



ANTIMICROBIAL, CYTOTOXIC AND PHYTOCHEMICAL ANALYSIS OF *MUSSSAENDA FRONDOSA*

Anitha C.*¹ and Sajina Thomas²

Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliakkavilai, 629153.

***Corresponding Author: Anitha C.**

Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliakkavilai, 629153.

Article Received on 15/03/2018

Article Revised on 04/04/2018

Article Accepted on 25/04/2018

ABSTRACT

Medicinal plants play an important role in the discovery of novel drugs used in medicine. The *Mussaenda frondosa* was screened for phytochemical, antimicrobial and cytotoxic activities of the methanol, hexane, petroleum ether and chloroform crude extracts were studied in this work. The preliminary screening of the various extracts revealed the presence of tannin, alkaloids, carbohydrate, saponin, terpenoids, flavonoids and cardiac glycosides. The antimicrobial screening was Carried out using the following organisms; *Bacillus subtilis*, *Klebsiella pneumonia*, *Bacillus thuringiensis*, *Proteus vulgaris*, *Agrobacterium rhizogens*, *Lactobacillus acidophilus*, *Bacillus megaterium*, *Candida tropicalis*, *Aspergillus niger*, *Aspergillus aculeatus*, *Rhizomucor miechei* and *Saccharomyces cerevisiae*. Phytochemical analysis of aqueous extract showed that the presence of tannin, alkaloids, carbohydrate, saponin, terpenoids, flavonoids and cardiac glycosides. Hexane extract showed highest antibacterial activity against *Lactobacillus acidophilus* and highest antifungal activity against *Candida tropicalis*. An invitro cytotoxicity test using Test on Extraction method was performed with test sample *Mussaenda frondosa* and based on ISO 10993-5. This study offers a scope for developing medicines.

KEYWORDS: *Mussaenda frondosa*, phytochemical analysis, antimicrobial activity, cytotoxicity.

INTRODUCTION

Plants have been used for the treatment of diseases for thousands of years. Terrestrial plants have been used as medicine in China, India and Egypt from ancient time for number of modern drugs are developed.^[1] In recent years, herbal medicines have become an integral part of the primary health care system of many nations.^[2] In 300 BC man was fully aware of the medicinal value of plants.^[3] *Mussaenda* (Rubiaceae) are increasingly popular for their different colour. Many species of this plant have been found to be biologically active.^[4-6] Iridoids and flavonoids are more common in this plant.^[7-9] The variety of *Mussaenda* is a large shrub with medium sized oval leaves. It has large velvety sepals surrounding small star shaped leaves.^[10] The leaves and flowers of *Mussaenda frondosa* was used in external applications for ulcers. A weak decoction of dried shoots is given to children to relieve cough. The bitter root is used in the treatment of leprosy and eye troubles. *Mussaenda frondosa* is distributed in central Nepal, India and Srilanka.^[11]

MATERIALS AND METHODS

Preparation of plant extract

Fresh leaves of *Mussaenda frondosa* was collected were washed under running tap water, air-dried at room

temperature and then reduced to coarse powder using an electric blender. The powders obtained were stored in airtight containers prior to extraction. The dried powder of leaves was successively extracted using Soxhlet apparatus involving different solvents. The soxhlet extractor was placed on to a flask containing 250ml of solvent. Solvents such as methanol, hexane, petroleum ether and chloroform separately for extraction. They were run in a soxhlet apparatus for 12-24 hours after 5 cycles the extracts were collected from the distillation flask. The extract obtained was evaporated completely for dryness. 2g of dried sample were mixed with 10ml of DMSO and stored for further studies.^[12]

Screening of phytochemicals

Qualitative chemical tests were carried out using the extracts obtained from plant in solvents namely methanol, hexane, petroleum ether and chloroform using standard procedures to identify the constituents.^[13-17]

Antimicrobial screening

Antibacterial screening of plant extracts

The antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method. Pathogenic bacteria (Gram positive and Gram negative) were obtained from Microbial Type Culture Collection (MTCC), Institute of

