



ORIGINAL RESEARCH ARTICLE

OPEN ACCESS

Antifungal properties of selected plants of Apocynaceae family against the human fungal pathogen *Candida albicans*

Savita Bajrang Wankhede, Madhushree Madhumita Routh, Sandeep Balsingh Rajput, *Sankuny Mohan Karuppaiyl

DST-FIST and UGC-SAP Sponsored School of Life Sciences, SRTM University, Nanded 431-606, M.S, India

ABSTRACT

The ethnobotanical evaluation of plant based chemicals has proven themselves greatly in last few decades. Plants have been a rich source of therapeutic agents and form the basis of traditional medicine system. On the basis of this, *in vitro* antifungal activities of extracts of *Catharanthus roseus*, *Nerium oleander* and *Tabernaemontana divaricata* leaf extracts were evaluated in the present study by 96 well microtiter plate assays using human fungal pathogen *Candida albicans* ATCC90028 strain. The activity was measured by spectrophotometric methods to determine the Minimum Inhibitory Concentration (MIC). The Minimum Fungicidal Concentrations (MFC) for the extracts was also determined by a plate assay. The distilled water extract, petroleum ether extract, methanolic extract, ethyl acetate extract and sequential distilled water leaf extracts of all the plants showed potential activity against *C. albicans* ATCC 90028. The study ascertains the value of Apocynaceae plants, which could be of considerable interest to the development of new safer antifungals.

Key Words: *Catharanthus roseus*, *Nerium oleander*, *Tabernaemontana divaricata*, antifungal, *Candida albicans*.

INTRODUCTION

Plant use in treating diseases is as old as human civilization and traditional medicines are still a major part of regular treatments of different maladies (Alviano and Alviano, 2009). Plants are no doubt one of the main sources of biologically active materials. According to a recent report medicinal herbs are used by 80% of world population living in rural areas for their primary health-care (Sakarkar and Deshmukh, 2011). In Europe and USA 50% of the prescriptions are prescribing natural products including plants or their derivatives for different maladies (Cordell, 2002; Newman *et al.*, 2007). Interestingly out of the 250,000-500,000 plant species on earth, only 1-10% have been studied chemically and pharmacologically for their innumerable medicinal values (Verpoorte, 2000).

Recently the synthetic chemistry has dominated the market of drug discovery and production still there is enormous scope for bioactive principles or extracts derived from plants (Raskin *et al.*, 2002). If we compare them with synthetic drugs than plant derived natural products are surely good source of biologically active agents since they are natural and cost effective (Ghosh *et al.*, 2008). Again plant molecules may have different mode of action than conventional drugs derived agents may have different mechanisms than conventional drugs so as they may be better for health care improvement (Eloff *et al.*, 1998). Plant derived natural products are generally bioactive secondary metabolites with better potential to treat different diseases. Some examples of these compounds include terpenoids, alkaloids, phenols, unsaturated lactones, phenolic glycosides, saponins, sulphur compounds, cyanogenic glycosides and glucosinolates (Mukherjee *et al.*, 2001; Quiroga *et al.*, 2001). These plant based natural products are recently of interest among many scientists to design new remedies for different afflictions like microbial infections.

During the last two decades scientists have tried to develop antimicrobial drugs with low side effects from the natural sources and it may be due to the increasing development of drug resistance to human pathogenic organisms. *Candida albicans* is a human fungal pathogen which causes severe infections in cancer, HIV AIDS, diabetic, surgery and organ transplantation patients. In the present study we have evaluated the *in vitro* antifungal properties of the plants from Apocynaceae family described below:

Catharanthus roseus is an important medicinal plant of family Apocynaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities (Jaleel *et al.*, 2009, Aslam *et al.*, 2010). Antibacterial potential of crude extracts of different parts (*viz.*, leaves, stem, root and flower) of *Catharanthus roseus* against clinically significant bacterial strains is also reported. The present investigation is focused on the screening of leaf extracts of *Catharanthus roseus* for antifungal activity.

