Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 4, Suppl 2, 2011 ISSN - 0974-2441

Research Article

BIOLOGICAL SYNTHESIS OF SILVER NANOPARTICLES BY USING STEM OF SHOREA TUMBUGGAIA ROXB. AND ITS ANTIMICROBIAL EFFICACY

S. ANKANNA* AND N. SAVITHRAMMA

Department of Botany, Sri Venkateswara Unviersity, Tirupati – 517 502, A.P. India, Email: ankanna2010@gmail.com

Received: 29 August 2011, Revised and Accepted: 19 October 2011

ABSTRACT

Metal nanoparticles have been using as an ingredients in the preparation of complementary medicines to cure different diseases is an age old medicinal practices. The plant based Ayurvedic preparations are preferred by 80% of the world population and WHO is encouraging the green medicine due to its less side effects. Hence an attempt has been made to screen this important medicinal plant for secondary metabolites; and biogenesis of silver nanoparticles (SNPs) and test their antimicrobial efficacy. The stem of *Shorea tumbuggaia* was selected for the study as it has been using in traditional medicines. The results indicate that the stem of *Shorea tumbuggaia* is source for different secondary metabolites like anthroquinones, emodins, flavonoids, lignins, leucoanthocyanins, phenols, reducing sugars, tannins and triterpenoids. The SNPs formation from stem extract was confirmed with the help of UV-VIS spectroscopy and characterized by AFM. The size of the SNPs was measured 50 to 60 nm with various shapes. The SNPs are having high toxic effect towards microorganisms than that of plant extract. Moreover the SNPs of *Shorea tumbuggaia* stem having high toxicity towards bacterial species than that of fungal species. The secondary metabolites of *Shorea tumbuggaia* will be helpful to the phytochemists and pharmacologists for identification of new active principles and the environmentally benign SNPs can be used effectively against drug resistant bacterial and fungal strains.

Key words: medicinal plants; silver nanoparticles; atomic force microscope (AFM); inhibition zone; secondary metabolites; anti microbial efficacy.

INTRODUCTION

Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents present in the plant play a significant role in the identification of crude drugs. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenols, saponins, steroids, tannins, terpenoids etc.,[1]. Previously the crude drugs were identified by comparison only with the standard descriptions available, but recently due to advancement in the field of Pharmacognosy various techniques have been following for the standardization of crude drugs[2].

The silver nanoparticles (SNPs) have various important applications, historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items[3]. It has been reported that SNPs are non-toxic to human and most effective against bacteria, virus and other eukaryotic microorganisms at low concentrations[4]. Several salts of silver and their derivatives are commercially manufactured as antimicrobial agents[5]. Antimicrobial capability of SNPs allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices[6]. The most important application of SNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds[7]. Biological synthesis of nanoparticles through plant extracts is under exploitation as they are using extensively in medical field as drug delivery agents.

For the last two decades extensive work has been done to develop new drugs from natural products because of the resistance of microorganisms to the existing drugs. The pathogens like *Klebsiella pneumoneae*, *E. coli* and *Bacillus* species developed resistance to number of antibiotics[8]. Empirical therapy is needed to overcome this problem. *S. tumbuggaia* is a tree taxon with economic and medicinal values. Plant parts are used as an external stimulant and plant extracts used to cure ear-aches[9]. The stem is a source of resin, which is used to cure duodenal ulcers and amoebic dysentery. It is also used in indigenous medicine as an external stimulant and a substitute for arbutus[10]. The present work is aimed for qualitative analysis of phytochemical constituents by using stem of *Shorea tumbuggaia*; and biological synthesis of SNPs and screening of SNPs for microbial efficacy.

MATERIAL AND METHODS

Plant Materials

The fully matured healthy stem of *Shorea tumbuggaia* was collected from Tirumala hills, Chittoor District of Andhra Pradesh, India during December 2010. The material was washed thoroughly and shade dried.

