



Preparation of activated carbon by chemical activation method from the plant *Wrightia tinctoria* and its application

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Abstract

Activated carbon (AC) has been widely used in wastewater treatment for the removal of various pollutants because of its large surface area and high adsorption capacity. In the present study, the phytochemical analysis of the indigenous medicinal plant *Wrightia tinctoria* revealed the presence of Alkaloids, Flavonoids, Sterols, Glycosides, Terpenoids, and Phytosterols. The activated carbon was prepared using *Wrightia tinctoria* and characterized by Field Emission-Scanning Electron Microscopy (FE-SEM), Fourier Transform Infra-Red Spectroscopy (FT-IR) & Energy-dispersive X-ray spectroscopy. Parameters analyzed for effluent treatment analysis include Alkaloid, Chloride, Hardness, COD (chemical oxygen demand), TDS (total dissolved solids), and pH. The application of AC was tested towards methylene dye degradation and the best result with 0.09mg concentration. The antimicrobial activity using the agar well diffusion method against the five bacterial cultures was studied by comparing silver nitrate, silver nitrate+AC, and regular ampicillin (25 mcg). Silver nitrate+AC with a 23 mm zone of inhibition produced the best results. *In vitro* tests revealed that AC has a positive impact on mung seed germination.

Keywords: *Wrightia tinctoria*, activated carbon, antimicrobial activity, phytochemical analysis, dye degradation, effluent treatment.

Introduction

Activated carbon (AC) or activated charcoal is a form of carbon-containing small low pores which increase the exterior area for the adsorption process. AC surface area is suitable for methods like metal extraction from industrial waste purification, treatment of sewage, air conditioning filters, and gas mask manufacturing [1]. Physical and chemical methods used to prepare the AC material. The activated carbon was separated using physical methods at high temperature. The thermal decomposition of raw material with chemicals is used to perform chemical activation in a single phase [2]. ZnCl₂, H₃PO₄, HCl, and H₂SO₄ are acidic reagents used in chemical activation, while Na₂CO₃, KOH, and K₂CO₃ are basic reagents [3].

AC was prepared from animal bones [4], algae [5], sawdust [6], chitosan [7], the mixture of orange peels-coffee grounds-melon seeds [8] and sorghum grains [9]. The fibrous protein collagen makes up 30% of the weight of animal bones, while the remaining 70% is made up of inorganic hydroxyapatite with defect poorly crystalline cation and anion substitutes [4].

AC and various low-cost materials such as peat, coal and coconut shells have been studied for their ability to adsorb various hazardous materials, especially heavy metals [10]. The AC through phosphoric acid chemical activation method from latex producing plant rubberwood sawdust has a high surface area [11]. The chemical activation method was used for the preparation of AC from coir pith and adsorption of toxic heavy metals - mercury(II), lead (II), cadmium(II),

nickel(II), and copper(II) were reported and studied. The adsorption pH increases from 2 to 6 and remains constant up to 10. The cost-effective removal of toxic heavy metals from industrial wastewater using discarded coir pith from coir manufacturing industries [12].

The granular AC adsorbs pharmaceutical components namely acetaminophen, diclofenac, and sulfamethoxazole [13]. The AC polymeric coating or microencapsulation of charcoal within polymers has improved their bond compatibility. The activated carbon is encapsulated within chitosan (ACCB) beads were comprehensively investigated for the removal of various toxins such as urea, creatinine, uric acid & bilirubin [14].

Wrightia tinctoria belongs to the family, Apocynaceae. It is a small deciduous tree having pale grey color, smooth bark, distributed in tropical Africa, Southeast Asia, and Australia. Pala Indigo Plant, Sweet Indrajao and Dyer's Oleander are some of the other names for this plant. In India, medicinal preparations use the whole plant or plant-specific sections such as bark, leaf, seed, and root [15]. Bark and seeds are used in the treatment of bilious fever, patches of abnormal skin, Hansen's disease, asthma, and other skin diseases [16]. The leaves juice is used widely in the treatment of jaundice, and the crushed fresh leaves relieve toothache. Its bark ethanol extract showed immune, modulatory activity, and protective activity against an acute gastric ulcer in experimental rats [17].

It has been traditionally used with special emphasis on pharmacological action like analgesic [18], anti-

inflammatory, anthelmintic [19], antiulcer [20], antidiabetic [21], anti-cancer, antipyretic, antidysenteric activity [22], aphrodisiac, astringent and antimicrobial properties. Silver nanoparticles had been synthesized and characterized from *Wrightia tinctoria* [23]. It has the pharmacological potential to be used as an anti-inflammatory agent when tested in several animal models [24]. It is an important ethnomedicinal plant widely used to treat skin diseases in folk medicine [25]. The effects of *Wrightia tinctoria* leaves extract and *Morinda citrifolia* fruit powder on HIV-1(IIIB) replication in MT-4 cells and HCV replication in Huh 5,2 cells were investigated. In MT-4 cells, *Wrightia tinctoria* chloroform extract showed 48% defence against cytopathic effects on HIV-1 (IIIB) [26].

