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CONTENTS

VIEWS

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Antibacterial Activity of Gold Nanoparticles Synthesized from Euphorbia hirta and Euphorbia hetrerophylla on Enteric Bacterial Pathogens

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ABSTRACT

The antibacterial assay of gold nanoparticles (AuNPs) was carried out against *Escherichia coli, Salmonella typhi and Klebsiella pneumoniae* at various concentrations (40mg/ml, 80mg/ml, 120mg/ml, 160mg/ml and 200mg/ml) using agar dilution method. Ciprofloxacin (at 40mg/ml) was used as positive standard antibiotic against the test organisms. UV-Vis spectroscopy was used to confirm the synthesis of the AuNPs (peak wavelength recorded at 530nm and 540nm). The study revealed plants constituents such as alkaloids, tannins, saponin, carbohydrates and flavonoids as the active phytochemical components present in the plants extracts used for synthesis of AuNPs. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined against the test organisms. The MIC results of AuNPs synthesized from *Euphorbia hirta* against *Escherichia coli, Salmonella typhi and Klebsiella pneumoniae* were 40mg/ml, 80mg/ml and 80mg/ml respectively. The MBC results using *Euphorbia hirta* synthesized AuNPs were 120mg/ml, 200mg/ml and 160mg/ml for *Escherichia coli, Salmonella typhi, and Klebsiella pneumoniae* respectively. The MIC results from *Euphorbia heterophylla* AuNPs against *Escherichia coli* and *Klebsiella pneumoniae* was at 40mg/ml, *Salmonella typhi* was at 80mg/ml. The MBC of *E. heterophylla* AuNPs was at 160mg/ml for *Escherichia coli, Salmonella typhi and Klebsiella pneumoniae.* These plants seem to be candidates for the development of new drugs against intestinal bacterial pathogens

Key words: Antibacterial Activity, Gold Nanoparticles, *Euphorbia hirta, Euphorbia hetrerophylla,* Enteric Bacterial Pathogens.

Introduction

Nanotechnology first started in 1959 with a lecture delivered by Richard Feynman. Nanotechnology is a branch of science which deals with production, manipulation and use of materials ranging in nanometers. The emergence of nanotechnology in recent years has provided an extensive research by intersecting with various other branches of science and forming impact on all forms of life. Nanoparticles have been intensively studied over the last decade due to their physical, chemical, electronic, electrical, mechanical, magnetic, thermal, dielectric, optical and biological properties[1] (Daniel *et al,*2004). With applications in many industrial sectors, nanoparticles are considered as building blocks of the next generation of technology. An array of physical, chemical and biological methods has been used to synthesize nanomaterials. Specific methodologies have been used to synthesize noble metal nanoparticles of particular size and shape. Most of these methodologies remain expensive and involve the use of hazardous chemicals (ultraviolet irradiation, aerosol technologies, lithography, laser ablation, ultrasonic fields, and photochemical reduction techniques have been used successfully to produce nanoparticles). Therefore, there is a growing concern to develop simple, cost-effective, and sustainable methods.

Nanoparticles have more penetration powers into microorganisms due to their large surface areas and more activity could be recorded if the active plant extracts can be delivered into the 'interior' of the microbes. Many chemical methods exist for synthesis of nanoparticles but have been found to be toxic since nanoparticles could be used in humans and other animals or plants which may eventually end up in human system. It therefore, became extremely important

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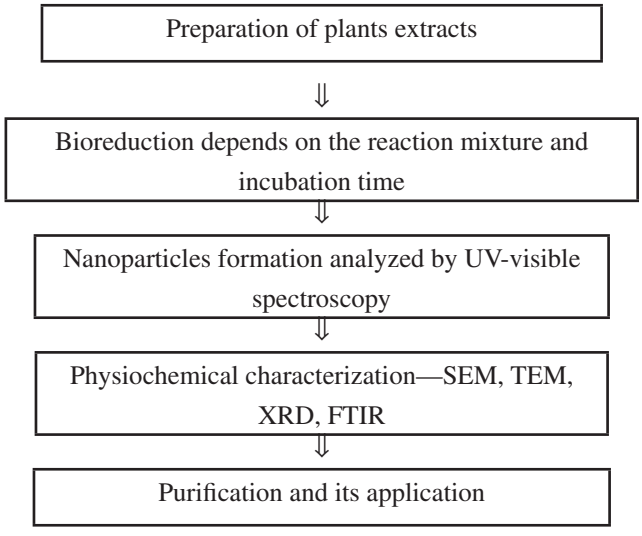
to focus on the biological methods for the production of nanoparticles[2] (Abalaka *et al,* 2014).

Biological synthesis of nanoparticles is a green chemistry approach that interconnects nanotechnology and biotechnology. The use of plants, microorganisms and algae as helped in the synthesis of materials in the nano range and the reduction in the toxicity of by-products is an alternative to these toxic and expensive physical nanoparticles fabrication methods. Biosynthesis of silver, gold, gold-silver alloy, platinum, tellurium, selenium, palladium, silica nanoparticles by bacteria[3] (Wen *et al*, 2009).Although biosynthesis of gold and silver nanoparticles by plants such as *Hibiscus rosasinensis, Aloe vera, Emblica officianalis, Carica papaya, Alfalfa, Diopyros kaki, Parthenium hysterophorus, Azadirachta indica, Eucalyptus hybrid, Cinnamomum camphora, Capsicum annuum* and *tamarind* have been reported, the potential plants as biological materials for the synthesis of nanoparticles is yet to be fully explored because of the rich biodiversity of plants and microbes.

The present study was carried out using *Euphorbia hirta* and *Euphorbia heterophylla* for green synthesis of gold nanoparticles. *Euphorbia hirta* is a slender-streamed, annual hairy plant with many branches from the base to the top, spreading up to 40cms tall, reddish or purple in colour. Leaves are opposite, elliptic oblong to oblong-lanceolate, it is about 1-2.5cms long, blotched with purple in the middle, toothed at the edge[4][5] (Huxley, 1992) (Huxley, 1992). The plant is mostly found in waste places and open grasslands[6](Joshi, 2000). *Euphorbia heterophylla Euphorbia heterophylla*is an annual plant with milky latex in all plant parts. It grows 30 to 100 cm tall and has simple or branched hollow stems with angular ribs. The leaves have variable shapes (as the species name suggests) both within and between populations. The lower leaves are alternate and the upper leaves are opposite and often have a whitish or bright red base[7].

Steps involved in the biosynthesis of nanoparticles



Materials and Methods

Plant Collection and Identification

Fresh whole plants of *Euphorbia hirta* and *Euphorbia heterophylla* were collected from around Bosso and Gidan Kwano Campuses of Federal University of Technology, Minna, Nigeria. The plants leaves were picked, washed thoroughly and air-dried at room temperature (28 °2c) for two weeks. The dried plants leaves were grounded to obtain fine powder using a mortar and pestle. This was done to enhance the permeability of the extracting solvents into the cells, thus facilitating the release of active ingredients.

