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Green Synthesis of Magnesium Oxide Nanoparticles Using *Brassica oleracea* and *Punica granatum* Peels and their Anticancer and Photocatalytic Activity

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Experiments were carried out on the synthesis of magnesium oxide nanoparticles using the water extract of cauliflower and the water extract of pomegranate peels. The primary particles were of sizes 30-45 and 50-65 nm in the powder synthesized using the water extract of cauliflower and the water extract of pomegranate peels respectively. Polycrystalline powders were obtained when synthesis was carried out using the water extract of pomegranate peels, white crystalline powders were obtained when using the water extract of cauliflower. The green-synthesized magnesium oxide nanoparticles exhibited good anticancer activity, with 31.2 µg/mL being the nanoparticles concentration required for destruction of 50 % of HeLa cells. The green-synthesized magnesium oxide nanoparticles were found to possess photocatalytic activity both under UV irradiation and sunlight.

Keywords: MgO, Nanoparticles, Green synthesis, Anticancer, Photocatalytic.

INTRODUCTION

It is well recognized that the nanoparticles have wide ranging applications in the field of agriculture, optical fibres, antimicrobials, electronics, catalyst, biomedical, bio labelling, sensitized solar cell, pharmaceuticals, *etc.*[1,2]. The performance of a nanomaterial can be tuned depending on the size, shape and crystal structure. Nanomaterials can be prepared through green, chemical or microbial synthesis[3]. Magnesium oxide is one of the widely used metal oxide for catalytic applications. Magnesium oxide is nontoxic and biodegradable. However, MgO is toxic to microbes, through its binding to intracellular protein at low concentration[4]. Magnesium oxide is also used in remediation of toxic waste and as superconductor products[5]. Gas and humidity sensors have been made with MgO as the key constituent. Magnesium oxide is also a good adsorbent for vapours and gases such as acetone, ammonia, carbon dioxide, methanol, dimethylamine, trimethyl acetaldehyde, benzaldehyde and acetaldehyde[6].

Chemical methods used for the preparation of MgO nanoparticles include microwave-induced combustion, precipitation, hydrothermal, aerosol combustion, flame spray pyrolysis, chemical vapour deposition, flame metal combustion, sonication, sol-gel method and lysine nitrate combustion[5,7,8]. Magnesium oxides have been synthesized in different shapes leading to nanoplates, nanocubes, nanoparticles and nanowires[9-11]. Green synthesis is an attractive technique for preparation of nanomaterials predominantly owing to its eco-friendliness. When the green synthesis of nanoparticles is successfully scaled up, they are likely to be cost-effective when compared to chemical and physical methods[12]. This cost-effectiveness is attributed to fact that the green synthesis is performed at near-ambient conditions without the requirement of use of high pressure, temperature or heat energy[13,14].

Pomegranate (*Punica granatum*) belongs to the family of Lythraceae. It is one of the oldest edible fruit and contains polyphenols at higher concentrations. The other major components are flavones, flavan-3-ols and anthocyanidin. The important tannins in the pomegranate are hydrolazable tannins including gallo tannins, ellagic tannins and condensed tannins. It may be recalled that the tannins are good antioxidants[15]. The peels of pomegranate too contain polyphenols such as ellagic tannins, gallic acid and ellagic acid. Pomegranate has therapeutical applications, apart from being used in preparation of cosmetics, food recipes, tinctures, *etc*[16].

Cauliflower (*Brassica oleracea*) contains a number of phytonutrients such as glucosinolate, ascorbic acids, polyphenols, anthocyanins, sulphoraphane and indole-3-carbinol, which are well-known antioxidants. Anthocyanins are known to provide protection against free radicals[17]. It has been reported that the glycosinolate derived product possess anticancer and antioxidant activity, while indole-3-carbinol inhibit growth of cancer cells[18].