Microbial Technology and Chandigarh. The bacterial strains were *Bacillus subtilis*, *Klebsiella pneumonia*, *Bacillus thuringiensis*, *Proteus vulgaris*, *Agrobacterium rhizogens*, *Lactobacillus acidophilus*, *Bacillus megaterium* are spread over the medium. Filter paper disc of uniform size (4mm) are impregnated with specified concentrations of plant extract and then placed on the surface of Muller Hinton agar plates that has been seeded with organism to be tested. Label the each plate with the name of the test organism to be inoculated. Bacterial colonies were allowed to grow overnight at 37°C, then the inhibition zone around the disc was measured.^[18]

Antifungal screening of plant extract

Antifungal screening was followed by the method Cappucciino et al., 2004. Fungal cultures were obtained from Microbial Type Culture Collection (MTCC), inoculated with respective fungi. The pathogenic strains include *Candida tropicalis*, *Aspergillus niger*, *Aspergillus aculeatus*, *Rhizomucor miechei* and *Saccharomyces cerevisiae*. The filter paper disc of uniform size (6mm) are impregnated with specified concentrations of plant extract and then placed on the surface of Rose Bengal agar plates that has been seeded with organism to be tested. Label the each plate with the name of the test organism to be inoculated. Fungal colonies were allowed to grow 7 days at 20°C and then the inhibition around the disc was measured.^[19]

Cytotoxicity test

An in vitro cytotoxicity test using Test on Extract method was performed with test sample of *Mussaenda frondosa* and based on ISO10993-5. Extract was prepared by incubating test materials with culture medium containing serum at 37±2 °C for 24-26 hours at an extraction ratio of 0.1 g/ml. extract was acidic in nature and pH of the extract was adjusted to 7.4 with 0.1 M NaOH. 100% extracts were diluted to get concentrations of 50% and 25% with media. Different dilutions of extracts on test sample, negative control and positive control in triplicate were placed on subconfluent monolayer of L-929 cells. After incubation of cells with extracts of test sample and controls at 37±2 °C for 24 ± 1 hour, cell culture was examined microscopically for cellular response. Cellular responses were scored as 0, 1, 2, 3 and 4 according to none, slight, mild, moderate and severe based on USP28.

RESULT AND DISCUSSION

Phytochemical analysis

The phytochemistry of *Mussaenda* species has been studied extensively since 1990s. Iridoids, flavanoids and triterpenes are the common chemical ingredients distributed in *Mussaenda* species. The present study showed the preliminary phytochemical screening of seven compounds (tannin, flavanoids, saponin, alkaloids, carbohydrate, cardiac glycosides and terpenoids) were tested for their presence or absence in five different extracts of *Mussaenda frondosa*. The results were summarized in table 1.

Table 1: Phytochemical Screening of *Mussaenda frondosa*.

Sl No.	Test conducted	Chloroform	Petroleum ether	Hexane	Methanol	Aqueous
1	Tannin	+	-	-	+	+
2	carbohydrate	-	+	+	+	+
3	Saponin	+	+	+	+	+
4	Alkaloids	-	-	+	-	-
5	Cardiac Glycoside	+	-	-	+	+
6	Terpenoids	+	+	+	-	+
7	Flavanoids	-	-	-	+	+

‘+’ indicates positive and ‘-’ indicates negative.

Antimicrobial activity

Antibacterial activity and antifungal activity

Ole Pederson et al., 1999 carried out invitro antibacterial and antifungal activity of plant extracts from *Mussaenda*. The present study showed the results of antibacterial activities of plant, *Mussaenda frondosa* were studied against human pathogenic bacteria and fungi. Among the five extracts, hexane extract showed highest antibacterial activity against *Lactobacillus acidophilus* and highest antifungal activity against *Candida tropicalis*. The results of antibacterial activity and antifungal activity screening are summarized below in figure 1 and 2.