Phytochemical Screening of Plants

Phytochemical screening was carried out on the aqueous crude extract to determine the active components present using the method of [8](Prashant*et al,* 2011).

Preparation of Stock Solution of Gold Chloride from $AuCl_4$

To make 50ml of 10mM stock solution the following calculations were made:

Molecular weight of AuCl4 = 393.83g

1M =393.83g in 1

To convert this to mM = 0.39383g/mM

To convert g to mg = 393.83mg/mM /L

10mM = 10 × 393.85mg/L = 3938.5mg/L

$$50\text{ml} = \frac{3938.5\text{mg} \times 50\text{ml}}{100\text{ml}} = 196.25\text{mg}$$

This (196.925mg $AuCl_4$) was then dissolved in 50ml distilled water and stored in a dark brown bottle and labeled 10mM AuCl4.

Now the working solution of 2.5mM was prepared using the formula,

RV/O

Where;

R = required concentration,

V = volume,

O = original concentration

Now, R =2.5mM

V = 50ml O = 10Mm

Then

$$\frac{2.5\text{mM} \times 50\text{ml}}{10\text{Mm}} = 12.5\text{ml}$$

Twelve point five (12.5ml) millilitre of stock solution taken and made up to 50ml with distilled water[2] (Abalaka *et al*, 2014).

Extraction Procedure (Aqueous Extraction)

Thirty gram (100g) each of the powdered leaves of the plants was weighed into 300ml of distilled water in a 500ml conical flask. The conical flask was covered with foil paper and allowed to stay for three days. The resulting mixture was filtered using a muslin cloth in order obtain the filtrate.

Green Synthesis of Gold Nanoparticles (AuNPs)

Four milliliter (4ml) each of the filtrate was pipetted into four (4) test tubes. The pH of each test tube was adjusted to 4, 7 and 11 using appropriate buffer solutions (i.e. dilute NaCl and HCl), the 4th test tube served as the control. Into each test tube 2ml of 2.5mM gold chloride was added (except the control). The test tube were mixed thoroughly and observed for colour change. Ultraviolet light (UV) was used to carry out the spectrophotometric analysis to determine the wavelength[2] (Abalaka*et al*, 2014).

Source of Bacterial Isolates

The micro-organisms used for this study were bacterial strains of *Escherichia coli, Salmonella typhi and Klebsiella* were obtained from the stock culture of the Department of Microbiology Federal University of Technology Minna, Nigeria. The microorganisms were maintained on nutrient agar (NA) slants at 4°C prior to subculture. The organisms were then transferred into slants and incubated at 37°C for 24 hours and the pure of the test organisms were then transferred into slants and stored at 4°C. Gram staining and biochemical tests were carried out for confirmation of these bacterial isolates.

Standardization of Microorganisms

A loopful of the stock culture of the micro-organisms was inoculated into 5ml of sterile nutrient broth and incubated for 24 hours. 0.2ml of the overnight culture of the organisms was inoculated into 19ml of sterile broth and incubated 3-5hours to standardize the culture to 106cfu/ml. a loopful of the standardized culture was used for the antibacterial assay[9] (Babayi*et al*, 2004).

Organism viability control (OVC)

This is carried out to determine if the test organisms used for the experiment were viable, each of the test organisms were streaked on a sterile Muller hinton agar and then incubated for 24hrs at 37oC. Observable growth shows that the organisms are viable while no growth show that organisms are not viable (i.e. unable to grow).

Extract sterility control (ESC)

This is carried out to determine the sterility of the extracts i.e. if the extract is free from contaminants. Each of the extracts was plated on sterile nutrient agar, incubated at 37^{o}C for 24hrs. The extract was free from any contaminant because there was no any growth on the agar.

Medium sterility control (MSC)

This is done to determine if the medium is free from any contaminant. Sterile nutrient agar was dispensed in sterile plates, incubated for 24hrs at 37oC. The medium remain clear which signifies that it is free of contaminant.

Concentration/standardization of AuNPs

The filtrate obtained was evaporated to dryness using a steam bath. 0.2g, 0.4g, 0.6g, 0.8g and 1.0g of the aqueous crude extracts were reconstituted into 5ml of AuCl4 to obtain standardized AuNPs solution. 0.2g of Ciprofloxacin antibiotic was dissolved into 5ml of distilled water and used as standard control antibiotic.

Evaluation of the Antibacterial Activity of AuNPs

Agar dilution method was used for antimicrobial assay: a weighed gram of nutrient agar was prepared and it was sterilized with the autoclave. 1ml of each of the standardized AuNPs solutions was introduced into 19ml of molten nutrient agar was dispensed into sterile petri dishes, allowed to gel and labeled appropriately. Sterile swab sticks was used to smear the surface of the gelled agar with the standardized inoculum 106cfu/ml aseptically and incubated at 37°C for 24 hours. Ciprofloxacin was used as the positive control antibiotic for the test organisms. After incubation, the efficacy of the AuNPs was observed for activity on the plates and compared with the control antibiotic. The control plate also includes organism viability control, extract sterility control and media sterility control. All the tests were conducted aseptically and in duplicates[9] (Babayi*et al,* 2004). The minimum inhibitory concentration (MIC) was the lowest concentration of the AuNPs with no bacterial growth.

Determination of Minimum Bactericidal Concentration (MBC)

For determination of minimum bactericidal concentration (MBC) of the AuNPs, agar dilution method was employed. The lowest concentrations from the plates that did not show visible growth after incubation for 24 hours were subculture into freshly prepared sterile nutrient agar. The least concentration that did not produce growth after 24 hours was recorded as the MBC.

Results

Phytochemical analysis

The results from the phytochemical analysis of *Euphorbia hirta* and *Euphorbia heterophylla* are shown in the tables below:

Table 1
Phytochemical constituents of *Euphorbia hirta*

Group Constituents	Test	Results
Alkaloids	Mayer's test	
	Dragendroff's test	+
Carbohydrates	Fehling's test	+
Flavonoids	Lead acetate test	+
Tannins	Gelatin's test	+
Saponin	Froth test	+

Key: present (+), absent (-)

Table 2
Phytochemical constituents of Euphorbia heterophylla

Group Constituents	Test	Results
Alkaloids	Mayer's test	
	Dragendroff's test	+
Carbohydrates	Fehling's test	+
Flavonoids	Lead acetate test	+
Tannins	Gelatin's test	+
Saponin	Froth test	+

Key: present (+), absent (-)

Antibacterial Activity of AuNPs

Table 3
Antibacterial activity of *Euphorbia hirta* AuNPs against the test organisms

Concentration of AuNPs	Test Organisms Escherichia coli	Salmonella typhi	Klebsiella Pneumoniae
40mg/ml	+	-	-
80mg/ml	+	+	+
120mg/ml	+	+	+
160mg/ml	+	+	+
200mg/ml	+	+	+
Control antibiotic (Cipro-floxacin) 40mg/ml	+	+	+

