



RESEARCH ARTICLE

Antioxidant and antimicrobial properties of methanol extracts from underutilized fruits of Bireuen Regency, Aceh, Indonesia

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Abstract

Underutilized fruits benefit humans through their adaptability to the local environment. However, their low economic value and limited utilization contribute to their gradual disappearance from the local community. Therefore, studying their biological properties and potential applications may support conservation efforts and encourage their reintroduction into local communities. Three commonly found fruit plants in Bireuen Regency Aceh, namely *Lepisanthes rubiginosa*, *Mangifera quadrifida* and *Annona reticulata*, were once valued by local communities. Still, nowadays the presence is often ignored due to low economic value. As fruit is usually rich with bioactive compounds, research has been conducted to elucidate the biological potency as a means of conservation and reintroduction of the fruit to the community. DPPH free radical scavenging activity, antibacterial potential, total flavonoid and phenolic content of methanol extracts of *L. rubiginosa*, *M. quadrifida* and *A. reticulata* were conducted. Results showed that all three extracts exhibited significant free radical scavenging activity, with *M. quadrifida* demonstrating the highest potency, as indicated by the lowest IC₅₀ value of 0.0012 mg/ml. Flavonoid content and TPC results also in correspondence with *M. quadrifida* showed the highest flavonoid content 282.11 ± 0.1 mg QE/g extract and 655.26 ± 0.1 mg GAE/g extract, consecutively, *M. quadrifida* also showed the strongest growth inhibition of *S. aureus* and *E. coli*. These results suggest that underutilized fruit may serve as valuable sources of flavonoids and exhibit promising biological potential.

Keywords: *Annona reticulata* ; antibacterial activity; antioxidant activity; flavonoids; *Lepisanthes rubiginosa*; *Mangifera quadrifida*; phenolic content

Introduction

Fruits, as seed dispersal agents, have a wide variability in structure and function and often contain beneficial compounds for humans. Health-promoting substances such as flavonoids, phenolic acids, carotenoids, stilbenes, tannins and anthocyanins, among others, have been reported to be in abundance in various fruits. Research has reported various biological activities, including antioxidant, antimicrobial, antidiabetic, antiobesity, anticarcinogenic and anti-inflammatory, related to health-promoting substances (1).

Several underutilized fruits from local plants in Bireuen Regency, Aceh, hold a special place in the community due to their common use for consumption in the past. Nowadays, these plants are rarely cultivated and their consumption is limited to locals who have inherited from older generations or to occasional sales catering to sentimental buyers. Whereas an underutilized plant is important in addressing food security problems related to climate change. It is an excellent choice for crop diversification (2). It is generally adapted to diverse ecological niches and exhibit resilience to varying conditions compared to commercial crops (3).

Some local species are rare, while others grow wild or remain uncultivated. A recent study that has been conducted identifies the plants and opens windows of information regarding the plant species. *Lepisanthes rubiginosa* (synonym *Erioglossum rubiginosum*), locally known as keulayu (Aceh language) or boni, buni (in Indonesian language); found in South Asia, South East Asia, to Papua New Guinea (4). In Aceh, the ripe fruits were loved by many and was often beaten by birds that feasted on the whole tree just within days. There are very limited studies on the biological properties of the fruit pulp of *L. rubiginosa*. The presence of amino acids, phenolic acids and flavonoids of different stages of maturity of *L. rubiginosa* fruits, as well as antioxidant activities, has been previously reported (5). Further study pointed out the high amount of lycopene and cyanidin-3-O-glucoside, emphasizing the potency of *L. rubiginosa* as the source of bioactive compounds (6).

Mangifera quadrifida, also known as kumbang, belongs to the Anacardiaceae family and is classified as a wild Sumatran Mango species. It is now threatened due to rapid and massive deforestation, as well as low economic market value due to low demand for consumption (7). In

Aceh, kumbang's mature fruit is popular as a component of local fruit salad (called rujak); and the use of the fruit is limited solely to that. Thus, the economic value is not good and is not known to grow commercially.