In the present work, attempts have been made to utilize the potential of this pomegranate peels and cauliflower for preparation of magnesium oxide nanoparticles. While the extracts of cauliflower and pomegranate peels have been used to synthesize silver nanoparticles[19,21,22] and gold nanoparticles[19,20,22], to the best of our knowledge, this is the first report on the synthesis of magnesium oxide nanoparticles using pomegranate peel and cauliflower. The prepared nanomaterial has been characterized and its anticancer and photocatalytic activity are being reported.

EXPERIMENTAL

Magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$) was obtained from Merck, Mumbai. Fresh *Brassica oleracea* (Cauliflower) and *Punica granatum* (Pomegranate peels) were obtained from local market. Double distilled water was utilized for the preparation of extracts.

Reactive black dye was purchased from Indian dyes and chemicals, Coimbatore. *HeLa* cell lines were obtained from National Centre for Cell Sciences, Pune (NCCS). Minimum Essential Medium was purchased from Hi Media Laboratories, Mumbai. Fetal Bovine Serum (FBS) was purchased from Cistron Laboratories, Andhra Pradesh. Trypsin, methylthiazolyl diphenyl-tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were procured from Sisco Research Laboratory Chemicals, Mumbai.

Preparation of water extract and nanoparticles: About 500 g of fresh *Brassica oleracea* (cauliflower) and 24 g dry powder of *Punica granatum* (pomegranate peels) was taken into separate beaker and double distilled water added to make up to 250 mL. This mixture was boiled for about 15-20 min. The mixture was then cooled, followed by filtration to separate solid matter from the extract. The extract was collected and stored for subsequent use.

About 250 mL of magnesium sulphate heptahydrate solution of 0.1 M concentration was prepared using double-distilled water. To this solution, 250 mL of water extract of cauliflower and pomegranate peels was added drop by drop with continuous stirring in a magnetic stirrer. The total time consumed for addition of 250 mL of water extract of cauliflower and pomegranate peels was about 12 h. Solid-liquid dispersion was obtained at the end of the reaction, which was then centrifuged at 7000 rpm for about 10 min. While the supernatant was discarded, the pellet was washed, dried and calcined at 450 ºC for 1 h. The mass of sample was measured before and after calcination to determine the weight loss during calcination.

Characterization of extract and the synthesized product: The ethanol extract of pomegranate peels was analysed using (GC-MS) gas chromatograph coupled with mass spectrometer (Clarus 500, PerkinElmer, USA). The water extract of cauliflower was subjected to LC-MS/MS Spectrometer (MicrO TOF-Q II, Bruker Daltonik GmbH, Germany).

Electron microscopy is a valuable tool to determine the morphological features of materials. In the present work, a field emission scanning electron microscope (JSM, 6701F, JEOL, Japan) has been used to determine the morphological features and the particle size of the prepared samples. A small amount of the synthesized powder was carefully sprinkled on the stub used to place the specimen for imaging. The stub with the synthesized powder was coated with gold before imaging was performed at the acceleration voltage of 3 kV.

The crystalline nature of the sample was ascertained using a powder X-ray diffractometer (D8 Focus, Bruker, Germany), by carrying out scan in the '2θ' range of 25-55. The wavelength of the radiation used for X-ray diffraction experiment was 1.54 Å.

A dispersion of synthesized product was made in water to enable determination of hydrodynamic size distribution and zeta potential. A zetasizer (Nano-ZS, Malvern, UK) was utilized for this purpose. A standard operating procedure designed for particle dispersions in water was used for the measurements.

Photocatalytic activity: One hundred millilitre of dye solution containing 50 ppm of reactive black dye was taken as the feed solution for experiments carried out to ascertain the photocatalytic activity of synthesized powders. A photochemical reactor fitted with a lamp of 125 W was used as the source for ultraviolet radiation. The wavelength of UV radiation emitted by the lamp was 254 nm.

