Anti-mycobacterial activity of nanoparticles from *Psidium guajava* L.

Jivan Rameshroa Kote¹, Ramjan. M. Mulani¹, Ambadas. S. Kadam¹ and Bhimrao. M. Solankar²

1School of Life Science, Swami Ramanand Teerth Marathwada University, Nanded
2DST-FIST sponsored UGC-SAP School of Life Science, Swami Ramanand Teerth Marathwada University Nanded, (Maharashtra) India
3Dynopasak Sikshan Mandale Jintur, Taluka-Jintur Dist-Parbhani(Maharashtra), India
4Swami Ramanand Teerth Marathwada, University, Nanded(Maharashtra), India

ABSTRACT

The leaves of *Psidium guajava* L. used in present investigation were collected from Nanded district of Maharashtra India. Non pathogenic strains used in this study are *Mycobacterium tuberculosis*, *M. spegmatis*, *M. phlei* were obtained from, Institute of Microbial Technology, Chandigarh (PB) India. All the strains were subcultured and maintained on Lowenstein Jensen Media. The plant material was ground in the mortar and pestle and extracted with methanol. The plant extract were added with 1 mM AgNO₃ and 1mM CuCl₂ and incubated for the synthesis of plant based silver and copper nano particles. It was confirmed by UV-visible spectroscopy. After that the anti-Mycobacterial activity was determined by disc diffusion method. Anti-Mycobacterial discs were prepared by dipping them in plant base nano particles of 1 mM AgNO₃ and 1mM CuCl₂. Five different concentrations such as 100µl, 200µl, 300µl, 400µl, 500µl of plant based nano particles extract were used respectively. The Anti-Mycobacterial activity of particles from *Psidium guajava* L. showed both silver (14.23± 0.69) and copper (13.13± 0.19) nanoparticles positive activity. It was observed that plant based silver nanoparticles showed highest activity than copper nanoparticles. The activity depends on the concentration of nanoparticles in plant extract.

Key words: Silver and copper nano particles, Non pathogenic bacteria, *Psidium guajava* Anti-Mycobacterial activity.

INTRODUCTION

Tuberculosis is an infection caused by *M.tuberculosis*, characterized by chronic inflammatory changes with formation of tubercles on lungs. Even though more than 250,000 children develop TB each year, no sufficient anti-TB agents are available in suitable pediatric formulations [1]. Focusing on treatment for active TB, there is paramount need to develop new and effective anti TB therapeutic regimen, focusing on not only development of the new natural scaffolds, but also repurposing existing anti-TB agents in clinical development. According to World Health Organization (WHO) report, 8.8 million incident cases of TB were recorded, of which 1.1 million deaths were documented among HIV-negative group and an additional 0.35 million deaths from HIV-associated TB infections [2]. Present study development of novel natural compounds those are effective, non toxic, the suitability of the synthesized compounds as potential anti-TB agents. In previous era the silver and copper nano particles are used fully to the human being. Green synthesis of silver and copper nano particles using environmentally acceptable plant extract and eco-friendly reducing and capping agents.
nanoparticles using microorganisms or plants can potentially eliminate this problem by making the nanoparticles more biocompatible. Present study was carried out on *Psidium guajava* L. The leaves of *P. guajava* L. contains the flavonolmorin, morin-3-O-lyxoside, morin-3-O-arabinoside, quercetin and quercetin-3-O-arabinoside [5]. The matured leaf is fibrous, and it usually used as a functional food in Asia. *Psidium guajava* L., family Myrtaceae, is used widely in traditional medicine throughout the world for a number of gastrointestinal ailments [6]. In Thailand, Guava was introduced in early seventeenth century and in present era 167,000 metric tons of Guava is produced annually [7]. Guava is good source of antioxidants such as ascorbic acid [8-9], carotenoids, and phenolic compounds such as ellagic acid, quercetin, gallic acid [10-11] and flavonoids [12]. The peel portion of *Psidium guajava* L. contains higher amount of polyphenols. Than its pulp [13]. The extraction process and the solvents utilized influence the recovery of polyphenols. The polarity of the solvent also plays a key role in increasing the solubility of phenolic compounds [14]. The TPC of ripe and unripe guava fruits (Kampuchean variety) have been found less in polyphenol content (unripe = 270, ripe = 138 mg GAE/100 g) which causes a loss in astringency during the ripening process of the fruit [15].

