes a transformation to black when exposed to sunlight and when the pericarp contains a high level of 3-deoxyanthocyanidins (Taleon et al. 2012, 2014; Dykes et al. 2013). The 3-deoxidanthocianidines compounds in black sorghum cause black sorghum to have important antioxidant and anti-cancer properties (Dykes et al. 2013; Fedenia et al. 2020). Human colon cancer was prevented by phenols extracted from red sorghum bran using a 70% ethanol and 5% citric acid solvent (Lee et al. 2020). The presence of phenol in sorghum was also reported to reduce the activity of *Bacillus* glucosidase found in human salivary amylase, making sorghum an anti-diabetic food (Meena et al. 2022). Based on the results of this study, further research is needed to identify and characterize the properties of the phenol compounds in colored sorghums, especially the red and black colors.

Membrane stabilization profile (Anti-inflammatory activity)

The results of in vitro membrane stabilization of seven seed sorghums extracts from the dry land area of ENT Province are shown in Figure 3. Brown Sabu showed the lowest membrane stabilization activity (IC_{50}=70.92 µg/mL), followed by red Sumba, black Sumba, black Sabu, and black Flotim, while the highest were red Sumba and brown Sumba. Red blood cell membrane stabilization activity in brown Sabu, with a lower IC_{50} value, shows the highest anti-inflammatory activity value. The lower the IC_{50} value, the higher the anti-inflammatory activity. This is in line with the antioxidant activity value found in brown Sabu of 4.22 µg/mL. Antioxidants influence the anti-inflammatory activity of sorghum, which may be influenced by the color of sorghums. The anti-inflammatory activity values produced by the seven different sorghums may be due to the different colors of sorghum grains, which can have an influence on the resulting tests. Different colors of sorghum seeds have different impacts because each color of sorghum seeds shows the phytochemical content, polyphenol content, tannin content and different bioactive compounds of the sorghum (Desta et al. 2023).

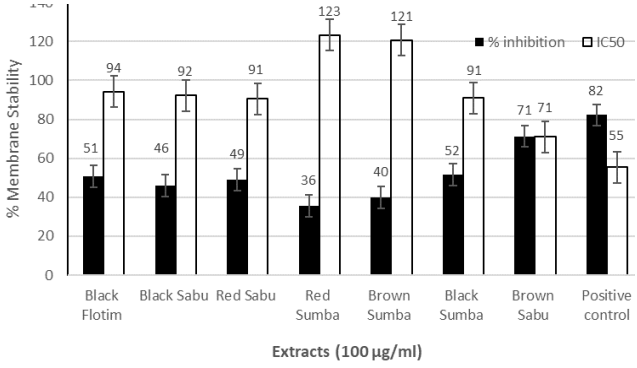

Figure 3. Anti-inflammatory activity and IC_{50} of seven seed sorghums extracts

The brown Sabu may contain flavonoids, affecting the anti-inflammatory activity value produced. Flavonoids have characteristic anti-inflammatory activity, which can inhibit the metabolic process of arachidonic acid in sorghum. When arachidonic acid levels fall, the levels of acids that produce pro-inflammatory prostaglandins have an anti-inflammatory impact (Naibaho et al. 2023). Prior research has indicated that the free phenolic extract and specific phenolic compounds, such as phenolic acid and flavone, derived from sorghums exhibit strong inhibitory effects against pro-inflammatory activities (Agah et al. 2017). Other research found that bread supplemented with sorghum bran extract and sorghum leaf extract (2.24 g per 300 g of flour) significantly improved the bio accessibility and bio efficiency of anti-inflammatory compounds in bread (Afrin and Sidhu 2021).

Bioactive composition of colored seed sorghums

The GC-MS chromatograms of all seven sorghum samples are presented in Figure 4, while retention time, peak area (%), and type of bioactive compounds contained in the sample are shown in Table 7.

All compounds were detected at 6.24 to 21.81 minutes. Benzofuran compound, 2.3-dihydro- was the first compound detected with a retention time of 5.21 minutes, while the last was lup-20(29)-en-3-ol, acetate, (3beta). The data from Table 7 indicate that sorghum varieties exhibit a range of 8-20 different bioactive components. Red sorghum Sumba had a total of 20 distinct bioactive chemicals, whereas red Sorghum from Sabu only contains six types of compounds. Six major chemicals were found in the sorghum extracts, as indicated by the peak area of the GC-MS histogram (Table 8).