The dye sample was withdrawn at intervals of 10 min to determine the absorbance at 580 nm, which is the wavelength corresponding to the maximum absorption coefficient for the dye solution. The dye degradation was studied in the presence of sunlight, without the use of external UV source also. The mass of nanoparticles used for degradation studies was 50 and 100 mg, respectively for degradation using photochemical reactor and sunlight, respectively.

Anticancer activity: The *HeLa* cells were maintained as per standard protocol. *HeLa* cells were plated in 24-well plates at the rate of 1×10^5 cells per well and incubated at 37 ºC and 5 % CO_2 condition. Once the cells reached confluence, the synthesized powder were added at different concentrations and incubated for 24 h. The sample was removed from the well after incubation and washed with pH 7.4 phosphate-buffered saline or MEM without serum. 100 µL/well, corresponding to 5 mg/mL of 0.5 % 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 h. Upon completion of incubation, DMSO was added to all the wells at an amount of 1 mL each. The cell concentration was determined by measuring the absorbance at 570 nm using a UV-spectrophotometer. DMSO was used as the blank for these measurements. The concentration of synthesized powder investigated for the anticancer activity ranged between 7.8 and 1000 µg/mL. The studies on anticancer activity were outsourced to Life Teck Research Centre, Chennai.

RESULTS AND DISCUSSION

Components present in ethanolic extract of pomegranate peels and water extract of cauliflower: Fig. 1 shows the results of GC-MS analysis of the ethanolic extract of pomegranate peels. The important components of the extracts were found to be *n*-hexadecanoic acid (256), 2-furancardoxaldehyde 5-(hydroxymethyl)- (126), furfural (96), 4*H*-pyran-4-one 2,3-dihydro-3,5- dihydroxy-6-methyl- (144), D-allose (180) *etc*., The classes of antioxidant compounds identified from LC-MS/MS analysis of water extract of cauliflower are 6-mercap-

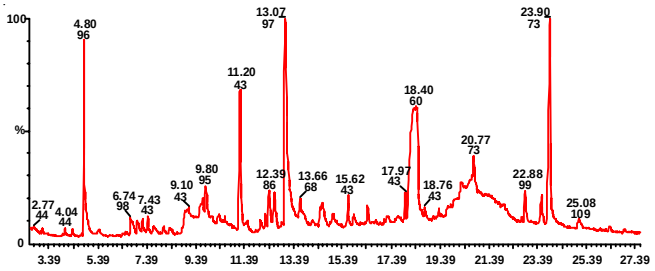

Fig. 1. GC-MS analysis of water extract of pomegranate peels

topurine (152.01), resveratrol (228.07), 7-hydroxyflavone (238.06), ipriflavone (280.10), kaempferol (286.04), catachin (290.07), herperetin (302.07), quercitrin (448.10), glycyrrhetinic acid (470.38), naringin (580.17) *etc*.,

Characteristics of the product: Fig. 2a,b show the scanning electron micrograph of the as-synthesized, calcined powders using the two water extracts. It is evident from Fig. 2a that the product synthesized using water extract of cauliflower contains nearly spherical nanoparticles with diameter ranging between 30 and 45 nm. Very few particles of relatively diameter are also seen, but they are relatively less in number upon comparison with particles in the size range of 30-45 nm. The product synthesized using the water extract of pomegranate peels contains particles in the size range of 50-65 nm (Fig. 2b). The difference in particle size could be attributed to the difference in the nature of water extracts. The powder X-ray diffraction patterns (Fig. 3) reveal the presence of MgO in the product synthesized using water extract of cauliflower, as the spectra matches with that of reference spectra for MgO (JCPDS No-270759). The broad nature of peaks indicates the presence of nanocrystalline MgO. The crystallite size was calculated using Scherer's formula and was found to be 24.13 nm for MgO synthesized using water extract of cauliflower. The MgO nanoparticles synthesized using water extract of pomegranate peels were found to be polycrystalline.