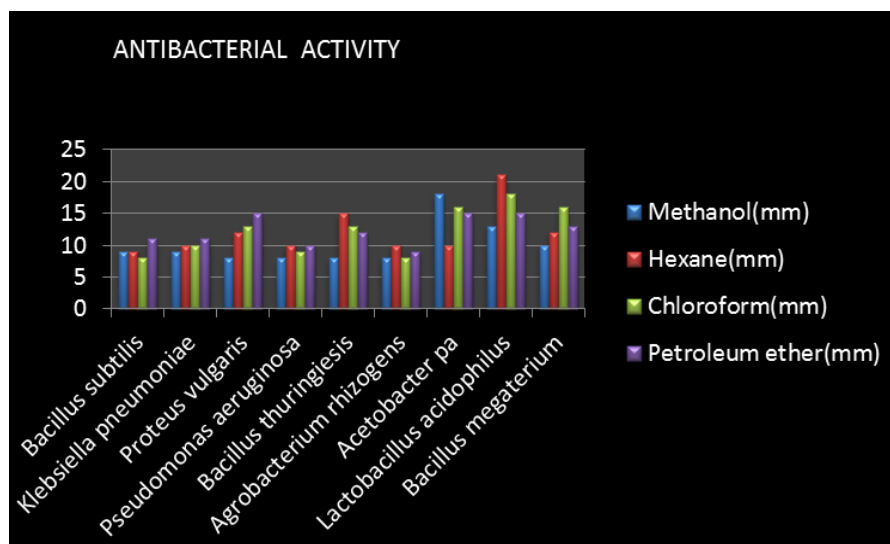


Figure 1: Antibacterial activity of different extracts of *Musssaenda frondosa* against human pathogens.

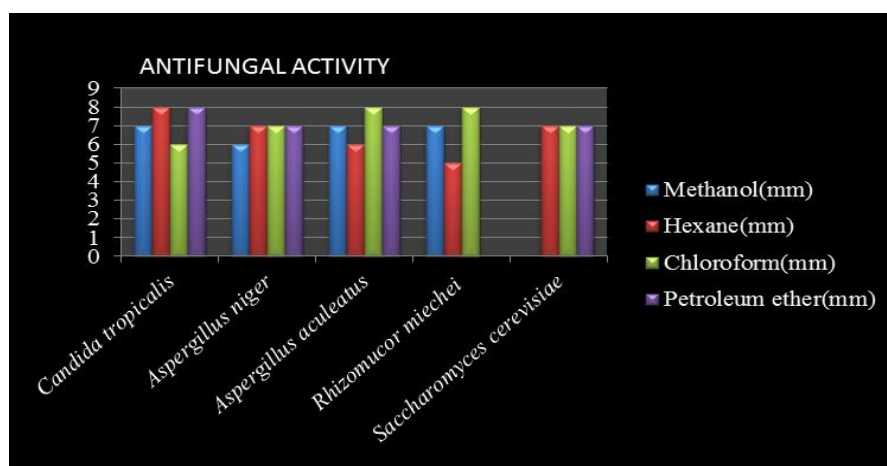


Figure 2: Antifungal activity of different extracts of *Musssaenda frondosa* against human pathogens.

Cytotoxicity test

Patcharawan *et al.*, 2003 studied about twenty plants in the family rubiaceae collected from Northern Thailand were extracted and compared for their cytotoxic activities against a human cervix carcinoma cell line (KB-3-1). These plants were classified into three groups as active, moderately active and inactive plants according to their cytotoxic activity. The present study

test material *Musssaenda frondosa* showed severe cytotoxic response to fibroblast cells (strain-L-929) at an extraction ratio 0.1 g/ml. The negative and positive controls used were High density poly ethylene and dilute phenol. Extracts of negative control gave none cytotoxic response and positive control gave severe cytotoxic response. The results were summarized in table 2.

Table 2: microscopic observations of cytotoxic activity in fibroblast cells (strain-L-929).

S.NO	Sample	cytotoxic scale	interpretation
1	Negative control	0	None cytotoxic
2	Positive control	4	Severe cytotoxic
3	<i>Musssaenda frondosa</i>	4	Severe cytotoxic

CONCLUSION

In the present study *Musssaenda frondosa* was screened for phytochemicals, antimicrobial activity and cytotoxicity. The study revealed that the plant, *Musssaenda frondosa* have severe cytotoxic activity and moderate antimicrobial activity. The phytochemical such as tannin, alkaloids, carbohydrate, saponin, terpenoids, flavonoids and cardiac glycosides are present in the

plant. This study offers a scope for developing medicines.

