Pak. j. sci. ind. res. Ser. B: biol. sci. 2014 **57**(2) 76-80

Thrombolytic and Antimicrobial Activities of *Andrographis paniculata* – A Preliminary Investigation

Md. Mamun Al-Amin[a]*, Mir Muhammad Nasir Uddin[b], Md. Siddiqul Islam[c], Muhammad Ibrahim Chowdhury[c] and Mohammad Shohel[a]

[a]Department of Pharmaceutical Sciences, North South University, Bashundhara, Dhaka-1229, Bangladesh
[b]Department of Pharmacy, University of Chittagong, Chittagong-4331, Bangladesh
[c]Department of Pharmacy, School of Science and Technology, Manarat International University, Mirpur, Dhaka-1216, Bangladesh

(received April 1, 2013; revised February 9, 2014; accepted February 11, 2014)

Abstract. An attempt has been made to investigate thrombolytic and antimicrobial activities of ethanolic extracts of *Andrographis paniculata* whole plant. Phytochemical constituents of *A. paniculata* were assessed by human erythrocyte and the results were compared with standard streptokinase (SK). Moreover, the plant extracts were compared with the antibiotic kanamycin to investigate antibacterial activity against several microorganisms. Glycosides, steroids, phenols, alkaloid and tannins were found in the ethanol extract of whole plant. Crude ethanol extract ($P<0.05$) and soluble fraction of ethanol extract ($P<0.05$) have shown thrombolytic properties. Crude ethanol extract, *n*-hexane soluble fractions and carbon tetrachloride soluble fraction of ethanol extract of the whole plant have shown antimicrobial activities against common gram positive and gram negative microorganisms. The results of current study justify thrombolytic and antimicrobial activities of *A. paniculata*.

Keywords: *Andrographis paniculata*, thrombolytic activity, antimicrobial activity

Introduction

The demand for searching pharmacological activities of plant extract is a long term practice. Nature has been providing the ideas for developing novel drugs against the diseases (Hughes *et al*., 2011). The usage of medicinal plants has drawn avalanche of interest as they could provide therapeutic activities and are promising candidate to be developed as pharmaceutical products in large scale production. The development of new disease accelerates for investigating new chemical entity from medicinal plants (Aderogba *et al*., 2005; Rabaud *et al*., 1997), which may contribute in life threatening diseases such as stroke (which is caused by thrombosis). In case of stroke, new thrombolytic agent is needed to be isolated from the medicinal plants.

Thrombolytic property of a plant extract could be useful for arresting the damages caused by the obstruction or occlusion of a blood vessel, which occurs during myocardial infarction (heart attack), thromboembolic strokes, deep vain thrombosis and pulmonary embolism. Medicinal plants containing phenols, flavonoids, alkaloids, and saponins have shown potential antibacterial activity (Mahenic *et al*., 2007; Pereira *et al*., 2007; da Silva *et al*., 2006), whereas, alkaloids and saponins have shown thrombolytic activity.

Andrographis paniculata has been widely used for the treatment and prevention of common cold especially in the subcontinent (Thisoda *et al*., 2006). *A. paniculata*, a plant of Acanthaceae family and locally known as kalmemg is an erect annual herb extremely bitter in taste in all parts of the plant body. It grows erect to a height of 30-110 cm in moist, shady places. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles. The lance-shaped leaves have hairless blades measuring up to 8 cm long by 2.5 cm wide. The small flowers are borne in spreading and the fruit is a capsule around 2 cm long and a few millimeters wide. It contains many yellow-brown seeds. Aerial parts of the plant contain a large number of chemical constituents, mainly lactones, diterpenoids, diterpene glycosides, flavonoids and flavonoid glycosides (Akbar *et al*., 2011).

Previous studies reported many pharmacological activities of the extract of *A. paniculata* (Bhatnagar *et al*., 1961). Being motivated with the reported biological activities, its thrombolytic and antimicrobial activities were evaluated by the established methods.

*Author for correspondence; E-mail: alamin@northsouth.edu

Pharmacological uses of this plant especially, in thrombosis and antibacterial has not yet been established, therefore, the present study was aimed to investigate the thrombolytic and antimicrobial activities of the whole plant extract of *A. paniculata*.