Table 5. Oxidant activity and IC_{50} of colored seed sorghums

Sorghum types	Oxidant activity (%)	IC_{50} (µg/mL)
Black Flotim (01)	56.13 ± 0.39	5.31
Black Sabu (02)	52.94 ± 2.29	5.15
Red Sabu (03)	49.23 ± 0.17	5.31
Red Sumba (04)	69.23 ± 0.06	3.99
Brown Sumba (05)	82.21 ± 0.39	3.19
Black Sumba (06)	41.41 ± 0.06	6.32
Brown Sabu (07)	66.74 ± 0.06	4.22
Vitamin C	97.23 ± 0.15	4.70

Table 6. Total phenol content (TPC) of colored sorghum

Sorghum types	% GAE dry basis			
	n-1	n- 2	n-3	Average
Black Flotim (01)	61.83	65.98	64.54	64.12 ± 1.21
Black Sabu (02)	61.10	56.72	61.29	59.71 ± 1.49
Red Sabu (03)	61.73	61.77	61.77	61.76 ± 0.01
Red Sumba (04)	56.42	56.70	65.91	59.68 ± 3.11
Brown Sumba (05)	75.39	56.25	53.92	61.85 ± 6.80
Black Sumba (06)	61.83	65.98	64.54	64.12 ± 1.22
Brown Sabu (07)	54.44	63.90	54.72	57.69 ± 3.11

Note: n: replication

Table 7. Retention time, peak area (%), and type of bioactive compounds of crude extracts of sorghum

Rt (mins)	Compounds (%)	Peak area (%)						
		Black Flotim (01)	Black Sabu (02)	Red Sabu (03)	Red Sumba (04)	Brown Sumba (05)	Black Sumba (06)	Brown Sabu (07)
6.24	Benzofuran. 2,3-dihydro-				0.6			
7.12	2-Methoxy-4-vinylphenol				0.06			
7.49	Benzaldehyde, 4-hydroxy-				0.09			
7.93	Vanillin				0.06			
8.23	Acetophenone. 4'-hydroxy-				1.98			
8.41	Methylparaben				0.1			
8.56	Phenol, 3,5-dimethoxy-				0.53			
8.89	Benzoic acid, 4-hydroxy-				0.54			
9.13	Flamenol				3.97			
9.44	Vanillic acid				0.05			
9,44	Dodecanoic acid		0.54					
12,54	Daphnetin		0.33					
13,01	(Z)-15-Octadecenoic acid methyl ester	0.17			0.04		0.05	
13.22	Hexadecanoic acid, methyl ester	6.21	1.64	2.07	1.86	2.28	2.25	2.28
13.35	11,13-Dimethyl-12-tetradecen-1-ol acetate	0.35	0.35		0.24			
13.35	Disparlure						0.2	
13.55	n-Hexadecanoic acid	6.38	25.12	31.35	13.57	16.64	20.9	16.64
14.82	11,14-Octadecadienoic acid, methyl ester	14.66	3.22	4.24	4.04	5.21	4.07	5.21
14.88	4-Octadecenoic acid, methyl ester	11.25	2.5	3.06	2.83	3.76	3	3.76
14.93	4-Octadecenoic acid, methyl ester (overlape)	0.32			0.08			
15.12	Methyl stearate	0.85					0.44	
15.14	Heptadecanoic acid, methyl ester			0.59				
15.20	9,12-Octadecadienoic acid (Z,Z)-	19.79		25.91	64.04		33.97	35.46
15.23	cis-13,16-Docasadienoic acid			31.94		35.46		
15.24	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-	40.37	63.4			36.64	33.98	36.64
15.42	Docosanoic acid				3.4			
15.42	Dasycarpidan-1-methanol, acetate (ester)		1.51					
19.01	Phenol, 4,4'-methylenebis[2,6-dimethyl-				1.05			
20.80	Supraene			0.85				
21.81	Lup-20(29)-en-3-ol, acetate, (3. beta.)-		1.38		0.89		1.14	

The compound hexadecanoic acid, methyl ester, was found in all analyzed types of sorghum, the highest content found in black Flotim (6.38%), while the lowest content was found in black Sabu (1.64%). n-hexadecanoic acid is also detected in all types of colored sorghum, with the highest concentration found in red Sabu (31.35%), followed by brown Sumba and Sabu, and the lowest concentration found in black Sabu. The methyl ester of hexadecanoic acid and n-hexadecanoic acid have been shown to possess antioxidant, antibacterial, and anti-inflammatory properties (Abu-Rumman 2018; Asnaashari et al. 2023). The other main chemical compounds include 11.14-octadecadienoic acid, methyl esters, 9.12-octadecadenoic acids (Z, Z)-, and 4-octadecenoic acid and methyl esters. The chemicals possess antibacterial, antioxidant, snailicidal, and anticancer activities (Pu et al. 2010). The red Sumba type had the largest proportion of 9.12-octadecadienoic acid (Z, Z), reaching to 64.04%, while brown Sabu, black Sumba, red Sabu and black Flotim contain 35.46, 33.97, 25.91, and 19.79% of 9,12-octadecadienoic acid (Z, Z), respectively. This compound is also known as omega-6 polyunsaturated fatty acid. The other main chemicals found in colored sorghums were aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy. This study shows that red sorghum had a high level of aspidospermedin-17-ol, 1 acetyl-19,21-epoxy, at a concentration of 63.4%. Similarly, other varieties of sorghum range from 33.98 to 40.37%. Hence, further study is required to elucidate the specific role of the predominant chemicals in pigmented sorghum to optimally utilize its naturally occurring capabilities.

