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Research Article

Coleus atropurpureus (L) Benth. Leaves as a New Promising Drug for Abscesses Caused by Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus aureus*

Resmi Mustarichie[1,*], Yoppi Iskandar[2], Nyi Mekar Saptarini[1]

Resmi Mustarichie[1,*], Yoppi Iskandar[2], Nyi Mekar Saptarini[1]

[1]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, 45363, INDONESIA.

[2]Department of Biology Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, 45363, INDONESIA.

Correspondence

Resmi Mustarichie

Pharmaceutical Analysis and Medicinal Chemistry Department, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, INDONESIA.

Email: resmi.mustarichie@unpad.ac.id

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ABSTRACT

Background and Objective: *Staphylococcus aureus* and *Methicillin-Resistant Staphylococcus aureus* (MRSA) can cause an abscess, a skin condition with a build-up of pus due to a fluid or pus-filled tissue covering. This study aimed to determine the antibacterial activity of the ethanolic extract and its fraction from jawer kotok (Indonesian), *Coleus atropurpureus* (L) Benth.) against abscess-causing bacteria, *S. aureus* and MRSA. **Materials and Methods:** Laboratory Experimental Design. *C. atropurpureus* was collected, macerated using 96% ethanol extract, then fractionated using ethyl acetate and n-hexane. Antibacterial properties of ethanol extract and its fraction using S. aureus ATTC 25923 and MRSA. Statistical analysis used: descriptive statistics. **Results:** It was found that the MIC values *for S. aureus* ATTC 25923 and MRSA were in the range of 0.78% - 1.56% w/v and the MBC value for the two test bacteria was 1.56% **Conclusions:** Ethanol extract and n.hexane fraction from *C. atropurpureus* were new drugs for abscess treatment. It is necessary to research the formulation and evaluation of the ethanolic extract and the n-hexane fraction from *C. atropurpureus* against the two bacteria that cause abscesses first.

Key words: Antibacterial, Maceration, *Coleus atropurpureus* (L) Benth., *Methicillin-Resistant Staphylococcus aureus* (MRSA), *Staphylococcus aureus* ATTC 25923.

INTRODUCTION

Abscesses is soft mass filled with pus caused due to infection. It can develop in any part of the body. They are usually red, warm, and painful. One of the causes of infectious diseases is bacteria. Bacteria are microorganisms that cannot be seen with the naked eye but can only be seen with the aid of a microscope.[1,2] Pathogenic bacteria cause sporadic and endemic infections, including Staphylococcus aureus and Methicillin-resistant Staphylococcus aureus (MRSA).[3]

S. aureus is a normal human microflora. The upper respiratory tract and skin are breeding grounds for these bacteria. *S. aureus* infection is associated with several pathological conditions, including boils, acne, pneumonia, meningitis and arthritis. Most of the diseases caused by these bacteria produce pus; therefore, these bacteria are called pyogenic.[4] MRSA is *S. aureus* bacteria that has become resistant to antibiotics that can generally kill *S. aureus*, for example, Methicillin, a type of antibiotic that belongs to the penicillin group. MRSA can cause infections of the skin, bones, lungs and heart. If the antibiotics given do not kill MRSA, the infection will continue and spread widely and endanger the sufferer's life. Skin infections by MRSA often occur and cause symptoms of swelling, pus, redness and pain.[5]

Antibacterial is a natural chemical compound that can inhibit the growth of bacteria at low levels. Organisms can produce natural antibacterial by making a compound similar to the original, obtained directly from the organism that produces the compound by carrying out the extraction process.[6]

C. atropurpureus (L) Benth. is an ornamental plant as a traditional medicine originating from Southeast Asia. The pattern, shape and color of the *C. atropurpureus* vary, but the medicinal ones are the brownish-red leaves.[7] The *C. atropurpureus* plant contains efficacious compounds such as antibacterial, diarrhea, ulcers, ear infections, hemorrhoids and an appetite enhancer.[8]

Ajizah mentioned in addition to the concentration factor, the type of antimicrobial material also determines the ability to inhibit the growth of germs.[9] In this study, the antibacterial activity of *C. atropurpureus* is thought to be due to the presence of nutritious compounds, such as flavonoids, polyphenols, saponins, alkaloids and essential oils. This study reports *c. atropurpureus* (L) Benth. leaves as a new promising drug for abscesses caused by *Methicillin-resistant Staphylococcus aureus* and *Staphylococcus aureus* ATTC 25923.

MATERIALS AND METHODS

This research was conducted with the following stages: first, Determination of plants and preparation of simplicial, followed by extraction, fractionation, characterization of the extract and testing of the antibacterial activity of the extract. Then the antibacterial activity of the fraction was carried out, Determination of the Minimum Growth Inhibitory Concentration (MIC) and Minimum Kill Concentration (MBC) of the most active fraction. The research continued with a phytochemical screening of the extract, the most active fraction and Thin Layer Chromatography Profile (TLC) of the fraction.