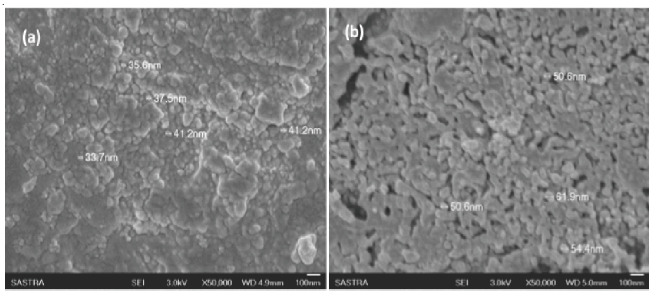

Fig. 2. Scanning electron micrograph of green-synthesized nanoparticles using [a] water extract of cauliflower; [b] water extract of pomegranate peels

Role of water extract of cauliflower and pomegranate peels in product synthesis: There are two widely used routes for synthesis of MgO from $MgSO_4 \cdot 7H_2O$. In one of the routes, a carbonate such as sodium carbonate is reacted with $MgSO_4 \cdot 7H_2O$ leading to the formation of $MgCO_3$. The calcination of $MgCO_3$ results in the formation of MgO. Another route involves reaction between $MgSO_4 \cdot H_2O$ and a strong base such as NaOH or KOH leading to the formation of $Mg(OH)_2$, which upon calcination results in MgO.

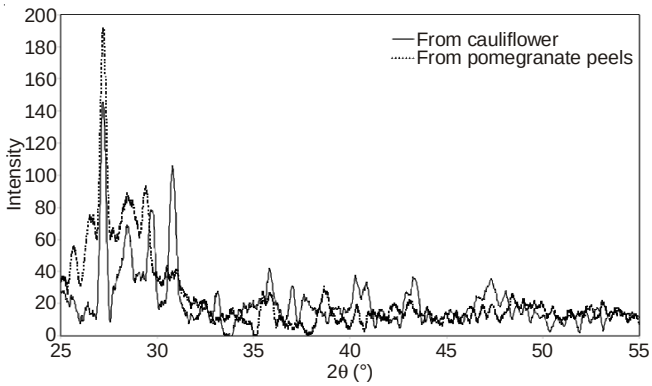

Fig. 3. Powder X-ray diffraction patterns of MgO nanoparticles using water extracts of cauliflower and pomegranate peels

Several components of water extract have hydroxyl groups that may react with $MgSO_4$. H_2O leading to the formation of $Mg(OH)_2$. The evidence for this pathway stems from the weight loss observed during calcination. While the theoretical weight loss calculated from stochiometry during the transformation of $Mg(OH)_2$ to MgO is 30 % , the actual weight loss observed was 31 and 31 % for intermediates obtained during the reaction between $MgSO_4 \cdot 7H_2O$ and water extract of cauliflower and between $MgSO_4 \cdot 7H_2O$ and water extract of pomegranate peels, respectively, which are closer to the theoretical value.

Hydrodynamic size distribution: Hydrodynamic size refers to the diameter of the particles and the surrounding liquid layer formed when particles are dispersed in a liquid. Though the hydrodynamic diameter depends on the nature of particle-liquid interaction and the presence of ions in the liquid also apart from the primary size of the particles, hydrodynamic diameter is widely used to characterize the state of aggregation in nanoparticles dispersion[23-25]. A lower value of hydrodynamic diameter denotes lower extent of particle aggregation, while a higher value is indicative of higher degree of aggregation.

The hydrodynamic size distribution for MgO nanoparticles synthesized using the two extracts in water is shown in Fig. 4. It is evident that the size distribution is monodisperse, with the average hydrodynamic diameter of 206 and 350 nm, respectively for samples prepared using the water extract of cauliflower and pomegranate peels, respectively. While comparing the average hydrodynamic size with the particle size measured using scanning electron microscopy (30-45 and 50-65 nm) and the crystallite size determined by Scherer's formula (24.13 and 24.64 nm), the ratio of average aggregate size to primary particle size is about 5.

