AU J.T. 15(4): 239-245 (Apr. 2012)

Antibacterial Activity of *Chrysanthemum indicum*, *Centella asiatica* and *Andrographis paniculata* against *Bacillus cereus* and *Listeria monocytogenes* under Osmotic Stress

Nateepat Pitinidhipat and Patchanee Yasurin

Faculty of Biotechnology, Assumption University, Bangkok, Thailand
E-mail: <patchaneeYsr@au.edu>

Abstract

Bacillus cereus *and* Listeria monocytogenes *have an ability to survive in high osmotic stress. Three Thai herbs;* Chrysanthemum indicum, Centella asiatica *and* Andrographis paniculata, *are chosen to study their antibacterial activity on* B. cereus *and* L. monocytogenes *10403S under normal and osmotic stress (5% NaCl) condition by agar disc diffusion method. The in vitro antibacterial screening results of crude 95% ethanolic extracted under normal stress showed* A. paniculata, *the highest antibacterial activity,* C. asiatica *and* C. indicum *crude 3.33 ± 0.47, 1.67 ± 0.94, and 1.17 ± 0.85 mm, respectively, against* B. cereus. *Only* C. asiatica *and* A. paniculata *crude showed antibacterial activity against* L. monocytogenes*; 1.67 ± 0.24, and 1.83 ± 0.24 mm, respectively. Under osmotic stress, the antibacterial activity of all crude 95% ethanolic extracted was increased two-fold. The MICs of* A. paniculata, C. asiatica *and* C. indicum *showed 4, 16, and 16 µl /ml against* B. cereus *while* A. paniculata *and* C. asiatica *showed 16 and 8 µl /ml, respectively, against* L. *monocytogenes, respectively. The MCBs of* A. paniculata, C. asiatica *and* C. indicum *showed 4, 16, and >32 µl /ml against* B. cereus *while* A. paniculata *and* C. asiatica *showed 16 and >32 µl /ml, respectively, against* L. monocytogenes.

Keywords*: Antibacterial activity, herb, crude ethanolic Extract,* Bacillus cereus, Listeria monocytogenes *10403S, osmotic stress.*

1. Introduction

Bacillus cereus is a Gram-positive, may change to Gram-negative when get older, rod-shaped with endospore and aerobe (Todar 2008a). *B. cereus* themselves not tolerate to physical condition, basic or acidic, but their spores are heat resistant and active in wide range of pH (Todar 2008a). Growth of *Bacillus* sp. will gives an enterotoxin. Even *B. cereus* outbreaks is only 2% of all foodborne illness (Todar 2008a; Davis 2010), but they are still found and cause illness to citizen in developing countries, especially in hot climate countries like Thailand.

Listeria monocytogenes is a gram-positive, rod-shaped, non-sporeformer bacteria, and cause listeriosis disease. In elders, children, pregnant women, and immune-compromised patient listeriosis can cause serious effects to nervous system which lead to death (Davis 2010). The mortality percent of the patients infected by this disease is around 30 (Todar 2008b). 5-10% of human populations carry *Listeria* in intestinal tract without any symptoms. *L. monocytogenes* is marked resistant to classic methods of food preservation such as low pH, high salt (osmotic stress) and low temperature (cold stress). About 2,500 cases of listeriosis found in U.S. each year (Todar 2008c). In Canada, the Public Health Agency of Canada reported in 2008 outbreak of listeriosis which killed 23 Canadian from 57 confirmed cases. About 40% mortality was observed in San Antonio, Texas, USA, in 2010, which killed 2 from 7 infected from consuming contaminated fresh cut celery (Davis 2010). The latest outbreak was in early

September-November 2011; a total of 139 persons infected with any of the four outbreak-associated strains of *L. monocytogenes* from whole cantaloupes, Jensen Farms, Colorado have been reported to Centers for Disease Control and Prevention, CDC, USA, from 28 states, 29 deaths have been reported. In addition, 1woman pregnant at the time of illness had a miscarriage.