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RESULTS AND DISCUSSION

Material collection

Collection of C. atropurpureus leaves from Manoko, Lembang, West Java. determination at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. showed that the plants used in this study belonged to the kingdom Plantae, division Magnoliophyta, class Magnoliopsida, order Lamiales, family *Lamiaceae*, genus Plectranthus and species *P. scutellarioides* (L.) R.Br. with its synonymous name *C. atropurpureus* Benth.

Extraction

The leaves of *C.atopurpureus* were washed and dried to be free of foreign materials and other impurities, then cut into small pieces to increase the surface area. Furthermore, the maceration method for extraction *C.* atropurpureus leaves using 96% ethanol. Maceration was a safe method for all substances, both resistant to the heating process and not heat-resistant.[10] 96% ethanol could attract almost all chemical compounds in plants, both polar, semi-polar and non-polar. In addition, ethanol was a universal solvent and was not toxic.[11] The concentration of 96% ethanol had a small water content, namely 4%, where the water serves to help penetrate the solvent into the plant cell wall. The choice of 96% ethanol solvent because fresh *C. atropurpureus* leaves had a lot of water content ethanol with a low water concentration was needed.[12]

The fresh leaves of *C. atropurpureus* were soaked in 96% ethanol for 3 x 24 hours in a dark brown macerator and occasionally stirred during immersion and kept away from sunlight. The macerate was collected every day. The aqueous extract obtained was then concentrated using a rotary evaporator at a temperature of 50 – 60°C to separate the ethanol from the active substance of interest. Ethanol is a disinfectant that can inhibit and kill bacteria, so it must be separated from the extract to not interfere with the antibacterial activity test.[13] Evaporation of the extract using a rotary evaporator followed by a water bath at a temperature of 50-60°C until a thick extract with constant weight and calculated yield.

Phytochemical screening

Table 1 shows the phytochemical screening of the most active extracts and fractions. Saraswati et al reported the presence of flavonoids, glycosides, volatile constituents, phenolics and many other compounds.[14] Mutiatikum *et al.* stated that the secondary metabolites contained in Miana fruit (Plectranthus scutellaroides (L) R.Btlz) originating from 3 places grew from three different islands in Indonesia, namely Manado (North Sulawesi), Kupang (East Nusa Tenggara and Papua) showed the presence of tannins, alkaloids, saponins, steroids and terpenoids and flavonoids.[15] The difference in the results of phytochemical screening was most likely due to plant origin.

Thin Layer Chromatography (TLC) profile

Determination of thin-layer chromatography (TLC) profile of ethanol extract of *C. atropurpureus* leaves to ensure various secondary metabolites contained in it through the pattern of compound separation. Table 2 shows the results of determining the TLC profile of the ethanol extract of the leaves of *C. atropurpureus*. Table 2 shows the presence of 6 spots from the ethanol extract *C. atropurpureus*. They were patches of polyphenolic compounds, flavonoids, terpenoids, steroids and triterpenoids, as well as quinones.

Extract antibacterial activity test

The antibacterial activity test of the extract to determine the antibacterial potential of the ethanolic extract of Jawaer kotok leaves against the bacteria that cause abscesses, namely *S. aureus* ATTC 25923

Table 1: Results of phytochemical screening of ethanol extract of *C. atropurpureus* leaves.

Secondary Metabolite	Ethanol extract
Alkaloid	+
Polyphenol	+
Tannin	-
Flavonoids	+
Monoterpenoid dan Sesquiterpenoid	+
Steroids and Triterpenoid	+
Quinone	+
Saponins	-

Notes: + = detected; - = not detected

Table 2: Thin Layer Chromatography (TLC) ethanol extract of *C. atropurpureus* leaves.

No. spots	Rf	Visible light	UV	
			254nm	366nm
1	0.42	-	-	blue
2	0.46	yellow	-	pink
3	0.51	yellow	yellow	pink
4	0.65	yellow	-	pink
5	0.80	green	yellow	pink
6	0.87	green	-	blue

Table 3: Antibacterial activity of ethanol extract of *C. atropurpureus* Leaves against s. aureus ATTC 25923 and MRSA.

Extract concentration (%)	Inhibition zone diameter (mm)	
	Staphylococcus aureus ATTC 25923	Methicillin resistant Staphylococcus aureus
50	21.1±0.1	16,2± 0,057735027
25	15.1±0.1	13,3± 0,028867513
12,5	13.6± 0.115470054	11,5±0,1
6,25	11.5± 0.115470054	10,3± 0,115470054

Remarks: Diameter of perforator = 8.5 mm= 8,5 mm, data obtained from 3 measurements

Methicillin-resistant S. aureus. (see Table 3). Table 3 showed that the ethanol extract of the leaves of *C. atropurpureus* had good antibacterial activity against *S. aureus* ATTC 25923 and MRSA 50%, 25%, 12.5% and 6.25%. The greater the concentration of the extract solution, the greater the zone of inhibition.

