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Antibacterial and Antioxidant Potential of Herbal Nanoparticles Produced from the Shells of *Jatropha Curcas*

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Abstract

Shells of Jatorpha curcas is commonly used as fuel and as soil fertilizer. In our study, we investigate the use of shells to synthesize nanoscale particles by mechanical grinding via top down approach. The shade-dried shells are subjected to high energy milling to produce nanoparticles and the collected powder is characterized for their particle size, crystallinity, functional group and elemental composition. Antimicrobial activity of the prepared nanoparticles reveals the significant inhibitory effect against Gram positive bacteria (S. aureus and S. epidermis) and gram negative bacteria (E. coli and K. pneumoniae). Growth of gram positive organism is found to be reduced by 50-60 % when the particle size is increased from 1 to 20 mg/ml whereas gram negative bacteria has 30-45 % of growth reduction. When compared to control, shell nanoparticles exhibit an excellent antioxidant activity about at 78 % at 20 mg/ml against DPPH reagent. This preliminary evaluation for the bioactivity of shell nanoparticles from Jatropha lead the future biorefinery processing of Jatropha plant materials into cost effective biological product development.

Keywords: *Jatropaha curcus*, shells, nanoparticles, antibacterial activity, antioxidant

1. Introduction

Jatorpha curcas is a commonly available plant in India, has many potential applications from energy to medicine. It does not require sophisticated agronomic cultivation, rather grows in arid and semi-arid conditions. *Jatropha* is also a drought and pest-tolerant plant and unpalatable by animals [1]. All parts of the plant are beneficial in any one or many products developments. Dried fruits of *Jatropha* have about 35–40% shell (by weight) which are available during the harvest [2]. Shells are also known rich in lignin that is used for the particle board and energy production [3,4]. They are also rich in inorganic sources and hence, biorefinery processing of shell is commonly used as feedstocks and soil fertilizer applications [5,6]. As it has high ash content, its influence on the type of conversion technology can be used to obtain energy from the shells [7].

Nanotechnology is one of the fast-growing technologies which cover wide range of applications due to its high surface area and size dependant transport in living cells. The interaction of nanoparticles in living cells has either toxic or beneficial effect which depends upon the structure, composition and dosage of the nanoparticles. Plants are generally rich in organic compounds and hence, use of crude plant compounds as dried

powders to administer for therapeutic purpose is one of the modes of drug delivery. Nowadays, new discoveries have helped to develop herbal drugs that have no side effects and have high therapeutic activities [8]. Green chemistry plays an important role in the field of nanoscience and nanotechnology, which are very important in synthesizing nanoparticles for different applications [9].

Preparation of crude plant nanoparticles from *Jatropha* shells via top down approach is one of the cost-effective routes along with free of chemicals and reagents for processing. Screening the antibacterial and antioxidant properties of the shell nanoparticles aids the novel application of shell waste material to many cosmetic and therapeutic applications. Mechanical attrition is one of the effective methods to synthesize homogenous particles of plant powders at nanoscale. Even though plenty of studies have been previously made on the phtyochemical analysis and antibacterial activity of leaf, latex, seed and oil of *Jatropha* as reported by Abdelgadir and Van Staden [10], no work has been reported on the use of shells for medicinal property except their applications in fuel [3-6]. Studies on evaluating the medicinal applications of nanoscale shell particles from *Jatropha* are very scanty. Therefore, in our study, it is aimed to synthesize plant nanoparticles from *Jatorpha curcas* shells and characterize its physico-chemical properties. The prepared nanoparticles are evaluated for their antibacterial and antioxidant properties for medicinal additives and agricultural applications.

2. Experimental

2.1 Materials

Jatropa curcas fruits were collected in and around the area of Tiruchengode, Tamil Nadu, India. The shells were mechanically removed from the ripened fruit and weighed. Dried shells were cleaned and thoroughly washed with deionized water. The composition of shells is given in Table 1. Before milling, the nutshells were sun dried to reduce the moisture content.

