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Screening for Antibacterial Activity of *Andrographis paniculata* Used in Malaysian Folkloric Medicine: A Possible Alternative for the Treatment of Skin Infections

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Abstract

In this study non-polar (dichloromethane) and polar (MeOH & aqueous) extracts of *A. paniculata* (whole plant) were evaluated for *in vitro* antibacterial activity against 12 skin disease causing bacterial strains (7 gram positive strains; *Staphylococcus saprophyticus, Staphylococcus epidermis, Staphylococcus aureus, Streptococcus pyogenes, Bacillus anthracis, Micrococcus luteus, Enterococcus faecalis*) and 5 gram negative strains; *Proteus mirabilis, Proteus vulgaris, Klebsiella pneumoniae*, *Neisseria meningitis*, *Pseudomonas aeruginosa*) using the disc diffusion method at three concentrations; 1000, 500, and 250 µg/disc respectively in order to ascertain its folkloric claim to treat skin infections. The extracts showed significant antibacterial activities against both the Gram-positive and Gram-negative bacterial strains tested. Highest significant antibacterial activity was exerted by the MeOH extract against *E. faecalis* at 1000 µg/disc (24.00 ± 0.00 mm) and the least activity by the DCM extract against *N. meningitis* at 250 µg/disc (6.00 ± 0.00mm). The minimum inhibitory concentration ranged between 150 µg /mL and 300 µg /mL depending on microorganism and various extracts. Presence of phytochemicals such as terpenoids, tannins, flavonoids, saponins, alkaloids, amino acids and steroids were observed. These results candidly suggest the presence of promising antibacterial substances in the polar as well as non-polar extracts which could be potential phytomedicine for the treatment of skin

infections caused by pathogenic bacterial strains. These findings explicitly support its traditional claims and form a strong basis for further efforts to explore *A. paniculata's* antibacterial potential to treat skin frailties efficaciously. Our results confer the utility of this plant extracts in developing a novel broad spectrum antimicrobial agent.

Keywords: *Andrographis paniculata*, Acanthaceae, antimicrobial activity, skin infections, MIC.

Introduction

There have been high rise in the frequency of certain skin infections in developing countries including Malaysia. Indeed, skin infections are among the most prevalent in the world. Bacterial skin infections are common outpatient problems and the 28th most common infections diagnosis in hospitalized patients (Elixhauser *et al*., 2001). Studies have stated that it may account for up to 17% of clinical visits (Sadick, 1997). Therapies of bacterial skin infections are frequent problems due to the emergence of resistant bacterial strains to numerous antibiotics (Marimoto *et al*., 1999). Some plants have shown the ability to overcome resistance in some organisms and this has led to researchers' investigating their mechanisms of action and isolating active compounds (Ncube *et al*., 2007). Nowadays, researches on medicinal plants have attracted a lot of attention globally. A number of evidences have been accumulated to demonstrate the promising potentials of medicinal plants used in various traditional, complementary and alternative systems (Fabricant and Fansworth 2001; Kanokwan *et al*., 2008).

Andrographis paniculata (Burm.f.) Wall. ex Nees., also known commonly as "King of Bitters (English) or Hempedu Bumi (Malay)," is a member of the plant family *Acanthaceae*. It is an annual herbaceous plant which is widely cultivated in southern Asia, Scandinavia, China and some parts of Europe. *Andrographis paniculata* extract is traditionally used as a medicine to treat different diseases in India, China and Southeast Asia including Malaysia. The leaves and roots have traditionally been used over the centuries in Asia and Europe as a folklore medicine for a wide variety of ailments or as herbal supplements for health promotion. In traditional Chinese medicine, it is widely used to get rid of body heat, as in fevers and to dispel toxins from the body. In Scandinavian countries, it is commonly used to prevent and treat common cold (Caceras *et al*., 1997). Previous studies have explicitly revealed that *A. paniculata* has a wide range of pharmacological effects and some of them extremely beneficial such as anti-inflammatory (Shen *et al*., 2002), anti-diabetes (Syahrin *et al*., 2006), antidiarrhoeal (Gupta *et al*., 1990), antiviral (Wiart *et al*., 2005), antimalarial (Rahman *et al*., 1999), hepatoprotective (Trivedi and Rawal, 2005), anticancer (Zhou *et al*., 2006), antihuman immunodeficiency virus (HIV) (Calabrese *et al*., 2000), immune stimulatory (Iruretagoyena *et al*., 2005), and antisnakebite activity

(Samy *et al*., 2008). Diterpenoids and flavonoids are the main chemical constituents of *A. paniculata* which are believed to be responsible for the most biological activities of this plant (Tang and Eisenbrand, 1992).

