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IN VITRO ANTIBACTERIAL EFFICACY OF LEAVES EXTRACT OF *Centela asiatica* AGAINST *Vibrio harveyi* AND *Aeromonas hydrophila*

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ABSTRACT

Disease infection is one of the limiting factors that affect productivity in aquaculture and has caused economic losses. Luminescent vibrios and motile aeromonas septicemia (MAS) are diseases caused by *Vibrio harveyi* and *Aeromonas hydrophila* bacteria, respectively. Certain plants have antimicrobial compounds and can potentially be used to treat the diseases, such as *Centella asiatica*. In the present study, the crude leaves extracts of *C. asiatica* were examined for its antibacterial potential using methanol solvents against *V. harveyi* and *A. hydrophila* bacteria. Different concentrations of 50 mg/mL and 100 mg/mL were checked for its antibacterial activity. The crude extract was also tested for phytochemistry content and LC_{50} using Brine Shrimp Lethal Assay. The crude extracts of *C. asiatica* showed a remarkable antibacterial activity with inhibition zone of 10.57 mm against *A. hydrophila* and 21.14 mm against *V. harveyi*. The phytochemistry test result showed that *C. asiatica* leaves contain alkaloid, phenol, and tannin compounds. The acute lethal concentration (LC_{50}) of *C. asiatica* after 24 hours exposure to the extract mixture was 254 mg/L. The results confirmed the potential use of *C. asiatica* extracts as a source of antibacterial compounds.

KEYWORDS: **antibacterial; *Centella asiatica*; *Vibrio harveyi*; *Aeromonas hydrophila***

INTRODUCTION

Aquaculture is the fastest-growing food-producing sector in the world with annual growth reaching 9%. This growth is driven by the increased of seafood demand along with global population growth (Allan & Burnell, 2013). Aquaculture activities are not only required to be able to provide food source but also to sustain aquaculture production and product trade. The growth of aquatic biota was influenced by infection of diseases. Disease infection is one of limiting factors that affect productivity in aquaculture and has caused economic losses due to various diseases caused by bacteria, fungi, parasites, and viruses (Flegel *et al*., 2008).

Here, we focus on disease caused by luminescent vibrios (i.e., *Vibrio harveyi*) and motile aeromonas septicemia (MAS) diseases caused by *Aeromonas hydrophila*. *Vibrio harveyi* is a bacterium that causes shrimp mass mortality. These bacteria attack shrimp larvae in hatcheries and shrimp cultivated in on growing ponds and known as luminescent shrimp disease. The shrimp infected by this bacteria will glow in the dark and usually attack the larvae at *zoea*, *mysis* and post larvae stages. *V. harveyi* are gram-negative, usually motile rods, facultative anaerobes and types of bacteria that live in the sea and have high salinity resistance (Thompson *et al*., 2004). While *A. hydrophila* attacked all types of freshwater fish in the tropics and were often isolated from diseased catfish. This disease usually occurs when the fish condition decreases due to stress and poor water quality (Sarono *et al*., 1993).

There are many approaches and mitigation efforts to reduce the negative impacts caused by pathogenic agents, including the use of high quality seed, best culture management, and application of biotechnology. In addition, many farmers, especially in developed countries, have improved their capacity to respond quickly and effectively to emergent disease situations. In most developing countries, the use of antibiotics is common to control fish disease distribution. However, antibiotics to treat bacterial diseases has been prohibited because uncontrolled antibiotics use may lead to resistant bacteria. This is

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viewed as potential health issue related to the transmission of resistant bacteria from aquaculture environment to humans (Cabello, 2006). Other related risk is the introduction of non-pathogenic bacteria in the environment which contain antimicrobial resistance genes causing genetic contamination on the aquatic environment (Serrano, 2005). Because of their toxic and carcinogenic side effects, the use of modern therapeutic drugs is being restricted. Thereby, interest in finding alternative medicinal ingredient for fish diseases from natural source without undesirable effects has increased greatly (Bothon *et al*., 2013).