Components	Ash	Crude protein	Moisture	Content of shell
%	7.2	5.1	9.3	38

Table 1. Compositions of *Jatropha curcas* shell powder

2.2 Top down approach

The dried shells were initially ground in a mixer to get a coarse powder. Then, the coarse powders are subjected to high energy milling using 20 mm sized ball (Zirconia) in a Planetary Ball mill (PM100; Retsch, Germany) for 1 h. Then, the obtained fine powders were equally (nearly 5 mg) divided into four parts. The separated fine powders were again milled (10 mm balls: 300 rpm) for 1 h. The average particle size of the shells was thus reduced within the milling system to nanoscale.

2.3. Characterization

The particle size distribution was studied using a sub micrometer particle size analyser (Nanophox; Sympatec, Zellerfeld, Germany) based on dynamic light-scattering technique. The particle size distribution was analysed employing three-dimensional photon cross-correlation techniques. The morphology and the purity of shell nanoparticles was studied using scanning electron microscope coupled with energy dispersive X-ray spectrometer (SEM-EDAX) (JEOL JSM-6390LV, Japan). Jatropha shell nanoparticles were

observed for its morphology and particle size using transmission electron microscopy (TEM) (CM 200, Philips, Netherlands). In addition, SAED pattern is used to determine the crystallinity of the prepared sample.

2.4. Antimicrobial activity

The bacterial cultures namely gram positive (*S. aureus* and S. *epidermis*) and gram negative (*E. coli* and *K. pneumoniae*) organisms were obtained from the National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, Maharashtra, India. The test bacterial cultures were periodically sub-cultured at room temperature 310 K for 24 h. The bacterial cultures were maintained on a nutrient agar slant (HiMedia, India) for further experiments at 281 K.

2.5. Growth Reduction Test

Bacterial inoculum was prepared by inoculating a loop of test organisms into a nutrient broth and incubating it at 310 K for 5-8 h until a moderate turbidity was developed. A loop of culture was inoculated in a Luria Bertani broth and added with different concentrations of well dispersed shell nanoparticles. The broth was allowed to incubate in an arbitrary incubated shaker. The qualitative assessment of the antibacterial activity of *Jatropha* shell nanoparticles was carried out every two hours interval by Turbidometric analysis in UV-Vis Spectrophtotmeter (Cary 8454; Agilent, Singapore). Then, the growth reduction percentage of bacterial cultures treated with shell nanoparticles are calculated by applying the following formula:

$$\text{Growth Reduction percentage} = \frac{\text{Control - Test}}{\text{Control}} \text{ X } 100 \qquad \text{(i)}$$

2.6. Antioxidant activity

Antioxidant activity was studied using the di(phenyl)-(2,4,6-trinitrophenyl) imino azanium (DPPH) method for hydrophobic particles [11]. Nanoparticles of different masses (1, 5, 10 and100mg) were used for analysing the dose-dependent activity as reported by Karunakaran et al [12]. 1.7ml DPPH reagent was added followed by vortexing for 3 min. The 1.7 ml DPPH vial without particles served as control (C) and the vials with particles acted as test (T). Then, the mixture was allowed to stand for 30 min incubation at room temperature. The supernatant was collected at 11,963*g* for 2 min after incubation, and then, the optical density (OD) was measured at 517 nm using UV–Vis spectrophotometer (U-2900/2910; Hitachi, Japan). The percentage free radical scavenging was calculated with respect to the control by applying the following formula:

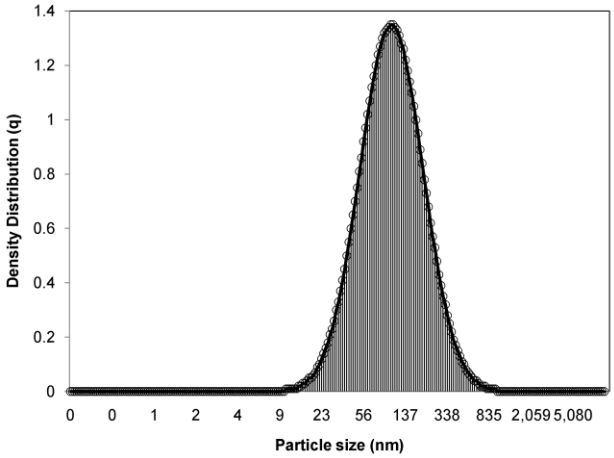
$$\text{DPPH scavenging } (\%) = \frac{CA - TA}{CA} \text{ X } 100 \qquad \text{(ii)}$$

where, CA and TA are respectively the control absorbance and test absorbance.

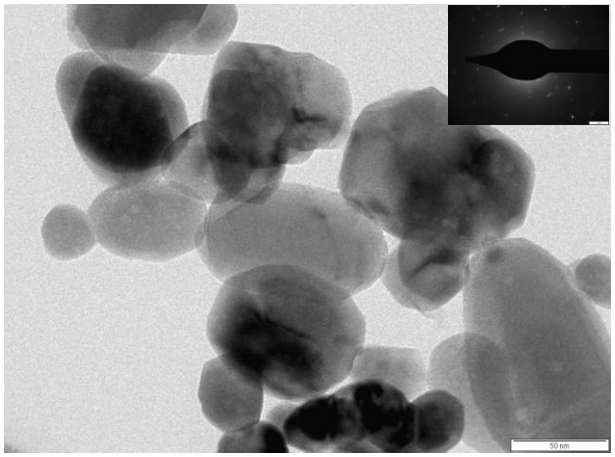
3. Results and discussion

The shell nanoparticles are synthesized from high energy ball milling as fine brown coloured homogenous powders. Ball milling of the samples does not alter the chemical composition of the shell particles and hence, it is studied for their particle size, shape and morphologies. The particle size distribution of the prepared powders is characterized and it is found to be in the range from 80 to 96 nm that is observed from Figure 1a. These nanoparticles are further analysed under transmission electron microscope which confirms the amorphous structures with slight crystallinity

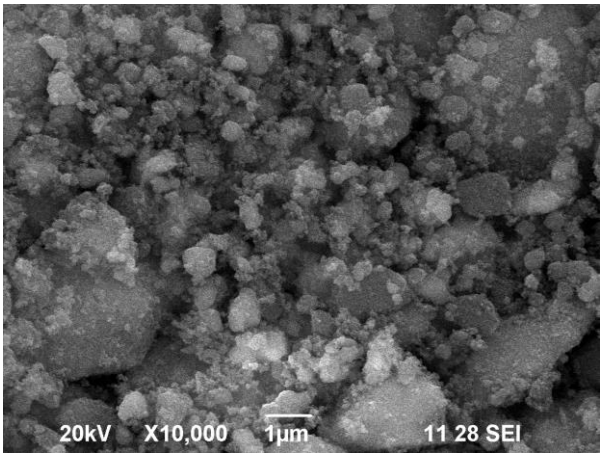
(Figure 1b). In addition, the morphology seen in figure clearly depicts the irregular morphology with a mixture of spherical and rod shapes. Dispersion of the particles are also observed to be fine and discrete that gives the uniform dispersion while using shell nanoparticles for biological applications. The range of particle size measurements are made and found to be in the range of 50 - 95 nm (Figure 1b). The SEM image which is seen in Figure 1c shows the dispersed shell nanoparticles with irregular shapes and size.