A. paniculata has been used in the treatment of some skin infections in India and China by folkloric medicine practitioners. It is considered beneficial to the skin and is used both internally and externally for this purpose (Jain, 1991). Evidences on its wide use by the traditional clerics in treating some infections of the skin (Tapsell *et al*., 2006) have prompted us to choose and confirm this plant for further evaluation in order to ascertain its antibacterial potential to treat skin infections caused by some pathogenic bacterial strains.

Materials and Methods

Collection and preparation of plant material

Fresh plant material (5kg) of *Andrographis paniculata* was procured from the botanical gardens of the Forest Research Institute of Malaysia (FRIM), Kuala Lumpur, Malaysia. Specimen sample was authenticated by Dr. Richard Chang (Taxonomist, FRIM) and deposited (voucher specimen number: NMPC-KOS-025) in the Herbarium, Kulliyyah of Pharmacy, IIUM, Malaysia. All parts of the plant material were dried in a protech laboratory dryer (LDD-720) at 37°C in the dark for 7 days and grounded to powdered form using the Fritsch Universal Cutting Mill. This was then stored in a desiccators at 2ºC until further use.

Preparation of non-polar and polar extracts

500g dry powder of *A. paniculata* (whole plant) was sequentially extracted with dichloromethane and methanol using the Soxhlet apparatus on the water bath for 12 h each (Harborne, 1998). Each of the mixtures was carefully filtered using filter paper (Whatman No. A-3) and concentrated using a rotary evaporator (Buchi Rotary Evaporator, R-210) at 40^0C. The final concentrated extracts were stored at -18°C in labeled sterile bottles and kept as aliquots until further evaluation.

Another 500 g of powdered sample of the herb was extracted by soaking in 1 L double distilled water in a round bottom flask, stirred for about 6 min, closed tight using a rubber cork and left overnight at room temperature. Thereafter, the solution was filtered using filter paper (Whatman No. A-1) and extract was freeze dried and carefully stored at -18°C in labeled sterile bottles.

Microorganisms

Twelve skin disease causing bacterial strains were taken into consideration, viz., (7 Gram-positive: *Staphylococcus saprophyticus*-IMR S-1242, *Staphylococcus epidermis*-IMR S-947, *Staphylococcus aureus*-IMR S-277, *Streptococcus pyogenes*-IMR S-526, *Bacillus anthracis*-IMR B-132, *Micrococcus luteus*-IMR B-7, *Enterococcus faecalis*-IMR E-150 and 5 gram negative strains: *Proteus mirabilis*-IMR P-76, *Proteus*

vulgaris-IMR P-147, *Klebsiella pneumonia*-IMR K-6, *Neisseria meningitis*-IMR N-349, *Pseudomonas aeruginosa*-IMR P-84. All bacterial strains were purchased directly from the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia. The test organisms were sub-cultured at 37 °C for 24 h and maintained on nutrient agar media.

