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SYNTHESIS OF SILVER NANOPARTICLES USING ORANGE PEEL EXTRACTS AND THEIR ANTIBACTERIAL ACTIVITY

Dr. S. Usharani* and B. Ruba Devi

Department of Biochemistry, Idhaya College for Women – Sarugani.

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***Corresponding Author**
Dr. S. Usharani
Department of Biochemistry,
Idhaya College for Women – Sarugani.

ABSTRACT

Green synthesis of metallic nanoparticles has gained significant attention in the field of nanomedicine as an environment-friendly and cost-effective alternative in comparison with other physical and chemical methods. Several metals such as silver, gold, iron, titanium, zinc, magnesium and copper have been subjected to nanoformulation for a wide range of useful applications. Silver nanoparticles (AgNPs) are playing a major role in the field of nanomedicine and nanotechnology. They are widely used in diagnostics, therapeutic and pharmaceutical industries. Studies have shown potential inhibitory antimicrobial, anti-inflammatory and antiangiogenesis activities of AgNPs. AgNPs have been synthesized using silver nitrate and methanolic root extract of orange peel that belongs to the *Rutaceae* family. Synthesized nanoparticles were characterized by Fourier transforms infrared spectroscopy. Furthermore, the antibacterial effect of the plant extract and the nanoparticles were evaluated against gram-positive (*Bacillus subtilis*) and gram-negative (*Escherichia coli*) bacteria. The average size of AgNPs synthesized, was 20 nm with the spherical shape. Orange peel extract based nanoparticles exhibited improved antibacterial activity against both gram-positive and negative strains.

KEYWORDS: FT-IR, Orange peel extract, SNP, Antibacterial activity.

INTRODUCTION

Silver nanoparticles are used in various applications such as biomedical devices, biosensors, catalysis, electronics and pharmaceuticals. Many of these materials are not suitable for critical applications such as in medicine. Furthermore, they represent an environmental hazard. Eco-friendly methods for the synthesis of metal nanoparticles are needed to avoid or minimize such problems. Nowadays, metal nanoparticles are synthesized using eco-friendly natural sources such as plant extracts, fruits, fungi, honey and microorganisms (Aina, 2012).

The biological molecules undergo highly controlled assembly in order to make themselves suitable for nanoparticles synthesis. These nanoparticles are reliable and eco-friendly when compared with nanoparticles synthesized by other methods. The use of environmentally benign materials for the synthesis of nanoparticles offers numerous benefits in pharmaceutical and biomedical applications (Jain *et al.*, 2009), as toxic chemical substances are not employed in their synthesis. The size and shape of the nanoparticles forms the basis for its usage as antimicrobial agents. Antibacterial activity of silver nanoparticles on antibiotic resistant, non-resistant and multidrug resistant strains disrupts bacterial enzyme activity, capable to penetrate bacterial cell membrane, creates structural imperfections thereby preventing bacterial proliferation (Caroling *et al.*, 2013). Extensive work has been carried out on the biological synthesis of nanoparticles by using plant extracts (Subbaiya *et al.*, 2014, Prathiba *et al.*, 2015, Kirubha *et al.*, 2015). Very few studies are available on the biosynthesis of silver nanoparticles from peel extracts (Kokila *et al.*, 2015) and seed extracts (Pandit, 2015).

Citrus sinensis (Rutaceae) trees most important commercial fruits crops widely cultivated in tropical and subtropical climate for their sweet fruits(Deschner *et al.*, 2001). Citrus family had rich sources of phytochemicals. The peel of citrus fruit is having abundant source of flavanones and many polymethoxylated flavones which are very rare in other plants (Rekha S swapna *et al.,* 1993). The peel is the byproduct of citrus juice processing with the high potential use two different tissues are found in citrus peel flavedo and albedo (Janati somayeh sadat fakoor 2012). Beta-carotene is a strongly colored red-orange pigment abundant in plants and fruits (Kirubha *et al*., 2015) The present study illustrates the biofabrication of silver nanoarticles from fruit waste materials especially citrus fruit peel extracts. Characterisation of silver nanoparticles was done by using UV- Visible spectroscopy, which gives a preliminary confirmation of silver nanoparticles. An attempt has been made to compare the antibacterial activity of silver nanoparticles prepared from citrus fruit peel extract.