Materials and Methods

Collection of plant material. The whole plants of *A. paniculata* were collected from Mirpur Botanical Garden, Dhaka, Bangladesh, in July 2012. A voucher specimen for this plant had been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no DACB-32888). The collected plant materials were chopped, dried and powdered and about 1200 g of the powdered material was soaked in 4 L of ethanol at room temperature for 7 days. The extract was filtered by Whatman (No. 1) filter paper and concentrated with a rotary evaporator. An aliquot of the concentrated ethanol extract was fractionated by the modified Kupchan method (Saha *et al*., 2012) and the resultant partitioned were evaporated to dryness with a rotary evaporator to yield hexane, carbon tetrachloride and chloroform soluble materials. The residues were then stored in a refrigerator until further use.

Preliminary phytochemical screening. One gram of the ethanolic extract of *A. paniculata* was dissolved in 4 L of ethanol and was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents (Paterson *et al*., 1999). The freshly prepared crude extract was qualitatively tested for the identification of chemical constituents, such as, alkaloids, flavonoids, steroids, glycosides, saponins, terpenoids, gums and tannins.

Test for alkaloids. Dragendroff's test: 2 mL solution of the extract and 0.2 mL of dilute hydrochloric acid were taken in a test tube. After adding 1 mL of Dragendroff's reagent, orange brown precipitate appeared indicating the presence of alkaloids.

Test for glycosides. Molisch test: Alpha naphthol and concentrated H_2SO_4 were added to 2 mL solution of the extract. Development of reddish violet ring at junction of two layers indicates the presence of glycosides.

Test for flavonoids. A few drops of concentrated hydrochloric acid were added to a small amount of extract solution. Immediate appearance of a red colour indicated the presence of flavonoids.

Test for steroids. The ethanol extract was treated with 50% sulphuric acid and a few drops of acetic anhydride were added. Reddish brown ring indicated the presence of steroids.

Test for saponins. One mL solution of the extract was diluted to 20 mL distilled water and shaken in a graduated cylinder for 15 min, 1 cm layer of foam indicated the presence of saponins.

Test for tannins. Ferric chloride test: About 0.5 g of extract was dissolved in 5 to 10 mL of distilled water and filtered. A few drops of 5% ferric chloride solution were added to the filtrate. A greenish black precipitate was formed which confirmed the presence of tannins.

Test for phenols. Small quantity of extract was diluted with 5% ferric chloride solution. Intense colour indicated the presence of phenols.

Streptokinase (SK). Commercially available lyophilised Altepase (Streptokinase) vial (Beacon pharmaceutical Ltd., Dhaka, Bangladesh) of 15,00,000 I.U., was collected and 5 mL sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µL (30,000 I.U) was used for *in vitro* thrombolysis.

Blood sample. Blood was drawn from six healthy human volunteers without a history of oral contraceptive or anticoagulant therapy and 1 mL of blood was transferred to the previously weighed micro centrifuge tubes and was allowed to form clots.

Thrombolytic activity. The thrombolytic activity of all extracts was evaluated by the method developed by Prasad *et al*. (2006) and modified by Kawsar *et al*. (2011) using streptokinase (SK) as the standard.

***Antimicrobial activity*.** The antimicrobial test was performed by the disc diffusion method (Luo *et al*., 2000) against gram positive and gram negative bacteria collected as pure cultures from the Department of Microbiology, University of Dhaka, Bangladesh. Standard disc of kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition (Bauer *et al*., 1966).

Statistical analysis. The results of statistical analysis for animal experiment were expressed as mean ± SEM. Data were analysed by paired sample t test. The results obtained were compared with the control group. The

criterion for statistical significance was considered as ***P< 0.01, and *P< 0.05. All the statistical tests were carried out using SPSS (for Windows version 16.0) statistical software.

Results and Discussion

The results of phytochemical test are summarized in Table 1, which demonstrated that ethanol extract of *A. paniculata* contains glycosides, steroids, phenols, alkaloid and tannins.

Table 1. Phytochemical compounds present in the ethanol extract of *Andrographis paniculata*

Test	Observation
Alkaloids	+
Glycosides	++
Flavonoides	-
Steroids	++
Saponins	-
Tannins	+
Phenols	++

++ = indicates presence in moderate concentrations; + = indicates presence in trace concentration; - = indicates absence.

Thrombolytic activities were analysed with water, crude ethanol extract, *n*-hexane soluble fraction, ethanol soluble fraction and streptokinase. Antibacterial activity was also assayed with kanamycin and various extracts (crude ethanol extract, *n*-hexane soluble fraction, carbon tetrachloride soluble fraction) of the whole plant of *A. paniculata*.

