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The Scientific Base of *Myrmecodia pendans* as Herbal Remedies

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Authors' contributions

This work was carried out in collaboration between all authors. Author JS designed the study, wrote the protocol and wrote the first draft of the manuscript. Author JS managed the literature searches, analyses of the study, performed the spectroscopy analysis and author CTO managed the experimental process and author PT identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To find out the scientific base of the traditional plant *Myrmecodia pendans* as a new natural source for herbal remedies in aspect of its therapeutic compounds and cytotoxic effect on normal cells.
Study Design: Experimental laboratory, *in vitro* study.
Place and Duration of Study: Laboratorium Bio Core Faculty of Dentistry Trisakti University, Jakarta, Balai Penelitian Tanaman Rempah dan Obat (BALITRO), Bogor and Pusat Studi Satwa Primata, Bogor, between March to August 2014.
Methodology: Several extraction methods of *Myrmecodia pendans* using maceration technique was done to evaluate their phytochemical contents and cytotoxic effects using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium (MTT) assay method.
Results: The phytochemical test of both ethanol 70% extract and boiling water extraction produce active phenolic compounds, especially those of flavonoids. There is no cytotoxic effect of the

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ethanol 70% dried extract on fibroblast cells by MTT assay method. One way analysis of variance (ANOVA) test showed significant differences of % inhibition cells growth effect between *M. pendans* extracts and control group ($p=0.00<0.05$). Tukey' high significant difference (HSD) test showed significant differences of % inhibition cells growth effect between each concentrations of 500; 250; 100; 50; 25; 12,5; 6,25; 3,125; 1,56 to 1.000 ppm and also between 1.000 ppm to control ($p=0.00<0.05$).

Conclusion: *Myrmecodia pendans* can be used as herbal remedies and moreover, the water boiling extraction can be employed as a simple manner for community herbal medicine without any toxic effect on cells.

Keywords: Myrmecodia pendans; community herbal medicine; phytochemical content; cytotoxic effect; MTT assay.

1. INTRODUCTION

Ant nests plant (*Myrmecodia pendens*) is an epiphytic plant of *Hydnophytinae* (*Rubiceae*) family with 5 genus; two of which are associated with ants. They are *Myrmecodia* (45 species) and *Hypnophytum* (26 species). From those species only *Hypnophytum formicarum*, *Myrmecoda pendens* and *Myrmecodia tuberosa* that are considered to have medicinal value. [1] However, scientific literature on these plants are still very limited and usually only cover their ecology, taxonomy, and ethnobotany [2].

The importance of finding a new natural source in aspect of its therapeutic compounds has urged this research to study its potential phytochemical contents and investigate its biocompatibility on cells.

Traditionally, some tribes in Wamena island (Papua New Guinea) consumed it as tea by boiling the dried part of this plant into water and used it as herbal remedies for various mild to severe diseases, such as nausea and breast cancer. *M. pendens* exhibits a strong anti-tumor activity in human oral tongue squamous cell carcinoma (B88 cell) through the induction of p27Kip1 and suppression of cyclin E. [3-4]. *M. pendens* can treat a variety of systemic diseases such as leukemia, heart diseases, tuberculosis, kidney and prostate dysfunction, various allergies, migraine, rheumatism, hemorrhoid, and infectious diseases [5].

M. pendans species used in this study contains essential nutrients, namely flavonoid and tanin. Flavonoid may role directly as antibiotic by disturbing the microorganism or viral function [3], and as antioxidant against free radical [6]. Such phenolic compound is a potent antimicrobial agent [7] that could be extracted by several methods. Heat reflux is a common method for the extraction of bioactive compounds from natural products. This extraction method is chosen for the first preliminary study because of its simplicity and manageability. A traditional method that is quite simple is by immersing the dried tuber part of this plant in the boiling water. However, this method is only able to extract 5% of the active compound. It is suggested that extraction through the mixture solution of alcohol and water yields more potential substances. [8] Based on the fact that there are many commercial products of ant nest plant in spite of the limitation of its scientific publication, this study is conducted to evaluate the phytochemical contents of several extraction methods and their effects on normal cells.

1.1 Extraction

The purpose of extraction is to investigate certain part of material which has bioactive compound. Extraction is a process to put the active component out of the material using certain solution which can be done by simple method, such as immersing in boiling water to obtain the semi liquid material. The heat reflux extraction is the most common method and is chosen for the first preliminary study for the extraction of bioactive components from natural products because of its simplicity and manageability. By this method, the dried and powdered plant material (1 g per batch) is extracted using water bath at 55ºC [9].

