Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(19):572-580
(http://derpharmachemica.com/archive.html)

Synthesis of activated carbon, magnesium oxide, magnesium oxide impregnated activated carbon nanoparticles and their antibacterial activity

D. Geethapriya* and S. Barathan

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

ABSTRACT

Citrus limetta fruit is commonly known as sweet lime, sweet lemon and sweet limetta or mosambi. The outer skin of the citrus limetta fruit acts as a precursor for the preparation of activated carbon by chemical activation with sulphuric acid. Activated carbon was prepared by the carbonization of citrus limetta at different combinations of temperatures and time periods. The different activated carbons prepared were washed for several times with $NaHCO_3$ additionally distilled water to remove the presence of excess acid. The resulting product transferred to hot air oven at $110^oC \pm 5^oC$ for more than 3 hours to obtain an entire fine black activated carbon. The effective activated carbon was chosen from among the different activated prepared based on the porous structure analyzed using scanning electron microscope (SEM). Thus chosen activated carbon was converted into nano sized activated carbon (ACNP) using planetary ball mill. Metal nanoparticle namely MgO nanoparticles (MgONP) were chemically synthesized and that MgONP was impregnated onto the ACNP to form MgONP-AC nanoparticles. The ACNP, MgONP and MgONP-AC compounds were treated with selected seven bacteria such as Staphylococcus aureus, Escherichia Coli (E.Coli), Klebsiella pneumoniae, Proteus mirabilis, salmonella typhi, Pseudomonas aeruginosa, Vibrio cholerae for their antibacterial activity.

Keywords: Citrus limetta, activated carbon, bacteria, Thermo gravimetric-Differential Thermal Analysis, scanning electron microscope, X-ray powder diffraction, Fourier transform infrared spectroscopy.

INTRODUCTION

In recent years, municipal wastage is being a major problem for world as well India. Many ways can be adopted for the disposal of that wastage but almost all the ways leads to the environmental pollution. In our day to day life, environmental pollution is also a major problem and various methods were employed to minimize its adverse effects but it is small part. Importance given by various countries to keep their country clean shows the seriousness of this issue. Permanent and feasible solution for this issue is recycling of waste material and converts it into value added product without polluting the environment. Activated carbon is one such value added product in the treatment and prevention of bacterial infection [1] [2]. Activated carbon is a fine carbonaceous material which is widely used as adsorbent [2] [3], and in separation process [4] [5] or catalysis [6] [7]. Activated carbons play a major role in adsorption process because of its high surface area and porous texture. Due to this property it has been used in many disciplines such as medical, agriculture and so on.

Activated carbon can be produced from all carbon containing ligno-cellulosic materials such as rice husk [8], wastes of vegetable origin [9], palm shells [10], pomegranate seeds [11], fruit peels [12], olive stones [13], peat [14], saw dust [15], Bagasse [16], waste tea [17], coffee grounds [18], desert plants [19], rice bran [20]. They were prepared in two different methods, physical and chemical activation. In the first method, the raw materials is carbonized by thermal treatment and activated in presence of gasifying agent such as CO_2, steam, air [21]. Chemical activation is the second method in which the raw material is impregnated with chemical such as NaOH, KOH, H_3PO_4, $ZnCl_2$, H_2SO_4, etc. for a period of time [22]. The sample is washed several times and a thermal treatment is given finally.

There are several studies on activated carbon using different raw materials and identified it as a carrier of antibacterial chemical but very limited studies have been taken Citrus limetta or Mosambi peel as raw material for activated carbon preparation and analyze the antibacterial effect.

In the field of medicine, nanoparticles are being explored extensively because of their size dependant chemical and physical properties. The size of nanoparticles is similar to that of most biological molecules and structures. This makes them an interesting candidate for application in both *in vivo* and *in vitro* biomedical research. The result of their studies in the field of medicine has led to their increasing number of application. Adding to these another interesting avenue for their exploration in medicine is their use as antimicrobials to target highly pathogenic microbes. it has been demonstrated that highly reactive metal oxide nanoparticles exhibited excellent biocidal action against Gram-positive and Gram-negative bacteria [23]. It is proven that highly ionic nanoparticulate metal oxides can be prepared with extremely high surface areas and unusual crystal morphologies having numerous edge/corner and reactive surface sites [23]. But, for the application of nanoparticles in biology, biocompatibility is a highly desired trait. Biocompatibility is the materials ability to perform medically without exertion of undesired local or systemic effects [24], to avoid these effects it has to been used to treat with low cost adsorbent.

Citrus limetta peel is being one of the major municipal wastage due to the increasing consumption of Mosambi juice. The Mosambi fruits are commonly known as sweet lime. It is native to South East Asia and cultivated in Mediterranean basin. At present the majority of world population has accepted that Mosambi fruit and its juice enhances energy of the human. After the usage, the peels of the fruits were thrown away as waste material which causes major disposal problems in the environment and causes pollution. Even though there are very many chemicals used as an activating agent H_2SO_4 is the chemical which does not pollute the environment. Hence, H_2SO_4 which is the strong dehydrating agent, which reacts with both organic and inorganic compounds [25], is taken as the activating agent to react with the raw material. In order to convert these waste materials into a value added product without polluting environment mosambi peel has been taken as a precursor and H_2SO_4 as the activating agent of the present study.

