

ASSESSMENT OF PHYSICOCHEMICAL PROPERTIES AND PHYTOCHEMICAL CONSTITUENTS ALONG WITH ANTIBACTERIAL POTENTIAL OF *TARENNA POLYCARPA* LEAF FRACTIONSBUNGA RIMTA BARUS^{1*}, PANAL SITORUS¹, MASFRIA²¹Department of Biological Pharmacy, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia. ²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia. Email: bungarimtabarus@gmail.com

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ABSTRACT

Objective: The aim was to investigate the physicochemical analysis, preliminary phytochemical screening and further evaluation of its antibacterial activity from morbesi-besi (*Tarenna polycarpa*) leaf ethyl acetate and hexane fractions (HF).

Methods: The physicochemical parameters were carried out as per the World Health Organization (WHO) guideline from its ethanolic extract. The phytochemical screening was performed as per Indonesia Pharmacopoeia and conventional method on its ethyl acetate and HF. Ethyl acetate and HF at different concentration were evaluated using agar well diffusion assay.

Result: The plant was found to be free from contaminations. Morbesi-besi leaf fractions are composed of flavonoids, glycosides, saponins, tannins, steroids, and triterpenoids. The phytochemical screening showed only ethyl acetate of lotus leaf extract contains the flavonoids. The antibacterials assay showed that ethyl acetate fraction was better than HF from the inhibition zone.

Conclusion: From the outcomes of this study, morbesi-besi leaf (*T. polycarpa*) possess potential antibacterial activity.

Keywords: *Tarenna polycarpa*, Fractions, Antimicrobial, Phytochemical, Physicochemical, Agar well diffusion method.

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INTRODUCTION

Nowadays, the use of herbal drugs to bacterial infection and is gaining popularity due to their effectiveness, fewer side effects, low cost, and availability [1]. Nature has been the source of many medicinal agents for thousands of years and large of the population of the people living in the developing countries almost exclusively using the traditional medicines [2]. One of the developing countries is Indonesia. Indonesia has a lot of medicinal plants, but despite this abundance, only around 300 species that have been explored for the beneficial properties [3]. One of them is morbesi-besi (*Tarenna polycarpa* (Miq.) Koord. ex Valetton) leaf. Morbesi plant belongs to the *Rubiaceae* genus which widespread in the Central Tapanuli Region in North Sumatera, Indonesia [4].

There is a wide range of reported therapeutic effects which have been proved by Middle Tapanuli society such as hypoglycemia, anti-cholesterol, antidote, and antimicrobial. In recent years, with increasing technology and medical knowledge, people have been becoming aware of the quality of antimicrobial medical care. This can happen because of the resistant process from the microbial agent [5].

In this study, the physicochemical parameters were studied, and the ethyl acetate and hexane fractions (HF) of dried leaf of morbesi-besi (*T. polycarpa*) were subjected to phytochemical screening and their effect on antibacterial properties. This plant may provide the new source of plant drugs to fight against the bacterial infections.

METHODS**Plant material**

The leaf of morbesi-besi leaf was collected in the month of November 2016 from the local area of Sibolga (North Sumatera, Indonesia) and authenticated by Indonesian Institute of Sciences: Research Center For Biology (No: 615/IPH.1.01/If.07/III/2016. Voucher specimen was

deposited in the Pharmacognosy Laboratory, Faculty of Pharmacy, University of Sumatera Utara for future reference and project.

Plant extraction preparation

Ethanolic extract of the powder was obtained by maceration method for 5 days followed by filtration. The ethanolic solvent was evaporated on a rotary evaporator to obtain crude ethanolic extract and dried using freeze dryer to get the dried crude ethanolic extract. The ethanolic extract was then suspended in distilled water and partitioned with hexane and ethyl acetate to obtain fractions of these solvents. The solvents were removed on rotary evaporator to obtain dried fractions [6,7].

Phytochemical screening of various lotus leaf extract

Phytochemical screening carried out on various morbesi-besi leaf fractions which are hexane, and ethyl acetate includes examine the chemical secondary metabolites of alkaloids, flavonoids, glycosides, tannins, saponins, triterpenoids, and steroids [8-10].

Physicochemical evaluation

Various physicochemical parameters such as total ash value, acid insoluble ash value, moisture content, alcohol, and water-soluble extractive value were calculated as per the WHO and Indonesia Materia Medika guidelines from morbesi-besi leaf powder [8,11].

Antibacterial activity assay**Microorganisms used**

The bacteria used are Gram-positive bacteria (*Staphylococcus aureus*/ATCC6538 and *Staphylococcus epidermis*/ATCC 12228) and Gram-negative bacteria (*Salmonella thypi*/ATCC 14028 and *Pseudomonas aeruginosa*/ATCC 9027) which were obtained from Microbiology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, with each concentration of culture test was 10⁶ cfu/ml which has been lined to the turbidity standard solution of McFarland [4].