Bacteria is supposed to breed on the surface of activated carbon fibres, ultimately becoming a pollutant. As a result, silver-loaded fibre could effectively combat and destroy bacteria [27, 28] and will benefit from the high adsorption potential of activated carbon fibre while avoiding biological contamination [29]. Activated carbon had an application in plant tissue culture and was used in the germination of orchid [30]. Adding activated charcoal to the medium in plant tissue culture encourages growth by adsorbing toxic metabolites, according to studies [31].

In light of this, the aim of this study was to prepare and characterise activated carbon from *Wrightia tinctoria* in order to investigate its antibacterial properties, dye adsorption, and seed germinating potential. The phytochemicals were screened for secondary metabolites.

Materials and methods

Reagents and Chemicals

Hi-media and Sigma chemicals supplied all of the reagents and media elements.

Plant materials Collection

Wrightia tinctoria is popularly known as the jaundice curative plant was collected from the local area in and around Erode, Erode District, Tamil Nadu, India.

Phytochemical screening

Phytochemical screening was performed according to Harborne's (1984) [32] method. The dried leaves powder of *Wrightia tinctoria* was screened for the presence of Alkaloids, Tannins, Phytosterols, Phenols, Flavonoids, Sterols, Terpenoids, Saponins, Glycosides, Carbohydrates, Proteins, Gums and Mucilages.

Preparation of activated carbon

Harvested mature green leaves were extensively washed with double distilled water. The leaves were chopped into small pieces shade dried for a week and powdered 50 g of powder was taken and mixed with sulphuric acid and kept for two days. To obtain activated carbon, the carbonized material was filtered through a cotton cloth, washed with double distilled water, and dried in a hot air oven for two days at 180 °C [3].

Characterization of activated carbon

FE-SEM (Field Emission Scanning Electron Microscopy)

Topographic information attainable using the FE-SEM allows that surface features such as pore characteristics, the description of which has been a significant preoccupation of

AC chemicals studied and measured directly from FE-SEM micrographs.

FTIR (Fourier-Transform Infra-Red Spectroscopy)

The chemical structure and functional groups were calculated by analysing the infrared spectrum. The FTIR analysis of the prepared AC was performed.

EDX (Energy-Dispersive X-ray analysis)

It's used to figure out the elements present in the sample of specimen.

Antimicrobial activity

Sample Preparation

0.1gm of activated carbon was grained and mixed with 1ml of dimethyl sulfoxide (DMSO) (Kannan *et al.*, 2006) [33].

Preparation of Silver Nitrate

1ml of distilled water was mixed with 0.5mM silver nitrate.

Bacterial Strains

Bacillus cereus (MTCC No.– 430), *Escherichia coli* (MTCC No.– 1698), *Klebsiella pneumoniae* (MTCC No.– 10309), *Pseudomonas aeruginosa* (MTCC No.– 424), and *Staphylococcus aureus* (MTCC No. – 3160) were used for analysis.

Determination of Antimicrobial Activity

The antimicrobial activity of activated carbon was assessed using the proper diffusion technique by the Kirby-Bauer method [34]. Test organisms were swabbed on air-dried nutrient agar plates using sterile cotton swabs. The wells were loaded with 100 µl of dissolved activated carbon on the surface of nutrient agar plated swabbed with bacterial cultures and control was also maintained. The activity of AC, silver nitrate, and combination were determined and the zone of inhibition was measured. The plates were incubated at 37 °C overnight, and the activity was determined by measuring the zone of inhibition around each disc in mm (Excluding the diameter of the disc).

In vitro seed germination

Seed Collection

Mung bean (*Vigna radiate*) seeds were purchased from the local market.

Materials Preparation

Seeds were washed with sterile distilled water thrice and then the seeds were inoculated onto the culture medium surface containing AC.

Sterilization

The explants were rinsed for 10 minutes in running tap water to remove the soil and other foreign particles. The explants were rinsed with double distilled water and then with two drops of Tween 20. Later the sample was washed for 30 seconds with 70% ethanol, followed by 10 minutes of rinsing with freshly prepared mercuric chloride solution (0.1%). Until inoculation, the explant was washed 3-4 times with double distilled water.

