



1 **Green Synthesis of Nanosilver Particles from Extract of**
 2 ***Eucalyptus citriodora* and Their Characterization**

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9 The primary motivation for the study to develop simple eco-friendly green synthesis of silver nanoparticles using leaf extract of *E.*
 10 *citriodora* as reducing and capping agent. The green synthesis process was quite fast and silver nanoparticles were formed within 0.5 h.
 11 The synthesis of silver nanoparticles was investigated by UV-visible spectroscopy, X-ray diffraction, SEM and FTIR. The developed
 12 nanoparticles demonstrated that *E. citriodora* is good source of reducing agents. UV-visible absorption spectra of the reaction medium
 13 containing silver nanoparticles showed maximum absorbance at 460 nm. FTIR analysis confirmed reduction of Ag⁺ to Ag⁰ atom in silver
 14 nanoparticles. The XRD pattern revealed the crystalline structure of SNPs. The SEM analysis showed the size and shape of the nanoparticles.
 15 The environmental friendly method provides simple, easy and cost effective faster synthesis of nanoparticles than chemical methods and
 16 can be used in several areas such as food, medicine and medical application, etc.

17 **Key Words: Silver nanoparticles, *Eucalyptus citriodora*, Capping agents, Scanning electron microscopy, Nanotechnology.**

INTRODUCTION

18 Nanoparticles are being viewed as fundamental building
 19 blocks of nanotechnology. The optical electrical, magnetic and
 20 catalytic properties of metal nanoparticles have been intensively
 21 studied during the last two decades because of their
 22 unique properties¹. The development of biologically inspired
 23 experimental process for synthesis nanoparticles is evolving
 24 into an important branch of nanotechnology^{2,3}. Biogenic syn-
 25 thesis is useful not only because of its reduced environmental
 26 impact^{3,4} compared with some of the physiochemical produc-
 27 tion methods, but also because it can be used to produce large
 28 quantities of nanoparticles that are free of contamination and
 29 have a well define size and morphology⁵. Biosynthesis routes
 30 can actually provide nanoparticles of a better defined size and
 31 morphology than some of the physiochemical methods of pro-
 32 duction⁶. The antibacterial activities of silver nanoparticles are
 33 related to their size, with the smaller particles having higher
 34 activities on the basis of equivalent silver mass content⁷. Con-
 35 cerning the biological application of nanoparticles. It has been
 36 emphasized that methods of synthesis through biological system.
 37 There are different plant extracts have been used and reported
 38 for synthesis of gold, silver and biometallic nanoparticles⁷.

In the present study, *Eucalyptus citriodora* was used for
 source of reducing agent. The plant is easily available in all
 the regions in Pakistan. *Eucalyptus* extract show various
 biological effects, such as antibacterial, antifungal, antihyper-
 glycemic and antioxidant activities⁸. There are more than 500
Eucalyptus species, ranging from shrubs to several hundred-
 foot trees. *Eucalyptus* leaves and oil are utilized for medicinal
 and other uses, such as fragrance in perfumes. Volatile oils are
 derived principally from species that are rich in 1,8-cineol (eu-
 calyptol, α -monoterpene), such as *Eucalyptus globulus*
 Labillardiere (blue gum), *E. smithii*, or *E. fructicetorum*.
E. globules Labillardiere is the most common medicinal
 species⁹.

EXPERIMENTAL

Preparation of plant extract: 10 g of fresh leaves of *E.*
citriodora were washed thoroughly with double-distilled water
 and were then cut into small pieces. These finely cut pieces
 were then mixed with 100 mL doubled distilled water and this
 mixture was kept for boiling for a period of 15 min. After
 cooling, it was filtered through Whatman Filter paper No. 1.
 Filtrate placed at 4 °C for further experiment.

59 **Synthesis of silver nanoparticles:** 1 mM aqueous solu-
 60 tion of silver nitrate (AgNO_3) were prepared and used for the
 61 synthesis of silver nanoparticles. 10 mL of extract were taken
 62 and 100 mL of AgNO_3 solution was added to it. The colour
 63 change from pale green to dark brown due to surface plasmon
 64 resonance. This occurs due to the collective oscillation of the
 65 conduction electrons confined to metallic nanoparticles. They
 66 were incubated at room temperature for 24 h. The
 67 colourchange indicate the synthesis of silver nanoparticles.
 68 UV-visible spectra showed strong SPR band at 460 nm and
 69 thus indicating the formation of silver nanoparticles The silver
 70 nanoparticles (AgNPs) obtained by *E. citriodora* leaves
 71 extract were centrifuged at 13,000 rpm for 25 min and subse-
 72 quently dispersed in sterile distilled water to get rid of any
 73 uncoordinated biological materials.

