Section A-Research paper
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Preparation of lignin nanoparticles from coconut coir of Cocos nucifera and its antiviral potential

A. G. Potphode and R. C. Patil
1. Ph.D scholar and 2. Associate professor
Department Of Microbiology, Bharatiya Vidya Bhavan's, M.M College of Arts, N.M Institute of Science, H.R.J. College of Commerce, Bhavan's College [Autonomous], Andheri, Mumbai
Corresponding Author: Email address: aratiphdwork@gmail,com

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ABSTRACT
This research work mainly focuses on preparation and characterization of lignin nanoparticles from coconut waste of Cocos nucifera L and determination of its antiviral potential. Lignin nanoparticles were prepared successfully by alkaline extraction method. Further the nanoparticles were tested for antiviral potential against SARS- Covid 19 virus using silver nanoparticles as a control with the help of BioRad CFX 96qRT-PCR machine. Covid-19-delta variant when added with lignin nanoparticles values changed with a large difference, for FAM value changed from 24.21 to 34.66, for Texas red from 26.38 to 32.33 and for VIC from 25.62 to 33.08. This large increase in cq values suggesting that RNA genome is degraded after reacting with lignin nanoparticles. This type of degradation is also observed when covid-19-delta variant was added with silver nanoparticles but the maximum degradation was observed when lignin nanoparticles were used.

Keywords- Lignin nanoparticles, Cocos nucifera L, SARS- Covid 19, Antiviral

INTRODUCTION
Lignin, a major polymeric component in plants, arises as a promising candidate for some applications due to its chemical versatility. Lignin is one of the most abundant natural polymers, together with cellulose and hemicellulose, and it forms part of the secondary cell walls of plants and helps to maintain the integrity of the cellulose/ hemicellulose/ pectin matrix. Apart from this, lignin is obtained from paper industry, when extracting cellulose in the pulping process. The composition and ratio of lignin in plants depends on the botanical species. [15]

Lignin is mostly present in the middle lamella between wood cells and the secondary cell wall. Thus, it plays an important role in bio-based plants by adding strength to the cell walls, controlling the fluid flow as well as protecting the plant against bio chemical attacks. [14]

Among the recent technologies, nanotechnology takes a predominated position in many applications especially in the field of electronics, energy, and medicine, but its application to crop protection is still in its early stages. Nanoparticles have a great potential for the use as "magic bullets" that are loaded with herbicides, chemicals, or nucleic acids and then targeted at specific plant tissues or areas where they release their loads. In recent years the development of nanocarriers for the targeted delivery and controlled release of agrochemicals has been reported. The encapsulation of some pesticides into nanocarriers can lead to improvements during their application, and allows the slow and constant release of the active ingredient, reducing the use of organic solvent. [7].

The mini emulsion process is an efficient method for the preparation of polymer particles in the size range between about

30 and 500 nm. Using this technique, a wide variety of different materials ranging from liquid to solid, from organic to inorganic, and from molecularly dissolved to aggregated species can be encapsulated into polymeric solid nanoparticles or nanocapsules with a liquid core. The use of renewable sources like lignin in the miniemulsion process breakthrough might help to overcome the petrochemical source limitation in diverse applications. [8]

MATERIALS AND METHODS

Sample

The coconut plant from Coastal region of Maharashtra, India was selected. The coir of coconut was selected as a source for preparation of lignin nanoparticles. Cocos nucifera L. species was labelled as selected.

Preparation of Lignin Nanoparticle: Finely chopped Coconut coir was sundried for 7 days and was cleaned for impurities like leaves, sand particles and other solid waste. Clean and sundried coconut waste was packed and labelled in sterile polythene bag of 25 Micron thicknesses.

1 gm of coconut coir was treated with 20 ml of 2M NaOH solution and was kept at 90°C for 1.5hrs. After completion of incubation period sample was washed with 0.1M NaOH solution and filtered with nylon cloth followed by washing with distilled water. After that filtrate was incubated at 50°C for 24 hrs.

