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Full Length Research Paper

Bacteriostatic and bactericidal activities of *Andrographis paniculata* extracts on skin disease causing pathogenic bacteria

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Non-polar (dichloromethane) and polar (MeOH and aqueous) extracts of *Andrographis paniculata* (whole plant) were evaluated for *in vitro* antibacterial activity against 10 skin disease causing bacterial strains (6 gram positive strains; *Staphylococcus saprophyticus, Staphylococcus epidermis, Staphylococcus aureus, Streptococcus pyogenes, Bacillus anthracis, Micrococcus luteus*) and 4 gram negative strains *(Proteus mirabilis, Proteus vulgaris, Neisseria meningitis, Pseudomonas aeruginosa)* using disc diffusion method at three different concentrations; 1000, 500 and 250 µg/disc respectively. The extracts showed significant antibacterial activities against both Gram-positive and Gram-negative bacterial strains tested. Highest significant antibacterial activity was exerted by the aqueous extract against *M. luteus* at 1000 µg/disc and the least activity was exhibited by the DCM extract against *N. meningitis* at 250 µg/disc. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) observed were between 150 to 300 µg/ml and 250 to 400 µg/ml respectively, depending on microorganism and the nature of various extracts. Time-kill experiments indicated that *A. paniculata* extracts have bactericidal characteristic against most of the Gram positive bacteria and bacteriostatic activity against both Gram negative and Gram positive bacteria. These results candidly suggest the presence of promising antibacterial substances in the polar as well as non-polar extracts which could be the source of potential phytomedicine for the treatment of skin infections caused by the pathogenic bacterial strains. Our findings explicitly support its traditional claims and form a strong basis for further sincere efforts to explore *A. paniculata's* antibacterial potential to treat skin frailties efficaciously.

Key words: *Andrographis paniculata*, antimicrobial activity, skin infections, disc diffusion method, minimum inhibitory concentration, minimum bactericidal concentration, time to kill assay.

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 from them (Ncube et al., 2007). Nowadays,

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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 (Kanokwan et al., 2008).

Andrographis paniculata (Burm.f.) Wall. ex Nees., belongs to the family of Acanthaceae and is popular worldwide with the name of King of Bitters in English and Hempedu bumi in Malay. It is an annual herbaceous plant which is widely cultivated in southern Asia, Scandinavia, China and some parts of Europe. 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 (Cheung et al., 2005; Tan et al., 2005; Zhou et al., 2006), antihuman immunodeficiency virus (HIV) (Calabrese et al., 2000), immune stimulatory (Iruretagoyena et al., 2005), antimicrobial (Prajjal et al., 2003; Coon et al., 2004; Limsong et al., 2004) 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 infection that are caused by some pathogenic bacterial strains.

METHODOLOGY

Collection and preparation of plant material

Fresh plant material (5 kg) of *A. paniculata* was procured from the botanical gardens of the Forest Research Institute of Malaysia (FRIM), Kuala Lumpur, Malaysia. Specimen sample was authenticated by Dr. Saw Leng Guan (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 5 days and pulverized to powdered form using the Fritsch Universal Cutting Mill. This was then stored in a desiccators at 2°C until further use.

Materials

All the chemicals and standard antibiotics were purchased from Fisher scientific chemicals, UK and Oxoid Ltd England respectively. All the solvents used were of analytical grade. Precoated silica gel 60 F_{254} TLC plates were purchased from Merck, Germany.

Preparation of non-polar and polar extracts

500 g 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 extracts was carefully filtered using filter paper (Whatman No. A-3) and concentrated using a rotary evaporator (Buchi Rotary Evaporator, R-210) at 40°C. 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

Ten skin disease causing bacterial strains were taken into consideration, viz., (6 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) and 4 gram negative strains: *Proteus mirabilis* (IMR P-76), *Proteus vulgaris* (IMR P-147), *Neisseria meningitis* (IMR N-349), *Pseudomonas aeruginosa* (IMR P-84)). All bacterial strains were obtained 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.