These food-borne pathogenic bacteria like *B. cereus* and *L. monocytogenes* have ability to survive, grow and proliferate in those classic preservation methods; salting freezing. Introducing of natural antibiotics to food product might be another attraction candidate process for food preservation method and keep the desirable flavor and texture. Plants and herbs are good candidate source for natural antibiotics. Chrysanthemum *(Chrysanthemum indicum* L.) is a well known Thai and Chinese herbal tea. The whole plant has health benefit but the famous part is the flower used in chrysanthemum tea (Jung 2009). *C. indicum* always used in traditional drug formula for the treatment of several infectious disease such as pneumonia, colitis, stomatitis, cancer, fever, sore and used to treat vertigo, pertussis and hypertensive symptom (Jung 2009). Active compounds in *C. indicum* are glycosides, adenine, and flavanoids. Previous research work also showed that *C. indicum* has the ability to act as antibiotic to many species of bacteria (Jung 2009).

Centella asiatica (L.) Urban. (Tiger Herbal, pennywort, *gotu kola*) is well known as local herb of Southeast Asia and China. *C. asiatica* has good aroma, a little bit bitter taste. Traditional drug formula use stem and leaves, aerial parts to decrease blood pressure, cure the fresh wound, heal bruised and diuretic (Ullah *et al.* 2009). Active compounds in *C. asiatica* are many types of terpenes or terpenoids (Oyedeji and Afolayan 2005; Gershenzon and Dudareva 2007).

Andrographis paniculata (Burm.f.) Wall.ex Nees (Kariyat, Creat, *Chuanxinlian*) is a seasonal plant in the Family of Acanthaceae. The whole plant has bitter taste. *A. paniculata* also contained many of flavanoids and polyphenol as their active compounds (Chao and Lin 2010).

The objectives of this experiment are to study: (i) the extraction condition of antibacterial compound from three Thai local herbs, *C. indicum*, *C. asiatica* and *A. paniculata*; and (ii) the antibacterial activity of crude extract of these three local herbs on *B. cereus* and *L. monocytogenes* under normal and osmotic stress (5 % NaCl) condition.

2. Materials and Methods

2.1 Plant sample preparation and extraction

C. indicum, *C. asiatica*, and *A. paniculata* were obtained from local fresh market in Bangkok, Thailand. Herbs were cut into small pieces and air dried in oven (Memmert, UM500) at 45^{o}C for overnight. Then dried herbs were blended in food blender to reduce the size. Herb powder was stored in refrigerator at 6^{o}C until use. 20 g herb powder was weigh on top-loaded balance, 1decimal (ZEPPER model ES-300) then 180 ml ethanol (Rung-Sap Co.,Ltd.) with different concentration 0 (water), 25, 50, 75 and 95% was added and soaked for 48 hours at room temperature and stirred every 12 hours. After 48 hours, the liquid part was separated by filter pass through thin cloth. Then crude extract was centrifuged (Chermle model Z230A) at 5,000 rpm for 5 min. Supernatant was collected and concentrated in water bath (Schutzart DIN40050 - IP20) at 45^{o}C until become very concentrate slurry. Crude extract was kept in freezer at -20^{o}C until use. Crude extract was diluted 100 mg/ml by Ethanol 95% as final concentration. Diluted crude extracted were 0.2 µm CE filter sterile (Minisart®). Then keep in freezer at – 20^{o}C.

2.2 Antibacterial Assay and Growth Condition

B. cereus, stock culture of the Faculty of Biotechnology, Assumption University, and *L. monocytogenes* 10403S (Gift of G.M. Smith, UCD) were used to test antibacterial activity of three herbs crude extract in this experiment. 1-looped *B. cereus* was inoculated into Nutrient Broth (NB) and Brain Heart Infusion (BHI) broth for *L. monocytogenes*, incubated at 37^{o}C for 24 hours (Jouan incubator, model EB280). 1% v/v of incubated broth was transfer to fresh NB, BHI broth and incubated at 37^{o}C by

Culture tube Rotator SCI (Stuart Scientific), until OD_{600} reach 0.1 (SPECTRONIC, model GENESYS 5) which is their early log phase. BSAC Disc Diffusion Method for Antimicrobial Susceptibility Testing version 8 by The British Society for Antimicrobial Chemotherapy was used for antibacterial activity assay.100 µl of culture was swabbed on the agar. Sterile paper disc contained 15 µl of 100 mg/ml crude extract concentration, 100 mg/ml Penicillin-G, and ethanol 95% was placed. All plates were incubated at 37^{o}C for 24 hours. Clear zone result was measured as antibacterial activity then the data were collected. Positive antibacterial activity, using clear zone as criterion, was selected for next osmotic stress experiment. Osmotic stress condition was prepared by adding 5% NaCl (w/v) to media and incubation time was increased from 24 hours to 48 hours at 37^{o}C, then the data were collected, mean and standard deviation of data were calculated. All experiment was performed in duplicate and repeated three times

