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Research Article

IN VITRO ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT FROM SOME HERBAL PLANTS

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ABSTRACT

Traditionally plants have been used as medicine for treatment of dermatophytes, burns and infectious diseases is common in traditional medicine. Based on ethnopharmacological and taxonomic information, antibacterial activities of methanol extracts of some herbal plants were determined by *in vitro* by agar diffusion-method against some human pathogenic bacteria. In this study, methnolic extract of four plants, viz *Carum copticum* (Ajwain), *Bacopa monnieri* (Brahmi), *Rauvolfia serpentina* (Sarpagandha) and *Curcuma longa* (Haldi) , which have some ethnomedicinal applications were screened for their antbacterial activity against four bacterial pathogen; two Gram's positive *Staphylococcus aureus* and *Bacillus amyloliquifaciens* and two Gram's negative; *Escherichia coli* and *Pseudomonas aeruginosa*. The plant extract were more active against Gram-positive bacteria than against Gram-negative bacteria. Data showed methanolic extract of *T. copticum* and *B. monnieri* showed the more antibacterial activity compared to *C. longa* and *R. serpentina* at 1mg/mL, 0.8mg/mL, 0.5mg/mL, 0.3 mg/mL. Hence these plants can be further subjected to isolation of the therapeutic antimicrobials and pharmacological evaluation. This study supports the use of herbal extract as traditional medicines to cure many diseases like intestinal tract, ear infections, throat, diarrhoea, skin diseases and fever.

Key words: Antibacterial property, herbal extract, methanol, MIC, zone of inhibition.

INTRODUCTION

During the last decade, development of antibiotic resistance as well as undesirable side effects of some drugs has led to the search for new antimicrobial agents. Antimicrobial activity and other biological effects have been shown in plants and their essential oils by many researchers[1]. Microbial resistance problem and the outlook for the use of antimicrobial drugs in the future is still not certain. Therefore, some actions must be taken to reduce the problem of control in the use of antibiotics and carrying out research for the better understanding of the genetic mechanism of resistance. This evoked us to evaluate plants as the source of antimicrobial agent, their ethno medicinal use and as potential chemo therapeutic agent[2].

All over the world herbal medicine represents one of the most important disciplines of traditional medicine. Herbs have been identified as source of traditional medicines which are used to cure illness and are still extensively used all over the world. For various health problems herbal treatment is still used. Herbs are economical, safe, less toxic, and a reliable key natural resource of drugs all over the world. In Saudi Arabia, use of traditional medicine among the tribal local people and medicinal healers (*Hakim*) is a significant part of their tradition and till date it is widely practiced[3].

It is essential to study medicinal plants in order to promote the proper use of herbal medicine and also to determine their potential as sources for new drugs, which have folklore stature in a more intensified way[4]. The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparations have been found to be potent in the treatment of diseases. Therefore the need of the medicinal plants has tremendously increased, to meet the increasing demand of manufacturing modern medicines and export. This demand is generally met by cultivating and uprooting medicinal plants[5].

Contrary to the synthetic drugs, antimicrobials originated from plant are not associated with the side effects and have enormous therapeutic use to heal many infectious diseases. Plant-based antimicrobials have proved to have great therapeutic potential as compared to synthetic antimicrobials as the later causes less side effects and serve the purpose well[6]. Since secondary metabolites from natural resources have been elaborated within living systems, they often show more "drug - likeness and biological friendliness than totally synthetic molecules" making them good candidates for further drug development[7].

C. copticum (Umbelliferae), an annual plant which grows in India, has white flowers and small fruits. The fruits of *C. copticum* (Ajwain) were traditionally used as antihelmentic, carminative and diuretic. Some biological effects of ajwain such as anti-inflammatory, antifilarial, antifungal, antipyretic, analgesic, antinociceptive, antioxidant and antiviral activity have been confirmed. There are some reports on chemical composition of ajwain oil. Four chemotypes -

a) thymol, γ-terpinene; b) thymol, carvacrol; c) carvacrol, γ-terpinene and d) thymol for ajwain oil have been reported so far.

B. monnieri, also referred to as *Herpestis monniera*, water hyssop. For several centuries, the name Brahmi has been used in the Ayurvedic system of medicine[8]. *B. monnieri*, a member of Scrophulariaceae family, is a small, perennial, creeping herb with numerous branches, small long leaves and produces light purple or white colour flowers[9]. The purpose of the present study was to evaluate the anti microbial activities of various extracts of *B. Monnieri*[10].