Antibacterial activity screening

The agar disc diffusion method was employed for the determination of antibacterial activities of the polar (MeOH and aqueous) and non-polar (DCM-dichloromethane) extracts of *A. paniculata* (NCCLS, 2004). 6 Gram-positive (*S. saprophyticus, S. epidermis, S. aureus, S. pyogenes, B. anthracis, M. luteus*) and 4 Gram-negative (*P. mirabilis, P. vulgaris, 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°C 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°C 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 MIC of the crude extracts of *A. paniculata* was determined by agar dilution method as validated by EUCAST, 2000. The growth media, Mueller Hinton Agar were first prepared and sterilized by autoclaving (Webco GmbH and Co. KG Bad Schwartau, Germany). The sterilized media was allowed to cool to 50°C and 18 ml of the molten agar was added to test tubes which contained 2 ml of different concentration of the test crude extract and the control (10% DMSO). The mixture of the media and the test crude extracts were thoroughly mixed and poured into 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 organisms. The concentrations of the extracts used in this test ranged from 150 to 300 µg/ml. The plates were then set at room temperature and dried. The suspensions of the respective microorganisms having density adjusted to 0.5 McFarland turbidity standards were inoculated onto the series of agar plates. Three loopful of the suspension were transferred into each plate and spread evenly. The plates were then incubated at 37°C for 24 h. The lowest concentration of the extract which inhibited the growth of the respective organisms was taken as MIC.

Determination of minimum bactericidal concentration (MBC)

The MBC was determined by the micro broth dilution method (NCCLS, 2003, 2004). Plant extracts were resuspended in 10% DMSO (which had no activity against test microorganisms) to make final concentration of 500 µg/ml, this was then serially diluted by adding to the broth media in a 96-wells microtiter plates to obtain 450, 400, 350, 300, 250, 200,150 and100 µg/ml. Thereafter, 100 µl inoculum was added into each well. Bacterial suspensions were used as negative control, while broth containing standard drugs (Tetracycline and Gentamicin) were used as positive control. The micro titer plates were incubated at 37°C for 24 h. Each extract was assayed in triplicates and each time two sets of micro plates were prepared, one was kept for incubation while another set was kept at 4°C for comparing the turbidity in the wells of micro plate. The turbidity of the wells in the micro titer plate was interpreted as visible growth of microorganisms. The MBC was determined by sub culturing 50 µl from each well showing no apparent growth. Least concentration of extract showing no visible growth on subculturing was taken as MBC.

Time to kill assay

Time-kill assay were performed as described by Ernst et al. (2002) with little modifications to determine the rate of killing of the chosen bacteria by the extracts of *A. paniculata*. 1000 µg/ml concentrations of each extract were prepared. Inoculums of the test microganisms were prepared from 24 h cultures grown on Mueller-Hinton Broth (MHB) and suspensions adjusted to a turbidity equivalent to 10^8CFU/ml. Equal volumes of the diluted inoculums and the extracts to be tested were mixed and incubated at 37°C. At time intervals of 2 to 24 h, 50 µl of the mixed suspension was spread on two separate nutrient agar plates and incubated for 24 h at 37°C. The mean number of colonies were obtained and compared with that of control in which the plant extracts was replaced with DMSO. Each experiment was repeated thrice. The colony count results were expressed as a percentage of control (that is, percentage of bacteria killed compared to the control).

Phytochemical screening

Phytochemical screening of plant extracts was carried out qualitatively for the presence of alkaloids, terpenoids, tannins, flavonoids, saponins, cardiac glycosides and steroids. Alkaloid detection was carried out by extracting 1 g powdered sample with 5 ml methanol and 5 ml of 2N HCl; and then treating the filtrate with Meyer's and Wagner's reagents. The samples were scored positive on the basis of turbidity or precipitation. Flavonoids were tested by heating 1 g powdered sample with 10 ml ethyl acetate over a steam bath (40 to 50°C) for 5 min; filtrate was treated with 1 ml dilute ammonia. A yellow colouration demonstrated positive test for flavonoids. The presence of tannins was confirmed by boiling 0.5 g powdered sample in 20 ml distilled water, followed by addition of 3 drops of 5% $FeCl_3$ to the filtrate. Development of brownish-green or blue black colouration was taken as positive for the presence of tannins. Saponins content was determined by boiling 1 g powdered sample in 10 ml distilled water for 15 min and after cooling, the extract was shaken vigorously to record froth formation. Cardiac glycosides were identified by extracting 2 g sample in 10 ml methanol. Five ml of this methanolic extract was treated with 2 ml glacial acetic acid containing 1 drop of 5% $FeCl_3$ solution. This solution was carefully transferred to surface of 1 ml conc. H_2SO_4. The formation of reddish brown ring at the junction of two liquids was indicative of cardenolides/cardiac glycosides (Harborne, 1998).