Upon completion of incubation period samples were filtrated so as to collect filtrate and solid residues was discarded. Collected filtrate was neutralized with concentrated HCL for adjustment of pH to 5.5, immediately after neutralization filtrate was treated with 3 volumes of 95% ethanol.

Sample was again filtered with the help of nylon cloth for collection of filtrate and solid residues was discarded. Ethanol was allowed to evaporate and pH was adjusted to 1.5. At pH 1.5 solid particles was settled and collected, further washed with HCL to adjust pH 2.0. These solid particles are dried and used for further study.

Antiviral activity against SARS-COVID-19-DELTA

Antiviral activity was determined using BioRad CFX 96 qRT-PCR machine. Thermo Scientific Covipath Kit was used as a Master mix for testing. Silver nanoparticles was used as a control. All the viral tests were conducted in a controlled environment with a negative pressure room and in biosafety level-3 and level-4 cabinets.

The test solution of lignin nanoparticles was used in two different concentrations. First concentration is of 1:1 proportion (lignin nanoparticles: virus lysate) and other is of 1:2 proportion (lignin nanoparticles: virus lysate).

In first set only virus lysate was taken in VTM (virus transfer media). In second set virus lysate and silver nanoparticles was taken in a ratio of 1:1 proportion. In third set virus lysate and silver nanoparticles was taken in a ratio of 1:2 proportion. In fourth set lignin nanoparticles and virus lysate was taken in 1:1 proportion. In fifth set lignin nanoparticles and virus lysate was taken in 1:2 proportion. Positive control and control from test kit was used.

Automated mixing of test solution negative with viruses and extraction of mRNA was done using Promoter 96 set with magnetic beads. To quantify the viral mRNA which is converted into cDNA the fluorescence probes were administered with suitable mastermix of 10µl with 15 µl of template mRNA, making the total volume of 25 µl in 96 plat

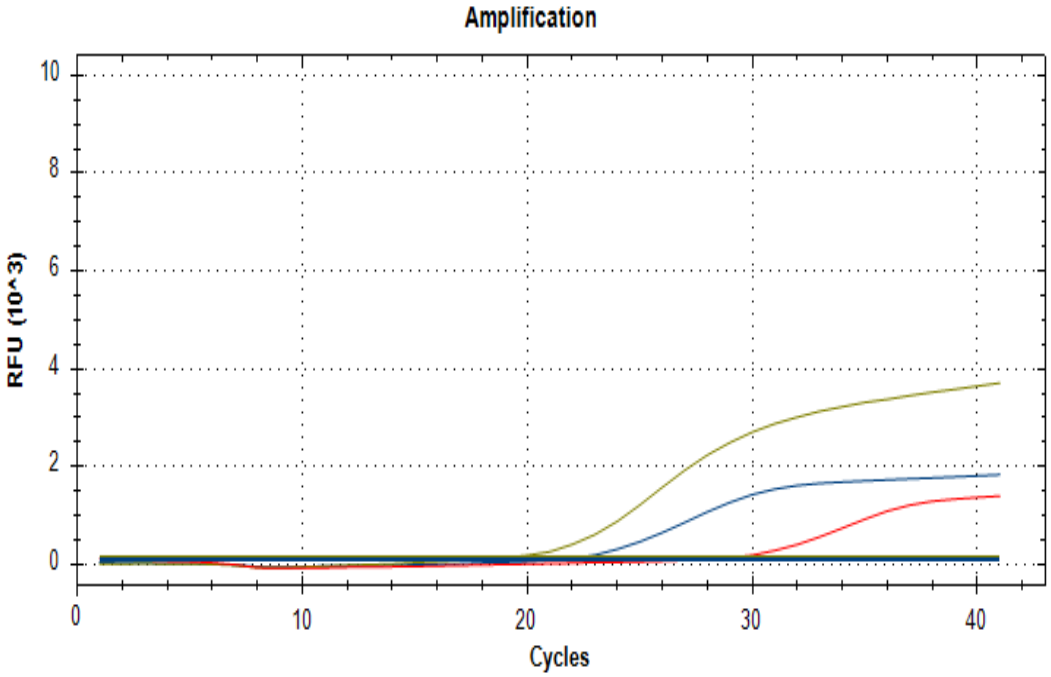
RESULTS AND DISCUSSION

Lignin nanoparticles were prepared successfully with alkaline precipitation method.