Magnesium oxide (MgO) nanoparticles are arranged in an extensive porous structure with considerable pore volume [26] and hence MgONP has been widely used in various areas and recently it has been report that MgONP has a good bactericidal performance in aqueous environments [27] [28]. Not only in aqueous environments, MgONP also exhibited biocidal activity against certain vegetative Gram-positive bacteria, Gram-negative bacteria and the spores in all conditions [29]. Compared with TiO_2, silver, copper and other kinds of solid bactericides, MgONP are found to possess many properties that are desirable for a potent disinfectant [30]. MgONP have a stronger and faster effect on the killing action of both bacteria and spores [30]. MgONPs has the advantage of being prepared from readily available and economical precursors and solvents, and therefore has considerable potential as a solid bactericidal material under simple conditions [31] has been chosen as a bactericidal agent and thus it was chemically synthesised in this present study. Also synthesised MgONP was impregnated on to prepared ACNP to form MgONP-AC nanoparticles. Also it is proposed to study the antibacterial activity of prepared ACNP, MgONP, MgONP-AC nanoparticles using Kirby Bauer method. The MIC and MBC were also been studied. And hence the major objectives of this study are taken as

1. To identify the effective MPAC among different activated carbons prepared on various combination of time and temperature using SEM analysis
2. To synthesis ACNP, MgONP and to form MgONP-AC nanoparticles.
3. To test the anti bacterial effect of the obtained ACNP, MgONP and MgONP-AC nanoparticles.

MATERIALS AND METHODS

2.1 Synthesis of activated carbon nanoparticles

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. Thermo gravimetric experiments were carried out by a Thermal analyzer, NETZSCH-STA 449F3-JUPITER, available at Centralized Instrumentation and Service Laboratory (CISL), Department of Physics, Annamalai University, Tamilnadu, India. In order to measure the pyrolysis behavior of mosambi fruit peel. Then it is crushed in to small pieces and soaked with the chemical activating agent H_2SO_4, in the ratio of 1:1 for twenty four hours. The resulting black products was kept in hot air oven for about three hours at 110^oC and then it is carbonized to different temperatures, within the ranging obtained as a result of TG-DTA analysis at different time periods from 30 minutes to 2 hours in muffle furnace. This activated part of the carbon is washed several times with $NaHCO_3$ followed by distilled water to remove the excess acid present. Then the resulting product is once again kept in hot air oven at $110^oC \pm 5^oC$ for more than 3 hours to obtain a complete fine black activated carbon. Finally, it is preserved in desiccators for further use. Many activated

carbons have been prepared on various combination of time and temperature.The morphology of prepared activated carbons were examined by a scanning electron microscope JEOL, JSM-5610 LV, available at Centralised Instrumentation and Services Lab, Department of Physics, Annamalai University, Tamilnadu, India. By this process among different activated carbon produced, the effective carbon was chosen for further study.

The milling experiment is conducted in a planetary ball mill (Insmart systems, Hyderabad) [32] chosen effective activated carbon. 25 grams of AC were placed in 250 ml tungsten carbide mixing jar together with 5 tungsten carbide milling balls (10mm diameter) and one tungsten carbide milling ball (15mm diameter); giving a ball-to-powder weight ratio of 10:1. The jars were agitated using a high energy planetary ball mill at 300 rpm for 40 hours. 2 ml of methanol was added as a process control agent (PCA) in order to prevent powders sticking to the balls and the jar walls. The powders becomes hot during milling therefore it allowed to cool for every 15 min [33]. Finally at the end of the process the nanosized activated carbon (ACNP) was obtained.

2.2 Synthesis of MgO nanoparticles

Magnesium oxide nanoparticles were prepared by wet chemical method using magnesium nitrate and sodium hydroxide as precursors and soluble starch as stabilizing agent. Starch (0.1 % concentration) solution was prepared in 100 ml of distilled water and Magnesium nitrate 7.42 g (0.05 M) was added to the above solution. Then the solution was kept under constant stirring using magnetic stirrer for complete dissolution of contents. After complete dissolution, 2g of (0.05M) sodium hydroxide solution (25 ml) was added in drops along the sides of the container under constant stirring for 2 hours and allowed to settle for 24 hours. The supernatant liquid was then discarded carefully and the remaining solution was centrifuged (10,000 rpm at 25°C) for 10 minutes. Centrifugate was washed three times using distilled water to remove the byproducts and the excessive starch that bound with the nanoparticles. After washing, the nanoparticles were dried at 80^{o}C for overnight and then the nanoparticles of magnesium hydroxide were placed in furnace at 200°C for 2 hours. During this process, conversion of magnesium hydroxide into magnesium oxide takes place. The following reaction explains the formation of magnesium oxide nanoparticles [34].