Agar well diffusion method

Antibacterial activity was tested by the agar well diffusion method. Mueller Hinton Agar was prepared and autoclaved for 15–20 min and poured in Petri plates and then cooled. The different concentrations (0, 20, 40, 60, 80, 100, 200, 300, 400, and 500 mg/mL) were used for this study. The Petri plates were kept for 3–4 h at low temperature and incubated at 36–37°C for 24 h. Antibacterial activity was recorded by measurement of the zone of inhibition around each disc in the plate using zone reader. Each assay was using triplicate for determination of antibacterial test [4].

Determination of minimum inhibitory concentration (MIC)

The MIC of the ethanolic extract was determined according to the macro broth dilution technique. Standardized suspensions of the test organism were inoculated into a series of sterile tubes of nutrient broth containing two-fold dilutions of leaf extracts and incubated at 37°C for 24 h. The MICs were read as the least/minimum concentration that inhibited the growth of the test organisms [12,13].

Statistical data analysis

The results were given as mean \pm standard deviation (SD) from 3 times measurement. Each assay was used triplicate for determination of antibacterial test.

RESULTS AND DISCUSSION

It is believed that the history of humankind medicine treatment already using herbal medicine to treat with many diseases. Some of the advantages of herbal medicines are that they have fewer side effects and safe to use over time. They also inexpensive compared to the formulated drugs and they are readily available [14]. One of the common diseases is an infection from microbial. Recently, the antibiotic drugs mostly have been resistance and become an increasingly serious problem [5] and this case, making the development of alternative antibiotics a very urgent issue. One of the rising plants is morbesi-besi from Central Tapanuli. Central Tapanuli society uses it as antimicrobial and antidote. Due to this ethnopharmacological usage and the lack of the information about its biological activities led us to investigate the phytochemical compound and the antibacterial activity of the ethanolic extract from morbesi-besi leaf.

Phytochemical screening analysis

Medicinal plants represent a rich source of chemical compounds. The phytochemical screening of ethyl acetate and HF of morbesi-besi leaf showed the presence of flavonoids, glycosides, anthraquinone glycosides, saponins, tannins, triterpenoids, and steroids compounds (Table 1). Morbesi-besi leaf contains almost all the phytochemical content except alkaloids. These showed that morbesi-besi ethanolic leaf extracts effective as an antibacterial agent.

Physicochemical evaluation

The result of physicochemical evaluation shows that morbesi-besi leaf powder was made with good quality and high purity level, which accepted by the WHO [15]. The result from the physicochemical evaluation is presented in Table 2.

Antibacterial activity

Antibacterial activity of ethanolic extract of different bacteria such as Gram-positive bacteria (*S. aureus*/ATCC6538 and *S. epidermis*/ATCC 12228) and Gram-negative bacteria (*S. typhi*/ATCC 14028 and *P. aeruginosa*/ATCC 9027) was evaluated and compared by a zone of inhibition in disc diffusion method. The ethyl acetate and HF exhibited maximum activity on 500 mg/ml concentration (Table 3 and Figs. 1 and 2). The data showed the ethyl acetate was more potential than HF from morbesi-besi leaf.

From the antibacterial assay, the data from the zone of inhibition showed the ethyl acetate fraction from morbesi-besi leaf significant activity against bacteria such as Gram-positive bacteria (*S. aureus*/ATCC6538 and *S. epidermis*/ATCC 12228) and Gram-negative bacteria (*S. typhi*/

ATCC 14028 and *P. aeruginosa*/ATCC 9027). This was expected because of the chemical compounds that contained in morbesi-besi leaf drawn on ethyl acetate provides an antibacterial activity which is very strong (David and Stout, 1971). According to David and Stout (1971), if the diameter of the zone of inhibition more than 20 mm, it can be concluded as a very strong antibacterial agent. With the lowest concentration (40 mg/ml), morbesi-besi leaf ethyl acetate fraction showed the super effective as an antibacterial agent rather than hexane fraction.

The results are almost similar to Karthikkumara study (2014) when they studied the antibacterial activity of *Tarenna asiatica* extract [16]. This

Table 1: Phytochemical screening result of various fractions of morbesi-besi leaf

| No | Screening | HF | EA |
|----|-----------------------|----------|----------|
| 1 | Alkaloids | Negative | Negative |
| 2 | Flavonoids | Negative | Positive |
| 3 | Glycosides | Negative | Positive |
| 4 | Tannins | Negative | Positive |
| 5 | Saponins | Negative | Positive |
| 6 | Triterpenoid/steroids | Positive | Negative |

HF: Hexane fraction, EA: Ethyl acetate fraction

Table 2: Physicochemical evaluation result of morbesi-besi leaf powder

| Physicochemical parameters | Value |
|--------------------------------|-------|
| Total ash value (%) | 4.75 |
| Acid-insoluble ash (%) | 1.97 |
| Moisture content (%) | 6.65 |
| Alcohol extractive soluble (%) | 26.31 |
| Water extractive soluble (%) | 36.06 |