Alavijeh *et al*., (2012) observed the comparative study on the antibacterial efficacy of the methanolic extract of leaves of four medicinal plants[11]. *R. serpentina* is a member of Apocynaceae family which is native to the Indian subcontinent and East Asia. These plants have medicinal importance and few of these are used for different purpose which is already established. *R serpentina* extract has been used for the treatment of anxiety, eczema, epilepsy, fever, insanity, intestinal disorders, snake bite, rheumatism, bacterial infections, cardiovascular disorder, nervous disorders, psychiatric disorders, and in the management of hypertension schizophrenia.

The current investigation was carried for screening the antibacterial activity of four herbal plants used for herbal treatment by local communities against some pathogenic bacterial strains.

MATERIAL AND METHODS

Collection of Plant Materials

The fresh and healthy leaves of *B. monnieri* and *R. serpentina*, roots of *C. Longa* and seeds of *C. copticum* were brought to the laboratory and thoroughly washed in distilled water collected from Uttarakhand, India. Plant parts were selected based on the information provided in the ethnobotanical survey of India. Each plant material was labelled, numbered and information regarding collection date, their medicinal use and locality from were collected were recorded. All the plant materials were identified and contaminated particles were removed. Then the plant materials were air dried and were powdered for further use.

Processing and extraction of plant material

The plants were air dried, chopped and again shade dried for 14 days at room temperature and then grounded to coarse powder using a homogenizer for ease of extraction of active compounds. The powdered plant material (100g) was packed into a soxhlet apparatus and extracted up to 4 h with petroleum ether (60-80 °C) for defatting. Then it was extracted for further 4 h with methanol. The extract was filtered, and the solvent was evaporated under reduced pressure using a rotary vacuum evaporator. These purified extracts were then dissolved in DMSO and stored at 4°C until further use.

Antibacterial Assay

Based on their clinical and pharmacological importance, *S. aureus*, *E. coli*, *P. aeruginosa* and *B. amyloliquifacienes*, were chosen for evaluating antibacterial activity.These bacterial strains obtained from Institute of Microbial Technology, Chandigarh (IMTECH). The bacterial stock cultures were incubated at 37°C for 24 hours on nutrient agar followed by storage at 4°C until further use.

Determination of zone of inhibition method

Methanolic extracts of herbal plants were examined *in vitro* for antibacterial activities. Four pathogenic bacteria *B. Amyloliquifaciens, E.coli, P. Aeruginosa and S. aureus* (two Gram-positive and negative) were used for evaluation of antibacterial activity using agar disk diffusion method.

About 50µL of bacterial culture were spread thoroughly with sterile glass spreader on presterilized petriplates containing 23-25mL molten sterile LB agar. After drying the plates for 5minutes 6.0mm sterile cork borer was used to bore wells at spaced position on the agar plates. The wells were filled with 100µL of methanolic extract of the plants diluted in DMSO at different concentrations such as 400mg/mL, 300mg/mL, 200mg/mL, 100mg/mL, 80mg/mL, 50mg/mL, 30mg/mL, 20mg/mL, 10mg/mL, 8mg/mL, 5mg/mL, 3mg/mL, 1mg/mL, 0.8mg/mL, 0.5mg/mL, 0.3mg/mL and 0.1mg/ml of the crude extract for determining minimum inhibitory concentration. DMSO was used as solvent control. These agar plates were kept at room temperature for some time for diffusion to take place and then were kept for incubation at 37ºC for 18-24 h. After incubation period is over, the diameter of zone of inhibition (mm) was measured.

Minimum inhibitory concentration (MIC)

Agar well diffusion method was adopted to determine the minimum inhibitory concentration of the effective plant extracts. 50µl of overnight grown bacterial cultures were spread thoroughly on LB agar plates. Then different dilution of the plant extracts viz 100 mg/ml, 80mg/ml, 50mg/ml, 30mg/ml, 10mg/ml, 8mg/ml, 5mg/ml, 3mg/ml, 1mg/ml, 0.8mg/ml, 0.5mg/ml, 0.3mg/ml and 0.1mg/ml were made and were poured in the wells. The plates were then incubated at 37 ºC for 18-24 hours. The lowest concentration at which the plant extract showed visible inhibiting growth of microorganisms was considered as MIC.

RESULTS AND DISCUSSION

Antibacterial activity was assessed by analysing the diameter of the zone of inhibition and the methanolic extract of four plants showed best results. However, the plants differed in their antimicrobial activity against test organism at various concentrations.

