

## ***In vitro* Antifungal Potentials of *Hamelia patens* Jacq. (Rubiaceae) Aqueous Extracts of Leaves, Flowers and Fruits**

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***Hamelia patens* Jacq. of Rubiaceae is a medicinal shrub. The aqueous leaf, flower and fruit extracts were tested for antifungal potentials against *Aspergillus fumigatus* NCBT 112, *Candida albicans* NCBT 140, *Fusarium oxysporum* NCBT 156 and *Rhizoctonia solani* NCBT 194. The leaf and fruit extracts at 10.0% concentration resulted 100% growth inhibition for *A. fumigatus* as well as for *C. albicans*. The leaf, flower and fruit extracts at 10.0% concentration revealed 100% growth inhibition for *F. oxysporum* and *R. solani*. The fruit extract at 5.0% inhibited 100% growth in *F. oxysporum* and the same concentration of leaf extract inhibited 100% growth in *R. solani*. The 100% inhibition of leaf, flower and fruit aqueous extract can be comparable with positive control (Control-1) with bavistin 0.5% concentration.**

**Key words:** Antifungal potentials, aqueous extract, growth inhibition, *Hamelia patens*.

Herbal medicines are in great demand in the developed as well as in developing countries for primary health care because of their wide biological and medicinal activities, higher safety margin and lower costs. Since the time immemorial, our traditional system of medicine and folklore claiming that medicinal plants as a whole or their parts are being used in all types of diseases successfully including anti-bacterial, anti-helminthic and anti-inflammatory (Sapana Khandelwal *et al.*, 2012). The medicine practiced by our forefathers has been the precursor of modern pharmaceutical medicine. This includes herbal medicine and phytotherapy, which is prevalent in Chinese, Ayurvedic (Indian), and Greek medicine (Ahmad, 1992). Now-a-days the medicinal preparation

available in the market from which most of them either not effective upto the mark or has to develop resistance resulting in reoccurrence again. Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines (Mann *et al.*, 2008). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plants products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997; Ogundipe *et al.*, 1998).

*Hamelia patens* Jacq. (Rubiaceae) is commonly known as Scarlet Bush or Firebush. It is a large perennial shrub or small tree. Also, the plants are used in folk medicine against a range of ailments. A number of active compounds have been found in firebush, such as apigenin, ephedrine, flavanones, isomaruquine, isopteropodine, maruquine, narirutins, oxindole alkaloids, palmirine, pteropodine, rosmarinic acid, rumberine, rutin, seneciophylline, speciophylline, stigmast-4-ene-3, 6-dione and tannin (Rios and

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Aguilar-Guadarrama, 2006; Duke, 2007). The bark also contains significant amounts of tannins, firebush contains 17.5 per cent crude protein and has *in vitro* digestibility of 61.1 per cent (Benavides, 2001).

The plant possesses analgesic, anti-septic, anti-inflammatory, febrifuge, refrigerant properties. Firebush is loved and planted as an ornamental almost worldwide in warm, moist areas. The fruit is edible (Little *et al.*, 1974). Firebush is used in herbal medicine to treat athlete's foot, skin lesions and rash, insect bites, nervous shock, inflammation, rheumatism, headache, asthma and dysentery (Liogier, 1999).

All parts of *H. patens* tree are used in natural medicine. It has been reported to exhibit that this plant possesses anti-diarrhoeal activity (Salud Perez *et al.*, 1996). Insect bites, menstrual disorders, uterine and ovarian affliction (Shrisha *et al.*, 2011). Cytostatic and cytotoxic activity against tumor cell lines (Taylor *et al.*, 2013) and wound healing activity (Sandhya, 2011) with *H. patens*, but no information is available with respect to anti-fungal activities. In view of this, the present study was designed to evaluate the anti-fungal potentials on fungi.