Annona reticulata, locally known as teubarasa (in Aceh), mulwo, or buah nona, is native to Bireuen Regency and has been cherished for its sweet, creamy fruit flesh. However, it is now less known and has become less recognized by the younger generation. *A. reticulata* is a productive but considered very invasive tree, with up to 45 kg fruit production per year and equipped with hard-shelled seeds which remain viable for more than one year (8). *A. reticulata* contains approximately 17 % sugar, 1.6 % protein and 0.26 % fat (9). However, limited information is available on the bioactive compounds of *A. reticulata*, despite its long-standing popularity.

However, the information regarding the biological potencies of these fruits is still limited, especially those from the Bireuen region. Whereas these fruits showed promising potencies through their vibrant colour, refreshing taste and unique aroma, which are often associated with the presence of beneficial compounds such as flavonoids, anthocyanins, terpenoids and esters, to name a few. The mentioned class of compounds has been linked with antioxidant, antimicrobial and anti-inflammatory activities, to name a few. Moreover, consumption patterns have shifted to a healthier lifestyle, in which food selection is made based on its effect on the human body. Thus, validation of the potent beneficial effect of the underutilized plant might lead to an increase in economic value and the use of the fruit in general.

Materials and Methods

Fruit sample preparation

Fruit samples (*L. rubiginosa*, *A. reticulata* and *M. foetida*) were collected at the ripe stage and washed. Ripeness stages were determined according to fruit colour changes (*L. rubiginosa*; dark red to purple red); *A. reticulata* (red fruit skin; soft fruit texture); and fruit colour and texture (*M. foetida*; dark yellow, soft fruit texture); Flesh and pith were then separated; flesh was then dried at 50 °C for 8 - 10 hr. Dried fruit samples were then homogenized and stored at 4 °C.

Sample extraction

Extractions were performed using maceration with methanol. Dried and homogenized samples were macerated in methanol (1:3; w/v) for 24 hr and filtered. The filtrate was collected and evaporated to dryness using a dryer. The remaining residue was air-dried to remove the solvent.

Phytochemical screening

Prepared methanol extracts were subjected to phytochemical screening tests to determine the presence of groups of secondary metabolites. Tests that were carried out and their respective methods are as follows: alkaloids were tested using Dragendorff's, Mayer's and Bouchardat's reagents; flavonoids were detected using Shinoda's test; phenolic compound and polyphenol were tested using potassium dichromate test and ferric chloride test, respectively; and terpenoid/steroid were detected using Salkowski's test. All procedures were carried out as per the

previous protocols (10).

The total flavonoid content

Total flavonoid content was determined using the colorimetric $AlCl_3$ method (11). An aliquot of 1 mL of extract solution or quercetin was mixed with 0.2 mL of 10 % (w/v) $AlCl_3$ solution in methanol, 0.2 mL of 1 M potassium acetate and 5.6 mL distilled water. The mixture was incubated for 30 min at room temperature, followed by the measurement of absorbance at 434 nm against a blank. The outcome data were expressed as mg QE (quercetin equivalent)/100 g of extract.

Total phenolic content

Determination of total phenolic content was performed using the Folin-Ciocalteu method (12). Absorbance was recorded at 740 nm using a spectrophotometer (Shimadzu). A standard curve of gallic acid (0.05-1 mM) was used to determine the total phenolic content of fruit samples and the results are expressed as mg GAE (gallic acid equivalents)/100 g.

Antioxidant activity test

The antioxidant activity of MeOH fruit extract was determined using the 1,1-diphenyl 1,2-picrylhydrazyl (DPPH) method. Initially, DPPH stock solution (50 ppm) was prepared in methanol of pro-analysis grade. A stock solution of each of the MeOH fruit extracts was made by diluting 1 mg/mL in methanol. A series of extract concentrations (0.002-0.01 mg/mL) were prepared in methanol. Ascorbic acid, as a positive control, was also prepared at the same concentration as the tested extracts. Tested solutions were prepared by adding 2 mL of each prepared extract concentration and then were mixed with 2 mL of DPPH solution, followed by incubation in the dark condition for 30 min at 37 °C. The absorbance value was measured using spectrophotometry at 517 nm. The inhibition percentage was calculated using the formula:

$$\% \text{ inhibition} = \frac{(\text{Control absorbance} - \text{Sample absorbance})}{\text{Control absorbance}} \times 100 \quad (13)$$

The IC_{50} value of each extract was then determined using GraphPad Prism Software (version 10.2.2).

