

# In-vitro antifungal activity of the essential oil of flowers of *Plumeria alba* Linn. (Apocynaceae)

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**Abstract:** The aim of the present investigation was to evaluate the antifungal efficacy of essential oil of the flowers of *Plumeria alba*. It was tested for its Minimum Inhibitory Concentration (MIC) against the fungi *Candida albicans* ATCC 10231, *Candida albicans* 5, *Aspergillus niger* MTCC 281, *Penicillium chrysogenum* MTCC 2725, *Phaenorochoete chrysosporium* MTCC 787 and *Ralstonia entrophia* MTCC1255. Further, zones of inhibition produced by the crude extract against the fungal strains were measured and compared with those produced by standard antifungal agent Griseofulvin. The extract was proved that it was highly toxic against *Aspergillus niger* MTCC 281, *Candida albicans* 5 and *Penicillium chrysogenum* MTCC 2725. The essential oil showed very less toxicity against *Ralstonia entrophia* MTCC1255. The extract was found to be fungistatic in its action.

**Keywords:** antifungal; fungal strains; MIC; essential oil; *Plumeria alba*.

## Introduction

In recent years there has been an increasing interest in the use of natural substances, and some questions concerning the safety of synthetic compounds have encouraged more secondary metabolism, have a wide application in folk medicine, food flavoring and preservation as well as in fragrance industries. The antimicrobial properties of essential oils have been known for many centuries.<sup>1</sup> A survey of literature reveals that there are many essential oils which possess antifungal activity.<sup>2</sup> The plant *Plumeria alba* is a

species of the genus *Plumeria* (Apocynaceae). This large evergreen shrub has narrow elongated leaves, large and strongly perfumed white flowers with a yellow center. Native from Central America and the Caribbean, it is now common and naturalized in southern and southeastern Asia.<sup>3</sup> Traditionally, many parts of the plant considered medicinal. The bark is used as purgative, emmenagogue, and febrifuge. The latex of the plant has rubefacient and purgative properties. The flower oil mainly consists of primary alcohol, viz. geraniol, citronellol, farnesol and phenyl ethyl alcohol and some linalool. The flowers contain

quercetin and kaempferol.<sup>3,4&5</sup> The extract of the bark shows antifungal activity against *Helminthosporium sativum*.<sup>6</sup> The present investigation was undertaken to test the antifungal potentiality of the essential oil of *Plumeria alba* Linn. flowers.

## **Materials and Methods**

### **Plant Material**

The flowers and leaves of *P. alba* were collected from the surrounding areas of Greater Noida in the month of March. The plant was identified and authenticated (voucher no. NHCP/NBPGR/2010-18 dated 13<sup>th</sup> April' 2010) by Dr. Anjula Pandey, Principal Scientist, NBPGR, Pusa Campus, New Delhi. A voucher specimen has been retained in the Dept. of Pharm. Technology, N.I.E.T., Greater Noida for future references. The fresh flowers were collected, washed and cut into small pieces.

### **Method for essential oil extraction<sup>6</sup>**

The extraction of essential oil by hydro-distillation method using Clevenger apparatus was performed using pieces of flowers in a round bottom flask and small amount of distilled water was added by taking care that the temperature did not rise above 50° C. The obtained essential oils were kept in sealed glass tube at 4 °C until analysis. The crude essential oil (yield 0.08% w/w) thus obtained was tested for its antifungal potentiality.

### **Test micro-organisms**

The test fungi used included *Candida albicans* ATCC 10231, *Candida albicans* 5, *Aspergillus niger* MTCC 281, *Penicillium chrysogenum* MTCC 2725, *Phaenorochoete chrysosporium* MTCC 787 and *Ralstonia entrophia* MTCC1255. These fungal strains included various drug resistant hospital isolates collected and characterized in Department of Pharmaceutical Technology, Jadavpur University, India. All strains were maintained on Sabourauds's Dextrose Agar (SDA) slants at 4° C prior to use for antifungal activity.

