

Optimisation of the conditions involved in the preparation of activated carbon from mosambi peel and the evaluation of antibacterial activity of nano-sized activated carbon, silver nano-particles, and silver impregnated activated carbon

D.Geethapriya* and S.Barathan

Department of Physics, Faculty of Science, Annamalai University, Annamalai University-608002, India

Abstract : The present research work aimed to optimise the condition for the preparation activated carbon from mosambi peel. Variables involved in the preparation such as heating temperature, and heating time were optimised by keeping the missing ratio of H_2SO_4 and precursor as constant. The surface area, methylene blue adsorption capacity, iodine adsorption capacity and the yield of the final products were determined and compared. Silver nanoparticles were prepared using the chemical reduction method. They loaded into the activated carbon to form nano composite. The ability of the activated carbon, silver nano particles, and silver impregnated activated carbon were tested for its antibacterial activity using disc diffusion technique. Experimental results revealed that the activated carbon showed the highest BET surface area, methylene number iodine number was produced at the heating temperature of $350^{\circ}C$ and the heating time 90 mins. Silver impregnated activated carbon as nanocomposite showed the highest antibacterial activity than other two compounds.

Introduction

A recounting of past events have shown that a lot of efforts have been made to discover a new antimicrobial compounds from various kinds of sources such as soil, plants, microorganisms, animals and their wastages. Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search to develop new compounds¹. Discoveries in the past decade have shown that once materials are prepared in the form of very small particles, they change their physical and chemical properties significantly, sometimes to the extent that completely new phenomenon are established². Reducing the particle size of the materials is an efficient and reliable tool for improving biocompatibility^{3, 4}. In particular, the nanoparticles have some very attractive quality of properties such as an ultra-light weight, ordered structure with a high aspect ratio, high mechanical strength, electrical and thermal conductivity. Since they have a specific surface area hence they are used as sorbents for organic and inorganic pollutants removal. And also, these have a large number of unsaturated atoms on their surfaces that can bind readily with most of other atoms^{5, 6, 7}. However, little is known about how the biological activity of certain materials changes as the size of the constituting particles decreases to nanoscale dimensions. There are some reliable reports demonstrated the encouraging results about the activity of different drugs and antimicrobial formulations in the form of nanoparticles^{8, 9}. Efficient methods and different techniques can be used for the production of nanoparticles and they are as follows, chemical, aerosol, electrochemical, laser irradiation, sonochemical deposition, photochemical reduction and biological techniques^{10, 11}. Even though there are many

different possibilities for the production of particles, Wet chemical synthesis of nanoparticles is a valuable alternative to conventional process and gas phase synthesis with known commercial applications¹².

In the world emerging nanotechnology, one of the primary concerns is the potential environment impact of nanoparticles (NPs)¹³. Monitoring the response of bacteria exposed to these particles is a more efficient way to estimate nanotoxicity. Resistance of bacteria to bactericides and antibiotics has increased in recent years due to the development of resistant strains. Some antimicrobial agents are extremely irritant and toxic and there is much interest in finding ways to formulate new types of safe and cost-effective biocidal materials. Previous studies have shown that the Metal nanoparticles can be used in different applications due to their typical optical, electrical and magnetic properties¹⁴⁻²⁶ and the antimicrobial formulations in the form of nanoparticles could be used as effective bactericidal materials^{9,27}. Considering the above recently it has been demonstrated that highly reactive metal oxide nanoparticles exhibited excellent biocidal action against Gram-positive and Gram-negative bacteria²⁸. Among the metal oxide nanoparticles, silver nanoparticles (AgNP) are gaining more importance because of its antimicrobial and antiviral properties²⁹. These nanoparticles exhibits action through the mechanism is that AgNPs attach to the surface of cell membrane, which disturbing the permeability and respiratory functions of the cells and its leads to microbial cell death³⁰. Thus, the preparation, characterization, surface modification, and functionlization of nanosized inorganic particles open the possibility of formulation of a new generation of bactericidal materials³¹. However, metallic nanoparticles tend to either react with surrounding media or agglomerate to result in significant loss of reactivity³². Out of economic and duration consideration, it has been used to treat with low cost adsorbent for prolonged activity. The most popular and commonly used adsorbent throughout the world is activated carbon (AC)³³ because of its relatively high surface area, large porosity, total pore volume and presence of wide spectrum of functional groups on its surface. This also provides a strong affinity for even low concentration organics to attach to itself³⁴. So it is foremost important to produce low cost AC from waste material. Nanocomposites are materials that incorporate nanosized particles into a matrix of standard material. The result of the addition of nanoparticles is a drastic improvement in properties. Nanocomposite will be proved as an efficient adsorbent because of the increase in their surface-to-volume ratio with the reduction of the size of the adsorbent particle from bulk to nano dimensions³⁵.

