Green Synthesis of Silver Nanoparticles using *Azadirachta indica* (Neem) Leaf Extract

W.H. DE SILVA[1], K. VIVEHANANTHAN[1] and C.A.N. FERNANDO[2]

[1]*Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP)*

[2]*Department of Electronics, Faculty of Applied Science, Wayamba University of Sri Lanka, Kuliyapitiya*

ABSTRACT

Development of eco-friendly process for the synthesis of metallic nanoparticles is an important aspect in the field of nanotechnology. In this study, silver nanoparticles were synthesized using the leaf extracts of *Azadirachta indica* and different process parameters such as reductant concentration, pH of the medium and mixing ratios were considered on nanoparticle synthesis. *Plectranthus amboinicus* (Kapparawalliya) leaf extract was also used to biosynthesize silver nanoparticles as a control. The biomolecules found in plants induce the reduction of Ag^+ ions from silver nitrate to silver nanoparticles (AgNPs) and it also acts as a capping and stabilizing agent for silver nanoparticles. Synthesized silver nanoparticles were initially characterized by colour change and further confirmed by Ultra Violet Spectroscopy. Neem leaf extract was prepared in two different ways as crude extract and modified extract which obtained by adjusting the pH into 10. The ultraviolet-visible spectrum of the synthesized silver nanoparticles by neem leaf extract showed strong absorption peak ranged from 384 nm to 465 nm. However the absorbance values were significantly varied with the process parameters. The UV-Visible Spectra of 2.5 g/ml of both crude and modified showed the best results with increased absorbance after 24 hours with mixing ratio of (Neem and $AgNO_3$) 1:2 and 1:9 respectively. Maximum absorbance of Kapparawalliya leaf extract was found to be lower than neem extract. Therefore it can be concluded that neem leaf extract is more effective in producing silver nanoparticles than Kapparawalliya to be used in a wide variety of applications.

KEYWORDS: *Azadirachta indica, Plectranthus amboinicus*, Silver nanoparticles, UV-Visible Spectroscopy

INTRODUCTION

The synthesis of noble metal nanoparticles like silver received greater attention due to their distinctive potential applications in the fields electronic, antimicrobials, magnetic, information storage and drug delivery. A number of approaches are available for the synthesis of silver nanoparticles by physical and chemical methods and currently via biological methods (Begum *et al.*, 2009; Song and Kim, 2009a).

Biological methods are considered safe and ecologically sound for the nanomaterial synthesis as an alternative to conventional physical and chemical methods which were previously used for the nanoparticle synthesis. Biological routes to the synthesis of these particles by plant materials are more advantageous, because they are easily available, safe to handle and possess a broad variability of metabolites that may aid in producing silver nanoparticles. In an attempt to find natural, environmental friendly and easily available plant-based agents for synthesis of metal nanoparticles, several plants have been used in the synthesis of nanoparticles (Shankar *et al.*, 2004).

Gold nanoparticles have been synthesized using live alfa alfa plants (Torresday *et al.*, 2002). Silver nanoparticles have been synthesized using various plant leaf extracts such as *Camellia sinensis* (Nestor *et al.*, 2008), *Magnolia kobus* and *Diopyros kaki* leaf (Song *et al.*, 2009b), *Acalypha indica* leaf (Krishnaraj *et al.*, 2010) and *Gliricidia sepium* (Rajesh *et al.*, 2009). Other nanoparticles such as nickel, cobalt, zinc and copper have been synthesized by using the live plants of *Brassica juncea* (Bali *et al.*, 2006). *Azadirachta indica* leaf extract has also been used for the synthesis of silver, gold and bimetallic (silver and gold) nanoparticles as it is commonly available medicinal plant and the biosynthesized silver nanoparticle has efficient antibacterial activity as it was capped with the neem leaf extract (Shankar *et al.*, 2004; Tripathy *et al.*, 2009).

As previous studies indicated that the modified neem leaf extract has efficient antibacterial activity than the crude neem leaf extract (Warusawithana and Vivehananthan, 2010), present study was further extended to compare the synthesis of silver nanoparticles for both types of extract to be used for future applications.

This study also focused on *Plectranthus amboinicus* (Kapparawalliya) leaf extract for synthesizing silver nanoparticles to compare with neem leaf extract on silver nanoparticle synthesis.

