

## Antibacterial Activity of *Andrographis paniculata* and *Euphorbia hirta* Methanol Extracts

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### ABSTRACT

*Two species of plants, Andrographis paniculata and Euphorbia hirta were screened for antibacterial activities against three Gram positives and Gram negatives. The leaves from both plants were extracted by methanol extraction. The antibacterial activity was detected with spread plate well diffusion method. The extracts of both plants demonstrated inhibitory activity against both Gram negative and positives bacteria. Staphylococcus aureus, Bacillus subtilis, Streptococcus epidemidis, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa. The minimum inhibitory concentration (MIC) determination using micro dilution method showed that the MIC of A. paniculata for the tested bacteria were 1.56 mg/ml (Staph. aureus), 3.13 mg/ml (Bacillus subtilis), 3.13 mg/ml (Strept. epidemidis), 1.56 mg/ml (Escherichia coli), 12.50 mg/ml (Klebsiella pneumoniae) and 3.13 mg/ml (Pseudomonas aeruginosa) respectively. The MIC value for E. hirta was 6.25 mg/ml (Staph. aureus) and 3.13 mg/ml (Bacillus subtilis), 3.13 mg/ml (Strept. Epidemidis), 3.13 mg/ml (Pseudomonas aeruginosa), 12.5 mg/ml (Escherichia coli), and 6.25 mg/ml (Klebsiella pneumoniae). Both plants represent a potential for pharmaceutical and agricultural applications and are worthy of further study.*

*Key words: Antibacterial, Andrographis paniculata, Euphorbia hirta, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC)*

### ABSTRAK

*Dua spesis tumbuhan herba Andrographis paniculata dan Euphorbia hirta telah disaring untuk melihat kesan antibakteria terhadap tiga bakteria Gram positif dan tiga Gram negatif. Kaedah ekstrak metanol telah digunakan untuk mengekstrak daun daripada tumbuhan. Aktiviti antibakteria dikesan dengan kaedah penjerapan perigi agar sebaran. Ekstrak daripada kedua tumbuhan tersebut telah memerencat kedua dua kumpulan bakteria ujian Gram positif dan negatif seperti Staphylococcus aureus, Bacillus subtilis, Streptococcus epidemidis, Escherichia coli, Klebsiella pneumoniae dan Pseudomonas*

*aeruginosa*. Ujian nilai perencatan minimum atau MIC yang dilakukan terhadap ekstrak *A. paniculata* adalah seperti berikut, 1.56 mg/ml (*Staph. aureus*), 3.13 mg/ml (*Bacillus subtilis*), 3.13 mg/ml (*Strept. Epidemidis*), 1.56 mg/ml (*Escherichia coli*), 12.50 mg/ml (*Klebsiella pneumoniae*) dan 3.13 mg/ml (*Pseudomonas aeruginosa*). Manakala nilai MIC untuk *E. hirta* pula adalah 6.25 mg/ml (*Staph. aureus*) 3.13 mg/ml (*Bacillus subtilis*), 3.13 mg/ml (*Strept. Epidemidis*), 3.13 mg/ml (*Pseudomonas aeruginosa*), 12.5 mg/ml (*Escherichia coli*), dan 6.25 mg/ml (*Klebsiella pneumoniae*). Kedua-dua tumbuhan herba tersebut telah mempamerkan potensi untuk kegunaannya dalam sektor farmaseutikal dan aplikasi industri pertanian dimasa akan datang.

**Kata kunci:** Antibakteria, *Andrographis paniculata*, *Euphorbia hirta*, nilai perencatan minimum, kepekatan bakterisidal minima.

## INTRODUCTION

*Andrographis paniculata* is found in Southeast Asia, India, China and Pakistan. It has the distinguishably terrible bitter taste, due to a chemical known as andrografolida or diterpene lactone (Rajagopal et al. 2003). The herb has been used for almost hundred of years in Asia for treating upper respiratory tract infection, herpes and gastrointestinal tract infection. It has also been used to treat hypertension, diabetic or as an anti-malarial (Sheeja et al. 2007 and Dual et al. 2004).