MATERIALS AND METHODS

CITRUS SINENSIS

TOXANOMICAL CLASSIFICATION OF *CITRUS SINENSIS*

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Subfamily	Rosidae
Order	Sapindales
Family	Rutaceae
Genus	Citrus
Species	Sinensis

Collection of plant material

The fresh peels of citrus sinensis were collected from the place of paramakudi. Ramnad district. The peels were washed thoroughly with tab water, shade, dried, homogenized to fine powder.

Preparation of powder

The peel part of citrus sinensis was collected and dried under shade. These dried materials were mechanically powdered, sheaved using 80 meshes and stored in an airtight container. These powder materials were used for further phytochemical analysis and.

Chemicals and reagents

Ethanol, nutrient agar, silver nitrate were purchased from balaji scientific company from karaikudi, sivagangai, Tamilnadu.

Preparation of extracts

300ml ethanol, methanol, chloroform, aqueous added to 50g of powder sample. Extraction was allowed to stand for 2 days at 27^0c, after which they were filtered using whatmann filter paper. Extract were stored at 4^0c in refrigerated until use and further study used ethanolic extract.

PHYTOCHEMICAL SCREENING

The for carbohydrates

Molish's reagent was added to 2 ml of extract. A little amount of concentrated H2SO4 was added and allowed to stand for few minutes. Purple precipitate ring showed the presence of carbohydrate.

The for alkaloids

To 0.5 ml of extract, add dilute H 2SO4. It was boiled and filtered. Perform Hager's test.

Test for saponins

0.5 ml of extract was boiled and filtered. 10 ml of distilled water was added. Formation of honey comb indicated the presence of saponins.

Test for tannins

To 3ml of extract was added few drops of 10% FeCL3 solution for deep blue colour.

Test for steroids

0.5 ml of the extract added 3ml of chloroform and 2ml concentrated H2SO4.

Test for phenols

1ml extract was added 5ml Folin-Ciocalteu reagent and 4ml of Na2CO3. Blue color indicates the presence of phenols.

Test for flavonoids

A Small quantity of the extract is heated with 10ml of ethyl acetate in boiling water for 3 minutes. The mixture is filtered differently and the filtrate was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed at ammonia layer. This indicates the presence of the flavonoids.

Test for proteins and amino acids

Millions Test-Few drops of Million's reagent added to extract and heat, reddish-brown coloration or precipitation indicates presence of tyrosine residue, Which mostly occurs in proteins.

Synthesis of Silver Nanoparticles

In a typical reaction procedure, 5ml of peel extract was added to 1mM aqueous solution of AgNO3 dissolved in 50ml of double distilled water. The solution was maintained at 60^0c for few minutes until the color changes to yellowish-brown indicating the formation of AgNPs. The result solution was monitored at different time intervals using UV-Visible spectrophotometer.

PREPARATION OF MEDIA

Nutrient agar media for Bacteria.

Composition of media

Peptone	- 5.0 gm
Beef extract	- 3.0 gm
Yeast extract	- 3.0 gm
NaCl	- 5.0
Distilled water	- 100 ml
PH	- 7.2

Microorganism

Pure culture of Escherichia coli, Bacillus subtilis procured from the were purchased from Madurai kamarajan university research laboratory.

Preparation of 24 hours pure culture

A loop full of each of the microorganism was suspended was suspended in about 10 ml of physiological saline in a roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37^0c for 24-48 hours. After completion of incubation period. When growth was observed the tubes were kept in to 2-8^0c until use.

Preparation of dried paper discs

Whatmann filter paper (No:1) was used to prepare disc approximately 6mm in diameter, which are placed in hot air for sterilization after sterilization, the disc were loaded with different concentration of prepared peel extract solutions and again kept under refrigeration for 24 hours.