In the present study, AC have been prepared from Mosambi fruit peel and they are easily available in abundance which are disposed as a waste material. They have been collected, processed at different operating conditions and converted into an effective low cost adsorbent. AgNPs are chemically synthesized using AgNO₃ and tri-sodium citrate. The synthesized nanoparticles are incorporated onto AC to produce a nanocomposite (AgNP-AC). Where the combination of the AC and AgNP would take an important antibacterial advantage, due to the strength of these two nanoparticles, they are used widely for many applications in a prolonged manner. The main objectives of the present study are to optimise the activation time and temperature for the preparation of AC from mosambi fruit peel, to elucidate the adsorption characteristics of AC prepared from mosambi peel and to evaluate the antibacterial efficacy of the prepared AC nanoparticles, AgNP, and AgNP-AC against bacterias such as *Staphylococcus aureus*, *Escherichia Coli* (*E.Coli*), *Klebsiella pneumoniae*, *Proteus mirabilis*, *salmonella typhi*, *Pseudomonas aeruginosa*, *Vibrio cholerae*.

Materials and methods

Preparation of activated carbon

The peels of mosambi fruit were obtained after extracting the juice from the households and juice shops. Those peels were washed in distilled water and dried in the sunlight for several days. Then it is crushed in to small pieces and soaked with the chemical activating agent H₂SO₄, at a ratio of 1:1 for 24 hours. The resulting black product was kept in hot air oven for about three hours at 110°C and then it is carbonized at different temperatures ranging from 150°C to 500°C at 50°C interval for different time periods ranging from 30 minutes to 2 hours with 30 mins interval. This activated part of the carbon is washed several times with NaHCO₃ followed by distilled water to remove the excess acid present. Then the resulting products were kept in hot air oven at 110 ± 5°C for more than 3 hours to obtain a complete fine black AC. Finally, it was stored in desicator until further use. A well developed porous structure is obtained, depending on the time, temperature, and impregnation ratio. Among the various prepared ACs the effective AC has been chosen based on its adsorption characteristics, the chosen AC was converted into nanoparticles using planetary ball mill.

Synthesis of silver nano particles

The AgNP was prepared as per the chemical reduction method adopted by Fang, et al³⁶. Heat the 50 ml of prepared 1×10^{-3} M silver nitrate till it boils and added 5 ml of 1% tri-sodium citrate drop by drop. Mixed the solution forcefully and heated it until the colour changed to pale brown. Cooled it by stirring at room temperature and finally powdered form of AgNP has been obtained by drying the aqueous solution on air for 4 days.

Preparation of nanocomposite

For coating, 50gm of powdered AC added with 250 ml of AgNPs solution was stirred vigorously at a concentration of 500ppm in room temperature for overnight and then cured at 110°C for minimum of 2hrs in vaccum oven. Finally the AgNP-AC of 2.5 mg/g composition was obtained. The SEM analysis was carried out to confirm the coating of AgNPs.

Adsorption studies

The adsorption characteristic of AC was studied by identifying Iodine number³⁷ methylene blue number³⁸, and BET surface area³⁹.

Methylene blue adsorbtion

The methylene blue number is defined as the milligram of methylene blue adsorbed onto 1.0 gm of adsorbent⁴⁰. As per the method adopted by Sahira Joshi and Bhadra Prasad Pokharel⁴¹ the Methylene blue number was determined according to Standard Method⁴². In this method Erlenmeyer flask containing 100 ml of MB solutions with initial concentrations of 50-500 mg/L was added with equal mass of 0.1 g of the AC at room temperature, kept for 24 hrs in shaker at 30°C and centrifuged to reach equilibrium. To minimize the interference of carbon particles, samples were filtered prior to analysis. Each experiment was carried out in triplicate under the same conditions. UV-Visible Spectrophotometer (Shimadzu-UV1800) at 663 nm was used to find out the concentrations of MB in the supernatant solutions before and after adsorption. The percentage removal of dye from the solutions and the amount of adsorption at equilibrium were calculated by the formula

$$\% \text{ of removal} = \left(\frac{C_0 - C_e}{C_0} \right) \times 100$$

Amount of adsorption (Q_{1e}) = $(C_0 - C_e) V/W$ (mg/g)

Where, C_0 and C_e (mg/L) are the liquid-phase concentrations of the dye at initial and equilibrium state; V is the volume of the solution (L) and W is the mass of dry adsorbent used (g).