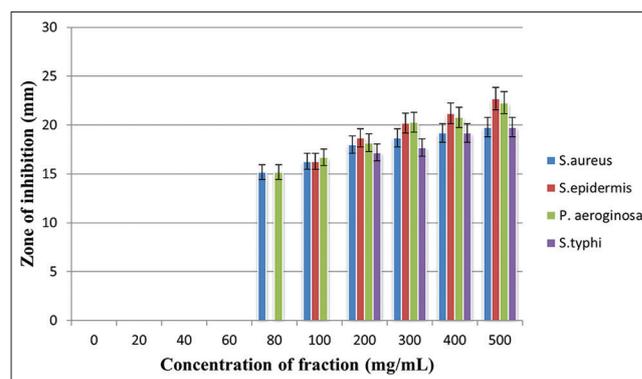


Fig. 1: Antibacterial activity of morbesi-besi leaf hexane fraction

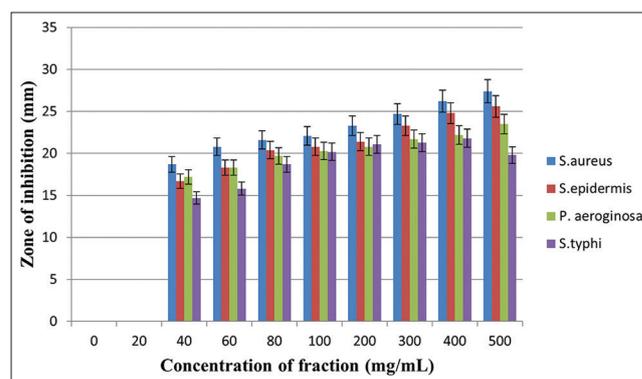


Fig. 2: Antibacterial activity of morbesi-besi leaf ethyl acetate fraction

Table 3: MIC (mg/mL) of hexane and EA from morbesi-besi leaf

| No | Concentration of fraction (mg/mL) | Zone of inhibition (in mm)* | | | |
|----|-----------------------------------|-----------------------------|---------------------|----------------------|-----------------|
| | | <i>S. aureus</i> | <i>S. epidermis</i> | <i>P. aeruginosa</i> | <i>S. thypi</i> |
| 1 | HF 500 | 19.8±0.14 | 22.7±0.75 | 22.3±1.0 | 19.8±0.14 |
| | EA 500 | 27.4±0.25 | 25.6±0.25 | 23.5±0.5 | 22.7±0.5 |
| 2 | HF 400 | 19.2±0.75 | 21.2±0.18 | 20.8±0.14 | 19.2±0.25 |
| | EA 400 | 26.2±0.14 | 24.8±0.14 | 22.2±0.25 | 21.8±0.14 |
| 3 | HF 300 | 18.7±0.75 | 20.2±0.14 | 20.3±0.5 | 17.7±0.25 |
| | EA 300 | 24.7±0.25 | 23.3±0.3 | 21.7±1.0 | 21.3±0.5 |
| 4 | HF 200 | 18±0.75 | 18.7±0.24 | 18.2±0.65 | 17.2±0.75 |
| | EA 200 | 23.3±0.75 | 21.4±0.5 | 20.8±0.27 | 21.1±0.75 |
| 5 | HF 100 | 16.3±0.75 | 16.3±1.0 | 16.7±0.24 | 16.3±0.24 |
| | EA 100 | 22.1±0.25 | 20.8±0.5 | 20.3±1.0 | 20.2±0.48 |
| 6 | HF 80 | 15.2±0.14 | - | 15.2±0.5 | - |
| | EA 80 | 21.6±0.18 | 20.4±0.75 | 19.7±1.0 | 18.7±0.3 |
| 7 | HF 60 | - | - | - | - |
| | EA 60 | 20.8±0.27 | 18.3±0.25 | 18.3±0.07 | 15.8±0.75 |
| 8 | HF 40 | - | - | - | - |
| | EA 40 | 20.7±0.25 | 16.7±0.75 | 17.2±0.25 | 14.7±0.18 |
| 9 | HF 20 | - | - | - | - |
| | EA 20 | - | - | - | - |
| 10 | HF 0 | - | - | - | - |
| | EA 0 | - | - | - | - |

*The results were given as mean±SD from 3 times measurement, HF: Hexane fraction, EA: Ethyl acetate fraction. MIC: Minimum inhibitory concentration, *S. aureus*: *Staphylococcus aureus*, *S. epidermis*: *Staphylococcus epidermis*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *S. thypi*: *Salmonella thypi*, SD: Standard deviation

higher inhibition zone of the ethyl acetate fraction of morbesi-besi leaf showed the effect of flavonoid and tannin compounds [17,18]. The MIC showed that low concentrations of 40 mg/ml of the plant extract, in disc method, can inhibit the growth of Gram-positive bacteria (*S. aureus*/ATCC6538 and *S. epidermis*/ATCC 12228) and Gram-negative bacteria (*S. thypi*/ATCC 14028 and *P. aeruginosa*/ATCC 9027).

CONCLUSIONS

The present results, therefore, offer a scientific basis for traditional herbal medicine use of both ethyl acetate and hexane fraction separately against Gram-positive and Gram-negative bacteria. This fraction of morbesi-besi (*T. polycarpa*) may provide a promising antimicrobial agent for therapeutic applications against Gram-positive and Gram-negative bacteria.

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AUTHORS CONTRIBUTION

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none.

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