Screening for Antibacterial Activity

The agar disc diffusion method was employed for the determination of antibacterial activities of the extracts of *A. paniculata* (NCCLS, 2004). 7 gram-positive (*S. saprophyticus, S. epidermis, S. aureus, S. pyogenes, B. anthracis, M. luteus, E. faecalis*) and 5 gram-negative (*P. mirabilis, P. vulgaris, K. pneumoniae, N. meningitis, P. aeruginosa*) standard bacterial strains of human skin disorders were used. All bacterial cultures were first grown on nutrient agar plates at 37 ºC for 24 h. Few colonies (2 to 3) of similar morphology of the respective bacteria were transferred to a liquid medium (Mueller Hinton Broth) and incubated until adequate growth of turbidity equivalent to McFarland 0.5 turbidity standard was obtained. The inocula of the respective bacteria were streaked on to the Mueller Hinton plates. The dried plant extracts were dissolved in 10% aqueous dimethyl sulfoxide (DMSO) and sterilized by filtration through a 0.45 mm membrane filter. Sterile filter paper discs (5 mm) (Whatman no. 1) were punched and impregnated with 10 µl of the DCM, MeOH and aqueous extracts (corresponding to 1000, 500, and 250 µg/disc) and allowed to dry at room temperature. These were placed on the Mueller-Hinton agar plates inoculated with the test strains. The plates were then allowed to stay for 1 h at room temperature and finally incubated at 37 ºC for 24 h (Heraeus GmbH, D-6450, and Germany). The assessment of antibacterial activity was based on the measurement of diameter of inhibition zone (mm) formed around the disc. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Tetracycline (30 µg) and gentamicin (30 µg) were used as positive controls while 10% DMSO was taken as negative control.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the crude extracts of *A. paniculata* was determined by agar dilution method (EUCAST, 2000). The growth media, Mueller-Hinton agar (MHA) was first prepared and sterilized by autoclaving (Webco GmbH & Co. KG Bad Schwartau, Germany). The sterilized MHA was allowed to cool to 50 ºC and 18 ml each of the molten agar was added to test tubes which contained 1 ml of different concentrations of the test crude extracts (150 to 300 µg/ml). The mixture of the media and the crude extract were thoroughly mixed and poured onto pre-labeled sterile petri-dishes on a level surface. Additional petri-dishes containing only the growth media were prepared in the same way so as to serve for comparison of growth of the respective bacteria. The plates were then set at room temperature and dried. The suspensions of the respective bacteria (corresponding to 10^8 CFU/ml) were inoculated onto the series of agar plates. The plates

were then incubated at 37 ºC for 24 h. Experiments were performed in duplicate and MIC values expressed as the lowest concentration of the plant extracts that produced complete suppression of colony of respective bacteria.

Phytochemical screening

Phytochemical screening of plant extracts was carried out qualitatively for the presence of terpenoids, steroids, tannins, flavonoids, amino acids, glycosides, saponins, and alkaloids (Harborne, 1998).

Result and Discussion

The dichloromethane, methanolic and aqueous extracts of the whole plant of *A. paniculata* were investigated at 3 different concentrations by disc diffusion method against 12 bacterial strains notable for causing skin infections. The antibacterial activity was expressed as the average diameter of the zone of inhibition of bacterial growth around the disc. The minimum inhibitory concentration (MIC) of active extracts was determined by using the agar dilution assay. Most of the extracts displayed relatively high antibacterial activity against most of the tested microorganisms with the diameter of inhibition zones ranging between 6.00 ± 0.00 to 24.00 ± 0.00 (Table1). The gram-positive strains were found to be the most susceptible to growth inhibition by the plant extracts forming zones of inhibition ranging from 7.00 ± 0.00 to 24.00 ± 0.00 (Table1). The DCM extract was the least potent against *S. saprophyticus* (7.00 ± 0.00) at 250 µg/disc and the methanolic extract revealed the most potent antibacterial activity against *E. faecalis* (24.00 ± 0.00) at 1000 µg/disc (Table1). However, no activity was observed with the DCM, methanolic and aqueous extracts of the plant at 250 µg/disc against *M. luteus*, *S. pyogenes*, *E. faecalis* and *S. saprophyticus* (Table1). The gram negative strains were less sensitive to the plant extracts as compared to the gram positive, forming zones of inhibition ranging from 6.00 ± 0.00 to 20.33 ± 0.58 (Table1). The aqueous extract was the least potent against *N. meningitis* (6.00 ± 0.00) at 250 µg/disc and the methanolic extract showed the most potent activity against *P. mirabilis* (20.33 ± 0.58) at 1000 µg/disc (Table1). No activity was observed with the DCM, methanolic and aqueous extracts of the plant at 250 µg /disc against *P. aeroginosa* and *K. pneumoniae* (Table1). *P. aeroginosa* and all *Staphylococcus* strains used for the study were found to be resistant to tetracycline (Table1).