Statistical analysis

All tests were conducted in triplicates. All values have been expressed as mean ± standard deviation and the comparison of the antibacterial activity of the samples with standard antibiotics was evaluated by applying t-test. $P \leq 0.05$ values were considered to indicate statistically significant difference.

RESULTS AND DISCUSSION

The results of present study are encouraging as all the tested extracts revealed antibacterial potential, although the inhibitory activity was strain specific and concentration dependant. The dichloromethane, methanolic and aqueous extracts of *A. paniculata* were assessed at 3 different concentrations by using disc diffusion method against 10 bacterial strains notable for causing chronic skin infections and expressed as the average diameter of the zone of inhibition of bacterial growth around the disc. The MIC and the MBC of active extracts were determined by the agar dilution and micro broth dilution assays respectively. The extracts displayed relative antibacterial activity against most of the tested microorganisms with the diameter of inhibition zones ranging between 6.00 ± 1.00 to 23.17 ± 0.76mm (Table 1). The Gram-positive strains used for this study were the most susceptible to growth inhibition by the plant extracts forming zones of inhibition ranging from 7.00 ± 0.00 to 23.17± 0.76mm. The DCM extract was found to exhibit the least potent antibacterial activity against

Table 1. Antibacterial activity of polar and non-polar extracts of *A. paniculata*. Numbers indicate the mean diameters of inhibition of triplicate experiments ± (SD).

Zone of inhibition diameters in mm											
Extracts	**DCM**			**MeOH**			**Aqueous**			**Gentamicin**	**Tetracycline**
Concentration	**1000 µg/disc**	**500 µg/disc**	**250 µg/disc**	**1000 µg/disc**	**500 µg/disc**	**250 µg/disc**	**1000 µg/disc**	**500 µg/disc**	**250 µg/disc**	**30 µg**	**30 µg**
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
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
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

S. saprophyticus (7.00 ± 0.00mm) at 250 µg/disc and the aqueous extract displayed the most potent activity against *M. luteus* (23.17 ± 0.76 mm) at 1000 µg/disc (Figure 1). The highest MIC value was observed at 300 µg /ml exerted by the aqueous extract against *M. luteus*, DCM extract against *M. lutues* and *B. anthracis* respectively. The least MIC was 150 µg/ml exerted by the aqueous extract against *S. aureus* and the methanolic extract on *B. anthracis* respectively.The highest MBC was observed at 400 µg/ml exerted by the DCM extract against *S. pyogenes* and aqueous extract against *S. saprophyticus* and *S. epidermis*.The least MBC was 250 µg/ml exerted by the DCM extract against *S. saprophyticus* and the methanolic extract against *M. luteus* and *S. pyogenes* (Table 3). However, no activity was observed with the DCM, methanolic and aqueous extracts of the plant at 250 µg/disc against *M. luteus, S. pyogenes* and *S. saprophyticus*. 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 ± 1.00 to 20.00 ± 1.26mm. The aqueous extract was found to be the least potent against *N. meningitis* (6.00 ± 1.00mm) at 250 µg/disc and the methanolic extract showed the most potent activity against *P. mirabilis* (20.00 ± 1.26mm) at 1000 µg/disc (Figure 2). The highest MIC value was found to be 300 µg/ml exerted by the DCM extract against *P. aeroginosa* and the least was 150 µg/ml exerted by the aqueous extract against *P. vulgaris* and the methanolic extract extract on *N. meningitis* respectively (Table 2). MBC values were undetected against all gram negative strains tested (Table 3). No activity was observed with the DCM, methanolic and aqueous extracts of the plant at 250 µg /disc against *P. aeroginosa*. *P aeroginosa* and all *Staphylococcus* strains used for the study were found to be resistant to tetracycline (Table 1).