Extraction of Plant Material for Phytochemical screening

10 g of air dried powder was used to prepare plant aqueous extract[11] and preliminary phytochemical screening was carried out for flavonoids[12]; steroids, alkaloids and phenols[13]; triterpenoids and glycosides[14]; tannins, anthroquinons, leucoanthocyanins and emodins[15]; saponins[16]; and reducing sugars and anthocyanins[17].

Synthesis of silver nanoparticles

SNPs were synthesized by using stem extract of *Shorea tumbuggaia*[18]. The reduction of pure Ag^{2+} ions were monitored by measuring the UV-Vis spectrum of the reduction media at 5th h after diluting a small aliquot of the sample in distilled water by using Systronic 118 UV-Vis Spectrophotometer. The size and shape of SNPs were confirmed with AFM (Nanosurf ® AG, Switzerland; Product: BTO2089, Vl. 3RO).

Microorganisms

Pure cultures of *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Proteus vulgaris* and *Klebsiella pneumoneae* species of bacteria and *Fusarium oxysporum, Curvularia lunata, Rhizopus arrhizus, Aspergillus niger* and *Aspergillus flavus* species of fungi were procured from the Department of Microbiology of Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, Andhra Pradesh, India.

Antimicrobial activity

The antimicrobial activities of SNPs were carried out with paper disc diffusion method using nutrient agar medium and potato dextrose agar medium for bacterial and fungal cultures respectively. Zones of inhibition for control, SNPs and silver nitrate were measured after 24 h and 7 days for bacterial and fungal growth. The experiments were repeated thrice and mean values of inhibition zone diameter were presented.

RESULTS AND DISCUSSION

The phytochemical investigation of *Shorea tumbuggaia* showed that the stem is rich in anthroquinons, tannins, terpenoids, leucoanthocyanins, emodins, flavonoids, phenols, reducing sugars and lignins and lacking anthocyanins, saponins, glycosides, alkaloids, coumarins, fatty acids and steroids (Table 1). The secondary metabolites like anthroquinones, flavonoids, emodins and phenolic compounds are medicinally used as antistomatic, diarrhoea, anti-inflammatory, anti cancer and antioxidative[19-22].

Table 1: Secondary metabolites of aqueous extract from stem part of *Shorea tumbuggaia*

S. No.	Secondary metabolites	Stem
1.	Alkaloids	-
2.	Anthocyanins	-
3.	Anthroquinones	+
4.	Coumarins	-
5.	Emodins	+
6.	Fatty acids	-
7.	Flavonoids	+
8.	Glycosides	-
9.	Lignins	+
10.	Lueco anthocyanins	+
11.	Phenols	+ +
12.	Reducing sugars	+
13.	Saponins	-
14.	Steroids	-
15.	Tannins	+
16.	Triterpenoids	+

Note: '+' indicates presence, '++' indicates presence of more amount '-' indicates absence

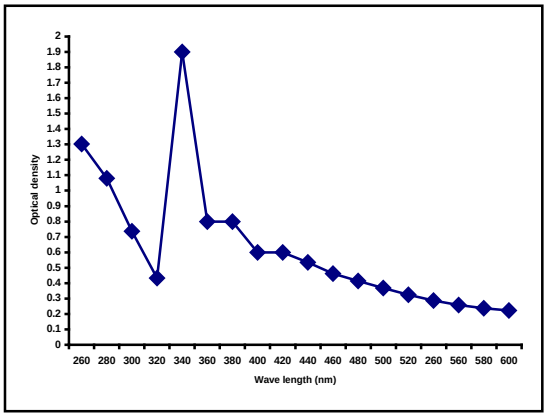
The compounds lignin, leucoanthocyanins, tannins and triterpenoids present in the stem of *Shorea tumbuggaia* showed the properties of persistence to microbial degradation, improve the beer quality and; anti microbial and anti viral activity[23].