2.3 MIC and MBC Determination

MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) methods were modified from BSAC Disc Diffusion Method for Antimicrobial Susceptibility Testing version 8 by The British Society for Antimicrobial Chemotherapy. For MIC test, crude extracted were add to the 1ml fresh broth in different concentration as following 32, 16, 8, 4, 2, 1, and 0.25 µl/ml. One hundred µl of culture 0.1 OD_{600} was inoculated then incubated at 37^{o}C for 24 hours. The MIC test negative result tubes were chosen for MBC Test then incubated at 37^{o}C for 24 hours. All experiments were performed in triplicate and repeated three times

3. Results and Discussion

3.1 The Extraction Condition and Anti-bacterial Activity

Among 5 ethanolic extraction conditions, only 75% and 95 % crude ethanolic extracted concentration on both *B. cereus* and *L. monocytogenes* of all three crude ethanolic extracted at different under normal and osmotic stress (5% NaCl) showed the antibacterial activity in all three plants. The 95% crude ethanolic extracted showed the highest significant antibacterial activity resulting in inhibition growth of *B. cereus* and *L. monocytogenes* under both normal and osmotic stress (5% NaCl) in all plants. The previous GC-MS study (Jung 2009) showed that 73 active compounds were identified from *C. indicum*'s essential oil extracted which had stronger antibacterial activity than its individual antibacterial active compounds (Jung 2009); monoterpene hydrocarbons α-pinene, oxygenated monoterpenes camphor, 1,8-cineole, terpinen-4-ol, borneol and sesquiterpene hydrocarbon β-caryophyllene against oral bacterial strains; *Streptococcus* sp., *Prevotella intermedia* ATCC 25611, *and Porphylomonas gingivalis* ATCC 33277 (MICs, 0.1 to 1.6 mg/ml; MBCs, 0.2 to 3.2 mg/ml) (Jung 2009). *C. indicum* have germacrene D which is known to be strong antimicrobial (Oyedeji and Afolayan 2005; Jung 2009). Its essential oil is terpenoids rich which exert inhibitory action against microorganisms such as *S. aureus*, *E. coli* and *Streptococcus pneumoniae* by disrupting their membranes (Arldogan *et al.* 2002). This study confirmed the previous works (Oyedeji and Afolayan 2005; Jung 2009) that the *C. indicum* crude 95% ethanolic extracted have antibacterial activity.

Table 1 showed that *C. asiatica* crude 95% ethanolic extracted have antibacterial activity against the growth of both *B. cereus* and *L. monocytogenes* under normal and osmotic stress. Previous study (Panthi and Chaudhary 2006)showed that *C. asiatica* has antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Shigella boydii* (Panthi and Chaudhary 2006). Those result (Panthi and Chaudhary 2006) also confirms the result from this experiment that *C. asiatica* crude 95% ethanolic extracted has antibacterial activity. The chemical profile of *C. asiatica* essential oil also found three types of germacrene which are germacrene A (0.24%), germecrene B (6.29%) and germacrene D (4.01%) (Oyedeji and Afolayan 2005).

Table 1. Antibacterial activity as clear zone (mm).

75 % ethanolic extraction				
Crude extract	*B. cereus*		*L. monocytogenes*	
	0% NaCl	5 % NaCl	0% NaCl	5 % NaCl
C. indicum	1.00 ± 0.00	1.17 ± 0.24	1.00 ± 0.82	-
C. asiatica	1.17 ± 0.24	2.58 ± 1.40	1.00 ± 0.00	-
A. paniculata	1.33 ± 0.47	-	1.33 ± 0.47	-
95 % ethanolic extraction				
Crude extract	*B. cereus*		*L. monocytogenes*	
	0% NaCl	5 % NaCl	0% NaCl	5 % NaCl
C. indicum	1.17 ± 0.85	2.67 ± 0.75	1.67 ± 0.24	8.50 ± 1.26
C. asiatica	1.67 ± 0.94	5.67 ± 1.11	1.83 ± 0.24	3.92 ± 1.74
A. paniculata	3.33 ± 0.47	4.17 ± 0.94	1.00 ± 0.00	-
	B. cereus		*L. monocytogenes*	
95% Ethanol	0.67 ± 0.47	1.67 ± 0.24	1.00 ± 0.00	23.50 ± 1.61
Penicllin-G	12.83 ± 2.20	19.67 ± 2.34	1.5 ± 0.50	32.00 ±2.38