Iodine adsorbtion

It is the amount of iodine adsorbed (in milligrams) by 1gm of carbon when the iodine concentration of the filtrate is 0.02 N⁴³. The iodine number was determined as per the standard method⁴⁴ which was previously adapted by O. A. Babatunde et.al⁴⁵. Under this method, 10 mL of 5% HCL was added in a 250 mL flask containing (0.2-1.0 g) of different powdered AC samples and made the carbon as wet by swirling the flask. Added 100 mL of stock iodine solution (2.7 g of Iodine (Merck) and 4.1 g of potassium iodide (Merck) in 1L of de-ionized water) into the mixture and shaken it in an orbital shaker for 5 minutes. The samples were filtered using Whatman No.1 filter paper. Then titrate 50 mL of filtrate with 0.1 M sodium thiosulphate until the solution color changed into pale yellow. Added 1 mL of starch indicator solution (1%) in that solution and continue the titration with sodium thiosulphate until the solution become colorless. The amount of iodine adsorbed per gram of carbon (iodine number) and the residual iodine concentration in the filtrate were calculated using below equation

$$IN = \frac{VB - VS}{VB} \times \frac{V1}{WM1}$$

Where IN is the iodine number, VB is the volume of $\text{Na}_2\text{S}_2\text{O}_3$ used for blank titration, VS is the volume of $\text{Na}_2\text{S}_2\text{O}_3$ used for test titration, M1 is molarity of iodine solution, V1 is volume of iodine solution used and W is weight of AC.

BET surface area

Nitrogen adsorption and desorption isotherms were measured at 77 K on a Micromeritics ASAP 2020 volumetric adsorption system as used by Sevilla et.al⁴⁶. The samples were out gassed overnight at 180 °C prior to adsorption measurements. The BET model was applied to fit the nitrogen adsorption isotherms and evaluated the specific surface area of the samples⁴⁷.

Anti bacterial study

The anti bacterial activity of AC, AgNP, AgNP-AC was tested against various microorganisms such as gram positive strain *Staphylococcus aureus* and gram negative strains like *Escherichia Coli* (*E.Coli*), *Klebsiella pneumoniae*, *Proteus mirabilis*, *salmonella typhi*, *Pseudomonas aeruginosa*, *Vibrio cholerae* obtained from RMMCH, Annamalai University Annamalai Nagar, India. It was maintained at 4°C on nutrient agar slant.

As similar to the adaption of C. Karthik and K. V. Radha⁴⁸ the antibacterial activity was performed by adapting disc diffusion method⁴⁹ otherwise called as Kirby-Bauer method. The three test solutions have been prepared individually by dissolving a known weight of different compounds (AC, AgNP, AgNP-AC) with 50% of Dimethyl sulfoxide (DMSO) to give a concentration of 250µg/ml each. Inoculums have been prepared by taking 24hrs old culture of above mentioned seven bacteria and mixed it with physiological saline in a seven different containers individually until the turbidity was corrected to McFarland standard (i.e.) 1(10⁶cfu/ml). The mixture of MHA was sterilized and poured into 7 petridishes with a uniform surface thickness of 4-5mm. Allow the agar to solidify at room temperature. The seven prepared inoculums have to be applied on seven different MHA plates and spread it evenly using standard swaps. The inoculated plates have to be kept aside for few minutes in order to dry. The Whatmann filter paper disc dipped in test solutions and plain DMSO solution have been placed on all solidified plates at an equal distance from the centre point of plates using sterilized forceps. The plates were incubated at 37°C for 24hrs and the zone of inhibition of bacterial growth for test solutions and DMSO solution (i.e.) control against each bacteria was obtained by measuring the zone diameter. The experiment was carried out in triplicate and the mean values are presented.