## MATERIAL AND METHODS

### Collection and Identification of Plant Material

Fresh leaves, flowers and seeds of *Hamelia patens* Jacq. (Rubiaceae) were collected from a private garden in Tiruchirappalli, Tamil Nadu (Fig. 1a). Fresh leaves flowers and matured fruits (Fig. 1b, c and d) were collected and washed under tap water, air dried in shade and then homogenized to fine powder and stored in sterile air tight bottles for the experimental work.

### Fungal Cultures

The fungal cultures tested in this work *Aspergillus fumigatus* (NCBT 112), *Candida albicans* (NCBT 140), *Fusarium oxysporum* (NCBT 156) and *Rhizoctonia solani* (NCBT 194) were maintained in immobilized condition in polyurethane foam in Microbiology Lab, Department of Biotechnology, National College, Tiruchirappalli.

### Culture Medium

The antifungal activity of leaves, flowers and fruits were conducted using Sabourand

Dextrose Agar (SDA) medium (HI media - M063) formulated by Cavangh (1963) for human pathogenic fungi *A. fumigatus* and *C. albicans*. Whereas Czapek Dox Agar medium was used (Raper and Thom, 1949) for plant pathogenic fungi *F. oxysporum* and *R. solani*. Sabourand Dextrose broth (HI media - MU 033) and Czapek Dox broth was used for the determination of Minimal Inhibitory Concentration (MIC) for human pathogenic and plant pathogenic fungi tested for this work.

### Experimental Procedure

For the preparation of leaf, flower and fruit extracts to test anti-fungal activity, Singh and Geetha Singh's (1997) methodology was followed. Ten grams of leaf, flower and fruit dried powder were ground with 30 ml of distilled water to obtain aqueous extracts. The extracts were passed through double-layered muslin cloth and filtered through filter paper and then centrifuged at 3000 rpm for 10 minutes. The supernatant liquid was filtered through Whatman No.1 filter paper and then sterilized by passing through Millipore filter.

The sterilized leaf, flower and fruit extracts was added to Sabourand Dextrose Agar Medium / Czapek Dox Agar medium to get different concentrations as given below:

97.5 ml of medium consisting of 2.5 ml of extract (2.5%)

95 ml of medium consisting of 5.0 ml of extract (5.0%)

90 ml of medium consisting of 10.0 ml of extract (10.0%)

Only 100 ml of medium as Control

95.5 ml of medium consisting of 0.5 ml of bavistin mixture (0.5%) Control-1

For the evaluation of antifungal effect various concentrations, *i.e.*, 2.5%, 5.0% and 10.0% concentrations as well as Control, Control-1 was done in triplicate. Petriplates were inoculated with the test fungi individually. The plates were incubated at  $28 \pm 1^\circ\text{C}$  temperature in dark.

### Determination of the Minimum Inhibitory Concentration (MIC)

MIC has determined by the liquid dilution method (Irobi *et al.*, 1996). Dilution series were prepared with 0.25 to 10.0 mg/ml of Sabourand Dextrose broth medium. To each tube 0.1 ml of standardized suspension of fungal spores ( $4 \times 10^6$  spores/ml) were added and incubated at  $28^\circ \pm$

2°C for 24 hr. The lowest concentration which did not show any growth of the tested fungi after microscopic evaluation was determined as MIC.

## RESULTS AND DISCUSSION

The aqueous extract of dried powder of *H. patens* leaf flower and fruit has shown varied antifungal properties for all the four fungal strains tested in this work (Table 1, 2, 3, and 4). The growth of *A. fumigatus*, *C. albicans*, *F. oxysporum* and *R. solani* were totally inhibited at 10.0%. Concentration for leaf aqueous extract (Fig. 2a and d, Fig. 3a and d). The total inhibition can be comparable to Control-1, a standard antifungal agent bavistin at 0.5% concentration, whereas only 25% growth inhibition was noticed at 2.5% concentration for *A. fumigatus* 50% growth inhibition for *R. solani*, 75% growth inhibition for *C. albicans* and *F. oxysporum*. For flower extract

the total inhibition was obtained for *F. oxysporum* and *R. solani* at 10.0% concentration (Fig. 3b and e). The other fungi namely *A. fumigatus* and *C. albicans* only 50% and 75% growth inhibition was noticed (Fig. 2b and e). The fruit extract showed total growth inhibition at 10.0% concentration for all the four fungi tested for this work (Fig. 2c and f, Fig. 3c and f).

