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An Investigation on the Antimicrobial Activity of *Andrographis paniculata* Extracts and Andrographolide *in vitro*

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Abstract: An aqueous and two ethanolic extracts of *Andrographis paniculata*, used in traditional Chinese, Thai and Indian medicine and andrographolide, an active principle of *Andrographis paniculata*, were investigated for their antimicrobial activity against nine bacterial species including *Salmonella typhimurium*, *Escherichia coli*, *Shigella sonnei*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Legionella pneumophila* and *Bordetella pertussis*, using the disc diffusion method. Of all tested concentrations, direct antimicrobial activity of the two ethanolic *Andrographis paniculata* extracts was observed for only two human pathogens, *Legionella pneumophila* and *Bordetella pertussis*. However, no antibacterial activity of andrographolide against any of the pathogens tested in this investigation was observed. Given that the TLC of *Andrographis paniculata* extracts showed that andrographolide was present in all the three *Andrographis paniculata* extracts, it was concluded that the observed antimicrobial activity was due to other active principle(s) present in the extracts used in this investigation.

Key words: *Andrographis paniculata*, andrographolide, antimicrobial, disc diffusion, medicinal plant extracts

INTRODUCTION

The development of bacterial resistance to currently available antibiotics has made it necessary to search for new antibacterial agents. New sources, especially natural products from plants, are being investigated because medicinal plants have been widely used for treatment of many types of acute and chronic diseases in Asia and many plants with antimicrobial activity have been reported (Cowan, 1999). The crude aqueous or alcohol extractions of *Andrographis paniculata* (AP) and one principle of AP viz. andrographolide (AND) have been reported to be effective in the treatment of dysentery, diarrhoea and upper respiratory tract infection (Gupta *et al*., 1990; Yin and Guo, 1993; Chturvedi *et al*., 1983; Chang *et al*., 1986; Hancke *et al*., 1995; Melchior *et al*., 1997, 2000; Thamlikitkui *et al*., 1991; Poolsup *et al*., 2004; Coon and Ernst, 2004). The antibacterial activity of aqueous, ethanol or methanol extracts of *Andrographis paniculata* has been tested *in vitro* against *Escherichia coli*, *Staphylococcus aureus*, *Proteus vulgaris*, *Salmonella typhi*, *Shigella* species and *Pseudomonas aeruginosa in vitro* (George and Pandalai, 1949; Nakanishi *et al*., 1965; Prajjal *et al*., 2003) with no antibacterial activity observed with the aqueous extract tested against *E. coli*, *S. aureus*, *S. typhi* or *Shigella* species (Leelarasamee *et al*., 1990). We decided to extend these investigations using a broader spectrum of microbial pathogens of relevance to human health. We discovered that neither the aqueous extract nor AND were bacteriostatic or bactericidal against *Salmonella typhimurium*, *E. coli*, *Shigella sonnei*, *S. aureus*, *P. aeruginosa*, *S. pneumoniae*, *S. pyogenes*, *L. pneumophila* or *B. pertussis* but the two ethanolic extracts of AP were bacteriostatic against *L. pneumophila* and *B. pertussis*.

MATERIALS AND METHODS

Plant materials: AP whole plant coarse powder was purchased from Woods plus Woods Co. Ltd., Australia. The AND was purchased from Sigma (St Louis, MO).

Preparations of extracts: AP powder (100 g) was placed in flasks, then soaked and stirred in distilled water, 80% or absolute ethanol (1:10 w/v) overnight. After filtration through cotton wool, the filtrates were centrifuged at 10000 x g for 20 min at room temperature (Cowan, 1999; Puri *et al*., 1993). The extract solutions were evaporated in a rotary evaporator at 60°C until dry. Dried extracts were weighed and stored in labelled sterile screw capped bottles at -20°C.