In the present study SNPs were synthesized by using stem extract of *S. tumbuggaia* rapidly within 15 min of incubation period and yellowish brown colour was developed by addition of Ag $(NO_3)_2$ to the plant extract (Fig.1a) similar results was observed in *Svensonia*[24]. The appearances of yellowish-brown colour in the reaction vessels suggest the formation of SNPs[25]. The time duration of change in colour and thickness of the colour varies from plant to plant. The reason could be that the quantitative variation in the formation of SNPs (or) availability of H^+ ions to reduce the silver. It is well known that SNPs exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles[26]. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The synthesis of SNPs had been confirmed by measuring the UV-Vis spectrum of the reaction media. The UV-Vis spectrum of colloidal solutions of SNPs synthesized from stem of *S. tumbuggaia* have the characteristic absorbance peaks at 320 nm and 420 nm (Fig. 1b); and the broadening of peak indicated that the particles are poly-dispersed. The weak absorption peak at shorter wavelengths due to the presence of several organic compounds which are known to interact with silver ions same results observed in *Boswellia ovalifoliolata*[27]. AFM analysis the SNPs were clearly distinguishable owing to their size difference. The synthesized SNPs size is ranging from 50 to 60 nm. SNPs attached with one another and looks like a cluster in an area of 15 μm with rod shape in 3D view (Fig.1c-f). The physico chemical properties of nanoparticles differ dramatically from fine particles of the same composition [28, 29]. Nanoparticle usually forms the core of nano-biomaterial. It can be used as a convenient surface for molecular assembly, and may be composed of inorganic or polymeric materials. It can also be in the form of nano-vesicle surrounded by a membrane or a layer[30]. Nanoparticles can offer significant advantages over the traditional delivery mechanisms in terms of high stability, high specificity, high drug carrying capacity, ability for controlled releases, possibility to use in different types of drug administration and the capability to transport both hydrophilic and hydrophobic molecules[31].

Figure 1:

(1) **(2)**

(a)



(b)