2.3. Preparation of MgONP-AC nanoparticles

For impregnation, 50gm of powdered ACNP added with 250 ml of MgONP solutions at a concentration of 500ppm were stirred vigorously in room temperature for overnight and then cured at 400^{o}C for minimum of 2hrs in muffle furnace. Finally the MgONP-AC was obtained.

2.4 Antibacterial Activity

2.4.1 Determination of zone of inhibition

The Kirby-Bauer test known as the disc diffusion method is the most widely used antibiotic susceptibility test to determine the zone of inhibition. This test is used to determine the susceptibility of bacteria to specific particle. In Kirby-Bauer method of determining the sensitivity of bacteria, the test solutions of compounds such as ACNP, MgONP, MgONP-AC have been prepared individually by dissolving a known weight of compounds such as ACNP, MgONP, MgONP-AC into 50% of Dimethyl sulfoxide (DMSO) to give a concentration of 250µg/ml. Inoculum has been prepared by mixing the 24hrs old culture of selected seven bacteria such as *Staphylococcus aureus, Escherichia Coli (E.Coli), Klebsiella pneumoniae, Proteus mirabilis, salmonella typhi, Pseudomonas aeruginosa, Vibrio cholerae* with physiological saline in a seven different containers individually until the turbidity was corrected to McFarland standard (i.e.) 1(10^6 cfu/ml). The mixture of MHA and sterile solution was poured in 7 petridishes with surface level of 4-5mm uniform thickness. Allow the agar to solidify at room temperature. Apply the seven inoculum prepared using seven cultured bacteria such as *Staphylococcus aureus, Escherichia Coli (E.Coli), Klebsiella pneumoniae, Proteus mirabilis, salmonella typhi, Pseudomonas aeruginosa, Vibrio cholerae* evenly spread using standard swaps on seven different MHA plates and kept aside the inoculated plates 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 dry plates at equal distance from the centre point of plates using sterilized forceps. The plates were incubated at 37ºC for 24hrs and the area of inhibition of bacterial growth for test solutions and DMSO solution (i.e.) control against each bacteria were measured from plates with different bacteria have been recorded individually.

2.4.2 **Determination of Minimum Inhibitory and Bactericidal Concentration (MIC and MBC):**

The MIC and MBC values were determined using Macrodilution broth method. In this method, One ml of compounds such as ACNP, MgONP, MgONP-AC (1mg/ml) was incorporated into 1ml of nutrient broth was serially diluted to obtain concentration of 1000µg/ml, 500µg/ml, 250µg/ml, 125 µg/ml and 62.5 µg/mlrespectively. 20µl/ml of the bacterial inoculum was added to each of the test tubes. The tube without compounds served as control. The tubes were incubated at 37ºC for 24hrs. The readings were recorded. MIC was recorded as the lowest concentration of a compound at which no visible growth of bacterial isolates occurred after a period of time.

After the recording of MIC, the culture was transferred from the each test tube containing 1000µg/ml, 500µg/ml, 250µg/ml, 125 µg/ml and 62.5 µg/ml compounds to theMueller- Hinton Agar plate separately for the presence and absence of growth. After incubation period the lowest concentration of the compounds that inhibit the complete growth of bacterial isolates recorded as MBC.

RESULTS AND DISCUSSION

3.1 TG – DTA Analysis (Thermo gravimetric-Differential Thermal Analysis)

The thermal decomposition of mosambi fruit peel showed the following behavior when the temperature is increasing from 100^{o}C to 1000^{o}C at a rate of 10^{o}C per minute. In TG curve, the dried mosambi fruit peel gets decomposed in three stages and this was shown in fig 2

