Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 08, pp.72594-72598, August, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

MICROWAVE-ASSISTED SYNTHESIS OF GOLD NANOPARTICLES USING NEEM LEAVES (AZADIRACHTA INDICA)

*[1]Abha Anand and [2]Murthy Chavali

[1]Department of Biotechnology, Jawaharlal Nehru Technological University Hyderabad (JNTUH) 500 085 Telangana State, India
[2]MCETRC, Chinnaravuru, Tenali, Guntur 522 201 AP, India

ARTICLE INFO

Article History:
Received 25th May, 2018
Received in revised form
10th June, 2018
Accepted 07th July, 2018
Published online 30th August, 2018

Key Words:
Gold Nanoparticles (GNPs), Phytoextraction, Azadirachta indica

ABSTRACT

Ecological generation and isolation of heavy metals such as Ag, Au, Cu, etc by Phytoextraction one of the best methods in biotechnology and nanotechnology field for various applications for human use. Since Gold (Au) is one of the expensive heavy metal, so Gold Nanoparticles (GNPs) or compounds are also available in market at a very high price. Therefore the isolation of GNPs by Phytoextraction methods cost comparative very low. GNPs are easily prepared using neem (Azadirachta indica) leaves by phytoextraction procedure using $HAucl_4$. This research work provides a more possible option for generation of GNPs in lesser time, very low cost and non-toxic procedure without using large and complex apparatus. This paper deals with the generation of eco-friendly and cost effective gold nanoparticles by using leaf extract of neem (Azadirachta indica) as reducing agent in short period of time by microwave assistance showing improvement particularly for reduction of time, modulation of size and shape.

Citation: ***Abha Anand and Murthy Chavali.*** **2018.** "Microwave-Assisted Synthesis of Gold Nanoparticles using Neem Leaves (Azadirachta Indica)", *International Journal of Current Research*, 10, (08), 72594-72598.

INTRODUCTION

Phytoextraction is a secondary procedure of phytoremediation in which plants remove wanted and unwanted elements such as metal, heavy metal, compound and other contaminants from soil or water. Phytoremediation is an environmentally forthcoming, protected and inexpensive technique used to get rid of pollutants from an environment (Zhang *et al*., 2010). Generally, heavy metals, metals which are extracted have a high density, may be toxic to organisms even at relatively low concentrations and are toxic to the plants as well. This is one of the effective methods in the field of biotechnology and nanotechnology for ecological generation of heavy metals nanoparticles such as gold, Hg, Cu, etc for various applications for human use at very low cost. It is a cost effective, long-term, environmentally and aesthetically friendly method of immobilizing/stabilizing and transferring contaminants such as metals, pesticides and chlorinated hydrocarbon without causing any disturbance to the land (Zhang *et al*., 2010; Tai *et al*., 2011). Au NPs have been synthesized using various fruit and plantextracts (Tai *et al*., 2011).

Corresponding author: **Abha Anand ,**
Department of Biotechnology, Jawaharlal Nehru Technological University Hyderabad (JNTUH) 500 085 Telangana State, India.
DOI:https://doi.org/10.24941/ijcr.31947.08.2018

Azadirachta indica is commonly known as Neem belonging to family Meliaceae. It is typically grown in tropical, semi-tropical regions and in islands located in the southern part of Iran. Neem oilis obtained from its fruits and kernel of seeds (Paul, 2011). Neem is one of a very few shade-giving trees that thrive in drought-prone areas. The trees are not at all insubstantial about water quality and thrive on the merest trickle of water, whatever the quality (Nahak, 2011). Neem products are believed as a major component by Siddha and Ayurvedic (Lekshmi *et al*., 2012) practitioners to be Anthelmintic, antifungal (Anjali *et al*., 2013; Shrivastava, 2014), antidiabetic (Patil *et al*., 2013; Dholi *et al*., 2011), antibacterial (Yerima *et al*., 2012), antiviral (Arumugam *et al*., 2014), contraceptive, and sedative (Baligar *et al*., 2014). Neem oil is also used for healthy hair, to improve liver function, detoxify the blood (Nahak, 2011), and balance blood sugar levels (Nahak, 2011; Patil *et al*., 2013; Dholi *et al*., 2011). The biopesticide produced by extraction from the tree seeds contains Limonoids. Plant chemical ingredients concentration is limited by the soil concentration and by suitable ligands. This is a natural process in which environmentally occurring chemicals will cause plants to accumulate Au. This is also a known process biogeochemical exploration (Kiranmai *et al*., 2011).