a) Particle size distribution



b) TEM



c) SEM

Figure 1. Characteristic result of prepared *Jatropha* shell nanoparticles

Size-dependant changes on biological activity of the shell nanoparticles are studied in terms of their antibacterial activity against gram positive (*S. aureus* and *S. epidermis*) and gram negative (*E.coli* and *K. pneumoniae*) organisms, antioxidant property against DPPH reagent at different concentrations of particle treatment. Bacterial cultures treated with different concentration of particles are analysed for their inhibition in growth based on culture turbidity by spectrometric analysis in different time intervals. Growth is reduced in all the tested bacteria as reflected from growth reduction percentage in Figure 2-4 when the particle concentration increases. However, after

360 and 480 min, it continues to increase in their turbidity of bacterial culture. It may be due to the formation of biofilms after they enter into the logarithmic phase of its growth. It is evident that bacteria persist to grow as biofilm on surfaces and tolerate to environmental stresses [13,14].

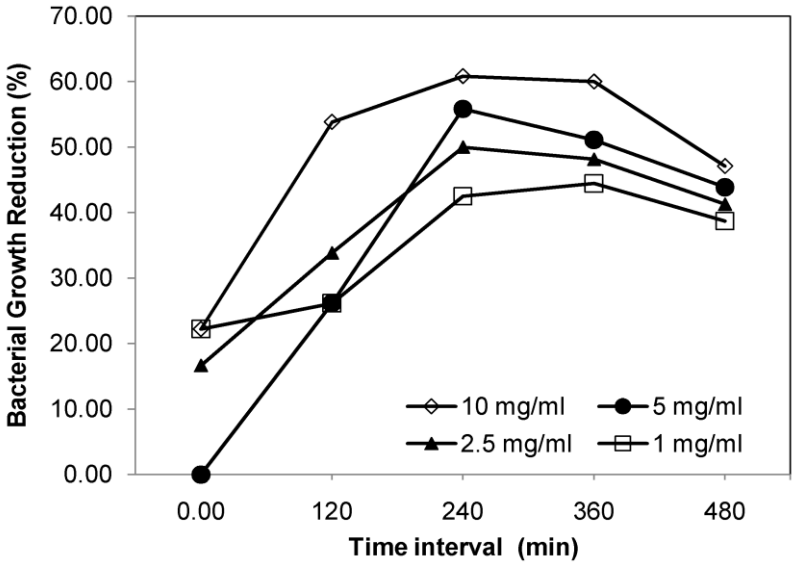


Figure 2. Antibacterial activities of shell nanoparticles against *S.aureus*

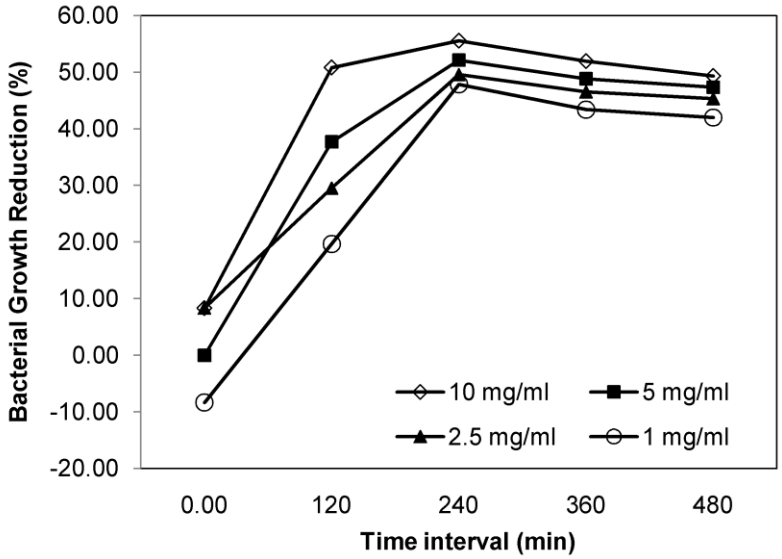


Figure 3. Antibacterial activities of shell nanoparticles against *S.epidermis*

The results observed from Figure 2 and 3 reveal that *S. aureus* is found to be more susceptible to particle treatment than S. *epidermis* by 10 %. Over all, the growth of gram positive organisms is reduced by 50-60 % when the particle sizes are increased from 1 to 20 mg/ml. At low concentration, shell powders are not enough to inhibit the bacterial growth. It is interesting to note

that gram positive organisms such as *S. aureus* and *S. epidermis* are prominent causative agents of nosocomial infections associated with medical devices and implants [15,16] and they are significantly inhibited by the shell nanoparticles. As other parts of *Jatropha* such as extracts of leaf, seed and seed oil, shells are also found to have non-specific interactions with bacterial cell wall and inhibit the bacterial growth [17].