The temperature has strong influence on antioxidant activity of ant nest extract. Higher temperature can dissolve more active compound in the solution and decrease its viscosity. Smaller particle has larger surface area between solid and liquid phase. This will shorten the distance of diffusion and increase the speed mass migration. As a result, the total active phenolic compounds can be extracted faster [10]. Longer extraction process and smaller size of material may increase the advantage of extract due to extend of the molecule mobility from solid phase to solution [11].

1.2 Ant Nest Plant (*Myrmecodia pendens*)

The *M. pendans* plant is called *sarang semut* (Fig. 1) in local language (*sarang* is nest and *semut* is ant in Indonesian) since the inner part of its hypocotyls is used as nest by ants. *Sarang semut* contains glycoside, vitamin, mineral, flavonoid, tocopherol, polyphenol and tanin. In general, flavonoid can inhibit the growth of Gram positif and negatif bacteria. Flavonoid represents a highly diverse class of secondary plant metabolites with about 9000 structures [12]. Flavonoid is polyphenolic compounds derived from 2-phenylchromane commonly found in many plants, vegetables, and flowers. In literatures, flavonoid receives considerable attention specifically due to its biological and physiological importance [14,15]. In medical field, flavonoid acts as antioxidant [16], anti-carcinogenic, antibacterial, anti-inflammatory, anti-allergic, and anti-viral. Therefore flavonoid may prevent and treat several diseases such as diabetes, hemorrhoid, rheumatoid, migraine, periodontitis and cancer [17-21]. At the concentration of 12 ppm, flavonoid contains 313 ppm tocopherol against 96% free radical. This inhibition rate is stable up to higher concentration of free radical [3]. Flavonoid also functions as an antimicrobial agent either by binding the cell membrane to form complex binding, which can destroy microorganism membrane, or as extracellular dissolve protein due to its lipophylic nature [22].

Fig. 1. Ant nest plant (*Myrmecodia pendens*) [24]

Tanin has antibacterial role by destroying the bacterial membrane that induces the complex binding with enzyme or microbial substrate through its astringent nature. This complex binding with metal ion increases its toxicity capacity [23]. Therefore the inhibitory mechanism of tanin on the growth of bacteria is through bacterial protein denaturization decreasing the cell membrane function as substrate transport from one to other cells and nucleic acid synthesis.

2. MATERIALS AND METHODS

The plant material used in this study is *sarang semut* plant (ant nest) or *Myrmecodia pendans* species, obtained from a traditional plant medicine store in Jakarta, Indonesia (Fig. 2). This study evaluated three methods of extraction, namely the heat reflux by boiling in water, ethanol 70% semi liquid extraction, and ethanol 70% dried extraction.

Phytochemical test was done on each extraction in order to find out the active compounds. The ethanol 70% dried extract was then tested for its cytotoxicity effect on fibroblast cell culture using MTT assay.

2.1 Sample Preparation

The dried commercial ant nest plant was washed with running tap water and then rinsed by distilled water to remove any absorbed contaminant from sample surface. The clean sample was chopped and dried using freeze dryer, and then placed in an oven at 40°C for 12 h to remove any remaining moisture. The dried material was grounded by a blender into powder and passed through a sieve (120 meshes) and collected for extraction. The fibroblast cell culture was prepared for the ant nest plant's cytotoxicity test.

2.2 Extraction Using Maceration Technique

2.2.1 Boiling water extraction

As much as one spoon (10 g) of dried ant nest plant immersed in 250 mL distilled water was boiled in stainless bowl until half of mixture solution was left (125 mL). It was stirred several times during boiling process and kept cool for 15 minutes. The supernatant was used as sample for phytochemical test (Fig. 3). Three times water reflux of 200 g dried powder with 2 L distilled water resulted in 44.32 g (22.16%) water extraction.

2.2.2 Ethanol extraction

In this study, the ethanol extract was done at Balai Penelitian Tanaman Aromatik dan Obat (BALITRO), Bogor, using maceration technique. As much as 50 g dried powder was macerated using 500 mL ethanol 70% (with the ratio of 150

H_2O:350 ethanol absolute) for 5 days. The solution was stirred to prevent saturated condition. The supernatant was tested for phytochemical contents (Fig. 4).

The supernatant was then evaporated by vacuum rotary evaporator at 57ºC, 90 rpm, and frozen dry resulted in powder extract (Fig. 5) and finally was tested for its phytochemical compounds.