Antibacterial activity test

The bacteria used (*E. coli* and *S. aureus*) were clinical isolates from the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Syiah Kuala University. Bacteria were cultured in Nutrient Agar (NA) medium. Before the test, bacteria were suspended in a tube containing 5 mL of physiological NaCl. Turbidity of the cells was then adjusted with 0.5 % McFarland solution (1.5×10^8 CFU/mL). Then, bacterial cells were inoculated on MHA agar media. The antibacterial test was carried out using the disc diffusion method. MeOH extracts of samples were prepared by weighing the extract to 1 mg/mL concentration dissolved in methanol. Sterilized discs (6 mm) were loaded with 15 μ L of the corresponding extract with a pipette. The discs were then left to dry at room temperature before placement onto the surface of inoculated Mueller-Hinton (MHA) agar. Amoxicillin

and ofloxacin (the same as tested concentration) were used as positive control, while methanol was used as negative control. The experiments were conducted in triplicate. A clear zone around the disc indicated the inhibition of bacterial growth by the extract.

Statistical analysis

The results were expressed as mean \pm SD. The results were statistically analysed using SPSS v25.0 software (SPSS Inc., Chicago, IL, USA). Normality of the data was determined using the Kolmogorov-Smirnov test and equality of variance was determined based on the Levene test. Significance of differences between samples was determined using one-way ANOVA, followed by Tukey's test for post hoc analysis. $P < 0.05$ was considered significantly different.

Results and Discussion

Total flavonoid content and total phenolic content

Total flavonoid content (TFC) was measured using a colorimetric assay, in which the formation of chelate of Al (III)-flavonoids from binding with oxo and hydroxyl groups of flavonoid compounds was measured spectrophotometrically at 340 nm (14). TFC of MeOH extracts of the tested samples is presented in Table 1. *M. quadrifida* showed the highest flavonoid content (282.11 ± 0.10 mg QE/g extract). There are no previous reports on total flavonoid content. However, quercetin and its glycosides have been reported as the main flavonoid of *M. indica*, as well as other minor flavonoids such as catechin, apigenin, cyanidin, delphinidin, pelargonidin, luteolin, kaempferol and myricetin (15).

TPC was measured using the Folin-Ciocalteu method, which is based on the reaction of reduction of phosphomolybdate heteropoly acids' Mo(IV) centre in the heteropoly complex to Mo(V), which produced blue coloration measured at 740 nm (12). TPC of MeOH extracts of the tested samples is presented in Table 2. *M. quadrifida* showed the highest total phenolic content compared to other tested extracts (655.26 ± 0.1 mg GAE/g extract). There is no previous study reported on the TPC of *M. quadrifida*; however,

the records on some Mangifera genus are available. *M. pajang* fruit has been reported with a TPC value of 19.30 mg GAE/100 g (16). While the TPC value of various mango cultivars ranged between 15.25 ± 0.28 - 44.47 ± 0.78 mg GAE per 100 g FW, the value is closely related to various phenolic compounds identified (17). Information on the TPC of *L. rubiginosa* is quite limited. However, a study reported that the extraction of mature fruit of *L. rubiginosa* resulted in 5.00 ± 0.57 mg GAE/g DW (5). TPC of *L. rubiginosa* has been previously reported, used an 80 % MeOH extract, but the result reported here was much higher. The difference in the results might be due to various reasons, including different experimental conditions and the fruit sample used in each study. Thus, it is interesting to explore more about the TPC of *L. rubiginosa*'s fruit. Studies reporting the bioactive content of *A. reticulata* are limited. However, an investigation reported moderate TPC (199.1 ± 8.2 mg GAE/100g fresh fruit) of *A. reticulata* of Srilanka (18).

Flavonoid content of extract methanol is in correlation with DPPH free radical scavenging activity. A strong correlation has been reported between antioxidant activity, TPC and flavonoid content (19).

