Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Botany

Elixir Appl. Botany 78 (2015) 29699-29702

ISSN: 2229-712X

# Antimicrobial activity of Organic and Alcoholic extracts of Medicinal Plants against clinically important Microbial pathogens

V.Parivuguna[1] and A.Doss[2]

[1]Department of Microbiology, RVS College of Arts and Science, Coimbatore.

[2]PG & Research Department of Botany, National College, Tiruchirapalli, Tamil Nadu, South India.

**ARTICLE INFO**

**Article history:**

Received: 5 November 2014;

Received in revised form:

21 December 2014;

Accepted: 9 January 2015;

**Keywords**

Medicinal plants,

Folklore, Antimicrobial activity,

Microbial pathogens, MIC.

**ABSTRACT**

In this study, we used ten medicinal plants (*Azima tetracantha* Lam. (Salvadoraceae ), *Corchorus aestuans* Linn.(Tiliaceae), *Garcinia mangostana* Linn. (Guttiferae), *Guazama ulmifolia* Lam. (Sterculiaceae), *Cardiospermum halicacabum*Linn. (Sapindaceae), *Pimenta dioica* Lindl.(Myrtaceae), *Phyla nodifera* (L) E.Greene (Verbenaceae), *Scoparia dulcis* Linn. (Scrophulariaceae), *Croton bonplandianus* Baillon (Euphorbiaceae), and *Clerodendrum inerme* Linn. (Verbenaceae) were screened for its potential antibacterial activity against clinically important microbial pathogens like *Staphylococcus aureus, Streptococcus pyogenes, E. coli, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* The antimicrobial activity was determined in organic (Petroleum ether, Benzene and Chloroform), alcoholic (methanol) and aqueous extracts of these ten medicinal plants. The aqueous extracts showed minimum antimicrobial activity when compared to other extracts. The methanolic extracts of *P.dioica* showed the maximum activity against *Staphylococcus aureus*. The present study suggested that these medicinal plants represent may be used to find bioactive compounds from natural products that might lead to the development of new drugs against bacterial diseases.

© 2015 Elixir All rights reserved.

## Introduction

In the worldwide as well as in the developing countries, the frequency of life threatening infections caused by pathogenic microorganisms has increased and is becoming an important cause of morbidity and mortality in immune compromised patients (Al-Bari *et al*., 2006). The microbial pathogens including Gram positive and Gram negative like different species of *Bacillus* sp., *Staphylococcus* sp., *Salmonella* sp. and *Pseudomonas* sp. are the prime source to cause severe infections in humans. Because these organisms have the ability to survive in harsh condition due to their multiple environmental habitats (Ahameethunisa and Hoper, 2010). Antibiotic resistance has increased widely in the recent years and is posing an ever increasing therapeutic problem. In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut and mucosal microorganism, immune suppression and allergic reactions. However, only one third of the bacterial diseases known have been treated from these synthetic products. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants (Kim *et al*., 1995; Alagesaboopathi, 2011). The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. Medicinal plants, as a source for new potential drugs is still largely unexplored and only a small percentage of them has been subjected to phytochemical investigation and the fractions submitted to pharmacological screening is very low. It is in view of this, that the present research was set up to evaluate the antimicrobial activity of selected medicinal plants using different plant extractions against some microbial pathogens.

## Materials and methods

### Collection of plants

The fresh plant materials were collected from three different regions like, The Anaimalai hills, The Nilgiris and Coimbatore regions, Tamil Nadu State, South India. The collected plants were authenticated by Botanical Survey of India (Southern Circle), Coimbatore and voucher specimens are deposited at Department of Microbiology, RVS College of Arts and Science, Coimbatore, Tamil Nadu State, South India. The collected plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in air tight bottles

### Crude extracts preparation

50 gm of powdered each plant material was extracted by Cold maceration method with different solvents like petroleum ether, benzene, chloroform, methanol and water (24 hours). All the extracts were evaporated in vaccum under reduced pressure. All the plant extracts were stored in sterile glass bottles at room temperature until used.

### Microorganisms

Pure cultures of all microbial pathogens were obtained from Microbial Type Culture Collection Centre (MTCC), Chandigarh (Table 1). The pure microbial cultures were maintained on nutrient agar medium. Each culture was further maintained by subculturing regularly on the same medium and stored $4^0$C before use in tests.