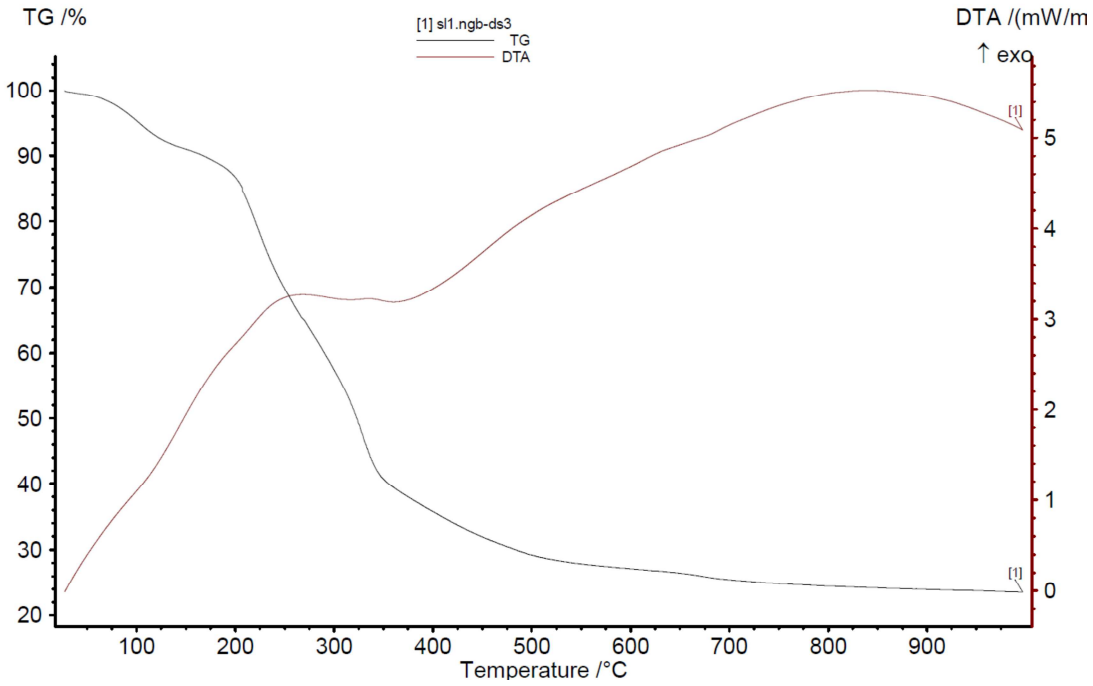
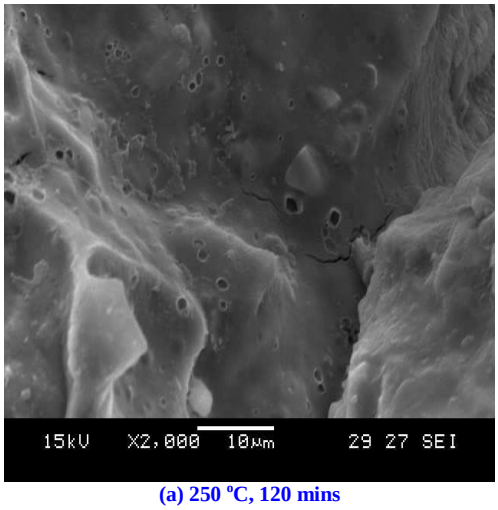


Fig 1 Thermal analysis of raw mosambi peel

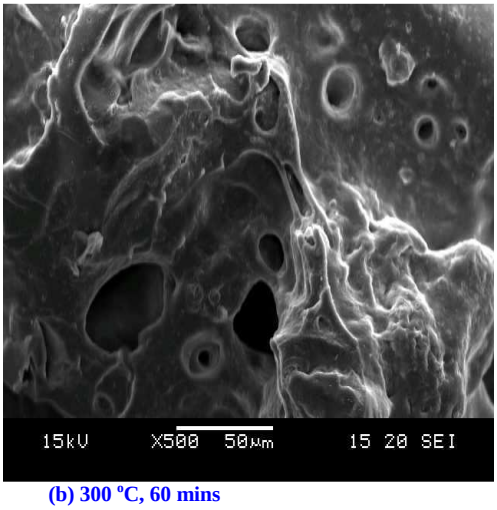
Fig 1 reveals that the weight loss takes place at three stages. In the first stage below 200 °C, the weight loss of about 16% takes place due to the elimination moisture content. In the second stage (200 °C to 360 °C), maximum devolatisation takes place with a weight loss of (44%). This is due to decomposition of hemicelluloses followed by degradation of celluloses. In the third stage (360 °C to 850 °C), a decomposition rate of 18% is observed which is due to the degradation of lignin content. From the figure 4.1 it is noted that almost 90% of weight loss have been takes place between 100 ^{o}C and 500 ^{o}C of the activation temperature. Hence, for the precursor (MosambiPeel) the optimum activation temperature lies between 100 ^{o}C and 400 ^{o}C and it may be 350 °C because at this temperature considerable weight loss were taken place

3.2 Preparation and Optimisation of time and temperature by Surface Morphology of Activated carbon

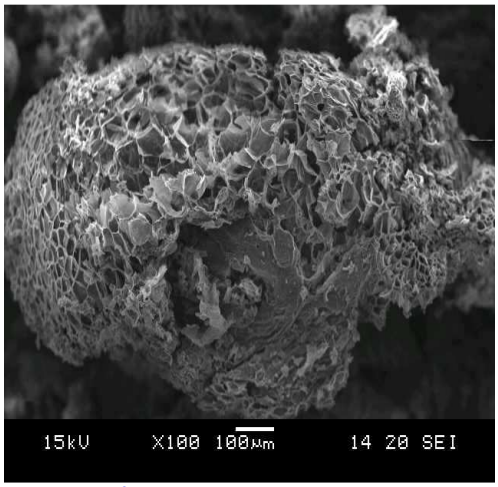
The scanning electron microscopy (SEM) technique is one of the methods to examine the morphological properties of the prepared AC. In this study, the raw material gets carbonized and activated at different temperatures (100 ^{o}C-500 ^{o}C) and time periods ranging from (30-120) minutes. The SEM photographs of the prepared AC at different temperatures and time intervals with a constant impregnation ratio 1:1 were shown in Fig 2 (a) – (e) respectively. By analysing the surface morphology of prepared AC from SEM photographs, the optimum temperature and time for the preparation of MPAC were identified. The sample is heated in a muffle furnace at a rate of 10^{o}C/ min. First the sample is heated from (100 ^{o}C- 200 ^{o}C) for a time interval of (30–120) mins. At this temperature only the surface of the sample gets heated. By keeping the time intervals constant, the temperature is increased to 250 ^{o}C, the heat slowly enters into the layers of the sample. At 120 min, the outer surface of the sample shows a slight change to form holes. At 300 ^{o}C and 60 mins time interval the carbonization takes place, small holes named as the micropores were formed. Large numbers of micropores were formed on the surface of the raw material for the same temperature but time period is varied up to 120 mins. At low temperature, the pore structure mainly consisted of micropores. The sample is partially carbonized but not activated.



