International Journal of Research in Engineering, Science and Management
Volume-2, Issue-8, August-2019
www.ijresm.com | ISSN (Online): 2581-5792

Screening of Medicinal Plants Against Clinical Pathogens and its Antimicrobial Activity

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***Abstract*: Various medical plants have been used for years in daily life to treat disease all over the world. The present study was under taken to investigate Aleovera, Calotropis, Cinamomum, Adhatoda, Vitex for their potential activity against clinical pathogens. The plants extracts were prepared in butanol and acetone. The activity of plant extracts was evaluated against five clinical pathogens including Staphylococcus aureus, Streptococcus pyogens, klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa. Among these plants Aleovera and Cinamomum of butanol and acetone extract showed the potential activity against clinical pathogens. The antimicrobial activity of 50 mg/ml concentration of butanol and acetone of Aleovera and Cinamomum (9-11mm; 5-20nm) showed maximum inhibitory effect against almost all pathogens in disc diffusion method. The antibacterial activity of 50mg/ml concentration of butanol and acetone Aleovera and Cinamomum (11-13mm; 6-21mm); (10-13mm; 10-17mm) showed maximum inhibitory effect against almost all pathogens in agar well diffusion method. Further studies are needed to explore the novel antibacterial non-active molecules.**

***Keywords*: Antibacterial activity, Adhatoda, Aleovera, Calotropis, Cinamomum, Medicinal plants**

1. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The wide spread use of herbal remedies and health care preparations, such as those described in ancient texts like the Vedas and the bible, has been traced to the occurrence of natural products with medicinal properties. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural product origin (Stufness and Dourous, 1982) and natural products play an important role in drug development programme in the pharmaceutical industry (Baker et.al 1995). Medicinal plants are a source of great economic value in the Indian sub-continent. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country (Jigna parekh et al.,1995).It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents (Chung et al., 1995; Vilitinck et. al., 1995). The selection of crude plant extracts for screening sufficient quantities of these programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products (Kusumoto et. al.,1995).

2. Methodology

A. Selection of suitable medicinal plants

For the present study, alternative medicinal plants such as Aleovera barbedensis; Calotropis giganta; Cinnamomum cassia; Adhatodo vasica; Vitex negundo were selected.

B. Collection of medicinal plants

Sufficient quantities of these plants were collected in and around the regions of kanyakumari district, Tamilnadu. Specimens were cleaned of adhering soil / dust in the field by shaking and quick rinsing with tap water; plants were placed in paper bag and transferred to the laboratory

C. Isolation of bacterial strains

For present study, clinical pathogenic bacterial strains were isolated and identified from clinical samples such as blood, pus, sputum and urine.

D. Sample collection

The above samples were aseptically from the inpatients of various hospitals in and around Nagercoil, kanyakumari district Tamilnadu. The collected samples were packed in icebox with ice cubes, taken to the laboratory and kept in freezer until bacteriological examinations carried out.

E. Isolation and identification of bacterial microflora

From the individual clinical samples, serial dilutions were made separately up to 10-7 dilutions. 1 ml of each dilution was inoculated for 24 hrs at 30◦c. After 24 hrs of incubation,50 to 200 bacterial colonies were taken for enumeration. Using colony morphology, shape and colour major dominant flora was determined. Dominant species were selected and again restreaked in to nutrient agar plates individually for purification. After purification, the pure cultures were isolated and maintained as stock culture in plates and slants they were stored in refrigerator as stock culture. Further identification was done based on the morphological and physiological tests

described in bergey's manual of determinative bacteriology (Holt et al.,1996).

F. Identified organisms

- *Staphylococcus aureus*
- *Streptococcus pyogens*
- *Klebsiella pneumonaia*
- *Escherichia coli*
- *Pseudomonas aeruginosa*

G. Preparation of plant extracts

After shade drying, the dried plants were ground well individually by using a mixer grinder then sieved the powder individually by using a nylon sieve in order to remove plant fibres. The large particles were again ground with the mixture grinder and sieved through a fine cloth (mesh size <50 µm) to obtain the products with uniform particle size. The selected medicinal plants powders were mixed with liquid solvents such as acetone and n- butanol individually. Extraction was done by soaking one part of powder in to five parts of water / liquid solvent (1:5) separately and kept for perculation process for 7 days.The crude extracts were filtered individually. Extraction was done by soaking one part of powder in to five parts of water / liquid solvent (1:5) separately and kept for perculation process for 7 days. The crude extract were filtered individually and filterates were inoculated in to solid extract by allowing them for evaporation of solvent completely under room temperature. The powder form extracts were used further for antimicrobial studies

3. Primary screening of antimicrobial activity of plant extracts

The overnight broth culture of the identified five test strains were kept ready for antibiotic assay. Assay of the antimicrobial activity of plant extracts were done by agar diffusion methods

A. Agar diffusion method

Cotton swabs were charged with various stock cultures of test bacterial strains in muller hinton broth(MHB) suspension individually, then inoculated on muller hinton agar plates and were spread evenly over the surface of the agar medium. wells of 5mm diameter were aspetically cut and filled with the already prepared various concentration (10,20,30,40,50 mg/ml) of plant extracts individually. Then the plates were incubated at 32◦C for 24 hrs and examined for zone of inhibition. simultaneously triplicates were maintained for each sample. A positive result was interpreted as conforming presence of antibacterial substances (samania et al.,1995)

B. Disc diffusion method

The various concentrations (10, 20, 30, 40, 50 mg/ml) of extracts of the selected plants separately for their inhibitory activity against the test organisms by disc diffusion method (Kirby Baur method). The sterilized petriplates were poured previously with Muller Hinton Agar medium and labelled correspondingly to the sample. Then 0.1 ml of test organisms was taken from the stock (broth) and swabbed on the agar medium. Then the plates were incubated at 32◦C for 24 hrs. The anti-microbial activity of the test materials was observed through zone of inhibition (mm) on the plates