Washed with 70% ethanol for 30 seconds, followed by rinsing with freshly prepared mercuric chloride solution (0.1%) for 10 minutes. Finally, the explant was washed with double distilled water 3-4 times before inoculation.

Media Composition

Properly surface-sterilized seeds were inoculated on Murashige and Skoog basal media [35] supplemented with growth regulator hormone IAA acid (0.1/50 ml), 3.0 % sucrose, and 0.7% agar as a solidifying agent [36]. Three different concentrations of AC (0.1, 0.2 & 0.3 gm) were added in MS media and seed germination of the seed was analyzed. The pH of the media is maintained at 5.7 with 1N NaOH or HCl before 20 minutes of sterilization. The proliferation of germination seeds was observed [37].

Dye degradation

Preparation of Dye

About 0.1 gm of methylene blue was dissolved in a 1000ml volumetric flask and diluted up to the mark by the addition of deionized water.

Dye Degradation

Different concentration (0.07, 0.08, 0.09 & 0.1 mg) of AC was added and analyzed by using UV-Visible spectrometer at the wavelength of 665 nm [38].

Wastewater treatment plant

Filtration Method

Wastewater treatment analysis was done with the effluent collected from industries near Perundurai, Erode District, Tamil Nadu, India. The effluent treatment was carried out by the filtration method using the AC filter. The wastewater was allowed to pass through the filter and the filtrate obtained was collected for further analysis [39].

Results and Discussion

The Phytochemical screening of *Wrightia tinctoria* was performed according to the method of Harborne [32] showed the presence of alkaloids, flavonoids, sterols, glycosides, terpenoids, and phytosterols (Table-6). Terpenoids have anti-malarial, anti-cancer, anti-inflammatory, cholesterol-synthesis-inhibiting, anti-viral, anti-bacterial, and anti-viral properties [40]. The preparation of AC from the plant leaves was done by the chemical activation method using H₂SO₄ (Fig.-1). Similar studies were carried out using coconut shells [41] and agricultural waste [42]; where AC was oxidized by concentrated HNO₃. The presence of a dark black colour when *Wrightia tinctoria* dried leaves powder was combined with a concentrated sulphuric acid solution suggested the formation of AC primarily due to the decomposition of the plant cell wall by concentrated sulphuric acid. Since the efficacy and significance of AC are based on the adsorption mechanism, surface chemistry, and porosity, different characterization techniques (FE-SEM, EDX, and FTIR) were used to investigate the prepared substances. The FE-SEM studies confirmed that the formation of AC in the size range of 20µm showing, honeycomb shapes like structure which was found to be the same as reported earlier [43] and 5µm showing ice flood like structure (Fig.-2 a & b). Heating at various steps of the AC preparation led to the resulting structure & better used for the adsorption of various compounds. The analysis through FTIR helped to identify the chemical bond and the functional groups. The peak at 2353.16cm⁻¹ corresponded to the strong vibrations of C-N triple bond indicated the presence of Nitriles, the band at 3780.48cm⁻¹ referred to the OH stretching with strong vibrations revealed the Hydroxyl groups and was found to