MATERIALS AND METHODS

Experimental Site

The study was conducted at the Department of Biotechnology, Faculty of Agriculture and Plantation Management and Nanotech Laboratory, Wayamba University of Sri Lanka during the period of January to April 2013.

Preperation of Azadirachta indica and Plectranthus amboinicus Leaf Extract

Crude neem leaf extract was prepared by taking known amount of thoroughly washed and finely cut *Azadirachta indica* leaves in a 500-mL Erlenmeyer flask with 100 ml of sterile distilled water and then boiled the mixture for 2 min. Modified neem leaf extract was prepared by adjusting the pH of the crude neem leaf extract into pH 10 by using previously modified method (Warusawithana and Vivehananthan, 2010).

Crude and modified Kapparawaliya leaf extract were also prepared with same concentration series and mixing ratios as same as neem broth to be used as a control.

Biosynthesis of Silver Nanoparticles

Different concentrations (1, 2.5, 5 and 10 g/mL) of crude and modified leaf extracts (Neem and Kapparawalliya) were prepared. These extracts were mixed with 10^{-3} M $AgNO_3$ in four different ratios (1:1, 1:2, 1:3 and 1:9) without varying the other conditions.

Characterization of Nanoparticles

The samples were used for characterization by diluting the 0.2 ml of samples 20 times in sterile distilled water (Shankar *et al.*, 2004; Tripathy *et al.*, 2009). Sampling was done at different time intervals (30 min, 2 hr, 4 hr, 5 hr and 24 hr) and the maximum absorption was scanned by UV-Visible Spectrophotometer from 200 to 800 nm on Shimadzu double beam spectrophotometer (model U-1800). The sterile distilled water was used as the blank.

RESULTS AND DISCUSSION

Reduction of the silver ions to silver nanoparticles during exposure to the plant leaf extracts was followed by color change from pale yellow to yellowish brown colour. This is due to excitation of surface plasmon vibrations in silver nanoparticles (Shankar *et al.*, 2004; Ankamwar *et al.*, 2005; Chandran *et al.*, 2006). Synthesized silver nanoparticles were further confirmed by UV–Visible Spectroscopy by taking the absorbance values of silver nanoparticle solution at different time intervals. Wavelength for the maximum absorbance value (λ_{max}) was reported in the visible range of 400–500 nm indicated the presence of silver nanoparticles (Sastry *et al.*, 1997). UV–Visible spectra of the present study also showed that the appearance of a surface plasmon resonance (SPR) band within this range and increased in intensity with time and the maximum absorbance was reported after 24 hr.

The particles synthesized with 2.5 g/ml broth concentration gave the maximum absorbance value in the UV-Visible Spectroscopy for both crude and modified broth after 24 hr confirming the best concentration and time period to synthesize silver nanoparticles. In crude broth, all ratios gave a peak and the highest peak was given by 1:2 ratio over other three ratios at 426 nm with an absorbance value 0.327 (Figure 1).

Maximum absorption of the modified broth was detected only at 1:9 ratio which may explained through the alkaline pH of the broth (Figure 2). Because at alkaline pH values, Ag (I) ions in solution may partly hydrolyze to form Bio-organic–Ag(OH)x complex on the surface of the particles and $AgOH/Ag_2O$ colloid in the medium (Tripathy *et al.*, 2009). Therefore the amount of Ag (I) ions to synthesize silver nanoparticles are low in the modified extract and may require more amount of $AgNO_3$ to produce considerable amount of silver nanoparticles.

Although the peak was observed in the expected λ max range for the rest of the concentrations (1, 5 and 10 g/ml) of neem leaf extract there was no intense peak for both crude and modified broth as like in 2.5 g/ml modified broth.

Therefore, 2.5 g/ml of neem leaf extract was found to be effective in producing silver nanoparticles for both crude and modified broth in 1:2 and 1:9 ratios respectively. In addition, both crude and modified Kapparawaliya leaf extract were not much effective as neem extract in all concentrations. Though crude Kapparawalliya leaf extract gave maximum absorbance in 1:1 ratio compared to other ratios, it was found to be lower (0.123) than the crude neem leaf extract (Figure 3). The peak in the expected wavelength range was not observed even in the modified Kapparawalliya leaf extract indicating less efficiency in synthesizing silver nanoparticles (Figure 4).

