

## **Antioxidant, antibacterial and catalytic properties of *Mesona Blumes* gum stabilized Silver Nanoparticles**

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## 2. Experimental works:

### 2.1. Catalytic reduction of CV and Rh-B dyes

A reaction mixture of Rh - B (5mL, 1mM) and NaBH<sub>4</sub> (5mL, 10mM) was prepared in 20mL distilled water. About 3 mL of this solution was taken in a cuvette and 7mg of MB@AgNPs was added. The reduction of Rh-B was monitored using a UV-visible spectrophotometer [32]. A similar procedure was carried out for CV dye reduction.

### 2.2. Antioxidant activity of MB@AgNPs

DPPH assay was used to examine the antioxidant activity of synthesized AgNPs. The previously reported method was followed with a few modifications [33]. The AgNPs were prepared up to 60μL using various volumes (5,10, 15, 20 and 25μL) of AgNPs with dimethyl sulfoxide, and 205 ml of DPPH (0.15 mM) solution was added to each sample. The above reaction mixture was vigorously stirred for 40 minutes at room temperature. At 517 nm, absorbance measurements were made using DPPH as a blank and ascorbic acid as standard [30]. The following expression was used to estimate free radical scavenging activity:

$$DPPH \text{ scavenging effect } (\%) = \frac{A_0 - A_t}{A_0} \times 100$$

A<sub>0</sub> is the absorbance of control (standard) and A<sub>t</sub> is the absorbance of the sample.

### 2.6. Antibacterial activity of MB@AgNPs

To evaluate the antibacterial activity of MB@AgNPs, agar well diffusion was used. AgNPs were dissolved in the DMSO solvent. The antibacterial properties of MB@AgNPs were assessed against Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* & *Bacillus cereus*). Measured zones were compared to standard streptomycin (positive control) using the ruler scale procedure. A total of 100 litres of phase cultures were placed in a nutrient agar medium (beef extract, peptone, and agar) for bacterial culture. A sterile borer was used to create four wells in each Petri dish after solidification. MB@AgNPs

stabilized were loaded in three wells with 25, 50, 75 and 100 $\mu$ L concentrations (5 $\mu$ g/mL), respectively, and positive control was loaded with *streptomycin* (100  $\mu$ L). The Petri plates were incubated in an incubation chamber for 24 hours at 37°C. Zones of inhibition were measured with a ruler method.

### ***2.7. Characterization Techniques***

The formation of MB@AgNPs has been characterized using various techniques. UV-Visible spectrophotometer (Lab India UV-3200) was used for the identification of MB@AgNPs, and the dye reduction studies. FTIR spectral studies were carried out using Shimadzu IR Affinity-1. Rigaku Miniflex (powder XRD) was used to analyse the structure of synthesized MB@AgNPs. TEM (JEOL2000; ZEISS EVO instrument) was used to investigate the size and shape of MB@AgNPs.