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Microorganisms: The bacteria used included: *S. typhimurium* (bovine, 12313), *E. coli* (ATCC 25922), *E. coli* (Q 358), *S. typhimurium* (*aroA* SL 3261), *S. bovis-morbificans*, *S. sonnei*, *S. aureus* (ATCC 49476), *P. aeruginosa* (ATCC 27853), *S. pneumoniae*, *S. pyogenes*, *L. pneumophila* and *B. pertussis* (Tohama 1).

Analysis of the AP extracts: Thin Layer Chromatography (TLC) analysis was used to authenticate the AP extracts as judged by the detection of andrographolide (AND). The AP extracts were dissolved as per their preparation details above and 5 μL were used for each spot. The AND was dissolved in hot ethanol (5 mg mL^{-1}) and 5 μL were used. A pre-coated plate of silica gel 60F254 (Merck) and mobile phase (chloroform: methanol: ethyl acetate 8: 1.5: 1) were used. The extracts fractionated by TLC were detected by UV radiation (Electronic UV Transilluminator, Quantum Scientific). Rf (retardation factor) of AND was 0.58.

Screening for antimicrobial activity: The antibacterial activity of AND and three AP extracts was analysed by using disc diffusion assay (Dulger and Gonuz, 2004). The dry extracts were dissolved as per their preparation details above and diluted with distilled water to obtain concentrations at 100, 10 or 1 mg mL^{-1}. The AND was first dissolved in ethanol and then diluted with distilled water to obtain concentrations at 10, 1 or 0.1 mg mL^{-1} (w/v). Fifty microliters of each prepared dilution were added to each sterilized disc with a 6 mm diameter (Whatman, England). After adding the dilutions, the discs that had 5 mg, 0.5 mg or 50 μg of extracts and 0.5 mg, 50 or 5 μg of AND per disc, were placed in an oven and dried at 55°C overnight. The discs with 50 μL of 80% ethanol were also prepared in the same way as a control to account for any inhibition caused by any residual ethanol remaining after drying of the disc overnight.

All the bacteria mentioned above were grown in petri dishes with appropriate agar. *S. pneumoniae* and *S. pyogenes* were grown on horse blood agar plates. *L. pneumophila* was on Legionella CYE agar with BCYE growth supplement (Oxoid, England). *B. pertussis* was grown on Charcoal agar. All the other micro-organisms were grown on nutrient agar plates by spreading the entire agar surface with 0.1 mL of the bacterial suspensions prepared in distilled water at wavelength of 600 nm.

The discs with extracts or AND were placed on the agar plates on which the bacteria were spread, followed by incubation at 37°C for 18 h, except the plates of *L. pneumophila* and *B. pertussis* that were incubated for 3 days in a humidified atmosphere. The diameter of inhibition zone was measured in mm. Select reference antibiotic discs were used as positive controls, depending on the test micro-organisms. The 80% ethanol soaked and dried disc was also used as a control.

RESULTS

The AP extracts recoveries were obtained (Table 1). The thin layer chromatographs (TLC) of the AP ethanolic and aqueous extracts are shown in Fig. 1. The water extract showed two major bands whereas all the ethanol extracts showed more than 3 bands. The band representing AND (Rf = 0.58) was present in all the three AP extracts. The colour associated with the APE extracts was removed by absorption with activated charcoal (Sigma, St Louis, MO).

None of the concentrations at 5 mg/disc, 0.5 mg/disc or 50 μg/disc of AP extracts showed any antibacterial activity against *S. typhimurium* (bovine, 12313), *E. coli* (ATCC 25922), *E. coli* (Q 358), *S. typhimurium* (aroA SL 3261), *S. bovis-morbificans* (sheep, liver), *S. sonnei*, *S. aureus*, *P. aeruginosa*, *S. pneumoniae* or *S. pyogenes*. However, the concentrations of 5 mg/disc, 0.5 mg/disc and 50 μg/disc of 80% APE and 100% APE showed antibacterial activity against both *L. pneumophila* and *B. pertussis* (Table 2). The tested concentrations of different AP extracts showed no antibacterial activity towards any of the pathogens mentioned above. Antimicrobial activities of select antibiotics used as positive controls are shown in (Table 3).