Nerium oleander Linn. is a member belonging to Apocynaceae family. It is a herb with different colored flowers and is suited for dry locality (Lokesh *et al.* 2010). A wide spectrum of biological activities has been reported with various constituents isolated from different parts of the plant. *Nerium oleander* Linn. has been reported in ancient texts and folklore for many years and most of its parts and plant products such as flowers, leaves, leaf juice, latex and bark are used for the treatment of microbial diseases. Used traditionally by herbalists as a folk remedy for a wide variety of conditions, including dermatitis, abscesses, eczema, psoriasis, sores, warts, corns, ringworm, scabies, herpes, skin cancer, asthma, dysmenorrhoeal, epilepsy, malaria, abortifacients, heart tonics, leprosy and tumors (Singhal *et al.*, 2011; Santhi *et al.*, 2011).

Tabernaemontana divaricata (Family-Apocynaceae) is a beautiful evergreen shrub, about 54cm high, with large shiny leaves, crepe jasmine flowers, may appear sporadically all year. *T. divaricata*, a garden plant in tropical countries, is a rich source of alkaloids with various

*Corresponding Author:

Dr. Sankuny Mohan Karuppaiyl, Professor
School of Life Sciences
SRTM University, Nanded, MS
India, 431 606
E-mail: prof.karuppaiyl@gmail.com
Contact No.: +91 902 852 8438

pharmacological properties. It has been used in the folk medicine for anti-infection, anti-inflammation, analgesic, anti-tumour, antioxidative and for neurological disorders (Rumjhum *et al.*, 2012; Pratchayasakul *et al.*, 2008). In this study, the antifungal properties of leaf extracts are being reported to validate the traditional use of the crude drug through *in vitro* evaluation.

Global attention has been shifted towards finding new chemicals, specific the development of new drugs. These natural products can provide unique elements of molecular diversity and biological functionality, which is indispensable for novel drug discovery. The present investigation is focused on the screening of leaf extracts of *Catharanthus roseus*, *Nerium oleander* and *Tabernemontana divaricata* for its *in vitro* antifungal activity against human fungal pathogen *Candida albicans*.

MATERIALS AND METHODS

Cultures

One standard strain of *Candida albicans* ATCC 90028, was obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India, and maintained on yeast peptone dextrose (YPD) agar slants at 4°C.

Medium and culture condition

Activation of culture was done by inoculating a single colony from YPD agar plate (yeast extract 1%, peptone 2%, dextrose 2%, agar 2%) into 50 ml YPD broth in a 250 ml conical flask. The flask was incubated at 30°C at 100 rpm on an orbital shaking incubator for 24 h. Cells were harvested by centrifugation at 2000 × g and washed thrice with 0.1 M phosphate buffer saline (PBS), pH 7.4. The cell density was determined by hemocytometer count; cells were suspended in PBS and used as inoculum for further experiments. All the media components and chemicals were purchased from HiMedia laboratories Pvt. Ltd. Mumbai, India. Fluconazole (Forcan) was purchased from, Cipla Pharmaceutical Ltd., India.

Collection of plant materials

Fresh and mature leaves of *C. roseus*, *N. oleander* and *T. divaricata* were collected from Nanded District in the state of Maharashtra (India). The medicinal plants were collected and identified according to their habit, habitat and floral characteristics following the guidelines described in Flora of Marathwada (Naik, 1998). The leaves of were shade-dried and powdered. The powder was sieved and stored in airtight containers. The botanical identity of the plant was confirmed at the School of Life Sciences of SRTM University, Nanded and herbariums are deposited in Lab no 7 of this department.

Solvent extraction of plant materials

10-20 g powder of the leaves was used for extraction. Extraction of leaves with distilled water was carried out at 100°C for 2-3 hrs using a Borosil soxhlet apparatus, model 3840024. Extracts were filtered, evaporated, weighed, and re-dissolved in minimum volume of Methanol and used as a crude extract (Khan *et al.*, 2011).