Centella asiatica (L.) is a tropical medicinal plant from Apiaceae family native to Asian countries such as India, Sri Lanka, China, Indonesia, and Malaysia as well as South Africa and Madagascar (Jamil *et al*., 2007). The leaves of *C. asiatica* had traditional uses as treatment of asthma, ulcers, leprosy, lupus, vein diseases (Brinkhaus *et al*., 2000; Kartnig, 1988), memory improvement (Gupta *et al*., 2003; Rao *et al*., 2005), antidepressant (Chen *et al*., 2003), antibacterial, antifungal (Ullah *et al*., 2009), psoriasis (Sampson *et al*., 2001), and anti-cancer agent (Babu *et al*., 1995). *C. asiatica* contains a variety of compounds including asiaticoside, centeloside, madecassoside, brahmoside, glycosides, saponine, asiatic centoic acid, centelic acid, centoic acid, madecassic acid, and oxyacathicoside. Some of these compounds are known to have the most active antimicrobial capability which could even inhibit tuberculosis bacteria (Kurniawati *et al*., 2005). Methanol extract of *C. asatica* has been investigated for antimicrobial activity against *A. hydrophila* and *Edwardsiella tarda* (Purkait *et al*., 2018). However, there are currently no reports on the ability of *C. asiatica* extracts to inhibit the growth of *V. harveyi*. This study was aimed to evaluate the antibacterial activity of *C. asiatica* against *V. harveyi* and *A. hydrophila*.

MATERIALS AND METHODS

Materials

The chemicals used in this study were methanol, sulfuric acid (H_2SO_4), hydrochloric acid (HCl), potassium iodide, iodine, glacial acetic acid, ferric chloride, chloroform, and media cultures (nutrient agar, nutrient broth, tryptic soy agar, and thiosulfate citrate bile salts sucrose) obtained from Water Quality Laboratory, Faculty of Fisheries and Marine Science, Borneo Tarakan University. The bacterial strains were *V. harveyii* and *A. hydrophila* obtained from the collection of the laboratory of Nutrition and Fish Feed, Faculty of Fisheries and Marine Science, Borneo Tarakan University, Indonesia.

Collection of Plant Material

C. asiatica leaves were collected in the early morning hours in Tarakan rural areas. The plants were washed with cold distilled water and then dried at room temperature for seven days. The samples were crushed properly by a metal mortar until a fine homogeneous dried powder was obtained and then placed in paper bags under free humidity conditions (Cipriano, 2001).

Preparing Plant Extract

Dried powder of *C. asiatica* leaves (40 g) was placed in a 500 mL flask, mixed with 160 mL of methanol, and tightly wrapped with aluminum foil. Extraction was carried out using an orbital shaker at 180 rpm for 24 hours, after which the suspensions were filtered using Whatman No. 1 filter paper. The filtrate was evaporated using rotatory evaporator maintained at 65°C to get dry extracts. Solvent-free extracts were transferred to the extract vials and stored at 4°C for further used.

Phytochemical Screening of *C. asiatica* Leaves

Phytochemical screening of *C. asiatica* to determine the presence of alkaloids, saponins, tannins, flavonoids, and phenol hydroquinone was done by using a standard procedure of Samejo *et al*. (2013).

Test for Tannins

About 0.5 g of a dried powdered sample of plant was boiled in 10 mL distilled water in test tube and then filtered. A few drops 0.1% of $FeCl_3$ solution were added to the filtrate. Blue-black precipitate indicated the presence of tannins.

Test for Alkaloids

About 2 mL of 2N HCL was added to 5 mL extracts and then heated with stirring in a water bath for 10 minutes. The cooled solution was filtered and added with few drops of Wagner's reagent. Reddish-brown precipitate indicated the presence of alkaloids.

Test for Saponins

About 1 g of dried powdered sample was boiled with 10 mL distilled water. Frothing precipitate indicated the presence of saponins.

Test for Flavonoids

About 0.5 g dried powdered plant sample was boiled in 10 mL ethanol and filtered. Few pieces of magnesium ribbon and few drops of concentrated HCl were carefully added to the filtrate. The red color indicated the presence of flavonoids.

Test for Phenol Hydroquinone

One mL of extracted sample (ethanol, n-hexane-ethanol) was added with $FeCl_3$ 1%. Terpenoids compound was indicated by the appearance of blue to black or purple color.