The crude ethanol extract and the fraction of *n*-hexane and ethanol showed 65.97%, 30.38% and 60.92% lysis of clot, respectively. Distilled water which was treated as negative control exhibited negligible lysis of clot i.e., 50.14%. Streptokinase activity was the highest (96.58%) clot lysis activity. The results of thrombolytic activity are presented in Table 2.

Table 2. Thrombolytic activity of water (control), streptokinase and various extracts of the whole plant of *Andrographis paniculata*

Activity	Water	*n*-hexane soluble fraction	Ethanol soluble fraction	Crude ethanol extract	Strepto-kinase
Thrombolytic activity (% of Lysis)	50.14	30.38	60.92*	65.97*	96.58***

* = P < 0.05; *** = P < 0.01

The result also shows that crude ethanol extract of *A. paniculata* has significant thrombolytic activity. This indicates the presence of antiplatelet compounds in the ethanol extract of *A. paniculata*, which contribute to the thrombolytic activity. Few major chemical constituents have been isolated from *A. paniculata* in the past such as andrographolide, neoandrographolide, isoandrographolide and 14-deoxy-11,12-didehydro-14-doxy-11, 12-didehydroandrographolide (Chandrasekaran *et al*., 2011). Among them andrographolide inhibits blood platelet aggregation (Amroyan *et al*., 1999). Therefore, *A. paniculata* could be a potential option for the prevention and treatment of cardiovascular disorders e.g., thrombosis. Antiplatelet activity of the extract of *A. paniculata* was investigated in human model by Zhang and his colleagues and they suggested that *A. paniculata* can inhibit dense and alpha granules from platelet and dilate canalicular system. They also suggested that the mechanism of *A. paniculata* in providing antiplatelet effect is due to the increase level of platelet cAMP (Zhang *et al*., 1994).

Antimicrobial study was done against two gram positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and four gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella dysenteriae*). The zone of inhibition was highest for crude ethanol extract against all bacteria. Antimicrobial activity against all the bacteria was also found for carbon tetrachloride soluble fraction. Interestingly, *n*-hexane soluble fraction was not able to show antimicrobial activity against *B. cereus, S. aureus, S. typhi and S. dysenteriae* bacteria. The results of antimicrobial activity are presented in Table 3.

The study demonstrated that crude ethanol extract inhibits microbial growth. Possibly this antimicrobial activity was observed to the presence of arabino galactan proteins and andrographolides in the ethanol extract of *A. paniculata* (Singha *et al*., 2003). Mishra *et al*. (2009) also evaluated the antimicrobial activity of ethanol extract of *A. paniculata* by zone of inhibition method against 11 bacterial strains and compared the concentration against ciprofloxacin at a concentration 100 and 300 µg per milliliter and reported that the ethanol extract was able to inhibit the growth of both the gram positive and gram negative bacteria.

Table 3. Antimicrobial activity of kanamycin and various extracts of the whole plant of *Andrographis paniculata*[a]

Sample	Diameter of zone of inhibition (mm)					
	Gram positive bacteria		Gram negative bacteria			
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Shigella dysenteriae*
NHSF	-[b]	-[b]	15.2±0.91	10.7±0.31	-[b]	-[b]
CTCSF	13.2±1.00	12.9±0.61	15.5±0.65	11.9±0.57	15.6±0.34	14.7±0.21
CEE	18.1±1.21	16.6±0.73	17.7±0.82	18.4±0.93	18.9±1.0	17.9±0.34
Kanamycin disc	30.5±2.33	31.7±2.54	32.1±2.49	29.7±1.76	30.1±3.21	31.2±1.52

[a] = results are mean ± S.D. values; [b] = no activity (diameter of zone of inhibition less than 10 mm); CEE = crude ethanol extract, NHSF = *n*-hexane soluble fraction, CTCSF = carbon tetrachloride soluble fraction.

Conclusion

The extracts of the *Andrographis paniculata* can be used to develop different thrombolytic and antimicrobial agent. This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. Further studies on isolation of secondary metabolites are required to identify the active component of the extract and to confirm the mechanism of action, to facilitate the development of a potent thrombolytic and antimicrobial agent.

References

Aderogba, M.A., Okoh, E.K., T.O., I. 2005. Evaluation of antioxidant activity of the secondary metabolites from *Poliostigma reticulatum* (DC) hochst. *Journal of Biological Sciences*, **5:** 239-242.

