Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 4, 2011

Research Article

IN VITRO ANTIBACTERIAL SCREENING OF DIFFERENT EXTRACTS OF MORINA LONGIFOLIA ON PATHOGENIC MICROORGANISMS

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Received: 30 May 2011, Revised and Accepted: 8 July 2011

ABSTRACT

The antimicrobial activities of hexane(H), petroleum ether(PE) , acetone(AC),choloroform(C), ethanolic(E) and water(W) extracts of whole plant (1mg/ml) of Morina longifolia were determined against wide variety of pathogenic bacteria.The extracts were tested against various bacteria's like Escherichia coli(EC), Staphylococcus aureus(SA),Staphylococcus pyogens(SP), Bacillus subtilis(BS), Klebsiella pneumoniae (KP) and lactococcus(LC) by well diffusion method. Minimum inhibitory concentration (MIC) and Minimum lethal concentration(MLC) values of each extract were determined. It is concluded that ethanolic extract, Acetone extract and choloroform extract of whole plant of Morina longifolia exhibited significant antibacterial activity. These findings established the potential of the plant Morina longifolia as an effective antibacterial agent. However, further studies are needed to evaluate active compounds and probable medicinal benefits in chemotherapy among humans.

Keywords: Morina longifolia, Antimicrobial activity, MIC, MLC, Pathogenic microorganisms, Extracts.

INTRODUCTION

According to an estimate of the World Health Organization, approximately 80% of the people in developing countries depend on traditional medicine for primary health care needs; a major portion of these involves the use of medicinal plants[1].Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind. The different parts of several medicinal plants were used to cure specific ailments has been in vogue from ancient times[2] in India. Traditional healers claim that their medicine is cheaper and more effective than modern medicine. With the continuous use of antibiotics, microorganisms have become resistant. This has created immense clinical problem in the treatment of infectious diseases[3]. It is necessary to evaluate, scientifically, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases. Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganisms[4]. Plants also have been used in ethnopharmacy for various diseases such as hypertension, cholesterol, eczema and diarrhoea for centuries and today their scientific validation was provided by identification and isolation of bioactive phytochemicals[5]. Phytochemicals are the secondary metabolites that have several subgroups possessing various bioactivities such as antioxidant[6], antimicrobial[7and 8], antivirus, anticancer etc[9]. Exploration of the chemical constituents of the plants and pharmacological screening is of great importance which leads for development of novel agents[10]. First, it is very likely that the phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. It is reported that, on average, two or three antibiotics derived from microorganisms are launched each year[11]. It is estimated that there are 250,000 to 500,000 species of plants on Earth[12]. Terpenenes or terpenoids are active against bacteria[13] and fungi[14]. Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. One approach is to screen local medicinal plants for possible antimicrobial properties.

Morina longifolia belongs to family Morinaceae and is found at an altitude of 3000-4000m along Bhutan,Kashmir and himalayan region.The flowers are hermaphrodite (have both male and female organs) and are pollinated by Moths. The plant is self-fertile. The stem, leaves and flowers are used in Tibetan medicine, they are said to have a sweet and astringent taste with a healing potency. They are digestive, emetic and stomachic, and are used in the treatment of stomach disorders such as indigestion giving rise to vomiting and nausea[15]. The roots are used as poultice on boils and wounds .During flowering period, contact with the plants causes dizziness and giddiness [16].

Plants are the important source for free radical scavenging molecules. Intake of natural antioxidant has been associated with reduced risk of cancer; cardiovascular diseases, diabetes and other diseases associated ageing.

MATERIALS AND METHODS

Collection and Identification of Plant:

The plants of Morina longifolia were collected in the month of July 2009 from Binouk hill, Mussorie, Dehradun (U.K). The plants were authenticated at Forest Research India (FRI), Dehradun, India.

Preparation of Solvent extracts

The method [17] was adopted for preparation of plant extracts with little modifications. Briefly 20 g portions of the powdered plant material were soaked separately in 100 ml of each hexane(H) , petroleum ether(PE) , acetone(AC),choloroform(C), ethanolic(E) and water(W) for 72 h. Each mixture was stirred after every 24 h using a sterile glass rod. At the end of extraction, each extract was passed through Whatman filter paper no. 1 (Whatman, England). The filtrate obtained were concentrated in vacuo using rotary evaporator at 30^{o}C.

Test organisms used

The test organism's Escherichia coli(EC), Staphylococcus aureus(SA),Staphylococcus pyogens(SP), Bacillus subtilis(BS), Klebsiella pneumoniae (KP) and lactococcus(LC) were the bacterial strains obtained from Institute of Microbial Technology (IMTECH) Chandigarh, India. These were obtained from pure lab cultures of Dept. of Biotechnology, Graphic Era University, Dehradun, India.