Gold nanoparticles (GNPs) are used for various application viz, gold-colloid paints, electronics, as an industrial catalyst and biomedical purposes. Development of GNPs by this phytoextraction method is more cost-effective gold recovery system based on nanotechnology (Tai *et al*., 2011). Recently many researchers are providing much more prominence on biological or plant-mediated synthesis of nanoparticles as it is simple, eco-friendly and also low cost. Despite the fact that it has previously been reported the plant-mediated synthesis or phytoextraction of gold nanoparticles such as Terminalia catappa (Ankamwar, 2008), tea (Satish *et al*., 2009), Cymbopogoncitrates, lemongrass tree, (Shiv Shankar *et al*., 2004) Alliumcepa (Parida *et al*., 2011) and fruit extracts (Tai *et al*., 2011) but still upcoming researches are there.

Research on on neem (Azadirachta indica) (Birendra Kumar Bindhani, 2014) shows synthesis is time consuming almost 3-4 days for isolation of gold nanoparticles, therefore this research work shows improvement particularly for reduction of time for synthesis of nanoparticles by microwave assistance, modulation of size and shape. This experiment is a very appealing study to know the nature of nanoparticles which is produced by using leaves extracts. The selection of neem (Azadirachta indica) plant extracts for this study because of abundant availability and its numerous biomedical applications and so on (Birendra Kumar Bindhani, 2014). The main active ingredients are chemically similar and biogenetically derivable from a tetracyclicterpenes. These are also called limonoids (azadirachtin, meliantrol, salanin etc.) Neem limonoids belong to nine basic structures: azadiron (from seed oil), amoorastatin (from fresh leaves), gedunins (from kernels), salannin (from fresh leaves and seeds), and azadirachtin (from seed oil)(Paul, 2011). The activated neem leaves consist of mainly three different kinds of phenolic compounds such as 4-chlorophenol, 4-nitrophenol and phenol (P) was studied from fixed bed mode (Birendra Kumar Bindhani, 2014). 4-nitrophenol is a white to light yellow crystalline solid. Contact may severely irritate the skin and eyes. Poisonous by ingestion and moderately toxic by skin contact (Manohar *et al*., 2016).

Objective: The objective of the proposed research work is to develop a simple, green method for the isolation and separation of Gold (Au) nanoparticles (NPs) from the leaves extract of neem (Azadirachta indica). Unlike other biological methods for nanoparticles synthesis, the uniqueness of this method lies in its fast synthesis rates (~3 min for AuNPs)once the extract is ready through phytoextraction of neem and the capability to tune the nanoparticle size (and subsequently their catalytic activity(Manohar *et al*., 2016)) via the extract concentration used in the experiment. The phenolic ions (p-nitrophenol) present in the extract are largely responsible for the rapid reduction rates of Au^{3+} ions to AuNPs. Efficient reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) in the presence of AuNPs was observed and found to depend upon the nanoparticle size or the leaves extract concentration used for isolation of gold nanoparticles. This work aimed to the synthesis of the gold nanoparticle using neem leaves (Azadirachta indica) within an anticipated time period i.e. within a day and finally, gold nanoparticles where characterized through Uv-Visible and SEM techniques.

MATERIALS AND METHODS

Plant material: The fresh leaves of Neem (Azadirachta indica) were collected in the month of September from Balanagar, Hyderabad. Leaves were rinsed and repeatedly washed with water properly to remove dust particles followed by air dried at room temperature.

Chemical and Reagents: All chemicals used were analytical reagent grade and the solutions were prepared with Millipore purified distilled water. $HAuCl_4.XH_2O$ (Tetrachloroauric acid), ethanol (96%) was obtained from Sigma Aldrichwas used tocarry out experimental activities. All glassware such as a conical flask, measuring cylinder, test tubes burette, beaker, Petri dishes were thoroughly washed and rinsed with purified water followed by drying properly for the experiment. The experiments are carried out by using re-usable flat bottom glass cuvettes for absorbance mode.