Application of disc to inoculated agar plates

Previously prepared paper discs were dispensed on to the surface of the inoculated agar plate. Each disc was pressed down firmly to ensure complete contact with agar surface. The disc were placed on the medium suitably apart and the plate were incubated at 5^0c for 1hr to permit good diffusion and then transferred to incubator at 37^0c for 24 hours. After completion of 24 hrs, the plate were inverted and placed in an incubator set to respective temperature for 24 hrs.

Antibacterial assay

Disc preparation

The 6mm (diameter) discs were prepared from whatmann NO.1 filter paper. The discs were sterilized by autoclave at 121^0c. After the sterilization the moisture disc were dried on hot air oven 50^0c. Then various solvent extract discs and control disc were prepared.

Collection of test organism

The bacterial strain of *Escherichia coli* and *Bacillus substilis* were obtained from microbial type culture collection centre.

Antibacterial efficacy of AgNPS

The antibacterial activity was determined by agar well diffusion assay. The media was punctured with 6 mm diameter and filled with various concentrations (100, 200, 300, 400 µg/ml) of AgNPs. Tetracycline was used as a positive control. The diameter of zone of inhibition was indicated by clear area which was devoid of growth of microbes. It was measured using a ruler scale in mm. The silver compounds and silver ions had been applied in various therapeutic agents for preventing wound infections due to its excellent inhibitory action against pathogenic bacterial strains.

RESULT

Preliminary Phytochemical Analysis Of *Citrus Sinensis* Peel Extract

Qualitative analysis

The result of qualitative phytochemical are presented (Table1). The qualitative phytochemical analysis of ethanol, extract of *citrus sinensis* revealed that the preliminary identification of bioactive compounds such as carbohydrate, alkaloids, saponins, tannins & steroids, phenols, flavonoids are present and which could make the fruit useful in treating different ailments and having potential for providing useful drug for human use (Table1).

Synthesis of silver Nanoparticles

The current study was undertaken to exploit the hitherto un-utilized plant sources in the development of AgNPs. The *citrus sinensis* peel extract was subjected to FTIR analysis and antibacterial activity using the conventional methods to test for the silver nano particle was found to be present. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ion when exposed to herbal extracts was reduced in solution, there by leading to the formation of silver hydrosol (Figure: 2).

UV-Visible analysis

After addition of peel extract to AgNO3 solution initial yellow changes to Final reddish brown suggest AgNO3. Absorption spectrum of AgNPs formed in the reaction media has absorption maximum at 425 and 475nm.

FT-IR analysis of fruit peel extract of *citrus sinensis*

FTIR spectra of *citrus sinensis* peel extract samples. Absorption bands around 1715, 1255 and 915 cm^{-1} occur due to C=O stretching, C-O acid stretching, and O-H deformation of acid respectively stretching and –OH deformation primary, secondary, and tertiary alcohols result in peaks at 1150, 1100 and 1050 cm^{-1}. The absorption band around 2940cm^{-1} belongs C-H stretching. Absorption bands around 1700-1800cm^{-1} region, which corresponds to the C=O stretching mode.

The FTIR spectrum showed major absorption peaks at 3286cm^{-1}, which corresponded to -OH stretching of phenolic and carboxyl groups, respectively. Furthermore, the absorption band at 1636cm^{-1} was related to the presence of N-H bending of primary amines. The appearance of peak in FTIR was comparable with other researchers and ranged from 1217 to 3600cm^{-1}. The presence of phenolic OH, vibration carbonyls, alkanes and –C-O- stretch represented strong absorptions(Table 2) (Figure:3).

The weak band at 586cm^{-1} corresponds to C-C1 stretching due to alkyl halides. The bands at 586cm^{-1} and 720cm^{-1} corresponds to C-H aromatic compounds. Weak band at 720cm^{-1} can be observed in the C-H aromatic bond of the extract (Table3).

