ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Online - 2455-3891
Print - 0974-2441

Special Issue (May)

Research Article

# ANTIBACTERIAL ACTIVITY OF PAPUAN ANT-NEST (*MYRMECODIA PENDANS L.M. PERRY*) ETHANOL EXTRACT AGAINST *Z*

**ARIF SATRIA WIRA KUSUMA*, SRI AGUNG FITRI KUSUMA, SULISTIYANINGSIH**

**Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia. Email: arif.satria@unpad.ac.id**

***Received: 2 November 2016, Revised and Accepted: 24 January 2017***

**ABSTRACT**

**Background:** *Shigella dysenteriae* continues to be a major health problem in Indonesia, which usually leads to death, due to diarrhoea and dysentery, predominantly in children below the age of 5. Bacterial invasion of the colonic epithelium leads to severe inflammation together with bacterial dissemination generates abscesses and ulcerations. *Myrmecodia pendans*, also locally known by indigenous Papuans as ant-nest is native to Southeast Asia. This tropical plant has proven to be rich in bioactive constituents and highly valued as an alternative choice for cancer/tumor treatments and an efficacious herbal drug to prevent and cure diarrhea.

**Objective:** This research aimed to determine antibacterial activity of Ant-nest extract against *S. dysenteriae* and to determine its minimum inhibitory concentration (MIC) – minimum bactericidal concentration (MBC) range of concentration.

**Methods:** Ant-nest dried plant were obtained from Wamena, Papua. The extract was obtained by using maceration method with 70% ethanol as solvent. Antibacteria activity test were then performed by using perforated agar method with various extract concentration (10, 20, 40, and 60% (g/mL)). MIC-MBC concentration determinated by using microdilution method.

**Results:** From 500.42 g dried plant we can obtain 77.47 g dry extract (15.48% rendement). Phytochemical screening result showed that the ethanol extracts of ant-nest contains metabolites such as alkaloids, flavonoids, tanins, saponins, and steroids/terpenoids. Largest inhibition zone was shown by 60% extract concentration with 1.74 ± 0.021 cm diameter. MIC – MBC concentrations lays in range of 14 – 16 % (w/v).

**Conclusion:** The results of the present investigation suggest that the extracts of the studied plants can be used as potential leads to discover new drugs to control some *S. dysenteriae* infections..

**Keywords:** Ant-nest, *Shigella dysenteriae*, Antibacteria.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10s2.19485

## INTRODUCTION

Bacilli-related dysentery was first demonstrated by Shiga during 1898, and subsequent research showed that four serogroups (species), *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*, were responsible for causing this disease [1]. The World Health Organization estimates *Shigella* to cause at least 80 million cases of bloody diarrhea and 700,000 deaths each year [2]. Almost all infections caused by *Shigella* occur in developing countries, and the majority of cases and deaths occur among children <5 years old. Diarrheal disease associated with *Shigella* also occurs among travelers and military forces. *S. dysenteriae* type 1 is rarely endemic but can cause disease with severe complications and is historically associated with devastating pandemics with high case fatality rates in all age groups, described for Central America, Central Africa, and Southeast Asia [3].

*Shigella* ability to invade host cells involves attachment and internalization controlled by a large plasmid which contains multigene virulence factors. The organisms penetrate through the colonic mucosa, invade, and multiply in the colonic epithelium. Bacterial cells preferentially attach and enter through Peyer's patches by way of M-cells through membrane ruffling and macropinocytosis in a manner similar to *Salmonella* [4]. The bacteria also produce a very potent toxin (Shiga toxin) that adheres to small intestine receptors and blocks absorption of electrolytes, glucose, and amino acids from the lumen.

*Myrmecodia pendans* (a member of Myrmecophytes genus), which also locally known by indigenous Papuans as ant-nest plant, is a native plant that mostly grows in Southeast Asia region. Ant-nest has proven to be rich in bioactive constituents such as flavonoids, tocopherols, tannins, and essential oils. This plant is highly valued as an alternative choice for cancer/tumor treatments and an efficacious herbal drug to prevent and cure various illnesses include hemorrhoid, ulcer, allergy, gout, uric acid disorder, stroke, coronary heart, lung tuberculosis, rheumatism, and diarrhea [5]. Many researchers in pharmaceutical field strongly believe that the nutritional values as well as powerful *in vitro* anticancer, antibacterial, and antioxidant properties of ant-nest are sourced from flavonoid compounds [6].