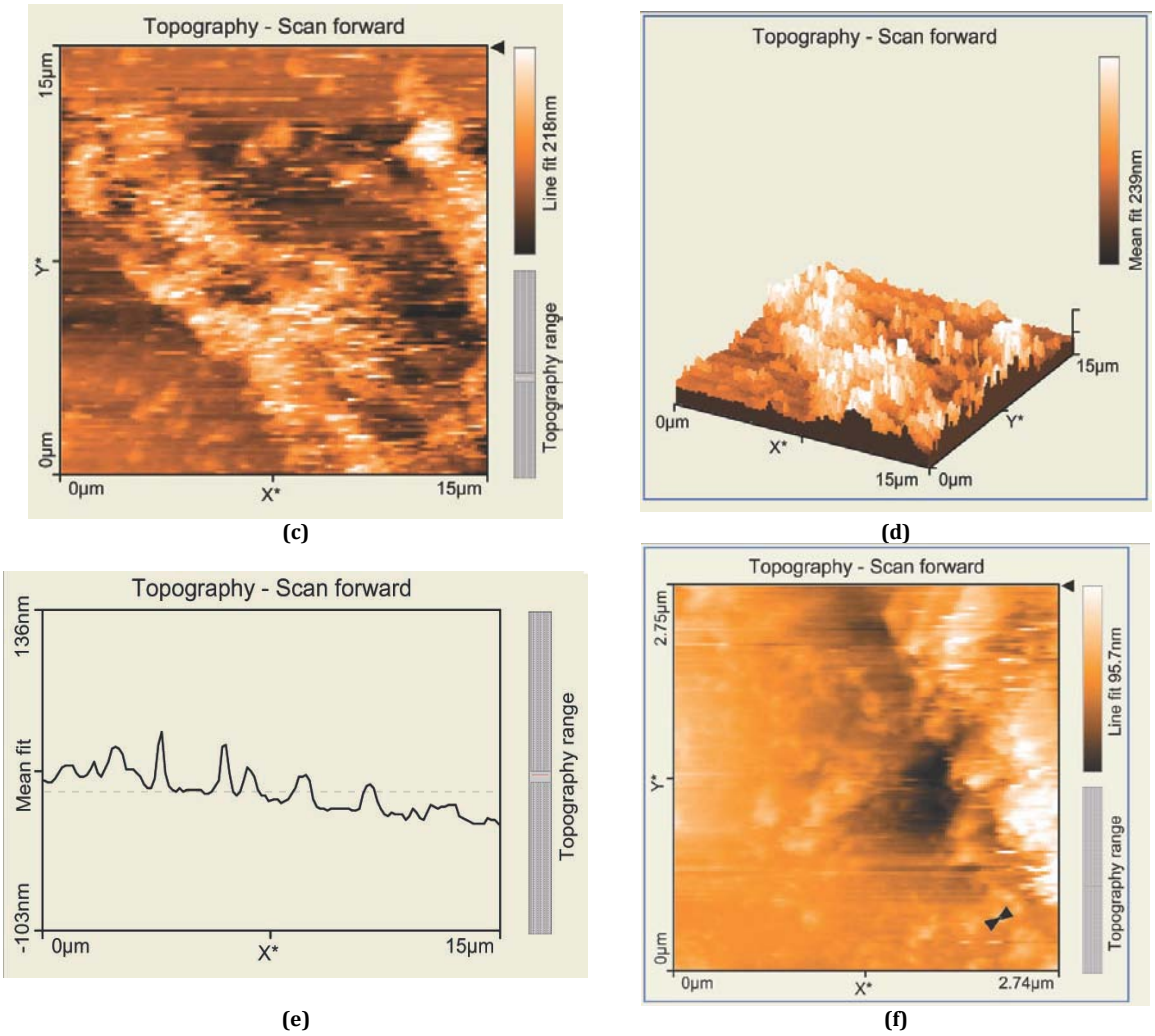


Fig 1: (a) The colour change of stem extracts of *Shorea tumbuggaia* (1) blank extract without silver nitrate (2) stem extract with 1 mM silver nitrate; (b) UV-VIS spectroscopy of synthesized silver nanoparticles; (c) Image, (d) Three dimensional structure, (e) Graph and (f) Size of SNPs.

