J. Phytol. Res. **22**(2) : 251-254, 2009

IN VITRO SCREENING FOR ANTIBACTERIAL ACTIVITY OF SOME INDIAN PLANTS

A. D. CHOUGALE, MD. SAIFUL-ARFEEN† and M. V. PADUL*
Biochemistry Department, NACAS College, Pune University, Ahmednagar-414001, Maharashtra-414001, India
†Biotechnology Department, NACAS College, Pune University, Ahmednagar-414001, Maharashtra-414001, India.
*E-mail: manoharpadul@yahoo.co.in

Present study conducted to evaluate the antibacterial activity of crude ethanolic extracts of seven edible and medicinal plants against *Bacillus subtilis*. Among tested plants, *Tinospora cordifolia* (TC) *and Syzygium aromaticum* showed antibacterial activity. The MICs of these plants were found to be 12.5µg and 25µg $disc^{-1}$, respectively by disc diffusion method. While, MICs from broth dilution methods were found to be 1.5 mg ml^{-1}, and 2.5 mg ml^{-1}, respectively. The MIC values reveled TC has more antibacterial potential than *S. aromaticum*. Antibacterial activity of TC was further evaluated against different microbes like *Staphylococcus aureaus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Pseudomonas desmolyticum, Proteus vulgaris and* yellow cells. Different crude solvent extracts of TC (Hexane, Dichloromethane, Acetone and Methanol) were screened for their antibacterial activity. In which acetone extract exhibited highest inhibitory activity for all tested microorganisms. The TLC of acetone extract of TC showed four different spots among which only 4th spot (R_f =0.14) from solvent front showed promising antibacterial activity.

Keywords: Antibacterial activity; Crude extracts; MIC; TLC.

Introduction

Different sources are in search by clinical microbiologists for new antimicrobial compounds from various kinds of microorganisms, animals, and plants[1] while, finding healing powers in plants is an ancient idea, which attract scientists in the topic of antimicrobial plant extracts. Every year two to three antibiotics derived from the microorganism getting launched in the market[2]. Such huge amount of the launching of antibiotics is due to getting resistance for the pathogen towards these antibiotics. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents[3]. The world health organization (WHO) estimated that around 80% of population of developing countries relies on traditional medicines, mostly plant drugs from their primary health care. Demand for using traditional plants is increasing both in developing and developed countries. These were due to growing reorganization of natural products, being non necrotic and having less side effects, easy availability and affordable prices[4]. In addition traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics[5]. Much work has been done on ethnomedicinal plants in India[6-8]. Interest in a large number of traditional natural products has increased[9]. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents[10, 11]. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products[12].

In the present study ethanolic extracts of seven edible and medicinal plants were screened for their antibacterial activity. The various solvent extracts of undertaken plant were tested for the antibacterial activity and TLC fractionation was done to detect the active antibacterial spot.

Material and Methods

Plant materials : Fresh plant materials of *Syzygium aromaticum, Pisum sativum, Amaranthus viridis, Cicer arientinum, Sesbania grandiflora, Tinospora cordifolia (TC) and Glycine max* were collected from the Ahmednagar district, Maharashtra state, India. The plants collected were authenticated at Department of Botany, Shivaji University, Kolhapur, India.

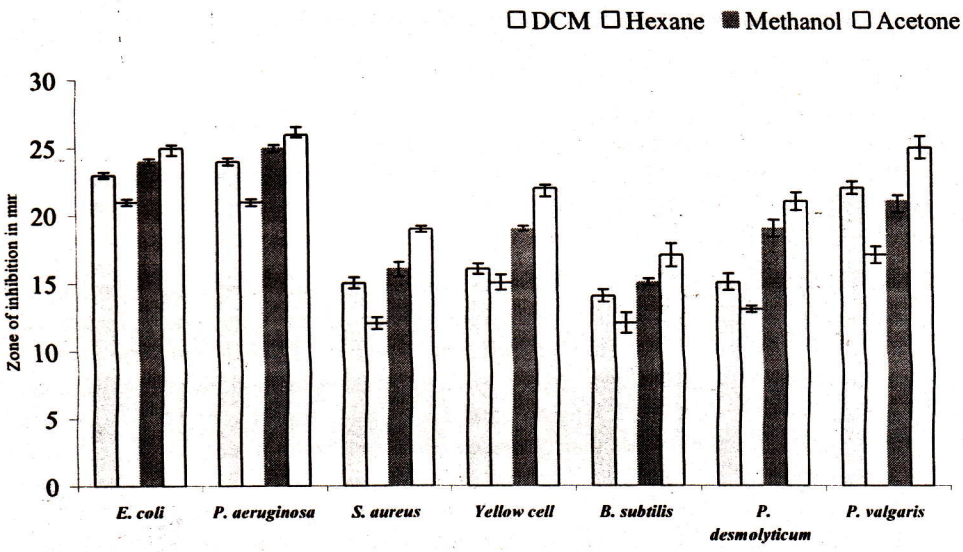

Fig.1. Antibacterial activity of different solvent extracts of *Tinospora cordifolia* at 5 mg concentration. Data representing three individual test ± SE.