Brine Shrimp Lethality Test

The extracts of *C. asiatica* leaves were routinely evaluated in a test for lethality to brine shrimp larvae, with minor modifications. Toxicities of the compounds were tested at 10, 50, 100, and 300 ppm in 10 mL sea-water. Ten of the one-day age nauplii were used in each test and survivors were counted after 24 hours. Three replications were used for each concentration. The blank control was always present. The lethal concentration for 50% mortality after 24 hours of exposure, the chronic LC_{50}, and 95% confidence intervals were determined using the probit method (Finney, 1971), as the measure of toxicity of the extract.

Antibacterial Activity

The antibacterial screening was carried out by agar disk diffusion method with the determination of diameters of inhibition zones made by *C. asiatica* extract against two bacterial strains (*V. harveyi* and *A. hydrophila*). Sterilized tryptic soy agar (TSA) and thiosulfate citrate bile salts sucrose (TCBS) were poured into plates individually and then inoculated with 100 μL suspension of tested bacteria (TSA for. *A. hydrophila*; TCBS for *V. harveyi*). One of five-millimeter discs of Whatman No. 1 filter paper was prepared and immersed in each of 1 mL extracts solution (50 mg/mL, and 100 mg/mL) in Aquadest. Chlorampenicol was used as positive control. The plates were incubated at 37°C for 18 hours. Antibacterial activity was evaluated by measuring the diameter (mm) of inhibition zones (Alabri *et al*., 2014).

Statistical Analysis

The antibacterial screening experimental results were run with four treatments in triplicate and expressed as mean ± SD and results were statistically evaluated using SPSS software version 16 at 5% significant level. Means were compared using Tukey's simultaneous test set at $P < 0.05$ (Dhiman *et al*., 2016).

RESULTS AND DISCUSSION

Determination of The Phytochemicals

The qualitative phytochemical analysis data of extracts of *C. asiatica* leaves showed that the phytochemicals were present in the methanolic extracts (Tabel 1). However, flavonoid and saponin were absent. Phytochemical screening in the plant samples determined the biologically active compounds, which have different benefits such as antioxidant, antimicrobial, antifungal, and anticancer (Alabri *et al*., 2014; Suresh & Nagarajan, 2009). All secondary metabolite components displayed antioxidant and antimicrobial properties through different biological mechanisms. Most of the secondary metabolite components were isolated and identified in the polar plant extracts (Alabri *et al*., 2014; Gonzales-Guevarra *et al*., 2004).

The phytochemical screening of methanol extracts of *C. asatica* leaves showed the major presence of active chemical constituents such as alkaloids, phenol hydroquinone, and tannins. Alkaloids have been reported as powerful poison, and many alkaloids derived from medicinal plants showed biological activities like antimicrobial (Iqbal *et al*., 2015; Benbott *et al*., 2012) that had bioactivity against gram-positive bacteria (Iqbal *et al*., 2015; Omar *et al*., 1992). Tannins and its derivatives are phenolic compounds which are also bioactive constituents and has a role in plant defense system due to their antimicrobial activity (Sudira *et al*., 2011).

Brine Shrimp Lethality Test (BSLT)

The mortality percentages of brine shrimp and the LC_{50} values after 24 hours (chronic) exposure to various extracts of *C. asiatica* are shown in Table 2. After 24 hours of exposure, increased mortalities of brine shrimps were recorded in the treatment using all prepared extracts. Maximum mortalities (50%) were observed in the 300 mg/L concentrations of the mixture extract tested. According to Meyer *et al*. (1982), LC_{50} lower than 1,000 ppm is considered cytotoxic (active) on the evaluation of plant extracts by BSLT, while non-toxic (inactive) if it is greater than 1,000 ppm. Meanwhile, Ramos *et al*. (2009) stated that a compound categorized highly toxic if the LC_{50} value is less than 1 mg/L, toxic if the LC_{50} value range is 1-250 mg/L, weak toxicity if the LC_{50} value is more than 250 mg/L. According to these results, *C. asiatica* extract as antimicrobial agent poses a low risk of harm against the brine shrimps. Phytochemical screening of the extract of *C. asiatica* showed that the extract contained secondary metabolites such as alkaloid, tannins and phenol hydroquinone. These phytochemicals have been implicated in the lower cytotoxicity activity demonstrated by the extracts of *C. asiatica*.