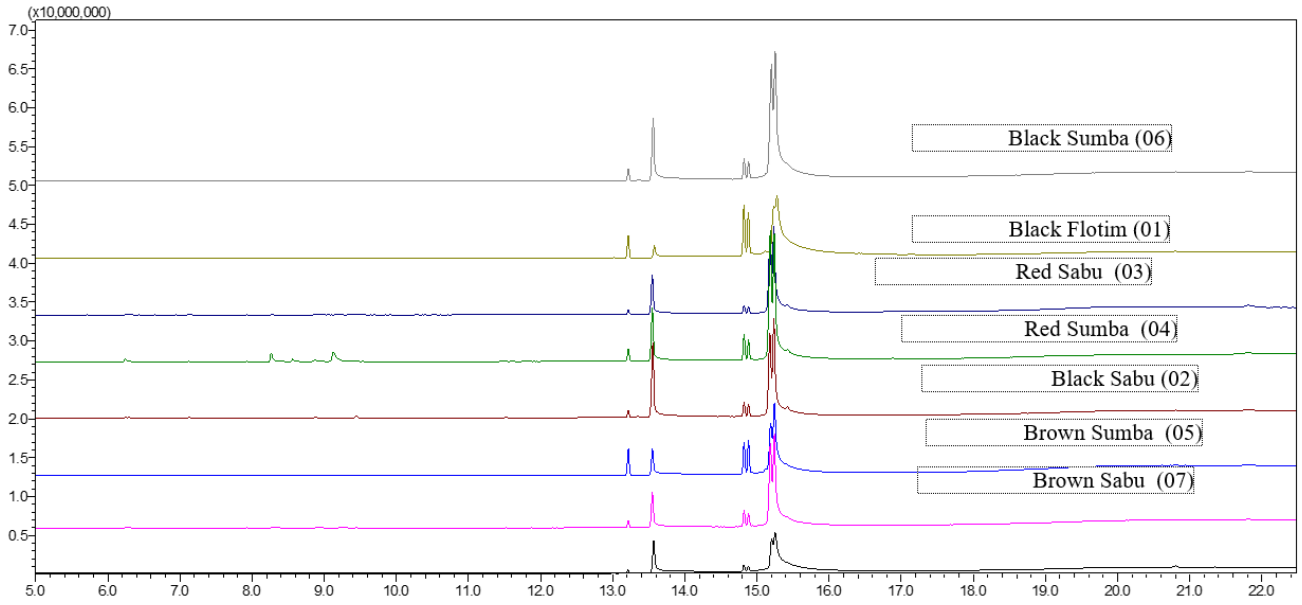

Figure 4. Chromatogram of crude extract samples

Table 8. Major bioactive compounds, molecular formula, and their possible health properties

Compounds name	Rt (min)	Molecular formula	Possible medical roles	References
Hexadecanoic acid, methyl ester	13.217	$C_{17}H_{34}O_2$	Antibacterial, antifungal	(Abubacker and Deepalakshmi 2013; Shaaban et al. 2021)
n-Hexadecanoic acid	13.548	$C_{16}H_{32}O_2$	Antioxidant, antimicrobial, anti-inflammatory, hypocholesterolemic, nematicide and pesticide activity	(Abubakar and Majinda 2016; Ganesan et al. 2022)
11,14-Octadecadienoic acid, methyl ester	14.821	$C_{19}H_{34}O_2$	Antimicrobial	(Asnaashari et al. 2023; Pu et al. 2010)
9,12-Octadecadienoic acid (Z,Z)-	14.884	$C_{18}H_{32}O_2$	Antibacterial, antioxidant	(Abu-Rumman 2018)
4-Octadecenoic acid, methyl ester	15.204	$C_{19}H_{36}O_2$	Snailicidal, antimicrobial, antioxidant and anticancer	(Karim et al. 2021)
cis-13,16-Docasadienoic acid	15.235	$C_{22}H_{40}O_2$	Antimicrobial, anti-fungi	(Abu-Rumman 2018; Abdel Karim et al. 2021)
Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-	15.241	$C_{23}H_{30}O_5N$	Antioxidant	(Dhanasezhian et al. 2018)

In conclusion, the findings of present study showed that colored sorghum extract cultivated in the dry areas of East Nusa Tenggara Province can effectively inhibit the proliferation of *P. acnes* and *S. epidermis*, responsible for *A. vulgaris* inflammation. The sorghum extracts also exhibited antioxidant and anti-inflammation properties. These biological functions were related to the TPC content and some dominant compounds that were detected by GC-MS. Further studies are needed on the antibacterial compounds in colored sorghums and their efficacy as anti-acne medications.

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