Antibacterial activity

The antibacterial activity of biosynthesized AgNPs against *Basillus subtilis, Escherichia coli* bacteria was successfully investigated. Our result clearly revealed that the *citrus sinensis* peel extract mediated AgNPs have potent antibacterial activity against bacteria with a higher zone of inhibition of 21mm for *Escherichia coli* (Figure: 4, 5) (Table 3). Clearly indicates that the AgNPs have shown a considerable amount of activity than that of the antibiotic tetracycline. Antibacterial activity for higher concentration of 400µl revealed dose dependent activity. Synthesized AgNPs by peel extract of citrus sinensis potentially eliminates the problem of using peril chemicals as reducing and stabilizing agent, thus making nanoparticles biocompatible with the eco-friendly approach. Therefore, in this study, AgNPs prepared from citrus sinensis peel extract were proved to be an excellent antibacterial agent which could be used in pharmaceutical industries.

Table: 1 Qualitative Phytochemical analysis of *Citrus sinensis* peel extract.

Secondary Metabolites	Appearance	Ethanol extract	Methanol extract	Chloroform extract	Aqueous extract
Carbohydrate	Purple ring	+	-	+	+
Alkaloids	Brown	+	+	-	+
Saponins	Comb	+	-	-	-
Tannins	Deep blue	+	-	+	+
Steroids	Reddish brown	+	+	-	-

Phenols	Blue	+	+	+	-
Flavonoids	Yellow	+	+	+	+
Protein and amino acid	Light yellow	-	-	-	-

Figure- 1: Phytochemical analysis for *citrus sinensis* peel extract.

Figure 2: Synthesis of Silver Nanoparticle From *Citrus Sinensis* Peel Extract.

AgNO3+citrus sinensis peel extract.

Synthesis of silver Nanoparticle

Table- 2: FT-IR analysis of silver nanoparticle from *citrus sinensis* peel extract.

S.NO	PEAK	FUNCTIONAL GROUP	TYPE OF VIBRATION	INTENSITY
1.	3347	C-O-S Phenolic group	stretch	Strong
2.	1639	N-H Amine	Bend	Strong
3.	1352	C-H alkane	Rock	Medium
4.	1219	C-C(In-ring) aromatics	stretch	Medium
5.	738	C-H aromatics	"oop"	Strong
6.	600	C-C1 alkayl halides	Stretch	weak
7.	557	C-Br or C-CL Alkyl halides	Stretch	weak

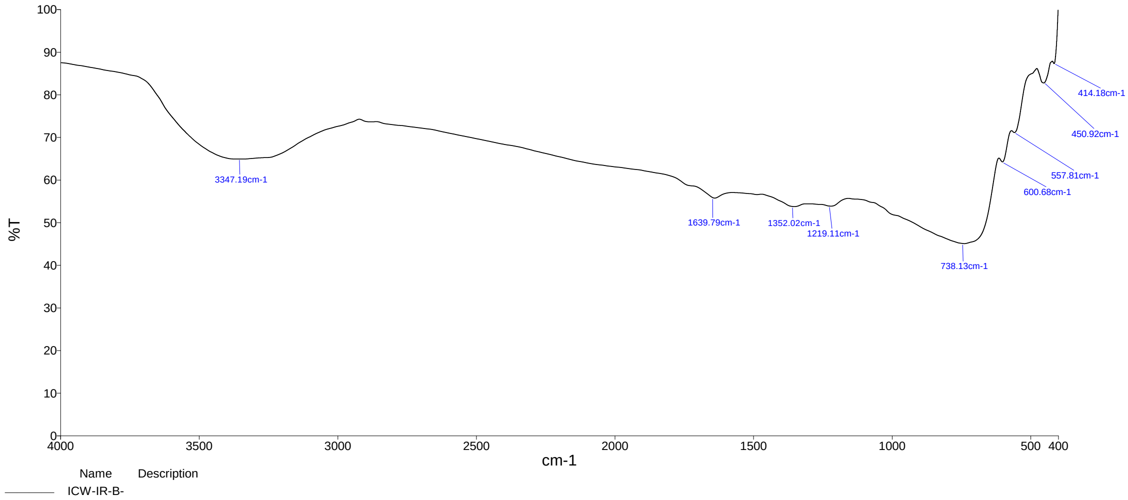

Figure: 3 FT-IR analysis of silver nanoparticle from *citrus sinensis* peel extract.