Results and discussion

Optimization of activated carbon preparation

In this study, 150-300°C with different time interval no carbonization was taken place. 350°C onwards and up to 450°C it becomes carbon. It becomes ash when it reached 500°C. Hence, 12 different carbons have been prepared on various combinations of time and temperatures (350-450°C and 30-120 mins). The prepared carbons were collected, activated and its characteristics were studied using standard procedures and the results are given in below Table 1.

Table 1 Adsorption Characteristics Activated Carbon Prepared on Various Combinations of Time and Temperatures

Temperature (°C)	Time (mins)	Yield (%)	Iodine number(mg/g)	Methylene blue number(mg/g)	BET surface area(m ² /g)
350	30	58.4	220.1	87.6	108.3
	60	53.9	233.8	101.9	112.6
	90	46.3	243.7	106.5	148.1
	120	42.6	218.6	92.0	97.3
400	30	39.5	201.8	91.1	81.2
	60	30.2	172.2	75.3	56.8
	90	23.6	138.5	66.8	45.1
	120	19.9	107.1	54.4	25.8
450	30	17.5	97.5	32.3	21.0
	60	13.8	76.4	24.1	16.7
	90	11.8	45.8	16.4	9.9
	120	8.75	19.3	10.5	5.7

From the above table it is noted that among 12 different carbons, the carbon prepared at 350°C and in 90mins was having higher iodine number, methylene blue number and BET surface area which indicates comparatively it was having higher adsorption characteristic. This showed the optimum temperature and time for preparing AC from mosambi peel are 350°C and in 90 mins respectively. U. V. Ladhe and P. R. Patil⁵⁰ produced activated carbon from mosambi peel by carbonization and activation with H₂SO₄ at 105°C for 12 hrs and reported the surface area of 189m²/g. In this work using the same raw material, the surface area of 143 m²/g, iodine number of 243.7 mg/g and methylene blue number of 106.5 mg/g were obtained at 350°C for 90 mins. . It also observed that the surface area is lower than ladhe's and compared the results with this study, their temperature for production of AC is lower, and the time for production of AC is higher on their study with the higher surface area. In this study the effective AC was obtained at 350°C for 90 mins and hence that carbon has been chosen for further experiments after converting it into nano particle.

Influence of temperature on the yield, iodine and methylene blue numbers and BET surface area of activated carbon

By keeping the activating time, the precursor and activating agent ratio(1:1) as constant and changing the activation temperature from 350 to 450°C, the effect of activating temperature on the yield, iodine number, methylene blue number and BET surface area were studied. The results are given in table1. The results shows that when the activating temperature increased the yield, iodine number, methylene blue number and BET surface area of the AC get decreased due to the pore formation and widening of pores. This indicates that the adsorption capacity of AC increased with reduced activation temperature. This is quite opposite to the findings of Xiaojun Ma et.al^{51,52}. This may due to the attainment of optimised temperature on carbonisation⁵³.

Influence of time on the yield, iodine, methylene blue numbers and BET surface area of activated carbon

By keeping the activating temperature (350°C), the precursor and activating agent ratio(1:1) as constant and varying the activation time from 30 to 120 mins the yield, iodine number, methylene blue number and BET surface area were studied and the results are given in table1. The results shows that, when the activation time increased the yield of the AC and iodine number get decreased, when the activation time increased methylene blue number and BET surface area of the AC get increased to certain time and suddenly get decreased at a particular point. The adsorption capacity of the AC prepared increasing with increases in activation time from 30 to 90 mins and then decreases at 120mins. The results are as similar to the findings of RinitaRajbhandari Joshi⁵² and SamornHirunpraditkoon et.al⁵⁴ upto the optimum time and suddenly changes reversely when it crosses the optimum time.



Fig 1A: Against *Staphylococcus aureus*



Fig 1B: Against *Escherichia coli* (E.Coli)

Fig 1C: Against *Klebsiella pneumoniae*Fig 1D: Against *Proteus mirabilis*Fig 1E: Against *Salmonella typhi*Fig 1F: Against *Pseudomonas aeruginosa*Fig 1G: Against *Vibrio cholera*

Fig. 1A-G. Antibacterial Activity Test Results of DMSO, Nano Sized Activated Carbon, Silver Nanoparticles, and Silver Impregnated Activated Carbon

The above 7 pictures shows the anti bacterial test results against *Staphylococcus aureus*, *Escherichia Coli* (*E.Coli*), *Klebsiella pneumoniae*, *Proteus mirabilis*, *salmonella typhi*, *Pseudomonas aeruginosa*, *Vibrio cholerae* respectively. The four disc in each plate represents the activity of plain DMSO solution which act as control (bottom), AC nano particle (left), AgNPs (right), and AgNP-AC (top). The results of Kirby-Bauer test revealed that the antibacterial activity of plain DMSO solution was null but other three compounds exhibited antibacterial activity against all the seven bacteria. The result was obtained by measuring the zone diameter. The experiment was done triplicates and the mean values of zone of inhibition are presented in table2.