The objective of this research was to test antimicrobial activity of ant-nest plant extract and found its effective concentration against *S. dysenteriae*. A series of microbiology-based procedure were performed during the research, including perforated agar method for antibacterial activity test and microdilution method for determining minimum inhibitory concentration (MIC) - minimum bactericidal concentration range.

## MATERIALS AND METHODS

### Chemicals and materials

The plant material used in this study was obtained from the stem tuber part of the plant. It is a traditional medicine plant from Wamena, Papua, Indonesia. Ethanol (Merck, Germany), ammonia (Merck, Germany), chloroform (Merck, Germany), hydrochloric acid (Merck, Germany), potassium iodide mercury (Merck, Germany), bismuth potassium iodide (Merck, Germany), magnesium (Merck, Germany), amyl alcohol (Merck, Germany), iron (III) chloride (Merck, Germany), gelatin solution (Merck), ether (Merck, Germany), 10% solution of vanillin in concentrated $H_2SO_4$, acetic acid anhydride in concentrated $H_2SO_4$, sodium hydroxide (Merck, Germany), distilled water, and dimethyl sulfoxide/dimethyl sulphoxide (DMSO) (Sigma Aldrich, Germany).

**Sample preparation**

Ant-nest was washed with running tap water and then rinsed with distilled water to remove any adsorbed contaminant from the sample surface. The cleaned sample was chopped and dried, then, placed in an oven at 40°C for 12 hrs to remove any remaining moisture. The dried material was ground by a blender into smaller parts and were collected for extraction.

**Extraction**

Maceration is a method that commonly used for extraction of bioactive components from natural products. This extraction method was chosen for the first preliminary study because of its simplicity and manageability. Small parts of dried ant-plant were macerated with 70% ethanol (1:20, w/v) at room temperature for 4 days and filtered through a Whatman no.1 filter paper. The extraction process was repeated until the last extract was colorless. Ethanol was then removed by using rotary evaporator at 68°C and water was removed by putting the crude extract on top of 40°C water bath for 24 hrs. The percentage of crude dry extract was determined as follows:

$$Y\text{extract}(\%)=\left(\frac{M\text{extract}}{m\text{feed}}\right)\times 100$$

Where Y extract is the extraction yield, M extract is the crude extract mass (g), and m feed is the feed mass (g).

**Phytochemicals screening**

The crude ethanolic extracts of ant-plant were tested for the presence of alkaloids, steroids, tannins, saponins, and glycosides. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

*Test for alkaloids*

Few mg (about 15 mg) of the extract was separately stirred with 1% HCl (6 ml) on a water bath for 5 minutes and filtered. These filtrates were divided into three equal parts.

a. Dragendorff's test: To one portion of the filtrate, Dragendorff's reagent (Potassium bismuth iodide solution) (1 ml) was added; an orange-red precipitate shows the presence of alkaloids.
b. Mayer's test: To one portion of the filtrate, Mayer's reagent (potassium mercuric iodide solution) (1 ml) was added. Formation of cream-colored precipitate gives an indication of the presence of alkaloids.
c. Wagner's test: Potassium iodide (2 g) and iodine (1.27 g) were dissolved in distilled water (5 ml), and the solution was diluted to 100 ml with distilled water. Few drops of this solution were added to the filtrate; a brown-colored precipitate indicates the presence of alkaloids [7,8].

*Tests for steroids and terpenoids*

a. Salkowski test: The crude extract (about 100 mg) was separately shaken with chloroform (2 ml) followed by the addition of concentrated $H_2SO_4$ (2 ml) along the side of the test tube, a reddish-brown coloration of the interface indicates the presence of terpenoid [9].
b. Liebermann–Burchard test: Extract (100 mg) was shaken with chloroform in a test tube; few drops of acetic anhydride were added to the test tube and boiled in a water bath and rapidly cooled in iced water. Concentrated $H_2SO_4$ (2 ml) was added alongside the test tube. Formation of a brown ring at the junction of two layers and turning the upper layer to green show the presence of steroids while the formation of deep red color indicates the presence of triterpenoids [7].