Bacillus Subtilis

Escherichia coli

Figure-4: Antibacterial activity of silver nanoparticle from *citrus sinensis* peel extract.

Table-3: Antibacterial efficacy of synthesized AgNP against human pathogenic bacteria.

S.NO	CONCENTRATION (µg/µl)	ZONE OF INHIBITION(mm)	
		Bacillus Subtilis	Escherichia coli
1.	Control	15	15
2.	100	14	17
3.	200	16	18
4.	300	19	20
5.	400	20	21

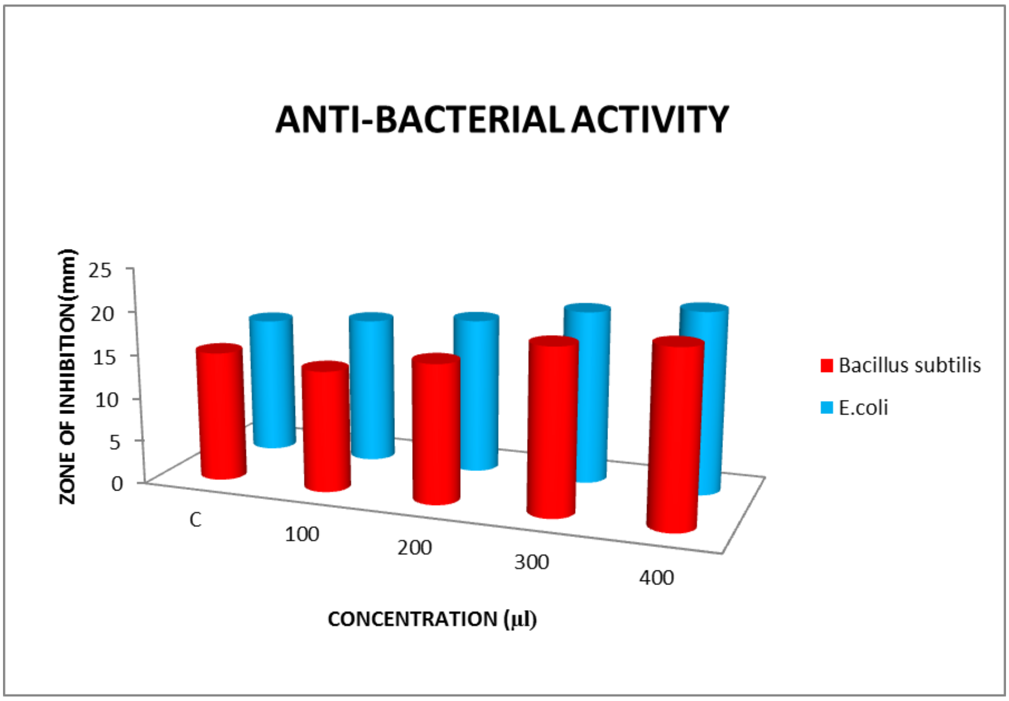

Figure: 5 Antibacterial activity of silver nanoparticle from *Citrus sinensis* peel extract.

DISCUSSION

The *Citrus Sinensis* fruit is low in calories, contains no saturated fats or cholesterol, but rich in dietary fiber, pectin. Pectin helps to protect the mucosa of the colon by its virtue as a bulk laxative. Oranges, especially the juice is rich Vitamin C (provides 48.5 mg per 100 g, about 81% of DRI) which helps in the antioxidant protection and Immune Support because helps the body develop resistance against infectious agents that come from the blood. Also, compounds in orange peel can lower the Cholesterol and act as a cleaner of the interior of the human body.