### **Determination of minimum inhibitory concentration by Serial Dilution Technique<sup>7, 8&9</sup>**

Calculated volume of the stock solution of essential oil were dispensed in a series of sterilized test tubes previously containing calculated volume of sterile cooled molten SDA media (40-45°C) to prepare final volume of 5ml each with dilutions of 100, 200, 400, 800, 1200, 1500 and 2000µg/ml. Then, these test tubes were kept in an inclined position for preparation of agar slants. These slants were then kept in refrigerator at 4°C for 24hrs to ensure uniform diffusion of essential oil. One loopful (loop diameter: 3mm) of an overnight grown fungal strains suspension (10<sup>5</sup> CFU/ml) were streaked. The inoculated tubes were incubated at 25±2°C for 3- 7days until growth were observed in the control tubes and MIC values were obtained.

### **Determination of Zones of inhibition of Disc Diffusion Method<sup>10, 11&12</sup>**

The essential oil and griseofulvin stock solution (10µg/ml) were taken. From these solutions two sets of four dilutions (1000, 1200, 1500, 2000 µg/ml) each of essential oil and griseofulvin (solvent: sterile distilled water) were prepared in sterilized McCartney bottles. Sterile SDA plates were prepared and incubated at 25±2°C for 24 hours to check for the presence of any sort of contamination. Then each sterilized agar plates were flooded with liquid culture of fungal strains, dried for 30 minute at 25±2°C. The sterile whatman filter paper disc (4mm diameter) were soaked in four different dilution of the crude extract and placed in appropriate position of the plates marked as quadrant at the backs of petridishes. All the flooded plates with corresponding paper discs soaked with appropriate dilution of extract were incubated at 25±2°C for 48 hours and diameter of zone of inhibition were measured in mm. Similar procedure was adopted for Griseofulvin and corresponding zone diameters were measured and compared accordingly.

### **Determination of mode of action of the extract**

To determine whether the extract were fungistatic or fungicidal in nature, plugs from the zone of inhibition were taken out and reincubated into fresh media which were then examined for their growth after 96 hours incubation at 25±2°C in an incubator.<sup>11&12</sup>

**Table 1: Determination of MIC of the essential oil of flowers of *P.alba* Linn. against different fungal strains**

S. No.	Name of Fungi	Dilution of methanolic leaf extract (µg/ml) in Sabouraud's Dextrose Agar(SDA) media							
		0*	100	200	400	800	1000	1500	2000
1.	<i>Aspergillus niger</i> MTCC 281	+	+	±	±	-	-	-	-
2.	<i>Candida albicans</i> 5	+	+	+	±	±	-	-	-
3.	<i>Candida albicans</i> ATCC 10231	+	+	+	±	±	±	-	-
4.	<i>Penicillium chrysogenum</i> MTCC 2725	+	+	+	+	±	-	-	-
5.	<i>Phaenorochoaete chrysosporium</i> MTCC 787	+	+	+	+	±	±	-	-
6.	<i>Ralstonia entrophia</i> MTCC1255	+	+	+	+	±	±	±	-

\* = Control (without extract), ± = Inhibited Growth, + = Growth, - = No Growth.

**Table 2: Determination of diameter of zone of inhibition (in mm) produced by the essential oil of *Plumeria alba* and its comparison with Griseofulvin against different sensitive fungal strains.**

S. No	Name of Fungi	Extract (µg/ml)				Griseofulvin (µg/ml)			
		1000	1200	1500	2000	1000	1200	1500	2000
1.	<i>Aspergillus niger</i> MTCC 281	10.0	11.0	12.0	13.0	9.0	11.0	12.0	14.0
2.	<i>Candida albicans</i> 5	8.0	8.5	10.0	12.0	8.5	10.0	12.0	14.0
3.	<i>Penicillium chrysogenum</i> MTCC 2725	7.0	7.5	9.0	10.0	8.0	9.0	11.0	12.0
4.	<i>Phaenorochoaete chrysosporium</i> MTCC 787	4.5	5.0	6.5	7.5	7.5	8.5	11.0	11.5
5.	<i>Candida albicans</i> ATCC 10231	4.0	4.5	6.0	6.5	7.0	8.5	9.0	11.5

**Fig.1: Sensitivity pattern of *Aspergillus niger* MTCC 281 against the essential oil of *P.alba* Linn. flowers.**



**Fig.2: Sensitivity pattern of *Penicillium chrysogenum* MTCC 2725 against the essential oil of *P.alba* Linn. flowers.**



**Fig.3: Sensitivity pattern of *Penicillium chrysogenum* MTCC 2725 against the essential oil of *P. alba* Linn. flowers.**