be similar to the earlier report on physical activation used to characterize activated carbon made from *Albizia lebeck* [43]. The peak at 439.77cm⁻¹ with C-H stretching weak vibrations confirmed the presence of hydrocarbons. The medium vibrations of C-H stretching were attributed to the confirmation of aromatic rings, and the carboxylic group was ratified with O-H stretching strong vibrations [44] (Fig.-3 & Table-1). A similar research on Xylenol Orange adsorption on *Kigelia africana* and *Melia azedarach* fruits based on the presence of carboxyl group supported the presence of carboxyl group [45]. The peak at 2877.77cm⁻¹ attributed to CH stretching revealed the presence of aldehydes and the peak at 555.5 cm⁻¹ indicated the presence of iodides. The concept of surface functional groups was recorded to offer insight into the adsorption capability of the activated chars generated [46]. The study on AC from various agricultural wastes by chemical activation with KOH had supported the current study in FTIR characterization and the functional groups identified [47]. EDX analysis revealed the contents as Carbon (52.95%), Oxygen (33.93%), Silicon (0.43%), Sulfur (8.49%), Calcium (4.20%), and the highest percentage of AC were identified as carbon (Fig.-4 & Table-2). Since the prepared AC has well-defined porosity of various shapes and sizes, as well as the presence of various high carbon functional groups, it could be used for dye removal [48]. The formation of activated carbon was rapid and the reaction was completed in 2 days when comparing to physical activation. This made the investigation highly significant for the rapid formation of activated carbon. As reported earlier, the objective of relatively well-developed porous AC by chemical activation method was achieved [49]. The antimicrobial activity of AC, silver nitrate, and combination were determined and the zone of inhibition was measured. AC & Silver nitrate profoundly inhibited the microorganism when combined especially against *Escherichia coli* and *Staphylococcus aureus* which revealed the zone of inhibition as 23 mm and 23 mm respectively (Table-3). It was earlier analyzed that activated carbon is the widely used nanoparticles for water purification because of its large surface area and high adsorption capacity [50]. Its reported that activated carbon was found to be a powerful antimicrobial agent and bacteria adsorbent, supporting the current study and suggesting that carbon with antimicrobial properties may be used in water filtration systems and cosmetics [51]. Surface sterilized seeds of mung beans were inoculated on MS basal media supplemented with varying concentrations of growth regulator hormone IAA (control). The germination of seeds was observed for about 7 days. The seeds sprouted in 3 days. The measurement of plant growth was noted, and the maximum growth of 7.5 cm was recorded in 0.1 mg AC supplemented media (Fig.-5 a to c). Using similar approaches, orchid seeds were germinated in vitro, supporting the current study's findings that activated carbon helps seed germination [52,30]. So it could be used in seed propagation as well as the breaking of dormancy. Methylene blue dye degradation was measured and analyzed using different concentrations (0.07, 0.08, 0.09, and 0.1 mg) of activated carbon. The high degradation of dye was observed with a 1mg concentration (Fig.-6 a to e; Table-4). A related analysis of methylene blue removal using AC from the *Bombax cieba* tree [53], pea shell [54], rice husk [55], and coir pith [56] served as support for the current work. The sample was allowed to move through the activated carbon filter during the wastewater treatment analysis. The filtrate was collected in separate beakers and tested for various parameters such as alkaloids, chloride, hardness, TDS, COD, and pH, and it yielded positive results across the board (Table-5).

Table 1: FTIR Analysis of Prepared Activated Carbon

Frequency (cm ⁻¹)	Bonding	Type of vibration	Functional group
2353.16	C-N triple bond stretch	Strong	Nitriles
3780.48	OH stretch	Strong	Hydroxy group
439.77	C-H stretch	Weak	Hydrocarbons
47063	C-H stretch	Medium	Aromatic ring
3711.04	O-H stretch	Strong	Carboxylic group

Table 2: EDX Analysis of Activated Carbon

S. No.	Element	Weight	Atomic %	Error
1.	C k	52.95	63.76	7.45
2.	O k	33.93	30.67	9.15
3.	Si k	0.43	0.22	26.98
4.	S k	8.49	3.83	4.74
5.	Ca k	4.20	1.52	11.66

Table 3: Comparative Antimicrobial analysis of Activated Carbon, Silver Nitrate & Combined with standard

Microorganisms	Zone of inhibition in diameter (mm)			
	Activated Carbon (0.1g/ 1ml DMSO) (100µl)	Silver Nitrate (0.5mM) (100µl)	Combination of Activated Carbon and Silver nitrate (100µl)	Ampicillin (Standard) (25mcg)
<i>Escherichia coli</i> (MTCC No. - 1698)	14	15	23	10
<i>Bacillus cereus</i> (MTCC No. - 430)	13	14	16	12
<i>Klebsiella pneumoniae</i> (MTCC No. - 10309)	15	17	16	15
<i>Pseudomonas aeruginosa</i> (MTCC No. - 424)	15	14	14	10
<i>Staphylococcus aureus</i> (MTCC No. - 3160)	16	18	23	11

Table 4: Dye degradation of activated carbon from *Wrightia tinctoria*

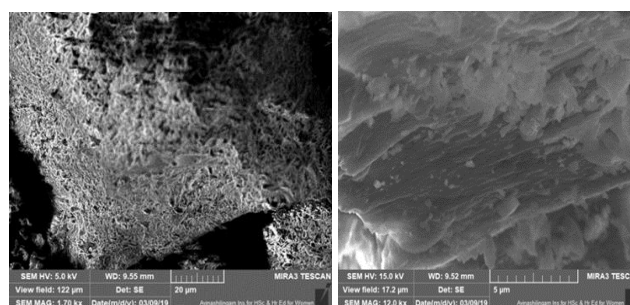
S.No.	Concentration of Activated Carbon (mg)	Percentage of Dye degradation
C	0.00	1.2
S1	0.07	48
S2	0.08	67
S3	0.09	82
S4	0.1	82

Table 5: Effluent treatment of activated carbon from *Wrightia tinctoria*

S.No.	Parameters	Before treatment of water	After treatment of water
1	Alkaloid	200 ppm	170 ppm
2	Chloride	110 ppm	90 ppm
3	Hardness	400 ppm	350 ppm
4	COD	20.96	20.48
5	TDS	212 ppm	171 ppm
6	pH	6.5	6.9