Key: activity (+), no activity (-)

Table 4
Antibacterial activity of *Euphorbia heterophylla*AuNPs against the test organisms

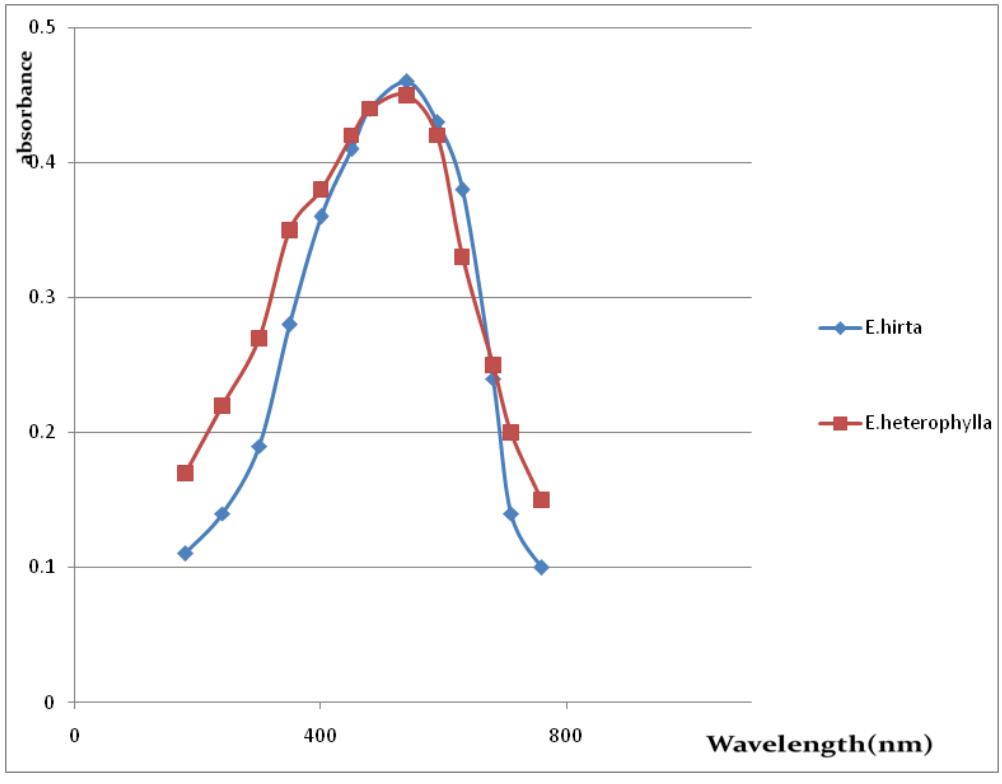
Concetration of AuNPs	Test Organisms Escherichia coli	Salmonella typhi	Klebsiella pneumoniae
40mg/ml	+	-	+
80mg/ml	+	+	+
120mg/ml	+	+	+
160mg/ml	+	+	+
200mg/ml	+	+	+
Control antibiotic (Cipro-floxacin) 40mg/ml	+	+	+

Key: activity (+), no activity (-)

Table 5
The minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Euphorbia hirta* and *Euphorbia heterophylla* AuNPs

Test organisms	*Euphorbia hirtas*		*Euphorbia heterophylla*	
	MIC	MBC	MIC	MBC
	(mg/ml)		(mg/ml)	
Escherich ia coli	40	120	**40**	160
Salmonella typhi	**80**	200	**40**	160
Klebsiella pneumoniae	**80**	160	**40**	160

Fig.1: UV-Vis spectroscopy analysis



Discussion

The result of phytochemical screening of *Euphorbia hirta* and *Euphorbia heterophylla* presented in **Table 1** and **Table 2** showed the presence of alkaloids, carbohydrates, flavonoids, tannins and saponins in the plants. The presence of these phytochemical constituents may account for the antibacterial activities of these plants. This is in agreement with the report of [10] (Prashant*et al,* 2011).

Gold nanoparticles (AuNPs) were synthesized using these plant extracts and AuCl4 solution. The plant extracts serve as a reducing agent (i.e. reducing the gold ion to gold) when mixed with the AuCl4 solution. Colour change was observed on addition of the AuCl4 to the aqueous extracts. The UV-Vis spectrum observed at 530nm and 540nm confirmed the synthesis AuNPs respectively. Gold has Plasmon resonance peak at 500–600nm[2] (Abalaka *et al,* 2014).

The inhibitory effect of the synthesized AuNPs on the test organisms were investigated in-vitro. The results revealed the antibacterial activity of the AuNPs at various concentrations against the test organism. At 40mg/ml of *Euphorbia hirta* AuNPs, *E. coli* was susceptible and was recorded as the minimum inhibitory concentration. *Salmonella typhi*a and *Klebsiella pneumoniae* were inhibited at a concentration of 80mg/ml using AuNPs from *Euphorbia hirta*[11][2] (Elumalai*et al*, 2010, Abalaka*et al,* 2014).It was also discovered that the AuNPs had bactericidal effect against the test organisms. The minimum bactericidal concentration (MBC) of *Euphorbia hirta* AuNPs against *Salmonella typhi, Escherichia coli, Klebsiella pneumonia* were also observed to be 200mg/ml, 120mg/ml and 160mg/ml respectively. AuNPs from *E. hirta* had same potency when compared with the positive control antibiotic used[12] [13] . This study revealed that nanoparticles from these plants possess antibacterial properties.

The antibacterial assay carried out using *Euphorbia heterophylla* AuNPs was most active against *E. coli* at 40mg/ml same the control antibiotic used. *Salmonella typhi,* and *Klebsiella pneumonia* showed no visible growth on the agar plates at 80mg/ml which was recorded as the MIC (from Table 5). At 160mg/ml, the *Euphorbia heterophylla* AuNPs had minimum bactericidal effect against *Salmonella typhi, Escherichia coli and Klebsiella pneumoniae.* 40mg/ml of Ciprofloxacin antibiotic used as positive control against the test organisms showed activity (inhibited the growth of the bacteria)[14-16] .

Conclusion

This study shows that synthesized AuNPs using plants extracts of *Euphorbia hirta*a and *Euphorbia heterophylla* against enteric bacterial pathogens have antibacterial affects and could be candidates for new drugs against enteric bacterial pathogens. The characterization analysis proved that the particle so produced in nano forms would be equally effective as that of antibiotics and other drugs in pharmaceutical applications. The use of gold nanoparticles in drug delivery systems might be the future thrust in the field of medicine. This study shows also that the "green" route (biosynthesis using plants extracts) for the synthesis nanoparticles (NPs) is of great interest due to economic prospects and feasibility, eco-friendliness, and wide range of applications in nano-medicine, catalysis medicine, nano-optoelectronics, etc. It is a new and emerging area of research in the scientific world where developments are still being made warranting a bright future for this field.