*Test for tannins*

Ant-plant extract (0.5 g) was separately stirred with distilled water (10 ml) and then filtered. A few drops of 5% ferric chloride were then added. Black or blue-green coloration or precipitate was taken as positive result for the presence of tannins [10].

*Test for saponins*

Ant-plant extracts (0.5 g) were separately shaken with distilled water (10 ml) in a test tube. The formation of frothing, which persists on warming in a water bath for 5 minutes, shows the presence of saponins [10].

*Tests for glycosides*

a. Anthraquinone glycoside (Borntrager's test): To the extract solution (1 ml), 5% $H_2SO_4$ (1 ml) was added. The mixture was boiled in a water bath and then filtered. The filtrate was then shaken with equal volume of chloroform and kept to stand for 5 minutes. Then, lower layer of chloroform was shaken with half of its volume with dilute ammonia. The formation of rose pink to red color of the ammoniacal layer gives indication of anthraquinone glycosides [7].
b. Cardiac glycoside (Keller-Killiani test): Extract (0.5 g) was shaken with distilled water (5 ml). To this, glacial acetic acid (2 ml) containing a few drops of ferric chloride was added, followed by $H_2SO_4$ (1 ml) along the side of the test tube. The formation of the brown ring at the interface gives positive indication for cardiac glycoside, and a violet ring may appear below the brown ring [9].

**Antibacterial activity**

*Test organisms*

The *in vitro* antibacterial activity of ant-plant ethanolic extract was tested against pathogen *S. dysenteriae*.

*Preparation of stock and working solutions*

The ant-plant ethanolic extract stock solutions were prepared at a concentration of 50 mg/ml, respectively, in 100% (DMSO, Sigma-Aldrich, Germany). The working solutions were prepared by diluting stock solutions in Mueller-Hinton broth.

*Determination of MIC*

The MIC was determined by broth microdilution method and as per the guidelines of Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2006, M7-A7), with some modifications. Briefly, 200 µl of the test sample was added to the wells of Column 1. All the remaining wells from column 2 to column 10 initially received 100 µl of MHB. Then, two-fold serial dilutions were performed by transferring 100 µl from column 1 to column 2 and continued through column 10. 100 µl of the excess medium was discarded from the wells in column 10. All the wells from column 1 to 10 received further 100 µl of drug-free MHB, whereas both columns 11 and 12 received 200 µl of drug-free MHB. For the preparation of bacterial inocula, 24 hrs cultures were suspended in 5 ml of sterile normal saline. The turbidity of each bacterial suspension was adjusted to 0.5 McFarland standards (1.5×108 CFU/ml). The bacterial suspension was further diluted in MHB, and 50 µl of the same was added to each well of microtiter plate to obtain a required inoculum of 5×105 CFU/ml in the well. The final concentration of ant-plant ethanolic extract ranged from 2.0 to 1000 µg/ml, respectively. Columns 11 and 12 served as growth and media controls, respectively. The plates were then incubated at 37°C for 24 hrs and were visually read for the absence or presence of microbial growth. The MIC was considered as the lowest concentration of the sample which completely prevented the visible growth.

**RESULTS AND DISCUSSION**

**Optimization of extraction condition**

To obtain an efficient extraction of target compounds, optimization of experimental conditions is a critical step in developing an extraction method. During this study, extraction was performed to obtain secondary metabolites contained by dried ant-plant. Since the active compound of ant-plant that acts as antimicrobial agent has not been

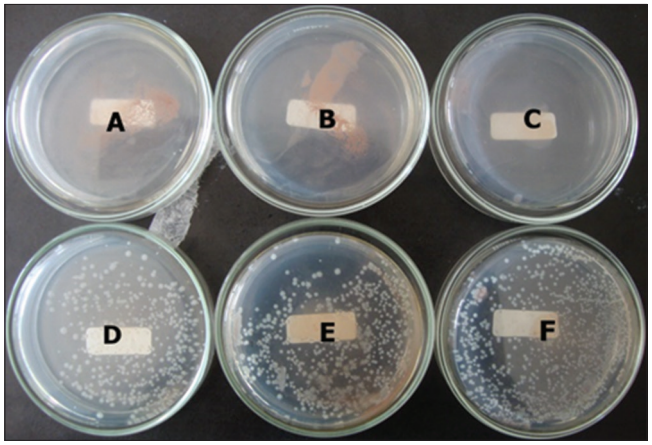


**Fig. 1: Subculture result (in % (w/v); a - 20, b - 18, c - 16, d - 14, e - 12, f - 10)**

discovered yet, the safest and simplest extraction method that can be used is maceration. By using maceration, we try to avoid the loss of thermolabile active compound that usually being degraded during heating process of extraction. After 4 days of extraction process using 5 liters of 70% ethanol as solvent, from 500.42 g dried plant, we can obtain 77.47 g dried extract. The characteristic of the extract was red-brownish powder and odorless.

