Anti-Bacterial Activity of Crude Extracts of Some Local Plants from Kashmir Valley

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ABSTRACT

Some crude extracts of different parts of *Artemissia* spp. *Berginia* spp. *Cannabis sativa*, *Euphorbia* spp. *Indigofera* spp. *Nepeta* spp. *Organum* spp. *Prunella* spp. *Punica granatum* and *Tragopogon* spp. the plants commonly found in the Kashmir valley, were screened for in vitro anti- Bacterial activity against Gram +ve bacilli and gram –ve colliforms. It was observed that two plants. e.g., *Cannabis* spp. *and Punica granatum* exhibited notable anti-bacterial activity while in the remaining plants the response could not be conclusively established.

INTRODUCTION

The Himalaya, situated between 75ºE-97ºE longitudes and 27ºN-37ºN latitudes, has different mean elevations of 6000m, 4500m and 1200m which provide it with both great longitudinal and altitudinal diversity. The resultant great diversity of climates, which in turn is manifested in great biological diversity. Kashmir, being a Himalayan state, also shares some of this biological wealth .Due to the absence of modern medical facilities until recently, people of the state were using certain plants in their folk medicine to treat different ailments. Though these plants have usually proved effective medicines but there has always been felt a need to scientifically establish their medicinal value. If we were able to prove these plants medically viable, they would be huge potential resource base of modern medicine.

The current study was aimed at establishing medicinal value of some plants, *Artemisia* spp., *Berginia* spp. *Cannabis sativa, Euphorbia* spp. *Indigofera* spp. *Nepeta* spp. *Origanum* spp. *Prunella* spp. *Punica granatum* and *Tragopogon* spp. reportedly used to treat different ailments such as sepsis, dysentery, diarrhea, cholera, tetanus, and skin eruptions (Kaul, 1997 and Bhattacharjee, 2004). The objective of this study was, therefore, to establish the reported medicinal value of these plants by determining the presence of anti-bacterial activity in crude extracts of these plants. For this purpose crude aqueous, methanol, ethanol and propanol extracts were tested against the pure cultures of gram-positive Bacilli and gram-negative coli forms.

MATERIAL AND METHODS

The standard method recommended for screening of plants was followed. The main steps involved were:

(i) Collection of plant material: The plants for study were mainly collected from Gulmarg area of northern Kashmir, an important site for medicinal plants in the valley. Some of the collected plant material was also air dried to reduce the moisture content.

(ii) Preparation of extracts: The fifty percent of aqueous extract of aerial parts of *Artemisia* spp. *Cannabis* spp. *Euphorbia* spp. *Nepeta* spp. *Origanum* spp. *and Prunella* spp. flower infusion of *Indigofera* spp. and fruit rind of *Punica granatum* was prepared by solvent reduction method. The 10% solvent (methanol, ethanol, isopropanol) extracts of the above mentioned plant parts were also prepared using cold process.

(iii) Micro-organisms and growth conditions: The test micro-organisms used in the study consisted of Gram +ve bacilli and Gram –ve colliforms, the pure culture of these organisms was obtained by isolating them on their selective media, Blood agar, and Mac Conkey's agar resp.ectively. The pure culture for test purposes was then maintained on Nutrient agar at 37° C and pH 7 (Anantnarayan and Panikar, 2000).

(iv) Bacterial susceptibility test: The anti-bacterial activity of extracts was measured in-vitro by disc-diffusion method (Brooks *et al.*, 2000). For this purpose, sterile test nutrient agar plates were first surface inoculated with suitable inoculums (Gram- positive Bacilli and Gram-negative Coli forms). Next sterile diffusion discs of diameter 6mm and thickness 1mm were transferred onto these plates. These discs were then laden with 25µl, 50µl, 75µl and 100µl of plant extracts. For each extract, the activity was tested on both Gram-positive Bacilli and Gram-negative coliforms. The plates were inoculated and at the end of incubation period were observed for the appearance of microbial inhibition zones around the discs.