Antioxidant activities

The free radical scavenging activities of methanol extracts of *L. rubiginosa*, *A. reticulata* and *M. quadrifida* were measured using a DPPH assay. The results were expressed in terms of free radical scavenging percentage and inhibitory concentration (IC_{50} value). Scavenging percentage indicates the ability of extract to quench the DPPH free radicals which correlated with the absorbance of the resulted solution measured at 517 nm (Fig. 1).

Methanol has been previously reported as an effective solvent to extract polar compounds, including flavonoids and phenolic acids, which are often linked with antioxidant activity (20, 21). Methanol extracts of several underutilized fruits of Bireuen showed strong antioxidant activity, as shown in Fig. 1. At the highest tested concentration, *L. rubiginosa* showed the highest DPPH free radical scavenging activity, followed by *M. quadrifida* and *A. reticulata*, consecutively. However, the IC_{50} analysis indicated that *M. quadrifida* had

Table 1. Flavonoid content (mg QE/g extract) and total phenolic content (mg GAE/g extract) of methanol extracts of fruits from Bireuen Regency, Aceh, Indonesia

No	Sample	Total Flavonoid Content (mg QE/g extract)			Total Phenolic Content (mg GAE/g extract)		
		Average	SD		Average	SD	
1	<i>L. rubiginosa</i>	153.16	\pm	1.23 ^c	339.47	\pm	0.01 ^b
2	<i>M. quadrifida</i>	282.11	\pm	0.10 ^c	655.26	\pm	0.10 ^c
3	<i>A. reticulata</i>	66.32	\pm	0.01 ^a	23.68	\pm	0.10 ^a

Values associated with different letters on each column differ significantly from one another at $p < 0.05$ based on post hoc Tukey's test

Table 2. Phytochemistry test results of methanol extracts of several underutilized fruit from Bireuen Regency, Aceh, Indonesia

No	Samples	Alkaloids	Flavonoids	Polyphenols	Sterols	Triterpene	Phenolic
1	<i>L. rubiginosa</i>	-	+	+	+	-	+
2	<i>M. quadrifida</i>	-	+	+	+	-	+
3	<i>A. reticulata</i>	-	+	+	+	-	+

+ = positive presence of the tested compound(s); - = negative presence of the tested compound(s)

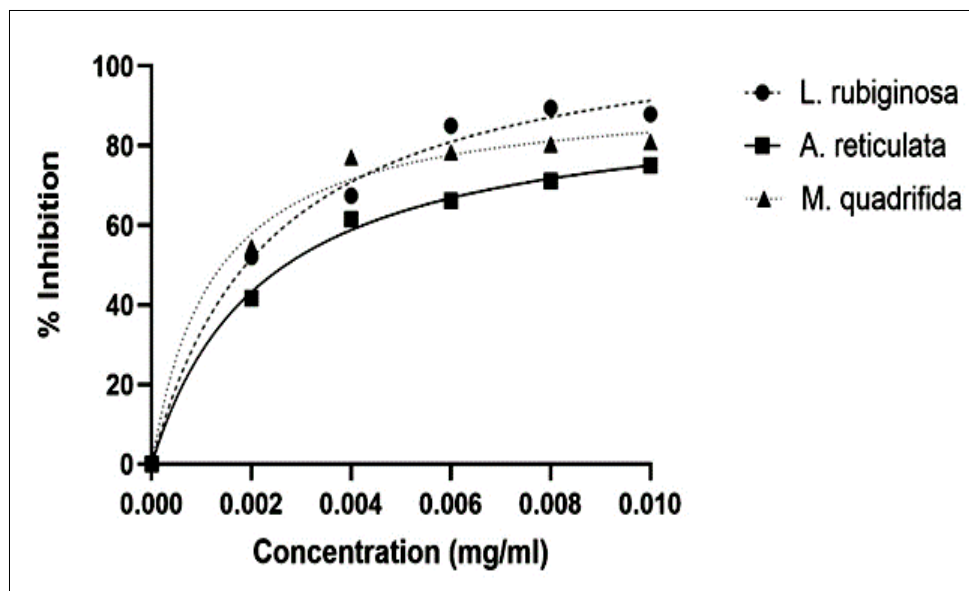


Fig. 1. Percentage of DPPH free radical scavenging activity of methanol extracts of several underutilized fruits from Bireuen Regency, Aceh, Indonesia at various tested concentration. Results expressed as mean \pm SD (in triplicate).