When shell nanoparticles are tested against gram negative bacteria such as *E. coli* and *K. pneumoniae,* their growth is reduced by 30-45 % as seen in Figure 4 and 5. Similar to gram positive organisms, the growth reduction percentage is increased with their rise in concentration of particles. In contrast to the previous observation, growth reduction undergoes saturated phase after 240 min of culture growth at 10 and 20 mg/ml concentration. It indicates the resistance of the organism to shell nanoparticles after 360 and 480 min at which bacterial growth is suppressed to cell doubling. *E.coli* is comparatively less susceptible to *K.pnuemoniae* to particle treatments. Because the growth of *K.pnuemoniae* is started reducing even at the lower concentration of particle treatment (1 and 2.5 mg/ml) (Figure 4) whereas *E.coli* exhibits negative values of growth reduction percentage (Figure 5). The outcome of this observation results the bacteriostatic action of shell nanoparticles against gram negative bacteria. As these bacterial pathogens predominantly cause pyogenic liver abscesses and highly resistant beta-lactum antibiotics [18,19], the present study is important to use eco-friendly herbal nanoparticles for biomedical and clinical device applications. *Jatropha curcas* contains higher steroids, terpenoids, flavonoids and alkaloids (mainly, jatrophine) compounds in their plant parts [20] which may responsible for the observed bacterial reduction. In addition, the presence of metal and lignin compounds in the shell nanoparticles may also responsible for rendering this antibacterial action.