*Euphorbia hirta* or known as cats hair or gelang susu in Malaysia, grows robustly well in the tropics and subtropics i.e. Africa, Asia and the Central America. This hairy plant grows up to 2 inches in height, has numerous small flowers clustered together with opposite oblong leaves. The young yellow fruit is a small hairy capsule with 3 reddish-brown seeds. The plant flowers and fruits all year long. *E. hirta* has many traditional medicinal values. Some herbalists use it to treat dysentery, diarrhea and colic (Lanhers et al. 1996) The latex is usually applied topically to treat small wound and also used as disinfectant (Sudakhar et al. 2006). Its extract was also found to active against *Helicobacter pylori* (Wang and Huang, 2005).

The extraordinary medicinal value of both plants has attracted special interest to investigate its natural antibacterial properties. The main objectives of this work are to collect methanol extract from the leaves of *Andrographis paniculata* and *Euphorbia hirta* and screen their antibacterial activities. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts are to be determined. This study could contribute to finding new antimicrobial substance that may control pathogens especially bacteria that are resistance to antibiotic.

## METHODS AND MATERIALS

### BACTERIA

Three Gram negatives and three positives, i.e. *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus epidemidis* were used throughout. The stock cultures were maintained on nutrient agar slopes at room temperature.

### METHOD OF EXTRACTION

Both *Andrographis paniculata* and *Euphorbia hirta*, were processed with similar method. The leaves were separated from bark, washed with cold water and left to dry at room temperature. Samples (1 kg) of dried leaves were grinded and mixed with a liter of absolute ethanol. The mixture was stirred thoroughly and left at room temperature for 24 hours. The insoluble particle from the mixture was removed from the solvent with slow filtration using Whatman paper no 1 and extracted liquid was collected in a clean conical flask. The flask was left for three to four days at room temperature until the solvent been evaporated partially. The remaining thick solution was further concentrated with a Rotary evaporator (R-114 Buchi) at 40°C. Finally the “jelly” like substance were carefully collected, weighed and kept in dark bottle at room temperature for storage. When required, the extract was dissolved in 10% DMSO solution.

### ANTIBACTERIAL SCREENING

Petri plates with 15 mL of Muller Hinton agar were inoculated with 0.1 mL of the microbial suspensions using sterile swabs that were spread on the medium, obtaining growth in junction. Three cavities (4 mm in depth and 4 mm in diameter) were made in each agar plate (total = 90) with a copper coil and completely filled with the plant extract. The plates were pre-incubated for 1 h at room temperature, and then incubated at 37°C for 24-48 h. Microbial inhibition diameters were measured. Positive and negative controls were done, keeping the plates inoculated and without inoculum, for the same periods and under identical incubation conditions. All assays were carried out under aseptic conditions.

### MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC)

The method tests the extract ability to prevent the growth bacteria in cultures. A series of tubes containing decrease concentrations of the extract is prepared, ranging from 50 mg/ml-0.0039 mg/ml. The tubes were inoculated with the test bacteria and incubated overnight at 30°C. Only the first clear tubes (without growth of bacteria) represents the MIC. Later the MBC was determined by taking

a drop of all clear tubes and subcultured on to a plate and the plates were incubated overnight. The minimum concentration of the extract with no sign of bacterial growth is accepted as the MBC.

## RESULTS

Methanol extracts of *Andrographis paniculata* and *Euphorbia hirta* inhibited all of the tested bacteria. Table 1 depicts the results of a typical experiment in which the effect of the two plants extracts were monitored and estimated by measuring the diameter of the zone corresponding to the area of bacteria growth inhibition. The diameter of the inhibition zone formed by dose for each bacteria is shown in Table 1. As the dose increases, the diameter of the inhibition zone was also increases. Most gram positives have much larger diameter than that of the gram negatives bacteria.