**Phytochemicals screening**

The phytochemical screening of crude methanolic extracts of *M. pendans* L.M. Perry revealed the presence of some secondary metabolites such as alkaloids, steroids, flavonoid, saponins, and tannins as shown in Table 1.

The phytochemical compounds detected are known to have medicinal importance. For example, alkaloids have been reported as powerful poison, and many alkaloids derived from medicinal plants show biological activities such as anti-inflammatory [11], antimalarial [12], antimicrobial, cytotoxicity, antispasmodic, and pharmacological effects [13,14]. Similarly, steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal properties [15]. They are very often used in medicines due to their well-known biological activities. Tannins, according to research, are known to have antibacterial [16], antitumor, and antiviral activities [17]. They work by precipitating microbial protein thus making nutritional protein unavailable for them. Other phytochemicals called cardiac glycosides have been used to treat congestive heart failure and cardiac arrhythmia [18]. Their mode of action starts by inhibiting Na+/K+ pump which then increases the level of calcium ion, so more Ca+ would be available for the contraction of heart muscles which recover cardiac output and reduce the distension of heart [10,19]. These phytochemical compounds identified in ethanolic extracts of ant-plant may be responsible for the biological activities shown by *M. pendans* L.M. Perry and the reason for their use as a traditional medicine by native Papuan.

**Antibacterial activity**

Preliminary antibacterial activity test was performed by doing agar diffusion method. This method uses ethanolic ant-nest extract with various concentration: 60, 40, 20, and 10% (w/v). Antibacterial activity of the extract is shown by the clear zone at about the hole in the test medium. The clear zone indicated that the ant-plant extract can inhibit *S. dysenteriae* growth as shown in Table 2. This test was only a qualitative test that only aim to identify the presence or absence of antibacterial activity.

Determination of MIC range was conducted by using various concentrations of ant-plant ethanolic extract: 20, 18, 16, 14, 12, 10, and 8% (w/v), as the result shown in Table 3. Since the extract has red brownish color, it is rather difficult to see the growth rate of *S. dysenteriae* by using naked eyes. Hence, it is necessary to do subculture from the microtiter plate into the surface of MHA to properly detect bacterial growth, as shown in Fig. 1.

**Table 1: Phytochemicals screening**

| Test | Result |
|---|---|
| Alkaloids | |
| Dragendorff's test | + |
| Mayer's test | + |
| Wagner's test | + |
| Steroids/terpenoids | |
| Salkowski test | + |
| Liebermann-Burchard test | + |
| Tannins | + |
| Flavonoids | + |
| Saponins | + |

+: Presence

**Table 2: Agar diffusion results**

| Extract concentration (%) | Inhibition diameter (cm) |
|---|---|
| 60 (w/v) | 1.74 |
| 40 (w/v) | 1.64 |
| 20 (w/v) | 1.51 |
| 10 (w/v) | 1.41 |

**Table 3: MIC result**

| Extract concentration in % (w/v) | Bacterial growth |
|---|---|
| 20 | - |
| 18 | - |
| 16 | - |
| 14 | + |
| 12 | + |
| 10 | + |
| 8 | + |

+: Presence, -: Absence, MIC: Minimum inhibitory concentration

The activity of plant extracts is considered as significant if MIC values are below 100 µg/ml, moderate when 100< MIC ≤625 µg/ml or weak when MIC >625 µg/ml. On the other hand, the activity of phytochemicals is significant if MIC <10 µg/ml, moderate if 1< MIC ≤1 µg/ml, and low or negligible when MIC >100 µg/ml [20]. Therefore, the activity recorded for the ethanolic ant-plant extract can be considered to be weak.