Preparation of Ethanolic Extract: Neem (Azadirachta indica) leaves (150 g) were separated, roughly chopped into pieces and again dried up by keeping in drying ovenat temperature 60-70°C. Dried Leaves were blended for extraction by soxhlet extraction method.96% ethanol was used as a solvent for extraction. The analytical balance was used for weighing neem leaves and heater Mantle was used for heating the solution during soxhlet extraction time. Soxhlet apparatus was used for the extraction process were maintained with temperature 70-90^{O}C till the extract is colour less. Filter papers were used for filtration of extract followed evaporation to the concentrated filtrate. 100 ml conical flasks and 100 ml volumetric flasks, beakers and pipette were used for measuring and solution preparation.

Phytoextraction of Gold Nanoparticles: Washed neem leaves (20g) were chopped finely for preparation of the stock which was used for reduction reaction (from Au^{3+} ions to Au^0). Chopped neem leaves were kept in a 500 ml conical flask with 60 ml of Millipore distilled water. The solution was boiled for 20min. Based on literature review and suggestion of expert in department of Pharmacognosy, this research was planned as 100ml of 10^{-3} M aqueous chloroauric acid (HAuCl4) was mixed with 0.3 ml of stock solution and kept inside domestic microwave (LG iwave working at 100% power of 600 watt (W))for irradiation for 90 Sec. as explained in fig.1.The colour of the solution was changed to a red colour within a few minutes(2-3 minutes). The reaction times were determined by the change in colour of the solution. The reaction time 2-3 min was adequate for the complete conversion of colloidal GoldNanoparticles. This stabilization of nitrophenol is not possible in the case of *m*-nitrophenol because there is no chance via resonance for delocalization onto the nitro group. Resonance is due to delocalization of negative charge at Ortho and Para position only whereas there is no delocalization at Meta position

RESULTS AND DISCUSSION

UV-Vis Spectrometric Method: Labindia UV3000 Spectrophotometer was used to take absorption of aqueous solution in the UV-visible spectral region. This instrument has holographic grating greatly reducing stray light of the instrument and making the analysis more accurate. The absorption during the observable range directly affects the perceived colour of the concerned chemicals. In this region of the electromagnetic spectrum, molecules undergo electronic transitions (Birendra Kumar Bindhani, 2014). Here in the experiment, 5 different concentrations of $HAuCl_4$ with neem extract and blank as shown in Fig. 2