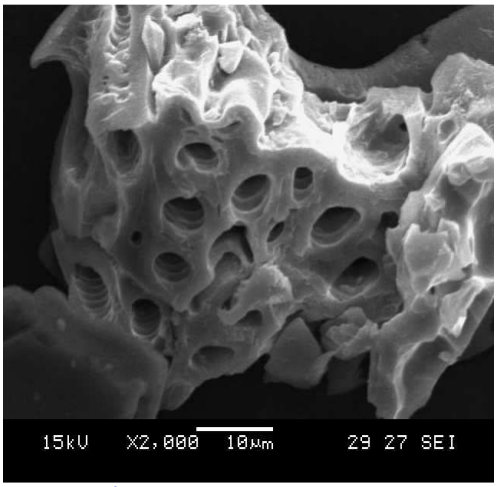
(a) 250 °C, 120 mins



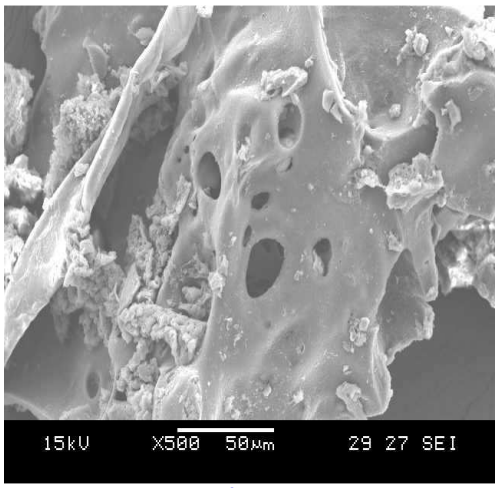
(b) 300 °C, 60 mins



(c) 350 °C, 90 mins



(d) 400 °C, 30 mins

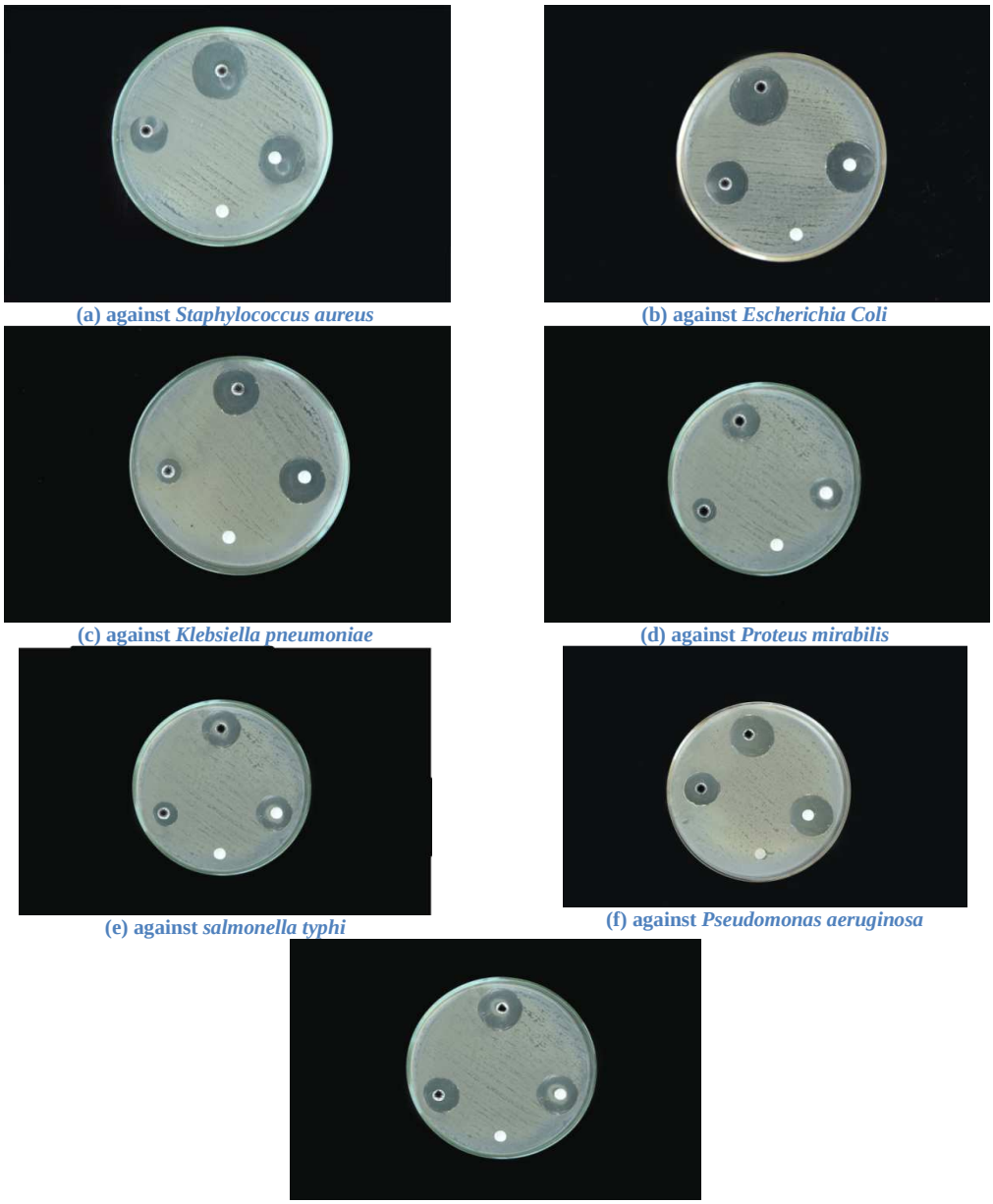


(e) 450 °C, 30 mins

Fig 2(a) - (e) SEM images of MPAC at different temperature and time period

For 350 °C temperature and 60 min time period, slowly the micropores starts to expand to form mesopores. The mesopores were formed due to breakup of the walls of the micropores. Exactly at 90 mins a well defined porous structure is obtained. In this stage the sample is completely activated. At temp 400 °C, for a time period of 30 minutes, the mesopore and micropore region combines to form macropores. When the time period is increased further full form of definite shape macropores were obtained. In this stage the carbonization process comes to an end. The temperature is further increased to 450 °C by keeping the same time period 30 mins, the macropores starts

to expand and get collapsed to form a big crater like structure. This high temperature affects the porous structure of the carbon. At 500 ^{o}C, and 30 mins time period the sample completely turns to ash. From this it clearly indicates that the activating temperature and time had significant effect on the porous structure of AC.



(a) against *Staphylococcus aureus* **(b) against *Escherichia Coli***

(c) against *Klebsiella pneumoniae* **(d) against *Proteus mirabilis***

(e) against *salmonella typhi* **(f) against *Pseudomonas aeruginosa***

(g) against *Vibrio cholera*

Fig 3 (a)-(g) Antibacterial activity test results of DMSO, ACNP, MgONP and MgONP-AC against selected bacterial strains

Fig 2(a) represents the presence of small holes on the surface of the AC at temperature 250 ^{o}C at a period of time 120 mins. Fig 2(b) represents the formation of micropores at 300 ^{o}C at time 60 mins. Fig 2(c) represents the formation of mesopores at 350 ^{o}C at time 90 mins. Fig 2(d) represents the macropore region at 400 ^{o}C at time 30 mins. Fig 2(e) represents the eroded macropore region at 450 ^{o}C at time 30 mins. Previously the efforts do not go beyond some primary interpretations of the performance of the adsorbents in terms of their textural properties (porosity, surface area). More recently, some