the lowest IC_{50} value among the tested samples. These values indicate the potency of *M. quadrifida* as a potent free radical scavenger, in which at low concentration, it can scavenge 50% free radicals in the tested solution, which is often regarded as a preferable indicator. A previous study reported a higher IC_{50} value of 0.0024 mg/ml of methanol extract of the baby fruit of *M. quadrifida* (22). The difference in maturity stages of the samples used in the study might contribute to the value. Reports on the DPPH free radical scavenging activity of *L. rubiginosa* are insufficient. A previous study reported the DPPH free radical scavenging activity of *L. rubiginosa* of 54.27 ± 0.13 mg Trolox/g DW (5). Different experiment designs resulted in different results, which are difficult to compare.

The determination of IC_{50} of methanol extracts of underutilized fruits is presented in Table 3. Pulp of *M. foetida* showed the lowest IC_{50} value, followed by *A. reticulata* and *L. rubiginosa*, consecutively. The result further highlighted the potency of underutilized fruits as a potent antioxidant agent, even though the values are higher than that of ascorbic acid as a positive control (0.0001 mg/mL).

Antibacterial activities

Sensitivity test using the disc diffusion method was employed in this study, using *S. aureus* and *E. coli*. *S. aureus* is a gram-positive bacterium often associated with skin infections and food poisoning (21). While *E. coli*, a Gram-negative bacterium, is often associated with fecal contamination of food samples (23). Samples of methanol extract of several underutilized fruits showed stronger antibacterial activity against Gram-positive bacteria than Gram-negative bacteria (Fig. 2). The difference in cell wall

Table 3. IC_{50} of DPPH free radical scavenging activity of methanol extracts of fruits from Bireuen Regency, Aceh, Indonesia

No	Samples	IC_{50} (mg/ml)
1	<i>L. rubiginosa</i>	0.0024
2	<i>M. quadrifida</i>	0.0012
3	<i>A. reticulata</i>	0.0023
4	Ascorbic Acid	0.0001

structure of both type of bacteria often resulted in different antimicrobial activity (24). Interestingly, *M. quadrifida* showed the widest inhibition area, indicating the strongest inhibition activity, both on *S. aureus* and *E. coli* (Table 4). There is no record of a previous study of the antibacterial activity of *M. quadrifida*. Information on other Mangifera fruit pulp or methanol extract of fruit pulp is also insufficient. *L. rubiginosa* and *A. reticulata* reports on the antibacterial study are still insufficient. However, *A. squamosa*'s weak antibacterial activity has been previously reported (25). Antibacterial activity of methanol extract tested is varied. However, all extracts showed inhibition activity against *S. aureus* and *E. coli*. Methanol, as a polar solvent, can extract a wide class of compounds with various biological potency. Flavonoids are known to exhibit antibacterial properties (26, 27). However, another class of compounds might be responsible for this activity.

Table 4. Antimicrobial activity of methanol extract of several underutilized fruit from Bireuen Regency, Aceh, Indonesia against *S. aureus* and *E. coli*

No	Samples	Diameter of inhibition zone (mm)			
		<i>E. coli</i>		<i>S. aureus</i>	
		Mean	SD	Mean	SD
1	<i>L. rubiginosa</i>	7.65 \pm	0.67	12.07 \pm	13.94
2	<i>M. quadrifida</i>	12.65 \pm	0.54	18.35 \pm	18.07
3	<i>A. reticulata</i>	8 \pm	0.39	11.2 \pm	9.1

Conclusion

Underutilized fruit showed significant potential as a source of bioactive compounds, which can be beneficial in health-promoting or other relevant aspects. *M. quadrifida*, *L. rubiginosa* and *A. reticulata* showed promising potency as antioxidant agents and bacterial growth inhibitors. Further study on specific bioactive compounds responsible for these activities, including their mechanisms of action, will be highly beneficial.