4. Secondary screening

A. Minimum inhibitory concentration (MIC)

MIC of an antibiotic expressed by determining the antibiotic activity quantitatively. Using the known sensitivity of a test organism towards a particular antibiotic can do it. Serial dilution of an antibiotic are pipetted out in to the antibiotic broth, which is inoculated with a defined quantity of the relevant test organisms. The test tube which does not show any turbidity due to suppression of microbial growth, indicates the presence of active antibiotic at a concentration corresponding to MIC. Based on the performance in primary screening tests like disc and agar diffusion methods, the plants extracts showing best growth inhibitory activity against the test pathogens were selected for MIC study. Accordingly two best active extracts such as A.barbedensis, C.cassia were selected for further studies. The individual plant extracts derived from acetone and butanolic extracts were individually solubilised in MHB serially diluted with (100,50,25,12.5,6.25,3.125,1:56 and 0 control ppm). Samples measuring 80 µl of each bacterial suspension were added to the serial dilution of the test substances. The MHB without plant extract was used as control . The inoculated test tubes were incubated at 35◦c for 24 hrs. After the incubation period, turbidity was evaluated. The MIC is defined as the lowest anti-microbial concentration of the test compounds which inhibits bacterial growth.

B. Minimum bactericidal concentration

To determine minimum bactericidal concentration, aliquots of one loopful of the above serially diluted and incubated concentration were streaked individually on petridishes containing Muller Hinton Agar and incubated the plates at 35◦C for 24 hrs. Then the plates were evaluated by comparing them with control plates containing test bacteria without test compounds. The lowest concentration that gave no visible growth was considered as MBC

5. Result and discussion

The five medicinal plant extracts were tested against five pathogens isolated from various clinical samples using disc and agar diffusion method. Among the tested plant extracts, the low concentrations (10-30 mg/ml) of certain plant extracts displayed nil or very less inhibitory activity against the tested pathogens except the extract of A.barbendensis and C.cassia. But almost all the tested medicinal plant extracts showed considerable level of growth inhibitory effect against all the pathogens at higher concentrations (40 and 50 mg/ml). Within these, butanol extract of C.cassia (12-26 mm) and acetone extract of some plant extract (9-16 mm) showed Considerable

effect again all the tested pathogens at 50 mg/ml concentration through disc diffusion method.

The activity of plant extracts against the tested clinical pathogens are solvent dependent. There are two solvents viz. butanol and acetone was used for extraction purpose. But the result obtained was varied. For instance the butanolic extract of C.cassia showed better activity against the test pathogens. Similarly the acetone extracts of certain plant A.vasica; A.barbedensis; V.negundo displayed better activity against the tested pathogens.

All the plant extracts were not effective against all the pathogens. For instance, the butanol extract of C.giganta and V.negudo were no effect against against S.aureus and S.pyogens respectively. Likewise, C.giganta has no effect against S.aureus and E.coli. Similary K.pneumoniae was resistant against the acetone extract of V.negudo. The MIC and MBC of both butanolic and acetone extracts of selected plant extracts C.cassia were tested.The result indicated that the MIC of butanolic extract of A.barbedensis was 100ppm against all the tested pathogen but the same extract of C.cassia against S.aureus and P.aeruginosa was 12.5ppm but the MIC of the same plant extract had 100ppm against E.coli and 50ppm against S.pyogens and K.pneumoniae. The MIC of acetone extracted showed 100ppm by A.barbedensis against all the tested pathogen except 50 ppm against E.coli. But C.cassia exhibited 25 ppm against S.aureus and P.aeruginosa respectively.

The MBC of butanolic extract of A.barbedensis against all the tested pathogens was 100 ppm, but the MBC of C.cassia against S.aureus and P.aeruginosa was 12.5 mm respectively. At the same time the result on acetone extract showed the MBC of A.barbedensis against E.coli was 50 ppm, whereas against other tested pathogens was 100 ppm. Likewise the MBC of C.cassia against S.aureus and P.aeruginosa was 25 ppm.

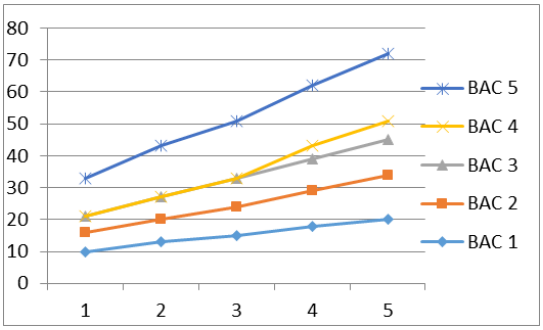

Fig. 1. Antimicrobial activity of butanol extract of different medicinal plants against various pathogen using disc diffusion method

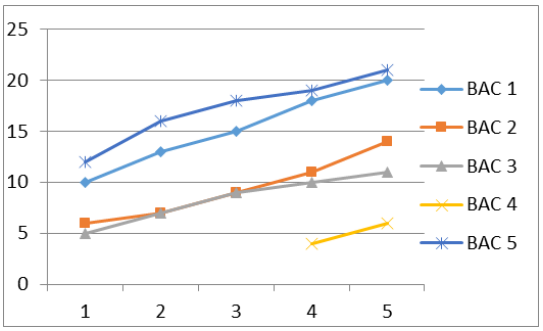

Fig. 2. Antimicrobial activity of acetone extract of different medicinal plants against various pathogen using agar well diffusion method

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