Table2 Zone of Inhibition Nano Sized Activated Carbon, Silver Nanoparticles, and Silver Impregnated Activated Carbon against Selected Bacterial Strains

Bacterial strains	Zone diameter of AC (mm)	Zone diameter of Ag (mm)	Zone diameter of Ag-AC (mm)
<i>Staphylococcus aureus</i>	15	35	37
<i>Escherichia Coli</i>	15	19	20
<i>Klebsiella pneumoniae</i>	3	4	5
<i>Proteus mirabilis</i>	5	12	13
<i>salmonella typhi</i>	18	25	25
<i>Pseudomonas aeruginosa</i>	19	21	22
<i>Vibrio cholerae</i>	20	23	26

Results of the study showed the inhibitory effect of AC, silver, and AgNP-AC against selected bacterial strains. The tested nanoparticles had an antibacterial effect exhibited by the zone of inhibition. AgNP-AC proved as the most effective antibacterial agent against *Staphylococcus aureus*, *Escherichia Coli* (*E.Coli*), *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Vibrio cholerae* with an inhibition zone of 37, 20, 5, 13, 22, 26 mm respectively followed by AgNPs and then AC. Sreeprasad and Pradeep⁵⁵ in their similar study investigated the reason for the superior antibacterial activity of nano composite and quoted synergistic effect of compounds presented in nano composite as the reason behind it. But against *Salmonella typhi*, the AgNPs and AgNP-AC showed same results (25 mm) followed by AC (18 mm). Eventhough there exist differences in zone of inhibition among AgNPs and AgNP-AC that differences are very narrow. In general the result of the study indicates that nano composite (AgNP-AC) showed superior anti bacterial activity than AgNP and AC due to the synergistic effect of AgNP and AC.

The bacteria adhere weakly with AC particles as compared with other two compounds namely AgNPs and AgNP-AC and the main reason behind this is the anti bacterial activity of AC depends on the number of attractive sites revealed upon traversing of a carbon particle through the outer bacterial surface layer⁵⁶. Once the nanoparticle comes in contact with the bacterial cell, it either inhibit the cell wall synthesis, damage the cytoplasmic membrane, inhibit nucleic acid and protein synthesis or inhibit specific enzyme systems which result in the complete bacterial inhibition⁵⁷. However the AgNP-AC proved to be the most effective antibacterial agent, the time taken to reach its 100% viability is greater for AC followed by AgNP-AC then by AgNPs. The bacterial reduction rate was greatly influenced by the increase of silver ion concentrations and contact time⁵⁸. In general, most of the bacterial reduction and inactivation took place during the first three hours of incubation, and the mortality rate increases continuously with the increase of nanomaterial concentration and time⁵⁹.

Conclusion

The preparation of AC from mosambi peel was optimised for better adsorption characteristics. AgNPs were synthesised successfully by chemical reduction method. The nanocomposite was prepared by incorporating synthesized AgNP onto AC. The anti bacterial effect of compounds was tested using disc diffusion method. The results of anti bacterial test showed that the AgNP-AC is possessing superior antibacterial activity than normal AC and synthesised AgNP against *Staphylococcus aureus*, *Escherichia Coli* (*E.Coli*), *Klebsiella pneumoniae*, *Proteus mirabilis*, *salmonella typhi*, *Pseudomonas aeruginosa*, and *Vibrio cholera*. Finally, silver nanoparticle loaded AC will be a desirable anti bacterial agent than other two nanoparticles against above mentioned bacterial strains.

Reference

1. Silver LL. Discovery and development of new antibiotics: the problem of antibiotic resistance. *Antimicrob. Agents Chemother.*, 1993, 37: 377-383.
2. Shah MA, Tokeer A. *Principles of Nanoscience and Nanotechnology* Naroosa Publishing House, New Delhi. 2010.