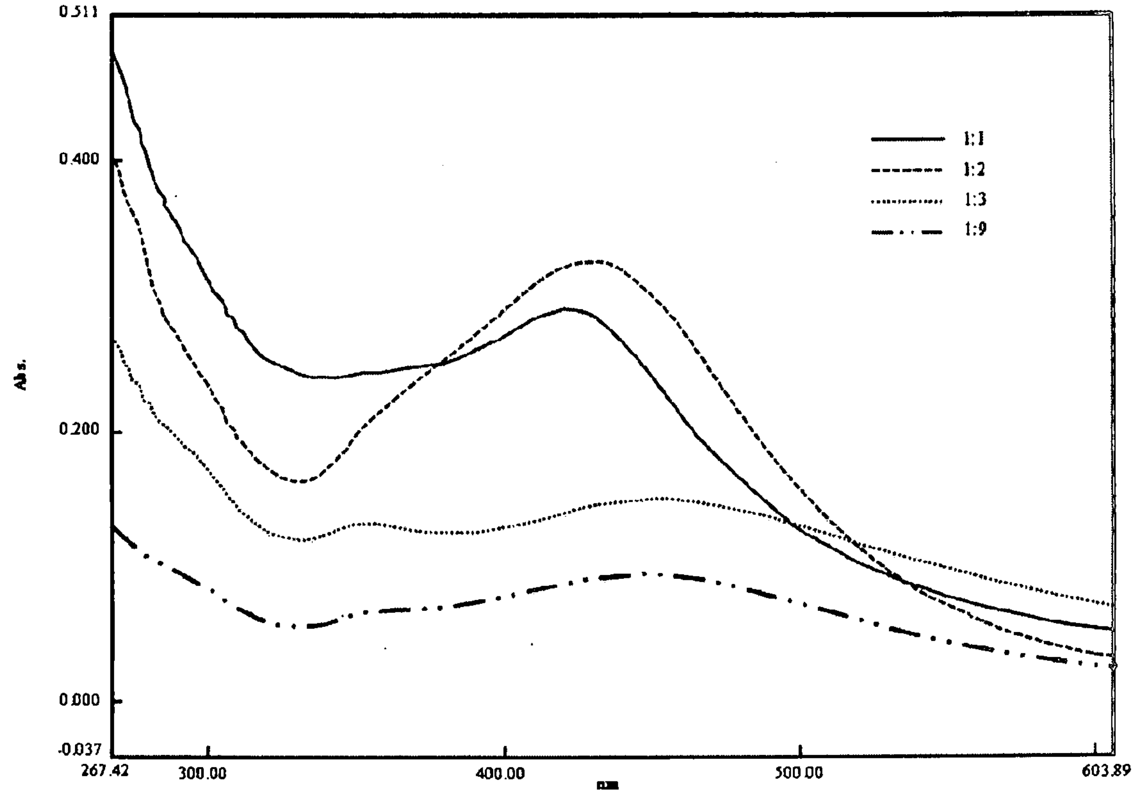

Figure 1. UV-Visible spectra recorded from the 10^{-3} M aqueous silver nitrate-2.5 g/ml crude neem leaf broth reaction as a function of time of reaction with varying mixing ratios after 24 hr