### Antimicrobial activity

The antibacterial activity of different plant species was evaluated by agar disc diffusion method using Mueller Hinton agar medium for the assay. The microorganism was activated by inoculating a loop full of the strain in the nutrient broth and incubated on a rotary shaker. Then the medium was poured into the sterile petri plate.

Tele:

E-mail addresses: androdoss@gmail.com

© 2015 Elixir All rights reserved

**Table 1. Bacterial cultures**

| S.No. | Name of the Microbial Pathogens | Type | MTCC No. |
|---|---|---|---|
| 1 | *Staphylococcus aureus* | Gram Positive | 3160 |
| 2 | *Streptococcus pyogenes* | Gram Positive | 442 |
| 3 | *Salmonella typhi* | Gram Negative | 733 |
| 4 | *Pseudomonas aeruginosa* | Gram Negative | 4676 |
| 5 | *Proteus mirabilis* | Gram Negative | 425 |
| 6 | *E. coli* | Gram Negative | 2961 |
| 7 | *Klebsiella pneumoniae* | Gram Negative | 432 |

**Table 2. Antibacterial activity of medicinal plants against clinically important microbial pathogens**

| Medicinal plants | Extracts | Zone of Inhibition (mm) Microorganisms | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ***Sa*** | ***Pms*** | ***Ec*** | ***Kp*** | ***St*** | ***Pa*** | ***Sp*** |
| *A.tetracantha* | P.ether | - | - | - | - | - | - | - |
| | Benzene | - | - | - | - | 12 | - | - |
| | Chloroform | - | - | - | - | - | - | - |
| | Methanol | 15 | - | - | - | - | 12 | - |
| | Water | - | - | - | - | - | - | - |
| *C. aestuans* | P.ether | - | - | - | - | - | - | - |
| | Benzene | 8 | - | 12 | 13 | 14 | - | - |
| | Chloroform | - | - | - | 9 | - | - | - |
| | Methanol | - | - | - | - | 14 | - | - |
| | Water | - | - | - | - | - | - | - |
| *G.mangostana* | P.ether | - | - | - | - | 10 | - | - |
| | Benzene | 10 | - | - | 11 | - | - | - |
| | Chloroform | 12 | - | - | - | - | - | - |
| | Methanol | - | - | - | - | - | - | - |
| | Water | - | - | - | - | - | - | - |
| *G.ulmifolia* | P.ether | - | - | 9 | 7 | - | 7 | - |
| | Benzene | - | - | 10 | 3 | - | - | - |
| | Chloroform | - | - | 9 | - | - | - | - |
| | Methanol | 7 | - | - | 10 | 11 | 10 | 12 |
| | Water | 4 | 7 | - | - | - | - | - |
| *C.halicabaum* | P.ether | 7 | 8 | 7 | 8 | 9 | 9 | 19 |
| | Benzene | 12 | 6 | 1 | 11 | 6 | 15 | 6 |
| | Chloroform | 5 | 7 | 1 | 6 | 6 | 7 | - |
| | Methanol | 7 | 9 | 5 | 7 | 5 | 13 | 7 |
| | Water | - | 2 | 1 | 9 | - | - | - |
| *P. dioica* | P.ether | - | - | - | - | - | - | - |
| | Benzene | 12 | 9 | - | 15 | - | - | 19 |
| | Chloroform | - | 15 | 21 | - | - | - | - |
| | Methanol | 25 | - | - | 20 | - | - | - |
| | Water | 9 | - | 18 | - | - | - | - |
| *S. dulcis* | P.ether | - | - | - | - | - | - | - |
| | Benzene | - | - | - | - | - | - | - |
| | Chloroform | - | - | - | - | - | - | - |
| | Methanol | 14 | - | - | - | - | - | - |
| | Water | - | - | - | - | - | - | - |
| *P. nodiflora* | P.ether | - | - | - | - | - | - | - |
| | Benzene | - | - | - | - | - | - | - |
| | Chloroform | - | - | - | - | 10 | - | - |
| | Methanol | 12 | - | - | - | - | - | - |
| | Water | - | - | - | - | - | - | - |
| *C. bonplandianus* | P.ether | - | - | - | - | - | - | - |
| | Benzene | - | - | - | - | - | - | - |
| | Chloroform | - | - | - | - | - | - | - |
| | Methanol | 10 | 12 | - | - | - | - | - |
| | Water | - | - | - | - | - | - | - |
| *C. inerme* | P.ether | - | - | - | 9 | 5 | 9 | 9 |
| | Benzene | 9 | 9 | 9 | 10 | - | 11 | 17 |
| | Chloroform | - | - | - | - | 9 | - | 8 |
| | Methanol | 10 | 9 | 10 | - | - | 8 | 4 |
| | Water | - | - | - | - | - | - | - |