TABLE 1. Diameter of inhibition zones with dose of *Andrographis paniculata* and *Euphorbia hirta* ethanol extract

| Bacteria                      | Diameter of inhibition zone $\pm$ sd (mm) |                |                        |                |
|-------------------------------|---|----------------|------------------------|----------------|
|                               | <i>Andrographis paniculata</i>            |                | <i>Euphorbia hirta</i> |                |
|                               | 25 mg/ml                                  | 50 mg/ml       | 25 mg/ml               | 50 mg/ml       |
| <i>Staph. aureus</i>          | 11.0 $\pm$ 0.2                            | 24.5 $\pm$ 0.2 | 0                      | 15.0 $\pm$ 0.8 |
| <i>Bacillus subtilis</i>      | 9.3 $\pm$ 0.1                             | 20.8 $\pm$ 0.5 | 8.3 $\pm$ 0.4          | 18.5 $\pm$ 0.4 |
| <i>Strept. epidemidis</i>     | 10.5 $\pm$ 0.2                            | 21.5 $\pm$ 0.2 | 9.5 $\pm$ 0.5          | 19.8 $\pm$ 0.2 |
| <i>Pseudomonas aeruginosa</i> | 9.5 $\pm$ 0.4                             | 16.0 $\pm$ 0.5 | 10.1 $\pm$ 0.3         | 20.0 $\pm$ 0.3 |
| <i>Escherichia coli</i>       | 10.0 $\pm$ 0.1                            | 19.4 $\pm$ 0.4 | 0                      | 15.5 $\pm$ 0.6 |
| <i>Klebsiella pneumoniae</i>  | 0   | 11.0 $\pm$ 0.4 | 9.5 $\pm$ 0.1          | 17.7 $\pm$ 0.2 |

### DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC value of *A. paniculata* extract against *Staph. aureus* was 1.56 mg/ml followed by *Bacillus subtilis* and *Strept. epidemidis* (3.31mg/ml). With Gram negative bacteria, particularly *Klebsiella pneumoniae* required high amount of the extract to inhibit growth, MIC value recorded was 12.5 mg/ml. *Escherichia coli* and *Pseudomonas aeruginosa* however has the MIC value of 1.56, and 3.13 mg/ml respectively. The MIC value for *E. hirta* on gram positive, *Staph. aureus* was 6.25 mg/ml, *Bacillus subtilis*, and *Strept. epidemidis*, were 3.13 mg/ml. As for the gram negatives, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* have MIC of 3.13 mg/ml, 12.5 mg/ml, and 6.25 mg/ml respectively. The results for both plant extracts against all the test bacteria are shown in Table 2.

TABLE 2. Minimum inhibitory concentration for plants extracts used in the study

| Bacteria                      | MIC (minimum inhibitory concentration) |                        |
|-------------------------------|--|------------------------|
|                               | <i>Andrographis paniculata</i>         | <i>Euphorbia hirta</i> |
| Gram positive                 |  |                        |
| <i>Staph. aureus</i>          | 1.56 mg/ml                             | 6.25 mg/ml             |
| <i>Bacillus subtilis</i>      | 3.31 mg/ml                             | 3.13 mg/ml             |
| <i>Strept. epidemidis</i>     | 3.31 mg/ml                             | 3.13 mg/ml             |
| Gram Negatives                |  |                        |
| <i>Pseudomonas aeruginosa</i> | 3.13 mg/ml                             | 3.13 mg/ml             |
| <i>Escherichia coli</i>       | 1.56 mg/ml                             | 12.5 mg/ml             |
| <i>Klebsiella pneumoniae</i>  | 12.5 mg/ml                             | 6.25 mg/ml             |

## DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION (MBC)

The results of MBC for both plant extracts against all the test bacteria have been shown in Table 3. The MBC value of *A. paniculata* extract against the Gram positive bacteria (Table 3) namely *Staph. aureus* was 3.31 mg/ml followed by *Bacillus subtilis* and *Strept. epidemidis*, 6.25 mg/ml. The bactericidal concentration for *Klebsiella pneumoniae* was 25 mg/ml, *Escherichia coli* and *Pseudomonas aeruginosa* has MBC value of 3.13 and 6.25 mg/ml respectively. The *E. hirta* extract against Gram positive has MBC value of 12.5 mg/ml for *Staph. aureus*, *Bacillus subtilis*, and *Strept. epidemidis*, shared similar MBC value, 6.25 mg/ml. As for gram negatives, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* was 6.25 mg/ml, 25 mg/ml, and 12.5 mg/ml respectively.