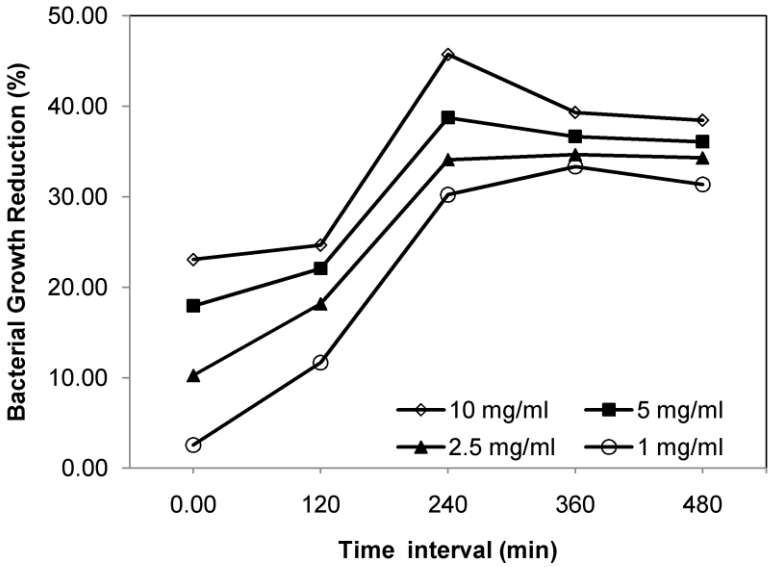


Figure 4. Growth reduction of *K.pneumoniae* treated with shell nanoparticles

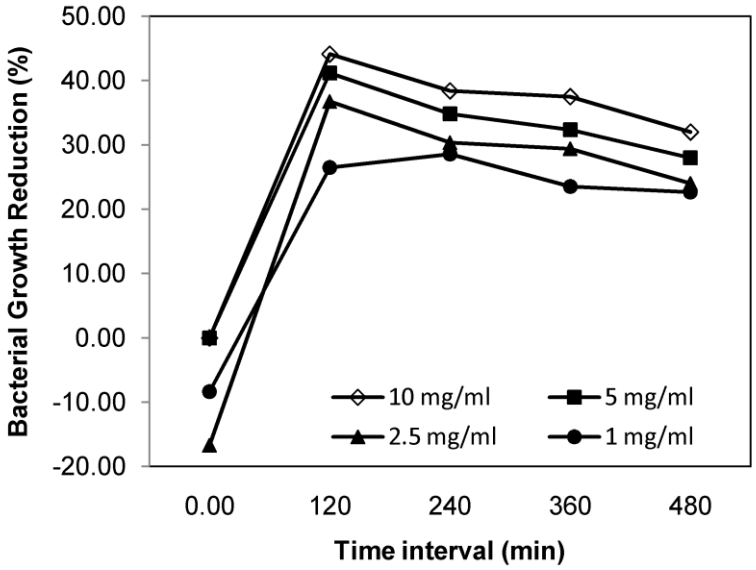


Figure 5. Growth reduction of *E.coli* treated with shell nanoparticles

Screening of antioxidant property of *Jatropha* shell nanoparticles of different concentration against di(phenyl)-(2,4,6-trinitrophenyl) imino azanium are shown in Figure 6. The response of shell nanoparticles to DPPH is observed to be the tangential increase with an increase is in particle concentration. Scavenging activity of jatropha shell particle is high at 20 mg/ml (78 ±1.5 %) which is comparatively high when compared to other metal oxide nanoparticles synthesized from natural resources. Generally, most of the fruits, vegetables and leaves of the plants are potent antioxidants. However, it is not either restricted to any part of the plant or specific family [21]. The antioxidant activity of the extracted compounds of *Jatropha* leaves, seed oil and fruits are studied and found to possess significant antibacterial and antioxidant activity at very higher concentrations [22, 23]. In addition, the phenol and methanol extracts of Mexican *Jatropha curcas* has been studied for their differences in antioxidant activity [24]. However, in this study, we observe an unprocessed shell powders along with phenolic and organic/inorganic components are being used as fuel source and its nanoscale powders have considerable antioxidant property.

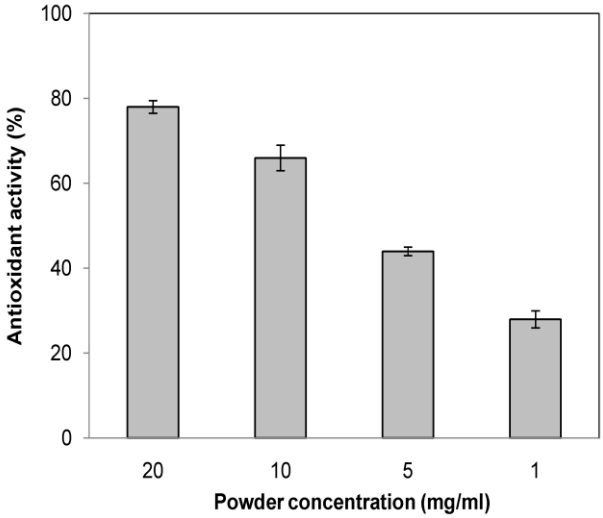


Figure 6. Study of antioxidant activity of shell nanoparticles against DPPH Reagent

4. Conclusion

The nutshells of *Jatropha* fruits are processed into crude nanoscale powders via ball milling

approach and it is comprehensively characterized to confirm their structure and particle size. The particles in the size range of 80-96 nm are found to exhibit considerable broad spectrum antibacterial activity against gram positive and gram negative microorganisms. In addition, evaluation of antioxidant property of shell powders also reveals the good response against DPPH. This observation is advantageous of using shell nanoparticles for biological and cosmetic applications.

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