**Sa:** *Staphylococcus aureus,* **Pms:** *Proteus mirabilis,* **Ec:** *E. coli,* **Kp:** *Klebsiella pneumonia,* **St:** *Salmonella typhi,* **Pa:** *Pseudomonas aeruginosa,* **Sp:** *Streptococcus pyogenes.*

**Table 3. Minimum Inhibitory Concentration of selected medicinal plants**

| Plant Name | Solvent | MIC value mg/mL | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ***Sa*** | ***Pms*** | ***Ec*** | ***Kp*** | ***St*** | ***Pa*** | ***Sp*** |
| *A.tetracantha* | Methanol | 2 | - | 1 | - | - | - | - |
| *C. aestuans* | Benzene | 0.25 | 1 | 4 | 1 | 1 | 1 | 0.5 |
| *C.inerme* | All solvent | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| *C.bonplandianus* | Methanol | 0.5 | 1 | 0.5 | 4 | 0.25 | 1 | 1 |
| *G.mangostana* | Methanol | 4 | 6 | 8 | 6 | 2 | 8 | 4 |
| *G.ulmifolia* | Methanol | 1 | 2 | 0.5 | 2 | 4 | 1 | 0.5 |
| *C.halicacabum* | Methanol | 4 | 2 | 0.5 | 1 | - | - | - |
| *P. dioica* | Chloroform | 6 | 8 | 4 | 4 | 2 | 4 | 2 |
| *P. nodiflora* | Methanol | 2 | 4 | 0.5 | 1 | - | - | - |
| *S. dulcis* | Methanol | 0.5 | 1 | 0.5 | 4 | 0.25 | 1 | 1 |

**Sa:** *Staphylococcus aureus*, **Pms:** *Proteus mirabilis*, **Ec:** *E. coli*, **Kp:** *Klebsiella pneumonia*, **St:** *Salmonella typhi*, **Pa:** *Pseudomonas aeruginosa*, **Sp:** *Streptococcus pyogenes.*

The test organism was swabbed in the medium. The sterile Whatman filter paper discs (6mm in diameter) impregnated with plant extracts (100mg/ml) were placed on the surface of the culture plates and incubated at $37^0$C for 24hrs and diameter of zone of inhibition were measured in mm. The controls were maintained in which pure solvents were used instead of the extract. Chloramphenicol was used as antibacterial positive control (Bauer *et al*., 1966)

**Minimum Inhibitory Concentration**

The Minimum inhibitory Concentration (MIC) of the extract was determined, using two-fold dilutions method. 1ml of broth was added into series of eight test tubes and 8ml of the test compound was then vortexed to mix thoroughly to make initial concentration (8 mg/ml). From the first, the procedure is repeated until 1ml of compound mixture is finally discarded from the $7^{th}$ tube to give a concentration of 1.0, 0.5, 0.25 mg/ml) concentrations. $8^{th}$ tube serves as the control (with no test compound). Each test tube was inoculated with 12 hrs culture of test organism with titre of $10^3$ -10 CFU/ml. The test tubes were incubated at 37°C to observe visible turbidity. The MIC is the lowest concentration of the test compound inhibiting the appearance of visible growth.

**Results and Discussion**

There is growing interest in the use of alternative therapy, generally known as natural substances, as phytodrugs. Recently the World Health Organization (WHO) has recommended the use of Arthemisinin derivatives derived from *Artemisia annua*, a chinese herb as a first line drug in the treatment of malaria. The present investigations, the inhibitory effect of different extracts of selected medicinal plants were screened against clinically important microbial pathogens. The antibacterial activity was determined using agar disc diffusion method and broth dilution method summarized in Table 2 & 3.

Different solvents and water extracts tested at 100 mg/ml concentrations against seven important clinically important microbial pathogens are presented in Table 2. Among five solvents (Petrolum ether, benzene, chloroform, methanol and water) tested against seven microbial pathogens, benzene and methanol extracts recorded significant antibacterial activity against one or all the test pathogens. Antibacterial activity was not observed in aqueous extracts against all the pathogens.