The highest MIC value was 300 µg /ml exerted by the DCM extract against *M. lutues* and *B. anthracis* and the aqueous extract against *M. luteus* and *E. faecalis* respectively. The least MIC was 150 µg/ml exerted by the aqueous extract against *S. aureus* and the methanolic extract against *B. anthracis* respectively (Table2). The highest MIC value was found to be 300 µg/ml exerted by the aqueous and DCM extracts against *K. pneumonia* and *P.aeroginosa* respectively and the least was 150 µg/ml exerted by the aqueous extract against *P. vulgaris* and the methanolic extract against *N. meningitis* respectively (Table 2).

Flavonoids, alkaloids and glycosides were present in all extracts of *Andrographis paniculata*.

Tannins, amino acids and saponins were present in methanol and aqueous extracts but were absent in dichloromethane extract. However, terpenoids and steroids were found to be present in dichloromethane and methanol extracts and were absent in aqueous extracts (Table 3).

Table 1. *In vitro* antibacterial activity of dichloromethane, methanol and aqueous extracts of whole plant of *Andrographis paniculata*. (values are mean of three replicates).

	Zone of inhibition diameters in mm										
Extracts	DCM			MeOH			Aqueous			Gentamicine	Tetracycline
Concentration	1000µg/disc	500µg/disc	250µg/disc	1000µg/disc	500µg/disc	250µg/disc	1000µg/disc	500µg/disc	250µg/disc	30µg	30µg
S.saprophyticus IMR S-1242	19.33±1.15	16.50±0.87	7.00±0.00	22.00±1.53	18.83±0.76	0.00±0.00	20.67±1.15	16.33±1.53	8.33±0.76	24.33±1.52	0.00±0.00
S. epidermis IMR S-947	18.00±0.50	18.00±0.50	8.33±1.04	20.67±0.58	18.67±1.15	8.00±1.00	19.00±0.00	17.67±0.58	12.00±0.50	18.67±1.52	0.00±0.00
S. aureus IMR S-277	20.00±1.50	17.00±0.00	14.00±0.50	22.00±0.00	17.50±0.50	13.50±0.87	19.00±0.00	15.67±0.58	10.00±1.00	22.33±1.08	0.00±0.00
B. anthracis IMR B-132	17.83±0.76	13.33±2.08	0.00±0.00	20.00±1.00	17.33±0.58	14.83±0.76	16.67±1.15	10.00±1.00	8.33±0.58	21.67±1.51	14.33±1.21
M. luteus IMR B-7	17.67±1.73	14.33±1.53	0.00±0.00	19.33±0.58	15.50±1.00	13.33±0.76	23.17±0.76	21.00±0.00	0.00±0.00	14.00±0.00	21.67±0.52
S. pyogenes IMR S-526	17.67±1.15	11.17±0.76	0.00±0.00	17.00±0.00	13.33±0.00	9.00±0.00	22.67±0.58	16.33±0.00	0.00±0.00	17.67±1.73	21.33±1.06
E. faecalis IMR E-150	21.33±1.53	14.17±1.89	0.00±0.00	24.00±0.00	16.00±0.00	12.00±0.00	22.00±1.00	15.67±1.00	0.00±00	25.00±1.73	24.67±1.97
P. mirabilis IMR IMR P-76	18.33±0.76	14.00±1.00	6.67±0.58	20.00±0.00	17.00±1.00	16.67±0.58	19.00±0.00	18.33±0.78	15.33±1.53	29.33±0.89	27.00±0.00
P. vulgaris IMR P-147	16.33±0.58	15.33±1.53	12.00±0.00	19.00±0.50	16.33±1.04	7.00±0.00	18.67±1.53	16.33±0.58	15.00±0.00	19.33±1.30	18.67±1.05
K. pneumoniae IMR K-6	15.67±1.53	14.00±0.00	10.33±1.15	14.00±1.00	11.67±0.58	11.67±0.58	19.33±1.15	10.00±1.00	0.00±0.00	21.67±1.08	15.00±0.00
P. aeruginosa IMR P-84	13.33±0.58	13.33±0.58	0.00±0.00	10.33±1.04	9.00±1.73	7.00±0.00	15.00±0.00	11.00±0.00	11.17±1.04	20.33±1.52	0.00±0.00
N. meningitis IMR N-349	17.67±0.76	15.33±0.58	10.00±0.00	18.33±0.58	9.67±0.58	7.00±1.00	12.17±0.76	14.67±0.58	6.00±0.00	12.00±0.00	18.67±0.52