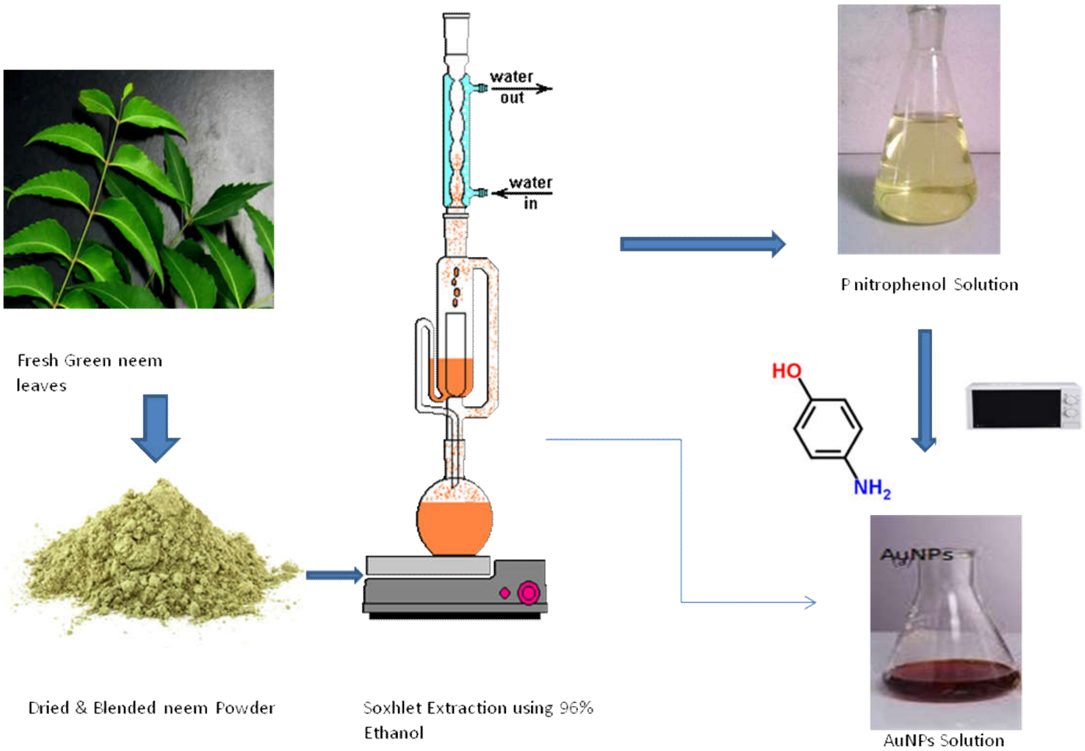

Fig. 1. Schematic diagram of Phytoextraction of Gold nanoparticles using $HAuCl_4$;Soxhlet extraction procedure was assembled using 96% ethanol for obtaining a colourless solution of p nitrophenol. Conversion of colour from pale yellow to red with the addition of HAuCl4 is due to delocalization of negative charge of nitrophenol

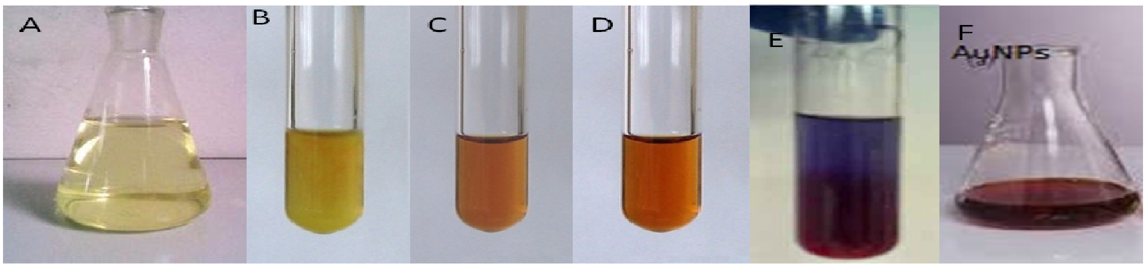

Fig.2. A Neem (Azadirachta indica) leaves extract, B-Gold ($HAuCl_4$) Aqueous Solution[Au], C- Au+1ml extract, D- Au+3ml extract, E-Au+5ml extract,F-Au+10ml extract of neem

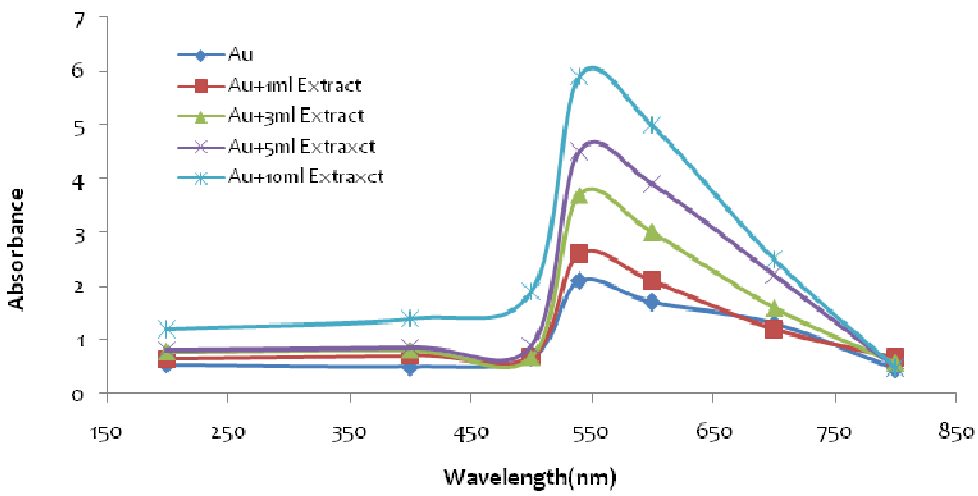

Fig.3. UV-Vis spectra of gold nanoparticles aqueous solution synthesized by adding a different volume of Neem (Azadirachta indica) extract to a fixed volume (10 ml) of HAuCl4 solution (10−3 M)