Table 1: AP extracts and their recoveries

Extract	Recovery (100 g of AP)
Absolute ethanol AP extract (100% APE)	9.8 g
80% ethanol extract (80% APE)	6.0 g
Water Extract (WE)	5.7 g

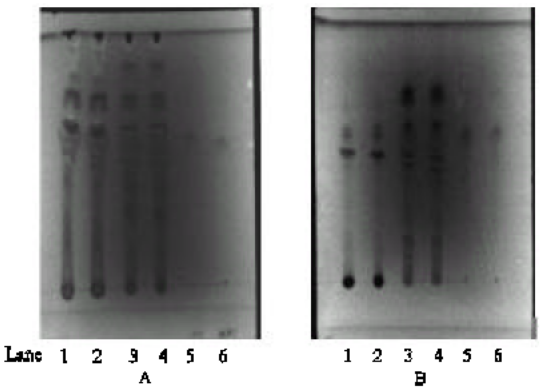

Fig. 1: TLC of AP extracts: from left to right, A: lanes 1 and 2: 80% APE; lanes 3 and 4: 100% APE; lanes 5 and 6: AND. B: lanes 1 and 2: water extract; lanes 3 and 4: APE; lanes 5 and 6: AND

Table 2: Antimicrobial activity of AND or different AP extracts (inhibition zone: mm diameter)

Micro-organisms	AND	Aqueous extract	80% ethanol extract	100% ethanol extract	Overnight dry of 80% ethanol
S. typhimurium (*aroA*, SL 3261)	-	-	-	-	-
S. typhimurium (bovine,12313)	-	-	-	-	-
E. coli (ATCC 25922)	-	-	-	-	-
E. coli (Q 358)	-	-	-	-	-
S. bovis-morbificans	-	-	-	-	-
Shigella sonnei	-	-	-	-	-
S. aureus	-	-	-	-	-
P. aeruginosa	-	-	-	-	-
S. pneumoniae	-	-	-	-	-
S. pyogenes	-	-	-	-	-
L. pneumophila	-	-	+22 (5 mg/disc)	+16 (5 mg/disc)	
			+22 (0.5 mg/disc)	+16 (0.5 μg/disc)	
			+22 (50 μg/disc)	+16 (50 mg/disc)	
B. pertussis	-	-	+18 (5 mg/disc)	+12 (5 mg/disc)	-
			+14 (0.5 mg/disc)	+12 (0.5 μg/disc)	
			+12 (50 mg/disc)	+10 (50 μg/disc)	

Notes: For medicinal plants, Resistant = <10 mm diameter zone of inhibition

Table 3: Antimicrobial activity of select antibiotics against common intestinal and respiratory tract pathogens

Micro-organisms	ST	P10	VA30	E15	FOX30	SXT	AM10	GM10
S. typhimurium (*aroA* SL 3261)	-	13	-	-	14	18	19	17
S. typhimurium (bovine, 12313)	18	15	-	-	19	22	20	15
E. coli (ATCC 25922)	20	-	-	-	21	24	18	17
E. coli (Q 358)	-	-	-	9	24	29	25	16
S. bovis-morbificans	16	14	-	-	19	21	19	17
Shigella sonnei	10	-	-	-	-	20	-	15
S. aureus	10	28	9	18	14	-	22	9
P. aeruginosa	7	-	-	-	-	-	-	16
S. pneumoniae	-	34	18	-	16	15	22	-
S. pyogenes	-	24	13	18	15	-	-	-
L. pneumophilia	-	-	-	40	31	27	12	20
B. pertussis	-	-	-	30	22	-	26	22

Note: ST: Streptomycin (100 μg), P10: Penicillin G (10IU), VA30: Vancomycin (30 μg), E15: Erythromycin (15 μg), FOX30: Cefoxitin (30 μg), SXT: sulfamethoxazole/trimethoprim (23.75:1.25 μg), AM10: Ampicillin (10 μg), GM10: Gentamicin (10 μg)