Determination of antibacterial

The agar well diffusion method [18] was modified.Nutrient agar medium (NAM) was used for bacterial cultures. The culture medium was inoculated with the microorganism separately suspended in Nutrient broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts (1mg/ml) and solvent blanks (hexane(H) , petroleum ether(PE) , acetone(AC),choloroform(C), ethanolic(E) and water(W) as the case may be). Standard antibiotic (Amoxicillin(A), concentration 1mg/ml) was simultaneously used as positive control. The bacterial plates were then incubated at 37^{o}C for 18 h. The antibacterial activity was evaluated by measuring the

diameter of zone of inhibition observed. The extracts that showed antimicrobial activity were subjected to minimum inhibitory concentration (MIC) and minimum lethal concentration(MLC) assay by two fold dilution method. The minimum dilution of the plant extract that kills the bacterial growth was taken as MLC (Minimum lethal count) while as MIC was interpreted as the lowest concentration of the sample,which showed clear fluid without development of turbidity.

RESULTS AND DISCUSSION

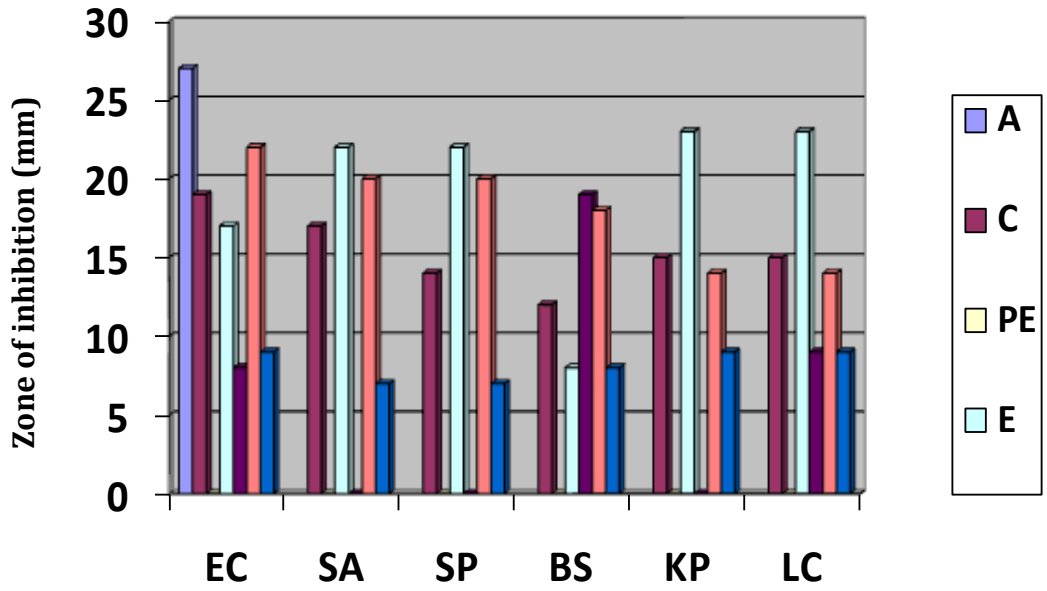
The antibacterial activities of the ethanolic, acetone and choloroform extracts of Morina longifolia showing significant variations as shown in Table 1.Among the three extracts tested, ethanolic extract had greater antibacterial potential followed by acetone and choloroform extracts. The largest zones of inhibition were observed for ethanolic extract against Klebsiella pneumoniae (23mm) and lactococcus(23mm).Acetone extract was very effective against E.coli(22mm) while as chloroform extract was also effective against E.coli(19mm).

Antimicrobial potency of the leaf extract of Morina longifolia against the tested bacteria were expressed in MIC as presented in Table 2. The MIC values against these bacteria strains ranged from 0.6 to 0.8 mg/ml while MLC values ranged from 0.7 to 0.9 mg/ml.

This indicates that the Morina longifolia extracts have broad inhibitory activities to pathogenic microorganisms and promising to act as potential antibacterial and antifungal agents from natural plant sources. The experiments were performed in triplicates. The results are indicated in Table 1 and Table 2.

Table 1: Antibacterial activity of various solvent extracts of Morina longifolia.

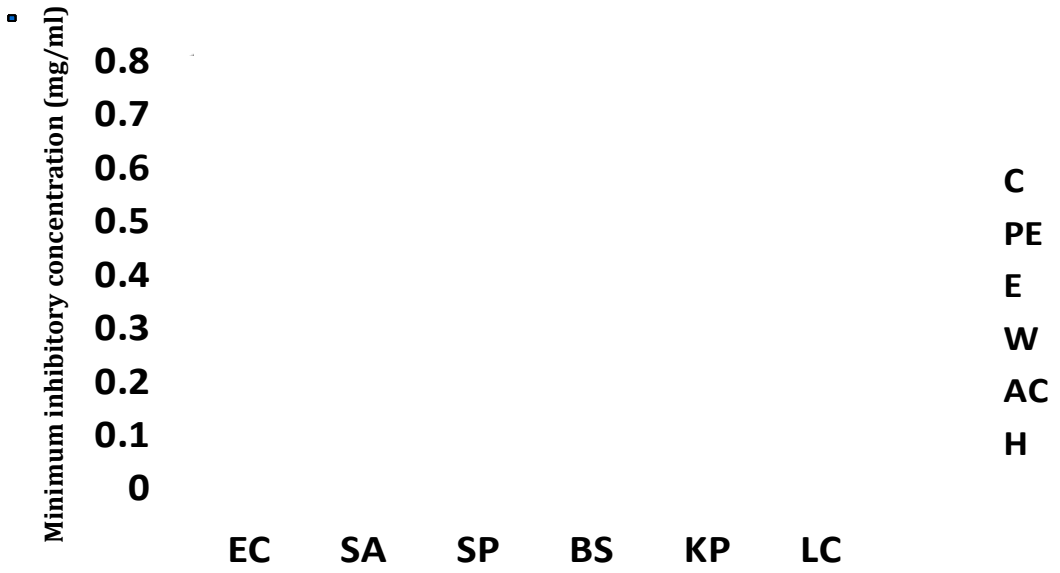
Micro-organism	Zone of Inhibition(mm)						
	A	C	PE	E	W	AC	H
Escherichia coli	27	19	NA	17	8	22	9
Staphylococcus aureus	23	17	NA	22	NA	20	7
Staphylococcus pyogens	21	14	NA	22	NA	20	7
Bacillus subtilis	25	12	NA	18	9	18	8
Klebsiella pneumoniae	22	15	NA	23	NA	14	9
Lactococcus	27	15	NA	23	9	14	9