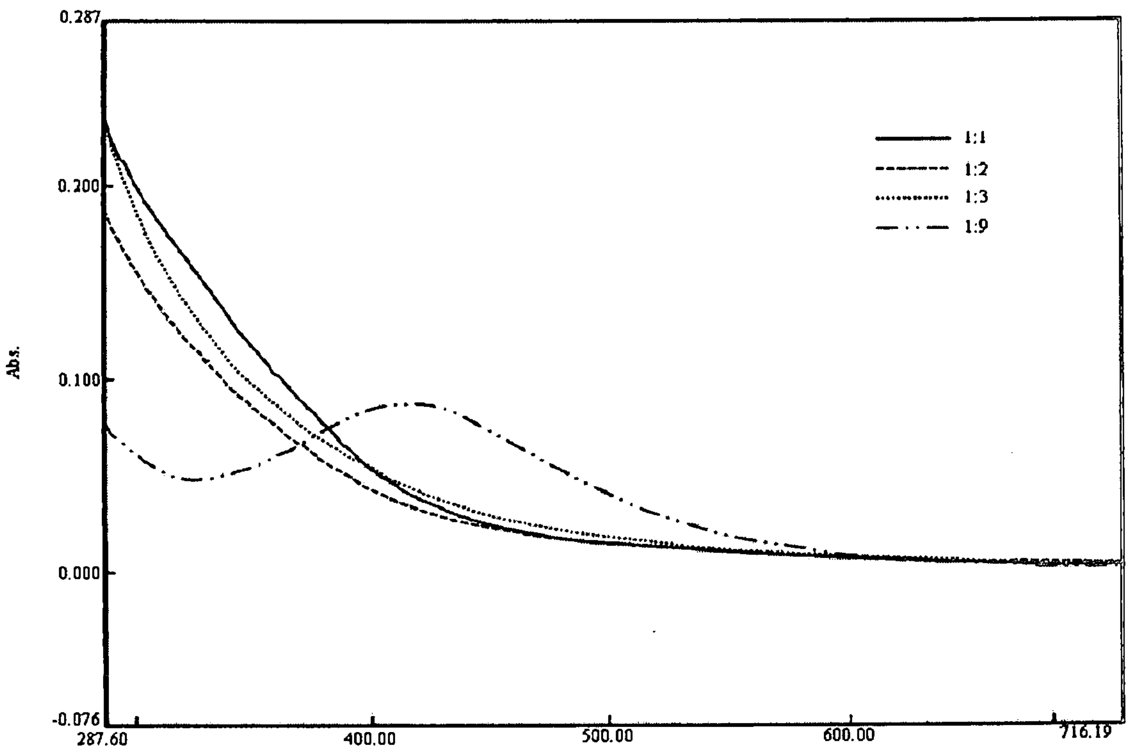

Figure 2. UV-Visible spectra recorded from the 10^{-3} M aqueous silver nitrate-2.5 g/ml modified neem leaf broth reaction as a function of time of reaction with varying mixing ratios after 24 hr