Among benzene and methanol water extracts, methanol extracts recorded significant antibacterial activity followed by benzene. *Staphylococcus aureus* found highly susceptible to methanol extract, whereas *Klebsilla pneumonia* and *Pseudomonas aeruginosa* was less susceptible to methanol extract. Methanol extract exhibited similar antibacterial activity against, *E.coli*, *Salmonella typhi* and *Proteus mirabilis*. Highest antibacterial activity was observed against *Staphylococcus aureus* (25 mm) followed by *E.coli* (21 mm) even though antibacterial activity was observed against other pathogens also it was not found significant. Of the ten candidate plants in this study *P.dioica* showed significant antibacterial activity against all the tested bacteria and the remaining plants showed moderate activity after alcoholic extraction.

Minimum Inhibitory Concentration (MIC) of the active extracts is shown in Table 3. *S.dulcis*, *C.bonplandianus* and *C. aestuans* showed the strongest antibacterial activity with MIC values of 0.250 mg/ ml, followed by *P. nodiflora*, *C.halicacabum* and *G.ulmifolia* (MIC of 0.500 mg/ ml).

Available literature results indicate a strong activity when MIC values are between 0.05-0.50 mg/ ml, moderate activity in values between 0.6-1.50 mg $mL^{-1}$ and weak activity above 1.50 mg/ $ml^{-1}$ (Diaz *et al*., 2009).

The organic extracts provided more powerful antimicrobial activity as compared to aqueous extracts. This observation clearly indicates that the existence of non-polar residues in the extracts which have higher both bactericidal and bacteristatic abilities. Cowan (1999) mentioned that most of the antibiotic compounds already identified in plants are reportedly aromatic or saturated organic molecules which can easily solubilized in organic solvents. Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria (Doss *et al*., 2009 and Parekh *et al.*, 2006). These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure. The results of the present study are quite encouraging as almost all spices exhibited antimicrobial activity against most of the pathogens, but the antimicrobial activity varies widely, depending on the type of solvents, test medium and microorganism. This study opens up the possibility for the search of new antimicrobials as an alternative to the antibiotics.

**References**


- Parekh, J. and S. Chanda, 2006. *In vitro* antimicrobial activities of extract of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* (Vitaceae) and *Cyperus rotundus* (Cyperaceae). *Afr. J. Biomed*. Res., 9: 89-93.
- Marisa A. N. Diaz, Ciro C. Rossi, Vitor R. Mendonca, Danielle M. Silva, Andrea de O. B. Ribon, Ananda P. Aguilar, Gaspar D. Munoz Screening of medicinal plants for antibacterial activities on *Staphylococcus aureus* strains isolated from bovine mastitis. Brazilian J Pharmacog 2009; http://www.scielo.br/pdf/rbfar/2010nahead/aop1310.pdf
- Al-Bari, M.A., M.A. Sayeed, M.S. Rahman, and M.A. Mossadik. 2006. Characterization and antimicrobial activities of a phenolic acid derivative produced by *Streptomyces*

*bangladeshiensis*, a novel species collected in Bangladesh. Res. J. Medicine & Med. Sci. 1: 77-81.

- Ahameethunisa, A.R. and Hoper, W. (2010). Antibacterial activity of Artemisia nilagirica leaf extract against clinical and phytopathogenic bacteria. BMC Complementry and Alternative Medicines,10:6.
- Kim H, Park SW, Park JM, Moon KH, Lee CK. Screening and isolation of antibiotic resistance inhibitors from herb material Resistant Inhibition of 21 Korean plants. Nat. Prod. Sci. 1995; 1: 50 - 54.
- Alagesaboopathi C. Antimicrobial potential and phytochemical screening of *Andrographis affinis* Nees an endemic medicinal plant from India. Int. J. of Pharma. and Pharmaceutical Sci. 2011; 3 (2): 157- 159.
- Doss, A., Muhamed Mubarack, H. and Dhanabalan, R. 2009. Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. *Indian Journal of Science and Technology*. 2 (2):41-43.
- Cowan M. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 1999; **12**: 564-582.
- Bauer, A. W., W. M. M. Kibry, J.C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 45: 493-496.