* Clear zones were measured from a paper disc edge to the end of a clear zone in mm.
** 95% ethanol and 100 mg/ml penicllin-G were used as positive control.

These three germacrenes might be a major active antibiotic compound of *C. asiatica* crude extract to inhibit the growth of both *B.cereus* and *L. monocytogenes* in this experiment. This hypothesis needs further investigation. Another previous study (Oyedeji and Afolayan 2005), *C. asiatica* essential oil showed the ability to inhibit growth of gram-positive bacteria. It was found that *C. asiatica* consist of triterpenoid glycosides, free acids, volatile oils and flavonoids (Mamtha *et al.* 2004). The triterpene plays importance rule in antibiotics activity in *C. asiatica* (Mamtha *et al.* 2004).The triterpenes weaken the membranous which results in dissolving the cell walls of the microorganisms (Mamtha *et al.* 2004). These results (Mamtha *et al.* 2004) candidly suggest the presence of promising antibacterial substances in *C. asiatica* crude 95% ethanolic extracted in this experiment. The germacrene and triterpenes might be antibacterial active compound candidates against *B. cereus* and *L. monocytogenes* in both normal and osmotic stress (5% NaCl). However this assumption need to be further investigated.

The result, as in Table 1, showed that *A. paniculata* gave the highest antibacterial activity among these three plants against both *B.cereus* and *L. monocytogenes*. *A. paniculata*'s active compounds have an antibacterial activity (Singhamutra 1993). The major active compound of *A. paniculata* are lactone group; andrographolide, deoxy-andrographolide, neoandrographolide, dehydroandrographolide (Singhamutra 1993). The andrographolide in *A. paniculata* soluble best in alcohol (Singhamutra 1993)which confirm the result in this experiment that 95% ethanolic extreaction gave the best antibacterial activity. Diterpenoids, flavanoids and polyphenols are also major active compound in *A. paniculata* (Chao and Lin 2010). However, it may be that individual antibacterial flavonoids have multiple cellular targets, rather than one specific site of action; inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism (Cushnie and Lamb 2005). The hydroxyl groups on A and B ring affect the antibacterial activity of flavonoids (Cushnie and Lamb 2005). There was a study indicated that 2´,4´- or 2´,6´ dihydroxylation of the B ring and 5,7-dihydroxylation of the A ring in the flavanone structure was important for anti-methicillin-resistant *S. aureus* activity (Tsuchiya *et al.* 1996). The former study investigated that no antibacterial activity of andrographolide against any of *E. coli*, *Shigella sonnei*, *S. aureus*, *P. aeroginosa*, *S. pneumoniae*, *S. pyogenes*, *Legionella pneumophila*, and *Bordetella pertusis* (Xu *et al.* 2006). While another independent lab had been reported that andrographolide in *A. paniculata* had

antibacterial activity against *B. subtilis* but there was no activity on *P. aeruginosa, S. aureus,* and *E. coli* (Singha *et al.* 2003). These results (Singha *et al.* 2003) confirmed result from this experiment that *A. paniculata* crude 95% ethanolic extracted have antibacterial activity against *Baciilus* sp. The *A. paniculata* crude methanolic extracted profile showed the presences of terpenoids, tannins, flavonoids, glysosides, saponins, alkaloids, amino acids and steroids, which also showed the antibacterial activity against both gram positive and negative bacteria (Sule *et al.* 2010).

It is interesting that even there was no presence of andrographolide but the crude extracted still showed antibacterial activity (Sule *et al.* 2010). *A. paniculata* show the presence of promising antibacterial substances. Andrographolide, terpenoids, flavonoid, glysosides might be the good candidates for antibacterial activity against *B. cereus* and *L. monocytogenes* in both normal and osmotic stress (5% NaCl) even the antibacterial activity mechanism of andrographolide is still unclear and need to be further investigated.