### **Results and Discussion**

The result in Table 1 and Fig. 1, 2 and 3 depicted the MIC values of the essential oil of the flower of *Plumeria alba* Linn. against various tested fungal pathogens. It is evident from figures 1, 2 and 3 that the essential oil is highly active against *Aspergillus niger* MTCC 281, *Candida albicans* 5 and *Penicillium chrysogenum* MTCC 2725. Whereas, it is moderately effective against *Candida albicans* ATCC 10231 and *Phaenorochoete chrysosporium* MTCC 787. The essential oil is found to be very less active against

*Ralstonia entrophia* MTCC1255. The observation suggests that antifungal principles in the essential oil have a broad spectrum of activity which is quite comparable with that of griseofulvin. The sensitivity pattern of the fungal organisms to the extract was found to decrease in following order: *Aspergillus niger* MTCC 281, *Candida albicans* 5, *Penicillium chrysogenum* MTCC 2725, and *Phaenorochoete chrysosporium* MTCC 787 as evident from Table 1&2.

## Conclusion

Plant oils are important source of fungitoxic compounds and they may provide a renewable source of useful fungicides that can be utilized in antimycotics drugs.<sup>13</sup> From this study it can be concluded that the essential oil of *P. alba* possess antifungal activity. We believe that the present investigation will provide a support to the

antimicrobial principle of the essential oil and can be used as antifungal supplement in the developing countries towards the development of new therapeutic agents. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of this oil as an antifungal agent in topical or oral applications.

## References

1. Bansod S. and Rai M., Antifungal Activity of Essential Oils from Indian Medicinal Plants Against Human Pathogenic *Aspergillus fumigatus* and *A. niger*, World J of Med Science, 2008, 3(2), 81-88.
2. Pinto E., Pina-Vaz C., Salgueiro L., Goncalves M.J., Costa-de-Oliveira S., Cavaleiro C., Palmeira A., Rodrigues A. and Martinez-de-Oliveira J., Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida*, *Aspergillus* and dermatophyte species, J. Med. Microbiology, 2006, 55, 1367-73.
3. Anonymous, The Wealth of India-Raw Materials, Council of Scientific and Industrial Research, New Delhi, 2005, V, 164-66.
4. Kirtikar K.R. and Basu B.D., Indian Medicinal Plants, International Book Distributors, Dehradun, 1984, 2, 768.
5. Prajapati N.D., Purohit S.S., Sharma A.K., and Kumar T., Handbook of Medicinal Plants, Agrobios India, 2004, 336-338.
6. Jamwal K.S. and Anand K.K., Antifungal activity of the bark extract of *Plumeria alba*, Ind J Pharm, 1962, 24, 218-19.
7. Kokate C.K., Practical Pharmacognosy, Vallabh Prakashan, Delhi, 2005, 4<sup>th</sup> edn., 116.
8. Mazumder R., Mendiratta T., Mandal S.C. and Mazumder A., Antimicrobial potency of the leaf stalk extract of *Curcuma longa* Roxb., Anc. Sc. of Life, 2000, XX, 92-96.
9. Mazumder A., Singh S.K., Mazumder R., Basu S.P. and Saha B.P., Antimicrobial action of the leaf extract of *Lagerstroemia parviflora* Roxb., Anc. Sc. of Life, 2001, XXI (3), 198-201.
10. Karuba R.M., Siboe G.M. and Dossaji S.F., Antifungal activity of *Schizogygia coffaeoids* Bail. (Apocynaceae) extract, J of Ethnopharmacol, 2001 74, 41-44.
11. Perucci S., Mancianti F., Cioni P.L., Flamini G., Morelli I. and Machioni G., In vitro antifungal activity of essential oils against some isolates of *Microsporum canis* and *Microsporum gypsum*, Planta Medica, 1993, 60, 184-187.
12. Zohra M. and Atik F., Antibacterial activity of essential oils from *Cistus ladaniferus* L. and *Lavandula stoechas* L., International Journal of Pharm Tech Research, 2011, 3(1), 484-487.
13. Balandrin M.F., Klocke J.A., Wutule E.S. and Bollinger W.H., Natural plant chemicals: Sources of industrial and medicinal materials, Science, 1985, 228, 1154-1160.

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