3. Mirkin CA, Taton TA. Semiconductors meet biology. *Nature.*, 2000, 405;626- 7.
4. Kim S, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK. Nanomedicine: Nanotechnology, Biology and Medicine., 2001, 3 (1); 95-101.
5. Sandoval R, Cooper A.M, Aymar K, Jain A, Hristovski K. Removal of arsenic and methylene blue from water by granular activated carbon media impregnated with zirconium dioxide nanoparticles, *Journal of Hazardous Materials*, 2011 193;296-303
6. Tuan TQ, Son NV, Dung HTK, Luong NH, Thuy BT, Anh NTV, Hoa ND, Hai NH. Preparation and properties of silver nanoparticles loaded in activated carbon for biological and environmental applications. *Journal of hazardous materials*, 2011, 192(3), 1321-1329.
7. Ghaedi M, Karimi F, Barazesh B, Sahraei R, Daneshfar A. Removal of reactive orange 12 from aqueous solutions by adsorption on tin sulfide nanoparticle loaded on activated carbon. *J. Ind. Eng. Chem.*, 2013, 19
8. Forestier F, Gerrier P, Chaumard C, Quero A, Couvreur P, Labarre C. Effect of nanoparticle-bound ampicillin on the survival of *Listeria monocytogenes* in mouse peritoneal macrophages. *J Antimicrob Chemother.*, 1992, 30: 173-179.
9. Fresta M, Puglisi G, Giammona G, Cavallaro G, Micali N, Furneri P M. Pefloxacin mesilate- and ofloxacin-loaded polyethylcyanoacrylate nanoparticles: characterization of the colloidal drug carrier formulation. *J Pharm Sci.*, 1995, 84:895-902.
10. Shahverdi AR, Minaeian S, Shahverdi HR, Jamalifar H, Nohi A. A novel approach, *Process Biochem*, 2007, 42; 919-923.
11. Raj LFAA, Jayalakshmy E. Biosynthesis and Characterization of Zinc Oxide Nanoparticles using Root Extract of *Zingiber officinale*. *Orient. J. Chem.*, 2015, 31;51-56.
12. Xu HY, Wang H, Zhang YC, He WL, Zhu MK, Wang B, Yan H. Hydrothermal synthesis of zinc oxide powders with controllable morphology. *Cer. Int.*, 2004, 30:93.
13. Brayner R. The toxicological impact of nanoparticles. *Nano Today.*, 2008, 3:48-55.
14. Umarani P, Venkatesan P, Radhakrishnan T. A Study of Antimicrobial Activity of High Fluorescent Cadmium Telluride Nanoparticles, *International Journal of ChemTech Research*, 2016, 9(01):313-317.
15. Seeram Hariprasad, Susheela Bai G, Santhoshkumar J, Madhu CH, Sravani D, Green synthesis of Copper Nanoparticles by Arecanata Leaves Extract and their Anti Microbial Activities, *International Journal of ChemTech Research*, 2016, 9(02):98-105.
16. Devasenan S, Hajara Beevi N, Jayanthi SS. Synthesis and characterization of Copper Nanoparticles using Leaf Extract of *Andrographis paniculata* and their Antimicrobial Activities., *International Journal of ChemTech Research*, 2016, 9(04):725-730.
17. Gnanasangeetha D and Sarala Thambavani D. Green Zinc Oxide Nanoparticle Incorporated on Activated Silica for the Removal of As(III) from aqueous solution using *Ocimum sanctum* and *Azadirachta indica*; *International Journal of ChemTech Research*; 2015, 8(8):44-52.
18. Manoj L, Vinita Vishwakarma. Green Synthesis and Spectroscopic characterisations of gold nanoparticles using invitro grown hypericin rich shoot cultures of *Hypericum hookerianum*; *International Journal of ChemTech Research*; 2015, 8(11):194-199.
19. Thamima M and Karuppuchamy S. Microwave Assisted Synthesis of Zinc Oxide Nanoparticles; *International Journal of ChemTech Research*; 2015, 8(11):250-256.
20. Joseph Sagaya Kennedy A, Johnson I. PL studies on NiO nanoflakes using natural *Tabernaemontana divaricata* plant Leaves; *International Journal of ChemTech Research*; 2016, 8(11):316-321.
21. Kavitha S, Shilpa R, Padmanabhan D, Angelin A. Preparation and characterization of SiO₂ nanoparticles doped carbonized *Zygosaccharomyces bailii* for arsenic detection; *International Journal of ChemTech Research*; 2015, 8(11):450-456.
22. Suganya R, Krishnaveni N, Senthil TS. Synthesis and Characterization of Zinc oxide Nanocrystals from Chemical and Biological methods and its Photocatalytic activities; *International Journal of ChemTech Research*, 2015, 8(11):490-496.
23. Gulam Rabbani, Shivaji Jadhav, Megha Rai and Mazhar Farooqui. Synthesis and Characterization of new precursor for conducting polymer-based thin film of PANI nanocomposites containing ZnO in aqueous solution.; *International Journal of ChemTech Research*, 2015, 8(12):386-394.
24. Savitha Elango, Kalainathan Sivaperuman. Sol-Gel mediated synthesis of tri-doped TiO₂ Nanoparticles towards application of photo catalysis and its kinetic study; *International Journal of ChemTech Research*, 2015, 8(12):588-597.