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Evaluation of In-vitro anti-inflammatory and Antioxidant activity of *Pergularia daemia* Linn

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ABSTRACT

Pergularia daemia is a well known traditional folklore medicinal plant. The plant has been found to possess diverse number of pharmacological properties. In the present study, aqueous extract of P.daemia leaves were used to evaluate in vitro antioxidant and anti-inflammatory activities. In vitro antioxidant activity was carried out by Total antioxidant activity, ABTS assay, Hydrogen peroxide assays, and anti- inflammatory activity by membrane stabilization method and heat induced hemolysis. Aqueous extract showed an effective pharmacological activity in all assays when compared with their respective standards.

Key words: Inflammation, Traditional medicine, Antioxidant activity, Protein denaturation.

Introduction

The use of medicinal plants as a source of new drug for pharmaceutical industry has been focused since last decades. Various traditional medicinal plants are nowadays focused for development of new alternatives for allopathic drugs. According to World Health Organization, about more than 80% of the world's population including developed countries still rely on use of medicinal plants for their primary healthcare. The pharmacological action is due to various secondary metabolites present in the plant. *Pergularia daemia* (Forsk.) Chiov (Apocyanaceae), commonly known as utaran (Hindi), Dustapuchettu (Telugu), Uttamarani (Sanskrit) is a slender, hispid, fetid smelling laticiferous twiner found in the plains throughout the hot parts of India. P. daemia is said to have more magical application than medical application as it posses diverse healing potential for a wide range of illnesses. Some of the Folklore people use this plant to treat jaundice, as laxative, anti-pyretic, expectorants and also in infantile diarrhea. The leaf latex is locally used as pain killer killer and for relief from toothache (Hebbar *et al*., 2010), the sap expressed from the leaves are held to cure sore eyes in Ghana. The plant reduces the incidence of convulsion and asthma. It is used to regulate the menstrual cycle and intestinal functions. The root is useful in treating leprosy, mental disorders, anemia and piles (Omale *et al*., 2011). Hence in this paper, the In-vitro anti-inflammatory effects of leaves of *Pergularia daemia* Linn. were investigated. Furthermore, the study also evaluated the antioxidant scavenging activities of the selected plant.

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Materials and Methods

Plant material

Fresh plant parts (*Pergularia daemia*) were collected randomly from the gardens and villages of Kovilpatti, Tamil Nadu from the natural stands. The botanical identity of these plants was confirmed by Dr. V. Nandagopalan, Associate Professor & Dean, PG & Research Department of Microbiology, National College, Tiruchirappalli, Tamil Nadu. A voucher specimen has been deposited at the Department of Botany, National College (Autonomous), Tiruchirapalli-620 001, Tamil Nadu, India.

Aqueous extraction

100 grams of dried powder were extracted in distilled water for 6 h at slow heat. Every 2 h it was filtered through What man no.1filter paper and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was repeated twice and after 6 h the supernatant was concentrated to make the final volume one-fifth of the original volume.

Solvent extraction

100 grams of dried plant powdered samples were extracted with 200 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume. It was stored at 4^{o}C in airtight bottles for further studies, *viz*. antimicrobial, antioxidant, anticancer and phytochemical analysis.

Antioxidant Activity

Determination of total antioxidant capacity

Total antioxidant activity of the plant from *Pergularia daemia* determined according to the method of Prieto *et al*. (1999). Briefly, 0.3 ml of sample was mixed with 3.0 ml reagent solution, (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95ºC for 90 minutes under water bath. Absorbance of all the sample mixtures was measured at 695 nm after 15 min. Ascorbic acid was used as standard.

Percentage scavenging (H_2O_2) = (A_1/ A_0) x 100)

A_o - Absorbance of control; A_1 - Absorbance of sample

Hydrogen peroxide scavenging assay

The free radical scavenging activity of the plant from *Peagularia daemia* determined by hydrogen peroxide assay (Gulcin *et al.,* 2004). Hydrogen peroxide (10mM) solution was prepared in phosphate buffered saline (0.1M, pH 7.4). 1ml of the extract containing samples of different concentration (100, 250, 500, 750 and 1000µg) was rapidly mixed with 2ml of hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer after 10 minutes of incubation at 37°C against a blank (without hydrogen peroxide). The percentage of scavenging of hydrogen peroxide was calculated using the formula,

Percentage scavenging (H_2O_2) = (A_1/ A_0) x 100)

A_o - Absorbance of control; A_1 - Absorbance of sample

ABTS inhibition assay

The ability of the extract to scavenge ABTS (2,2 azino bis (3-etheylbenzothiazoline-6-sulphonicacid) diammonium salt) radical scavenging was determined by the method of Re *et al.* (1999). ABTS was generated by mixing 5 ml of 7 mM ABTS with 88 µl of 140 mM potassium persulfate under darkness at room temperature for 16 hours. The solution was diluted with 50% ethanol and the absorbance at 734 nm was measured. The ABTS radical cation scavenging activity was assessed by mixing 5 ml ABTS solution (absorbance of 0.7± 0.05) with 0.1ml polysaccharide (100, 250, 500, 750 and 1000 µg). The final absorbance was measured at 743 nm with spectrophotometer. The percentage of scavenging was calculated by the following formula,

% of scavenging = (A_1/ A_0) x 100)

Where A_o - Absorbance of control; A_1 - Absorbance of sample

In vitro Anti-inflammatory activity

Membrane stabilization method

The SRBC membrane stabilization has been used as method to study the anti-inflammatory activity. Blood was collected from healthy volunteer who had not taken any NSAIDS for two weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2%dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.85%.pH 7.2) and a 10 %(v/v) suspension was made with isosaline. The assay mixture contained the drug (concentration as mentioned in the table 2), 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5ml of HRBC suspension. Diclofenac was used as reference drug. Instead of hyposaline 2ml of distilled water was used in the control. All the assay mixtures were incubated at 370C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in presence of distilled water as 100% (Gandhisan *et al*., 1991).

Heat induced hemolysis

A volume of 100 µL of 10% RBC was added to 100 µL of the extract. The resulting solution was heated at 56°C for 30 minutes followed by centrifugation at 2500 rpm for 10 minutes at room temperature. Supernatant was collected, and absorbance was read at 560 nm. Acetyl salicylic acid was used as a positive control. Percent membrane stabilization was calculated by the method of Saket *et al*. (2010)

% Inhibition=100− ((A1-A2)/A0)*100)

Where A1 is the absorbance of the sample, A2 is the absorbance of the product control and A0 is the absorbance of the positive control.