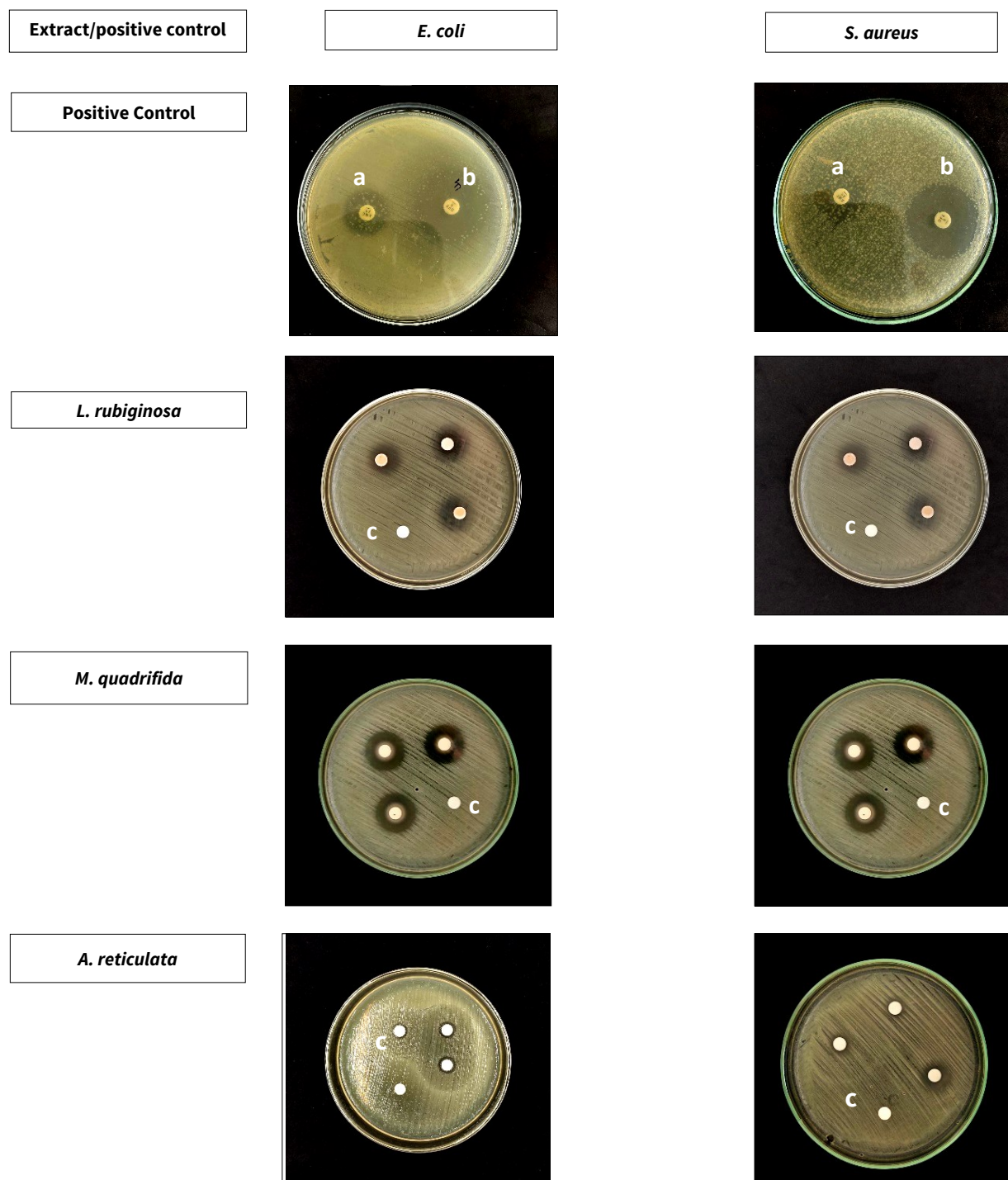


Fig. 2. Antibacterial activity of MeOH extracts of several underutilized fruit from Bireuen Regency, Aceh, Indonesia against *S. aureus* and *E. coli*, with amoxicillin and ofloxacin as positive controls. a. Amoxicillin; b. Ofloxacin; c. Negative control (methanol).

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Authors' contributions

E and ZF designed the experiment, coordinated it and wrote and finalized the manuscript. N wrote, statistically analyzed and sampled. AH conducted laboratory experiments and wrote the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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