The antibacterial activity of all crude extract was increased two-fold under osmotic stress (5%NaCl w/v) in both *B. cereus* and *L. monocytogenes*. The previous study revealed that at 5%NaCl (w/v) *B. cereus* still survive and grow (Besten *et al.* 2009). At osmotic stress, salt will induce protein and regulated protein in adaptation to osmotic stress (Besten *et al.* 2009). However, the mechanism of *B. cereus* that made them more sensitive to antibiotic under osmotic stress is still unknown and need more study. *L. monocytogenes* 10403S, this strain has a mechanism to transport compatible solutes (glycine betaine, carnitine) which are osmoprotectants to protect whole cell from osmotic stress (Angelidis and Smith 2003). Although the growth rate is slower but introducing of osmotic stress at 5% NaCl (w/v) is still fine condition of *L. monocytogenes* to grow (Angelidis and Smith 2003; Beales 2004). Previous research mentioned that at low water activity, lipid composition of bacterial cell membrane was changed (Beales 2004). This incident might leads to occur of more antibacterial binding site on cell membrane of *L. monocytogenes* and cause less resistance to antibacterial substance. Therefore, the presence of the salt triggered changes in the membrane lipid composition. This phenomenal is possible to increase the microbial activity of crude extract. Natural antibiotic are also providing a great benefit over other antibiotic in term of there working mechanism that mechanism of natural antibiotic are usually not specific binding sites (Lopez-Malo Vigil *et al*. 2005). This also made bacterial cell more difficult to produce resistance for natural antibiotic too, due to their random binding site (Lopez-Malo Vigil *et al*. 2005). However, previous research mentioned that salt condition might decrease bacterial cell ABR (scale of antibiotic resistance), then leads to lower activity of antibiotic on *E. coli*, and *S. aureus* (McMahon *et al.* 2007).

3.2 The Minimum Inhibitory Concentrations (MICs) and The Minimum Bactericidal Concentrations (MCBs)

Table 2. MICs and MBCs of crude extracted derived from herb sample (µl/ml), *B.cereus* was test on NB *and L .monocytogenes* 10403S was test on BHI broth.

B. cereus	**MIC (µl/ml)**	**MBC(µl/ml)**
C. indicum	16	> 32
C. asiatica	16	16
A. paniculata	4	4
L. monocytogenes		
C. asiatica	8	> 32
A. paniculata	16	16

The results from table 2 showed the minimum inhibitory concentrations (MICs) of *A. paniculata*, *C. asiatica* and *C. indicum*, using broth dilution method, showed 4µl/ml, 16µl/ml, and 16 µl /ml, respectively, against *B. cereus*. The MICs of *A. paniculata* and *C. asiatica* showed 16 µl /ml and 8 µl /ml, respectively, against *L. monocytogenes* 10403S. The Minimum Bactericidal Concentrations (MCBs), using broth dilution method, of *A. paniculata, C. asiatica* and *C. indicum* showed 4mg/ml, 16µl/ml, and >32 µl /ml, respectively, against *B. cereus*. The MCBs of *A. paniculata* and *C. asiatica* showed 16 µl /ml, and >32 µl /ml, respectively, against *L. monocytogenes* 10403.

4. Conclusion

C. indicum, *C. asiatica*, and *A. paniculata* showed the presence of promising antibacterial substances against *B. cereus* and *L. monocytogene* under normal and osmotic stress. The antibacterial substances, their individual antibacterial activity, combined antibacterial activity, and antibacterial mechanism under normal and osmotic stress in each crude extracted need to be further investigated for application in the food industry as one way for food safety.

5. References

Angelidis, A.S.; and Smith, G.M. 2003. Three transporters mediate uptake of glycine betaine and carnitine by *Listeria monocytogenes* in response to hyperosmotic stress. Appl.Envir. Microbiol. 69: 1,013-22.

Arldogan, B.C.L.; Bayda, H.; Kaya, S.; Demirci, M.; Ozbaw, D.; and Mumcu, E. 2002. Antimicrobial activity and chemical composition of some essential oils. Arch. Phar. Res. 25: 860-4.

Beales, N. 2004. Adaptation of microorganisms to cold temperatures, weak acid reservatives, low pH, and osmotic stress: A review. Comp. Rev. Food Sci. Food Safety 3(1): 1-20.