Results and Discussion

Steroidal and non-steroidal anti-inflammatory drugs are currently the most widely used drugs in the treatment of acute inflammatory disorders, despite their renal and gastric negative secondary effects. There is a need for the new safe, potent, nontoxicor less toxic anti-inflammatory drug. Plant medicines are great importance in the primary healthcare in many developing countries. According to World Health Organization (WHO) still about 80% of the world population rely mainly on plant-based drugs. The research is based on to evaluate for newer anti-inflammatory agents from herbal medicine with potent activity and lesser side effect substitutes for drugs. The results clearly showed that *P.daemia* extract had significant total antioxidant activity at the same concentration (1000 µl) (Table 1). The ABTS cation radical is formed by the loss of an electron by the nitrogen atom of ABTS (2,2'-azino-bis(3- ethylbenzthiazoline-6-sulphonic acid) which absorbs at 743nm giving a bluish-green colour in the presence of antioxidant, yielding the solution decolorization (Pisochi and Negulescu, 2011) . Result of present study reveals that the aqueous extract possesses good antioxidant activity which is equal to standards, ascorbic acid as depicted in table 2. The present results is also clearly indicating the percentage inhibition of ABTS radical scavenging activity

was concentration-dependent with increased in the reaction mixture for the extracts and the standards.

The scavenging ability of water extract of *P.daemia* on hydrogen peroxide is shown table 3 and compared with ascorbic acid as standards. The *P.daemia* extracts were capable of scavenging hydrogen peroxide in an amount dependent manner. The maximum hydrogen scavenging activity was noted at 1000 µl concentration (89.09 %). On the other hand, using the near amounts, ascorbic acid exhibited 92.63 % hydrogen peroxide scavenging activity.

Table 4 shows the results of inhibition of haemolysis and percentage of RBC membrane stabilization by the aqueous extract of *Pergularia daemia* at various concentrations. Maximum stabilization activity was observed (65.73%) at a concentration of 500µl. The present results provide an indication for membrane stabilization and protein denaturation as an additional mechanism of *Pergularia daemia* for anti-inflammatory activity. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 78.34% at the concentration of 500 µl.

The crude extract was effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti inflammatory effect. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The extract inhibited the heat induced hemolysis of RBCs to varying degree (Table 5). The maximum inhibitions (72.34%) were observed at 500µl concentration. The aspirin standard drug standard drug showed the maximum inhibition 84.66%.

Denaturation of proteins is a well-documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability to thermally induced protein denaturation (Mizushima and Kobayashi, 1968). The denaturation is used loosely to designate the change of proteins from a soluble to an insoluble form brought about by a large variety of chemical and physical agents, including acids, alkalies, alcohol, acetone, salts of heavy metals and dyes (Mann, 1906), and heat, light, and pressure (Robertson, 1918). Chick and Martin (1910) consider heat denaturation as a reaction between protein and water which implies in all probability a hydrolysis. Several author anti-inflammatory drugs have shown dose dependent ability to inhibit the thermally induced protein denaturation (Grant et al., 1970). Similar results were observed from many reports from plant extract (Sakat et al., 2010). The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The aqueous extract of *P. daemia* showed significantly higher anti-inflammatory and antioxidant activity at increasing concentration. Hence, *P. daemia* can be used as an anti-inflammatory agent. The investigation is based on the need for anti-inflammatory agents from natural sources with potent activity and lesser side effects as substitutes for chemical therapeutics.

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Table 1
Total antioxidant activity of *Pergularia daemia* Linn.

S.No.	Concentration (µl)	% scavenging activity	
		Ascorbic acid (standard)	Aqueous extract
1.	200µl	34.43	49.33
2.	400µl	52.17	60.49
3.	600µl	64.44	70.36
4.	800µl	76.11	74.46
5.	1000µl	87.58	82.3

Table 2
ABTS assay of aqueous extract of *Pergularia daemia* Linn.

S.No.	Concentration (µl)	% scavenging activity	
		Ascorbic acid (standard)	Aqueous extract
1.	200µl	20.37	16.34
2.	400µl	47.41	51.26
3.	600µl	63.7	56.68
4.	800µl	72.13	65.59
5.	1000µl	81.26	76.54

Table 3
H_2O_2 Scavenging activity of *Pergularia daemia* Linn.

S. No.	Concentration (µl)	% scavenging activity	
		Ascorbic acid (standard)	Aqueous extract
1.	200µl	27.43	25.43
2.	400µl	52.41	45.36
3.	600µl	67.38	54.18
4.	800µl	76.63	74.41
5.	1000µl	92.63	89.06

Table 4
Membrane stabilization of aqueous extract of *Pergularia daemia* Linn.

S.No.	Concentration (µl)	% Membrane stabilization	
		Aspirin (standard)	Aqueous extract
1.	100µl	45.61	34.24
2.	200µl	51.42	38.50
3.	300µl	59.20	46.59
4.	400µl	67.48	57.52
5.	500µl	78.34	65.73

Table 5
Heat heamolysis of aqueous extract of *Pergularia daemia* Linn.

S. No.	Concentration (µl)	% heat heamolysis	
		Aspirin (standard)	Aqueous extract
1.	100µl	46.49	28.42
2.	200µl	58.41	36.39
3.	300µl	67.33	51.44
4.	400µl	75.33	63.47
5.	500µl	84.66	72.34

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Measurement of Radon Gas Concentration in Ceramic Samples by Using Nuclear Track Detector (CR-39)

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ABSTRACT

In the present work, we have measured the radon gas concentration in many ceramic samples different in origin(UAE, Iran, Egypt, China, Spain), by registration alpha-emitters which are emitted from radon gas, using nuclear track detector CR-39, as a one important of building materials used in Iraq. The obtained results have shown that the highest average radon gas concentration in ceramic was found in Chinese samples, which was 59.66 Bq/m^3, while the lowest average radon gas concentration in ceramic was found in Spanish samples, which was 20.67 Bq/m^3. The present results show that the radon gas concentration in all ceramic samples is below the allowed limit from International Commission of Radiation Protection (ICRP) agency.

Keywords: Ceramic, Radon Gas, SSNTDs, CR-39.

Introduction

Radioactive materials produce ionizing radiation, which has sufficient energy to strip away electrons from atoms or to break some chemical bonds. Any living tissue in the human body can be damaged by ionizing radiation in a unique manner. The body attempts to repair the damage, but sometimes the damage is of a nature that cannot be repaired or it is too severe or widespread to be repaired. Also mistakes made in the natural repair process can lead to cancerous cells. The most common forms of ionizing radiation are alpha and beta particles, or gamma and X-rays [1].

Those radioactive particles when inhaled, can damage the cells that line the lung. And so, long-term exposure to radon can lead to lung cancer [2].

The raw material which is used in production of some ceramic is containing various amounts of natural radioactive elements. During processing this material, owing to chemical properties of radium, practically all (Ra^{226}) gets incorporated into ceramic and remains in disequilibrium status when it compared to radioactivity levels contained in the raw material. Most of the materials are considered waste and are stockpiled or discharged into the aquatic environment [3]. Potential issues of concern resulting from waste disposal are its environmental impacts; possible increases in radio- nuclides in soils or in groundwater and consequential ingestion by humans through exposure routes such as drinking water and food chain [4]. Once, deposited in bone tissue, (Ra^{226}) has a high potential for causing biological damage through continuous irradiation of human skeleton over many years and may induce bone sarcoma [5].