*A. fumigatus* a human pathogen cause *Aspergillosis*, oral mucosal lesions. *C. albicans* cause oral mucosal lesions, oral candidiasis and vaginal and urinary tract infections (Runyoro *et al.*, 2006). *F. oxysporum* a plant pathogenic organisms cause wilt disease, *R. solani* a plant pathogen cause root rot and leaf spot disease (Pandey, 1982). There are many reports regarding the antifungal effect of plant extracts on these test fungi (Lachoria *et al.*, 1999; Portillo *et al.*, 2001; Abubacker and Ramanathan, 2003; Rojas *et al.*, 2006; Abubacker *et al.*, 2008). As far as *H. patens* plant extracts

**Table 1.** Antifungal activity of leaf, flower and fruit aqueous extracts of *Hamelia patens* against *Aspergillus fumigatus* (NCBT 112)

Plant part	Control	Control-1	Concentration of plant extracts (%)		
			2.5	5.0	10.0
Leaf	++++	–	+++	+	–
Flower	++++	–	+++	+++	++
Fruit	++++	–	+++	+++	–

**Table 2.** Antifungal activity of leaf, flower and fruit aqueous extracts of *Hamelia patens* against *Candida albicans* (NCBT 140)

Plant part	Control	Control-1	Concentration of plant extracts (%)		
			2.5	5.0	10.0
Leaf	++++	-	+	+	-
Flower	++++	-	+++	+	+
Fruit	++++	-	+	+	-

**Table 3.** Antifungal activity of leaf, flower and fruit aqueous extracts of *Hamelia patens* against *Fusarium oxysporum* (NCBT 156)

Plant part	Control	Control-1	Concentration of plant extracts (%)		
			2.5	5.0	10.0
Leaf	++++	-	+	+	-
Flower	++++	-	++	+	-
Fruit	++++	-	++	-	-

**Table 4.** Antifungal activity of leaf, flower and fruit aqueous extracts of *Hamelia patens* against *Rhizoctonia solani* (NCBT 194)

Plant part	Control	Control-1	Concentration of plant extracts (%)		
			2.5	5.0	10.0
Leaf	++++	-	++	-	-
Flower	++++	-	+++	++	-
Fruit	++++	-	++	+	-

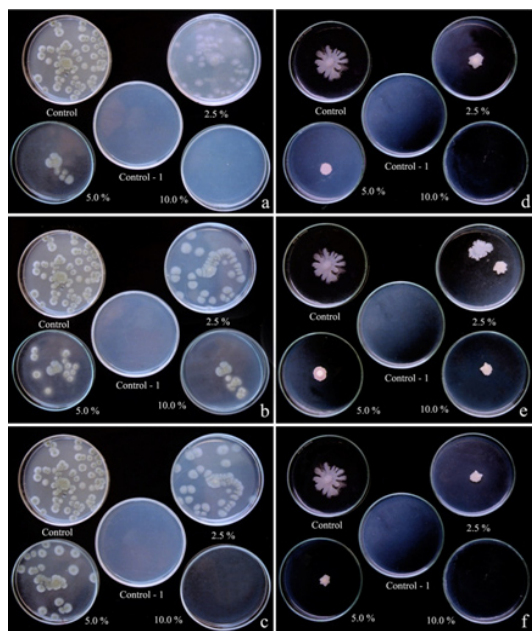
Control : Medium without plant extract  
 Control-1 : Medium with Bavistin (0.5%)  
 ++++ : Normal growth  
 +++ : 25% growth inhibition  
 ++ : 50% growth inhibition  
 + : 75% growth inhibition  
 - : Total (100%) growth inhibition

with antifungal potential is one of the first report, perhaps the bioactive compounds isopteropodine, rumberine, palmirine and maruquine (Reyes-Chilpa *et al.*, 2004) isolated from this plant might have acted as effective bioactive compound and acted as antifungal agent.