Akbar, S. 2011. *Andrographis paniculata*: a review of pharmacological activities and clinical effects. *Alternative Medicine Review*, **16:** 66-77.

Amroyan, E., Gabrielian, E., Panossian, A., Wikman, G., Wagner, H. 1999. Inhibitory effect of andrographolide from *Andrographis paniculata* on PAF-induced platelet aggregation. *Phytomedicine*, **6:** 27-31.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, **45:** 493-496.

Bhatnagar, S.S., Santapau, H., Desa, J.D., Maniar, A.C., Ghadially, N.C., Solomon, M.J., Yellore, S., Rao, T.N. 1961. Biological activity of Indian medicinal plants. I. Antibacterial, antitubercular and antifungal action. *The Indian Journal of Medical Research*, **49:** 799-813.

Chandrasekaran, C.V., Thiyagarajan, P., Deepak, H.B., Agarwal, A. 2011. *In vitro* modulation of LPS/calcimycin induced inflammatory and allergic mediators by pure compounds of *Andrographis paniculata* (King of bitters) extract. *International Immunopharmacology*, **11:** 79-84.

da Silva, J.F.M., de Souza, M.C., Matta, S.R., de Andrade, M.R., Vidal, F.V.N. 2006. Correlation analysis between phenolic levels of Brazilian propolis extracts and their antimicrobial and antioxidant activities. *Food Chemistry*, **99:** 431-435.

Hughes, J.P., Rees, S., Kalindjian, S.B., Philpott, K.L. 2011. Principles of early drug discovery. *British Journal of Pharmacology*, **162:** 1239-1249.

Kawsar, H.M., Sikder, M.A.A., Rana, M.S., Nimmi, I., Rashid, M.A. 2011. Studies of thrombolytic, antioxidant and cytotoxic properties of two asteraceous plants of Bangladesh. *Bangladesh Pharmaceutical Journal*, **14:** 103-106.

Luo, X.D., Wu, S.H., Ma, Y.B., Wu, D.G. 2000. Limonoids and phytol derivatives from *Cedrela sinensis*. *Fitoterapia*, **71:** 492-496.

Majheniè, L., Škerget, M., Knez, Ž. 2007. Antioxidant and antimicrobial activity of guarana seed extracts. *Food Chemistry*, **104:** 1258-1268.

Mishra, U.S., Mishra, A., Kumari, R., Murthy, P.N., Naik, B.S. 2009. Antibacterial activity of ethanol extract of *Andrographis paniculata*. *Indian Journal of Pharmaceutical Sciences*, **71:** 436-438.

Paterson, 1999. Phytochemical methods. A guide to modern techniques of plant analysis. *Plant Pathology*, **48:** 146.

Pereira, J.A., Oliveira, I., Sousa, A., Valentao, P., Andrade, P.B., Ferreira, I.C., Ferreres, F., Bento, A., Seabra R., Estevinho, L. 2007. Walnut (*Juglans regia* L.) leaves: phenolic compounds, antibacterial activity and antioxidant potential of different

cultivars. *Food and Chemical Toxicology*, **45:** 2287-2295.

Prasad, S., Kashyap, R., Deopujari, J., Purohit, H., Taori, G., Daginawala, H. 2006. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. *Thrombosis Journal*, **4:** 14.

Rabaud, C., Tronel, H., Fremont, S., May, T., Canton, P., Nicolas, J.P. 1997. Free radicals and HIV infection. *Annales de Biologie Clinique*, **55:** 565-571.

Saha, M.R., Ul Bari, F.M.S.N., Rahman, M.A., Islam, M.A. 2012. *In vitro* cytotoxic and anthelmintic activities of *Leonurus sibiricus* L. *Journal of Scientific Research*, **4:** 721-727.

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

Thisoda, P., Rangkadilok, N., Pholphana, N., Worasuttayangkurn, L., Ruchirawat, S., Satayavivad, J. 2006. Inhibitory effect of *Andrographis paniculata* extract and its active diterpenoids on platelet aggregation. *European Journal of Pharmacology*, **553:** 39-45.

Zhang, Y.Z., Tang, J.Z., Zhang, Y.J. 1994. Study of *Andrographis paniculata* extracts on antiplatelet aggregation and release reaction and its mechanism. *Zhonggue Zhong Xi Yi Jie He Za Zhi*, **14:** 28-30.