Antiviral activity against SARS-COVID-19-DELTA

Table 1: BioRAD analysis for virus analysis without Lignin nanoparticles

Cq value for FAM	21.23
Cq value for Texas red	28.11
Cq value for VIC	19.33
RFU for FAM	1794
RFU for Texas red	1325
RFU for VIC	3585



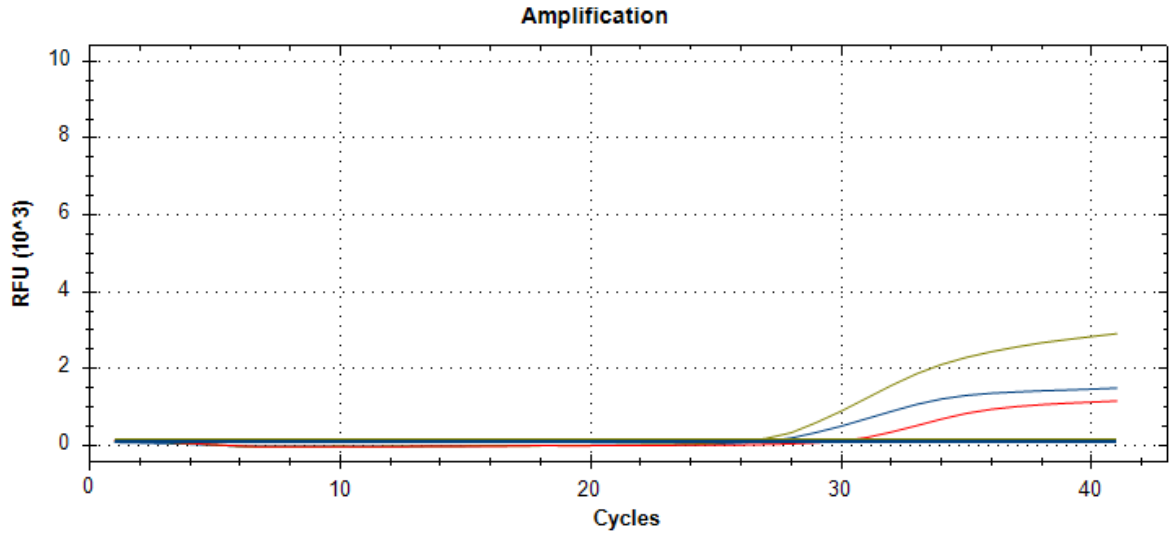
Graph: 01 BioRAD analysis for virus analysis without Lignin nanoparticles

Key-

FAM, VIC, Texas red

Table 2: BioRAD analysis for virus analysis with silver nanoparticles with 1:1 proportion

Cq value for FAM	26.58
Cq value for Texas red	29.67
Cq value for VIC	26.66
RFU for FAM	1447
RFU for Texas red	1095
RFU for VIC	2751