The extract of *C. copticum* with MIC value of 10mg/mL showed significant inhibition against *E.coli* with a diameter of zone of inhibition 1.2mm. While MIC value as 3mg/mL obtained against *S. aureus* and *P. aeruginosa* with diameter of zone 0.7mm and 1.1mm respectively and showed MIC value of 1mg/mL obtained against *B. amyloliquefaciens* with diameter of zone of inhibition 0.7mm (Table 2). The aqueous extract of *C. copticum* was earlier reported to have antibacterial effect on several bacterial strains such as *Escherichia coli, Enterococcus faecalis*, *P. aeruginosa, Staphylococcus aureus, S. typhimurium,* and *Shigella flexneri*[12]. The effect of *C. copticum* on fifty-five different bacterial strains showed antimicrobial activity with <2% (v/v) minimum inhibitory concentration except *Pseudomonas aeruginosa*[13]. It was also seen that ether fraction of *C. copticum* proved to have better antibacterial and antifungal activity against multidrug resistant (MDR) strains of *Escherichia coli, Candida albicans, Candida tropicalis, Candida krusei, Candida glabrata,* and reference strains of *Streptococcus bovis* and *Streptococcus mutans* than other fractions[14].

The extract of *B. monnieri* with MIC value 1mg/mL significantly inhibited *E.coli* and *S.aureus* with diameter of zone 1mm and 1.2 mm respectively, *B. amyloliquefaciens* with MIC value 0.3mg/ml and diameter of zone 0.9 while MIC value as 10mg/ml was observed with the diameter of zone of inhibition 1mm (Table 3). Simillary Ghosh *et al.,* 2007 reported investigation of antibacterial and antifungal activities of ethyl acetate and n-butanol fractions of ethanol extract of *B. monnieri* Linn. aerial parts which ended in conclusion that ethyl acetate fraction was found to be more potent than the n-butanol fraction, though both of them were endowed with antimicrobial activity. Despite many published reports dealing with treatment for neurological disorders, little was known about antimicrobial activity of *B. monnieri* prior to this study[16]. In *Bacopa monnieri*, methanolic extracts was found to possess maximum inhibitory effects against both Gram positive and Gram negative organisms.

The extract of *R. serpentina* showed MIC value of 3mg/mL against *E.coli*, 0.3 mg/mL MIC value against *B. amyloliquefacienes* at diameter of zone of inhibition 0.8 and 0.9mm respectively, while MIC value as 10mg/mL against *S. aureus and P.aeruginosa* with diameter of zone of inhibition 1.0 and 1.2mm respectively (Table 4). Recently, antimicrobial activity of ethanolic extract (30mg/ml) of *R. serpentina*[17] was also reported by Deshwal and Vig (2012). Similarly, Ahmed *et al.,* (2002) reported that leaf and root extract of *R. serpentina* showed a good antibacterial activity against *Salmonella typhi, Escherichia coli*, *Pseudomonas aeruginosa*, which may be due to the presence of alkaloid in the extract[18]. Jigna *et al.,* (2005) mentioned that aqueous extract of *R. serpentina* showed less antimicrobial activity as compared to ethanolic activity[19]. Some other report also showed that medicinal plant inhibited the growth of certain human pathogen[20].

The extract of *C. longa* showed MIC value of 3mg/mL against *E. coli*, *B. amyloliquefaciens, P. aeruginosa, S.aureus* strains with the diameter of zone inhibition 0.8, 0.7, 0.7, 0.8mm respectively (Table 5). Many *C. longa* species are known for their medicinal properties. Antifungal, antibacterial and antiflammatory activity has been reported for species such as *C. longa, C.zedoaria, C. Aromatic* and *C. Amada*[21]. It is evident from the results that *B. subtilis* was the most sensitive organism to *C. longa* extract of curcuminoid and oil. Wilson *et al.,* (2005) reported that antibacterial activity of ethanol extract of *C. zedoaria* (0.15mg/ml) and *C. Malabarica* (0.94mg/ml) showed higher inhibition against *B. subtilis* and their ethanol extracts were effective only at higher concentration of 3.75 mg/well[22]. Both the species of turmeric gave MIC against *B. Subtilis* was 8.0 mm in diameter. In has been reported that Gram positive bacteria are more sensitive to plant oil and extract[23]. Alzoreky & Nakahara, (2003) studied that among gram positive bacteria, *B. Cereus* was the most sensitive organism to *C. longa* extract and its ethanol extract gave MIC 12.0 mm in diameter[24].