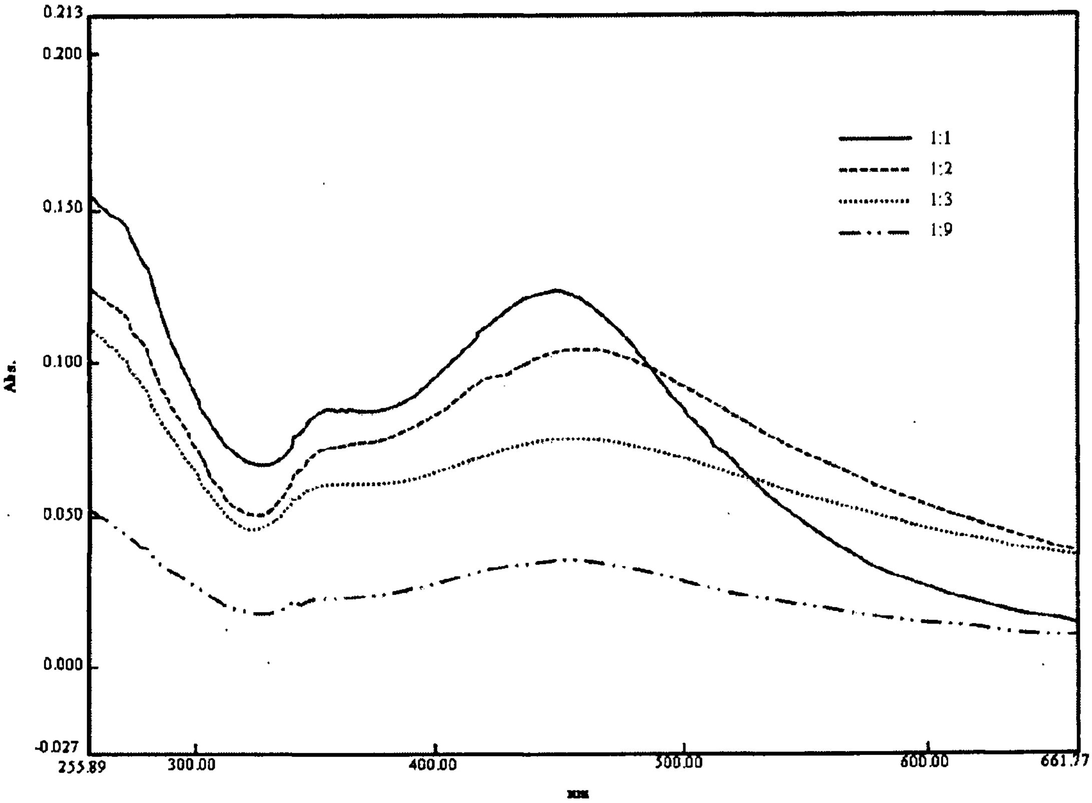

Figure 3. UV-Visible spectra recorded from the 10^{-3} M aqueous silver nitrate-2.5 g/ml crude Kapparawalliya leaf broth reaction as a function of time of reaction with varying mixing ratios after 24 hr

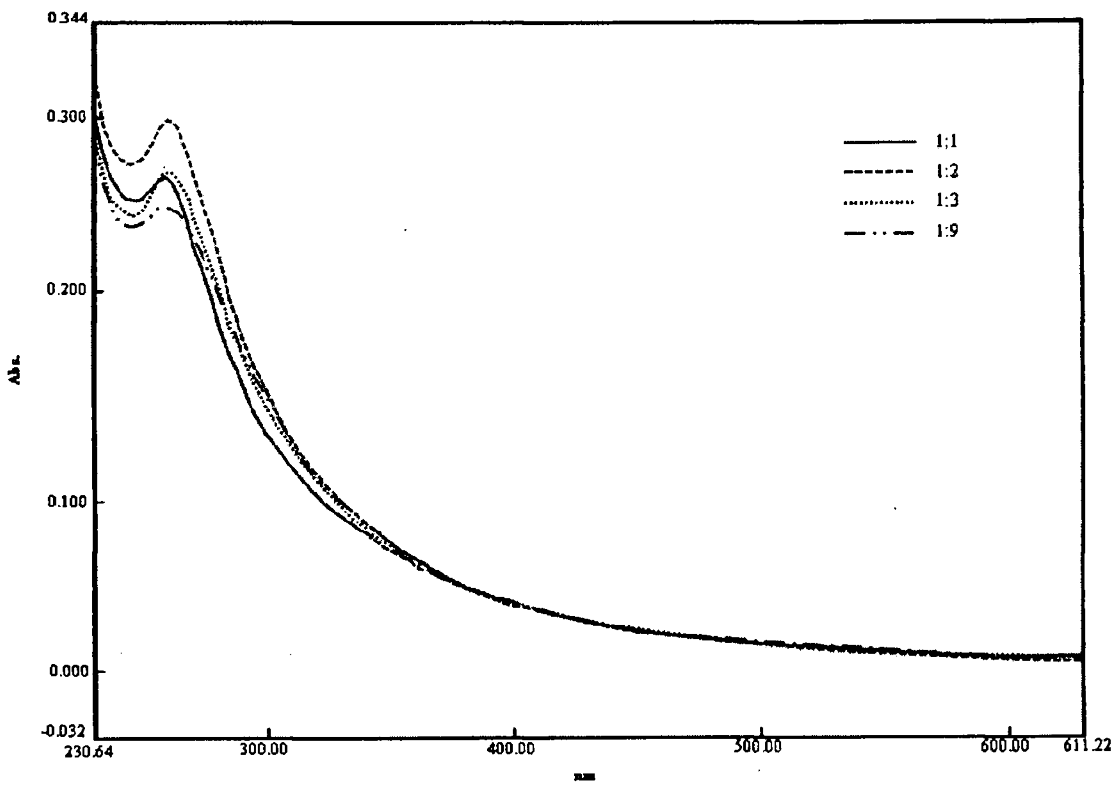

Figure 4. UV-Visible spectra recorded from the 10^{-3} M aqueous silver nitrate-2.5 g/ml modified Kapparawalliya leaf broth reaction as a function of time of reaction with varying mixing ratios after 24 hr

CONCLUSIONS

UV-Visible Spectroscopy confirmed the formation of silver nanoparticles. Best concentration for the synthesis of silver nanoparticles was found to be 2.5 g/ml in 1:2 ratio for crude broth and 1:9 for modified broth observed in the range of 384 nm to 465 nm. Although *Plectranthus amboinicus* leaf extract produced silver nanoparticles, rate of synthesis was lower than the neem leaf extract. Therefore it is possible to use both types of neem leaf extract for the synthesis of silver nanoparticles. However it is recommended to synthesize silver nanoparticles by using modified neem leaf extract for future applications as it has been previously confirmed to have the efficient antibacterial activity.