About 20 g powder of the leaves of *C. roseus* was sequentially extracted in solvents with increasing polarity viz. Petroleum ether > Ethyl acetate > Methanol and distilled water at their boiling temperature using Soxhlet's apparatus, for 2-3 hrs. Extracts were filtered, and evaporated; weights were taken and re-suspended in minimum quantity of Methanol. Concentrations of the stock solutions were adjusted to 100mg/ml.

Drug susceptibility

Minimum inhibitory concentration (MIC)

The susceptibility study was carried out by the standard methodology M 27 A2 as per CLSI guidelines (Pfaller *et al.*, 2006). Briefly, various concentrations of the anticancer drugs were prepared in RPMI-1640 medium by double dilution in the 96 well plates. Each well contained an inoculum of 1×10³ cells per ml and the final volume of RPMI-1640 medium maintained in each well was 200 µl. The wells without addition of drugs served as a control. Microplates were incubated at 35°C for 48 hours and read spectrophotometrically at 620 nm using microplate reader (Multiskan EX, Thermo Electron Corp. USA). The lowest concentration of the drugs which caused fifty percentage reduction in the absorbance compared to that of control was considered as MIC. Similarly, the fluconazole sensitivity of the two standard strains was determined. The activities of the drugs were compared to that of the standard antifungal drug fluconazole.

Minimum fungicidal concentration (MFC)

To determine the minimum *Candida*-cidal concentrations of the drugs, cells from MIC and the wells above that were selected. An aliquot of 10 µl cell suspension from each well was spread on YPD agar plates. The plates were incubated for 24 hours at 30°C temperature and observed for presence of colonies. Absence of visual growth (no appearance of colonies) was considered as fungicidal concentration of the drug (Routh *et al.*, 2011).

Statistical analysis

Values mentioned are the mean of triplicate observations with standard deviations indicated in parenthesis.

RESULTS

Effect of *Catharanthus roseus* (pink flower) extracts

Among the five extracts tested, petroleum ether and sequential distilled water extract of *Catharanthus roseus* leaf was found to be most effective growth inhibitors of *C. albicans*, inhibiting growth at 1 mg/ml. The minimum inhibitory concentration for distilled water extract of leaf was 2mg/ml, whereas methanolic extract of leaf and ethyl acetate extract of leaf was 4mg/ml. Petroleum ether extract was the most efficient *Candida*-cidal, 8 mg/ml of it caused killing of all the cells. MFC of distilled water extract of leaf was at 16 mg/ml, whereas sequential distilled water extract, methanolic extract and ethylacetate extract were not fungicidal up to 16 mg/ml concentration (Table 1).

Effect of *Catharanthus roseus* (white flower) extracts

The various leaf extracts of *C. roseus* leaf inhibited growth of *C. albicans* in a concentration dependent manner, in the range 0.25 to 4mg/ml. Methanolic extract of leaf was the most potent with MIC at 1mg/ml, followed by distilled water extract, ethyl acetate extract, petroleum ether and sequential distilled water extract having MIC at 2mg/ml concentration. Among the five extracts tested, petroleum ether had the most cidal and killed *Candida* cells at 8mg/ml. Methanolic leaf extract exhibited MFC at 16mg/ml concentration, whereas no MFC could be achieved for Distilled water extract, ethyl acetate extract and sequential distilled water extract up to 16mg/ml concentration (Table 1).

Effect of *Nerium oleander* extract

Ethyl acetate extract of leaf was the most efficient agent with MIC of 1mg/ml. At the concentrations of 2mg/ml, these drugs showed more than 80% inhibition of growth.

Distilled water extract and methanolic leaf extract exhibited MIC at 2mg/ml, whereas petroleum ether and sequential distilled water extract were comparatively less efficient with MICs of 4mg/ml. Efficient fungicidal activity was exerted by methanolic leaf extract at 8mg/ml. Ethyl acetate extract showed MFC at 16mg/ml. Among the others, distilled water extract and sequential distilled required 16mg/ml concentrations for *Candida*-cidal effect. No MFC could be achieved up to 16mg/ml concentration for petroleum ether extract (Table 1).