authors have stated that it will be beneficial to have an AC with sufficient amount of super mesoporosity for the enhanced solute adsorption. Hence it is concluded that as a result of SEM analysis the AC prepared at 350^{o}C and at time 90 mins represented in Fig 2(c) was the effective adsorbent. It was then converted into ACNP using the planter ball mill as described in section 2.1.

3.3 Antibacterial activity

(a) Kirby-Bauer Method

The experiment was carried out following the procedure described in section (2.3.1). The experiment was carried out in triplicate and the one disc shows best result of three was shown in Fig 3(a)-(g).

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 discs in each plate represent the activity of plain DMSO solution which act as control (bottom), ACNP (left), MgONP (right), and MgONP-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 table 1

Table 1 Zone of Inhibition of ACNP, MgONP and MgONP-AC against selected bacterial strains

Bacterial strains	Zone diameter of ACNP (mm)	Zone diameter of MgONP (mm)	Zone diameter of MgONP-AC (mm)
Staphylococcus aureus	15	20	24
Escherichia Coli	15	23	**26**
Klebsiella pneumoniae	3	20	**24**
Proteus mirabilis	5	10	**15**
salmonella typhi	18	23	27
Pseudomonas aeruginosa	19	25	26
Vibrio cholerae	20	24	25

From the table 1, it is noted that the anti-bacterial activity of ACNP showed 20 mm., 19 mm., 18mm., 15mm., 15mm., 5mmm., 3mm., against *Vibrio cholera, Pseudomonas aeruginosa, salmonella typhi, Staphylococcus aureus, Escherichia Coli (E.Coli), Proteus mirabilis,* and *Klebsiella pneumonia* respectively. Anti-bacterial activity of MgONP showed 25, 24, 23, 23, 20, 20, and 10mm against *Pseudomonas aeruginosa, Vibrio cholera, Escherichia Coli (E.Coli), salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae,* and *Proteus mirabilis* respectively. Anti-bacterial activity of MgONP-AC showed 27, 26, 26, 25, 24, 24, and 15 mm against *salmonella typhi, Escherichia Coli (E.Coli), Pseudomonas aeruginosa, Vibrio cholera, Staphylococcus aureus, Klebsiella pneumoniae,* and *Proteus mirabilis* respectively. These reveal that for all the selected bacterial strains the compound MgONP-AC exhibited higher activity than others and this may due to synergistic effect of compounds presented in MgONP-AC [35].