Besten, H.M.W.; Mols, M.; Moezelaar, R.; Zwietering, M.H.; and Abee, T. 2009. Phenotypic and transcriptomic analyses of mildly and severely salt-streesed *Bacillus cereus* ATCC 14579 cells. Appl. Enviro. Microbiol.75: 4,111-9.

Chao, W.W.; and Lin, B.F. 2010. Isolation and identification of bioactive compounds in *Andrographis paniculata* (Chuanxinlian). Chinese Medicine 5: 17.

Cushnie, T.P.T.; and Lamb, A.J. 2005. Review Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents 26: 343-56.

Davis, C.P. 2010. Listeriosis (*Listeria monocytogenes* Infection). What is listeriosis? Medicinenet website. Available: <http://www.medicinenet.com/listeria/article.htm>.

Gershenzon, J.; and Dudareva, N. 2007. The function of terpene natural products in the natural world. Nature Chem. Biol. 3:408-14.

Jung, E.K. 2009. Chemical composition and antimicrobial activity of the sssential Oil of *Chrysanthemum indicum* against oral bacteria. J. Bacteriol. Virol. 19: 61-9.

Lopez-Malo Vigil, A.; Palou, E.; and Alzamora, S.M. 2005. Naturally occurring compounds-plant sources. *In*: Antimicrobials in Food. 3rd ed. Davidson, P.M.; Sofos, J.N.; and Branen, A.L. (eds.) CRC Press, Taylor & Francis Group, Boca Raton, FL, USA. Pp. 429-51.

Mamtha, B.; Kavitha, K.; Srinivasan, K. K.; and Shivananda P.G. 2004. An in vitro study of the effect of *Centella asiatica* [Indian pennywort] on enteric pathogens. Indian J. Pharmacol. 36: 41-4.

McMahon, M.A.S.; Xu, J.; Moore, J.E.; Blair, I.S.; and McDowell, D.A. 2007. Environmental stress and antibiotic resistance in food-related pathogens. Appl.Envir. Microbiol. 73: 211-7.

Oyedeji, O.A.; and Afolayan, A.J. 2005. Chemical composition and antibacterial activity of the essential oil of *Centella asiatica* growing in South Africa. Pharmac. Biol. 43: 249-52.

Panthi, M.P.; and Chaudhary, R.P. 2006. Antibacterial activity of some selected folklore medicinal plants from west Nepal. Sci. World 4(4): 16-21.

Singha, P.K.; Roy, S.; and Dey, S. 2003. Antimicrobial activity of *Andrographis paniculata*. Fitoterapia 74(7-8): 692-4.

Singhamutra, S. 1992. Properties of 200 Herbs, 1st ed. SO Printing House, Bangkok (in Thai).

Sule, A.; Ahmed, Q.U.; Samah, O.A.; and Omar, M.N. 2010. Screening for antibacterial activity of *Andrographis paniculata* used in Malaysian folkloric medicine: A possible alternative for the treatment of skin infections. Ethnobot. Leafl. 14: 445-56.

Todar, K. 2008a. *Bacillus cereus* Food Poisoning. Available: <http://www.textbookof bacteriology.net/B.cereus.html>.

Todar, K. 2008b. *Listeria monocytogenes*. Natural Habitats of *Listeria* and Incidence of Disease. Available: <http://www. textbookofbacteriology.net/Listeria.html>.

Todar, K. 2008c. *Listeria monocytogenes*. Summary. Available: <http://www.textbookof bacteriology.net/Listeria_3.html>.

Tsuchiya, H.; Sato, M.; Miyazaki, T.; Fujiwara, S.; Tanigaki, S.; Ohyama, M.; Tanaka, T.; and Iinuma M. 1996. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. J. Ethnopharmacol. 50: 27-34.

Ullah, M.O.; Sultana, S.; Haque, A.; and Tasmin, S. 2009. Antimicrobial, cytotoxic and antioxidant activity of *Centella asiatica*. European J. Sci. Res. 30: 260-4.

Xu, Y.; Marshall, R.L.; and Mukkun, TKS. 2006. An investigation on the antimicrobial activity of *Andrographis paniculata* extracts and andrographolide in vitro. Asian J. Plt. Sci. 5: 527-30.