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Antimicrobial activity of *Hibiscus micranthus* Leaf and callus extract

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Abstract
Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, emergence and dissemination of multidrug resistant (MDR) strain in pathogenic bacteria have become a significant public health threat as there are fewer, or even sometimes no, efective antimicrobial agents available for the infection caused by pathogenic bacteria. In the present study aimed to investigate *in vitro* antibacterial and antifungal activity of *Hibiscus micranthus* leaf and best callus extract. Pathogenic microbes including bacteria as *Escherichia coli, Streptococcus pyogenes*, *Psudomonus aurginosa*, *Salmonella typhi* specie of bacteria was spread on Nutrient agar plates and fungi as *Aspergillus fumigates, Cryptococcus neoformans*, *Sporothrix schenckii* and *Phialophora verrucosa.* it can be concluded that the ethanolic extract of both the mother plant and its callus showed generally the great antimicrobial activities against 4 types of bacteria and 4 fungus species tested. It was observed that the callus extract was potent antimicrobial against all tested organisms than mother plan extract. The antimicrobial activity of callus extract due to the presence of higher content of phenol and flavonoids.

Keywords: Antimicrobial, *Hibiscus micranthus*, bacteria, fungi, leaf and callus

1. Introduction

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases (Bhatia R. Narain, (2010) [1]. However, emergence and dissemination of multidrug resistant (MDR) strain in pathogenic bacteria have become a significant public health threat as there are fewer, or even sometimes no, efective antimicrobial agents available for the infection caused by pathogenic bacteria (Boucher *et al*., 2009; Giamarellou, 2010) [2, 3]. Thus, in the light of the evidence of the rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates, *et al*., 2002; Marasini, *et al*., 2015) [4, 5].

A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of these problematic bacterial infections (Iwu, 1999) [6]. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs (WHO, 2002) [7]. Many plants have been used because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant (Medina, *et al*., 2005; Romero, *et al*., 2005) [8, 9]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and favonoids, which have been found in vitro to have antimicrobial properties {Duraipandiyan, *et al*., 2006; Djeussi, *et al*., 2013} [10, 11].

A number of Phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases as urinary tract infections, gastrointestinal disorders, respiratory disease, and cutaneous infections. The indigenous people of Nepal have been using many plant species as traditional medicines long ago, including treatment of infectious diseases, but there has been paucity in data regarding their *in vivo* and *in vitro* efficacy. In the present study aimed to investigate in vitro antibacterial and antifungal activity of *Hibiscus micranthus* leaf and callus extract.

2. Materials and Methods

2.1 Collection of plant materials and Sterilization of explants

H. micranthus plant was collected from Inamkulathur, Trichy district, Tamil Nadu, India. The specimen can be accessed as SJCBOT25981 department of Botany St. Joseph College (Autonomous), Tiruchirappalli-02. The plants washed with running tap water and rinsed in teepol (5 times dilute) for 2 minutes. Seeds were surface sterilized in 70% ethanol for 1 minute and immersed in 0.1% $HgCl_2$ for 2 minutes, then rinsed with autoclaved double distilled water (5 washes, each for 5 minutes).

2.2 *In vitro* Micro-propagation

MS medium (Murashige and Skoog, 1962) was used as the basal medium for the study. Auxin and Cytokinin were used. Various parts of *in vitro* seedlings have been used as explants. The cultures were incubated in culture chamber at 25±2 ºC with 16/8 h (light/dark) photoperiod. MS basal medium supplemented with 30gm of Sucrose, 7gm of agar. Growth regulators like auxins (NAA and IAA) and Cytokinin (BAP) were added to the basal medium either singly or in various combinations. The highest percentage (87.8±8.64%) of response in leaf explant callus treated with combination of BAP (2.5mg/l) IAA (0.5mg/l) and NAA (3mg/l) reported in earlier work and this callus used further studies. Ethanolic extract of best callus and leaf used for antimicrobial activity.

2.3 Antimicrobial activity

The antimicrobial activity was performed by disc diffusion method followed by NCCLS (1993) [12] and Awoyinka *et al*., (2007)[1[3]]. Agar disc diffusion method was followed for determination of antimicrobial activity. The bent glass rod is sterilized and used to spread the microbe-containing liquid uniformly on the nutrient agar (NA) and potato dextrose agar (PDA) plates using 24-48 hours culture of respective bacteria and fungus. Using sterile forceps, the sterile filter papers (6 mm diameter) containing each 50µl of samples extract and Standard solution were laid down on the surface of inoculated agar plate. The plates were incubated at 37 ºC for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate. Results were recorded as the presence or absence of inhibition zone. The inhibitory zone around the well, indicated absence of tested organism. The diameters of the zones were measured using diameter measurement scale. Triplicates were maintained and the average values were recorded for antibacterial activity.

3. Results and Discussion

The principle aim of the present work was to study the antimicrobial activity of *Hibiscus micranthus* leaf and leaf explant callus treated with combination of BAP (2.5mg/l) IAA (0.5mg/l) and NAA (3mg/l) against pathogenic microbes including bacteria as *Escherichia coli*, *Streptococcus pyogenes*, *Psudomonus aurginosa*, *Salmonella typhi* specie of bacteria was spread on Nutrient agar plates and fungi as *Aspergillus fumigates*, *Cryptococcus neoformans*, *Sporothrix schenckii* and *Phialophora verrucosa* (Table 1 and Plate 1).