74 Analysis of bio-reduced silver nanoparticles

75 **UV-Visible spectroscopy:** UV-Visible spectroscopic
 76 analysis was carried out on Shimadzu UV 1700. Cuvette of
 77 path length 10 mm was used. The measurements were carried
 78 out as a function of reaction time at room temperature.

79 **X-ray diffractometry:** XRD measurements were re-
 80 corded on PANalytical X'Pert PRO X-ray diffractometer. For
 81 XRD measurements, the AgNPs were dried in oven at 60 °C
 82 and such dried powder was further analyzed on XRD for their
 83 phase structure and exact material identification. The
 84 $\text{CuK}\alpha$ radiation ($\lambda = 1.582 \text{ \AA}$) was selected and the
 85 diffractogram was obtained in the 2θ range of 20-80°.

86 **Fourier transform infrared (FTIR) spectroscopy:** The
 87 binding properties of AgNPs synthesized by *E. citriodora* leaf
 88 extract were investigated by FTIR analysis. FTIR measure-
 89 ments were taken on MIDAC 2000M series. Dried and pow-
 90 dered AgNPs were palletted with potassium bromide (KBr)
 91 (1:10 proportion). The spectra were recorded in the
 92 wavenumber range of 4000-450 cm^{-1} and analyzed by sub-
 93 tracting the spectrum of pure KBr.

94 **SEM analysis:** Scanning electron microscopic (SEM)
 95 analysis was done using JSM-6480 SEM machine. Thin films
 96 of synthesized and stabilized silver nanoparticles were pre-
 97 pared on a carbon coated copper grid by just dropping a very
 98 small amount of the sample on the grid and sample was ana-
 99 lyzed for morphology and size of the silver nanoparticles.

RESULTS AND DISCUSSION

100 **U.V-Visible spectroscopy:** The formation of AgNPs was
 101 observed upon the colour change of the leaf extract of *Euca-*
 102 *lyptus citriodora* from transparent yellow into brown, as shown
 103 in Fig. 1, due to the coherent oscillation of electrons at the
 104 surface of nanoparticles, resulting in surface plasmon reso-
 105 nance⁹. The colour change into brown was noted within 20
 106 min and the colour intensity increased significantly with in-
 107 creasing the AgNO_3 concentration at a fixed volume of leaf
 108 extract of *E. citriodora*. The UV-visible spectrophotometry
 109 was also used to confirm the formation of the AgNPs as shown
 110 in Fig. 1. From Fig. 1, the SPR band steadily increased in
 111 intensity with a prominent peak at about 460 nm at 1 mM
 112 concentration. The change of colour and intensity of the SPR
 113 band might be due to the variation in concentration, size and
 114 shape of the resulting AgNPs¹¹.

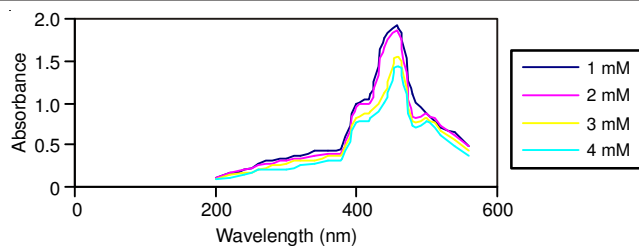


Fig. 1. UV-Visible spectroscopy of silver nanoparticles

115 **XRD:** The results of the XRD analysis showed 2θ in-
 116 tense values with various degree (31.769°, 37.605°, 43.83°,
 117 64.07° and 77.20°) these results are corresponds to (101),
 118 (111), (200), (220) and (311) Bragg's reflection based silver
 119 nanoparticles¹² (Fig. 2).