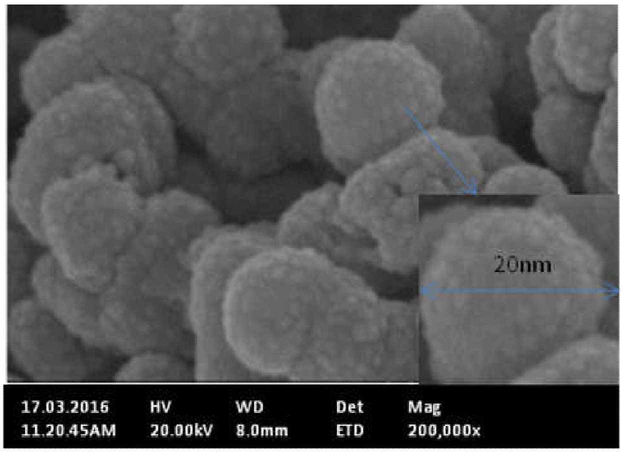

Fig.4. SEM images of the gold nanoparticles colloids (Spherical in shape & Size 10-20nm)

Absorbance's were observed at a different wavelength as shown in fig.3. The UV-Vis near infrared absorption spectra was used in aqueous suspension for the synthesis of gold nanoparticles and optical properties were exhibited closely associated with their shapes of such aqueous suspension metal nanoparticles (Birendra Kumar Bindhani, 2014). The peak (transverse plasmon resonance band) was observed at 540 nm shifted towards a higher wavelength, confirming a redshift along with amplified absorbance intensity. The UV-vis spectrum (Figure 3A) also showed a very broad b and towards the longer wavelength (IR) region.

The transform of colour was observed after the completion of each reaction of 5 samples under microwave irradiation is shown in Fig. 3A-F. The surface plasmon resonance (SPR) bands were observed using UV–Vis spectroscopy for the isolated colloidal suspensions of AuNPs clearly shows the gradual decrease in the intensity of absorption of SPR band. As the moving from the SPR band corresponding to a sample of Au NPs, the intensity of absorption decreases proportionally to wavelength revealing the decrease in particles size of the sample(Manohar, 2016). The reaction was observed as the conversion nitrophenol to p-aminophenol may be attributed due to disturbance of morphology of nanoparticles and the agglomeration of nanoparticles. The gradual reduction of p-nitrophenol to p-aminophenol was monitored at regular time intervals by taking 0.5 mL of the reaction mixture and diluting it up to 50 mL with distilled water and recording its UV–Visible spectrum at ambient condition.

SEM (Scanning Electron Microscopy) Measurements: Scanning electron microscopy measurement was carried out by using Hitachi S - 4700 SEM machine. Samples were prepared on a carbon-coated copper grid as a thin and small oval-shaped film. Removal of the extra solution was done using an absorbent paper before keeping the sample under a mercury lamp for 3-5 min for drying. The colloidal gold nanoparticles obtained from the reaction of $HAuCl_4$ with extract of neem (*Azadirachta indica)* followed by microwave irradiation are primarily observed morphologically spherical in shape with size 10-20nm as shown in fig.4 below.

Conclusion

In summary, this experimental work shows a simplistic, fast and microwave-assisted process for the synthesis of gold nanoparticles from neem leaves (*Azadirachta indica*). The colloidal AuNPs were obtained within 3 minutes under microwave irradiation and the particle sizes were observed in the range of 10–20 nm. Furthermore, the reaction is a reduction of p-nitrophenol to p-aminophenol and finally resultant AuNPs using $HAuCl_4$. It can be concluded that the Au NPs synthesized from the $HAuCl_4$show that microwave assisted synthesis is a faster reduction of p-nitro phenol to p-aminophenol than the other activities, with proper modulation of size and shape of nanoparticles.

Acknowledgement

The author is grateful to Saleemulla khan, Principal & Professor, P.A. College of Pharmacy, Mangalore, for providing assistance to my research work as a Pharmacognosy expert. The author is also thanks to Haneef Lucky, Sura Lab, Hyderabad for relevant assistance for analytical tests carried out in research work.

REFERENCES

Anjali K., Ritesh K., Sudarshan M., Jaipal S. C., Kumar S. 2013. Antifungal efficacy of aqueous extracts of neem cake, karanj cake and vermicompost against some phytopathogenic fungi. *The Bioscan*, 8:671–674.

Ankamwar, B. 2010. Biosynthesis of Gold Nanoparticles (Green-Gold) Using Leaf Extract of Terminalia Catappa. *E-Journal of Chemistry*. 7:1334 - 1339.

Arumugam A., Agullo P., Boopalan T. 2014. Neem leaf extract inhibits mammary carcinogenesis by altering cell proliferation, apoptosis, and angiogenesis. *Cancer Biology and Therapy*, 15(1):26–34.