Effect of *Tabernemontana divaricata* extract

Ethyl acetate extract of leaf exhibited MIC of 1mg/ml against growth of *C. albicans* 90028. In this strain, 8mg/ml of ethyl acetate was potent enough to kill all the cells. Distilled water extract, petroleum ether extract and methanol extract inhibited growth at 2mg/ml concentration in the strain 90028; however it did not possess fungicidal activity against *C. albicans* up to 16mg/ml. To inhibit the growth of *C. albicans* 90028, sequential distilled water extract required concentrations of 4 mg/ml. *Candida*-cidal activity was evident at 16 mg/ml for sequential distilled water extract of *T. divaricata* plant (Table 1).

DISCUSSION

Plants are important sources of potentially useful structures for the development of new drugs. Many reports are available on the antiviral, anticancer, antibacterial, antifungal, antihelmintic, antimolluscal and anti-inflammatory properties of plants. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use (Ithart *et al.*, 2007; Demain *et al.*, 2009; Mahesh *et al.*, 2008). However, not many reports are available on the antifungal properties of Apocynaceae family plants. In the present study, the distilled water extract, petroleum ether extract, methanolic extract, ethyl acetate extract and sequential distilled water leaf extracts of *Catharanthus roseus*, *Nerium oleander* and *Tabernemontana divaricata* showed activity against *C. albicans* ATCC 90028. Plant based products have been effectively proven for their utilization as a source for antimicrobial compounds.

In our study, various leaf extracts of *C. roseus* (Pink flower, White flower), *N. oleander* and *T. divaricata* were found to inhibit the growth in human fungal pathogen *Candida albicans*. The effect of various extracts was varying as per the plants. In *Catharanthus roseus* pink colour flower variety, petroleum ether and sequential distilled water extract most efficiently inhibited growth at 1 mg/ml whereas Petroleum ether was also the most effective *Candida*-cidal at 8 mg/ml (Table 1). In comparison various leaf extracts of *C. roseus* white flower variety, methanolic extract of leaf was inhibiting growth at 1 mg/ml and the most potential *Candida* cidal activity was exhibited by petroleum ether extract at 8 mg/ml. White flower extract was found to be more effective than pink flower extract of *C. roseus*. As described in the previous studies the vinca alkaloid present in this plant is the active principle of anticancer drugs Vincristine and Vinblastin and it acts by disrupting microtubulin network in cell division process. This may be the probable reason why this extracts are inhibiting *C. albicans* growth at lower concentrations and viability at higher concentrations because *C. albicans* is an eukaryotic organisms having many similar targets as human cells (Cardenas *et al.*, 1999).

N. oleander leaf extracts showed concentration dependent inhibition of growth. Ethyl acetate extract of leaf significantly inhibited growth of *C. albicans* at 1mg/ml

Table 1: MIC & MFC values of plant extracts against *C. albicans*.

Plant name	Extract name	MIC (mg/ml)	MFC (mg/ml)
<i>Catharanthus roseus</i> (Pink Flower)	Distilled water	2	16
	Petroleum ether	1	8
	Methanol	4	N/A
	Ethyl acetate	4	N/A
	Sequential Distilled water	1	N/A
<i>Catharanthus roseus</i> (White Flower)	Distilled water	2	N/A
	Petroleum ether	2	8
	Methanol	1	16
	Ethyl acetate	2	N/A
	Sequential Distilled water	2	N/A
<i>Nerium oleander</i>	Distilled water	2	16
	Petroleum ether	4	N/A
	Methanol	2	8
	Ethyl acetate	1	16
	Sequential Distilled water	4	16
<i>Tabernemontanas</i> <i>divaricata</i>	Distilled water	2	N/A
	Petroleum ether	2	N/A
	Methanol	2	N/A
	Ethyl acetate	1	N/A
	Sequential Distilled water	4	16

N/A - Not Achieved

whereas it was cidal at 16mg/ml. Methanolic extract of leaf was the most efficient fungicidal killing all the cells at 8mg/ml. For this plant we could achieve MFC for four leaf extracts only excepting petroleum ether. Ethyl acetate extract of *T. divaricata* leaf was the most efficient growth inhibitor of *C. albicans* 90028 followed by petroleum ether and methanolic extracts and the sequential distilled water extract killed all the cells at 16mg/ml proving to be the most efficient *Candida*-cidal.