Table 6: Phytochemical Analysis of *Wrightia tinctoria*

S.No.	Phytochemicals	Result
1	Alkaloids	Positive
2	Flavonoids	Positive
3	Saponins	Negative
4	Sterols	Positive
5	Tannins	Negative
6	Phenols	Negative
7	Glycosides	Positive
8	Terpenoids	Positive
9	Carbohydrates	Negative
10	Phytosterols	Positive
11	Proteins	Negative
12	Gums & Mucilages	Negative

**Fig 1:** Activated Carbon from dried leaves powder of *Wrightia tinctoria***Fig 2:** Characterization of the Prepared Activated Carbon by FE-SEM Analysis

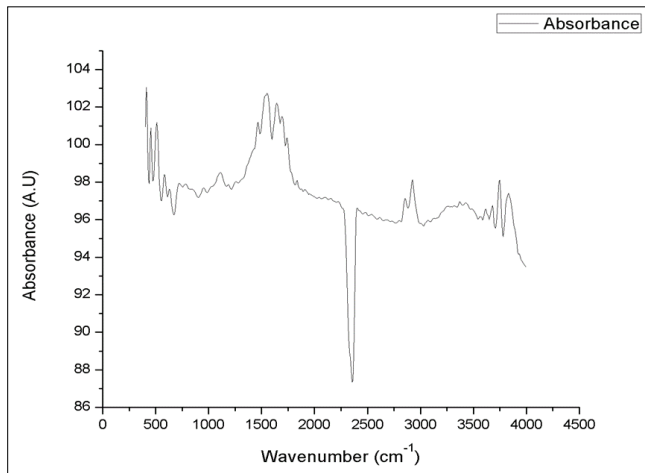


Fig 3: FTIR Analysis of Synthesized Activated Carbon

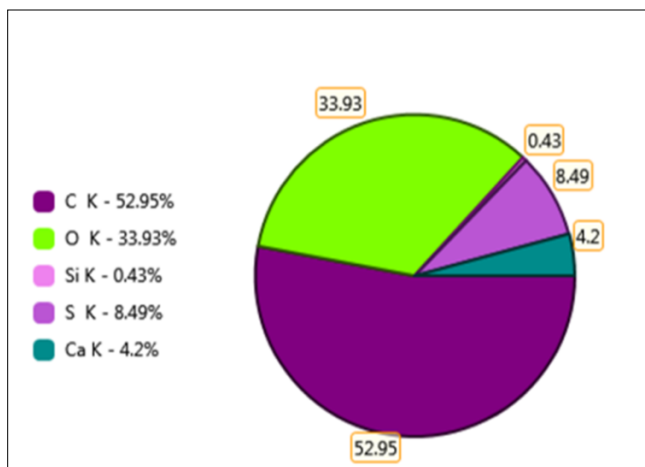


Fig 4: EDX Analysis of Activated Carbon

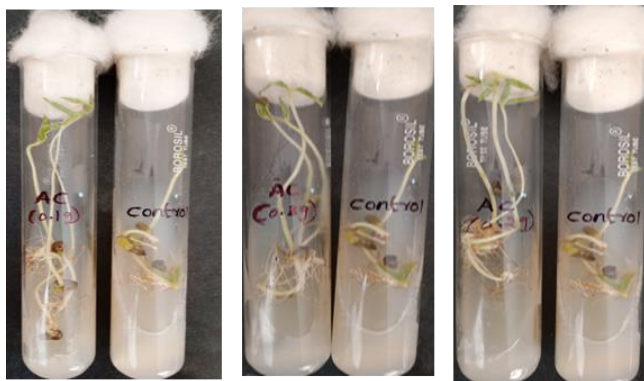


Fig 5: *In vitro* Seed Germination Analysis of Activated Carbon from *Wrightia tinctoria*

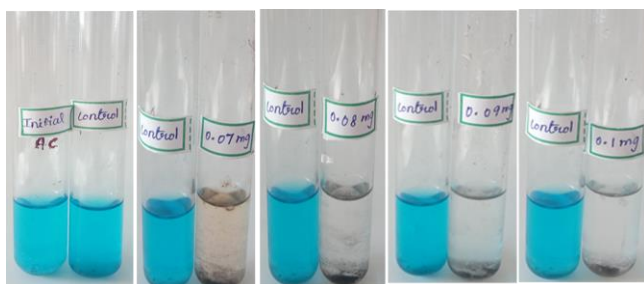


Fig 6: Dye degradation analysis of activated carbon from *Wrightia tinctoria*

Conclusion

We have established a green eco-friendly and expeditious biological method towards the preparation of activated carbon using *Wrightia tinctoria* leaves and it has various biological applications. The existence of various secondary metabolites was revealed through phytochemical screening. SEM studies have confirmed the formation of activated carbon in the size range of 20 μm with honeycomb-like structure and 5 μm shows Ice flood-like structure. The infrared spectrum helped to identify the chemical structure and functional groups in the activated carbon. So the use of activated carbon in various applications would favour the demand in dye, agricultural and medical industries.