One orange provides about 7% of the daily requirement of potassium needed of the body. Especially citrus sinensis juice are excellent to remove phlegm and clear the congestion in the nasal and chest passages (sweet orange 2015) Intake of vitamin C is associated with a reduced risk of colon cancer. Vitamin C also prevents us from asthma, osteoarthritis, and rheumatoid arthritis. Oranges are rich in citrus limonoids, proven to help fight a number of varieties of cancer including that of the skin, lung, breast, stomach and colon. Orange fruit also contains some minerals as potassium and calcium, potassium is an important component of cell and body fluids that helps control heart rate and blood pressure through countering pressing effects of sodium. The skin of sweet orange is used to increase appetite, reduce phlegm and treat coughs, colds, intestinal gas, acid indigestion and cancerous breast sores (Morton J, 1999).

Preliminary phytochemical analysis of *Citrus sinensis*

The present investigation preliminary phytochemical studies on peel of citrus sinensis extract showed the presence of phytochemical such as carbohydrate, alkaloids, saponins, tannins, steroids, phenols, flavonoids. The present study coincides with previous report by (Mehmood B., 2015). That the preliminary phytochemical analysis of the ethanol extract of citrus sinensis peel revealed the presence of carbohydrate, alkaloids, saponins, tannins, fixed oil, steroid, phenol.

Synthesis of silver nanoparticles

Silver nanoparticles were synthesis using *citrus sinensis* peel extract by synthesis method. The synthesis was effective in terms of stability and time of reaction. It does not involve any of chemical reducing agent. The synthesized silver nanoparticles were characterized using UV-visible spectroscopy. The maximum peak of absorption of silver nanoparticle using citrus sinensis was observed at 360-340nm respectively. This method of synthesis is cost effective, rapid, ecofriendly and safe (Kokila T, 2015). In future optimization can be carried out at different condition. Also, as it plays a role in environment and biomedical research.

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR Study of peel extract showed sharp absorption peaks located at about 1500 and 1650/cm represents C-C stretch (in-ring) and N-H bend may be assigned to the amide I bond of the proteins and peaks at 3430/cm are assigned to OH stretching in alcohol and phenolic compound (Kong J., 2007).

The FTIR analysis carried out to characterize the AgNPs obtained from of plant extract. The AgNP solutions, prominent bands of absorbance were observed peaks around 650, 1500, 1600, 1550-1650, 3500/cm. The observed peaks denote –C-O-C-, ether linkages, -C-O-, germical methyl or strong stretching due –C=C- bond of aromatic rings and alkyne bonds, respectively.

The functional groups of compounds adsorbed on the AgNPs were identified using FTIR studies. The peak near in FTIR 3347,1639,1352, 1219, 738, 600 and 557cm^{-1} assigned to Phenolic group C-O-S stretching, amine N-H stretching and alkane C-H rock, aromatic C-C stretching, alkylhalides C-C1 stretching respectively (Jain *et.al.*, 2009). The total disappeance of this band after the bioreduction may be due to the fact that the polyols are mainly responsible for the reduction of Ag ions. Thus result suggests that AgNPs might be capped by water soluble secondary plat metabolites.

Antibacterial activity

The antibacterial activity of ethanolic extract of *citrus sinensis* was evaluated and determined by using disc diffusion method. The antibacterial activity of ethanolic extracts was tested against *Basillus substilis*, *Escherichia coli*. The result showed that the *citrus sinensis* was found to be more effective.

The result show that citrus sinensis were found to be more effective against the bacteria tested. In the present study, ethanolic extract obtained from citrus sinensis showed significant activity against bacterial strain. The results of the present study showed that ethanolic extract of *citrus sinensis* has potent anti bacterial activity. Thus the ethanol extract of peel of *citrus sinensis* may be attributed to the presence of phenolic compounds and saponins etc., therefore, further investigation is needed to isolate and identify the active compounds present in the extract and its efficacy. However, detailed study is required to find out that specific bioactive compound responsible for antibacterial property through various advanced techniques.