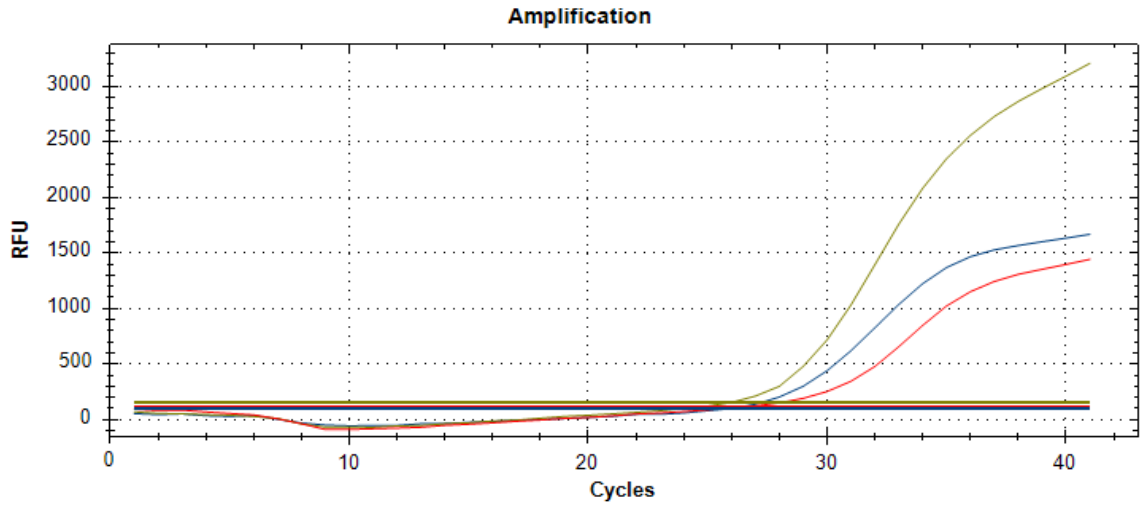
Graph: 02 BioRAD analysis for virus analysis with silver nanoparticles with 1:1 proportion

Key-

FAM, VIC, Texas red

Table 3: BioRAD analysis for virus analysis with silver nanoparticles with 1:2 proportion

Cq value for FAM	25.79
Cq value for Texas red	26.05
Cq value for VIC	26.08
RFU for FAM	1602
RFU for Texas red	1350
RFU for VIC	2977



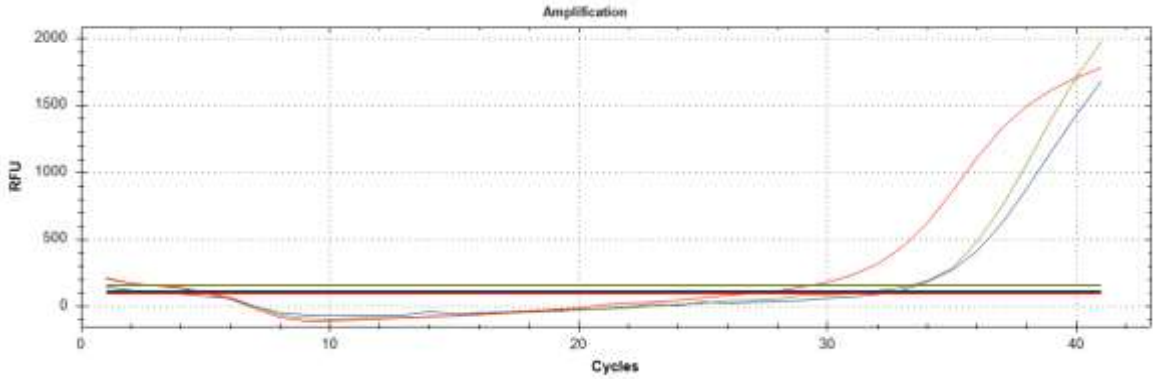
Graph: 03 BioRAD analysis for virus analysis with silver nanoparticles with 1:2 proportion

Key-

FAM, VIC, Texas red

Table 4: BioRAD analysis for virus analysis with lignin nanoparticles with 1:2 proportion

Cq value for FAM	32.81
Cq value for Texas red	26.93
Cq value for VIC	33.31
RFU for FAM	1156
RFU for Texas red	1588
RFU for VIC	1381