References

1. Grima-Olmedo C, Ramirez-Gomez A, Gomes-Limon D, Clemente-Jul C. Activated carbon from flash pyrolysis of eucalyptus residue. *Heliyon*,2016;2(9):e00155(1-18).
2. Dheeban Shankar P, Basker S, Karthik K, Karthik S. Adsorption of methylene blue dye using *Passiflora foetida* activated carbon on various parameters. *Research Journal of Chemical and Environment Sciences*,2017;5(2):58-66.
3. Hassanin M Ali. Characterization of activated carbon that's synthesis from green bean peels by using H_2SO_4 agent: Implementation in reactive blue dye removal. *International Journal of Chem Tech Research*,2016;9(12):688-694.
4. Chahrazed Djilani, Rachida Zaghdoudi, Faycal Djazi, Bachir Bouchekima, Abdelaziz Lallam, Pierre Magri. Preparation and characterisation of activated carbon from animal bones and its application for removal of organic micropollutants from aqueous solution. *Desalination and Water Treatment*,2016;57(52):1-10.
5. Altenor S, Carene B, Emmanuel E, Lambert J, Ehrhardt JJ, Gaspard S. Adsorption studies of methylene blue and phenol onto vetiver roots activated carbon prepared by chemical activation. *Journal of Hazardous Materials*,2009;165(1-3):1029-1039.
6. Sreejalekshmi KG, Anoop Krishnan K, Anirudhan TS. Adsorption of Pb (II) and Pb (II)-citric acid on sawdust activated carbon: Kinetic and equilibrium isotherm studies. *Journal of Hazardous Materials*,2009;161(2-3):1506-1513.
7. Wu FC, Tseng RL, Juang RS. A review and experimental verification of using chitosan and its derivatives as adsorbents for selected heavy metals. *Journal of Environmental Management*,2010;91(4):798-806.
8. Djilani C, Zaghdoudi R, Modarressi A, Rogalski M, Djazi F, Lallam A. Elimination of organic micro pollutants by adsorption on activated carbon prepared from agricultural waste. *Chemical Engineering Journal*,2012;189-190:203-212.
9. Diao Y, Walawender WP, Fan LT. Activated carbon prepared from phosphoric acid activation of grain sorghum. *Bioresource Technology*,2012;81(1):45-52.
10. Laine J, Simoni S, Calles R. Preparation of activated carbon from coconut shell in a small-scale cocurrent flow rotatory kiln. *Chemical Engineering Communications*,991:99(1):15-23.