Antimicrobial Activity

C. asiatica extracts were investigated for antimicrobial activity assay against both the gram-negative (i.e., *A. hydrophila* and *V. harveyi*) bacteria by agar dif-

Table 1. Phytochemical constituents of *C. asiatica* leaves extracts

Active material type	Result	Information
Alkaloid	+	If red sediment exists
Phenol hydroquinone	+	If the solution turn to black
Flavonoid	-	If red color presence
Saponin	-	If frothing precipitate presence
Tannin	+	If color turn to blue-black

Table 2. LC_{50} values of the *C. asiatica* leaves extracts against the brine shrimp, *Artemia salina*

Concentration (mg/L)	Brine shrimp mortality (%)	LC_{50} 24 hours
10	5	254 mg/L
50	35	
100	40	
300	50	

fusion method. Different concentrations of the sample extracts (i.e. 50 mg/mL and 100 mg/mL) were used for the antimicrobial tests. The extracts showed antimicrobial activity against all the microorganisms (Table 3) in both concentrations. The methanol extracts of *C. asiatica* leaves concentration of 50 mg/mL showed the maximum antibacterial activity against *A. hydrophila* and the diameter of the inhibition zone was 10.57 mm. *V. harveyi* was found to be more sensitive to *C. asiatica* with 14.16 and 21.14 mm inhibition zone concentration of 50 and 100 mg/mL, respectively. Most of the studies revealed the in vitro antimicrobial activities of plant extracts against different pathogens are difficult to compare due to the use of different extraction, solvents, microbial strains, and antimicrobial test methods (Dhiman *et al*., 2016).

The antibacterial activity of the leaves extracts of *C. asiatica* is caused by the presence of alkaloid, tannin, and phenol hydroquinone, which are known as potential antimicrobial agents. The alkaloid mechanism as an antibacterial is by intruding the peptidoglycan in bacterial cells causing unforming the cell walls layer, and subsequently the death of cells. Another mechanism for alkaloid is it inhibits topoisomerase enzyme of bacterial cell (Karou *et al*., 2005). The mechanism of phenolic compounds such as phenol hydroquinone is by attacking cell walls and cell membranes, and thus affecting their permeability and releasing intracellular constituents. Phenolic compounds also interfere with membrane functions such as electron transport, enzyme effect, and nutrient uptake. Thus, active phenolic compounds might have several targets, which could lead to the inhibition of bacterial growth (Amensour *et al*., 2010). Tannins are organic compounds that inhibit microbial growth by damaging cell walls of microbial and forming bonds

Table 3. Antimicrobial activity of *C. asiatica* leaves extracts against gram-negative bacteria

Plant	Concentration (mg/mL)	Diameter of zone of inhibition (mm)	
		Aeromonas hydrophila	***Vibrio harveyi***
Control	0	0^{a}	0^{a}
Chloramphenicol	1	28.00 ± 2.02^{c}	22.80 ± 0.72^{c}
C. asiatica	50	10.57 ± 3.84^{b}	14.16 ± 2.82^{b}
	100	8.67 ± 1.15^{b}	21.44 ± 1.57^{c}

Note: Each value is the mean standard deviation of minimum three observations. Different letters at the top of each bar showed significant differences among treatments ($P < 0.05$)

with functional proteins of microbial cell (Sudira *et al*., 2011). The mechanism of tannins is also through producing hydrogen bonds with proteins, which convert the structure and block the protein synthesis. Tannins were considered as a phenolic compound of plants with anti-oxidative effects (Khder, 2008).

CONCLUSION

The phytochemical qualitative analysis of *C. asiatica* by the methanol extract revealed the presence of three major bioactive compounds such as alkaloids, phenol hydroquinone, and tannins. This study suggests that those compounds are responsible for its antibacterial effect which lead to the conclusion that *C. asiatica* leaves extracts could be used antibacterial ingredient in treating *V. harveyi* and *A. hydrophila* infections.

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REFERENCES

Alabri, T.H., Al Musalami, A.H., & Hossain, M.A. (2014). Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. *J. King Saudi Univ. Sci*., 26, 237-243.