Our current study is aimed to determine and compare the anti-mycobacterial activity of both silver and copper nanoparticles capacity from the plant leaves extracts of Guava variety available in Maharashtra India by using methanol. The results of the anti-mycobacterial studies were also found to be in agreement with experimental studies focusing the significance of the synthesized compounds from leaf of plant Guava as potential growth inhibitors of three *Mycobacterium* species. The study may exalt the scope of developing these synthesized compounds from leaf of plant Guava as promising anti-tuberculare agent.

**MATERIALS AND METHODS**

**Collection of pathogens**
The *Mycobacterium* stain like *M. tuberculosis*, *M. spegmatis*, *M. phlei* (MTCC300) was obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh (PB) India. And was subculture and maintained into Lowenstein Jensen media.

**Preparation of plant extract**
The methanolic extracts of *Psidium guajava* L. were screened for their anti-Mycobacterium activity against *M. tuberculosis*, *M. spegmatis*, *M. phlei*. 50 grams of *Psidium guajava* L. were ground separately and filtered by using What man No.1 filter paper and the large solid particles were removed by centrifugation at 4000 rpm for 20 minutes.

**Synthesis of silver nanoparticles**
The fresh leaf of *Psidium guajava* L. broth solution was prepared by taking 20 g of thoroughly washed and finely cut leaves in a 300-ml Erlenmeyer flask along with 100 ml of sterilized double distilled water and then boiling the mixture for 5 min before finally decanting it. The extract was filtered with What man filter paper no. 1. The filtrate was treated with aqueous 1 mM AgNO3 (21.2 mg of AgNO3 powder in 125 ml distilled water) solution in an Erlenmeyer flask and incubated at room temperature.

**Synthesis of copper nanoparticles**
The fresh leaf extract of *Psidium guajava* L. *add to cuci2-2H2o solution(1mM 10 ml)* with rapid stirring at 50ºC adjusted pH -10 by addition of 10mM NAOH.

**UV-visible spectroscopy analysis**
The reduction of silver and copper chloride was recorded through visual observation. Synthesis of AgNPs and CuCl2 solution with leaves extract may be easily observed by UV–vis spectroscopy. The bioreduction of the Ag+ ions and Cu+ in solutions was monitored by periodic sampling of aliquots (1 mL) of the aqueous component after 20 times dilution and measuring the UV–vis spectra of the solution. UV–vis spectra of these aliquots were monitored as a function of time of reaction on a Schimadzu 1601 spectrophotometer in 300–700-nm range operated at a resolution of 1 nm.

**Preparation of disc diffusion method**
The sterile discs approximately 4mm in diameter was placed on Lowenstein Jensen media. Plates treated with *Psidium guajava* L. nanoparticles. The disc was then placed over the swabbed Lowenstein Jensen media plates and incubated at 37ºC for overnight to study the anti- mycobacterial activity.

**Anti-Mycobacterial activity of plant based silver and copper nanoparticles against Non pathogen**
The anti-mycobacterial assays were done on non pathogenic *M. tuberculosis*, *M. spegmatis*, *M. phlei* by standard disc diffusion method. Fresh overnight cultures of inoculums (100 µl) of each culture were spread on to Lowenstein Jensen media plates. Sterile paper discs of 4mm diameter containing silver nanoparticles were placed in each plate.