All tested extracts of *A. paniculata* exhibited bacteriostatic action against both Gram positive and negative bacterial strains (MIC) but revealed bactericidal action against Gram positive strains only except for *B. anthracis* (MBC). The colony count method was used to ascertain the time taken for the extracts to completely kill the bacterial cell. The strains which displayed considerably good sensitivity to plant extracts were selected further to determine bactericidal/ bacteriostatic activities by the time-kill assay viz., *M. luteus* (Gram positive) and *P. mirabilis* (Gram Negative); the results explicitly indicated a significant decrease in mean colony count of the tested strains as compared to the control at each time interval leading to the complete killing of *M. luteus* cells by the aqueous extract at 12 h, the MeOH extract at 14 h and the DCM extract at 20 h respectively, but these extracts were unable to kill the cells of *P. mirabilis* totally even though there was much decrease in mean colony count, *P. mirabilis* cells were still viable after 24 h. The results indicated that all extracts were bactericidal against *M. luteus* but exhibited bacteriostatic action against *P. mirabilis* (Figures 3 and 4).

Phytochemical screening of plant extracts was carried out qualitatively for the presence of terpenoids, tannins, flavonoids, saponins, cardiac glycosides and steroids (Harborne, 1998). All extracts showed the presence of terpenoidal and

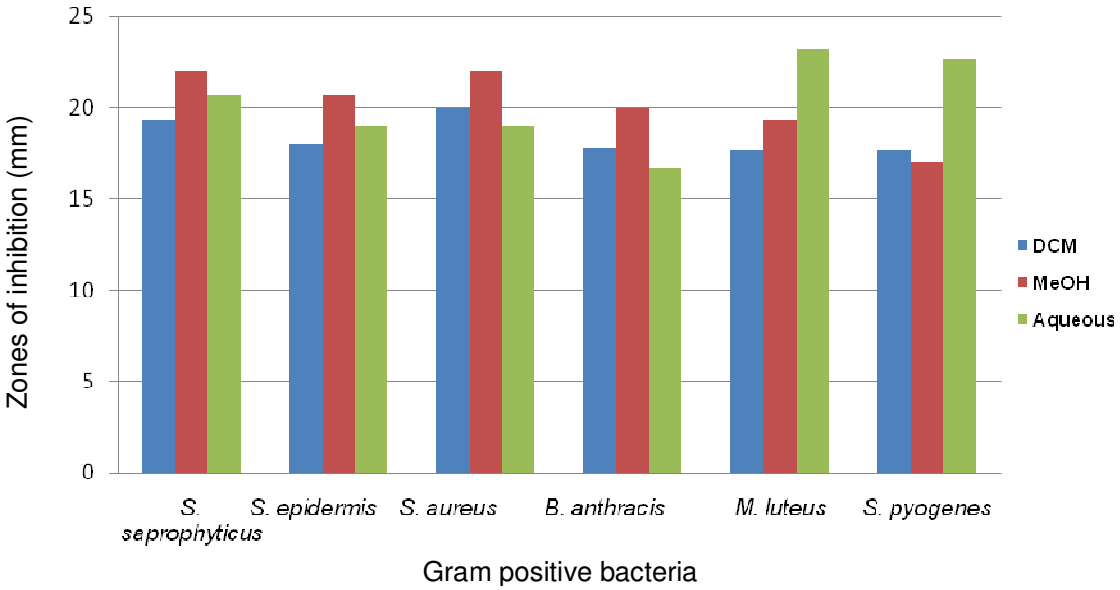

Figure 1. Zones of inhibition (mm) of the DCM, MeOH and aqueous extracts of *A. paniculata* at 1000 µg/disc on Gram positive bacterial strains.