To asses the effect of solvents utilized for extraction and to account for any other untoward factor affecting the culture, reference plates with only solvents applied onto discs were also incubated alongside test plates.

RESULTS

Of the various extracts prepared from different parts of the selected plants, only the extracts of *Cannabis* spp. and *Punica granatum* exhibited visible anti-bacterial activity against the test organisms. The anti-bacterial activity of the rest of the plant extracts against the test organisms could not be conclusively established. Of the different extracts

tested only methanol and iso-propanol extracts of aerial parts of *Cannabis* spp. were found to be active against the test organisms (Elsohloy and Turner, 1981). In case of *Punica granatum* only the aqueous and methanol extracts of fruit rind showed a good activity against the test organisms (Phulan and Khular, 2004). Further it was observed that for all the extracts with positive anti-bacterial activity the diameter of zone of inhibition around the discs laden with these extracts increased as the amount of extract applied on the discs was increased as depicted in the Tables 1 and 2.

Table 1: Details of the anti-bacterial activity of the crude extracts of aerial parts of *Cannabis* spp. with respect to the test organisms

Organisms	Extract	Amount applied on discs (µl)	Diameter of inhibition zone (mm)
Gram-positive Bacilli	Methanol (10%)	(i) 25 (ii) 50 (iii) 75 (iv) 100	(i) 0 (ii) 7 (iii) 11 (iv) 15
Gram-negative Coli forms	Methanol (10%)	(i) 25 (ii) 50 (iii) 75 (iv) 100	(i) 0 (ii) 6 (iii) 11 (iv) 13
Gram-positive bacilli	Iso-propanol (10%)	(i) 25 (ii) 50 (iii) 75 (iv) 100	(i) 0 (ii) 11 (iii) 17 (iv) 20
Gram-negative Coli forms	Iso-propanol (10%)	(i) 25 (ii) 50 (iii) 75 (iv) 100	(i) 0 (ii) 8 (iii) 11 (iv) 15

Table 2: Details of the anti-bacterial activity of the crude extracts of fruit rind of *Punica granatum* with respect to the test organisms

Organisms	Extract	Amount applied on discs (µl)	Diameter of inhibition zone (mm)
Gram-positive Bacilli	Aqueous (50%)	(i) 25 (ii) 50 (iii) 75 (iv) 100	(i) 10 (ii) 14 (iii) 18 (iv) 23
Gram-negative Coli forms	Aqueous (50%)	(i) 25 (ii) 50 (iii) 75 (iv) 100	(i) 9 (ii) 13 (iii) 16 (iv) 20
Gram-positive bacilli	Methanol (10%)	(i) 25 (ii) 50 (iii) 75 (iv) 100	(i) 6 (ii) 8 (iii) 10 (iv) 14
Gram-negative Coli forms	Methanol (10%)	(i) 25 (ii) 50 (iii) 75 (iv) 100	(i) 9 (ii) 12 (iii) 15 (iv) 20

DISCUSSION

The test organisms selected for this study, i.e. gram-positive Bacilli and gram-negative Coli forms, are responsible for various common ailments such as gastro-enteritis, urinary tract infections, diarrhea and septicemia (Anantnarayan and Panikar, 2000). The trend of increasing zone of inhibition around the discs applied on the bacterial cultures as the amount of extract applied on these was increased could be explained on the basis of increased diffusion of active principle from the discs as more extract was applied on the disc, more active components were released from it which inhibited the growth of bacteria. This shows the anti-bacterial activity of these extracts was concentration dependent since the rate of diffusion is directly proportional to the concentration. Thus, more the concentration of active principle within the discs, more they will diffuse out onto the culture plates.

The results also indicate the extracts to be in general more active towards Gram +ve bacilli as compared to Gram -ve forms. This is to be expected, since outer membrane of Gram -ve bacteria is known to present a barrier to penetration of numerous biologically active molecules and the peri-plasmic space contains enzymes which are capable of breaking down foreign molecules introduced from outside.

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