(b) Macrodilution broth method

The compounds that were found effective, as antimicrobial agent, were later tested to determine the MIC and MBC values for each strain. The MIC is the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after overnight incubation [36] and the MBC is the lowest concentration of antibiotic required to kill a particular bacterium [36] [37]. In this study the MIC and MBC values of the prepared ACNP and metal nanoparticles such as MgONP, AgNP, CuONP, ZnONP against *Staphylococcus aureus, Escherichia Coli (E.Coli), Klebsiella pneumoniae, Proteus mirabilis, salmonella typhi , Pseudomonas aeruginosa, Vibrio cholerae* respectively were identified following the procedure described in section (2.3.1) and presented in table 2.

Table 2 MIC and MBC values of ACNP, MgONP and MgONP-AC against selected bacterial strains

Bacterial strains	ACNP		MgONP		MgONP-AC	
	MIC (µg)	MBC (µg)	MIC (µg)	MBC (µg)	MIC (µg)	MBC (µg)
Staphylococcus aureus	125	250	125	125	125	125
Escherichia Coli	125	250	125	125	125	125
Klebsiella pneumoniae	250	250	125	125	125	125
Proteus mirabilis	250	250	250	250	250	250
salmonella typhi	125	125	125	125	125	125
Pseudomonas aeruginosa	125	125	125	125	125	125
Vibrio cholerae	125	125	125	125	125	125

The Minimum Inhibitory concentration value of ACNP was 125 µg against *Staphylococcus aureus, Escherichia Coli (E.Coli), salmonella typhi, Pseudomonas aeruginosa,* and *Vibrio cholerae* and 250µg against *Klebsiella pneumoniae, Proteus mirabilis.* The Minimum Bactericidal Concentration value of ACNP was 125 µg aganist *salmonella typhi, Pseudomonas aeruginosa, Vibrio cholera* and 250µg aganist *Staphylococcus aureus, Escherichia Coli (E.Coli), Klebsiella pneumoniae,* and *Proteus mirabilis.* The Minimum Inhibitory concentration value and the Minimum Bactericidal Concentration value of MgONP and MgONP-AC were 125 µg against *Staphylococcus*

aureus, Escherichia Coli (E.Coli), Klebsiella pneumoniae, salmonella typhi, Pseudomonas aeruginosa, Vibrio cholerae and 250µg against *Proteus mirabilis*. These reveal that the MIC and MBC values do not differ between MgONP and MgONP-AC. For all the compounds the MIC and MBC value lies only on two categories (125µg and 250µg).

CONCLUSION

From this study, it is clear that Sulphuric acid is a strong activating agent which reacts with the mosambi fruit peel to produce a well developed porous structured activated carbon. The characteristic study reveals that the mosambi fruit peel is a suitable raw material for preparing porous carbons at the temperature range between 100 and 400 ^{o}C. The efficient activated carbon was obtained at the optimum temperature 350^{o}C and the optimum time of 90 minutes and it was identified by studying surface morphology of activated carbon using SEM analysis. Then it was converted into ACNP. The nanoparticles such as MgONP and MgONP-AC were also synthesized. All the three compounds exhibits antibacterial activity against selected bacterial strains such as *Staphylococcus aureus, Escherichia Coli (E.Coli), Klebsiella pneumoniae, Proteus mirabilis, salmonella typhi , Pseudomonas aeruginosa, Vibrio cholerae* and against all the bacterial strains the compound MgONP-AC exhibited higher activity than others. The MIC and MBC values do not differ between MgONP and MgONP-AC. For all the compounds the MIC and MBC value lies only on two categories (125µg and 250µg). Hence it is suggested that instead of using MgONP as antibacterial agent individually they can be used by impregnating into ACNP in order to enhance their effectiveness and to reduce their adverse effects.