DISCUSSION

AP is an herbal medicine and has been used for therapy of respiratory tract infection as well as acute diarrhoea with reported efficacy of 75-100% in Thailand (Leelarasamee *et al*., 1990). To investigate whether anti-bacterial activity was responsible for the reported therapeutic successes of AP, direct anti-bacterial activity of AP extracts and AND was determined using disc diffusion tests. Direct anti-bacterial activity of two ethanolic *Andrographis paniculata* extracts against *Legionella pneumophila* and *Bordetella pertussis* was observed. No direct anti-microbial activity of the aqueous extract of AP and AND against the other microorganisms tested viz., including *S. typhimurium*, *E. coli*, *S. sonnei*, *S. aureus, P. aeruginosa*, *S. pneumoniae*, *S. pyogenes*, *L. pneumophila* and *B. pertussis* was observed.

Present results obtained with the aqueous extract of AP did not support those reported by Prajjal *et al.* (2003) with respect to *S. aureus*, *E. coli* or *P. aeruginosa*. Although it is possible that the lack of antimicrobial activity of the aqueous extract in this study may be attributable to a lesser concentration of the aqueous extract used per disc than that used by Prajjal *et al.* (2003) viz., 5 mg/disc instead of 10 mg/disc, it is highly unlikely because (a) no zone of inhibition against the pathogens tested was observed in this study at all and (b) Leelarasamee *et al.* (1990) also did not observe any antimicrobial activity of the aqueous extract either *in vitro* against *Salmonella*, *E. coli*, *Shigella*, *S. aureus* even at concentration as high as 25 g L^{-1} of AP powder suspended in water.

Although APE or AND did not show any direct antibacterial activity against intestinal pathogens tested in this study, extracts of AP have been claimed to have significant effects against the diarrhoea associated with *E. coli* infections using rabbit as the model system (Gupta *et al*., 1990). Yin and Guo (1993) found that a dose of 500 mg per day for six day of AND was effective in controlling or treating acute bacterial diarrhoea in human patients. In another study, AP was used to treat 1611 cases of bacterial dysentery and 955 cases of diarrhoea with overall effectiveness of 91.3% (Chaturvedi *et al*., 1983). However, we discovered that AP had no direct bacteriostatic or potential bactericidal reaction against *S. typhimurium* (bovine, 12313), *E. coli* (ATCC 25922), *E. coli* (Q 358), *S. typhimurium* (*aroA* SL 3261), *S. bovis-morbificans* or *S. sonnei*.

Andrographis paniculata has also been used clinically for symptomatic treatment of the common cold and uncomplicated sinusitis, pharyngotonsillitis, pneumonia and bronchitis (Chang *et al*., 1986; Hancke *et al*., 1995; Melchior *et al*., 1997; Thamlikitkui *et al*., 1991; Melchior *et al*., 2000; Poolsup *et al*., 2004; Coon and Ernst, 2004). Chinese clinical studies involving oral administration of AP or AND to patients suffering from bacterial and viral respiratory infections reported good effects. Investigations from the Sichuan Traditional Medicine Research Institution showed a lowered body temperature after treatment with AP: of 84 cases of common cold, 70 achieved normal body temperature within 48 h Melchior *et al*. (1997). Thamlikitkui *et al*. (1991) demonstrated that a dose of 1200 mg per day for 4 days of AP extract (standardized to 4% AND) was effective in reducing the symptoms of common cold in human patients. We found that AP extracts, but not AND, were inhibitory *in vitro* only for those respiratory pathogens that were associated with bronchitis or pneumonia viz., *L. pneumophila* and *B. pertussis*, respectively. It is thus clear that a component/principle other than AND may be responsible for the observed antimicrobial activity. Research aimed at identifying the component(s) responsible for the observed antimicrobial activity against the respiratory pathogens tested in this investigations is in progress as is the testing of the hypothesis that the antimicrobial activity claimed in the use of AP against microbial infections *in vivo* may be due to the immunomodulatory effects of an AP extract and one of its active compounds including AND since it has been suggested that the main clinical benefit of AP could be due to a possible immune-enhancing effect (Mills and Bone, 2000).