The antibacterial activity of leaf and callus extract were investigated by measuring zone of inhibition. The maximum activity (7.30±0.51mm) was observed in callus followed by leaf extract (3.75±0.26mm) against *Streptococcus pyogenes* and standard was 12.35±0.86mm. The zone of inhibition was observed in the following order Leaf extract < Callus extract. Among the various extract, the highest zone of inhibition was observed in callus extract and nearest to the standard (Table 1).

Table 1: Antibacterial activity of leaf and callus extract of *Hibiscus micranthus*

Bacterial strains	Leaf extract (50µl)	Callus extract (50µl)	Standard (30µl)	Control (30µl)
Escherichia coli (mm)	4.20±0.29	7.60±0.53	12.85±0.89	0.00±0.00
Pseudomonas aeruginosa (mm)	3.50±0.24	7.15±0.50	12.10±0.84	0.00±0.00
Salmonella typhi (mm)	3.90±0.27	7.45±0.52	12.60±0.88	0.00±0.00
Streptococcus pyogenes (mm)	3.75±0.26	7.30±0.51	12.35±0.86	0.00±0.00

Values expressed as Mean ± SD; Standard: Fluconazole; Control: D. Water; mm: Millimeter

The antifungal activity of leaf and callus extract were investigated by measuring zone of inhibition. The maximum activity (5.70±0.39mm) was observed in callus followed by leaf extract (3.35±0.23mm) against *Aspergillus fumigatu* and standard was 10.50±0.73mm. The zone of inhibition was observed in the following order Leaf extract < Callus extract. Among the various extract, the highest zone of inhibition was observed in callus extract and nearest to the standard (Table 2).

Table 2: Antifungal activity of leaf and callus extract of *Hibiscus micranthus*

Fungal strains	Leaf extract (50µl)	Callus extract (50µl)	Standard (30µl)	Control (30µl)
Aspergillus fumigatus (mm)	3.35±0.23	5.70±0.39	10.50±0.73	0.00±0.00
Cryptococcus neoformans (mm)	3.45±0.24	5.95±0.41	10.70±0.74	0.00±0.00
Phialophora verrucosa (mm)	3.20±0.22	5.40±0.37	10.35±0.72	0.00±0.00
Sporothrix schenckii (mm)	3.15±0.22	5.10±0.35	10.20±0.71	0.00±0.00

Values expressed as Mean ± SD; Standard: Fluconazole; Control: D. Water; mm: Millimeter

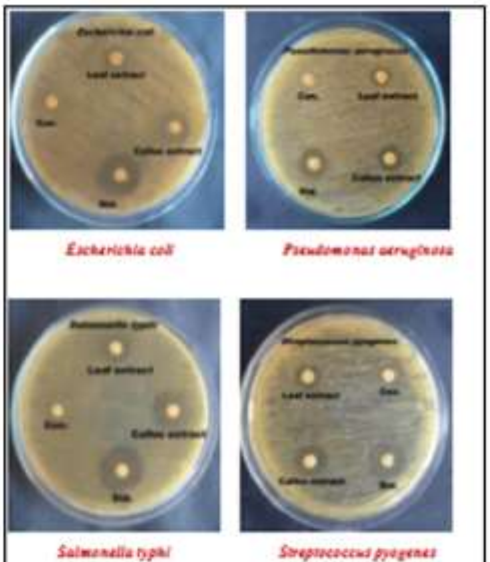

Plate 1 Antibacterial activity of leaf and callus extract of *Hibiscus micranthus*

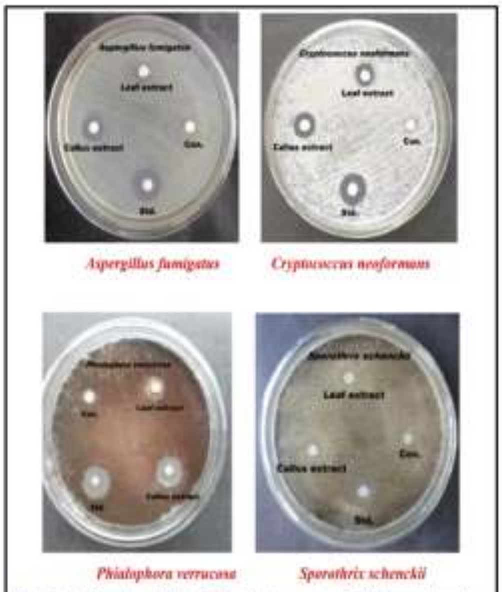

Plate 2: Antifungal activity of leaf and callus extract of *Hibiscus micranthus*

Present finding agreement with Nagarajan, *et al.* [14] who compared the natural plant and callus extracts of *Solanum trilobatum* L. was studied against two bacteria and fungi, for their antimicrobial activity using cup diffusion method. Various solvents such as chloroform, petroleum ether and ethanol were used. The leaf and stem segments of the plant were cultured on Murashige and Skoog basal medium supplemented with various growth regulators. Maximum callus was recorded on medium containing 0.5 mg/l NAA and 0.5 mg/l Kinetin. The results reveals that the stem and leaf callus extracts has shown significant activity against the tested microorganisms than the natural sample.

4. Conclusion

Over all, it can be concluded that the ethanolic extract of both the mother plant and its callus showed generally the great antimicrobial activities against 4 types of bacteria and 4 fungus species tested. It was observed that the callus extract was potent antimicrobial against all tested organisms than mother plan extract. The antimicrobial activity of callus extract due to the presence of higher content of phenol and flavonoids.

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