The sizes of aggregates are lower when the component of repulsive forces between the colloidal objects is higher than the magnitude of attractive forces. Zeta potential is a measure of charge strength on the particle surface. Higher magnitude of zeta potential indicates higher magnitude of repulsive interactions and hence indicates size stability of colloids. The zeta potentials of dispersion of green-synthesized MgO nanoparticles, prepared using cauliflower and pomegranate peels, in water was found to be -29.2 ± 1.5 and -30.5 ± 1 mV, respectively indicating the presence of negative charges on the particle surface when the same was dispersed in water.

Anticancer activity: The infleunce of MgO nanoparticle concentration of viability of HeLa cells was carried in the nano-

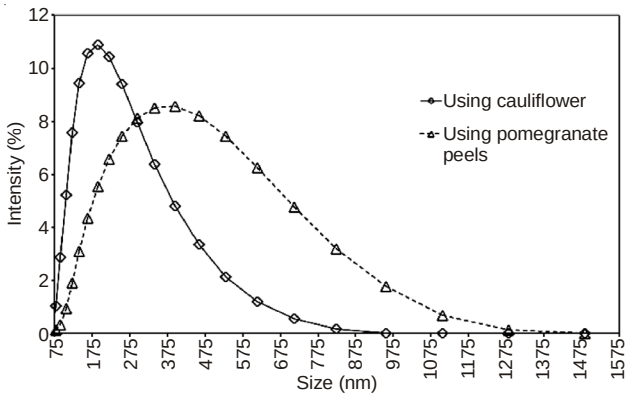

Fig. 4. Influence of source of water extract on hydrodynamic size distribution of dispersions of MgO nanoparticles in water

particle concentration range of 7.8-1000 µg/mL. When compared to the control, the cell viability decreased significantly with the addition of nanoparticles (Fig. 5). At about a nanoparticle concentration of 31.2 µg/mL, the cell viability was reduced to about 50 %. The fact that the cell viability was found to be independent of green reagent, indicates that both the green sources (cauliflower and pomegranate peels) contain a number of components with anticancer activity, which could be adsorbed on the particle surface.

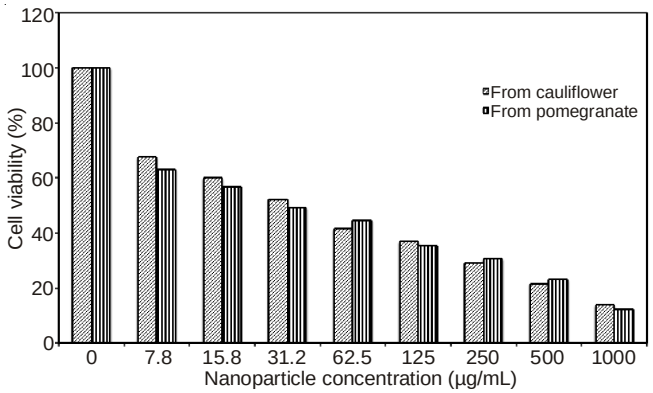

Fig. 5. Influence of MgO nanoparticle concentration and the green source on cell viability of HeLa cells

The FTIR spectra of the synthesized nanoparticles (Fig. 6) shows absorption bands characteristics of several functional groups associated with organic compounds. For instance, peaks around 3400, 2900 and 1600 cm^{-1} may be attributed to the presence of phenols and alcohols, alkanes and amides functional groups. The anticancer activity is also confirmed through microscopy (Fig. 7a,b), in which the extensive damage to cellular morphology is evident for nanoparticle concentrations of 31.2 µg/mL and above. All these testify the anticancer activity of green synthesized MgO nanoparticles.