Recent studies of people exposed to radon have confirmed that radon in homes represents a serious health hazard. The main health risk associated with long-term, elevated exposure to radon is an increased risk of developing lung cancer, which depends on the radon concentration and the length of exposure [6]. The hazard of Radon comes from its radioactive progeny, which use their physical properties to spread or attach like aerosols do, trapped in the lung and depositing their alpha-particle energies in the tissue, producing higher ionization density than beta particles or gamma-rays. Lung cancer, skin cancer, and kidney diseases are the health effects attributed to inhalation of radon-decay products [7]. The sources of radon gas are the building materials and its components, ground water, and soil [8]. The radiological impact from the above nuclides is due to radiation exposure of the body by the gamma rays and irradiation of the lung tissues from inhalation of Radon and its progeny [9]. From the natural risk point of view, it is necessary to know the dose limits of public exposures and to

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measure the natural environmental radiation level provided by ground, air, water, foods, building interiors, etc., for the estimation of the exposures to natural radiation sources. Low level gamma-ray spectrometry is suitable for both qualitative and quantitative determinations of gamma-ray emitting nuclides in the environment [10].

Experimental Part

The determination of the concentrations of alpha particles emitted from radon gas in ceramic samples were performed by using the nuclear track detector (CR-39) of thickness 250 μm and area of about 1×1 cm^2. The radon gas concentration in ceramic samples was obtained by using the sealed-cup technique as shown in figure (1).

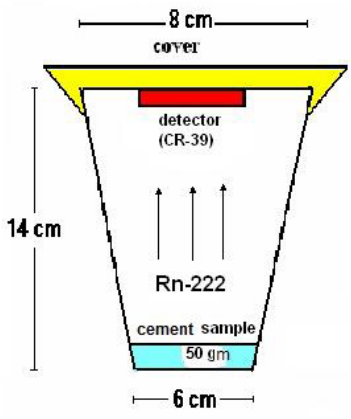

Figure 1. A schematic diagram of the sealed-cup technique in ceramic sample.

After the irradiation time (60 days), the track detectors (CR-39) were etched in 6.25 N of NaOH solution at temperature of 70 °C for 5 hr, and the tracks density were recorded using an optical microscope (type ALTAY BIO-1007) with magnification of 400X. The density of tracks (ρ) in the samples was calculated according to relation (1) [11]:

track density(ρ) = (1),

The radon gas concentration in ceramic samples were obtained by the comparison between track densities registered on the detectors of the samples and that of the standard ceramic samples which are shown in figure (2), using the relation (2) [12]:

$$C_X = \rho_X \quad (2).$$

where: C_X: alpha particles concentration in the unknown sample.

C_S: alpha particles concentration in the standard sample.

ρ_X : track density of the unknown sample (track/mm^2).

ρ_S : track density of the standard sample (track/mm^2).

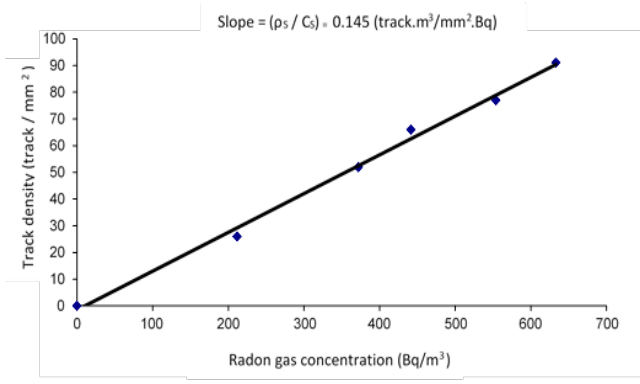

Figure 2. Relation of radon gas concentration and track density in standard samples [13].

Results and Discussion

Our present investigation is based on the study of many samples from different origin of ceramics which were available. It can be found that the radon gas concentrations by using alpha-emitters registrations which are emitted from radon gas in nuclear track detector (CR-39).

The solid state nuclear track detectors (SSNTDs) when exposed to a certain dose of radiation one or more than one measurable parameters will change. The passage of heavy ionizing nuclear particles (such as alpha particles) through most insulating solids creates narrow paths of intense damages on an atomic scale [14]. These damages (tracks) can be revealed and made visible indirectly by chemical etching (using NaOH solution) and using an ordinary optical microscope.

Table (1) represents the radon gas concentrations for ceramic samples in different countries. It can be noticed that, the highest average radon gas concentration in ceramic was found in Chinese samples, which was 59.66 Bq/m^3, while the lowest average radon gas concentration in ceramic was found in Spanish samples, which was 20.67 Bq/m^3. These results more clearly shown in figure (3).

It might be mentioned that, thoron gas (^{220}Ra has half-life 55.6 sec) and action gas (^{219}Ra has half-life 3.96 sec) are an alpha emitter which is also present in soil and the other investigated materials, whereas ^{222}Rn has 3.82 days half-life have much longer half-lives. Their emanation from building materials (shorter half-lives), as well as, its infiltration from the ground and further migration is restricted to a few centimeters only [15]. Thus, ^{220}Rn and ^{219}Rn are given less importance in environmental studies.

Table - 1

Radon gas concentration for ceramic samples from different countries.

No. of sample	Origin of sample		Samples			
			1	2	3	Mean
1	UAE	Track density (Track .mm^{-2})	5	4	4	4.33
		Radon Concentration (Bq/m^3)	34.45	27.56	27.56	29.83
2	Iran	Track density (Track .mm^{-2})	7	8	5	6.66
		Radon Concentration (Bq/m^3)	48.23	55.12	34.45	45.88
3	Egypt	Track density (Track .mm^{-2})	6	5	6	5.66
		Radon Concentration (Bq/m^3)	41.34	34.45	41.34	38.99
4	China	Track density (Track .mm^{-2})	10	8	8	8.66
		Radon Concentration (Bq/m^3)	68.9	55.12	55.12	59.66
5	Spain	Track density (Track .mm^{-2})	2	4	3	3
		Radon Concentration (Bq/m^3)	13.78	27.56	20.67	20.67

The rocks of substances with a high concentration of radiation, depending on the type of these rocks, then the results that obtained refer to the variation of rocks nature of deferent regions in world. In general, it can show the rising values of alpha emitters concentration in ceramics.

The present results indicate that the radon gas concentrations in all ceramic samples is below the allowed limit from International Commission of Radiation Protection (ICRP) agency which is 200 Bq/m^3 in soil sample.

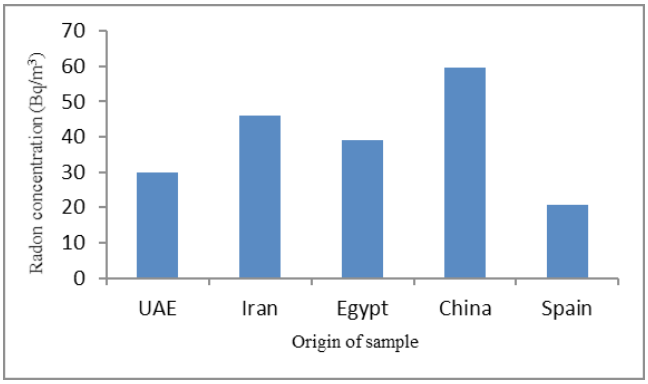

Figure 3. Radon gas concentration for ceramic samples.