2.3 Cytotoxicity Test

The test was done at Pusat Studi Satwa Primata, Bogor. *Myrmecodia pendans* ethanol 70% dried extract was tested for its cytotoxicity on fibroblast cell culture using MTT (3-(4,5-*dimethylthiazol*-2-yl)-2,5-*diphenyltetrazolium bromide*, tetrazolium) *assay.* MTT *assay* is a laboratoric and standard colometric test to investigate the cell viability. This test is also used to evaluate medicament substances nature potency and toxicity. Micro plate reader was used to quantitatively measure the color changes that based on absorbance degree of viable cells at certain wave length, which is commonly of 500-600 nm [25].

3. RESULTS AND DISCUSSION

Phytochemical test of several extracts is shown at Table 1. Heat reflux is the most common method for the extraction of bioactive components from natural products [9]. It is proven in this study that maceration technique within distilled water without heat reflux shows the least phytochemical content. Three independent variables and three levels solvent composition, extraction time, and solvent to sample ratio, might affect the diffusion of substances from sample matrix to solvent [26]. Water bath extraction method at constant temperature (55°C) and optimum conditions of solvent composition (80% ethanol), extraction time, 4 h; ethanol/water composition, 80%; and solvent to sample ratio 50 ml/g were an optimum condition for extraction, resulted in maximum yield as much as 13.82% antioxidant. The solution needs to be stirred several times during the process to prevent saturation within the mixture solution [27].

High temperature extraction process of wider surface contact area resulted in incomplete extraction process due to dissolve of unexpected components within solution. Based on the result of phytochemical test of this study, dried extraction of *M. pendans* showed better result (Table 1). It is assumed that in the extraction process, the low and medium temperature give better result instead of those of high temperature. This condition is not appropriate if we use the crude plant or solid form with their bioactive component contents. In this case, the extraction process using high temperature may attract more bioactive components inside the solid material.

Ant nest plant is rich in phenolics that have potential value added products [26]. The efficacy of this plant is due to the content of active substances as shown in this study, such as polyphenol, flavonoid, tanin, alkaloid, triterpenoid and glycoside. Flavonoid is a polyphenol compound that has the potency as antimicrobial and anticancer that are also able to do programmed cell death or apoptosis, while saponin has strong antibacterial effect [28].

The previous *in vitro* study of ant nest plant ethanol extract started from the concentration of 100µg/ml had shown the decrease of human oral tongue squamous cell carcinoma cell line (SP- C1) and an increase following the increase of extract concentration up to 1.000 µg/ml. The highest inhibition (74.6%) occurred on the concentration of 1.000 µg/ml on 24 hours of application [29]. Another in vitro study showed that ant nest plant extract was able to inhibit the squamous cell carcinoma proliferation through induction of p27Kip1 protein and suppression of cyclin E. Protein p27Kip1 is a gene that induces the arrest of cancer cell cycle and apoptosis, and moreover the decrease of growth, invasion and metastasis of cancer cells. The cyclin E over expression which commonly occur in the progress human cancer was suppressed by the extract [30].

Fig. 2. Simplisia

Fig. 3. Boiling water extract

Fig. 4. Ethanol 70% extract

Fig. 5. Dried ethanol 70% extract

Table 1. Phytochemical compounds of several extracts of dried commercial ant nest plant

No	Sample	Extract	Phytochemical test		Methods	
1.	Dried ant nest plant	Ethanol 70% (mL)	150		Maceration	Qualitative
			saponin	+		
			tanin	+		
			phenolic	+		
			flavonoid	+		
			alkaloid	+		
			triterpenoid	+		
			steroid	-		
			glycoside	+		
2.	Dried ant nest plant	Ethanol 70% (mL) Rendemen (%)	65.98		Maceration	Qualitative
			saponin	+		
			tanin	+		
			phenolic	+		
			flavonoid	+		
			alkaloid	+		
			triterpenoid	+		
			steroid	-		
			glycoside	+		
3.	Dried ant nest plant	Boiling water (mL)	150		Percolation	Qualitative
			saponin	+		
			tanin	+		
			phenolic	+		
			flavonoid	+		
			alkaloid	+		
			triterpenoid	+		
			steroid	-		
			glycoside	+		
4.	Dried ant nest plant	Water (mL)	150		Maceration	Qualitative
			saponin	-		
			tanin	+		
			phenolic	+		
			flavonoid	+		
			alkaloid	+		
			triterpenoid	-		
			steroid	-		
			glycoside	+		

Cytotoxicity test is one of the initial research procedures in order to find out whether the new material can be explored as treatment substances by evaluating its toxic effect on cells *in vitro*. MTT assay is a common method for cytotoxicity test by measuring the viable of cells such as cells' response toward mitogen, growth factor and other growth substances. MTT assay is a colorimetric assay for assessing cell viability. MTT as a yellow tetrazole is reduced by dehidrogenase to purple formazan within mitochondria viable cells. A solubilization solution is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by spectrophotometer at a certain wavelength. This absorbance degree or optical density (OD) value will be in line to the number of viable cells within culture medium.