11. Srinivasa kannan C, Mohamad Zailani Abu Bakar. Production of activated carbon from rubberwood sawdust. *Biomass and Bioenergy*,2004;27(1):89-96.
12. Kadirvelu K, Thamaraiselvi K, Namasivayam C. Removal of heavy metals from industrial wastewaters by adsorption onto activated carbon prepared from agricultural solid waste. *Bioresource Technology*,2001;76(1):63-65.
13. Chang EE, Jan-Chi Wan, Hyunook Kim, Chung-Huei Liang, Yung-Dun Dai, Pen-Chi Chiang. Adsorption of Selected Pharmaceutical Compounds onto Activated Carbon in Dilute Aqueous Solution Exemplified by Acetaminophen, Diclofenac and Sulfamethoxazole. *Scientific World Journal*,2015;186501:01-11.
14. Chandy T, Sharma CP. Activated Charcoal Microcapsules and their Applications. *Journal of Biomaterials Applications*,1998;13(2):128-157.
15. Reddy YSR, Sama Venkatesh, Ravichandran T, Subburaju T, Suresh B. Pharmacognostical studies on *Wrightia tinctoria* bark. *Pharmaceutical Biology*,1999;37(4):291-295.
16. Veerapur VP, Palkar MB, Srinivasa H, Kumar MS, Patra S, Rao PGM *et al.* The effect of ethanol extract of *Wrightia tinctoria* bark on wound healing in rats. *Journal of Natural Remedies*,2004;4(2):155-159.
17. Papiya Bigoniya, Avtar Chand Rana. Antidiarrheal and antispasmodic activity of *Wrightia tinctoria* bark and its steroidal alkaloid fraction. *Pharmacologyonline*,2009;3:298-310.
18. Aleykutty NA, Bindu AR, Sangeetha S, Jiljit G. Evaluation of Anti-inflammatory and Analgesic Activity of *Wrightia tinctoria* Leaves. *Journal of Biologically Active Products from Nature*,2011;1(1):33-41.
19. Rajalakshmi GR, Jyoti Harindran. *In-vitro* Anthelmintic activity of *Wrightia tinctoria*. *International Journal of Pharm Tech Research*,2013;5(2):308-310.
20. Madhu C Divakar, Lakshmi Devi S. Antiulcer activity of *Wrightia tinctoria* (Roxb.) R. Br. *Der Pharmacia Sinica*,2011;2(2):355-360.
21. Sandhya Rani M, Rao S Pippalla, Krishna Mohan G, Gangaraju M. Anti-Diabetic activity of methanolic and ethyl acetate extracts of *Wrightia tinctoria* R.Br. *Fruit. International Journal of Pharmaceutical Sciences and Research*,2012;3(10):3861-3866.
22. Ashish Dixit, Jain AK, Pawan Tiwari, Nidhi Gupta, Priyanka Gangele. A phytopharmacological review on an important medicinal plant *Wrightia tinctoria*. *Current Research in Pharmaceutical Sciences*,2014;04(03):70-76.
23. Bharani M, Thirunethiran Karpagam, Varalakshmi B, Gayathiri G, Lakshmi Priya K. Synthesis and Characterization of Silver Nanoparticles from *Wrightia tinctoria*. *International Journal of Applied Biology and Pharmaceutical Technology*,2012;3(1):58-63.
24. Jain PS, Bari SB. Anti-inflammatory effects of wood stem extracts of *Wrightia tinctoria*. *Asian Journal of Traditional Medicines*,2010;5(4):132-137.
25. Ramchandra P, Basheermiya M, Krupadanam GLD, Srimannarayana G. Wrightial, A new Terpene from *Wrightia tinctoria*. *Journal of Natural Products*,1993;56(10):1811-1812.
26. Selvam P, Muruges N, Witvrouw M, Keyaerts E, Neyts J. Studies of Antiviral Activity and Cytotoxicity of *Wrightia tinctoria* and *Morinda citrifolia*. *Indian Journal of Pharmaceutical Sciences*,2009;71(6):670-672.
27. Patricia C Mackeen, Stanley Person, Susan C Warner, Wallace Snipes, S. Edward Stevens, Jr. Silver-Coated Nylon Fiber as an Antibacterial Agent. *Antimicrobial Agents And Chemotherapy*,1987;31(1):93-99.
28. Park SJ, Jang YS. Preparation and characterization of activated carbon fibers supported with silver metal for antibacterial behavior. *Journal of Colloid and Interface Science*,2003;261(2):238-243.
29. Oya A, Wakahara T, Yoshida S. Preparation of pitch-based antibacterial activated carbon fiber. *Carbon*,1993;31(8):1243-1247.
30. Moraes LM, Faria RT, Cuquel FL. Activated charcoal for *in vitro* propagation of Brazilian orchids. *Acta Horti*,2005;683:383-390.
31. Wang PJ, Huang LC. Beneficial effects of activated charcoal on plant tissue and organ cultures. *In Vitro*,1976;12(3):260-262.
32. Harborne JB. *Phytochemical Methods*, Chapman & Hall, New York, NY, USA, 11th edition, 1984.
33. Kannan P, Shanmugavadivu B, Petchiammal C, Hopper W. *In vitro* Antimicrobial Activity of *Wrightia tinctoria* Leaf Extract against Skin Microorganisms. *Acta Botanica Hungarica*,2006;48(3-4):323-329.
34. Biemer JJ. Antimicrobial Susceptibility testing by the Kirby-Bauer disc diffusion method. *Ann Clin Lab Sci*,1973;3(2):135-40.
35. Rezali NI, Jaafar Sidik N, Saleh A, Osman NI, Mohd Adam NA. The effects of different strengths of MS media in solid and liquid media on *in vitro* growth of *Typhonium flagelliforme*. *Asian Pacific Journal of Tropical Biomedicine*,2017;7(2):151-156.
36. Pan M, Van Staden J. The effect of activated charcoal and auxins on root formation by the hypocotyl segment of *Daucus carota*. *South African Journal of Botany*,2002;68(3):349-356.
37. Priyanka Das, Alok Kumar Srivastav. To Study the Effect of Activated Charcoal, Ascorbic Acid and Light duration on *In vitro* Micropropagation of *Aloe vera* L. *International Journal of Innovative Research in Science, Engineering, and Technology*,2015;4(5):3131-3138.
38. Malik PK. Dye removal from wastewater using activated carbon developed from sawdust: adsorption equilibrium and kinetics. *Journal of Hazardous Materials*,2004;113(1-3):81-88.
39. Sajjan CP, Suresh Kumar BV, Amol Naik. Comparative Study of Hydrothermally synthesized AIPO₄-5, Activated Carbon, and the Combination of Activated Carbon and AIPO₄-5 Filters in the Treatment of Wastewater and Industrial Effluent. *Water Conservation Science and Engineering*,2016;1(3):177-195.
40. Mahato SB, Sen S. Advances in triterpenoid research 1990-1994. *Phytochemistry*,1997;44(7):1185-1236.
41. Lixia Pei, Jingru Zhou, Lizhi Zhang. Preparation and properties of Ag-coated activated carbon nanocomposites for indoor air quality control. *Building and Environment*,2013;63:108-113.
42. Chahrazed Djilani, Rachida Zaghoudi, Ali Modarressi, Marek Rogalski, Fayçal Djazi, Abdelaziz Lallam. Elimination of organic micropollutants by adsorption