Photocatalytic activity: The photocatalytic activity of MgO nanoparticles synthesized using the water extract of cauliflower and pomegranate peels, for degradation of dye under ultraviolet irradiation is shown in Fig. 8. It is evident from Fig. 8 that MgO nanoparticles synthesized from both the green sources possess photocatalytic activity. However, the MgO nanoparticles synthesized using water extract of cauliflower have shown better photocatalytic activity (93 % dye degradation after 50 min) than those prepared using the

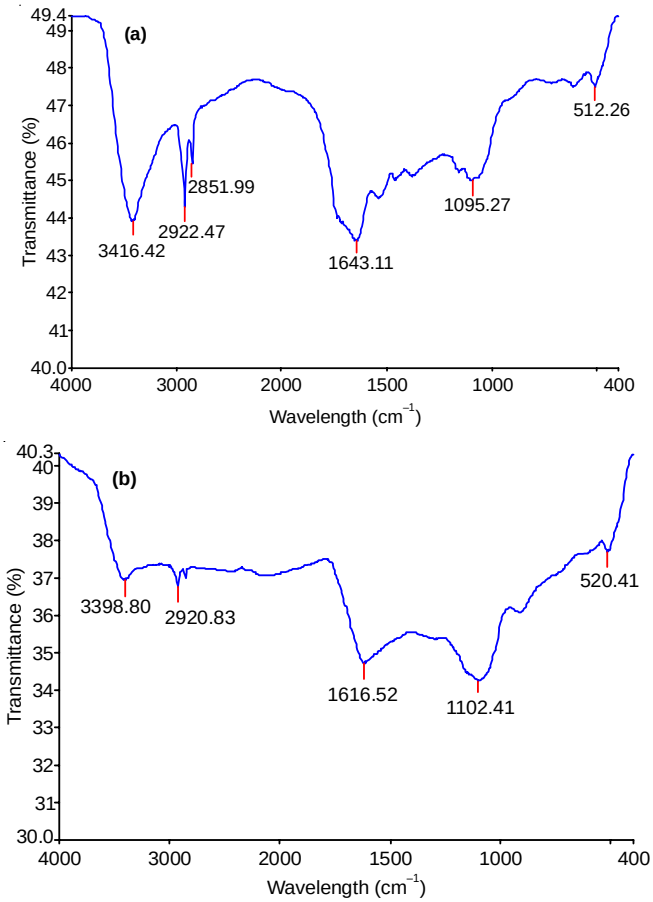

Fig. 6. FTIR spectra of MgO nanoparticles synthesized using [a] water extract of cauliflower; [b] water extract of pomegranate peels

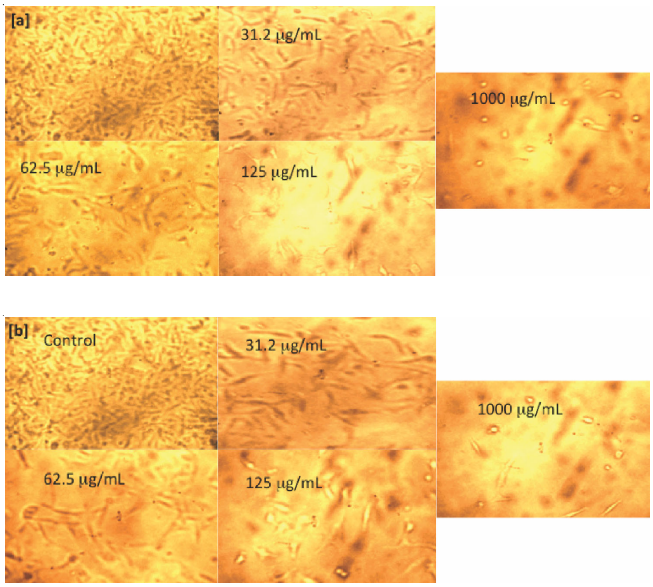

Fig. 7. Microscopic features of HeLa cells (control) and cells treated with various concentrations of MgO nanoparticles prepared using [a] water extract of cauliflower; [b] water extract of pomegranate peels

water extract of pomegranate peels (82 % dye degradation after 50 min). The photocatalytic activity of MgO nanoparticles synthesized using water extract of cauliflower (Fig. 9), under sunlight (30 % dye degradation after 1 h) is better than that of MgO prepared using the water extract of pomegranate peels (26 % dye degradation after 1 h). The surface area of particles