REFERENCES

[1] *NHS Direct* (UK electronic health service), 2010.
[2] Jump up *European Centre for Disease Prevention and Control*, **2014**.
[3] Serkar Timur, Ismail Cern Kantauli, Sermin Onene, Jale Yanik, *Journal of Analytical and Applied Pyrolysis*, **2010**, 89, 129 – 136.
[4] Hung, Y.T, Lo, H.H, Wang, L.K, Taricska, J.K, Li, K.H, Shammas, N.K, *Handbook of Environmental Engineering*, **2005**, 3, 573 – 634.
[5] Taylor, R, Marsh, H, Heintz, E.A, Rodriguez-Reinoso, F, *Universidad de Alicante, Secretariado de Publications, Alicante*, **1997**, 167 – 210.
[6] Rodriguez-Reinoso, F, Sehuth, F, Sing, K.S.W, Weitkamp, J, *Wiley – VCH, Weinheim Germany*, **2002**, 1766 – 1827.
[7] Dabrowski, A, *Advanced Collid Interface Science*, **2001**, 93, 135 – 224.
[8] Lee, J, Kaim, J, Hyeon, T, *Advanced Matter*, **2006**, 18, 2073 – 2094.
[9] Reimeink, W.M.T.M, *Stud, Surf. Sci. Catal*, **1999**, 120A, 751 – 759.
[10] Aygun, A, Yenisoy – Karakas, S, Dumal, I, *Microporous and Mesoporous Materials*, **2003**, 66, 189 – 195.
[11] Sumathy, S, Bhatia, S, Lee, K.T, Mohamed, A.R, *Bio Resources Technology*, **2009**, 100, 1614 – 1621.
[12] Suat Vcar, Murat Erdem, Turgay Tay, Selhan Karagoz, *Applied Surface Sciences*, **2009**, 255, 8890 – 8896.
[13] Sudaryanto, Y, Hartono, S.B, Irawaty, W, Hindarso, H, Isnadji, S, *Bio Resources Technology*, **2005**, 96, 1578 – 1583.
[14] Kula, I, Ugurlu, M, Karaoglu, H, Celik, A, *Bio Resources Technology*, **2008**, 99, 492 – 501.
[15] Veksha, A, Sasoake, E, Azharuddin, M.D, *Carbon*, **2009**, 47, 2371 – 2378.
[16] Karthekeyan, T, Rajagopal, S, Lima Rose Miranda, *Journal of Hazardous Materials*, **2005**, 124, 192 – 199.
[17] Valix, M, Cheung, W.H, Mckay, G, *Chemosphere*, **2004**, 56, 493 – 501.
[18] Yagmur, E, Ozmak, M, Aktas, Z, *Fuel*, **2008**, 87, 3278 – 3285.
[19] Reffas, A, Bernardet, V, David, B, Reinert, L, Lehocine, M.B, Dubois,M, Batisse, N, Duclaux, L, *Journal of Hazardous Materials*, **2010**, 175, 779 – 788.
[20] Benderdouche, N, Bestani, B, Benstaali, B, Derriche, Z, *Adsorption of Science and Technology*, **2003**, 21, 739 – 750.
[21] Suzuki, R.M, Andrade, A.D, Sousa, J.C, Rollemberg, M.C, *Bio Resources Technology*, **2007**, 98, 1985 – 1991.
[22] American Standard for testing materials (ASTM), D 3173 – 3175.
[23] Stoimenov, P. K., Klinger RL, Marchin GL, Klabunde KJ, *Langmuir*, **2002**, 18, 6679–6686.
[24] Samia ACS, Dayal S, Burda C, *Photochemistry and Photobiology*, **2006**, 82,617-625.
[25] Ould, A, Driss, I, Stitou, M, Cuerda - Correa, E.M, Fernandez – Gonzalez, C., Macias – Garcia, A, Alexandere – Franco, M.S, Gomez – Serrano, V, *Fuel Processing Technology*, **2011**, 92, 261-265.
[26] Klabunde KJ, Stark J, Koper O, Mohs C, Park D, Decker S, Jiang Y, Lagadic I, Zhang D, *J. Phys. Chem.* **1996**,100, 12142-12153.
[27] Cui. Z., G.W. Meng, W.D. Huang, G.Z. Wang, L.D. Zhang, *Materials Research Bulletin* **2000**, 35, 1653–1659.

[28] Kim H.W., Seung Hyun Shim and Chongmu Lee, *Journal of the Korean Physical Society*, **2006**, 49(2), pp. 628-631.
[29] Richards R, Li W, Decker S, Davidson C, Koper O, Zaikovski V, Volodin A, Rieker T, Klabunde K., *J. Am. Chem. Soc*, **2000**, 122, 4921-4925.
[30] Koper O, Klabunde J, Marchin G, Klabunde KJ, Stoimenov P, Bohra L, *Current Microbiology*, **2002**, 44, 49-55.
[31] Shah M. A, *African Physical Review*, **2010**, 4:0004, 21-23.
[32] Gujjala Raghavendra, Shakuntala Ojha, Samir Kumar Acharya, S K Pal, *International Journal of Material Science Innovations (IJMSI)*, **2014**, 2(3), 59-68.
[33] Babu RJ, Catherin GJ, Narasimha Murthy I, Venkata Rao D,Nooka Raju B, *International Journal of Engineering, Science and Technology*, **2011**, 3(4), 82-88.
[34] Sundrarajan , Suresh, Rajiv Gandhi, *Digest journal of nanomaterials and biostructures*, **2012**, 7(3), 983 – 989.
[35] Sreeprasad, T, and Pradeep T, *Inter. J. Modern. Phy*, **2012**, 21-26.
[36] Kaya O, Akçam F, Yaylı G., *Turk J Med Sci*, **2012**, 42, 145–148.
[37] Hoşgor Limoncu M, Ermertcan Ş, Eraç B, Taşlı H, *Turk J Med Sci*, **2011**, 41, 719–724.