Results of our *in vitro* study suggested a potential inhibitory effect of various leaf extracts of above mentioned plants against *C. albicans* growth and viability. These plants may be further investigated for their anti *Candida* properties under *in vivo* conditions. This may give insights into use of these plants belonging to Apocynaceae family for therapeutic purposes. *N. oleander* can be topically used in *Candida* infections such as skin or vaginal candidiasis. The results of present investigation clearly indicate that the antifungal activities vary with the species of the plants and plant material used. Thus, the study ascertains the value of Apocynaceae plants, which could be of considerable interest to the development of new antifungals.

CONCLUSION

Our present study demonstrates the antifungal potentialities of *C. roseus*, *N. oleander* and *T. divaricata*, which would improve our understanding to the biological role of the plant and future avenue to develop new pharmacological studies and antifungal therapies.

ACKNOWLEDGEMENT

Authors are thankful to Prof. S.B. Nimse, Honorable Vice chancellor, Swami Ramanand Teerth Marathwada University, Nanded for his support.

REFERENCES

- Alviano, D. and Alviano, A. (2009). Plant extracts: search for new alternative to treat microbial diseases. *Current Pharmaceutical Biotechnology*, Volume 10, Pages106-121. [\[DOI\]](#)
- Aslam, J. Khan, S.H. Siddiqui, Z.H. Fatima, Z. Maqsood, M. Bhat, M.A. Nasim, S.A. Ilha, A. Ahmad, I.Z. Khan, S.A. Mujib, A. and Sharma, M.P. (2010). *Catharanthus roseus* (L.) G. Don. An important drug: its applications and production. *International Journal of Comprehensive Pharmacy*, Volume 4(12), Pages 1-16.