## CONCLUSION

This work reports for the first time, the antibacterial activity of *M. pendans* L.M. Perry ethanolic extract against *S. dysenteriae*. The results of this study provide scientific basis for the traditional use of ant-plant against bacterial-administered diarrhea. The results of the present investigation suggest that the extracts of the studied plants can be used as potential leads to discover new drugs to control some *S. dysenteriae* infections.

## ACKNOWLEDGMENTS

This work was supported by Faculty of Pharmacy, Universitas Padjadjaran, Indonesia.

## REFERENCES

1. Shears P. Shigella infections. Ann Trop Med Parasitol 1996;90(2):105-14.
2. WHO. Guidelines for the Control of Shigellosis, Including Epidemics Due to *Shigella dysentaeriae* Type 1. Geneva, Switzerland: WHO; 2005.
3. Bercion R, Demartin M, Recio C, Massamba PM, Frank T, Escribà JM, *et al.* Molecular epidemiology of multidrug-resistant *Shigella*

*dysenteriae* Type 1 causing dysentery outbreaks in Central African Republic, 2003-2004. Trans R Soc Trop Med Hyg 2006;100(12):1151-8.
4. Blank TE, Nougayrede JP, Donnenberg MS. Enterophatogenic *Eschericia coli*. In: Donnenberg MS, editor. *Eschericia coli*: Virulence Mechanisms of a Versatile Pathogen. San Diego, CA: Academic Press; 2002. p. 81-118.
5. Hamsar MN, Mizaton HH. Potential of ant-nest plants as an alternative cancer treatment. J Pharm Res 2012;5(6):3063-6.
6. Soeksmanto A, Subroto MA, Wijaya H, Simanjuntak P. Anticancer activity test for extracts of Sarang semut plant (*Myrmecodya pendens*) to HeLa and MCM-B2 cells. Pak J Biol Sci 2010;13(3):148-51.
7. Joshi A, Bhobe M, Saatarkar A. Phytochemical investigation of the roots of *Grewia microcos* Linn. J Chem Pharm Res 2013;5:80-7.
8. Abdullahi MN, Ilyas N, Ibrahim H. Evaluation of phytochemical screening and analgesic activity of aqueous extract of the leaves of *Microtrichia perotitii* dc (*Asteraceae*) in mice using hotplate method. Med Plant Res 2013;3:37-43.
9. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, *et al*. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop J Pharm Res 2008;7(3):1019-24.
10. Banso A, Adeyemo S. Phytochemical screening and antimalarial assessment of *Abutilon mauritianum*, *Bacopa monnifera* and *Datura stramonium*. Biokemistri 2006;18:39-44.
11. Souto AL, Tavares JF, da Silva MS, Diniz Mde F, de Athayde-Filho PF, Barbosa Filho JM. Anti-inflammatory activity of alkaloids: An update from 2000 to 2010. Molecules 2011;16(10):8515-34.
12. Dua VK, Verma G, Singh B, Rajan A, Bagai U, Agarwal DD, *et al*. Anti-malarial property of steroidal alkaloid conessine isolated from the bark of *Holarrhena antidysenterica*. Malar J 2013;12:194.
13. Ameyaw Y, Duker-Eshun G. The alkaloid contents of the ethno-plant organs of three antimalarial medicinal plant species in the eastern region of Ghana. Int J Chem Sci 2009;7(1):48-58.
14. Thite SV, Chavan YR, Aparadh VT, Kore BA. Preliminary phytochemical screening of some medicinal plants. Int J Pharm Chem Biol Sci 2013;3:87-90.
15. Bagrov AY, Shapiro JI, Fedorova OV. Endogenous cardiotonic steroids: Physiology, pharmacology, and novel therapeutic targets. Pharmacol Rev 2009;61(1):9-38.
16. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother 2001;48(4):487-91.
17. Kumari M, Jain S. Review paper, tannins: An antinutrient with positive effect to manage diabetes. Res J Recent Sci 2012;1:70-3.
18. Kren V, Martínková L. Glycosides in medicine: "The role of glycosidic residue in biological activity". Curr Med Chem 2001;8(11):1303-28.
19. Aiyelaagbe OO, Osamudiamen PM. Phytochemical screening for active compounds in *Mangifera indica* leaves from Ibadan, Oyo state. Plant Sci Res 2009;2:11-3.
20. Kuete V. Potential of Cameroonian plants and derived products against microbial infections: A review. Planta Med 2010;76(14):1479-91.