Allan, G. & Burnell, G. (2013). Advances in aquaculture hatchery technology. Cambridge: Woodhead Publishing Limited, p. 223-273.

Amensour, M., Bouhdid, S., Fernández-López, J., Idaomar, M., Senhaji, N.S., & Abrini, J. (2010). Antibacterial activity of extracts of *Myrtus communis* against food-borne pathogenic and spoilage bacteria. *Int. J. Food*, 13(6), 1215-24.

Babu, T.D., Kuttan, G., & Padikkala, J. (1995). Cytotoxic and anti-tumor properties of ceratin taxa of Umbeliferae with special reference to *Centella asiatica* (L.) Urban. *J. Ethnopharmcol*., 48, 53-57.

Benbott, A., Yahyia, A., & Belaidi, A. (2012). Assesment of the antibacterial activity of crude alkaloids extracted from seed and roots and plant *Peganum harmala* L. *J. Nat. Prod. Plant. Resour*., 2, 568-573.

Bothon, F.T.D., Debiton, E., Avlessi, F., Forestier, C., Teulade, J., & Sohounhloue, D.K.C. (2013). In vitro biological effects of two anti-diabetic medicinal plants used in Benin as folk medicine. *BMC Complementary and Alternative Medicine*, 13(51).

Brinkhaus, B., Lindner, M., Schuppan, D., & Hahn, E.G. (2000). Chemical, pharmacological and clinical profile of the East Asian medicinal plant *Centella asiatica*. *Phytomedicine*, 7, 427-448.

Cabello, F.C. (2006). Minireview heavy use of prophylactic antibiotics in aquaculture/ : A growing problem for human and animal health and for the environment. *Environmental Microbiology*, 8(7), 1137-1144.

Chen, Y., Han, T., Rui, Y., Yin, M., Qin, L., & Zheng, H. (2003). Effects of total triterpenes of *Centella asiatica* on the depression behavior and concentration of amino acid in forced swimming mice. *Zhong Yao Cai*, 26, 870-873.

Cipriano, R.C. (2001). *Aeromonas hydrophila and motile aeromonas septicemias of fish. Fish Disease Leaflet*. Washington D.C: United States Department of The Interior Fish and Wildlife Service Division of Fishery Research.

Dhiman, R., Aggarwal, N., Aneja, K.R., & Kaur, M. (2016). In vitro antimicrobial activity of spices and medicinal herbs against selected microbes associated with juices. *International Journal of Microbiology*, 9 pp. http://dx.doi.org/10.1155/2016/9015802.

Finney, D.J. (1971). Probit analysis (3th ed.). Cambridge: Cambridge University Press, p. 76-80.

Flegel, T.W., Lightner, D.V., Lo, C.F., & Owens, L. (2008). Shrimp disease control: Past, present and future. *In* Bondad-Reantaso, M.G., Mohan, C.V., Crumlish, M., & Subasinghe, R.P. (Eds.), *Diseases in Asian Aquaculture VI*. Philippines: Fish Health Section Asian Fish Soc.

Gonzales-Guevara, J.L., Gonzales-lavaut, J.A., Pinorodriguez, S., Garcia-Tores, M., Carballo-Gonzales, M.T., Eschemendia-Arana, O.A., Molina-Tores, J., & Prieto-gonzales, S. (2004). Phytochemical screening and in vitro antiherpatic activity of four Erythtroxylum species. *Act. Farm Bon*., 23(4), 506-509.

Gupta, Y.K., Veerendra Kumar, M.H., & Srivastava, A.K. (2003). Effect of *Centella asiatica* on pentylenetetrazole-induced kindling, cognition and oxidative stress in rats. *Pharmacol. Biochem. Behav*., 74, 579-585.

Iqbal, E., Salim, K.A., Linda, B.L., & Lim. (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutimus* (Airy Shaw) from Brunei Darussalam. *J. King Saudi Univ. Sci*., 27, 224-232.

Jamil, S.S., Nizami, Q., & Salam, M. (2007). *Centella asiatica* (Linn.) urban: A review. *Natural Product Radiance*, 6(2), 158-170.