- Cardenas, M.E. Cruz, M.C. Del Poeta, M.Chung, N. Perfect, J.R. and Heitman, J. (1999) Antifungal activities of antineoplastic agents: *Saccharomyces cerevisiae* a model system to study drug action. *Clinical Microbiology Reviews*, Volume 12 (4), Pages 583-611. [\[LINK\]](#)
- Cordell, G. (2002). Natural products in drug discovery– creating a new vision, *Phytochemistry Reviews*, Volume 1 (3), Pages 261-273. [\[DOI\]](#)
- Demain, A. L. and Sanchez, S. (2009) Microbial drug discovery : 80 years of progress; *The Journal of Antibiotics*, Volume 62, Pages 5-16. [\[DOI\]](#)
- Eloff, J. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants?, *Journal of Ethnopharmacology*, Volume 60, Pages 1–8. [\[DOI\]](#)
- Ghosh, A. Das, B. Roy, A. Mandal, B. and Chandra, G. (2008). Antibacterial activity of some medicinal plant extracts. *Journal of Natural Medicines*, Volume 62, Pages 259–262. [\[DOI\]](#)
- Itharat, A. and Ooraikul, B. (2007). Research on Thai medicinal plants for cancer treatment. *Advances in Medicinal Plant Research*, Volume 2, Pages: 287-317.
- Jaleel, C.A. Gopi, R. and Pannseerselvam, R. (2009). Alterations in non-enzymatic antioxidant components of *Catharanthus roseus* exposed to paclobutrazole, gibberellic acid and *Pseudomonas fluorescens*, *Plant Omics*, Volume 2 (1), Pages: 30-40.
- Khan, M.S.A. (2011). Gastroprotective effect of *Tabernaemontana divaricata* (Linn.) R. Br. Flower methanolic extract in wistar rats. *British journal of pharmaceutical research*, Volume 1(3), Pages 88-98.
- Lokesh, R. Leonard, B. E. Madhuri, P. Saurav, K. and Sundar, K. (2010) Larvicidal activity of *Trigonella foenum* and *Nerium oleander* leaves against mosquito larvae found in Vellore city. *Current Research Journal of Biological Sciences*, Volume 2(3), Pages 154-160.
- Mahesh, B. Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World journal of agricultural sciences*, Volume 4(5), Pages 839-843.
- Mukherjee, A. Basu, S. Sarkar, N. and Ghosh, A. (2001). Advances in cancer therapy with plant based natural products, *Current medicinal chemistry*, Volume 8, Pages 1467-1486. [\[DOI\]](#)
- Naik, V.N. (1998). *Flora of Marathwada*. 2nd ed. Amrut Prakashan, Station Road, Aurangabad, India.
- Newman, D. and Cragg, G. M. (2007). Natural products as sources of new drugs over the last 25 years, *Journal of natural products*, Volume 70 (3), Pages 461-477. [\[DOI\]](#) PMID:17309302
- Pfaller, M.A. and Diekema, D.J. (2007). Epidemiology of invasive candidiasis: a persistent public health problem. *Clinical Microbiology Reviews* Volume 20, Pages 133–163. [\[DOI\]](#)
- Prachayasakul, W., Pongchaidecha, A. Chattipakorn, N. and Chattipakorn, S. (2008). Ethnobotany & ethnopharmacology of *Tabernaemontana divaricata*. *Indian Journal of Medicinal Research*, Volume 127, Pages 317-335. PMID: 18577786.
- Quiroga, E. Sampietro, A. and Vattuone, A. (2001). Screening antifungal activities of selected medicinal plants, *Journal of Ethnopharmacology*, Volume 74, Pages 89-96. [\[DOI\]](#)
- Raskin, I. Ribnicky, D. Komarnytsky, S. Ilic, N. Poulev, A. Borisjuk, N. Brinker, A. Moreno, A. Ripoll, C. Yakoby, N. Cornwell, T. Pastor, I. and Fridlender, B. (2002). Plants and human health in the twenty-first century, *Trends in Biotechnology*, Volume 20 (12), Pages 522-531. [\[DOI\]](#)
- Routh, M.M., Raut, J.S. and Karuppaiyl, S.M. (2011). Dual properties of anticancer agents: An exploratory study on the in vitro anti-Candida properties of thirty drugs. *Chemotherapy*. Volume 57, Pages 372–380. [\[DOI\]](#)
- Rumzhum, N.N. Rahman M.M. Kazal, M.K. (2012). Antioxidant and cytotoxic potential of methanol extract of *Tabernaemontana divaricata* leaves. *International current pharmaceutical journal*, Volume 1(2), Pages 27-31. [\[DOI\]](#)
- Sakarkar, D. and Deshmukh, V. (2011). Ethnopharmacological review of traditional medicinal plants for anticancer activity. *International Journal of Pharma Tech Research*, Volume 3, Pages 298-308.
- Santhi, R., Lakshmi, G., Priyadarshini, A. M. and Anandaraj, L. (2011). Phytochemical screening of *Nerium oleander* Linn. leaves and *Momordica charantia* leaves. *International research journal of pharmacy*, Volume 2(1), Pages 131-135.
- Singhal, K.G. and Gupta, G.D. (2011). Some Central Nervous System Activities of *Nerium Oleander* Linn (Kaner) Flower Extract. *Tropical Journal of Pharmaceutical Research*, Volume 10 (4), Pages 455–461. [\[DOI\]](#)
- Verpoorte, R. (2000). Pharmacognosy in the new millenium: lead finding and biotechnology. *Journal of Pharmacy and Pharmacology*, Volume 52, Pages 253-262. [\[DOI\]](#)