

with replicates in each treatment. Surface sterilized explant (0.5–1.0 cm) segments were placed on the solid MS basal medium, supplemented and incubated under dark ($25 \pm 2^\circ\text{C}$, 5 d), then transferred into light conditions.

16.2.6 ORGANOGENIC CALLUS

Well grown callus pieces (1.0 g, 25 d old) were transferred to MS medium containing different concentrations of cytokinins; BAP (1.0–2.0 mg/L) in combination with auxins; IAA (1.0–2.0 mg/L) and NAA (1.0–2.0 mg/L) for shoot regeneration. These include IAA+BAP (1+2mg/L), IAA+BAP (2+1mg/L), NAA+BAP (1+2mg/L) and NAA+BAP (2+1mg/L). The organogenic calli were subcultured at 20 d intervals on the same treatment of fresh medium until shoot regeneration commenced. The cultures were incubated in light ($25 \pm 2^\circ\text{C}$ for four weeks). Remaining calli harvested for the analysis of compounds.

16.2.7 ROOT INDUCTION

The roots were developed from callus nodes and shoot tips, the callus was transferred to the MS basal medium supplemented with 2,4-D + BAP (3 + 1 mg/L), IBA (0.5 mg/L), IBA (1.5 mg/L), IBA (2.5 mg/L) and GA₃ (1.0 mg/L) for root induction. The cultures were incubated in dark ($25 \pm 2^\circ\text{C}$) for two weeks. The cultures were subcultured every 28 d onto the fresh half-strength MS medium supplemented with plant growth regulators. The length of individual roots (in cm) was measured and recorded after six weeks of culture and the mean root length was determined. All treatments were conducted thrice with replicates.

16.2.8 ORGANIC SOLVENT EXTRACTION OF PLANT MATERIALS

The seeds and *in vitro* calli were shade-dried and powdered; the powdered samples were cold macerated with different solvents (hexane, ethyl acetate, chloroform, acetone and methanol) for 3 d with occasional stirring. Then all the extracts were filtered through Whatman filter paper and solvent contents were removed at the low temperature (40–45°C) in a rotary evaporator. The evaporated samples were kept in fridge (4°C) for further analysis (Divya et al. 2014).

16.2.9 MICROORGANISMS

Six microorganisms including five bacteria (gram-positive and gram-negative) and one fungal species were used. This includes *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Vibrio cholera*, *Salmonella typhi* and *Candida krusei*. All the ATCC cultures were purchased from Pune, India.

16.2.9.1 Antimicrobial Activity

The *in vitro* antimicrobial activity of different solvent extracts from leaves, stems and *in vitro* calli were determined by the well diffusion method described by