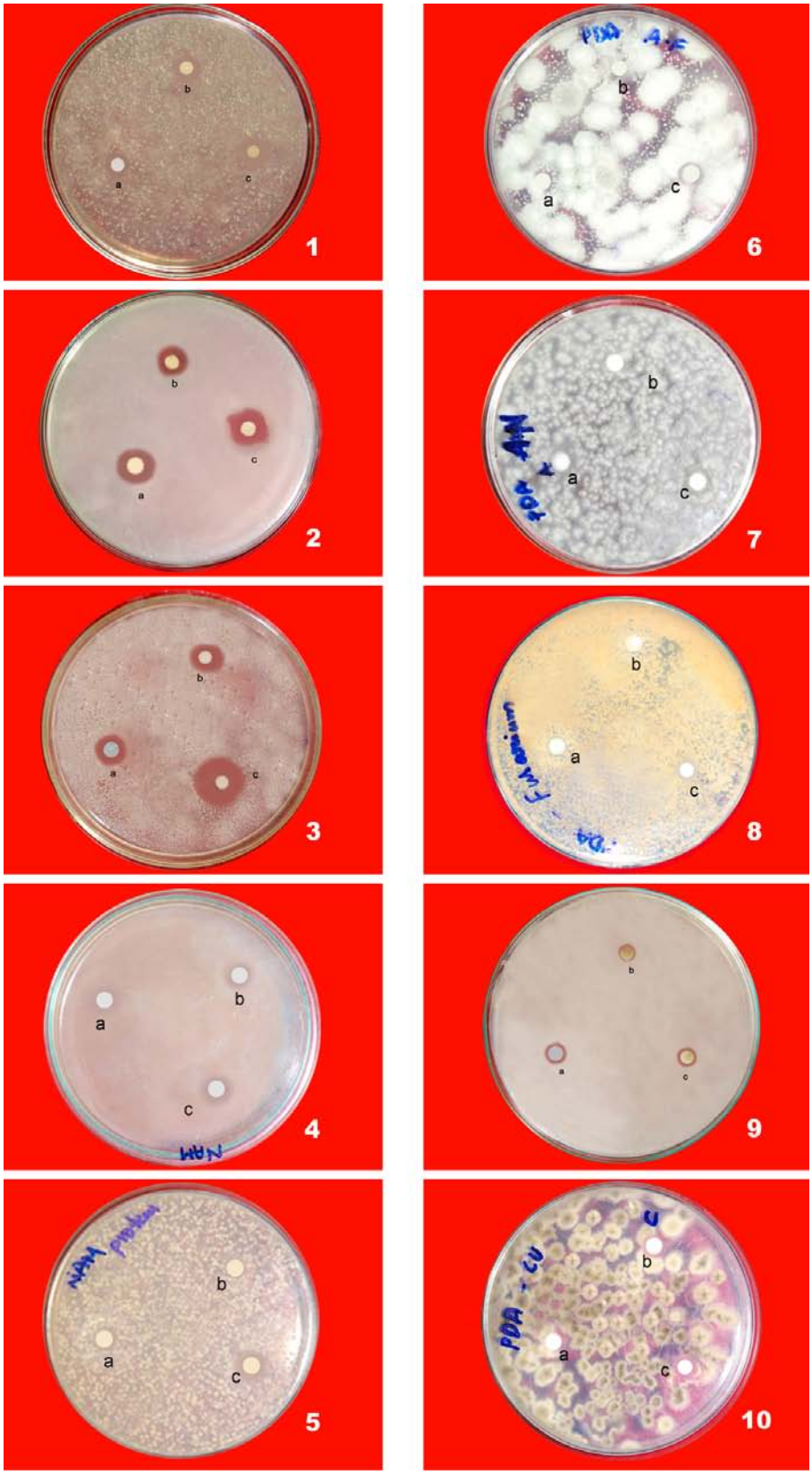
The stem of *S. tumbuggaia* SNPs showed highest percentage of bacterial inhibition to *E. coli* followed by *Bacillus, Pseudomonas, Klebsiella* and *Proteus* and antifungal activity against *A. flavus* and *Fusarium* followed by *A. niger*, *Rhizopus* and *Curvularia* (Table.2, Fig.2). The maximum toxicity was observed in SNPs treated cells than $AgNO_3$ and plant extract. The reason could be that the smaller size of the particles which leads to increased membrane permeability and cell destruction. Similar results were found in *Boswellia ovalifoliolata*[24]. The antimicrobial effect of green synthesized SNPs is attributed that the micro-organisms having of peptidoglycan, which is a complex structure and often contains teichoic acids or lipoteichoic acids and these are having strong negative charge. This charge may contribute to the sequestration of free silver ions. The findings of Sereemaspun et al.[32] suggested that the inhibition of oxidation based biological process by penetration of metallic nano sized particles across the microsomal membrane. SNPs have an ability to interfere with metabolic pathways and bacterial growth signaling pathway by modulating tyrosine phosphorylation of putative peptide substrates critical for cell viability and division[33]. The SNPs synthesized from stem of *Shorea tumbuggaia* are toxic to multi-drug resistant microorganisms. It shows that they have great potential in biomedical applications.

Table 2: Antimicrobial activity of SNPs isolated from stem part of *Shorea tumbuggaia*

S. No.	Pathogens	Inhibition Zone (mm)		
		$AgNO_3$	Stem extract	SNPS
1.	*E. coli*	10	7	15
2.	*Pseudomonas*	12	8	14
3.	*Klebsiella*	13	10	15
4.	*Bacillus*	11	8	14
5.	*Proteus*	11	10	13
6.	*A. flavus*	10	6	12
7.	*A. niger*	8	6	10
8.	*Fusarium*	10	7	12
9.	*Rhizopus*	6	-	8
10.	*Curvularia*	8	-	8

Note: '-' indicates no inhibition

Figure 2:



Antimicrobial activity of *Shorea* Stem 1. *E. coli*, 2. *Pseudomonas*, 3. *Klebsiella*, 4. *Bacillus*, 5. *Proteus*,6. *A. flavus*, 7. *A. niger*, 8. *Fusarium*, 9. *Rhizopus*, 10. *Curvularia*