25. Rajeshkumar S. Green Synthesis of Different Sized Antimicrobial Silver Nanoparticles using Different Parts of Plants – A Review, *International Journal of ChemTech Research*, 2016, 9(04):197-208.
26. Boualouache Adel, Belamri Laid, Boucenna Ali, KhaliliBenyoucef. Preparation and Characterization of Metals nanostructures supported on zeolitic and clay, application in the transformation of glycerol, *International Journal of ChemTech Research*, 2016, 9(03):491-499.
27. James, G. V. *Water treatment*. 4th ed. CRC Press, Cleveland, 1971, 38–45.
28. Stoimenov P K, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. *Langmuir*, 2002, 18: 6679–6686.
29. Chen Y, Chen H, Zheng X, Mu H. The impacts of silver nanoparticles and silver ions on wastewater biological phosphorous removal and the mechanisms. *J Hazard Mater.*, 2012, 239–240: 88–94
30. Anandhakumar S, Raichur AM. A facile route to synthesize silver nanoparticles in polyelectrolyte capsules. *Colloids and Surface B: Biointerfaces*, 2011, 84: 379-383.
31. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J Colloid Interface Sci.*, 2004 275:177 - 82.
32. Chen Z, Jin XY, Megharaj M and R Naidu. Removal of methyl orange from aqueous solution using bentonite-supported nanoscale zero-valent iron. *J. Colloid, Interface Sci.*, 2011, 363:601–607.
33. Bhatnagar, A, Hogland W, Marques M, Sillanpaa M. An overview of the modification methods of activated carbon for its water treatment applications. *Chem. Eng. J.*, 2013, 219: 499-511.
34. Ahmad M A, Alrozi R. Removal of malachite green dye from aqueous solution using rambutan peel-based activated carbon: equilibrium, kinetic and thermodynamic studies. *Chemical Engineering Journal*, 2011, 171(2):510-516.
35. Ghaedi, M, Ghayedi M, Kokhdan SN, Sahraei R and Daneshfar A. Palladium, silver, and zinc oxide nanoparticles loaded on activated carbon as adsorbent for removal of bromophenol red from aqueous solution. *J. Indust. Eng. Chem.*, 2013, 19: 1209-1217.
36. Fang J, Zhong C and Mu R. The Study of Deposited Silver Particulate Films by Simple Method for Efficient SERS. *Chem. Phy. Lett.*, 2005, 401: 271 275
37. Ahmedna M, Clarke SJ, Rao RM, Marshall WE, Johns MM. Use of filtration and buffers in raw sugar color measurements, *J. Sci. Food. Agric.*, 1997, 75:109-116.
38. Tan IAW, Hameed BH, Ahmad AL. Equilibrium and kinetic studies on basic dye adsorption by oil palm fibre activated carbon, *Chem. Eng. J.*, 2007, 127:111-119.
39. Esfandiari Ali, KaghazchiTahereh and SoleimaniMansooreh. Preparation of high surface area activated carbon from polyethyleneterephthalate(PET) waste by physical activation. *Research Journal of Chemistry and Environment.*, 2011, 15(2):432-437
40. Cleiton A, Nunes MC, and Guerreiro. Estimation of surface area and pore volume of activated carbons by methylene and Iodine number, *Quim. Nova.*, 2011, 34(3):472-476.
41. Sahira Joshi and Bhadra Prasad Pokharel. Preparation and Characterization of Activated Carbon from Lapsi (*Choerospondiasaxillaris*) Seed Stone by Chemical Activation with Potassium Hydroxide, *Journal of the Institute of Engineering.*, 2013, 9(1):79–88.
42. Standard testing method of methylene blue number of activated carbon, Japanese industrial standard test method for activated carbon, Japanese Standard Association, JIS K, 1991, 1470.
43. Cleiton A, Nunes MC, and Guerreiro. Estimation of surface area and pore volume of activated carbons by methylene and Iodine number, *Quim. Nova.*, 2011, 34(3):472-476.
44. ASTM D 4607-94. Standard Test Methods for the Determination of Iodine Number of Activated Carbon ASTM, Race Street, Philadelphia, PA., 2006, 19130
45. Babatunde OA, Garba S, Ali ZN. Surface Modification of Activated Carbon for Improved Iodine and Carbon Tetrachloride Adsorption. *American Journal of Chemistry.*, 2016, 6(3): 74-79
46. Sevilla M, Fuertes AB, Mokaya R. Preparation and hydrogen storage capacity of highly porous activated carbon materials derived from polythiophene. *International journal of hydrogen energy.*, 2011, 36(24):15658-15663
47. Brunauer S, Emmett PH, Teller E. Adsorption of gases in multimolecular layer, *J. Amer. Chem. Soci.*, 1938, 60(2):309-319.
48. Karthik C and Radha KV. Silver Nanoparticle Loaded Activated Carbon: An Escalated Nanocomposite with Antimicrobial Property. *Oriental Journal of Chemistry.*, 2016, 32(1):735-741
49. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J ClinPathol.*, 1966, 45(4):493–496.