Conclusion

From the present work, it can be concluded that the highest average radon gas concentration in ceramic was found in China samples, which was (59.66 Bq/m^3), while the lowest average radon gas concentration in ceramic was found in Spain samples, which was (20.67 Bq/m^3). The present results show that the radon gas concentration in all ceramic samples is below the allowed limit from International Commission of Radiation Protection (ICRP) agency.

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Green Technology for Production of Microcrystalline Cellulose

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ABSTRACT

In this paper, a green, zero-discharge technology for production of microcrystalline cellulose (MCC) was proposed. The hydrolysis of pulp was carried out with boiling 1-1.5 M sulphuric acid at liquid/solid ratio 5 for 1-1.5 h. The obtained MCC had average particle sizes of 50-150 x 15-20 μm, degree of polymerization of 150-170 and crystallinity of 71-73%. The proposed green technologies provide the complete utilization of acidic wastewater and their use for the production of valuable by-products, the selling of which covers main part of the production cost of MCC. Furthermore, all washing water is returned in the production line. Another version of green technology was used for the production of composite containing microparticles of MCC and $CaSO_4$. Thus, the cheap cellulose products, wetcake of MCC or MCC & $CaSO_4$ composite, were obtained without discharge of production waste into the environment. The obtained microcrystalline products can be used as auxiliary additive in pharmaceutics, dental remedies, cosmetics, personal care and food products, as well as in various technical applications.

Keywords: Microcrystalline cellulose, Green technology, Waste utilization, By-products.

1. INTRODUCTION

As is known, fibrils of cellulose consist of built of ordered crystallites and low ordered non-crystalline domains (NCD) statistically alternated along the fibril [1]. The crystallites having three-dimensional order are strong and inaccessible structural elements. As against, the low-ordered NCD having twisted and curved segments are weak and accessible places of the fibrils. Thereby, cleavage of glycosidic bonds at the hydrolysis with dilute mineral acids occurs mainly in non-crystalline domains of cellulose, which facilitates the release of rod-shape crystalline aggregates called microcrystalline cellulose (MCC).

The term microcrystalline cellulose was proposed by Battista and Smith as a powdered product of the cellulose hydrolysis with boiling 2.5 M hydrochloric acid having level-off degree of polymerization, LODP [2]. Currently MCC is produced by various companies such as FMC Bioproducts, Asahi Kasei, JRS Pharma, etc. World production volume of MCC is about 120,000 tons per year [3].

Main application fields of MCC are an inactive ingredient for tablets, cosmetic formulation and food products, as well as filler and special additive for some technical applications [4]. To MCC produce, cellulose feedstock is hydrolyzed with dilute (1-3 M) mineral acids at increased temperatures up to LODP, which approximately corresponds to average length of individual nano-crystallites of cellulose [5]. After acidic treatment, MCC is separated from the acid, neutralized, washed, dried and grinded. Another technology is that washed hydrolyzed cellulose is diluted to 1-3% and then spray dried to obtain beads of MCC [6]. Acidic wastewater is neutralized and discharged to sewage system. Both existing MCC-technologies pollute the environment; moreover, the production cost of solid MCC is high and reaches $7,000-9,000 per ton [7].

To turn a chemical processing really into “green”, it is necessary to implement the basic principles of green chemistry formulated by Anastas and Warner [8]. Along with these principles, there are also some other ideas related specifically to green chemistry to produce MCC, namely:

- In order to reduce production expenses, the microcrystalline cellulose must be manufactured directly at a pulp mill using its infrastructure, energy, water and inexpensive pulp as a feedstock.
- Production wastes should be used as raw materials for manufacturing of valuable by-products, the sale of which can cover part of production expenses of the primary product, MCC.
- It should be provided the complete recycling of used water and its return to the production line.
- Optimal production conditions should be found to increase the yield, prevent feedstock losses and reduce the consumption of chemicals and energy.

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- Extremely costly and low-productive process of fine comminuting should be avoided.
- In addition, the drying process should be also avoided because it requires increased energy consumption

The main purpose of this research was the development of some green technology for production of MCC at a low cost without discharge of production waste into the environment. General schemes of green process have been proposed and experimentally tested. Samples of MCC were obtained and their characteristics were studied. In addition, the cost of microcrystalline products was also evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Bleached Kraft pulp of Weyerhaeuser (USA) having 92% α-cellulose and DP =1100 was chosen as an initial feedstock. Besides, the technical grade 95% sulfuric acid, sodium carbonate and hydroxylapatite were used.

2.2. Production methods of MCC

The feedstock, bleached Kraft pulp, was cut into 1-3 cm pieces that were mixed with 1- 1.5 M SA up to liquid/solid ratio 5 and then put into a reactor. The reactor was heated to boiling temperature of the acid solution and maintained at this temperature for 1-1.5 h while stirring to hydrolyze amorphous domains of cellulose. After acidic treatment the reactor was cooled to room temperature. The obtained MCC slurry was separated from the acid solution by filtration on a vacuum filter and washed to neutral pH value separating the acidic water by filtration. Moreover, all acidic wastewater was collected together and neutralized with powdered hydroxylapatite (HAP) to obtain the superphosphate by-product, as follows:

$$7H_2SO_4 + 2Ca_5(PO_4)_3OH \rightarrow 3Ca(H_2PO_4)_2 \times 7CaSO_4 + 2H_2O$$

Another production method was that after acidic treatment the required amount of calcium oxide or carbonate was added to the acidic slurry of MCC to neutralize the acid and obtain calcium sulphate (CAS) as by-product:

$$H_2SO_4 + CaO \rightarrow CaSO_4 + H_2O$$

$$H_2SO_4 + CaCO_3 \rightarrow CaSO_4 + H_2O + CO_2$$

After blending, the final product was obtained, namely a composite consisting of microparticles of cellulose and CAS.

2.3. Methods of analysis

The chemical composition of initial feedstocks and obtained products was studied by standard methods of chemical analysis [9]. The degree of crystallinity of the cellulose samples was determined by method of wide angle X-ray scattering, WAXS [10]. Size and shape of the nanoparticles were investigated by method of field emission gun scanning electron microscopy [11]. The average degree of polymerization, DP, was measured by the viscosity method using diluted solutions of cellulose in Cadoxen [12].

3. RESULTS AND DISCUSSION

Since existing MCC technologies are accompanied by the formation of liquid wastes polluting the environment, a green technology has been developed, the scheme of which is shown in Figure 1.