Available online at www.scholarsresearchlibrary.com
RESULTS

The present study show that the Erlenmeyer flasks with the Psidium guajava L. supernatants were pale yellow before the addition of Ag+ ions and it changed to a brownish on completion of the reaction with Ag+. The reduction of copper chloride to CuNPs was confirmed by the color changes of solution from colorless to wine red. This event clearly indicating that the reduction of the ions occurs extracellular through reducing agents released in to the solution of Psidium guajava L. It was analyzed by the UV-Vis spectrophotometer with 300–700-nm range respectively, observed by the AgNPs and CuNPs. In the present study, the Anti-mycobacterial aqueous crude leaf extracts and synthesized AgNPs and CuNPs of Psidium guajava L. is noted; however, the highest Anti-Mycobacterial activity was found in synthesized AgNPs as compared to the CuNPs against the Mycobacterium strain.

Table 1. Anti-mycobacterium activity of silver nanoparticles from Psidium guajava L against M. tuberculosis, M. spegmatis. M.pheli

<table>
<thead>
<tr>
<th>Conc. of AgNPs nanofrom Psidium guajava L. (µl/disc)</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>100</td>
<td>7.2±0.12</td>
</tr>
<tr>
<td>200</td>
<td>9.2±2.12</td>
</tr>
<tr>
<td>300</td>
<td>12.3±0.22</td>
</tr>
<tr>
<td>400</td>
<td>12.1±0.11</td>
</tr>
<tr>
<td>500</td>
<td>13.7±0.12</td>
</tr>
</tbody>
</table>

Table 2. Anti-mycobacterium activity of copper nanoparticles from Psidium guajava L against pathogen

<table>
<thead>
<tr>
<th>Conc. of CuNPs nanofrom Psidium guajava L. (µl/disc)</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M. tuberculosis</td>
</tr>
<tr>
<td>100</td>
<td>6.4±0.22</td>
</tr>
<tr>
<td>200</td>
<td>9.2±2.12</td>
</tr>
<tr>
<td>300</td>
<td>11.4±0.32</td>
</tr>
<tr>
<td>400</td>
<td>12.8±0.11</td>
</tr>
<tr>
<td>500</td>
<td>13.7±0.82</td>
</tr>
</tbody>
</table>

The Anti-mycobacterial activity of silver nano particles from Psidium guajava L.was given in Table-1.The nanoparticles showed highest activity against the M. tuberculosis (13.7±0.12), M. spegmatis(12.5±0.42) M.pheli (14.2±0.69) in 500 µl. Present study reveals that the silver nanoparticles are novel scaffold Anti-TB agent.

The Anti-mycobacterial activity of copper nano particles from Psidium guajava L.was given Table-2.The nanoparticles shows highest activity against the M. tuberculosis (13.7±0.82), M. spegmatis(13.5±0.12) M.pheli (13.1±0.19) in 500 µl. Present study investigate that the copper nanoparticles are novel scaffold Anti-TB agent, But as compared to silver and copper solution alone the ,silver and copper nanoparticles extracts showed highest activity against the Mycobacterium tuberculosis.

CONCLUSION

In conclusion, green synthesis of AgNPs in present study shows that the plant extracts of Psidium guajava L. can be used as an effective reducing agent for the synthesis of silver nanoparticles. CuNPs are considered to be potential for biological resistant effect; we intend to evaluate whether they could apply for anti-mycobacterium agent. The nanoparticles synthesis is important as the instability or aggregation of nanoparticles would lead to a decrease in their biological activities. Both nanoparticles synthesized from plants are non toxic.

Acknowledgement

We thankfully acknowledgement the director of school of life science facilities for intense help throughout the research work.

REFERENCES

[3]. NCJ Packi; S Lekshmi; S Benarcin Sumi; S Viveka; S Jeeva; and J Raja Brindha. 2012, Journal of Microbiology and Biotechnology Research.
[5]. R Pongsak and P Parichat. Fitoferapia. 2007, volume 78, number 6, pages 434-436.ddde