CONCLUSION

In the current work Silver nanoparticles was synthesized from *citrus sinensis* peel extract. The silver nanoparticles Eco-friendly and cost effective, non toxic, economically feasible technique. Worthwhile and promising approach toward environment protection. In UV-Visible, FT-IR spectroscopy techniques confirmed the formation of silver nanoparticles by citrus sinensis peel. The antibacterial activity was undertaken out for the disc-plate and agar diffusion assay using *Escherichia coli*, *Bacillus subtilis*. The silver nanoparticle synthesis from *citrus sinensis* peel of ethanolic extract. The disc-diffusion assay when compared with standard Tetracyclin. The result clearly revealed that the *citrus sinensis* peel extract mediated silver nanoparticles have potent antibacterial against both bacteria. It is excellent antibacterial agent which could be used in pharmaceutical industries. Thus, the author recommends that further intensive isolation procedure can be adopted on these potent *citrus sinensis* peel extract which may lead to new chemical entity for eradicating the microbial related disease and other pharmacological activity for serving the human society.

REFERENCES

1. Aina VO, Mustapha MB and Mamman OA. Extraction and characterization of pectin from peels of lemon (*Citrus Limon*), grape fruit (*Citrus paradisi*) and sweet orange (*Citrus sinensis*). *Brit*. *J Pharmacolo*. *Toxicolo*, 2012; 3: 259-62.
2. Caroling, G., Tiwari, SK., Mercy Ranjitham A., and Suja, R. 2013. Biosynthesis of silver nanoparticles using aqueous broccoli extract- characterization and study of antimicrobial, cytotoxic effects. Asian Journal of Pharmaceutical and Clinical Research, 6(4): 165-172.
3. Deschner E. E., Ruperto J., Wong G. and Newmark H. L. (1993) The effect of dietray quercetin and rutin on mominduced acute colonic epithelid abnormalities in mice fed a high-fat diet. *Nutritional Cancer*, 20(3): 199-204.
4. Jain, D., Daima, KH. Kachhwaha, S., and Kothari, SL.2009. Synthesis of plant mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. Digest Journal of Nanomaterials and Biostructures, 4(3): 557-563.
5. Janati somayeh sadat fakoor, Beheshti Hamed Reza Fahim niloofar khosbakht. chemical composition of lemon (citrus lemon) and peel its consideration as animal food. GIDA Journal of food, 2012; 37(5): 267271.
6. Kirubha, R., and Alagumuthu, G. 2015. Investigation of antibacterial properties of silver nanoparticles using *Aerva lanata* extract. Indo American Journal of Pharmaceutical Sciences, 2(3): 668-675.
7. Kokila, T., Ramesh, P., and Geetha, D. 2015. A biogenic approach for green synthesis of silver nanoparticles using peel extract of *Citrus sinensis* and its application. International Journal of Chem Tech Research, 7(2): 804-813.
8. Mehmood B., Dar K. K., Ali S., Awan U. A., Nayyer A. Q., Ghous T. and Andleeb S. (2015) In vitro assessment of antioxidant, antibacterial and phytochemical analysis of peel of Citrus sinensis. Pakistan Journal of Pharmaceutical Science, 28(1): 231-239.
9. Morton, J.(n.d.). Orange. Retrieved orange (2015).

10. Pandit, R. 2015. Green synthesis of silver nanoparticles from seed extract of *Brassica nigra* and its antibacterial activity. Nusantara Bioscience, 7(1): 15-19.
11. Prathibha, S., Packiyam, JE, Bhat, PR., Jayadev, K., and Shetty, S, 2015.
12. Rekha s.swapna, Bhaskar M.In vitro screening and identification of antioxidant activities of (citrus sinensis)peel extract in different solvents.Int.Jpharm Bio sci, 2013; 4(4): 405-412.
13. Subbaiya, R., Shiyamala, M., Revathi, K., Pushpalatha, R., and Masilamani, SM. 2014. Biological synthesis of silver nanoparticles from Nerium oleander and its antibacterial and antioxidant property. International Journal of Current Microbiology and Applied Sciences, 3(1): 83-87.