Extract and fraction antibacterial activity test results

Antibacterial activity test of ethanol extract of *C. atropurpureus* leaf and its various fractions to determine the most active fraction and compare its antibacterial activity with ethanol extract. The test used the agar diffusion method at a concentration (12.5% w/v) in a similar cup. Table 4 shows the activity test of the extract and fraction of *C. atropurpureus* leaves against *S. aureus* ATTC 25923 and MRSA. The n-hexane fraction produced an inhibitory diameter of 18.0 mm against *Staphylococcus aureus* ATTC 25923. It resulted in an inhibitory diameter of 21 mm against Methicillin-resistant Staphylococcus *aureus*, more significant than the antibacterial activity of the extract. There were more antibacterial compounds in the fraction than the extract.

Determination of Minimum Growth Inhibitory Concentration (MIC) and Minimum Killing Concentration (MBC) most active fraction

The results of determining the n-hexane fraction of MIC as the most active fraction were carried out by the microdilution method using

Table 4: Antibacterial activity of ethanol extract and fractions of *S. aureus* ATTC 25923 and MRSA.

Groups	Inhibition zone (mm)	
	Staphylococcus aureus ATTC 25923	MRSA
Ethanol extract	11.2±0.05773502	11,8±0.115470054
n-hexane fraction	18.0±0.11547005	21.0±0.115470054
Ethyl acetate fraction	15.8±0.05773502	18.0±0.11547005
Water fraction	11.0±0.05773502	10.6±0.057735027

Remarks: Diameter of perforator = 8.5 mm= 8,5 mm, data obtained from 3 measurements

Table 5: MIC of n-hexane fraction against S. aureus ATTC 25923 and MRSA.

Concentration of n-hexane fraction (% w/v)	Results	
	Staphylococcus aureus ATTC 25923	MRSA
12,50	-	-
6,26	-	-
3,12	-	-
1,56	-	-
0,78	+	+
0,39	+	+
0,195	+	+
0,0975	+	+
0,0487	+	+

Remarks: - = no bacterial growth; + = there was bacterial growth

Table 6: MBC fraction of n-hexane against *S. aureus* ATCC 25923 and MRSA.

Concentration of n-hexane fraction (% w/v)	Results	
	Staphylococcus aureus ATTC 25923	MRSA
12.50	-	-
6.26	-	-
3.12	-	-
1.56	-	-
0.78	+	+
0.39	+	+
0.195	+	+
0.0975	+	+
0.0487	+	+

Remarks: - = no bacterial growth; there is bacterial growth

various concentrations of fractions with concentrations of 12.50%, 6.25%, 3.12%, 1.56%, 0.78%, 0.39%, 0.195 %, 0.0975%, 0.0487% (w/v). Table 5 shows The results of determining the n-hexane fraction of MIC against *S. aureus* ATTC 25923 and MRSA by the microdilution method.

From Table 5, that the MIC fraction of n-hexane against *S. aureus* ATTC 25923 and MRSA was susceptible to 0.78% - 0.0487%. This concentration represented the lowest concentration capable of inhibiting 90% of the initial inoculum growth. Therefore, to ensure the results of observations of bacterial growth and determine the value of MBC, using a subculture on a Petri dish containing MHA media. Table 6 shows the determination of the n-hexane fraction of MBC against *S. aureus* ATTC 25923 and MRSA.

There have been many reports about the efficacy of *coleus sp* that were different from this report. Subhas Chnadrappa *et al.*, for example, reported that the antibacterial activity of ethanol extract and decoction of *C. aromaticus* leaves against *E. coli.*[16] Alghamdi *et al.* reported that *C. forskohlii* extract had antibacterial properties against Against Multi Drug-Resistant Acinetobacter baumannii Strains Isolated from Hospital.[17] *C. blumei* is efficacious as a potential antibacterial mouthwash.[18] Leaf essential oil of *C. aromaticus* from Cambodia was steam-distilled and investigated for its percentage composition and antibacterial activity against fifteen oral microflora pathogen strains.[19] Evaluation antibacterial C. sp. against *S. aureus*, *E. coli, S. typhi* and *pseudomonas aeruginosa*.[20] *C. amboinicus* was performed by cup plate method. Pure cultures of Mucor species, Aspergillus species, *A. niger*, Penicillium species and Fusarium species.[21] Antibacterial & antifungal potentiality of *Ricinus communis* & *C. forskohlii* on some human pathogen microorganisms has also been reported.[22,23] Antibacterial Potentiality of Water Extract of selected Honey Samples on Some Clinical Isolates,[24] Antimicrobial Effects of the Fruit Extracts of *Punica granatum*, *Actinidia deliciosa* and *Citrus maxima* on Some Human Pathogenic[25] and Antimicrobial effects of *boswellia carterii*, *glycyrrhiza glabra* and *rosmarinus officinalis* some Pathogenic Microorganisms.[26]

So far, no similar report to our report. Therefore, it concluded that: extract and fractions of *C. atropurpureus* could be a new drug for abscess treatment. However, further research needs to formulate and evaluate extracts and *C. atropurpureus* fraction against two abscess-causing bacteria.