TABLE 3. Minimum bactericidal concentration for plants extracts used in the study

| Bacteria                      | MBC (minimum bactericidal concentration) |                        |
|-------------------------------|--|------------------------|
|                               | <i>Andrographis paniculata</i>           | <i>Euphorbia hirta</i> |
| Gram Positives                |  |                        |
| <i>Staph. aureus</i>          | 3.31 mg/ml                               | 12.5 mg/ml             |
| <i>Bacillus subtilis</i>      | 6.25 mg/ml                               | 6.25 mg/ml             |
| <i>Strept. epidemidis</i>     | 6.25 mg/ml                               | 6.25 mg/ml             |
| Gram Negatives                |  |                        |
| <i>Pseudomonas aeruginosa</i> | 6.25 mg/ml                               | 6.25 mg/ml             |
| <i>Escherichia coli</i>       | 3.31 mg/ml                               | 25 mg/ml               |
| <i>Klebsiella pneumoniae</i>  | 25 mg/ml                                 | 12.5 mg/ml             |

## STATISTICAL ANALYSIS OF MIC

Student's t-test was carried out to determine whether the means of the MIC between Gram positive and Gram negative are distinct. The results were expressed as mean, (df = 10) and the  $P < 0.05$ , indicating that the MIC between Gram positive and Gram negative are significantly different.

## DISCUSSION

Two herbal plants, the *Andrographis paniculata* and *Euphorbia hirta* were screened for their antibacterial properties against three Gram negatives bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) and three Gram positives (*Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus epidemidis*). The results obtained by agar diffusion test showed that the concentration of 50 mg/ml of both extract presented the strongest antimicrobial activity, which gave a 100% kill towards all of the tested bacteria species. Minimum inhibitory concentrations (MIC) were determined, where values being largely below 12.5 mg/mL, although the *Escherichia coli* and *Klebsiella pneumoniae* used were found to be less susceptible to the inhibitory activity of the extracts.

Commonly in many studies *Pseudomonas aeruginosa* shows a remarkable capacity to resist antibiotics, either intrinsically (because of constitutive expression of beta-lactamases and efflux pumps, combined with low permeability of the outer-membrane) or following acquisition of resistance genes (e.g., genes for beta-lactamases, or enzymes inactivating aminoglycosides or modifying their target) (Mesaros et al. 2007). However, in this study, the bacteria was sensitive to *A. paniculata* and *E. hirta* extract, therefore supporting the worthiness of these extract in controlling resistance bacteria.

The average MIC values of both extract indicated that the Gram positive are more susceptible towards the two plants extract then the Gram negative. Which means that the concentration require to inhibit Gram negatives were higher compared to that of the Gram positives (Table 2). The main factors behind this can be explained from its well organized outer membrane structure, which makes many harmful substances difficult to penetrate into the bacteria cell (Gootz 2005). The Gram negatives are better protected with three layers of membrane. The outer most layer which consists of lipopolysaccharide and proteins creates an impermeable barrier which acts as a filter. It allows only nutrient and other much smaller molecules but prevent larger or hydrophobic molecules such as antibiotics, to permeate through (Howe et al. 2007). Other Gram negatives have efflux pumps to remove harmful substance out of the cells, which was found initially in *E.coli* and *P. aeruginosa* (Poole et al. 1993). Many similar efflux pumps have since been discovered in nearly all clinically relevant gram-negative bacteria.

The MBC values (Table 3) requires at least double fold more concentration to that of MIC. This is common in antibacterial testing because of a higher

concentration is required for bactericidal activity compared to inhibitory activity. Generally, the *Andrographis paniculata* and *Euphorbia hirta* extracts have broader spectra type of antibacterial. Both plants are potentially useful in controlling pathogenic bacteria namely *Pseudomonas aeruginosa* and other antibiotic resistant bacteria such as MRSA and VRE.

## CONCLUSION

*Andrographis paniculata* and *Euphorbia hirta* has antibacterial compound which inhibit both Gram positive and negatives bacteria. The MIC of ethanol extract of *A. paniculata* was between 1.56 mg/ml to 12.5 mg/ml and *E. hirta* ethanol extract were 3.13 mg/ml to 12.5 mg/ml. for a range of Gram-positive and negative bacteria. The MIC and MBC value were higher for Gram negative compared to that of Gram positives due to the well protected cell wall.

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