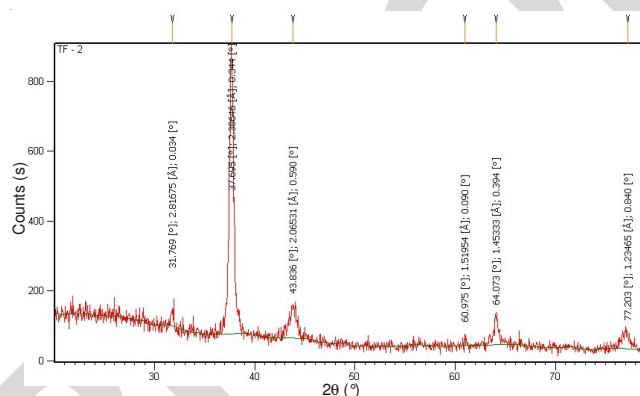


Fig. 2. XRD pattern of silver nanoparticle synthesis by leaf extract of *E. citriodora*

120 **FTIR:** The results of the FTIR used to identify the possi-
 121 ble bio molecules responsible for the stabilization of the syn-
 122 thesized silver nanoparticles. The prominent peaks of the FTIR
 123 results are showing the correspond values to the alcohol, phe-
 124 nol group (O-H stretching-3424), amides group (N-H stretch-
 125 ing-3357), carboxylic group (O-H stretch-3280) alkenes, aro-
 126 matics (C-H 3094), alkanes (C-H 2884), aliphatic saturated
 127 aldehydes (C=O 1729), unsaturated aldehyde (C=O 1667) and
 128 aromatic (C-C 1586). The observed peaks are considered as
 129 major functional groups in different chemical classes such as
 130 triterpenoids, flavonoids and polyphenols¹³. Hence, the terpe-
 131 noids are proved to have good potential activity to convert the
 132 aldehyde groups to carboxylic acids in the metal ions. Fur-
 133 ther, amide groups are also responsible for the presence of the
 134 enzymes and these enzymes are responsible for the reduction
 135 synthesis and stabilization of the metal ions, further, polyphen-
 136 ols are also proved to have potential reducing agent in the
 137 synthesis of the silver nanoparticles¹³⁻¹⁵.

138 **SEM:** According to SEM analysis the silver nanoparticles
 139 were spherical in shape with varied particle size in nm. The
 140 larger silver particles may be due to the aggregation of the
 141 smaller ones (Fig. 3).

Conclusion

142
 143 The present study demonstrated the extracellular biosyn-
 144 thesis of an isotropic silver nanoparticles using the leaf ex-
 145 tract of *E. citriodora*. We found that the leaves of *E. citriodora*
 146 can be a good source of synthesis of silver nanoparticles. The

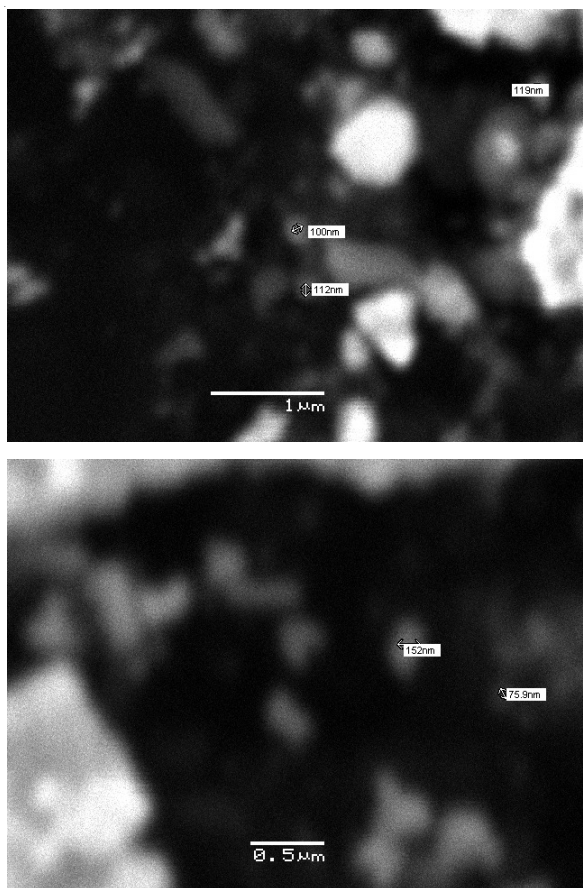


Fig. 3. SEM Micrograph of silver nanoparticles from *E. citriodora*

147 formation of AgNPs was well studied. The AgNPs character-
148 ization and morphology was studied with UV-Visible spec-

troscopy, XRD and SEM techniques. The FTIR examination 149
of the samples confirms the involvement of enzymes and amino 150
groups in the reduction and stabilization of the AgNPs. This 151
procedure is easy, cost-effective, energy saving and environ- 152
ment friendly. It can scaled up for large scale synthesis of silver 153
nano-particles. 154

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