Baligar N. S., Aladakatti R. H., Ahmed M., Hiremath M. B. 2014. Evaluation of acute toxicity of neem active constituent, nimbolide and its hepatoprotective activity against an acute dose of carbon tetrachloride treated albino rats. *International Journal of Pharmaceutical Sciences and Research*, 5(8):3455–3466.

Birendra Kumar Bindhani, Ashok Kumar Panigrahi, 2014. Green Synthesis of Gold Nanoparticles Using Neem (Azadirachta indica L.) Leaf Extract and Its Biomedical Applications. *International Journal of Advanced Biotechnology and Research*, 3:457-464.

Dholi S. K., Raparla R., Mankala S. K., Nagappan K. 2011. Invivo antidiabetic evaluation of Neem leaf extract in alloxan-induced rats. *Journal of Applied Pharmaceutical Science*, 1(4):100–105.

Huang X, Wu H, Liao X, Shia Bi 2010. One-step, size-controlled synthesis of gold nanoparticles at room temperature using plant tannin. *Green Chemistry* 12:395-399

Kiranmai M., Kumar M., Ibrahim M. 2011. Free radical scavenging activity of neem tree (Azadirachta indica A. Juss Var., Meliaceae) root barks extract. *Asian Journal of Pharmaceutical and Clinical Research,* 4:134–136.

Lekshmi N. C. P., Sowmia N., Viveka S., Brindha Jr., Jeeva S. 2012. The inhibiting effect of Azadirachta indica against dental pathogens. *Asian Journal of Plant Science and Research*, 2(1):6–10.

Manohar A. Bhosale, Shyam Sunder R. Gupta, Bhalchandra M. Bhanage 2016. Size-controlledthe synthesis of gold nanostructures using ketones and their catalytic activity towards the reduction of p-nitrophenol. Polyhedron 120: 96–100.

Nahak G., Sahu R. K. 201. Evaluation of antioxidant activity of flower and seed oil of Azadirachta indica A. juss. *Journal of Applied and Natural Science*, 3(1):78–81.

Parida UK., Bindhani BK., Nayak PL. 2011 Green Synthesis and Characterization of Gold Nanoparticles Using Onion (Allium cepa) Extract. *World Journal of Nano Science and Engineering*, 1:93 - 98.

Patil P. R., Patil S. P., Mane A., Verma S. 2013. Antidiabetic activity of alcoholic extract of Neem (Azadirachta indica) root bark. *National Journal of Physiology, Pharmacy and Pharmacology*, 3(2) 134-138

Paul R., Prasad M., Sah N. K. 2011. Anticancer biology of Azadirachta indica L (neem): a mini review. *Cancer Biology and Therapy*,12(6):467–476.

Premkumar T, Geckeler 2010. Cucurbit(7)uril as a tool in the green synthesis of gold nanoparticles. Chemistry,An Asian Journal 5:2468-2474

Satish KN, Nripen C, Ravi S, Kavita K, Rajesh RK, Subramanian T, Swapna M, Raghuraman K, KatteshVK. 2009. Green nanotechnology from tea: phytochemicals in tea as building blocks for the production of biocompatible gold nanoparticles. *Journal of Materials Chemistry*, 19:2912 - 2920.

Shiv Shankar S, Rai A, Ankamwar B, Singh A, Ahmad A, Sastry M. 2004. Biological synthesis of triangular gold nanoprisms. *Nature Materials*, 3:482 – 488.

Shrivastava D. K., Swarnkar K. 2014. Antifungal activity of leaf extract of neem (Azadirachta indica Linn) *International Journal of Current Microbiology and Applied Sciences*, 3(5):305–308.

Tai, Y., Tran, N.T.T., Tsai, Y., Fang, J., Chang. L. 2011. One-step synthesis of highly biocompatible multi-shaped gold nanostructures with fruit extract. IET *Nanobiotechnology*. 5: 52-59.

Yerima M. B., Jodi S. M., Oyinbo K., Maishanu H. M., Farouq A. A., Junaidu A. U. 2012. Effect of neem extracts (Azadirachta indica) on bacteria isolated from the adult mouth. *Journal of Basic and Applied Sciences*, 20:64–67.

Zhang, B. Y., Zhen, J. S. and Sharp, R. G. 2010 Phytoremediation inengineered wetlands: Mechanism and Applications. Procedia Environmental Science, 2: 1315-1325.

****