The feedstock can be, for example, bleached Kraft pulp supplied directly from the pulp mill at a reduced cost of

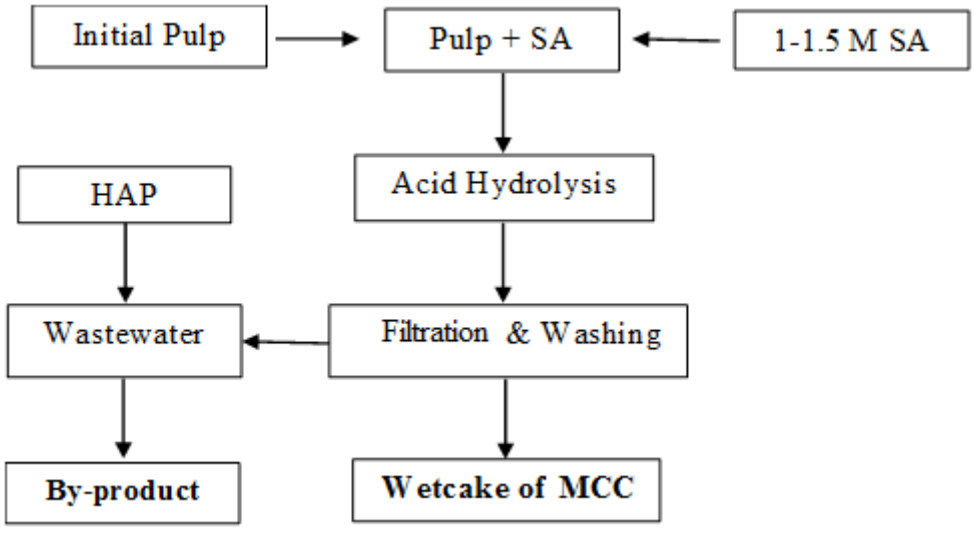

Fig.1: General scheme of green MCC technology

about $350 per ton. The initial pulp was treated with 1 M SA for 1.5 h or with 1.5 M SA for 1 h in accordance with method 2.2. As a result, about 40% wetcake of MCC was obtained along with by-product such as superphosphate. Neutral wastewater was returned in the production line to use for preparation of acid solutions and washing of hydrolyzed cellulose.

Structural studies showed that MCC wetcake contains rod-like particles with sizes of 50-150 x 15-20 μm (Fig. 2). Crystallinity degree of MCC was 71-73% and DP was 150-170.

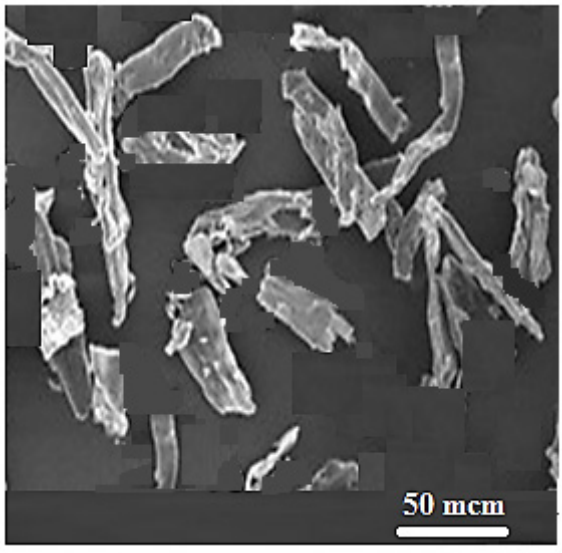

Fig. 2: SEM image of MCC particles

As a result of economic calculations [13], the production cost of 40% MCC wetcake was evaluated at $185-188 per ton wetcake or $460-470 per ton of solids MCC that is 15-17 times less that the price of commercial MCC.

The proposed green technology provides the complete utilization of acidic wastewater for production of valuable by-product such as superphosphate fertilizer, the sale of which covers part of the MCC production cost. The MCC was obtained without discharge of production waste into the environment. Besides, washing water was returned to the production line. As a result, production cost of MCC is relatively low. This microcrystalline product can be used as auxiliary additive in pharmaceutics, cosmetics, personal care, food products, etc.

Another version of green technology can be implemented in accordance with the scheme shown in Figure 3. This technology provides hydrolysis of initial cellulose with boiling sulfuric acid (1 M for 1.5 h or 1.5 M for 1 h) at LSR 5 and subsequent neutralization of the acid with calcium oxide or carbonate.

As a result, MCC & CAS composites are formed having 14-15% MCC, 11-15% CAS and 71-74 % Water (Table 1):

Table 1

Chemical composition of MCC & CAS composites, in %

Component	1 M SA	1.5 M SA
MCC	15	14
CAS	11	15
Water	74	71

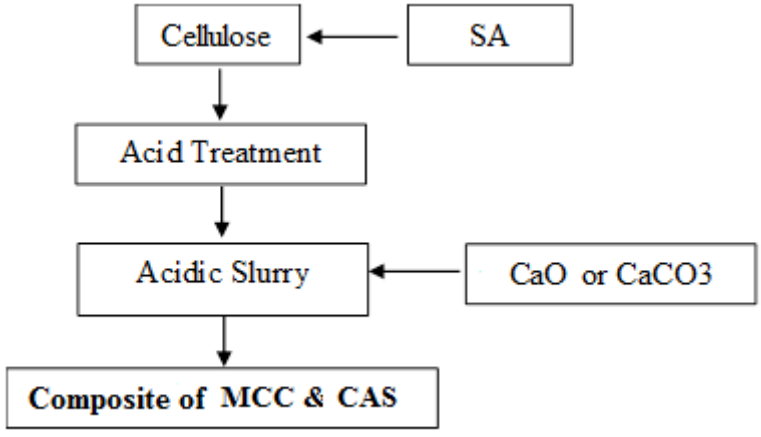

Fig. 3: General scheme of green production of MCC and CAS composite

The obtained composites contain rod-like MCC particles with sizes of 50-200 x 15-20 μm, along with round microparticles of CAS with diameter of 10-20 μm.

Economic calculations evaluated that production cost of micronized composites is about $95-100 per ton [13]. The proposed green technology provides the complete utilization of cellulose feedstock, chemicals and water. Furthermore, the used acid is converted into valuable by-product such as CAS. Thus, the cheap composites were obtained with zero-discharge of production waste into the environment. These composites containing microparticles of MCC and CAS can be used as thickeners, fillers and white pigments for dental pastes, cosmetic creams and dispersions, personal care remedies, as well as for various technical applications (putties, mastics, glues, liquid wallpaper, etc.).

4. CONCLUSION

Green technology of MCC production was explored using cellulose hydrolysis with dilute solutions of sulphuric acid. It was shown that MCC can be obtained together with conversion of liquid production wastes into valuable by-product such as superphosphate fertilizer, the sale of which can cover main part of production expenses of primary product, MCC. Besides, used water was recycled and returned to the production line. Another version of green technology was used for the production of composite containing microparticles of MCC and $CaSO_4$ without discharge of production waste into the environment. As a result, the production cost of microproducts can be significantly reduced.

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