Table 1. Antibacterial activity of the some plants against *Bacillus subtilis.*

Plant Name	Common name	Part used	Antibacterial activity
Syzygium aromaticum	Clove	Fruit	+
Pisum sativum	Pea	Seed	-
Amaranthus viridis	Amaranthus	Seeds	-
Cicer arientinum	Gram	Seeds	-
Sesbania grandiflora	Shevri	Flower	-
Tinospora cordifolia	Guduchi	Stem	+
Glycine max	Soya bean	Fruit	-

Table 2. Minimum inhibition concentration of the positive antimicrobial activity.

Sr. No.	Plant Name	MIC	
		BDM	DDM
1	*Syzygium aromaticum*	2.5 mg/ml	25 µg / disc
2	*Tinospora cordifolia*	1.25 mg/ml	12.5 µg / disc

MIC= minimum inhibitory concentration, BDM= broth dilution method, DDM= disc diffusion method, - = not determined

Preparation of extract: Dried plant material (20 g) was macerated with 80% ethanol (200 ml) for 24 hours and then centrifuged. The supernatant was evaporated to a thick residue at 40°C. The residue was dissolved in desired amount of DMSO (dimethyl sulfoxide). Same procedure was used for the extraction of TC by different solvents (Hexane, Dichloro methane, Acetone) separately.

Microorganisms tested and antibacterial assay: The following strains of bacteria were used: *Escherichia coli* ATCC 25922, *Bacillus sbtillus* ATCC 11778, *Pseudomonas aeruginosa* ATTC 27853, *Staphylococcus aureus* ATCC 25923, *Pseudomonas desmolyticum* NCIM 2112, *Proteus vulgaris* NCIM 2001 and yellow cells (clinical isolate). Antibacterial activity was screened by

well diffusion method. The Minimum inhibitory concertration (MIC) values were determined by broth dilution method[13] and disc diffusion method[14].

Thin layer chromatography: Acetone extract of TC was loaded on TLC (silica gel) plate and separated by using cyclohexane: diethyl ether : chloroform (8:10:6) solvent system. After complete run, chromatogram was developed in iodine chamber and marked. Iodine was completely evaporated and each spot was scraped and collected separately. The compounds were eluted from silica gel in acetone. Acetone was evaporated at 40°C and remaining residue dissolved in DMSO which was used for antibacterial assay.

Results and Discussion

Several pathogenic bacteria like *S. aureus, B. subtilis* etc, are known to exhibit resistance against antibiotics[15-19]. Plants are considered as potential sources of compounds with antibacterial activity[20]. A variety of phytochemicals belonging to different chemical groups like alkaloids, flavanoids, tannins, coumarins, quionens and proteins were reported to exhibit antimicrobial activities[20, 21].

In present study seven ethanolic extracts of different plants were screened for their antibacterial activity against *B. subtilis*. Out of which only two showed antibacterial activity *i.e. Syzygium aromaticum and Tinospora cordifolia* (Table 1). Both these plants were reported for many medicinal uses[6]. The MIC values of this two active plant extracts was determined by two different methods viz. broth dilution method (BDM) and disc diffusion method (DDM) (Table 2). The MIC values were found to be less in DDM than in BDM. The MIC of *Syzygium aromaticum* by BDM was found 2.5 mg / ml whereas the MIC by DDM for same plant was 25 µg / disc. The MICs of TC was 1.25 mg/ml and 12.5 µg / disc, respectively by BDM and DDM. Obtained MIC values reveal more sensitivity of DDM over BDM. The MIC values from table 2 indicate that TC is having more antibacterial activity than *Syzygium aromaticum*. Present finding is in concomitance with Jeyachandran and Anand[22].

As the plant TC showed more potential, attempts were made to thoroughly investigate its antibacterial potential against seven pathogenic bacteria. The different solvent extracts (Hexane, Dichloromethane, Acetone and Methanol) were tested for their antibacterial activity. Amongst which acetone fraction showed maximum zone of inhibition against all tested microorganisms. TC has been reported to possess multiple activities and it has been documented for treatment of several aliments such as diabetes[6], as immunomodulatury[23, 24], antioxidant[25] and radioprotective agent[26]. The plant extract has been reported to have hypolipidaemic action[7, 27].

The more active acetone extract of TC was fractionated by TLC method, which gives four different spots. It was observed that out of four fractions (R_f values 0.14, 0.41, 0.83 and 0.89) only 4th fraction (R_f value 0.14) from solvent was found to have potent antibacterial potential while rest of three fractions were unable to inhibit the bacterial growth.

Acknowledgements

Authors are thankful to the institution for providing necessary facilities.

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