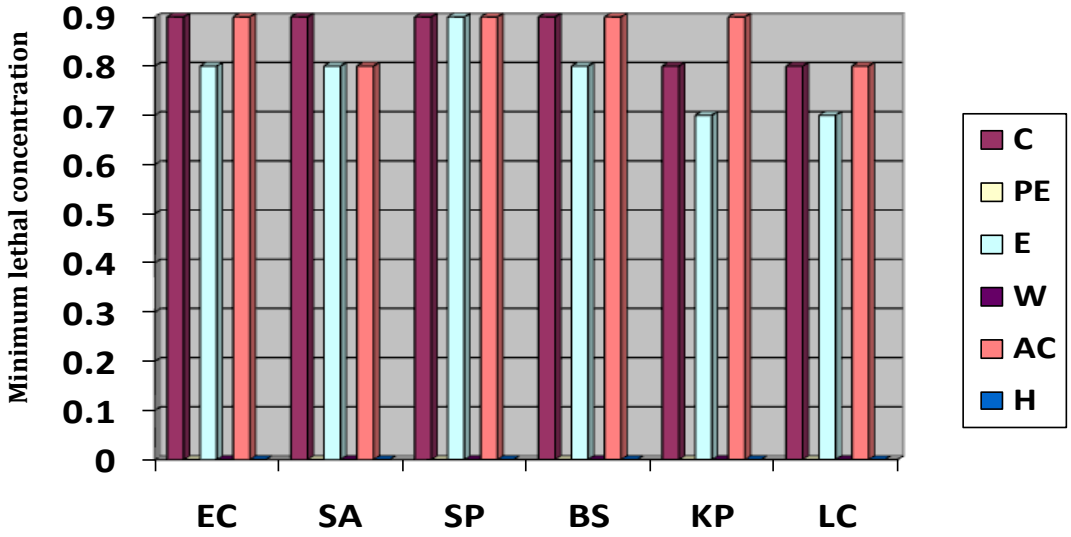
Graph 1: Zone of inhibition of various solvent extracts of Morina longifolia

Table 2: Antibacterial activity of various solvent extracts of Morina longifolia

Micro-organism	Minimum Inhibitory Concentration(MIC) (mg/ml)						Minimum Lethal Concentration (MLC) mg/ml					
	C	PE	E	W	AC	H	C	PE	E	W	AC	H
Escherichia coli	0.7	NA	0.6	NA	0.7	NA	0.9	NA	0.8	NA	0.9	NA
Staphylococcus aureus	0.8	NA	0.7	NA	0.7	NA	0.9	NA	0.8	NA	0.8	NA
Staphylococcus pyogens	0.8	NA	0.7	NA	0.7	NA	0.9	NA	0.9	NA	0.9	NA
Bacillus subtilis	0.8	NA	0.7	NA	0.8	NA	0.9	NA	0.8	NA	0.9	NA
Klebseilla pneumonia	0.7	NA	0.6	NA	0.8	NA	0.8	NA	0.7	NA	0.9	NA
Lactococcus	0.7	NA	0.6	NA	0.7	NA	0.8	NA	0.7	NA	0.8	NA



Graph 2: Minimum inhibitory concentration (MIC) of various solvent extracts of Morina longifolia



Graph 3: Minimum lethal concentration (MLC) of various solvent extracts of Morina longifolia

CONCLUSION

The extracts of the plant used in the present study showed prominent antibacterial activity against Escherichia coli, Klebsiella pneumoniae,lactococcus,staphylococcus pyogens and less activity against Bacillus bubtilis. Thus the use of these plants in the treatment of pathogenic diseases associated with the infection of these pathogens is validated and scientifically supported by the results obtained in this work.

ACKNOWLEDGEMENT

Sincere thanks for the cooperation to research staff of Department of Chemistry and Biotechnology, Graphic Era University, Dehradun (U.K), India for carrying out the research.

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