CONCLUSION

From the results of the study, it could conclude that: Ethanol extract of leaves *C. atropurpureus* had good antibacterial activity against *Staphylococcus aureus* and *Methicillin-resistant Staphylococcus aureus* at a concentration of 6.25%. The n-hexane fraction was the most active fraction from leaves *C. atropurpureus* and was good against *Staphylococcus aureus* and *Methicillin-resistant Staphylococcus aureus*. Extract and fractions of *C. atropurpureus* could be a new drug for abscess treatment. However, further research needs to formulate and evaluate extracts and *C. atropurpureus* fraction against two abscess-causing bacteria.

SIGNIFICANCE STATEMENT

This study discovers extract and fractions of *C. atropurpureus* could be a new drug for abscess treatment.

AUTHOR'S CONTRIBUTION

Resmi Mustarichie: carried out and supervised research, compiled the data and wrote manuscript; Nyi Mekar Saptarini: designed and supervised the study; Yoppi Iskandar: co-supervised the research especially collecting materials and helping in microbiological aspect of the research. All authors reviewed the manuscript.

CONFLICTS OF INTEREST

Authors declared that they have no conflicts of interest.

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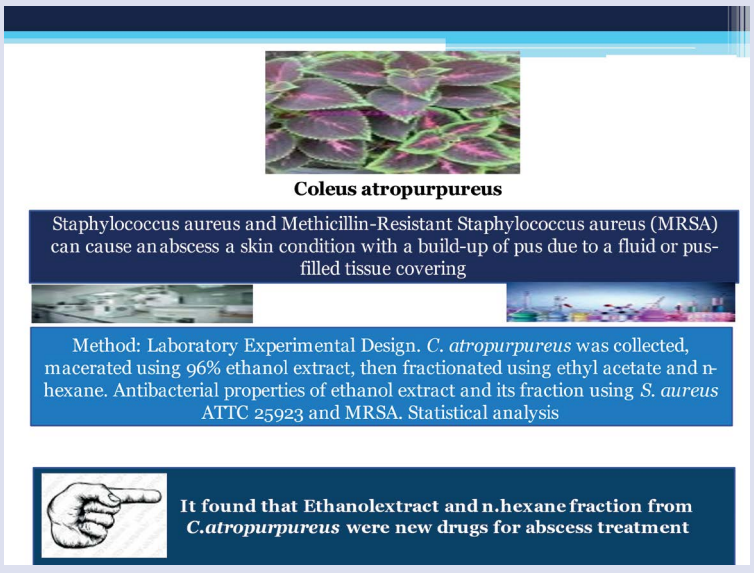
GRAPHICAL ABSTRACT



ABOUT AUTHORS

Resmi Mustarichie is Professor of Pharmaceutical Chemistry at the Department of Parmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia. His Ph.D. was awarded by Murdoch University, Perth, Western Australia. After graduating, he lived in Bandung, West Java, as a lecturer and researcher. He published many articles in reputed International Journals, wrote many ISBN books and conducted many types of research provided by the Government of Indonesia and on grants from Universitas Padjadjaran. His research interests are Anti alopecia from herbs, Analysis and determination of active compounds from plants, Anti-cancer compounds from herbs and *in-silico* research.

Dr. Yoppi Iskandar is BioPharmaceutical and Pharmacognosy Lecturer and Researcher at Biopharmacy Department, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia. His Doctorate in BioPharmacyst was awarded by School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia.
After graduating he lived in Bandung, serving at the Faculty of Pharmacy, as a lecturer and researcher. He has published many international articles, and books with ISBN, and conducted a lot of research funded by the Government of Indonesia and by the University of Padjadjaran. His research interest is in the field of pharmacognosy, elucidation structure and biochemical in herbs.

Dr. Nyi Mekar Saptarini is Pharmaceutical Biochemistry Lecturer and Researcher at Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia. Her Doctorate in Pharmaceutical Biochemistry was awarded by School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia. After graduating she lived in Bandung, serving at the Faculty of Pharmacy, as a lecturer and researcher. She has published many international articles and books with ISBN and conducted a lot of research funded by the Government of Indonesia and by the University of Padjadjaran. Her research interest is in the field of biochemistry in herbs, *in-silico* from compounds contained in plants.

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