Presence of phytochemicals flavonoids (Roa *et al.*, 2004) and diterpenoid lactones (Reddy *et al.*, 2003) in *Andrographis paniculata* have been reported.

Antibacterial activity of phytochemical andrographolide, a labdane diterpenoid (Xu *et al*., 2006) isolated from

plant materials has been studied. The antibacterial activity of the polar and non-polar extracts of the whole plant *Andrographis paniculata* suggests that every extract contains the effective active phytochemicals responsible for the elimination of microorganisms responsible for skin diseases.

Table 2. Minimum inhibitory concentration of of dichloromethane, methanol and aqueous extracts of whole plant of *Andrographis paniculata* against microorganisms. (values are mean of three replicates).

Organisms	Minimum Inhibitory Concentrations (µg/ml) Dichloromethane	Methanol	Aqueous
Gram Positive Strains			
S. saprophyticus	150	250	200
S. epidermis	200	250	250
S. aureus	250	200	150
B. anthracis	300	150	200
M. luteus	300	250	300
S. pyogenes	200	250	250
E. faecalis	250	250	300
Gram Negative Strains			
P. mirabilis	200	250	250
P. vulgaris	250	250	150
K. pneumoniae	300	200	250
N. meningitis	200	150	250
P. aeruginosa	300	250	250

Table 3. Qualitative analysis of the phytochemicals in the dichloromethane, methanolic and aqueous extracts of *Andrographis paniculata.*

Serial No:	Phytochemicals Constituents	Dichloromethane Extract	Methanolic Extract	Aqueous Extract
1	**Alkaloids**	+	+	+
2	**Amino acids**	-	+	+
3	**Flavonoids**	+	+	+
4	**Glycosides**	-	+	+
5	**Saponins**	-	+	+
6	**Steroids**	+	+	-
7	**Tannins**	-	+	+
8	**Terpenoids**	+	+	-

- = Negative (absent), + = Positive (present)

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization (Vogel, 1991). Although a significant number of studies have been used to

obtain purified phytochemicals, very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants (Veeramuthu *et al*., 2008). The greater susceptibility of gram-positive bacteria to plant extracts has been previously reported in South American (Paz *et al*., 1995), African (Kudi *et al*., 1999; Vlietinck *et al*., 1995) and Australian (Palombo & Semple, 2001) medicinal plant extracts. Susceptibility differences between gram-positive and gram-negative bacteria may be due to cell wall structural differences between these classes of bacteria. The gram-negative bacterial cell wall outer membrane appears to act as a barrier to many substances including antibiotics (Tortora *et al*., 2001). The significant results obtained in our study confirm the antibacterial potential of the plant investigated, and its usefulness in the treatment of skin infections. This *in vitro* study corroborates the antibacterial activity of *A. paniculata* used in folkloric medicine to treat skin infections (Jain, 1991; Ahmed *et al*., 1998). All these extracts were shown to exhibit inhibitory activity against most of the pathogenic bacteria which cause chronic bacterial skin infections. However, they were ineffective at low concentrations against *S. saprophyticus, E. faecalis, B. anthracis, M. luteus, S. pyogenes, K. pneumoniae* and *P. aeroginosa*. Hence, their medicinal uses in infections associated with these bacterial species are not recommended. *A. paniculata* could be a potential source of new antibacterial agents in the treatment of skin infirmities which are associated with these bacteria.

Conclusion

The present study explicitly exhibited the antibacterial effect of various extracts of *Andrographis paniculata* against skin infections causing bacterial strains. The inhibitory effect of the extracts justified the medicinal use of *Andrographis paniculata* in the treatment of skin infirmities by traditional practitioners and further study is mandatory to find out the active principles of medicinal value.

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