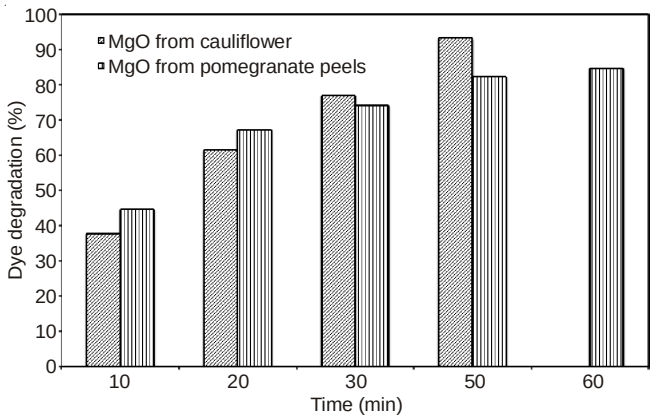

Fig. 8. Photocatalytic activity of green-synthesized MgO nanoparticles under UV irradiation

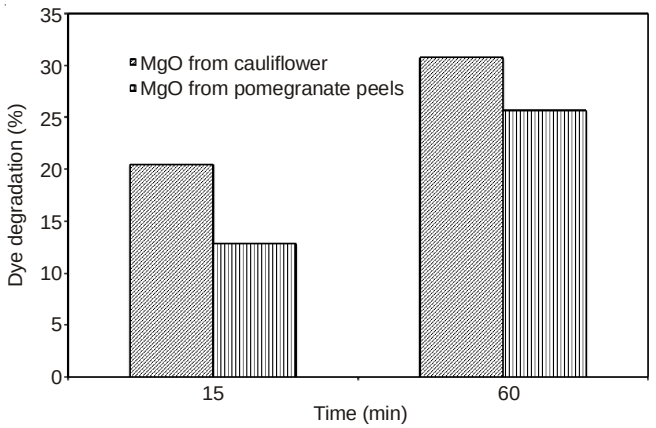

Fig. 9. Photocatalytic activity of green-synthesized MgO nanoparticles under sunlight

influences interactions between the fluid and solid media[26-31]. The nanoparticles synthesized using water extract of cauliflower are smaller than those synthesized using water extract of pomegranate peels. Therefore, the nanoparticles synthesized using water extract of cauliflower have high surface area per unit mass, when compared to those synthesized using water extract of pomegranate peels. Hence, the higher photocatalytic activity of MgO nanoparticles synthesized using water extract of cauliflower under UV irradiation and sunlight is attributed to their smaller particle size and higher surface area.

Conclusion

The water extracts of cauliflower and pomegranate peels were found to be suitable for preparation of MgO spherical nanoparticles from magnesium sulphate heptahydrate under optimized reaction conditions. The source was found to influence particle size and crystallinity of nanoparticles, with finer and crystalline particles obtained while using the water extract of cauliflower for preparation of nanoparticles. The anticancer activities of nanoparticles were found to be concentration dependent, while being independent of the green extract used for particle synthesis. The finer particles produced using the water extract of cauliflower made the MgO nanoparticles produced from this source, better photocatalysts under sunlight as well as UV irradiation, when compared to those prepared using the water extract of pomegranate peels. Hence, the green synthesis of MgO nanoparticles represents a facile, yet-simple and scalable method for preparation of MgO nanoparticles for a variety of applications.

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