REFERENCES

Chang, H.M. and P.P.H. But *et al*., 1986. Pharmacology and applications of Chinese materia medica. Singapore, World Scientific, 1: 918-928.

Chturvedi, G., G.S. Tomar, S.K. Tiwart and K.P. Singh, 1983. Clinical studies on Kalmegh (*Andrographis paniculata* Nees) in infective hepatitis. J. Internat. Inst. Ayurveda, 2: 208-211.

Coon, J.T. and E. Ernst, 2004. *Andrographis paniculata* in the treatment of upper respiratory tract infections: A systematic review of safety and efficacy. Planta Med., 70: 293-298.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.

Dulger, B. and A. Gonuz, 2004. Antimicrobial activity of certain plants used in Turkish traditional medicine. Asian J. Plant Sci., 3:104-107.

George, M. and K.M. Pandalai, 1949. Investigations on plant antibiotics. Part IV. Further search for antibiotic substances in Indian medicinal plants. Indian J. Med. Res., 37: 169-181.

Gupta, S., M.A. Choudhry and J.N.S. Yadava *et al*., 1990. Antidiarrioeal activity of diterpenes of *Andrographis paniculata* (Kal-Megh) agent *Escherichia coli* enterotoxin *in vivo* models. Intl. J. Crude Drug Res., 284: 273-283.

Hancke, J., R.A. Burgos and D.D. Caceres, 1995. A double-blind study with a new monodrug kan jang: Decrease of symptoms and improvement in the recovery from common colds. Phytother. Res., 9: 559-562.

Leelarasamee, A., S. Trakulsomboon and N. Sittisomwong, 1990. Undetectable anti-bacterial activity of *Andrographis paniculata* (Burma) wall. Ex ness. J. Med. Assoc. Thai, 73: 299-304.

Melchior, J., S. Palm and G. Wikman, 1997. Controlled clinical study of standardized *Andrographis paniculata* extract in common cold-a pilot trial. Phytomedicine, 3: 315-318.

Melchior, J., A.A. Spasov, O.V. Ostrovskij, A.E. Bulanov and G. Wikman, 2004. Double-blind, placebo-controlled pilot and phase III study of activity of standardized *Andrographis paniculata* Herba Nees extract fixed combination (Kan jang) in the treatment of uncomplicated upper-respiratory tract infection. Phytomedicine, 7: 341-350.

Mills, S. and K. Bone, 2000. Principles and Practice of Phytotherapy. Edinburgh: Churchill Livingstone, pp: 262-268.

Nakanishi, K. *et al*., 1965. Phytochemical surveyof Malaysian plants: Preliminary chemical and pharmacological screening. Chem. Pharmaceut. Bull., 13: 882-890.

Poolsup, N., C. Suthisisang, S. Prathanturarug, A. Asawamekin and U. Chanchareon, 2004. *Andrographis paniculata* in the symptomatic treatment of uncomplicated upper-respiratory tract infection: Systematic review of randomized controlled trials. J. Clin. Pharm. Ther., 29: 37-45.

Prajjal, K., S. Roy and S. Dey, 2003. Antimicrobial activity of *Andrographis paniculata*. Fitoterapia, 74: 692-694.

Puri, A., R. Saxena, R.P. Saxena and K.C. Saxena, 1993. Immunostimulant agents from *Andrographis paniculata*. J. Natl. Prod., 56: 995-999.

Thamlikitkui, V., T. Dechatiwongse and S. Theerapong, 1991. Efficacy of *Andrographis paniculata* Nees for pharyngotonsillitis in adults. J. Med. Assoc. Thailand, 74: 437-442.

Yin, J. and L. Guo, 1993. Contemporary traditional Chinese medicine. Beijing: Xie Yuan.