The cytotoxicity test in this study showed that there is no dead cell observed under microscope on each concentration of ethanol 70% extract treated medium well as shown in Figs. 6-9. These are the same as the control group in Fig 10, where the cells used are normal fibroblast cells culture with extract concentration of 1.56 ppm to 1.000 ppm. Elisa reader with wave length of 595 nm was used in this study to evaluate the viable cells by measurement absorbance degree of the purple formazan produced by viable cells. On Table 2, the number of viable cells (mean

value) increases following higher concentration. Negative inhibition percentage started to occur at the concentration of 250 ppm and increases following higher concentration. This means that outside of its toxic effect of the extract on cells' growth especially on those of bacterial and abnormal cells, i.e. cancer cells as shown on the previous studies in literature, there may be an induction process of extract on cells proliferation at certain concentration especially on those of normal cells such as used in this study. It is assumed that phytochemical contents within the extract such as flavonoid, tanin, pholyphenol, acting as antioxidant and antibacterial provide conductive environment for the normal cell growth within culture medium. This result was supported by the statistic analysis one way ANOVA test that showed significant difference of % inhibition viability cells between *M. pendans* extract and control group ($p=0.00<0.05$). Tukey' HSD test also showed the significant differences of % inhibition viability cells between concentrations of 1.000 ppm to 500; 250; 100; 50; 25; 12,5; 6,25; 3,125; and 1,56 ppm extract ($p=0.00<0.05$).

Table 2. Cytotoxicity test of ethanol 70% dried extract *Myrmecodia pendans* on fibroblast (wave length=595 nm)

***Myrmecodia pendans* ethanol 70% dried extract**	**Repeated**			**Mean**	**Inhibition (%)**
(ppm)	**ODI**	**ODII**	**ODIII**		
1000	0.496	0.347	0.365	0.403	-124.12
500	0.245	0.221	0.246	0.237	-32.10
250	0.221	0.206	0.203	0.210	-16.88
100	0.169	0.167	0.190	0.175	2.41
50	0.138	0.167	0.174	0.160	11.13
25	0.183	0.169	0.175	0.176	2.23
12.5	0.184	0.129	0.175	0.163	9.46
6.25	0.157	0.114	0.190	0.154	14.47
3.125	0.210	0.208	0.187	0.202	-12.24
1.56	0.176	0.179	0.154	0.170	5.57
Control group	0.178	0.179	0.182	0.180	0.00

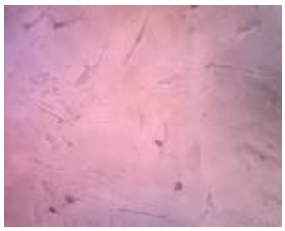

Fig. 6. There is no dead cell on concentration 1.56 ppm ethanol 70% extract treatment

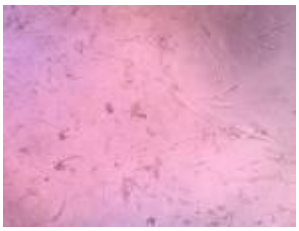

Fig. 7. There is no dead cell on concentration 25 ppm ethanol 70% extract treatment

Fig. 8. There is no dead cell on concentration 100 ppm ethanol 70% extract treatment

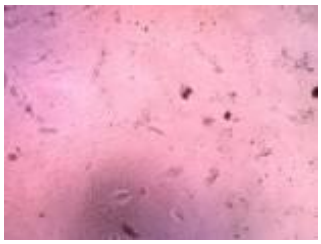

Fig. 9. There is no dead cell on concentration 1.000 ppm ethanol 70% extract treatment

Fig. 10. Control group (without extract treatment)

4. CONCLUSION

Heat reflux such as boiling water extraction is the most common method for extraction of bioactive components from natural products. Due to its simplicity and manageability, this method can be used by community as herbal medicine technique.

Phytochemical active compounds content within the ethanol 70% and boiling water extract of ant nest plant showed no differences, whereas these compounds play role as antibacterial, anticancer and antioxidant. Hence, the plant can be considered as a potential source for flavonoid particularly and phenolic compound generally.

Myrmecodia pendans ethanol 70% dried extract showed no toxic on fibroblast using MTT assay method. The higher concentration of extract showed the increase in viable cells and the decrease in inhibition percentage of cells proliferation. The findings from this work may add to the overall value of the herbal medicinal potential of ant nest plant that had been known previously as antibacterial and anticancer, yet does not show any toxic effect on normal cells.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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