50. Ladhe UV and Patil PR. Removals Of Sudan Red G Dye From Aqueous Solution By Adsorption On To Activated Carbon Prepared From Mosambi And Cotton An Agricultural Waste. International Journal Of Science, Environment And Technology., 2014, 3(2);546 – 555.
51. Xiaojun Ma, Hongmei Yang, Lili Yu, Yin Chen and Ying Li. Preparation, Surface and Pore Structure of High Surface Area Activated Carbon Fibers from Bamboo by Steam Activation Materials., 2014, 7: 4431-4441
52. RinitaRajbhandari Joshi. Optimization of Conditions for the Preparation of Activated Carbon from Lapsi (Choerospondiasaxillaris) Seed Stone Using ZnCl₂. Journal of the Institute of Engineering., 2015, 11(1):128-139
53. Tharapong Vitidsant, Terachai Suravattanasakul and Somsak Damronglerd. Production of Activated Carbon from Palm-oil Shell by Pyrolysis and Steam Activation in a Fixed Bed Reactor Science Asia., 1999, 25:211-222
54. Samorn Hirunpraditkoon, Nathaporn Tunthong, Anotai Ruangchai, and Kamchai Nuithitiku. Adsorption Capacities of Activated Carbons Prepared from Bamboo by KOH Activation International Journal of Chemical, Molecular, Nuclear, Materials and Metallurgical Engineering., 2011, 5(6).
55. Sreeprasad, T, and Pradeep T. Graphene for environmental and biological applications. Inter. J. Modern. Phy., 2012, 21:26
56. Busscher, H, Dijkstra R, Langworthy D, Collias D, Bjorkquist D, Mitchell M and Van der Mei H. Interaction forces between waterborne bacteria and activated carbon particles. J.Colloid.Inter.Sci., 2008, 322:351-357.
57. Sadeghi B, Jamali M, Kia Sh A, Amininia and Ghafari S. Synthesis and characterization of silver nanoparticles for antibacterial activity. Int.J.Nano.Dim., 2010, 1: 119-124.
58. Karnib M, Holail H, Olama Z, Kabbani A and Hines M. The Antibacterial Activity of Activated Carbon, Silver, Silver Impregnated Activated Carbon and Silica Sand Nanoparticles against Pathogenic E. coli BL21. Int.J.Curr.Microbiol.App.Sci., 2013, 2(4): 20-30
59. Liu S, Hu M, Zeng T, Wu R, Jiang R, Wei J, Wang L, Kong J and Chen Y. Lateral Dimension dependant antibacterial activity of Graphene oxide sheets. ASC. Nano. , 2012, 28:12364-12372.