Note: 'a' indicates $AgNO_3$, 'b' indicates stem extracts and 'c' indicates SNPs

CONCLUSION

The present study included the bio-reduction of silver ions through medicinal plants extracts and testing for their antimicrobial activity. The aqueous silver ions exposed to the extracts, the synthesis of SNPs were confirmed by the change of colour of plant extracts. These environmentally benign SNPs were further confirmed by using UV-Vis spectroscopy finally the size and shape of the SNPs was characterized by AFM analysis. The results indicated that SNPs have good antimicrobial activity against different microorganisms. It is confirmed that SNPs of *S. tumbuggaia* are capable of rendering antimicrobial efficacy and hence has a great potential in the preparation of drugs used against bacterial and fungal diseases. The phytochemical screening indicates that the plant part is a good source for bio active principle for pharmaceutical industry.

ACKNOWLEDGEMENTS

Authors are highly thankful to University Grants Commission for financial support; and to VIT University, Tamil Nadu for AFM studies; and Prof. P.Suvarnalatha Devi, Mahila University, Tirupati for antimicrobial experiments.

REFERENCES

1. Akindele AJ, Adeyemi OO. Antiinflammatory activity of the aqueous leaf extracts of *Byrsocarpus coccineus*. Fitoterapia 2007; 78: 25-28.
2. Savithramma N, Venkateswarlu P, Suhrulatha D, Basha SKM, Venkataramanadevi CH. Studies of *Boswellia ovalifoliolata* Bal. and Herny – An endemic and endangered medicinal plant. The Bioscan 2010; 5: 359-362.
3. Chikramane PS, Suresh AK, Bel-lare, JR, Kane SG. Extreme homeopathic dilutions retain starting materials: a nanoparticulate perspective. Homeopathy, 2010; 99: 231-242.
4. Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: Green synthesis and antimicrobial activities. Adv Coll Int Sci 2009; 145: 83-96.
5. Krutyakov YA, Kudrynskiy A, Olenin AY, Lisichkin GV. Extra cellular biosynthesis and antimicrobial activity of silver nanoparticles. Russ Chem Rev 2008; 77: 233.
6. Marambio-Jones C Hoek EMV. A review of the antibacterial effects of silver nano materials and potential implications for human health and the environment. J Nanopart Res 2010; 12: 1531-1551.
7. Ip M, Lui SL, Poon VKM, Lung I, Burd A. Antimicrobial activities of silver dressings: An in vitro comparison. J Med Microbial 2006; 55: 59-63.
8. Patil A, Krishna BVS, Chandrasekhar MR. Increasing burden of hospital acquired infections: resistance to cephalosporin antibiotic among *Klebsiella* and *Escherichia coli*. J Ind Med Assoc 2011; 109:158-160.
9. Savithramma N, Sulochana CH. Endemic medicinal plants from Tirumala hills of Andhra Pradesh. Fitoterapia 1998; 3: 253-269.
10. Savithramma N. *Shorea tumbuggaia* Roxb. An endemic endangered and medicinal tree taxon under threat. Non-wood News 2011; No. 22.
11. Parekh Jigna, Sumitra CV. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol 2007; 31: 53-58.
12. Peach K, Tracey MV. Modern methods of plant analysis. Vol.3, Springer Verlag, Berlin. 1956.
13. Gibbs RD. Chemotaxonomy of Flowering Plants. Vol.1, McGill Queen's University Press, Montreal and London. 1974.
14. Ayoola GA, Coker HAB, Adesegun SA, Adepoju – Bello AA, Obaweya K, Ezennia EC, Atangbayila TO. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria. Trop J Pharm Res 2008; 7: 1019-1024.
