Nagarajan et al. (2010). The Muller Hinton Agar (MHA) ingredients are beef infusion (300 g/L), casein acid hydrolysate (17.5 g/L), starch (1.5 g/L) and agar (1.7 g/L) at pH (7.3 ± 0.1) medium was poured onto sterile petriplates. Agar was allowed to set at ambient temperature. Fresh human pathogenic bacteria cultures of two gram-positive bacteria, *B. subtilis* and *S. aureus* and three gram-negative bacteria, *E. coli, S. typhi* and *V. cholera* were spread on the surface of MHA plates using cotton swabs. Wells were cut from the petriplates using a sterile cork (8 mm dia) borer. Different concentrations (25, 50 and 75 µL/mL) of the leaf, stem and *in vitro* calli were loaded into the wells using a sterile micropipette. The inoculated plates were initially incubated for 15 min at room temperature and then they were incubated at 37°C for 24 h. Turbidity was adjusted with sterile broth so as to correspond to 0.5 McFarland standards. Inhibition zones were recorded as the diameter of growth-free zones including the diameter of the well in mm at the end of incubation period.

16.2.9.2 Antifungal Activity

The antifungal activity of hexane, acetone, methanol and aqueous extracts were evaluated (Arjun et al. 2012). The Sabouraud Dextrose Agar (SDA) ingredients are meat peptone (5 g), casein peptone (5 g), dextrose (40 g), agar (15 g) and distilled water (1000 mL), poured onto sterile petriplates. The agar was allowed to set at ambient temperature. Antifungal activity of the extracts was tested against a human pathogenic fungi *C. krusei*. Fresh fungal culture was spread on surface of the SDA plates using a cotton swab. Wells were cut from SDA in the petridishes using a sterile cork (8 mm dia) borer. Different concentrations (25, 50 and 75 µL/mL) of solvent extracts were loaded into the wells using a sterile micropipette. The inoculated plates were initially incubated for 15 min at room temperature and then were incubated at 37°C for 24 h. Then, the plates were examined for any zone of growth inhibition. Inhibition zones were recorded as the diameter of growth free zones including the diameter of well in mm at the end of incubation period. The percentage of inhibition was calculated by the formula:

$$\text{Percentage of inhibition} = \frac{\text{I (Dia. of the inhibition zone in mm)}}{\text{90 (Dia of the petriplate in mm)}} \times 100 \qquad (16.1)$$

16.2.10 Antiangiogenesis: Chorioallantoic Membrane Assay (CAM)

Fifty leghorn eggs were purchased from TNUVAS, Chennai and Tamil Nadu. Seven day-old fertilized brown shell eggs were collected from the hatchery and the eggs were cleaned with ethanol (70%). A small window (10 cm^2) was made in the shell of the eggs. Next, air was sucked out from the eggs to bring their membrane down. Different solvent extracts were dissolved in phosphate buffer saline (PBS, 10 mg/mL). Through the window of each egg, a sterile disc of gelatine sponge containing different concentrations and pellets of these solutions (50, 100 and 150 µL/pellet) were added dropwise on gelatine sponges and applied onto the chorioallantonic