The MIC values of the aqueous extract of leaves, flowers and fruits varied from 2.50 mg/ml to 6.0 mg/ml for the fungi tested. The MIC value of *A. fumigatus*, *C. albicans*, *F. oxysporum* and *R. solani* were 3.75, 3.50, 3.75 and 3.0 mg/ml respectively for leaf and fruit extract. The flower

extract value varied from 4.25, 4.50, 5.0 and 5.50 mg/ml respectively. Further investigation was performed to demonstrate the action of the extract on these fungi at different concentrations. The growth of these fungi correspondingly decreased with increasing concentration of the extract and the growth was completely inhibited at these MIC values. The MIC determination is important in giving a guideline of the choice of an appropriate and effective concentration of anti-fungal therapeutic substance.

a) *Hamelia patens* twig with flowersb) *H. patens* leavesc) *H. patens* flowersd) *H. patens* mature fruits**Fig. 1.** Plant material used in Antifungal activity



a) *Aspergillus fumigatus* NCBT 112 (leaf extract)  
 b) *A. fumigatus* NCBT 112 (flower extract)  
 c) *A. fumigatus* NCBT 112 (fruit extract)  
 d) *Candida albicans* NCBT 140 (Leaf extract)  
 e) *C. albicans* NCBT 140 (Flower extract)  
 f) *C. albicans* NCBT 140 (Fruit extract)  
 Control without plant extract Control 1 bavistin (0.5%) 2.5 %, 5.0 % and 10.0% with leaf, flower and fruit extracts

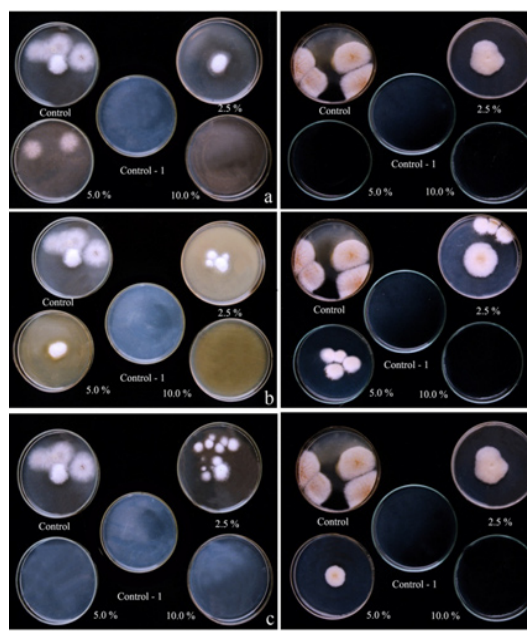
**Fig. 2.** Antifungal activity of *Hamelia patens*, Jacq. (Rubiaceae)

## CONCLUSION

*H. patens* is a well known medicinal plant for certain ethnic groups all over the world. The aqueous leaf and fruit extract is effective antifungal agent in controlling both plant and human pathogenic fungi screened for this study. Therefore, the identification of this potential plant as antifungal agent will help in environmentally safe herbal antifungal formulations to control *A. fumigatus*, *C. albicans* causing *aspergillus*, oral mucosal lesions in human and *F. oxysporum* and *R. solani* causing wilt and root rot diseases in plants.

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a) *Fusarium oxysporum* NCBT 156 (Leaf extract)  
 b) *F. oxysporum* NCBT 156 (Flower extract)  
 c) *F. oxysporum* NCBT 156 (Fruit extract)  
 d) *Rhizoctonia solani* NCBT 194 (Leaf extract)  
 e) *R. solani* NCBT 194 (Flower extract)  
 f) *R. solani* NCBT 194 (Fruit extract)  
 Control without plant extract Control 1 bavistin (0.5%) 2.5 %, 5.0 % and 10.0% with leaf, flower and fruit extracts

**Fig. 3.** Antifungal activity of *Hamelia patens*, Jacq. (Rubiaceae)

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