Table 1: Properties of herbal plant extract

Plant	Part of plant	Extract	Traditional use	Potentially bioactive compound	Colour	Nature	% yield
C.longa	Root	Methanol	Alzheimer's disease,cancer, arthritis,and other clinical disorders. anti-inflammatory agent and remedy for gastrointestinal discomfort	moterpen, zingiberene,alkaloid,cadiac glycosides	Yellow	Oily	7.87
C.Copticum	Seed	Methanol	abdominal discomfort due to indigestion and antiseptic	Alkaloids, carbohydrates, glycosides, phytosterols	Dark brown	Oily	8.68
B. monnieri	Leave	Methanol	epilepsy and asthma	alkaloids, tannins, flavonoids and phenolic compound	Green	Sticky/ Dry	9.44
R.serpentina	Leave	Methanol	Paranoia, schizophrenia, hypertension	serpentinine,yohimbine,reserpine,ajmaline,deserpidine,rescinnamine	Brown	Dry	8.76

Table 2: Antibacterial activity of plant extract from *C. copticum* at different dilution (diameter of zone of inhibition in mm)

Dilution of Plant extract (mg/mL)	*E.coli*	*B. amyloliquifacienes*	*S. aureus*	*P. aeroginosa*
100	2.4	2	2.5	1.8
80	2	1.8	2.4	1.7
50	1.8	1.5	1.9	1.6
30	1.4	1.3	1.3	1.3
20	1.2	1.2	1.3	1.3
10	1.2	1.2	1.1	1.2
8	No Zone	1.1	1	1.2
5		1	0.9	1.1
3		0.9	0.7	1.1
1		0.7	No Zone	No Zone
0.8		No Zone		
0.5				

Table 3: Antibacterial activity of plant extract from *B. monnieri* at different dilution (of zone of inhibition in mm)

Dilution of plant extract (mg/mL)	*E. coli*	*B. amyloliquifacienes*	*S. aureus*	*P. aeruginosa*
30	1.5	1.5	1.4	1.6
20	1.3	1.4	1.2	1.1
10	1.2	1.3	1.1	1
8	1.2	1.1	1.4	No zone
5	1.1	1.1	1.3	
3	1.1	1	1.3	
1	1	1	1.2	
0.8	No zone	1	No Zone	
0.5		1		
0.3		0.9		
0.1		No Zone		

Table 4: Antibacterial activity of extract of plant extract from *R. serpentina* at different dilution (diameter of zone of inhibition in mm)

Dilution of plant extract (mg/mL)	*E. coli*	*B. amyloliquifacienes*	*S. aureus*	*P. aeroginosa*
30	1.6	1.5	1.4	1.6
20	1.5	1.4	1.2	1.1
10	1.4	1.3	1.1	1
8	1.1	1.1	1.4	no zone
5	0.9	1.1	1.3	
3	0.8	1	1.3	
1	No zone	1	1.2	
0.8		1	No Zone	
0.5		1		
0.3		0.9		
0.1		No Zone		

Table 5: Antibacterial activity of plant extract from *C. longa* at different dilution (of zone of inhibition in mm)

Dilution of plant extract (mg/mL)	*E. coli*	*B. amyloliquifacienes*	*S. aureus*	*P. aeruginosa*
30	1.5	1.5	1.4	1.6
20	1.3	1.4	1.2	1.4
10	1.2	1.3	1.1	1.3
8	1.1	1.0	1.0	1.2
5	1.0	0,9	0.9	1.0
3	0.8	, 0.7	, 0.7	0.8
1	No zone	No zone	No zone	No zone
0.8				
0.5				
0.3				
0.1				

The current study showed the most of the plant extract have antibacterial activity against some of the common microorganism of human. This work could justify their traditional use in treatment of different diseases in human.

CONCLUSION

This study investigated that Ajwain (*C. copticum*) and Brahmi (*B. monnieri*) were more bacterial toxic as compared to Turmeric (*C. longa*) and Sarpgandha (*R. serpentina*) at 1mg/mL, 0.8mg/mL, 0.5mg/mL, 0.3mg/mL. The crude methanolic extract of plant showed significant antimicrobial activity against test strains. This indicated the great potential of these plant extracts as effective antimicrobial agents that can be used as single or in combination in medicines or can be used as natural food preservatives to retain the quality of food and prevent its spoilage.

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