ACKNOWLEDGEMENTS

The authors would like to offer their sincere thanks to Mrs. A.N.K. Perera and all the academic and non-academic staff members of the Department of Biotechnology and all the members of Nanotech Laboratory specially Mr. U.S. Liyanarachchi. Sincere thanks are also extended to Dr. N. A. Tennakoon, Head, Soil Division, Coconut Research Institute, Lunuwila for their assistance and support.

REFERENCES

Ankamwar, B., Chaudhary, M. and Sastry, M. (2005). Gold nanotriangles biologically synthesized using tamarind leaf extract and potential application in vapor sensing. *Synthesis and Reactivity in Inorganic Metal-organic and Nano-metal Chemistry*, **35** (1), 19–26.

Bali, R., Razak, N., Lumb, A. and Harris, A.T. (2006). The synthesis of metal nanoparticles inside live plants, IEEE *Xplore*, 4143372, 224-227.

Begum, N. A., Mondal, S., Basu, S., Laskar, R. A. and Mandal, D. (2009). Biogenic synthesis of Au and Ag nanoparticles using aqueous solutions of Black Tea Leaf Extracts. *Colloids and Surface B: Biointerfaces*, **71** (1), 113-118

Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A. and Sastry, M. (2006). Synthesis of gold nanotriangles and silver nanoparticles using aloe vera plant extract. *Biotechnology Progress*, **22** (2), 577–583.

Krishnaraj, C., Jagan, E.G., Rajasekhar, S., Selvakumar, P., Kalaichelvan, P.T. and Mohan, N. (2010). Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. *Colloids and Surface B: Biointerfaces*, **76**, 50-56

Nestor, A.R.V., Mendieta, V.S., Lopez, M.A. C., Espinosa, R.M.G., Lopez, M.A.C. and Alatorre, J.A.A. (2008). Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camellia sinensis*. *Materials Letters*, **62**, 17-18

Rajesh, W.R., Jaya, R.L., Niranjan, S.K., Vijay, D.M. and Sahebrao, B.K. (2009). Phytosynthesis of silver nanoparticle using *Gliricidia sepium*. *Current Nanoscience*, **5**, 117–122

Sastry, M., Mayyaa, K.S. and Bandyopadhyay, K. (1997). pH dependent changes in the optical properties of carboxylic acid derivatized silver colloid particles. *Colloids and Surface A*, **127**, 221–228.

Shankar, S.S., Rai, A., Ahmad, A. and Sastry, M. (2004). Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *Journal of Colloid and Interface Science*, **275**, 496–502

Song, J.Y. and Kim, B.S. (2009a). Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *The journal of Bioprocess and Biosystems Engineering*, **32**, 79 –84

Song, J.Y., Jang, H.K. and Kim, B.S. (2009b). Biological synthesis of gold nanoparticles using *Magnolia kobus* and *Diopyros kaki* leaf extracts. *Process Biochemistry*, **44**, 1133-1138

Torresday, J.L.G., Parsons, J.G., Gomez, E., Videa, J.P., Troiani, H.E., Santiago, P. and Yacaman, M.J. (2002). Formation and growth of Au nanoparticles inside live alfa alfa plants. *Nanoletters*, **2** (4), 397- 401

Tripathy, A., Raichur, A.M., Chandrasekaran, N., Prathna, T.C. and Mukherjee, A. (2009). Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of *Azadirachta indica* (Neem) leaves. *Journal of Biomedical and Nanotechnology*, **5**, 93-98.

Warusawithana, Y. and Vivehananthan, K. (2010). Upgrading the wastewater quality using neem (*Azadirachta indica*) plant parts. In Proceedings of 10th Agricultural Research Symposium, 12-13 August, 2010. Wayamba University of Sri Lanka. 40-44.