ACKNOWLEDGEMENT

We are grateful thanks to staff members of Biotechnology Department, Malankara Catholic College, Mariagiri for their encouragement throughout this work.

REFERENCE

- Samuelson G. Drugs of natural origin; A text book of pharmacognosy. 4th edition., Stockholm, Swedish pharmaceutical press., 1999.
- Fajimi AK, Taiwo AA, Herbal remedies in animals parasitic diseases in nigeria. A review. afr.j. biotechnology, 2000; 4(4): 303-307.
- Le Strange JN .the useful plants of west tropical Africa. The crown agents, LONDON, 1977; 325.
- Vidyalakshmi KS, Charles Dorni AI, Hannah P Vasanthi, Rajamanickam GV and Sukumar D, j appl. Sci., 2006; 6: 2251.
- Quin GW, XU RS, Medicinal Res REV, 1998; 18: 375.
- Kim NC, De Sjardins, Wu CD and Kinghorn. Activity of triterpenoid glycosides from the root bark of *Mussaenda macrophylla* against oral pathogens. J. Nat. prods, 1999; 62: 1379-1384.
- Weimn Zhao, Ping Wang, Rensheng XU, Guowei Qin, Shaokai Jiang, Houming Wu. Saponins from *Mussaenda pubescens* Phytochemistry, June, 1996; 42(3): 827-830.
- Ranarveloy, SkaltosunisAI, Andriantsiferana M, and Tillequin F, Ann.pharm.fr, 1990; 48: 273.
- Yoshio Takeda, Hiroshi Nishimura, Hiroyuki Inouye. Two new iridoid glucosides from *Mussaenda parviflora* and *Mussaenda shikokiana*. Phytochemistry, 1977; 16(9): 1401-1404.
- Rauch FD, and Weissich PR, Plants for tropical landscapes. A gardeners guide (Hawai press), 2000; 43.
- Jayasinghe ULB, Jayasooria CP, Bandra BMR. Antimicrobial activity of some sri lankan rubiaceae and meliaceae. Fitoterapia, 2002; 75(5): 424-7.
- Sheeba MS, Asha VV. Effect of *Cardiospermum halicacabum* on ethanol induced gastric ulcer in rats. J Ethnopharmacol, 2006; 106: 105-10.
- Muthumani PR, Meera S. Venkatraman S. Ganapathy P. Devi. Study of phyto chemical, analgesic and anti ulcer activity of extracts of aerial parts of *Cardiospermum halicacabum* Linn. I.J.P.S.R, 2010; 1(10): 128-137.
- Sukhdev Swami Handa, Suman Preet Singh Khanuja, Gennaro Longo, Dev Dutt Rakesh Extraction technologies for medicinal and aromatic plants, International centre for science and high technology, Trieste, 2008; 21-25.
- Harbone JB. Phytochemical methods. A guide to modern technique of plant Analysis. Chapinan and Hall, London, 1973; 33-185.
- Trease GE, Evans WC Pharmacognsy. 11th edn. Brailliar Tiridel Can. Macmillian publishers, 1989.
- Sofowara A. Medicinal plants and Traditional medicine in Africa, Spectrum Book LTD, Ibadan, Nigeria, 1993; 289.
- Okwu DE. Evaluation of the chemical composition of indigenous species and flavoring agents. Global J Pure Appl Sci., 2001; 7(3): 455-459.
- Bauer AN, Kirby WM, Sherries JC, Trucck M. Antibiotic susceptibility testing by standardized single disk method. Am J Clin Pathol, 1966; 45: 493-496.
- Ole Pedersen, Ameenah Gurib fakim, Hussein Subratty, Anne Andersen. Pharmacological properties of seven medicinal plants of the rubiaceae from Mauritius pharmaceutical biology, 1999; 37(3): 202-207.
- Patcharawan Tanamatayarat, Pornngram Limtrakul, Sunee Chunsakaow and Chadrat Duangrat. Screening of some rubiaceae plants for cytotoxic activity against cervix carcinoma(KB 3-1) cell line. The Thai Journal of pharmaceutical sciences, 2003; 27: 167-172.