Karou, D., Savadogo, A., Canini, A., Yameogo, S., *Montesano, C., Simpore, J., Colizzi,V., & Traore, A.S.* (2005). Antibacterial activity of alkoloids from Sida acuta. *African Journal of Biotechnology*, 4(12), 195-200.

Kartnig, T. (1988). Clinical applications of *Centella asiatica* (L) Urb. *In* Herbs, spices and medicinal plants. Cracker, L.E. & Simon, J.E. (Eds.). USA: Oxyx Press.

Khder, A.K. (2008). Effect of *Allium sativum* and *Myrtus communis* on the elimination of antibiotic resistance and swarming of *Proteus mirabilis*. *Jordan J. Biol. Sci*., 1(3), 124-8.

Kurniawati, A., Darusman, L.K., & Rachmawaty, R.Y. (2005). Growth, production and triterpenoid content of two types of *Centella asiatica* L. (Urban) as a medicinal ingredient at various levels of shade. *Bul. Agron*., 33(3), 62-67.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., & Maclaughlin, J.L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *J. Med. Plant Res*., 45, 31-34.

Omar, S., Chee, C.I., Fasihuddin, A., Ni, J.X., Jaber, H., Huang, J., & Nakatsu, T. (1992). Penanthrene lactams from *Goniothalamus velutinus*. *Phytochem*, 31, 4395-4397.

Purkait, S., Abraham, T.J., Karmakar, S., Dey, B., & Roy, A. (2018). Inhibition of fish pathogenic *Aeromonas hydrophila* and *Edwardsiella tarda* by *Centella asiatica* in-vitro. *Journal of Aquaculture Research and Development*, 9(2), 3 pp. DOI: 10.4172/ 2155-9546.1000524.

Ramos, A., Carvalho, B., Sena, M., Dethoup, T., Buttachon, S., Kijjoa, A., & Rocha, E. (2009). Crude extracts of marine-derived and soil fungi of the genus neosartorya exhibit selective anticancer activity by inducing cell death in colon, breast and skin cancer cell lines. *Journal of Pharmacognosy*, 8(1), 8-15.

Rao, S.B., Chetana, M., & Uma Devi, P. (2005). *Centella asiatica* treatment during postnatal periodenhnaces learning and memory in mice. *Physiol. Behav*., 86, 449-457.

Sarono, A.K.H., Nitimulyo, I.Y.B., Widodo, N., Thalib, E.B.S., Haryani, S., Haryanto, Triyanto, Ustadi, A.N., Kusumahasti, W., Novianti, S., & Setianingsih. W. (1993). Quarantine pests and diseases of the bacterial group. Yogyakarta: Cooperation between the Agriculture Quarantine Center and the Faculty of Agriculture, Department of Fisheries, Universitas Gajah Mada.

Samejo, M.Q., Sumbul, A., Shah, S., Memon, S.B., & Chundrigar, S. (2013). Phytochemical screening of *Tamarix diocia* Roxb. Ex Roch. *J. Pharm. Res*., 7, 181-183.

Sampson, J.H., Raman, A., Karlsen, G., Navsaria, H., & Leigh, I.M. (2001). In vitro keratinocyte antiproliferation effect of *Centella asiatica* extract and triterpenoid saponins. *Phytomedicine*, 8, 230-235.

Serrano, P.H. (2005). Responsible use of antibiotics in aquaculture. Issues 469. Rome: Food and Agriculture Organization of the United Nations.

Sudira, I.W., Merdana, I.M., & Wibawa, I.P., (2011). Inhibitory test of kedondong leaf extract (*Lannea grandis*) on the growth of *Erwinia carotovora* bacteria. *Buletin Veteriner Udayana*, 3(1), 45-50.

Suresh, S.N. & Nagarajan, N. (2009). Prelimenary phytochemical and antimicrobial activity analysis of *Begonia malabarica* Lam. *J. Basic App. Bio*., 3, 59-61.

Thompson, F.L., Iida, T., & Swings, J. (2004). Biodiversity of vibrios. *Microbiology and Molecular Biology Reviews*, 68(3), 403-431.

Ullah, M.O., Sultana, S., Haque, A., & Tasmin, S. (2009). Antimicrobial, cytotoxic and antioxidant of *Centella asiatica*. *Eur. J. Sci. Res*., 30, 260-264.