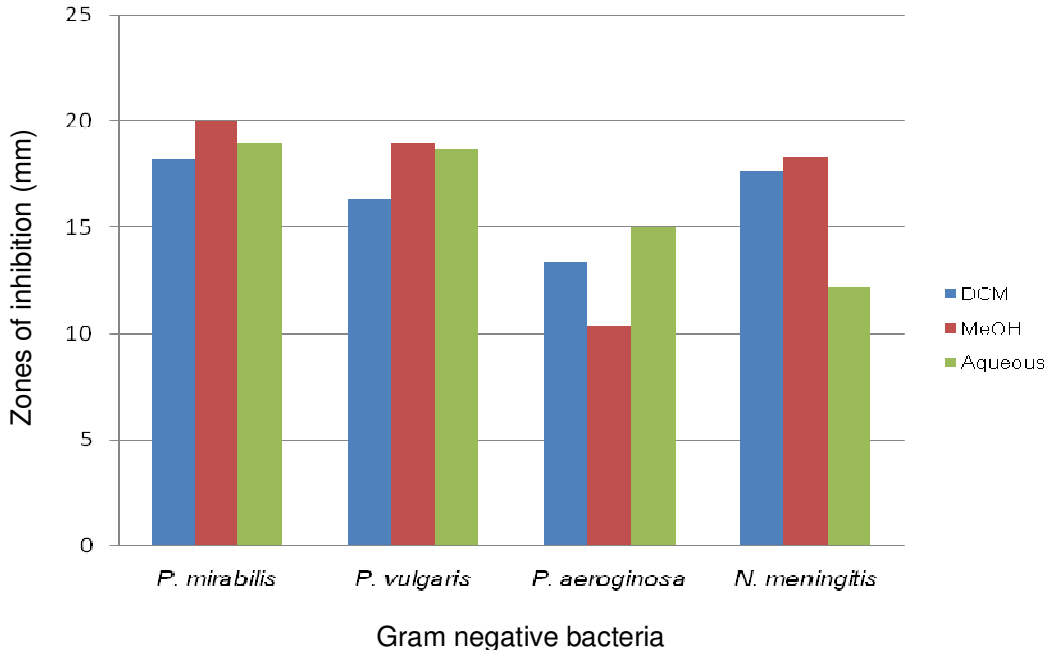

Figure 2. Zones of Inhibition (mm) of the DCM, MeOH and aqueous extracts of *A. paniculata* at 1000 µg/disc on Gram negative bacterial strains.

flavonoidal compounds. However, MeOH extract gave positive test for all compounds taken into consideration and showed the maximum number of aforementioned compounds on precoated silica gel 60 F_{254} TLC plate which could be responsible for its strong antibacterial activity against all microorganisms taken into account.

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

Table 2. MIC of the plant extracts on the bacterial strains.

Minimum inhibitory concentrations (MIC) (µg/ml)			
Bacterial strains	**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
Gram negative strains			
P. mirabilis	200	250	250
P. vulgaris	250	250	150
P. aeruginosa	300	250	250
N. meningitis	200	150	250

Table 3. MBC of the plant extracts on the bacterial strains.

Minimum bactericidal concentrations (MBC) (µg/ml)			
Bacterial strains	**Dichloromethane**	**Methanol**	**Aqueous**
Gram positive			
S. saprophyticus	250	300	400
S. epidermis	350	300	400
S. aureus	400	300	300
B. anthracis	UD	UD	UD
M. luteus	350	250	350
S. pyogenes	400	250	350
Gram negative			
P. mirabilis	UD*	UD	UD
P. vulgaris	UD	UD	UD
P. aeruginosa	UD	UD	UD
N. meningitis	UD	UD	UD

*UD=Undetected.

been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants (Veeramuthu et al., 2006). 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 and 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; Gurinder and Daljit, 2009). 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 manifested 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, B. anthracis, M. luteus*, *S. pyogenes,* and *P. aeroginosa*. Hence, their medicinal uses in infections associated with these bacterial species are not recommended. Antibacterial efficacy shown by these extracts provides a scientific basis and thus, validates their traditional uses as homemade remedies in the treatment of skin infirmities which are associated with these bacteria. The present study revealed that the crude polar and non-polar extracts contain a number of phytoconstituents whose isolation and purification may yield significant novel antimicrobial agents. Further,