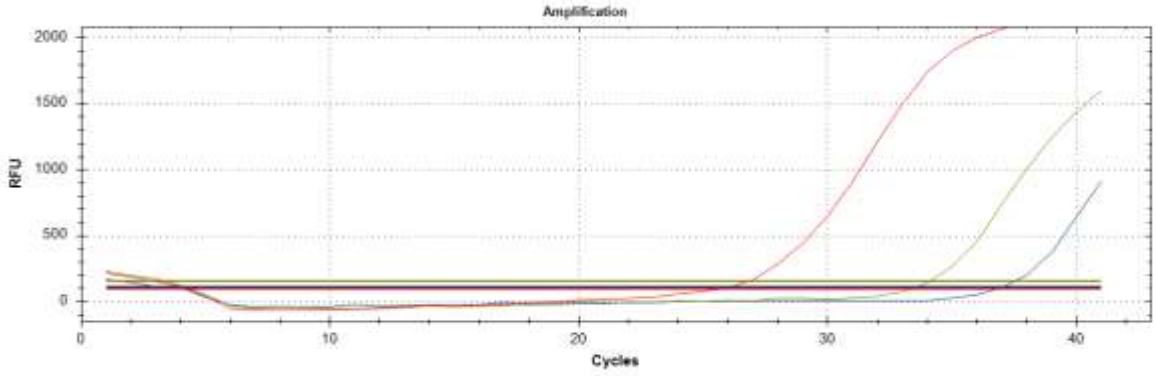
Graph: 04 BioRAD analysis for virus analysis with lignin nanoparticles with 1:2 proportion

Key-

FAM, VIC, Texas red

Table 5: BioRAD analysis for virus analysis with lignin nanoparticles with 1:1 proportion

Cq value for FAM	37.08
Cq value for Texas red	25.67
Cq value for VIC	34.13
RFU for FAM	448
RFU for Texas red	2145
RFU for VIC	1206



Graph: 05 BioRAD analysis for virus analysis with lignin nanoparticles with 1:1 proportion

Key-

FAM, VIC, Texas red

Antiviral activity of lignin nanoparticle was checked with the help of BioRad CFX 96 qRT-PCR machine. The test results are illustrated in Graph:01 FAM, VIC, and Texas red are showing start of pic in between 20 to 30 cycles it indicates the viral load is present in a sample. Graph:02 indicating the shift in sigmoidal curve as compare to Graph:01 due to treatment of silver nanoparticles in 1:1 proportion as a control. Curve shifted towards increased cycle number indicating maximum cycles are required to show fluorescence that means the nucleic acid is fragmented hence it is taking maximum time for anneal. This fragmentation of nucleic acid is due to treatment of silver nanoparticles. Graph:03 indicating shift in sigmoidal curve as compared to Graph:01 indicating fragmented nucleic acid due to treatment of silver nanoparticles in 1:2 proportion. Graph:04 and Graph:05 indicating shift in sigmoidal curve as compare to Graph:01 but with extra ordinary difference that is curve shifted above 30 cycles. This is indication of lignin nanoparticles in 1:2 proportion are degrading viral genome in a very short fragments as compare to silver nanoparticles. Shift above 30 cycles is indication of false positive results as per WHO guidelines. Hence these results are showing antiviral activity of lignin nanoparticles against SARS-COVID-19-DELTA variant.

DISCUSSION

The lignin is present in secondary cell wall of plant cell. The present research work mainly focuses on extraction of lignin from coconut plant waste. The lignin nanoparticles were prepared successfully from coconut species of Cocos nucifera L.

As per results lignin nanoparticles prepared from coconut coir of Cocos nucifera L showing good antiviral activity against SARS-COVID-19-DELTA variant. When compared with silver nanoparticles, lignin nanoparticles are showing promising results for antiviral activity. The results antiviral activity showing cq values for first tube that is covid-19-delta variant, for FAM it is 24.21, for Texas red it is 26.38 and for VIC it is 25.62, these values suggesting the test are positive and Covid-19- delta variant is present.

In tube number two covid-19-delta variant was added with silver nanoparticles, these tubes showing increase in cq values for all three fluorochrome, for FAM value changed from 24.21 to 26.58, for Texas red from 26.38 to 29.67 and for VIC from 25.62 to 26.66.

Interestingly in third tube that is with Covid-19- delta variant when added with lignin nanoparticles values changed with a large difference, for FAM value changed from 24.21 to 34.66, for Texas red from 26.38 to 32.33 and for VIC from 25.62 to 33.08. This large increase in cq values suggesting that RNA genome is degraded after reacting with lignin nanoparticles. This type of degradation is also observed when covid-19-delta variant was added with silver nanoparticles but the maximum degradation of viral genome was found when lignin nanoparticles were used.

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