- on activated carbon prepared from agricultural waste. *Chemical Engineering Journal*,2012:189-190:203-212.
43. Riaz Ahamed K, Chandrasekaran T, Arun Kumar A. Characterization of Activated Carbon prepared from *Albizialebbeck* by Physical Activation. *IJIRI*,2013:1(1):26-31.
 44. Fleming FF, Yao L, Ravikumar PC, Funk L, Shook BC. Nitrile-Containing Pharmaceuticals: Efficacious Roles of the Nitrile Pharmacophore. *Journal of Medicinal Chemistry*,2010:53(22):7902-17.
 45. Jugal K Puri, Amutha R, Radhe Shyam, Harminder Kaur, Akanksha Kapila. Adsorption of Xylenol Orange on *Kigelia africana* and *Melia azedarach* fruits based Adsorbents from aqueous solutions. *International Journal of Advanced Chemical Science and Applications*,2014:2(1):1-7.
 46. Sricharoenchaikul V, Pechyen C, Aht-ong D, Atong D. Preparation and Characterization of Activated Carbon from the Pyrolysis of Physic Nut (*Jatropha curcas* L.) Waste. *Energy Fuels*, 2008:22(1):31-37.
 47. Abdul Khalil HPS, Jawaid M, Firoozian P, Umer Rashid, Aminul Islam, Hazizan Md. Akil. Activated Carbon from Various Agricultural Wastes by Chemical Activation with KOH: Preparation and Characterization. *Journal of Biobased Materials and Bioenergy*,2013:7:1-7.
 48. Chubaakum Pongener, Daniel Kibami, Kaza S Rao, Rajib L Goswamee, Dipak Sinha. Synthesis and Characterization of Activated Carbon from the Biowaste of the Plant *Manihotesculenta*. *Chemical Science Transactions*,2014:4(1):59-68.
 49. Laine J, Calafat A, Labady M. Preparation and characterization of activated carbons from coconut shell impregnated with phosphoric acid. *Carbon*,1989:27(2):191-195.
 50. Ortiz-Ibarra H, Casillas N, Soto V, Barcena-Soto M, Torres-Vitela R, de la Cruz W and Gómez-Salazar S. Surface characterization of electrodeposited silver on activated carbon for bactericidal purposes. *J. Colloid. Inter. Sci*,2007:314:562-571.
 51. Yamamoto O, Sawai J, Sasamoto T. Activated Carbon Sphere with Antibacterial Characteristics. *Materials Transactions*,2002:43(5):1069-1073.
 52. Kull T, Arditti J. (Eds.). *Orchid Biology: Reviews and Perspectives*, VIII. Springer-Science & Business Media, B.V, 2002.
 53. Fazlullah Khan Bangash, Abdul Manaf. Dyes Removal from Aqueous Solution using Wood Activated Charcoal of *Bombax cieba* Tree. *Journal of the Chinese Chemical Society*2005:52:489-494.
 54. Gecgel U, Ozcan G, Gurpinar GC. Removal of Methylene Blue from Aqueous Solution by Activated Carbon Prepared from Pea Shells (*Pisum sativum*). *Journal of Chemistry*, 2013, 1-9.
 55. Rahman MA, Amin SMR, Alam AMS. Removal of Methylene Blue from Waste Water Using Activated Carbon Prepared from Rice Husk. *Dhaka University Journal of Science*,2012:60(2):185-189.
 56. Jeyanthi J, Dhinakaran M. Study of the removal of methylene blue from aqueous solution by using coir pith. *Journal of Experimental Sciences*,2012:3(9):21-26.