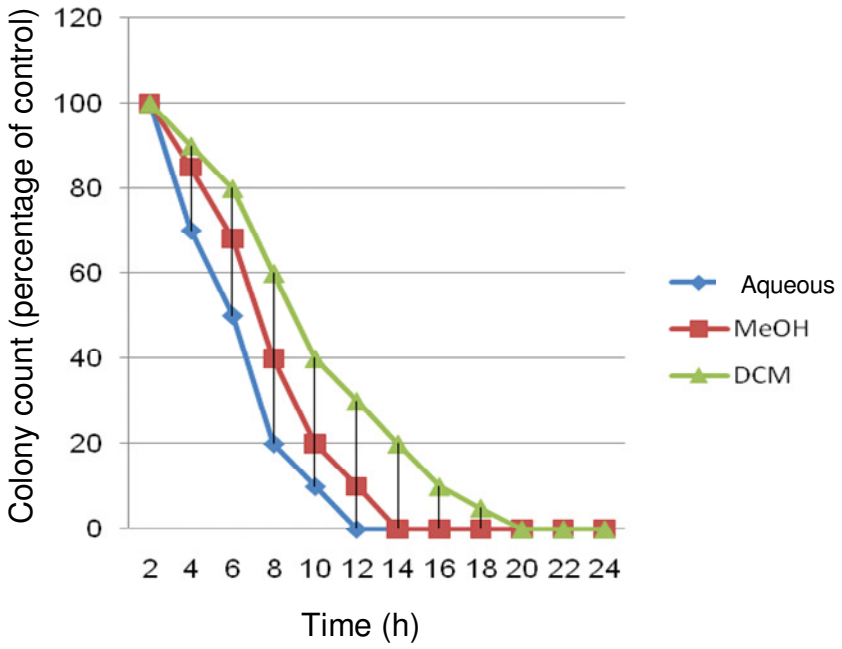

Figure 3. Colony count of *M. luteus* with *A. paniclata* MeOH, DCM and aqueous extracts.

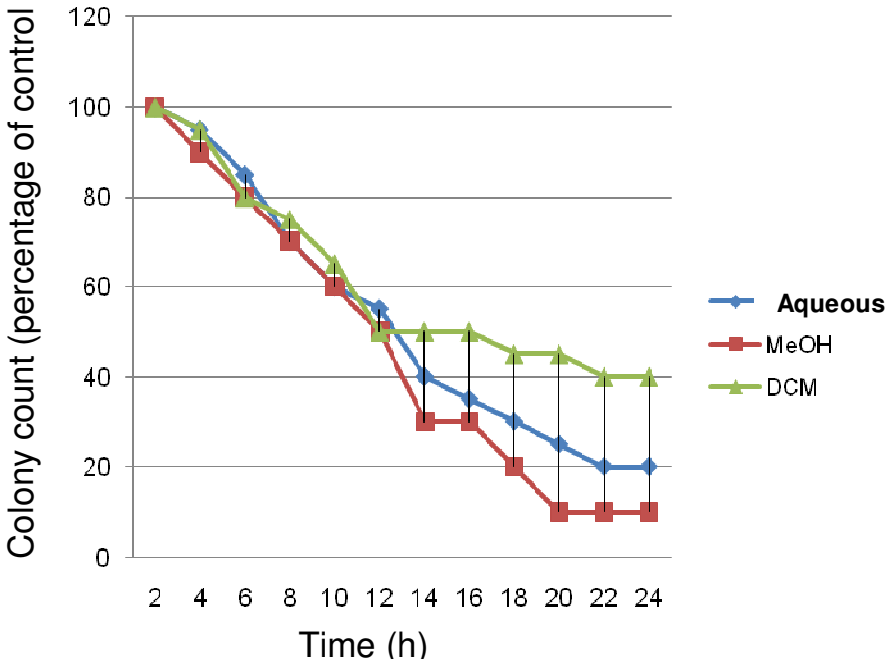

Figure 4. Colony count of *P. mirabilis* with *A. paniclata* MeOH, DCM and aqueous extracts.

investigation to obtain information on chemical composition, to purify and determine the structures of active principles in *A. paniculata* have been in progress in our laboratory.

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