15. Treare GE, Evans WC. Pharmacognosy 17th edn, Bahive Tinal, London, 1982; 149.
16. Kumar A, Ilavarasn R, Jayachandran T, Decaraman M, Aravindhan P, Padmanaban N, Krishnan MRV. Phytochemical investigation on a tropical plant. Pak J Nutri 2009; 8: 83-85.
17. Paris R, Moyse H. Precis de matiere medicinale. Paris: Masson. 1969.
18. Venkateswarlu P, Ankanna S, Prasad TNVKV, Elumalai EK, Naga Jyothy PC, Savithramma N. Green synthesis of silver nano particles using *Shorea tumbuggaia* stem bark. Int J Drug Dev Res 2010; 2: 720-723.
19. Jeong SJ, Wonil Koh, Eun-Ok Lee, Hyo-Jung Lee, Hyo-Jeong Lee, Hyunsu Bae, Junxuan Lu, Sung-Hoon Kim. Anti-angiogenic Phytochemicals and Medicinal Herbs. Phytother Res 2011; 25: 1-10.
20. Kubo I, Teruhiko Nitoda, Felismino E Tocoli, Ivan R Green. Multifunctional cytotoxic agents from *Anacardium occidentale*. Phytother Res 2011; 25: 38-45.
21. Chukwujekwu, JC, Van Heerden FR, Van Staden J. Antibacterial activity of flavonoids from the stem bark of *Erythrina caffra* Thunb, Phytother Res 2011; 25: 46-48.
22. Venkatesh R, Shanthi S, Rajapandian K, Elamathi, Thenmozhi, Radhan N. Preliminary study on antixanthomonas activity, phytochemical analysis, and characterization of antimicrobial compounds from *Kappaphycus alvarezii*. Asian J Pharma Clin Res 2011; 4(3): 46-51.
23. Chung KT, Wong TY, Wei CL, Huang YW, Lin Y. Tannins and human health: a review. Criti Rev Food Sci Nutr 1998; 6: 421-64.
24. Savithramma N, Linga Rao M, Rukmini K, Suvarnalatha devi P. Antimicrobial activity of silver nanoparticles Synthesized by using Medicinal Plants. Int J Chem Tech Res 2011; 3: 1394-1402.
25. Shankar SS, Rai A, Ahmad A, Sastry MJ. Rapid synthesis of Au, Ag and bimetallic Au shell nanoparticles using Neem, J Collid Interface Sci 2004; 275: 496-502.
26. Thirumurugan A, Tomy NA, Jai Ganesh R, Gobikrishnan S. Biological reduction of silver nanoparticles using plant leaf extracts and its effect an increased antimicrobial activity against clinically isolated organism. De Phar Chem 2010; 2: 279-284.
27. Ankanna S, Prasad TNVKV, Elumalai EK, Savithramma N. Production of Biogenic silver nano particles using *Boswellia ovalifoliolata* stem bark. Dig J Nano mat Bio-struct 2010; 5: 369-372.
28. Prow TW. Toxicity of nanomaterials to the eye, Wiley Interdisciplinary Reviews: Nanomed Nanobiotech 2010; 4: 317-333.
29. Charles L, Geraci, Vincent Castranova. Challenges in assessing nanomaterial toxicology: a personal perspective, Wiley Interdisciplinary Reviews: Nanomed Nanobiotech 2010; 6: 569-577.
30. Jitendra K, Meenakshi KC, Maheswari M. Nanobiotechnology: Application of nanotechnology in Diagnosis, drug discovery and drug development. Asian J Pharma Clin Res 2011; 4(1): 23-28.
31. Nilesh J, Ruchi J, Navneet T, Brham PG, Deepa KJ, Jeetendra B, Srendra J. Nanotechnology: A safe and effective drug delivery system. Asian J Pharma Clin Res 2011; 3(3): 159-165.
32. Sereemaspun A, Hongpiticharoen P, Rojanathanes R, Maneewattanapinyo P, Ekgasit S, Warisnoicharoen W. Inhibition of human cytochrome P450 enzymes by metallic nanoparticles: A preliminary to nanogenomics. Int J Pharmacol 2008; 4: 492-495.
33. Shrivastava S, Beva T, Roy A, Sing G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotech 2007; 18: 225103.